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PLENARY LECTURE

PL-01

Hemostasis; A Biochemical Overview

Asim Orem

Department of Medical Biochemistry, KTU Medical Faculty, Trabzon, Turkey

Blood plasma and the cells in vascular bed compatible with the vessel endothelial surface provide continuous perfusion of the tissues. Hemostasis is the process of blood clot formation at the site of vessel injury [1-3]. When a blood vessel wall is disrupted, the hemostatic response must be quick, localized, and carefully regulated. Abnormal bleeding and thrombosis are seen in the absence or dysfunction of the elements in this system. In this regard, we need to define two terms;

Hemophilia; Patients or clinical pictures or who do not stop bleeding or blood easily compared to a normal person due to hereditary or acquired reasons.

Thrombophilia; Patients or clinical pictures with more thrombotic episodes than a normal person due to hereditary and/or acquired reasons.

Hemostasis is examined under three main topics (Fig. 1);

1. Primary Hemostasis (weak plug): Vasoconstriction (immediately), platelet adhesion (in seconds), platelet aggregation (in minutes).
2. Secondary Hemostasis (rigid plug): Activation of coagulation factors, fibrin formation and termination of clotting by antithrombotic control mechanisms (in minutes).
3. Tertiary Hemostasis (fibrinolysis); Fibrinolysis stimulation (in minutes), dissolution of clot (in hours)

Three main components in vascular system play an important role to operate in a coordinated manner for carrying out hemostasis; Vascular endothelial cell,platelets and coagulation proteins.

Vascular endothelial cell; The robust, undamaged endothelial cell contributes directly to hemostasis by synthesizing and secreting many proteins and biomolecules. These factors include von Willebrand (vWF), thrombolomodulin, heparin like molecule, PGI2, nitric oxide (NO), endothelin (ET) and Tissue Factor (TF).

Platelets; It is the smallest blood cell and has no nucleus. It is synthesized from megakaryocytes in the bone marrow, has an average half-life of 10 days, founds as 200-400 thousand/µL in the bloodstream. One third of circulating platelets are found in the spleen. It plays an important role in primary hemostasis with membrane receptors and cytoplasmic granules. Glycoprotein receptors are found in platelet membranes (Gp Ia, Ib, IIb, IIIa).Platelets contains three types of granules ; alpha granules (Fibrinogen, fibronectin, factor V and VIII, PDGF, TGFb), dense bodies or delta granules: (ATP/ADP, ionized calcium, histamine, serotonin, epinephrine) and lysosomal granules [4].

Primary hemostasis: Arterial vasoconstriction is the first local response in the damaged area. It takes place with two mechanisms. The reflex neurogenic mechanism is transient and a longer-term vasoconstriction is achieved with TxA2 secreted from the platelets. The goal is to reduce the surface of the damaged area to reduce blood loss from this damaged area and increase the contact of platelets with this area. The platelet plug allows closure of the damaged area with platelets such as a plug to stop blood loss from the damaged area. Platelets achieve this in three stages [4-6].

1. Adhesion of platelets; It is the process of holding platelets to the subendothelial connective tissue components (collagen) exposed in the damaged area. The platelets attach to the collagen and other connective tissue components via their membrane surface proteins GPIa/IIa and GPIb/IX/V and vWF.
2. Activation of platelets; Following adhesion, membrane surface receptors (such as GPIIa/IIIb) are activated in platelets, while intracellular granules are secreted by activation. Many biomolecules such as ADP, epinephrine, thrombin and collagen play an important role in the activation of platelets. ADP and epinephrine are relatively weak platelet activators, while collagen and thrombin are the most potent platelet activators.
3. Aggregation of platelets; Platelets undergo significant shape changes, producing elongated pseudopods that make the platelets extremely adhesive. Platelet activation results in both exposure of and conformational changes in the GPIIb/IIIa receptor on the platelet surface, leading to binding of both immobilized VWF and fibrinogen Thus, locally activated circulating platelets collapse onto the platelets in the damaged area and form the thrombocyte plug.

Secondary hemostasis: In the damaged area, the plug formed by thrombocytes is a weak plug. In the face of strong blood flow, this plug cannot be long-term and may break. The coagulation proteins in the blood begin to take part in the event while platelet plugs are formed. The central feature of the clotting cascade is the sequential activation of a series of proenzymes or inactive precursor proteins (zymogens) to active enzymes, resulting in significant stepwise response amplification. As an example, the generation of a small number of factor VIIa molecules will activate many molecules of factor X, which in turn generates even larger numbers of thrombin molecules, which then converts fibrinogen to fibrin. The resultant local generation of fibrin, in turn, enmeshes and reinforces the platelet plug.

The endothelial damage-induced tissue factor initiates the extrinsic pathway in the coagulation cascade, while the intrinsic pathway may be activated with other interaction. Negative surfaces exposed on aggregated thrombocyte membranes in the damaged area provide the return to the active form of the coagulation proteins from the zymogen form by the effect of calcium in the blood. They are expressed as roman numerals. These factors (FI, FII, FIII) are in the form of zymogen in the blood, when the system is activated they return to the active forms (FIIa, FIIIa..). Some of the activated factors act as active serine proteases (F XII, F XI, F IX, F X, F II (Prothrombin)) while some of them act as cofactor (F V, F VIII). There is a need for vitamin K for posttranslational modification and maturation of coagulation proteins including F II, F VII F IX, F X, protein S and protein C. Gamma carboxylation provided in these proteins increase theirs negative charge, which allows the interaction of the negative surface of the thrombocyte membrane phospholipids with F IV (calcium). The common pathway of the coagulation cascade starts from F X, and produced F Xa leads to the conversion of prothrombin into thrombin. Thrombin is the most important molecule to show the activation of the coagulation cascade. Thrombin converts fibrinogen into fibrin monomers. The polymerization of this structure results in the formation of a strong fibrin network between and on the surface of aggregated platelets. Thus, a weak

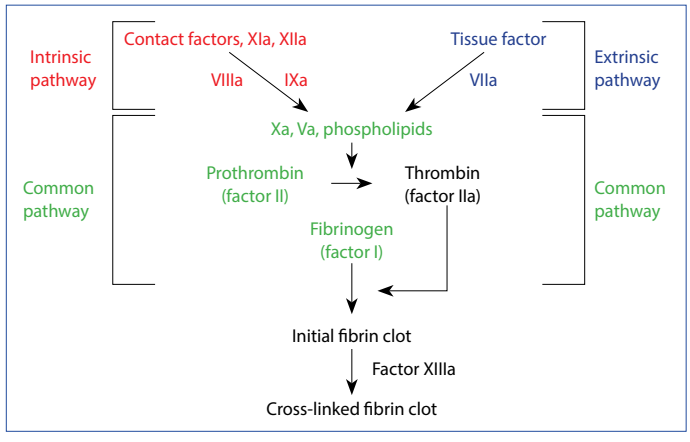

Figure 1. Intrinsik and extrinsic pathways in hemostasis.

platelet plug with primary hemostasis is converted into a solid plug with fibrin network [3,7].

Anti-coagulant mechanisms; With the activation of coagulation a stable plug is formed. However, the system should somehow stop itself and inactivate the increased active factors, especially the major factors of the common pathway (anticoagulant effect). Thrombin is the main product of the common pathway. The activation of antithrombin III in the blood with heparin results in thrombin binding. Thus, the return of fibrinogen to fibrin, the most important pillar of the cascade, is stopped. Tissue Factor Pathway Inhibitor (TFPI) also binds TF-VIIa and Xa complex. The C1 esterase inhibitor inhibits F XIIa and F IXa together with the active complement. Thus, an important pathway is stopped and thrombin formation is prevented. In addition, protein S and C the proteins expressed in the liver, provides the destruction of FVa and VIIIa. Thus, the common pathway is substantially become stopped [1-3,8].

Fibrinolysis (Tertiary hemostasis): Although blood loss has been prevented from the damaged vessel, there is now a strong plug on the surface of this area which has impaired endothelial integrity and occupies space towards the vessel lumen. Primary and secondary hemostasis occur within seconds and minutes, the complete elimination of this plug occurs within hours and days. The tissue plasminogen activator (t-PA), which is released from the endothelium, allows the plasminogen in the blood to return to the plasmin. Plasmin also returns fibrin to fibrin degradation products. When the plug is completely removed, the endothelial tissue progresses from the sides of the lesion and re-covers the area. Plasmin is neutralized with alpha2-antiplasmin in the blood [8,9].

Commonly used tests for hemostasis and thrombosis [9,10]:

1. Bleeding time; Demonstrates the interaction of thrombocytes with the vessel wall. When the platelet count is below 50.000, bleeding time becomes longer. The amount, structure and functions of platelets are checked. With the Duke test: 1-4 min, critical value <5 min.
2. Platelet count, structure and functions; functions can be examined by specific systems such as peripheral smear, number and structure with CBC, PFA 100.
3. Prothrombin time (PT) and INR; Shows the extrinsic pathway; tissue factor, FVII, FX, FV, FII, FI. It is used to monitor the effectiveness of oral anticoagulants (Warfarin). The reference range; 10-13seconds.International standardized ratio (INR) with standardization of reagents in measurement systems is more widely used in clinics: (Patient PT/Control PT) ISI=0.9-1.3, ISI: International Sensitivity Index (for the tissue factor used in the test).
4. Activated partial thromboplastin time (aPTT); Indicates the intrensek pathway (FVIII, FIX, FXII), the reference range is 25-35 seconds. Heparin usage is followed by aPTT test.
5. Thrombin time (TT): Shows the final stage of coagulation, the transformation of fibrinogen into fibrin. In the citrated plasma, clot formation is monitored by incubation of thrombin. Reference range: 14-9 sec. TT is prolonged in the presence of an anticoagulant that blocks thrombin or low levels of fibrinogen. Contrary to PT and aPTT, TT is not used as an initial screening test for hemostatic anomalies. It is used for evaluation of patients with prolonged PT and aPTT. It is used in hereditary fibrinogen disorders. It is also used to detect the presence of heparin in the sample. If heparin is present, TT is prolonged, and reptilase time (RT) is normal. The RT mechanism is similar to TT but cannot be inhibited by heparin.
6. Factor analysis; F VIII (hemophilia A), F IX (hemophilia B), F XI, fibrinogen.
7. Fibrinolysis analysis; Fibrinopeptides A and B, D-Dimer, Fibrin degradation products (FDP).
8. D-dimer; One of the fibrin fragmentation products which is degraded by plasmin. It is a dimer composed of F-XIIIa cross-linked D domains of two monomers side by side. The increase in D-dimer indicates newly initiated or ongoing intravascular coagulation and fibrinolysis. Reference <500 ng/mL. Compared to the other FDPs, D-dimer is the most studied and validated by clinical evaluations.

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PL-02

Pre-Pre-Analytic Phase in Hematological Tests

Dildar Konukoglu

Department of Biochemistry, Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Istanbul, Turkey

Diagnostic testing phase has been divided into five phases: pre-pre-analytic, pre-analytic, analytic, postanalytic, and post-post-analytic. Pre-pre-analytic steps contain initial procedures not performed in the clinical laboratory and outside the control of laboratory personnel (i.e. patient identification, the time of test order, appropriateness of the test, sample collection and sample transportation. The post-postanalytic steps are final procedures performed outside the laboratory, i.e. receiving, interpreting, and using laboratory information for patient management. It has been suggested that pre-pre-anaytic and post-post analytic phases are more error-prone than the other phases.

Studies confirmed that pre-analytical errors are estimated to account for up to 70% of all mistakes made in laboratory diagnostics. In particular, studies performed on the pre-pre-analytical phase show that failure to order appropriate diagnostic tests are related to the missed and delayed diagnosis. Laboratory related causes of diagnostic error are classified as; inappropriate test ordered, appropriate test not ordered, appropriate test result not used properly, appropriate test result delayed/missed or appropriate test result wrong/inaccurate. Hematology tests such as complete blood count (CBC), peripheral blood smear and coagulation analysis are commonly ordered tests in clinical laboratories. Interpretive testing can increase the value to laboratory services. Use of well-defined diagnostic algorithms algorithms can improve patient experience by decreasing sample volume necessary for diagnostics. It may also improve patient out- come by preventing misdiagnosis due to the effect of interfering drugs or incorrect clinical ordering. Overutilization of test is the common pre preanalytical error in laboratory tests as well as hematology test. On the other hand, the physician, biochemistry specialist and electronic medical record systems have an important role in the inappropri-

ate test orders. In relation to this, reflex testing and reflective testing are an important for the early intervation of the patients care. Althought reflex testing is performed automatically according to the guidelines without the intervention of the ordering physician reflective testing is a procedure in which the laboratory specialist evaluates abnormal test results and decides whether additional tests are needed. Therefore clinicians and biochemistry specialists should prepare and implement algorithms in collaboration. The communication and education between clinicians and biochemistry specialists are very important for patient safety.

PL-03

Preanalitical Errors in Hemostasis Testing: Phlebotomy, Transport, Centrifugation and Storage

Evin Ademoglu

Department of Medical Biochemistry, Istanbul University, Istanbul Faculty of Medicine, Capa, Istanbul, Turkey

Technological developments, effective internal quality control systems and external quality assuring systems with improved automated test methodologies and competency audits of laboratories have led to a considerable decrease in analytical errors. However, the test results that are either incorrect or incompatible with the clinical status of the patient are still being reported. It has been shown that erroneous test results are most frequently related to the preparation of the patient before the test, phlebotomy, handling and processing the samples. Performing the tests at the wrong sample or patient or at the wrong time or using inappropriate reference intervals are the less frequent reasons of erroneous test results. All the factors within these headings are associated with the pre-preanalytical and preanalytical stages and are called preanalytical variables. By the effects of preanalytical variables the test results may accurately reflect the status of the sample being tested, but often do not accurately reflect the clinical status of the patient being investigated and may lead to erroneous clinical decisions [1].

Among clinical laboratory tests, hemostasis tests are the most sensitive test group to preanalytical variables due to the nature of the biological sample (citrated plasma) being used. Since sample collection, transfer, centrifugation and storage steps are manual, multifactorial and complex, and are often outside the control of the laboratory performing the tests, hemostatic system testing is prone to random (or unpredictable) preanalytical errors. Furthermore, the laboratory is often unaware of the adverse events related to test results, which makes the control of the entire testing process more challenging. Leading causes of preanalytical errors in hemostatic system testing can be summarized as follows [2-12]:

I. Blood Collection

Patient-sample identification: This process should be performed with the active involvement of patient and at least two different patient identifiers should be used, such as name, date of birth, place of birth [9].

Posture: Blood samples should be always collected in the same reference position (seated or lying down) after the patient has been rested for at least 15-20 minutes. The transition from supine position to sitting position leads to significant bias in prothrombin time (PT) and an fibrinogen level.

Selection of the blood collection site: Due to the anatomic distribution of the cutaneous veins and nerves and, the diameter and pressure of the veins the most appropriate site for venous blood sample collection with minimal risk is the antecubital fossa. Collection from small veins, such as metacarpal veins or intravenous (i.v) catheters, are not recommended, they may significantly increase the risk of in vitro hemolysis. Central venous sets and i.v sets are not suitable for blood collection because they may lead to partially coagulated or activated samples or air-related incomplete filling, or diluted sample with i.v solution, or samples contaminated with heparin.

As it may cause cell injury, platelet activation, and contamination of cellular procoagulant substances the venipuncture should be atraumatic. When the first venipuncture attempt is unsuccessful, the procedure should be stopped and, repeated at another physical site.

Tourniquet application: Tourniquet should be applied less than 1 minute thus it should be released immediately when the first tube starts to fill. Prolonged applications may cause spurious changes in coagulation assays that may result in erroneous clinical decisions by the effects of hemoconcentration, hemolysis and endothelial cell activation. On the other hand, since it may cause false positive results in some specific tests related to thrombin formation, tourniquet should not be used.

Selection of blood collection devices: Tubes for coagulation assays are typically identified by the light blue cap, which signifies sodium citrate as anticoagulant. An intermediate size (19 to 21 gauge) needles and evacuated light blue caped blood collection tubes should be preferred for blood collection in hemostasis testing. Blood sample should be drawn directly into the primary tube and, should not be transferred from one tube to another. As they may induce in vitro clot formation or hemolysis and platelet activation, too small or too large size needles should not be used. Use of butterfly needle, i.v catheter and syringe should be avoided. They may adversely affect test results depending on the contact rate of blood with the tube surface, transfer method to the tube, anticoagulant addition time, and rate of hemolysis, clot formation and air-related incomplete filling.

Concentration of sodium citrate: The Clinical and Laboratory Standards Institute (CLSI) guidelines on the blood collection for coagulation testing recommend the use of tubes containing 3.2% (105-109 mM) buffered trisodium citrate and the pH of the anticoagulated plasma should be between 7.3 and 7.45. However, the tubes with 3.8% (129 mM) citrate concentration may also be used. Higher citrate concentration leads to greater calcium binding and longer clotting times. It should be noted that samples collected in 3.8% sodium citrate have prolonged PT and aPTT and underestimated fibrinogen in comparison to 3.2% citrated samples. Due to the variation in test results with regard to sodium citrate concentration, it is recommended that laboratories should standardize to one citrate concentration and develop appropriate reference intervals.

The order of blood draw: To avoid carry-over of additives between tubes, CLSI guidelines recommend that the order of draw during blood collection should be as blood culture tubes, then coagulation tubes, then plain tubes/gel separator tubes, then tubes containing additives other than citrate. According to the CLSI, blood drawing to a first discard tube (or a non-additive tube) is unnecessary for routine coagulation assays. In order to avoid air-related incomplete filling, a discard tube should be drawn when blood sample is collected using butterfly systems or other i.v catheter devices. A discard tube is also recommended when blood samples are collected for platelet function tests and thrombin generation measurements.

The appropriate filling of primary tube: Tubes should be appropriately filled as indicated on the tube or to not less than 90% of the total tube volume in order to produce appropriate blood-to-citrate ratio, which is fixed at one-part anticoagulant with nine-parts of blood. Under-filling may cause significant sample dilution and due to the excess ci¬trate, generates a significant bias, especially for APTT and underestimation in the levels of coagulation factors.

Mixing the samples: Following collection, blood samples should be promptly mixed with three to six complete end-over-end inversions of the tubes to ensure homogeneous distribution of anticoagulant in order to limit the risk of micro clots, clots or fibrin filament formation.

It should be noted that inappropriate mixing or leaving the blood sample unmixed causes partial clotting whereas vigorous shaking or vortexing causes hemolysis and, activation of platelets and coagulation factors.

II. Sample Transfer

The CLSI guidelines recommend transferring of the blood samples to the laboratory within 1 hour following collection at room temperature (15-22 °C) in the vertical position. During transfer, the blood samples should remain capped and excessive agitation should be avoided. Delays in transfer most often affect unstable factors such as factor V, factor VIII and causes prolonged clotting times and degradation of coagulation factors. If the transfer of blood samples will be delayed, separation of plasma followed by freezing and the transfer of the frozen plasma samples on dry-ice should be considered. Blood samples for hemostasis testing should not be transported on ice or in low temperatures. Low temperatures induce factor VII activity, platelet activation and may cause a reduction in fibrinogen, factor VIII, XIII, and vonWillebrand factor which may lead to a misdiagnosis of factor VIII and XIII deficiencies or, vonWillebrand disease. On the other hand, high temperatures cause rapid loss of temperature-sensitive hemostasis factors, such as factor V, VIII and protein S.

Depending on the acceleration and deceleration speeds and, the produced vibration, transfer of blood samples by pneumatic tube systems may have some adverse effects on hemostasis testing such as hemolysis, platelet activation. It is recommended that each hospital will validate their pneumatic tube system for hemostasis testing. In any case, due to the adverse effects on platelet function tests and thromboelastographic techniques, pneumatic tube system should not be used for these tests.

III. Centrifugation

For hemostasis testing blood samples should be ideally centrifuged once at 1500g no less than 15 minutes at room temperature (15-22 °C) to achieve platelet poor plasma (<10x10^3/µL platelets). Alternatively, refrigerated centrifuges may be used but, as low temperatures can cause platelet activation and induction in the activities of some clotting factors, temperature should be set to 15-22 °C. Using centrifugal forces greater than 1500 g is not recommended to avoid the risk of platelet activation and hemolysis, whereas; shortening the centrifugation time might be acceptable for routine coagulation tests that will be performed immediately after centrifugation. In order to eliminate platelet debris double centrifugation recommended for some plasma some samples, such as those for lupus anticoagulant testing.

IV. Sample Storage

It is recommended that to maintain sample stability, samples should be processed within 1 hour of collection, and analyzed within 4 hours after centrifugation. During the interval from collection to processing, whole blood samples should be stored at room temperature and remain capped to minimize loss of CO_2, which causes increase in pH and, as a result prolonged PT and/or aPTT. Whole blood samples should never be stored in the refrigerator since it may cause factor VII activation and a gradual degradation in vonWillebrand factor and factor VIII.

The plasma samples for hemostasis testing can mostly be stored at room temperature or in refrigerator for a few hours without any adverse effects. If the samples will not be analyzed within 4 hours following centrifugation, the plasma samples should be separated and frozen. It is reported that plasma samples for hemostasis testing should be stored two to four weeks at −20 °C and, several months at −80 °C. The frozen samples should be immediately analyzed, after completely thawed in a 37°C water bath and gently mixed. Repeated freeze and thaw cycles should be avoided.

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PL-04

Complete Blood Count, Blood Smear and Preanalytic Variables

Cıgdem Karakucu

Department of Biochemistry, Kayseri Education and Research Hospital, Kayseri, Turkey

Hematological measurement test are occasionally diagnostic tests those impact patient's diagnosis and treatment. For that reason, to know the preanalytical variables effecting results have more critical importance. Many physiological variables influence the results of complete blood count measurements. Also, specimen collection and processing variables, stability of cellular constituents affect hematological results. Abnormal concentrations of selected biochemical analytes can impact on the quality of hematological results.

1. Physiological Variables

Age and Gender

Hemoglobin (Hb) and Red blood cell (RBC) counts are at a maximum in neonates and they sharply change in newborn age even during days (Table 1). Age-related changes are also seen for leukocyte or white blood cell (WBC) counts and neutrophil and lymphocyte counts (Table 2). RBC count, hematocrit and Hb values are slightly lower in females when compared to males. For that reason, on Complete Blood Count (CBC) reports the true reference range information must be given according to age and gender.

Body mass index: The variability of body mass is not deeply influencing the stability of haematological parameters and Hb values.

Table 1. Progression of reference intervals from newborn to adult values

#s given reflect mean values. Adapted from reference*

Age	Hb (mmol/L)	RBC (x10^{12}/L)	MCV (fl)
1-3 days	115	5.3	108
2 weeks	103	4.9	105
2 months	72	4.0	96
2-6 years	78	4.6	81
12-18 years			
Male	90	4.9	88
Female	86	4.6	90

fl: Femtoliter; *Perkins SL. Normal blood and bone marrow values in humans. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, eds. Wintrobe's Clinical Hematology. 10th edition, Vol. 2, Baltimore: Williams and Wilkins 1999:2738–48.

Table 2. Range of WBC, neutrophils and lymphocyte counts: newborn to adult

#s reflect mean values. Adapted from reference*

Age	WBC (x10⁹/L)	Neutrophiis (x10⁹/L)	Lymphocytes (x10⁹/L)
12 hours	22.8	15.5	5.5
24 hours	18.9	11.5	5.8
1st week	12.2	5.5	5.0
6 months	11.9	3.8	7.3
2 years	10.6	3.5	6.3
6 years	8.5	4.3	3.5
21 tears	7.4	4.4	2.5

*Perkins SL. Normal blood and bone marrow values in humans. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, eds. Wintrobe's Clinical Hematology. 10th edition, Vol. 2, Baltimore: Williams and Wilkins 1999:2738–48.

Exercise: Increases WBC count. Strenuous exercise can also result in intravascular hemolysis.

Altitude: High altitude of 1.400 m has an effect of raising the Hb and hematocrit up to 8%.

Smoking: Long-term smoking has an impact on hematological constituents in blood. Cigarette contains carbon monoxide, which has a much greater affinity for Hb than oxygen, carbon monoxide levels in blood are increased. There is also an increase in h, WBC, and RBC counts. The MCV is also in-creased since the RBCs become larger.

Alcohol: Chronic alcoholism results in an increase in MCV which may be due either to a direct toxic effect of ethanol on erythropoiesis or due to folate deficiency.

2. Specimen Collection and Processing Variables

Whole venous blood should be collected. Alternatively, blood collected by skin puncture may be used. Standard precautions should be followed. Anticoagulant is used for specimen collection.

Which Anticoagulant?

Ethylene diamine tetraacetic acid (EDTA) is the most commonly used anticoagulant for routine hematological determinations. The free acid solution of EDTA shows a pH of 2.5±1.0. The pH of EDTA varies depending on the salt that is used. The disodium (Na_2) and di-potassium (K_2) EDTA salts have a lower pH compared to tri-potassium (K_3) EDTA whose pH is 7.5±1.0. K_2EDTA, in contrast, has a pH of 4.8±1.0. The EDTA salts are hyperosmolar, causing water loss from cells. The cell shrinkage is less apparent when K_2 and Na_2 EDTA are used. For that reason generally K_2EDTA salts are recommended.

Does the Concentration of Anticoagulant Impact?

The concentration is crucial. NCCLS recommended a K_2EDTA concentration of 1.5–2.2 g/L. When the concentration of EDTA is increased, the stability of cells decrease and MCV measured by automatic instruments is variably influenced, but it basically tends to rise. When the concentration of EDTA is decreased, the anticoagulant effect is also decreased and micro or macroscopically clots occur which effect mainly platelet (PLT) results. Macroscopically visible clots are cause for rejection of the specimen for analysis. Microscopically visible PLT clumps consisting only of PLT are acceptable, if their presence is noted.

Mixing

The mixing procedure of the tubes after the blood drawing and before analysis is also crucial for obtaining correct and valid data. For example, when compared with the reference specimens inverted 6 times, results on unmixed specimens revealed significant decreases RBC, Hb, haematocrit and PLTs count, whereas the mean PLT volume was significantly increased.

The proper preparation and treatment of the tube is fundamental for obtaining correct data: overfilling the collection tube leads to inadequate sample mixing and setting of cellular contents: all the parameters are altered.

Stability of Cellular Constituents:

Time-dependent morphological changes occur in cells upon collection of blood in. The stability of hematological parameters is high: Hb and haematocrit are stable for 48 hours, PLTs and erythrocyte indexes for 24 hours. The stability of Ret is lower than that of other haematological parameters and it is also dependent on analytical technique. Leukocytes are stable for 48 hours, but the differential count must be done before 6 hours from blood drawing.

The EDTA-Induced Pseudothrombocytopenia

EDTA-dependent pseudothrombocytopenia is an in vitro phenomenon of PLT agglutination that is encountered in blood collected in EDTA due to the presence of antibodies in blood that react with PLTs. These antigens become exposed when EDTA chelates calcium. At temperatures lower than 37° C the exposed PLT antigen further becomes modified to permit PLT-derived antibodies to agglutinate PLTs. The EDTA-induced pseudothrombocytopenia may be detectable on some automated systems by means of flags and graphics, and can be differentiated by comparing data obtained on EDTA- and sodium citrate-collected specimens. Peripheral blood smears prepared immediately from freshly collected EDTA anticoagulated or capillary blood can also solve this phenomen.

3. Effect of Chemical Analytes and Interferences

Hyperglycemia: An increase in MCV is observed. The osmotic effect of glucose causes water to enter the RBC as the cells are suspended in an isotonic diluent leading to a spurious increase in the MCV.

Hyperlipidemia: Spuriously high Hb values are obtained in hyperlipidemic specimens with a triglyceride concentration exceeding 10 g/L.

Cryoglobulins and erythrocyte parasites: The presence of cryoproteins, cryoglobulins and cryofibrinogen can lead to spurious increases in WBC and PLT counts since the cryoprotein aggregates are counted as WBC, while the cryoglobulin crystals are counted as PLTs. This phenomenon is abol- ished in blood warmed to 370 C. Due to the turbidity resulting from the precipitation of these proteins by the lysis reagents that are used to lyse RBC by the instrument high Hb values are also obtained.

4. Specimen Mix-Ups

Preanalytical errors that might affect hematology testing include specimen mix-ups, improper specimen acquisition, and compromise of specimen integrity between when the specimen is acquired and when it is received in the laboratory.

To detect specimen mix-ups in hematology, it is best to perform delta checks. on parameters that. Mean cell volume (MCV) and mean cell Hb concentration (MCHC), show the least short-term biologic variability, such that deviations are highly unlikely to be caused by changes in patient status. Both are extremely stable in a patient for the short term-that is 24 hours. For example, the diurnal biological coefficient of variation in MCV in healthy individuals is only 0.5 percent. Even in situations of rapid change in other red cell parameters, such as in acute hemorrhage, the MCV and MCHC will not change significantly in a 24-hour period since the reticulocyte response to acute blood loss does not begin for two to three days. With the exception of red blood cell transfusion and (rarely) acute intravascular hemolysis, there are no acute patient events that will change these indices appreciably in the short term. In the case of acute hemolysis with hemoglobinemia, the MCHC may be affected, but the MCV will not be.

Table 3. Desirable qualities of a blood film

- Sufficient working area.
- Minimum 2.5 cm in length terminating at least 1 cm from the end of the slide.
- Gradual transition in thickness from the thick to thin areas, ending in a feather edge.
- Acceptable morphology within the working area.
- Narrower than the slide on which the film is spread, with smooth continuous side margins that are accessible for oil immersion examination.
- No artifact introduced by the technique.
- Minimum distributional distortion.
- A far end that becomes gradually thinner, without grainy streaks, troughs, or ridges, all of which indicate an increased number of WBC carried into this area.

5. Blood Smears

The production of good blood films and their evaluation have been detailed in Clinical and Laboratory Standards Institute document H20-A2-Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods.

Specimen Condition

The WBC should be well-preserved, and anticoagulant effects, such as excessive vacuolization or changes in nuclear shape, must be minimal. Less than 2% of the WBC may be smudged, except in some lymphoproliferative disorders. As the time between blood collection and the preparation of a peripheral blood smear increases, changes occur such as swelling of neutrophils and loss of structure in the neutrophil lobes, loss of granulation in the cytoplasm and formation of vacuoles in the nucleus or cytoplasm of cells. The mononuclear cells undergo similar time-related and concentration-related changes. PLTs undergo changes such as swelling, which can give rise to giant PLTs.

Blood Film Preparation (Advised by CLSI- H20-A2)

1. Prepare three blood films from each specimen on clean, dry, and dust-free 25x75 mm (1x3 in), 0.8- to 1.2-mm thick, glass microscope slides of good quality. Label the slides A, B, and "spare." Two blood films will be used for the procedure and the third will be kept as a spare. (If the blood is leukopenic, prepare a larger number of blood films [e.g., six].)

2. Prepare blood films within four hours of blood collection. Do not store blood in the refrigerator. Adequate mixing (20 complete inversions by hand) is necessary, before blood film preparation.

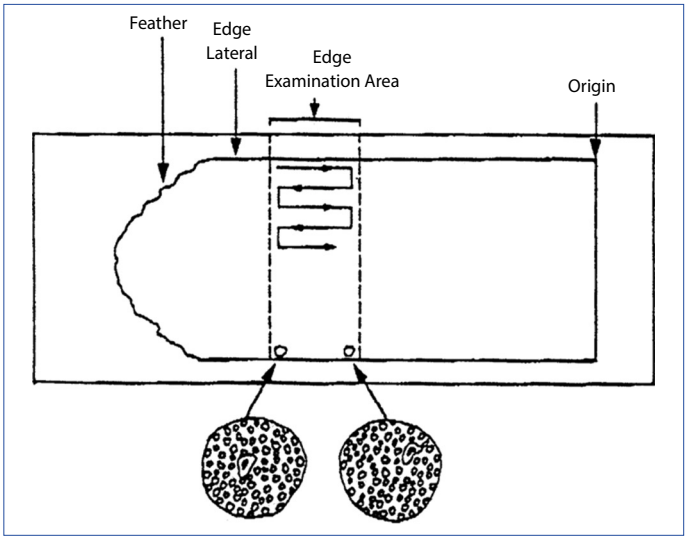

Figure 1. Blood film sample.

3. Prepare blood films using the manual wedge-pull film technique. Place one drop (approximately 0.05 mL) of well-mixed blood near one end of a glass microscope slide. Hold a second, narrower spreader slide with polished edges at about a 45° angle and immediately draw into the drop of blood. Allow the blood to spread almost to the width of the slide. Then rapidly and smoothly push the spreader slide to the opposite end of the slide, pulling the blood behind it. Alternatively, a mechanical spreader may be used (stand-alone or part of an automated spreader-stainer system), as this may offer greater uniformity; follow the manufacturer's directions.

4. Stain the film within one hour of preparation with a Romanowsky stain (see Section 6.4), containing fixatives, or fix within one hour with "water-free" (i.e., <3% water) methanol for later staining.

Desirable qualities of a blood film (Table 3) and a sample smear are demonstrated (Fig. 1).

Conclusions

True results without any preanalytic variable is important for hematological tests, however most of them are patient related or physiological variables that cannot be excluded. If the preanalytic error source can be corrected, then the sample must be rejected and a new sample must be requested. If not, at the end of analysis, any preanalytic factor recognized by laboratory must be recorded; such as any abnormal specimen condition; lipemia, icterus, or hemolysis in the supernatant plasma. It is inadvisable to let the cells settle or to centrifuge the specimen before analysis to observe the supernatant plasma.

PL-05

Preanalytic Problems in Pediatric Hematology Laboratory

Mine Kucur

Department of Medical Biochemistry, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey

Accurate laboratory results are essensial for diagnosis, treatment and follow-up of diseases. Today remarkable advances in analytical techniques, sufficient specimen collection and transport improve the performance of laboratories. Laboratory results affect approximately 70% of clinical decisions. However, it is impossiple to prevent laboratory errors that can occur in any phase during the process of blood sample. A reasonable definition for laboratory errors, acknowledged by the International Organization for Standardization (ISO), is "any defect from ordering tests to reporting results and appropriately interpreting and reacting on these.

Analytical phases are classified in laboratory practice as preanalytical, analytical, and postanalytical phase. Preanalytical phase is very impor-

tant component of laboratory medicine. Main comportments of this phase are specimen collection, handling, processing, physiological and endogenous variables. Among pediatric population the most common preanalytical error is due to incorrect phlebotomy techniques. Preanalytic errors relating to phlebotomy includes hemolyzed, insufficient, incorrect or clotted samples, and frequently empty tube and sample not protected from light (bilirubin) or not transported on ice (ammonia). Other reported types of preanalytical errors in pediatric hematology laboratory are ordering tests on the wrong patient, misidentifying the patient, ordering the wrong test, missing sample and/or test request, wrong or missing identification, contamination from infusion route, inappropriate containers, improper labeling of containers, inappropriate blood to anticoagulant ratio because of insufficient sample, and inappropriate transport (sample not protected from light or not transported on ice) and storage conditions.

Inadequate samples accounted for the majority of errors of pediatric hematology laboratory. This could be due to ignorance of phlebotomists, difficult sampling in pediatric group, patients with small and difficult veins. There is a risk of cell shrinkage and low mean corpuscular volume when less volume of blood is withdrawn than the recommended volume to tubes containing ethylenediaminetetraacetic acid (EDTA).

Clotted samples can be easily detected; however, microclots are difficult to detect, particularly anticoagulated blood samples. The presence of clots in EDTA samples can be explained primarily due to increased blood to additive ratio (inadequate EDTA) or improper mixing of the sample after collection. Diluted samples were observed only in inpatients' department where the samples can be diluted with intravenous (IV) fluids.

Since the preanalytical phase involves much more human handling compared to the analytical and postanalytical phases and commonly caused by human mistakes, these problems should be preventable by education and improvement awareness of healthcare specialists.

PL-06

Preanalytical Phase in Flow Cytometry Laboratory

Mesude Falay

Department of Hematology Laboratory, Ankara Numune Training and Research Hospital, Ankara, Turkey

Over the years, flow cytometry technology, with its multiple applications, has had a significant impact on our understanding of cell biology, immunology and haemopoietic ontogeny, allowing its application to the diagnostic challenges of clinical medicine. The basic principle of flow cytometry is inherent in the ability to analyse multiple characteristics of a single cell within a heterogenous population, in a short period of time. Modern flow cytometers have the capability to analyse. Several thousands of cells per second. Cells in suspensionpass through a beam of light (usually a laser beam) insingle file; signals generated are related to the size of thecell and the internal complexity or granularity of the cell,enabling the cytometer to identify different cell populationsdepending on these characteristics. There are a wide range of applications for flow cytometry in a number of different disciplines. However, in haematology it has become an important tool in the identification of haematological disorders from a wide range of diagnostic samples, such as peripheral blood, bone marrow, CSF, pleural effusion, ascitic fluid and lymph node aspirates. Furthermore, before this fascinating technology can be applied reliably and effectively, it is important to be aware of its limitations and preanalytical phase [1-12].

Patient information: The patient's identity information, pre-diagnosis, age, gender, treatment information (such as chemotherapy, radiotherapy) should be sent along with the patient sample. For example, the date and time of receipt, the name and type of the physician who made the request must be specified. Sample of peripheral blood/bone marrow aspiration

Samples handling and storage: All samples should be collected under aseptic conditions. In addition, all specimens (blood, bone marrow aspiration material, pleural fluid, acid fluid, cerebrospinal fluid, etc.) should be considered as potential sources of infection. The samples must be delivered to the laboratory as soon as they are received. If the samples are to be sent to another center, a stained or tissue imprint, for example, should be must be added. If the bone marrow aspiration material is to be examined, it should be compared with the sample of the peripheral blood sample and the smear to evaluate whether it has been diluted with sinus blood. Minimum 2 ml of peripheral blood and K1, 1 ml of apheresis, 1 ml of CSF should be sent. It is recommended to take CSF fluid into the sterile tube and to send it to the laboratory at 2-8 °C.

Anticoagulants: For peripheral blood samples, ethylene diamine tetra acetic (EDTA), heparin (50 U/mL) can be used. If the sample is to be disseminated for morphological evaluation, the EDTA sample is preferred. Heparin is not preferred because it affects the quality of smear. White blood cell counts should be performed within the first 6 hours after sampling. The disadvantage of the use of EDTA is that the light scattering of light scattering properties (FSC, SSC) is expected to deteriorate more quickly, while the advantage is that the mature myeloid cells cause less adhesion to the wall of the tube. Heparin or EDTA can be used, although there is no definitive definition for the anticoagulant to be used for the KI samples. If samples of body fluids (pleural, acid mai, etc.) are to be sent for losemi-lymphoma phenotyping, they should be sampled to contain 10 units/mL of non-condom heparin and the cell medium should be added and stored at 2-8 °C. EDTA/heparin/can be used for other body fluids. In order for some cell surface markers to be lost, the presence of calcium is necessary and EDTA is a risk, sodium heparin is recommended. Sodium heparin is also recommended for apheresis. The anticoagulant to be selected for specific studies may be different from that used in routine; For example, intracellular cytokine measurements should be performed with non-dedicated heparin, Ca binding agents should be avoided.

Transport of samples: All samples should be packaged and transported assuming that they carry the infection agent. Polypropylene tubes/syringes should be used for transport, and the polystyrene material should not be used because the cells may adhere to the wall of the tubes. Transport must be carried out at room temperature. As soon as possible, samples should be processed immediately after sampling. Specimens can be stored at +4 °C (preferably horizontal) in cases where it is mandatory. In this case, laboratories need to make sure that the expected samples give the same results as those that had not been expected (they should have already proved this).

Sample preparation procedures: Cells that cause cell loss should be avoided if it is known that the number of cells intended to be studied in the material is low (eg minimal residual disease follow-up).

Visual evaluation of samples: Hemolysis: a process that damages erythrocytes is likely to cause damage to leukocytes. Clot-type blood/KIA: Small clots can cause cell loss, however, if the target cells are found to be sufficient and no new sample is possible, the study can be continued. If there are visible visible clots, the sample should be rejected. Inadequate amounts of samples: especially in cases where ACD-A is used, the amount of anticoagulants is higher than desired and may cause damage due to hypertonic conditions in the cells. The temperature of the samples coming to the laboratory should be checked (freezing/overheating?). an abnormal observation must be recorded. Samples with incorrect labeling should not be accepted.

Erythrocyte lysing/selection of cells: Each procedure is a factor that increases the risk of cell loss. Tris buffered ammonium chloride or hypotonic solutions, commercial preparations, can be used as erythrocyte lysis solution (ELS) to remove erythrocytes from blood/KI samples. When using commercial preparations, refer to the manufacturer's instructions. dilution rate, amount to be used, time to wait,) must be adhered

to. Another method is to separate the targeted cells from the others using density gradients. This process may not be preferred because of the longer duration of the cell, which may cause cell losses. Specifically, the tolerance of the cells to the erythrocytes used in the losemic blood samples may be different. It must be known. Tissue biopsy or fine needle aspiration materials must be delivered freshly to the laboratory without detection. Biopsy specimens can be carried in a sterile environment, in a gauze soaked with physiological serum, at room temperature. Fat, connective tissue and normal tissues should be removed from the pathological stage before they are free of tissue. The tissue sample to be examined was taken into a plastic petri dish containing a medium that feeds the cells (eg, RPMI 1640, +4 °C); mechanically disassembled (cutting with scalpel) and nylon filters. Enzymes can also be used to release the cells, but the enzyme used has to be proven not to cause damage/alteration of the antigenic epitopes to be studied. The material obtained by fine needle aspiration can be immediately placed in a nutrient medium and delivered to the laboratory.

Adjustment of cell concentration: Although Uretifiers have defined the concentration at which concentrations of monoclonal antibodies should be used, these values are generally calculated by reducing the number of normal cells. If the number of cells exceeds the optimum values, all of the antigenic determinants on the surface of the cells cannot react with the target antibody to yield false-negative results. If the number of cells is too high, it may cause the device to click in the data collection process. Although each laboratory sets its own criteria, the following values may be indicative. (1): White Kure values (BK) <1x10^9/L starting with 200 L blood; The amount of ELS is accordingly increased (2): 1x10^9/L <BK <10 (20) In x10^9/L, 100 L of blood and standard amounts of ELS are used. (3): 50 L blood is used when BK> 10 (20) x10^9/L, or the blood sample is diluted with phosphate buffered saline (PBS, pH: 7.2) to the concentrations listed in point 2. When using bone marrow specimens, for example, following dilution with PBS (usually 1/10), it is recommended to perform CB counting and to establish the study in accordance with the above ratios. Cells collected with density gradient or cell culture are adjusted to a density of 1-5 x10^6/mL before use, the antibodies used at the concentrations recommended by the manufacturer.

Cell viability: Since non-live cells can bind non-specific antibodies, cell viability should be determined before starting work. For this purpose, vital dyes such as trypan blue, Propidium iodide and fluorescent dyes such as 7-aminoaktinomycin D (7AAD) can be used. An evaluation is carried out on the light microscope or flow cytometer, respectively, with respect to the desired paint. Before these studies, the samples must not be determined, and the dye concentrations to be used must also be tested by the laboratory and verified as appropriate.

Prevention of non-specific antibody binding by Fc receptors: In order to prevent non-specific antibody binding of some leukemic cells due to the Fc receptor transport above the normal level, it may be necessary to close these resptors using nedeniyle murine ü IgG (2mg/mL) before the addition of the antibody to the medium. Because immunoglobulins in plasma in whole blood or KI samples sell these receptors, an excess of Fc resptor may not be a problem when using unwashed samples. This risk exists when the samples are washed first. Fc receptors were not closed using murine IgG when immunoglobulin levels were to be determined on the surface of the cell. must. Especially in monocytic losemias, non-specific binding by Fc can not always be prevented. Different immunoglobulin subclass Fc receptors. As the affinity is different, there may be difficulties in evaluating reactions with antibody binding.

Determination of antibody groups: On the surface of the cells in different series of the hematopoietic system there may be antigens specific to that series as well as non-specific antigens (Etc: CD45 are found in all locos, HLA-DR eler B h lymphocytes and monocytes). When working with losemic cells, these cells usually have fewer, and rarely more than, normal-specific antigens to their series. (For example, CD33 expression in young myeloid series cells is greater than that of mature cells, whereas expression of CD38 in malignant plasma cells is weaker than that of non-malignant plasma cells). In malignant cells, there may be antigens in the same cell as well as antigens belonging to different series. For this reason, a cell is In order to define the series/series, it may be necessary to create panels in which many antigens of this series and other series can be observed together. Although it is not possible to restrict the panels to be used for diagnosis, it is advisable to create some panels based on the most common examples.

Selecting fluorochromes: In addition to the properties of the device to which the data will be collected while selecting fluorochrome, the amount of the antigen in the cell and the placement in the cell is important. In order to stain small amounts of antigens, it is recommended to select brighter fluorochromes such as Phycoerythrin (PE); Fluorescein isothiocyanate (FITC), with a molecular weight of at least as fluorochrome, is preferred for labeling cell antigens. The fluorochromes should not be changed frequently and the data should be compared with the results of the studies on the ancient historians.

Negative controls: The purpose of using negative control is to determine ground staining resulting from autofluorescence or non-specific binding in cells. The most preferred reagent for this purpose; antibodies (isotypic control) having the same Ig subtype as the antibody to be examined. Since the reactions of the antibodies produced by different companies show differences, the use of the same isotypic control for all reagents when the antibodies produced by different manufacturers are used together cannot provide the expected performance. In order to evaluate autofluorescence, it is recommended to perform the study without introducing antibodies into the medium. Auto-fluorescence in granular cells is expected to be higher than granular (eosinophils in not more than notrofilen, myeloid series than the lymphoid series). There are also observations that the best control is the normal cells of that series. Density gradient not applied; it will be useful to compare and compare normal and abnormal cells and cells of different series.

Positive controls: Positive control examples should be prepared to ensure that the method used is correct and that the system works. For this purpose, two kinds of studies can be done. i) A cell (reference cell) known to carry the target antigen is reacted with the antibody under test (most commonly CD45, HLA Class I antigens are used for this purpose). ii) An antibody/reagent that we are sure to carry or react to is examined. The cell, which is known to be normal with a certain amount of test, is tested in the same tuple with mixing; it can be confirmed that negativity is a true value. False negative results are often mistaken because of an inadvertent introduction of antibodies into a tub. For this purpose, improved control materials are commercially available. When evaluating the cell functions, positive control materials can be prepared with ten operations that cause the parameter to be positively positively evaluated. Such operations are generally described in the worksheets provided by the manufacturers.

Determination of intracellular antigens: Permeabilization and fixation performed prior to the introduction of antibodies to the medium for labeling antigens has led to the alteration of antigenic epitopes and inadequate binding of antibodies; false negative results may be obtained. For these processes, firstly the protocols proposed by the manufacturers should be tried. When the results are not satisfactory, comparative studies with different permeabilization/fixation solutions should be performed. Materials that can be used for this purpose include detergent-based solutions such as Triton X100, Tween20, saponin, methanol. Since the applied reagent has an effect on the results obtained and the temperature of the application, the process to be selected must be standardized.

Fixation of cells: In immunophenotyping studies, data collection may be delayed by adding formaldehyde/paraformaldehyde solutions (1%) on the cells if the data cannot be collected immediately after preparation of the cells. It is recommended that the cells waiting in this way should be read in the device without exceeding 24 hours. Especially when lysis of erythrocytes loses the reagent used in a certain amount of fixatives in

cases where data collection should be done quickly. For example, ELSs that are found to be fixative should not be used if they are contained in the presence of cells (or KIA). In special studies, the samples must be read immediately after preparation. (Or: CD34+cell count or other cell counting, determination of apoptosis, cell cytokine measurement, DNA ploidy analysis.

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PL-07

What Does Your Coagulation Analyser Tell You for Preanalytical Errors?

Sinem Firtina

Department of Biochemistry Laboratory, American Hospital, Clinical Laboratory, Istanbul, Turkey

The hemostasis laboratory allows diagnosis and monitoring of hemostatic thrombotic disorders, evaluation of the patient prior to surgical invasive procedures and monitoring of patients under anticoagulant therapy. So, laboratory report plays a major role in patient management. An important component of hemostasis laboratory medicine is preanalytical phase, the biggest source of laboratory error. Pre-analytical errors may occur throughout the testing process and mostly occurs during the collection of blood specimens such as misidentification of the samples, use of inadequate devices or needles, incorrect order of draw, prolonged tourniquet placing, unsuccessful to attempt to locate the vein, incorrect use of additive tubes, inappropriate mixing of a sample etc. Transportation, preparation and storage of blood specimens also plays role in preanalytical errors. Pre-analytical issues in haemostasis testing are an important cause of misdiagnosis and can lead to inappropriate treatment.

Continuous monitoring and management of pre-analytical errors is crucial in order to improve the quality of the pre-analytical phase. At this point interpretation of the warnings of the coagulation devices we use can be of great benefit. The Clot Curve which can also be called as Clot Signature is the final product of the whole testing process. With the help of the curves users can identify sample and instrument problems. The interpretation of clot signature curves can be simple or complex depending on the factors influencing the curve. Clot Signature curve may be influenced by sample quality and collection, reagents status, mechanical status and clinical condition of patient.

Coagulation testing is much more complex than other simpler test systems. Coagulation/hemostasis testing also requires additional technical and clinical expertise. In the presentation, the possible causes of pre-analytical errors identified by coagulation curves and the action steps to be taken will be explained in detail with case reports.

ORAL PRESENTATION

OP-01

The Effect of Pneumatic Tube System with Manual Transport on Hematology and Coagulation Tests

Ozlem Dogan

Department of Medical Biochemistry, Ankara University Faculty of Medicine, Ankara, Turkey

Objectives: Throughout the clinical laboratory testing process, the pre-analytical phase, including the test ordering, collection of diagnostic samples, handling, transportation, and storage of the samples, has been considered the most vulnerable phase to errors. It has been demonstrated that the great majority of laboratory errors in the total test process come from this preanalytical phase. Inappropriate transportation of samples may impede obtaining a valid and fast laboratory test result. Today, pneumatic tube transport systems (PTS) are widely used due to such advantages as speed, safety, and cost saving associated with less personnel employment. Nevertheless, it is suggested that PTS may alter the quality of the samples due to fast accelaration [1,2]. The present study aimed to observe if there were any differences in the test results of whole blood specimen (CBC), erythrocyte sedimentation rate (ESR), and routine coagulation tests (prothrombin time (PT), activated partial thromboplastin time (aPTT), D-Dimer, Fibrinogene) on the samples that were transported via PTS or carried manually.

Material and Methods: Randomly selected patients that presented to the Phlebotomy Unit of Cebeci Hospital, Ankara University Faculty of Medicine to be tested for hemogram, coagulation, and sedimentation were involved in the study. The blood drawing procedure was performed by one single nurse. Paired blood samples for 38 coagulation, 42 sedimentation, and 43 hemogram test requests were collected from the patients. The first tubes containing the samples were sent to the laboratory via pneumatic system, while the second tubes were manually delivered to the laboratory as carried by personnel. In order to reduce bias, both samples were analysed in parallel in the same haematology laboratory on the same analysers by the same specialised technicians and within 1 h after sample arrival. Hemogram tests were performed at Sysmex XN-9000 haematology analyser, were sedimentation tests were performed at YHLO Vision, and coagulation tests (PT, INR, aPTT, D-Dimer, Fibrinogen) at ACL TOP devices.

Results: Upon statistical assessment based on CBC measurement results, haemoglobin (Hg), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were significantly higher, where mean corpuscular volume (MCV) was significantly lower in the samples transported via PTS ($p<0.05$). ESR, prothrombin time (PT), and INR results for the samples transported via PTS were significantly higher ($p>0.05$). A review of the change (%) in different parameters suggested that all the tests were below the total acceptable error limits for CLIA and biological variation (Hg (0.76%), MCH (0.34%), MCHC (0.30%), MCV (0.11%), ESR (33%), PT (0.86%), and INR (1%).

In other words, despite the fact that there were significant differences in certain parameters in the CBC, sedimentation, and coagulation tests for the samples that were transported via PTS, the change values (%) were below the total acceptable error limits for CLIA, Ricos and biological variation. Therefore, all parameters were far from clinical significance.

Conclusion: The pre-analytical factors substantially determine the quality of test results in haematology. Reduction in the turnarounds time (TAT) and hence minimization of certain testing errors in the pre-analytical phase associated with an increased time of delay between blood sampling and blood analysis in conventional manual handling process, have been suggested the factors that contributes in opting for the PTT use [1-3]. Nevertheless, the PTS is characterized by such factors as air pressure changes, fast acceleration and/or deceleration, radial gravity forces, and vibrations that may disturb the blood samples causing damages in blood cells and haemolysis, and thus affecting various laboratory parameters. Consequently, although there were no significant changes in clinical terms, it should be noted that PTS may increase the total error rate. It should be considered that each hospital uses separate PTS systems with different specifications (speed, diameter, length of the line etc.). Therefore, the laboratories should perform validation studies based on the method of transportation of the samples for ensuring stability of the results.

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OP-02

Preanalytical Errors in Hematology Samples: Evaluation by Sigma Metrics

Aysegul Ozgenc[1], Nesibe Esra Yasar[2], Erkam Coskun[1], Dildar Konukoglu[1]

[1]Department of Medical Biochemistry, Istanbul Univesity-Cerrahpasa, Cerrahpasa Medical Faculty, Istanbul, Turkey

[2]Department of Medical Biochemistry, Ministry of Health, Kars Harakani Public Hospital, Kars, Turkey

Objectives: The aim of this study is to evaluate the performance of the preanalytical process in hematology samples by six sigma and Pareto analysis.

Material and Methods: This retrospective study was carried out from January 2018 to January 2019, in Fikret Biyal Medical Biochemistry Laboratory of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty. A total of 426.573 complete blood count (CBC) and coagulation samples which were received to Biochemistry, Stat and Pediatric Laboratories evaluated for preanalytical errors like request errors/unsuitable barcode/improper transport/wrong tube/inappropriate blood-anticoagulant ratio/hemolysis/clotted specimen/lipemia. Total number of errors were determined and converted into sigma scale (www.westgard.com/six-sigma-calculators); Pareto's chart (the 80/20 rule) was drawn.

Results: In CBC samples (n=333.270), the preanalytical error rate was 0.31% and sigma value of the process was 4.3. The preanalytical error rates were 0.12%, 0.56%, 0.3% and the process sigma values were 4.6, 4.1, 4.3 for the Biochemistry, Stat and Pediatric Laboratories, respectively. In coagulation samples (n=93.303), the preanalytical error rate was 0.55% and the sigma value of process was 4.1. Preanalytical error rates were 0.41% and 0.66%, the process sigma values were 4.2 and 4 for Biochemistry and Stat Laboratories, respectively. Pareto's chart showed that 80% of preanalytical errors was clotted specimen in both CBC (4.4 sigma) and

coagulation (4.3 sigma) samples. The clotted specimen were followed by inappropriate blood to anticoagulant ratio and wrong tube in both CBC (4.8 and 5.4 sigma) and coagulation (4.6 and 5.1 sigma) samples.

Conclusion: While a preanalytical quality indicator with a sigma value of ≤3 is considered as poor performance, a sigma value of ≥4 is considered as well-controlled process. In our study, an acceptable preanalytical process sigma value (>4) was found in hematology specimens. The most common type of preanalytical errors in hematology specimens was clotted samples. The major cause of clotted samples is the tubes that are not adequately mixed after blood collection. Another reason may be incorrect phlebotomy techniques (syringe draws). One of the major issues is the inappropriate filling for traditional syringe system. As a result, staff training on correct blood collection techniques can reduce these errors.

OP-03

Assessment of Hemogram Parameters in Cold Agglutinin Disease

Mehmet Kalayci

Elazig Fethi Sekin City Hospital, Laboratory of Biochemistry, Elazig, Turkey

Introduction

Technological developments in hematology analyzers have resulted in higher quality and improved accuracy of complete blood count results [1]. However, there are many pre-analytical and analytical factors, as well as clinical conditions, that might affect laboratory analysis results [2]. Among these are the cold agglutinins, which are autoantibodies that are activated in low temperature, causing agglutination by binding to 'I' antigens on the surface of erythrocytes. These autoantibodies are most commonly immunoglobulin (Ig) M isotype antibodies (and rarely IgA or IgG) [3].

Cold agglutinin disease (CAD) is an autoimmune hemolytic anemia that causes agglutination as a result of antibodies that are activated in low temperature and bind to the 'I' antigens on the surface of erythrocytes. Cold agglutinins cause discrepancies in complete blood count parameters (erythrocyte indexes). While erythrocyte agglutination causes clinical symptoms of hemolytic anemia, cold agglutinin-induced agglutination is a pre-analytical and analytical factor that leads to erroneous complete blood count results [3, 4].

This case report was written to inform laboratory specialists and clinicians of the effects of cold agglutinins on complete blood count parameters.

Material and Methods

A 19-year-old male patient who arrived at the emergency department with the complaints of cough and shortness of breath was admitted to the respiratory medicine inpatient unit. Routine biochemistry and complete blood count parameters were requested for the patient. The whole blood sample was collected into a tube containing K_2EDTA (BD Vacutainer SST II Advance, Becton, Dickenson). The patient's complete blood count analysis was performed in the Sysmex XN1000 analyzer. Upon the presence of discrepancies in the complete blood count results of the patient, cold agglutinin was suspected due to the observation of clearly visible precipitation in the patient's sample; the low Hematocrit levels that were not correlated with Hb concentrations; and the significantly low RBC levels. The patient's sample was incubated for 15 minutes at 37 ºC in a water bath.

Results

The patient's CBC parameters were measured as follows: RBC: 1.79 10^{12}/L, Hgb: 13.1 g/dL, HCT: 17.5%, MCV: 97.8 fL, MCH: 73.2 pg, MCHC: 74.8 g/dL, PLT: 245 10^9/L. After the patient's sample was incubated for 15 minutes at 37 ºC in a water bath, the agglutination that was observed in the tube disappeared, and the CBC parameters returned to normal levels (RBC: 4.51 10^{12}/L, Hgb: 13.0 g/dL, HCT: 40.5%, MCV: 89.8 fL, MCH: 28.8 pg, MCHC: 32.1 d/dL, PLT: 261 10^9/L) (Table 1).

Conclusions

The analysis of samples obtained from patients with cold autoimmune hemolytic anemia is a significant problem for hematology analyzers. The initial suspicion of cold agglutinin disease stems from the erroneous measurement of the parameters, even though the low RBC count and analyzed hemoglobin level are accurate [5].

Erythrocytes that are agglutinated may be perceived as single cells, or be too large to be considered as erythrocytes. Therefore, this results in a disproportionately low erythrocyte count and incorrectly high MCV count [2, 6]. Among the indices that are calculated, hematocrit is consequently calculated as low, while the MCH and MCHC are calculated as high. MCHC can provide an average for the quality control of these parameters. A high MCHC is a good indicator of an analysis or sample error [1].

In the first complete blood count of our patient, the number of erythrocytes was quite low, and we found a discrepancy between the hemoglobin and hematocrit levels, along with substantially high MCV, MCH and MCHC levels. Assuming that these results may have stemmed from cold agglutinin, the sample obtained from the patient was incubated for 15 minutes at 37 ºC. Reanalysis of the sample afterwards yielded normal results. The heating of the sample removes the autoantibodies on the erythrocyte cell membrane. In a similar case reported by Yasar et al., a female patient was diagnosed with CAD due to a low RBC count, low hematocrit count that was uncorrelated with Hb concentration, and increased RBC indices in the Beckman Coulter Unicel DxH800 analyzer [6]. This diagnosis was confirmed following the heating of the sample to 37 ºC. In another case report by Ercan S. et al., similar results were obtained using the ABXPentra 80 hematology analyzer [7]. An inaccurately low RBC count, a low Hematocrit count that is uncorrelated with Hb concentration, and RBC indices that increased in the presence of cold agglutinins were also reported by Nikousefat et al. with Sysmex hematology analyzers [5].

There is a need for pre-treatment protocols to eliminate the impact of cold agglutinins on the results generated by hematology analyzers. Informing the laboratory about CAD patients and providing the appropriate temperature conditions during transport will reduce workload and laboratory costs, while also leading to accurate results. At the same time, this information will prevent the loss of a substantial amount of time, and help make an early and accurate diagnosis.

In conclusion, there are many factors that may cause errors in the analysis of laboratory tests, one of which is cold agglutinins. Providing the appropriate temperature conditions when transporting samples obtained

Table 1. Results of samples at room temperature and after warming to 37°C

		Room temperature	After warming to 37°C
WBC	(10^9/L)	5.33	5.42
RBC	(10^{12}/L)	1.79	4.51
HGB	(gr/dL)	13.1	13
HCT	(%)	17.5	40.5
MCV	(fL)	97.8	89.8
MCH	(pg)	73.2	28.8
MCHC	(g/dL)	74.8	32.1
PLT	(10^9/L)	245	261
MPV	(fL)	11.7	12.0
RDW-CV	(%)	16.1	16.0
RDW-SD	(fL)	52.4	51.8

from cold agglutinin patients to the laboratory, and informing the laboratory about this situation, will contribute to achieving accurate results, while also reducing workload and laboratory costs.

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OP-04

Effect of Single Tube use on Test Turnaround Times and Sample Rejection Rates in Hematology Laboratories

Cemal Kazezoglu[1], Gokce Aktas Oguz[2]

[1]Department of Biochemistry Laboratory, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkey

[2]Department of Biochemistry Laboratory, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey

Objectives: In our study we aimed to evaluate the effect of single tube use on test turnaround times (TAT) and test rejection rates (tRR) in two different similar capacity hospitals.

Material and Method: We compared the laboratory information system data for TAT and tRR of two hospitals in which Kanuni Sultan Suleyman Training & Research Hospital one sample tube is drawn for complete blood count (CBC),, HbA1c and variant hemoglobin (VHb) analysis. However, in Sisli Etfal Training & Research Hospital 3 different tubes are drawn for the same tests. In Kanuni, the order of analysis from single tube is CBC, HbA1c and VHb analysis, respectively. In both hospitals the average TAT and tRRs were obtained for these three different tests involving the second half of 2018. Outpatients were included in the study. Inpatients and emergency room patients were excluded.

Results: Average TATs for the three tests (CBC, HbA1c, VHb) from lab acceptance to result verification in Kanuni and Sisli were 45.8 and 59.9 minutes for CBC, 48.6 and 65.8 minutes for HbA1c and 146 and 211 minutes for variant hemoglobin analyses, respectively. Insufficient sample rejection rates were 0.04% and 1.85% for CBC, 0.12% and 1.66% for HbA1c and 1.54% and 0.35% for variant hemoglobin analyses, respectively.

Conclusion: When we compare two hospitals in terms of TAT, single tube utilization for three tests performed on whole blood accelerated laboratory workflow. When compared in terms of insufficient sample rejection rates, using single tubes decreased sample rejection rates. In Sisli where blood drawn to separate tubes for three type of tests more tubes were rejected due to insufficient samples. We presume that this sRR increase is the result of too many tubes to be filled from the same patient. This study revealed that using single tube for tests performed on whole blood has a positive effect in terms of decreasing TATs and tRRs.

OP-05

Preanalytical Sigma Value of aPTT After Hospital Education

Sevim Esmedere Eren

Department of Biochemistry Laboratory, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey

Objectives: Preanalytical step should be evaluated by multidisciplinary approach as it can cause waste laboratory time and cost.The aim of this study was to see the effect of education over rejected activated partial thromboplastin time (APTT) samples by preanalytical six sigma and percentages. Central venous access devices (CVADs) sampling procedure was defined for intensive care unit in order to raise awareness about heparin interference also. Heparin saline lock prevents obstruction of CVADs but it can be a problem in APTT assays leading to false results.

Material and Methods: We estimated the types of preanalytical errors per number of APTT test results in coagulation laboratory between april 2014 and march 2016 at Nigde State Hospital. Hemolysed, clotted specimens, inappropriate sample-anticoagulant volume, the overdetection results, inappropriate containers for APTT were included. The overdetection results meant our intensive care unit APTT samples which Synthasil reagent could not perform a result (the error code of 'failed') after extended assay in ACL TOP 500 analyzer. Rejected aPTT sample percentages, sigmametric values were compared before and after hospital education about specimen handling.

Results: Our total sigma values of all rejected samples unchanged, calculated as 3.4 before and after education.The monthly total rejection percentages of APTT were between 2.28-4.85% before education and 2.10-5.21% after education.Total sigma values of five types of rejected APTT samples were between 3.8-4.9 and did not change significantly from 04.2014-03.2015 to 04.2015-03.2016. Inappropriate sample-anticoagulant volume (from 3.9 to 3.8); clotted (from 3.8 to 3.9) remained under 4 sigma. The effect of catheter sampling poster for intensive care unit was seen as the change from 4 to 4.1 sigma. Hemolysis remained 4.4 sigma and wrong container changed from 4.9 to 4.8 sigma value.In two years the change in sigmas was only 0.1 value better in overdetection results and clotted samples; meaning 1255 and 1061 less defects per million (DPM) respectively. On the other hand inappropriate sample anticoagulant volume rejections were 1872 more DPM regarding to laboratory technician education.

Conclusion: The change in sigma values are useful to evaluate our preanalytical phase and to plan our hospital staff educations. Staff educations should be organised with small groups in order to check the attendance. Every laboratory may add new preanalytical phase indicators problematic for their routine as sigma values are easy to follow the situation after corrective preventive actions.

OP-06

Experimental Investigation of Interference Effect on Hemogram Parameters Due to Intravenous Immunglobulin Therapy

Ataman Gonel[1], Ismail Koyuncu[1], Eray Metin Guler[2]

[1]Department of Biochemistry, Harran University, Medical Faculty, Sanliurfa, Turkey

[2]Department of Biochemistry, Bezmialem University, Medical Faculty, Istanbul, Turkey

Objectives: Intravenous immunoglobulins are parenteral drugs used in the treatment of immune deficiency and autoimmune diseases. It reduces the activation and inflammatory response of T lymphocytes by binding to CD4 receptors. This contributes to the reduction of the autoimmune reaction. Generally, they may show affinity to many macromolecules in the blood with the IgG and IgM they contain. Connecting antibodies to analytes during measurement can cause interference and hook effect. The aim of this study was to investigate the effect of intravenous immunoglobulin on hemogram parameters.

Material and Methods: Amounts of 10, 20, 30, 40, 50 uL were taken from parenteral immunoglobulin solution (KIOVIG 10 gr/100 ml IV) and then added to 2 different levels of hemogram control solution (Cell-dyn 26 Plus Control, lot: N 8365, Abbott, USA), respectively. The mixture was mixed by the rotator device. 15 parameters of hemogram test (Wbc, Neu, Lym, Mono, Eos, Baso, Rbc, Hgb, Hct, McV, Mch, Mchc, Rdw, Plt, Mpv) Abbott Ruby (USA) were measured through full automatic hemogram auto-analyzer (Abbott, Ruby, USA). The study was repeated by adding 10, 20, 30, 40, 50 µL of distilled water. Measurements were performed three times and mean values were recorded. Bias and percentage deviation from target values were calculated. Our experimental study using control solution instead of human blood requires no ethics committee approval.

Results: The deviation in the Wbc levels was between -9.8% and -16.7%. The deviation ratios of lymphocytes were from -22.2% to -66.89%. Amount of basophils deviated between -17.07% - (-) 54.83%. Neutrophil, monocyte, eosinophil levels were affected at lower rates (-8.33% - (+) 8.23%). The least deviation was seen in Rbc, Hgb, Hct, Mcv, Mch, Mchc, Rdw, Plt levels (-5.13% (+) 5,11%). The MPV amount ranged from -32.14% to -44.14%.

Conclusion: The binding of antibodies to the lymphocyte and basophil surface receptors after IVIG administration resulted in erroneous measurements. Negative interference in the measurement of hemogram in patients receiving IVIG therapy may conceal a secondary disease (infection, leukemia, etc.). Therefore, it is important to question the medications in the patients before the phlebotomy. It should not be forgotten that the false negativity is more dangerous than the false positivity.

OP-07

The Effect of Different Preanalytical Conditions on the Lymphocyte Vitality in the Patients with HIV and Healthy Individuals

Emre Yalcinkaya, Hayriye Erman, Rabia Korkmaz, Ferruh Kemal Isman

Department of Medical Biochemistry, Istanbul Medeniyet University Goztepe Training and Research Hospital, Istanbul, Turkey

Objectives: Flow cytometry is an important tool in evaluation of immune system disorders. Because the samples are usually analyzed in a central laboratory, storage conditions, time to analysis and properties of sample are important for stability and viability of cells. In our study, we evaluated the effect of different temperature and storage time on the stability and viability of samples from HIV+ and healthy individuals.

Material and Methods: The lymphocyte subgroup and viability analysis were performed on whole blood samples taken from 5 healthy and 5 HIV+ individuals with 2 EDTA tubes. One was kept at +25 °C and the other at +4°C. Each sample was analyzed at 0^{th}, 8^{th}, 24^{th} and 32^{th} hours. Lymphocyte subsets were studied in BD FACS CANTOII; hemogram was studied in the Mindray BC-6800 autoanalyser. The change in the analysis results was evaluated by Friedman ANOVA test. The percent change of lymphocyte subgroups and viability was determined. The results were compared with the total change limits recommended by ICSH.

Results: The mean change values for CD3, CD4, CD8 T lymphocytes, B lymphocytes, NK cells and decrease in viability in the healthy group were 1.6-4.5-4-6-9.9 and 14, respectively; in the HIV+ group, were 2.1-3-4.4-18.2-12.8 and 21.3. Loss of viability at 4 °C was lower in HIV+ individuals (0.5%) than in healthy individuals (7.1%). In HIV+ individuals at 25 °C, loss of vitality was higher (14-21.3%). Decrease in viability was statistically significant in these healthy and patient groups at 25 °C and 4 °C during the 32 hour study period.

Conclusion: Although changes in lymphocyte subgroups may be below 20% limit recommended by ICSH, the potential clinical effect of B lymphocytes and relatively high changes in NK cells should not be underestimated, even if stability indicates continued stability. Lack of statistical difference doesn't mean that there will be no clinical difference. The significant decrease in viability, especially in HIV+ samples at 25 °C, should be taken into consideration in the evaluation, although the percentage changes in lymphocyte subsets are not reflected.

OP-08

Effect of Autoverification Process on Hematology Laboratory Workflow

Cemal Kazezoglu

Department of Biochemistry Laboratory, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkiye

Objectives: Our aim was to compare and evaluate the effect of using auto verification process on turnaround times, test repeatability rates and test rejection rates in two different similar sized hospitals.

Material and Methods: Both of the hospitals performed the Complete Blood Count (CBC) analyses on Sysmex XN9000 series analyzers. Sysmex XN 9000 middleware (Extended IPU) is put in place in Kanuni Sultan Suleyman Training and Research Hospital while in Dr. Sadi Konuk Training and Research Hospital no auto verification process was used. We evaluated the analysis duration of CBC tests starting from laboratory acceptance to result verifications and also test rejection rates. Outpatients were included in the study and the emergency department patients and inpatients were excluded. Only CBC analyses were taken into account, the body fluid analyses were excluded.

Results: In Kanuni Sultan Suleyman Training and Research Hospital, out of 67287 CBC samples, 48155 samples were verified automatically (71.6%). In the same time period in Dr. Sadi Konuk Training and Research Hospital 89460 CBC samples were analyzed and resulted with manual verification. Average test resulting times were 51.5 and 68.6 minutes, respectively. Test repeatability rates were 0.38% and 1.22%, respectively.

Conclusion: Sysmex XN 9000 middleware (Extended IPU) is put in place in Kanuni Sultan Suleyman Training and Research Hospital for the first time. The middleware we use, questions 21 criteria in the pre analytical and analytical phase then verifies the result or redirects the result to the specialist. Auto verification process makes a significant contribution on the acceleration of hematology lab workflow. In the upcoming period auto verification process will take an active role on workflows and process managements of clinical laboratories.

OP-9

Evaluating the Measurement Uncertainty of Platelet

Musa Yilmaz[1], **Hakan Ayyildiz**[2]

[1]*Department of Medical Biochemistry, Hitit University, Faculty of Medicine, Corum, Turkey*

[2]*Department of Medical Biochemistry, Elazig Fethi Sekin City Hospital, Elazig, Turkey*

Objectives

Measurement uncertainty is a parameter associated with measurement results, which may influence the measured analyte and change the analysis content. According to the international criteria, a clinical laboratory is suggested to have procedures which may estimate the uncertainty of test results [1]. As the formulation of the components in the calculation of measurement uncertainty are hard and there is no accepted standard, various guidelines have been prepared [2]. Among them, Nordest Guide is one of the guidelines prepared to make the calculation of measurement uncertainty more understandable [3]. The calculation of measurement uncertainty is in roughly based on analytical processes and the uncertainty is provided for laboratory specialist in a numerical expression. Calibration, as an example of the factors existing in the analytical phase and affecting the uncertainty, is considered to be the changeability of calibrators and referential materials, the sample-oriented effects, lot differences in reactives, product calibrators and referential materials, variances among device users, the variance of the equipment and environmental changes [4]. Bleeding in the patient is not generally expected even in a major surgeon provided that the number of platelet is 100.000/mm^3 and over. When the value is between 50-100.000/mm^3 in severe traumas, bleeding may last longer [5, 6]. The aim of this study is to determine the measurement uncertainty of platelet done with automatized hematological analyzers and to evaluate the possible effects of the uncertainty of critical values (50.000/mm^3).

Material and Methods

The calculation sampling of measurement uncertainty defined in Nordest handbook [7] was utilized in the study. The analysis of Platelet test was studied using Beckman Coulter DxH 800 analyzer and reagents of the same company. The internal quality control and external quality control data of platelet test, done between the months September 2018 and February 2019 in Elazig Fethi Sekin City Hospital, were used in the calculation of measurement uncertainty. CLIA, RILIBAK and Fraser allowable total error values were determined for the platelet test.

1. CV% (Coefficient of variation) values of low, normal and high level-control materials were used to calculate the within-lab reproducibility (uRw).
2. Uncertainty of External quality assessment (uEQA) and relative uncertainty of calibration (uCref) values were used in the calculation of Ubia, which is a component of the uncertainty. Bias values obtained through external quality control were used in the calculation of Bias.
3. uCref is defined as an uncertainty component obtained calculating the true or expected value in the results of the certified reference material or external quality control. u(Cref) value of the uncertainty was calculated using CV% values obtained through external quality control data for each parameter and the number of the laboratories using the same method and the same device.
4. All of the uncertainty values were transformed into standard uncertainty [u (Bias)] value.
5. The composed standard uncertainty (Uc) value was calculated making use of all standard uncertainty components.
6. The composed standard uncertainty value was multiplicated with k factor to calculate an expanded uncertainty value (U) (k value is approximately 2, 95% reliability interval).

The expanded uncertainty value (U) was evaluated using total error limits allowed by Westgard (TEa%). The measured uncertainties were determined as lower than '88 of CLIA and % TEa values of RILIBAK and Fraser.

The measurement of the samples from the patients may be considered to be an entity in the real world, and it is impossible to find out the "true" value of measurements. Both MU and TEa theories are related to traceability. The purpose of TEa concept should be "comparability of the results among laboratories".

Results

Measurement uncertainty of platelet test was calculated as PLT±13.1% in 95% reliability interval. These results calculated in our laboratory were found as lower than total allowed error values determined by the international institutions (RILIBAK, Fraser principles).

Discussion

Uncertainty is a significant value indicating the distribution of measurement results. The inclusion of the uncertainty in reports started to gain significance in terms of both the reliability of laboratories and the quality of results. The result should be close to the "true" value and comparable to the uncertainty value so that any biochemical test results can be reliable [8]. The detection of an uncertainty value in any laboratory results is essential for clinicians to acknowledge the result restrictions in laboratory science.

The significance of measurement uncertainty in laboratory medicine and the studies related to this subject matter is increasing more and more [9-11]. In comparison to various calculation methods, Nordest guideline is on the foreground as a method to calculate easily [7].

Measurement uncertainty is a significant parameter as a current approach especially in the clinician decisions. Accordingly, Sentürk and et al. stated in their study that when the value of measurement uncertainty was added to or removed from the retrospectively examined results of 7259 patients, cannabis decision values in 161 patients and opiate decision values in 6 patients changed [12]. Furthermore, Ustundag and et al. examined blood ethanol levels of 1034 drivers in emergency laboratory and calculated the expanded uncertainty as 19.74%. Therefore, they noted that the results of blood ethanol concentration tests, close to the legal margins, should be reported in 95% reliability interval and a reliability interval including a real ethanol concentration [13].

Tekce and et al. researched measurement uncertainty of serum creatine concentrations using Nordest handbook in terms of its impacts on acute renal damage diagnosis. They stated that creatine concentrations are significant factors of measurement uncertainty in diagnosing acute renal damage accurately [14].

Critical platelet values are essential particularly for surgical indications. We determined measurement certainty of platelet as PLT±13.1% in the study. The acknowledgement of especially platelet measurements in critical values is crucial to know the uncertainty value and make clinician decisions.

Conclusions

A test or a measurement cannot be influential unless the test result or the reliability of the measurement is evaluated. Laboratories should calculate measurement uncertainty of each parameter, present the results in such a way that they may not exceed the targeted TEA values and take into consideration these factors while evaluating test results of the clinicians.

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OP-10

Unexplained D-Dimer Elevation; A Case Report

Berrin Oztas[1], Arzu Karafakilli[2], Erdinc Serin[1]

[1]Department of Biochemistry, Sisli Hamidiye Etfal Research and Training Hospital, Istanbul, Turkey

[2]Department of Pulmonary Disease, Sisli Hamidiye Etfal Research and Training Hospital, Istanbul, Turkey

Objectives: D-dimer is a fibrin degradation end product generated during fibrinolysis. D-dimer assays are commonly used in clinical practice to exclude diagnosis of deep vein thrombosis or pulmonary embolism. Besides, its increase is shown in cases with inflammation, cancer and after surgery. We tried to evaluate the reasons for the elevation of D-dimer in our case.

Case: Male patient, 74 years old admitted to our Pulmonary Diseases Outpatient Clinics with dyspnea. Thorax CT showed pleural effusion and pneumonia was the diagnosis. Also D-dimer analysis revealed a high result which was 8490 µg/L (reference range <654 µg/L). With a clinically medium high probability of pulmonary embolism his therapy was planned. Consecutive D-dimer requests revealed high results despite his dyspnea recovered with therapy and the last D-dimer result was 2980 µg/L. Due to lack of clinical and radiological finding to support the diagnosis of pulmonary embolism other than elevation of D-dimer pulmonologists requested a consultation from our laboratory. After assessment of the patients test results, plasma was treated with heterophilic blocking tube (HBT) to rule out the heterophilic antibodies. Upon treatment D-dimer analysis was repeated using same immunoassay method and the results before and after HBT use were 2390 µg/L and 1850 µg/L, consecutively. Then D-dimer analysis was repeated on another device using a different method (immune-turbidimetric method) however similar results were obtained (1175 µg/L and 1142 µg/L; reference range <500 µg/L). Rheumatoid factor was also analyzed to rule out its interference with D-dimer assays and again it was found in the reference range (3.94 kIU/L; reference range <14 kIU/L). Patient's creatinine values were over the reference range (3.83 mg/dl) which was the only probable reason for D-dimer elevation in this patient was chronic renal impairment (CRI).

Conclusion: D-dimer can be interfered from different pre analytical factors. Patient's age, presence of heterophilic antibodies, osteoarthritis, knee or hip joint replacement operations, renal impairments are the known factors that should be evaluated for D-dimer interferences. We suggest new D-dimer reference range studies should be done on CRI patients since it is hazardous to use contrast agent in pulmonary embolism for diagnosis.

OP-11

Resolution of Peak Separation Problem in Variant Hemoglobin Analysis

Ozgur Baykan

Department of Biochemistry, Faculty of Medicine, Balikesir University, Balikesir, Turkey

Objectives: Hemoglobinopathies are among the most important inherited diseases both in our country and in the world. Policies are carried out for the detection and prevention of this diseases. Chromatographic and electrophoretic techniques are frequently preferred in variant hemoglobin analysis. High performance liquid chromatography (HPLC) is the preferred method as rapid and reliable outputs can be obtained. We performed our analysis by the commercial kit (Recipe Chemicals, GmBH) applicated to the HPLC instrument (Ultimate 3000, Dionex, GmBH) in our laboratory. In this report, we aimed to find a solution to a problem that we have experienced during the application of commercial kits.

Material and Methods: In order to determine the variant hemoglobin, cation exchange colon is frequently used in routine laboratories. By this method, HbF and HbA2 forms can be determined quantitatively, and A1c, A0, D, C and S variants can be determined semi-quantitatively. In the analysis using a cation exchange column, the mobile phase pH is the basis of the separation. The tetrameric hemoglobin molecule undergoes separation in the column due to its conversion to dimer form at room temperature and neutral pH. However, situations that stop or delay this transformation may lead to peak separation problems.

Results: In our laboratory, during the application of the commercial kit, we encountered peak separation problem between HbA1c, F, A0, A2, D and C. All components such as gradient, column temperature and mobile phase pH were changed one by one in order to solve the problem. But the source of the problem could not be found. Then the variables of the device started to be examined. Finally, deactivation of the degasser unit, integrated into the device pump system, resulted in dramatic improvement of the peaks.

Conclusion: Degasser removes dissolves oxygen from the mobile phase. Because of this, it is thought to slow down the transformation to the dimeric form and to prevent the separation of the peaks.

POSTER PRESENTATION

PP-01

Evaluation of Rejected Samples for Coagulation Tests

Sehide Baz, Yuksel Gulen Cicek, Soner Erdin, Zeynep Levent Cirakli, Nilgun Isiksacan, Alev Kural

Department of Medical Biochemistry Laboratory, University of Health Sciences Bakırköy Dr Sadi Konuk Research and Training Hospital, Istanbul, Turkey

Objectives: Coagulation tests in emergency laboratories are very important because of the necessity giving accurate resuts with short turn around time. Aim of study was to retrospectively investigate the causes of rejected coagulation samples in emergency laboratories.

Materials and Methods: Between 1 January 2018-31December 2018, the number of samples that rejected in emergency laboratories and delayed test. Results were analyzed from laboratory information system (LIS) and retrospectively evaluated. Rejected samples were classified by errors sources.

Results: Number of samples for emergency laboratory coagulation tests was 87256 and total number of rejections was 2743 (%3.14). Error rates were found as insufficient volume 1674 (%61.02), clotted sample 596 (%21.72), inappropriate specimen container 135 (%4.91), hemolized sample 118 (%4.30), lipemic sample 92 (%3.35) and inappropriate order 128 (%4.66). The crucial issue was insufficient volume for emergency laboratory. Tubes with different vacuum levels caused the problem of obtaining insufficient volume. As a result of our demand, the firm supplied tubes with equal vacuum levels to our laboratory. Furthermore, aimed at reducing that certain point, emergency department phlebotomist teams have been regularly educated.

Conclusion: To minimize the most common preanalytical errors in laboratories is necessary for accurate and timely results for accurate patient. In the case of these preventive plans, training of the blood collection staff, the kinds of tubes (used in the laboratories for the coagulation tests) are important, especially in order to reduce the rejected sample rates.

PP-02

Cold Agglutinins: Should be Kept in the Mind

Ceyhun Gozukara[1], Sezen Irmak Gozukara[2]

[1]Department of Clinical Biochemistry, Sakarya Public Health Laboratory, Sakarya, Turkey

[2]Department of Clinical Biochemistry, Sakarya Training and Research Hospital, Sakarya, Turkey

Objectives: Cold agglutinins are autoantibodies that bind red blood cells at cold temperatures, cause agglutination. Autoantibodies may be idiopathic or secondary to infection, malignancy, and other autoimmune diseases. Cold agglutinins can interfere with laboratory tests such as complete blood count (CBC), glycated hemoglobin test and blood group test. This study is a case report of false CBC results and falsely rejected glycated hemoglobin test associated with cold agglutinins.

Material and Methods: A 77-year-old male patient who was operated for colon cancer admitted to the family physician for routine control on 14.11.2018. The patient had normal platelet and leukocyte counts, but the hemoglobin (Hb:145 g/L), haematocrit (HCT: 9.9%) and red blood cell (RBC: 0.99x10^{12}/L) results were incompatible. Also his glycated hemoglobin sample was rejected as a coagulated sample. Other preanalitic factors which can mismatch between hemoglobin and hematocrit values were evaluated. Complete blood count was performed with Beckman Coulter DXH-800 analyzer, glycated hemoglobin test was performed with Arkray HA8180V analyzer.

Results: When the patient's samples were examined, agglutinations were seen in the tube walls, which were different from the clot. The patient had a diagnosis of colon cancer and as we know secondary to malignancies cold agglutinins can interfere with laboratory tests. So we suspected cold agglutination. Then we incubated the patient's tubes for 1 hours at 37 °C. After incubation the agglutination was reversible in both tube. We analyzed the patient's tests and results were Hb:146 g/L, HCT: 39.2 % and RBC: 3.83x10^{12}/L. Glycated hemoglobin test result was 5.7%.

Conclusion: Many interferences and preanalytical errors in CBC measurement can be encountered in routine laboratory functioning. During the interpretation of the test, the interference of cold agglutinin should be kept in mind at low red blood cell counts which are incompatible with hemoglobin results. It should also be ensured that the sample is really clotted while rejecting the sample.

PP-03

Which is the Right Platelet Count?

Cigdem Karakukcu[1], Esra Yildizhan[2], Derya Kocer[1], Volkan Akdeniz[1]

Department of Biochemistry, Kayseri City Hospital, Kayseri, Turkey

Department of Hematology, Kayseri City Hospital, Kayseri, Turkey

Objectives: There are different platelet (PLT) counting techniques with automated analysers; Impedence, Optic and lastly Fluorescence. The most used Impedance platelet (PLT-I) analysis has limitations in the identification and discrimination of PLTs from interfering particles with the same size, especially in patients with low PLT count. PLT-O technique uses fluorescence marker those are quantifying mature and immature reticulocytes, labels also PLTs and gives in cases of interferences a more precise count. But the fluorescence marker is not dedicated to PLTs only, interferences can even happen. PLT-F is especially designed fluorescence marker, labeling intra PLT organelles, which gives more accurate results. Here we demonstrate a patient with PLT interferences.

Material and Methods: A Complete Blood Count (CBC) was requested from a 39 years old female patient admitted to the hematology clinic because of secondary acute myeloid leukemia who had completed chemotherapy treatment and in the process of donor searching for bone marrow transplantation.

Results: The patient had normocytic, normoblastic anemia with low white blood cells (WBC) (0.28x10^3/µL) and PLT count at lower reference range (158x10^3/µL). 24 hours later a new CBC+Reticulocyte request revealed PLT counts as (2x10^3/µL). We repeated the readings of the two samples both with PLT-I and PLT-O. PLT-I results of the both were at nearly 150x10^3/µL, however PLT-O were 2x10^3/µL. Indeed, PLT distribution and scattergram was normal, so there was no PLT flag with PLT-I; but abnormal WBC scattergram with blast/abnormal lymphocyte flag. Because there was no warning flag the analyzer gave PLT-I result. PLT-F result and blood smear was also correlated with PLT count 2x10^3/µL. The follow up of this patient also gave mostly higher results with PLT-I.

Conclusion: Conventional automated PLT counting methods are unable to provide consistently accurate results in this low thrombocytopenic range. We think that, fragmented WBCs at the same size with PLT are counted as PLT cells with PLT-I technique. WBC fragments are one of the reason of high PLT counts. So in hematology-oncology department patients with low PLT counts, PLT-O, PLT-F or blood smear counting should be preferred.

PP-04

Preanalytical Errors in Hematological Tests of Biochemistry Laboratory of Kayseri City Hospital

Derya Kocer, Cigdem Karakukcu, Selma Buldu, Leyla Oz, Gokcen Sert

Department of Biochemistry, Kayseri City Hospital, Kayseri, Turkey

Objectives: Errors in clinical laboratories, that the majority being outside the laboratory and a high proportion of these are avoidable, have an important effect on safety and care of patients. Thus, causes of specimen rejections should be identified and corrected to guarentee quality results. The objectives of this study were to identify and classify the causes of biological specimen rejections in hematological tests, according to rejection and patient type, to determine the specimen rejection rates for improvements at this stage, and contribute data to the literature.

Materials and Methods: In this retrospective study, data on rejected biological specimens in the laboratory information system (LIS) of Kayseri City Hospital (KŞH) from August 2018 and January 2019 were analyzed. Hematological specimen rejection rates according to the causes of sample rejections and collection area were determined.

Results: In total, 401.944 biological specimens were received to hematology laboratory during the period above and 3.233 (0.8%) specimens were rejected based on our laboratory sample rejection criteria. Specimen rejection rates were 0.46% and 2.38% for hemogram and coagulation tests respectively. Clotting of specimens was the most frequent reason for rejection (52.06% of total rejections), followed by incomplete/excessive volume (35.79% of total rejections) for hemogram tests. Incomplete/excessive volume was the most frequent reason for rejection (52.69% of total rejections), followed by clotting of specimens (34.80% of total rejections) for coagulation tests. Rejection rates of inpatient and outpatient services for hematological and coagulation tests were 0.44% and 0.37%, respectively.

Conclusion: The common causes of specimen rejection in our study included clotted samples, and incomplete/excessive volume for hematological and coagulation tests. Also, sample rejection rate was found to be higher in inpatients than in outpatients. Repetitive training programs about preanalytical errors were planned for phlebotomy staff and laboratory technicians. In this way, labor and financial loss due to preanalytical errors can be prevented.

PP-05

Verification of a New Hematology Analyzer: Unicel Dxh 900 Coulter Cellular Analysis System

Gunel Bahramzada[1, 2], Rana Turkal[2], Tulay Cevlik[2], Onder Sirikci[1, 2], Goncagul Haklar[1, 2]

[1]*Department of Biochemistry, School of Medicine, Marmara University, Istanbul Turkey*

[2]*Department of Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey*

Objectives: When a laboratory acquires a new hematology analyzer (HA), a verification process should be performed in which it is shown that the HA meets requirements and predefined criteria, specifically set for this laboratory method. The aim of this study was to evaluate the analytical performance of DxH 900 HA (Beckman Coulter, FL, USA) for its accuracy and imprecision, in which a new hematology parameter, monocyte volume width distribution (MDW), intended for use as an early sepsis indicator and offered as part of a routine cell blood count (CBC) with differential test, was included.

Material and Methods: Evaluation was performed according to recommendations of the H26-A2 of the CLSI during a one-month period in Marmara University Pendik E&R Hospital Laboratory. Venous blood samples were collected into EDTA anticoagulated tubes (Vacutainer-BD, NJ, USA). All samples were chosen randomly among the residual samples of the patients whose CBC was ordered and were analyzed within 4 hours of collection. Within-run precision coefficient of variations (CV%) for all parameters of CBC were assessed on DxH 900 by performing 10 consecutive measurements of the low, normal, and high level samples. Between-run precision were calculated by processing the manufacturer's three level control material twice daily over a period of 20 days. All parameters analyzed on DxH 900 were compared to DxH 800 (n=550).

Results: Within-run precision was satisfactory according to the manufacturer's specifications. CV for leukocyte count of 0–2 $\times 10^3/\mu L$ was 1.8%, and for the platelets 0-50x$10^3/\mu L$ it was 3.49%. Hb value 6-10g/dL also presented satisfactory results, with a CV of 0.67%. CV for within-run and between-run precisions of MDW were <6% and <2%, respectively. All the CVs observed in between-run precision were lower than the specification suggesting that the maximum intra-analyzer imprecision for hematological parameters must be lower than half of the intraindividual biological variability (CVanalytical or imprecision<0.5×CVintra). The comparison between two analyzers gave good agreement results for all parameters according to the criterion of biological variability.

Conclusion: As a conclusion, results provide a satisfactory acceptability of the DxH 900 in laboratory routine and accomplish an optimal reliability.

PP-06

Reducing Preanalytical Errors in Erythrocyte Sedimentation Rate Measurement

Ceyhun Gozukara[1], Sezen Irmak Gozukara[2]

[1]*Department of Clinical Biochemistry, Sakarya Public Health Laboratory, Sakarya, Turkey*

[2]*Department of Clinical Biochemistry, Sakarya Training and Research Hospital, Sakarya, Turkey*

Objectives: Establishing effective methods for the monitoring and control of preanalytical variables is difficult because this variables require coordinated effort of many individuals and departments, each of which must recognize the importance of these efforts in the maintenance of high-quality service. Especially in public health laboratories where samples are transferred from multiple centers, it is much more difficult to prevent preanalytical errors. The aim of this study is to investigate the effect of the tube change used in the measurement of erythrocyte sedimentation rate on our rejection rates.

Material and Methods: In the retrospective study, reject reasons of erythrocyte sedimentation rate samples were investigated between 01/01/2017-15/12/2017 and 01/01/2018-15/12/2018. In 2017 Black cap citrate tubes (Alaris, Turkey) were used in 2017 and they were replaced with purple cap EDTA tubes (Becton Dickenson, NJ, USA) in 2018. The rejection criteria are as follows: coagulated sample, insufficient sample, excess volume, inappropriate tube, wrong record.

Results: A total of 13211 samples were analyzed and 2555 errors detected in 2017. The total number of tests tested is 27300 while the rejected tests are 48, in 2018. The frequency of total errors in 2017 and 2018 were 19.3% and 0.17%, respectively. Rejection rates reduced dramatically just in a year.

Conclusion: In our laboratory rejection rates were decreased by performing root cause analysis of preanalytical errors. It is possible to dramatically reduce preanalytical errors by finding the correct root cause and making the necessary correction and prevention studies.