Chapter 1

Plasma for Laboratory Diagnostics

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Additional information is available at the end of the chapter

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Abstract

Main clinical and scientific aspects of methodology how to choose the right sample for testing to be involved in *Plasma for Laboratory Diagnostics* chapter as plasma is in use sufficiently widely. Different approach by clinical and research laboratories formed the necessity to discuss the basic laboratory terms and conditions to obtain correct results. Evaluation of preanalytical variables with impact on handling, processing and storage of samples with subsequent meaning for laboratory test results should be known by physicians, biomedical scientists, laboratory technicians and so on. The impact of chemical additives for diagnostics tools, the differences between plasma and serum samples for different laboratory tests and the subsequent analysis, management and traceability of specimens with standardisation value should be taken into account.

Keywords: plasma, laboratory, preanalytics, diagnostics

1. Introduction

Summarised results for *Plasma application for laboratory diagnostics* in one chapter for qualitative testing to be called "*Handbook.*" The understanding of laboratory phases, mainly preanalytics, is necessary both for clinical and research laboratories to obtain, compare and repeat or reproduce analysis. Main standardisation requirements are taken from clinical laboratories, and their approach should be useful for researchers to plan, organise the scientific work fulfilling their research interest. This is the first attempt to improve research by the requirements for clinical laboratories.

2. Challenges and limitation for plasma use in laboratory testing

A literature *review, metaanalysis and grounded theory* of known methods and obtained results in laboratory practice worldwide with value of quality assurance are taken. The suppositional contents are as follows.

2.1. Preanalysis. Factors before testing

Human blood with additives for plasma preparation is a subject for variables, affecting preanalytical process, which takes app 50% of total laboratory turnaround (see **Figure 1**).

The preanalytical phase is divided into *outside laboratory phase* and *inside laboratory phase* for samples processing. There are no standards defining the quality of preanalytical phase. Known and following criteria should be taken as quality criteria in each individual laboratory. See **Table 1**.

Quality of the sample must be desired through all laboratory phases, starting from the preanalytical, which covers more than 50% of the total laboratory investigation circle [1].

2.2. Influence of unavoidable factors

Unavoidable factors such as age, race, gender and pregnancy are important considering clinical chemistry and haematology subjects for testing and interpreting results. Biochemistry variables for serum iron [2], CK and creatinine [3] are always taken into account by gender performance; other various substrates and enzyme activity or concentration (uric acid, bilirubin, alkaline phosphatase, etc.) are taken into account depending on age. Race differences should

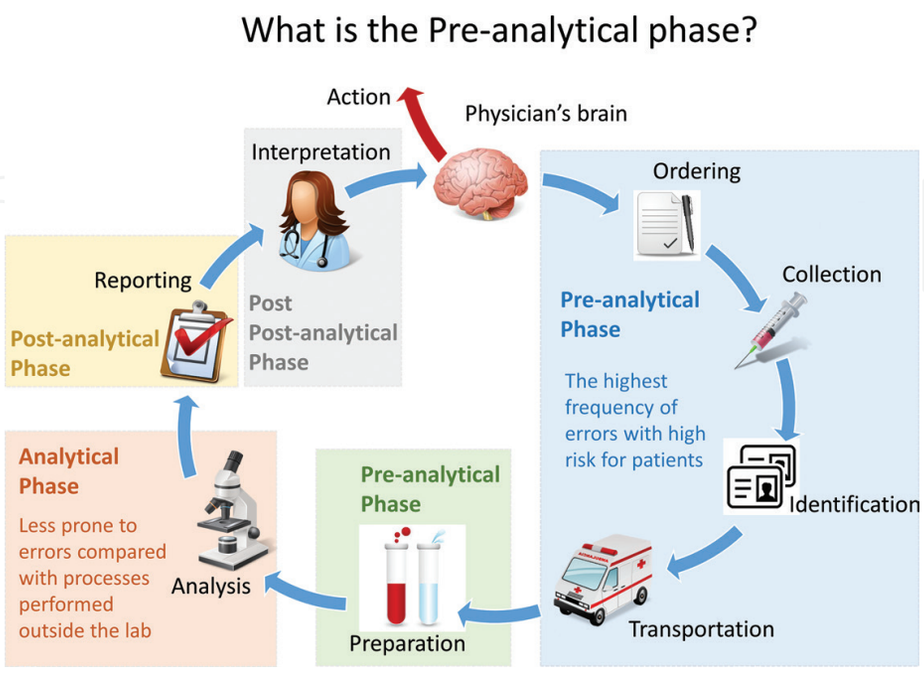

Figure 1. Preanalytical phase.

Process	Importance	Remarks for materials
Outside laboratory phase		
Unavoidable factors	Age, race, gender, pregnancy	Fill request form properly
Avoidable, variable factors	Caffeine, smoking, alcohol, drugs	Ask patient and mark
Patient preparation	Diet, starvation, exercise, altitude	Ask patient and mark
Preparation of sampling	Define and enter request into system, proper tube labelling	Request form and information system are mandatory. Patient and sample identification procedures
Sampling process	Patient's ID, timing, use of tourniquet, site of sampling selection, position of needle, run order of tubes	Use of tubes, needles, disinfection materials strongly according procedures.
Transportation	Difference of collecting and transporting tubes	Transporting containers, cooling systems according procedures
Laboratory phase		
Sample treatment	Registration, identification, centrifugation, distribution, extraction	Identification and registration procedures, authorised and secured laboratory information system
Sample/specimen storage	Selection of site and temperature, timing, utilisation	Storage and freezing devices with temperature control according procedures

Table 1. Preanalytical phase and its importance for quality testing.

be evaluated for blood count in haematology and enzymology within clinical chemistry, while the mean variable for plasma volume changes is pregnancy. During healthy pregnancy plasma volume increases by an average of about 1250 ml, with progressive increase up to 35th week of gestation, after which little or no further increase occurs. The frequently observed fall in plasma volume in the last 6 weeks of pregnancy is a false of measurement due to poor mixing of tracer when the woman lies supine, and this posture obstructs the circulation to the lower limbs [4]. The differential changes are biologically plausible. Erythrocytes mass rises proportionately to the need to carry the extra oxygen necessary in pregnancy [5]. The greater plasma volume is needed to cope with the very large increases in blood flow to organs which require different amount of extra oxygen. Increased plasma volume is greater than the increase in red blood cell mass, thus there is a fall in haemoglobin concentration, haematocrit and red blood cell count. It is worth to mention that despite this haemodilution, there is usually no change in mean corpuscular volume (MCV) or mean corpuscular haemoglobin concentration (MCHC) [6].

2.3. Meaning of variable subjects for plasma specimens

The variable diet, starvation, exercise are more important for serum analytes in clinical chemistry. Triglycerides, aspartate aminotransferase, bilirubin, glucose are very sensitive to diet and drinking habits. Starvation reduces blood cholesterol, triglycerides and urea concentrations. In contrast, creatinine and uric acid are elevated after long time (4 weeks) starvation period. Changes may occur to an increase in reabsorption of the measured analyte or metabolism changes [1].

Enzymes, such as pyruvate kinase, creatine kinase are raised from 2.5 to more than 4 times after a marathon rise. Sodium and potassium measurable in plasma after the same marathon rise elevate only by one fold. To reduce possible misinterpretation of laboratory findings, sampling after 12 h fasting and normal activity is highly recommended to ensure preanalytical procedures [1].

Significant changes may occur in blood at high altitude. β_2-globulin and C reactive protein (CRP) may rise 43–65%. Ht and Hb are less sensitive—they increase only by 8%. The opposite phenomenon with decreasing values is demonstrated at plasma osmolality, plasma renin analytes [7].

Studies concluded that caffeine, smoking, alcohol or drugs intake may either decrease or increase different analytes [8]. Thus, it is highly recommended for blood sampling—the early morning before coffee, cigarette or other intakes were eliminated.

2.4. Interference factors. Lipaemia, haemolysis

Haemolysis is defined as the release of blood cells content into plasma or serum. The colour intensity depends form haemoglobin released from erythrocytes. Sometimes this can occur due to platelet and or granulocyte lysis. An effect of haemolysis is classified according to the different mechanisms: increase of intracellular contents extracellularly; optical interference due to the haemoglobin colour; interference by intracellular contents with the different mechanisms of reaction. Laboratory should document haemolysed samples procedures as the responsibility of the laboratory diagnostics results belongs from the relevant interpretation of events [1].

After haemolysis, lipaemia is the most frequent interference factor that can influence results of clinical laboratory methods. Plasma or serum should be always considered due to varying degrees of an increased lipoprotein content. Turbidity is caused by an increased triglyceride concentration, which may variate from slight to milky, and these samples are called lipaemic. The most common preanalytical cause of lipaemia is short time from meal taken or parenteral administration of lipid emulsions to blood sampling. The best way to detect the degree of lipaemia is measuring lipaemic index on analytical platforms. Laboratory staff should keep preanalytical procedures under control. Unlike for other interferences, lipaemia can be removed, and measurement can be done in a clear sample, using a protocol for removing lipids. On the other hand, sample has to be chosen carefully, since its dependency on the analytes that have to be tested [9].

2.5. Plasma or serum samples *in vitro* diagnostics. Advantages and disadvantages for use

Scientists and clinicians are still on debates—what type of sample a laboratory should use. Serum is still considered to be the gold standard remaining the required sample matrix for some biochemistry, immunology assays.

Both plasma and serum are liquid parts of the blood. The main difference between them performs a clotting process. Plasma specimen is prevented from clotting and is more reflective of the systemic blood circulation in the body.

There are diagnostically relevant differences between the results obtained from serum or plasma in laboratory. This happens due to several physiological and technical reasons:

- clotting factors: fibrinogen, platelets, glucose affecting analyte;
- analytes (potassium, lactate dehydrogenase, phosphate, ammonia, lactate, neurone-specific enolase, dopamine and serotonin) may be released from the cells during clotting process;
- the anticoagulant may interfere with the assay or contaminate with its cations: Lithium (heparinate) with flame photometry, when calibrated with lithium [1, 10].

There are different tubes for plasma or serum collection (see **Figure 2**) and sometimes different applications due to the variable pathophysiological effects of platelets and clotting process to be involved for laboratory findings [11] (**Table 2**).

It is necessary to perform a rapid centrifugation to obtain more stable plasma. The extended contact with blood cells is complexable event including particles of the cells and metabolites after DNA, proteins degradation [12]. Thus, an immediate plasma centrifugation and extraction is desirable ASAP after blood collection.

There are proposed various methods and solutions for miniaturised blood plasma extraction. Macro-scale depending on the desired sampling volume: blood transfusion volume (500 ml), analytic venous sample (1–50 ml), or blood droplet from a finger-prick (up to 200 μl). Whatever the blood volume processed, the two conventional mechanisms—centrifugation or filtration—exploited for plasma separation at the macroscale remain as necessity. Different solutions are

Figure 2. Vacuum tubes for plasma collection. Images reproduced by kind permission of Becton, Dickinson and Company, All rights reserved. Unless otherwise noted, BD, the BD Logo and all other trademarks are property of Becton, Dickinson and Company.

Functions	Plasma	Serum
Time saving	✓	
Higher yield/specimen volume	✓	
Prevention of clotting effect	✓	
Prevention of changes induced with coagulation process	✓	
Contamination with NH_4^+, Li^+, Na^+, K^+		✓
Inhibition of metabolic reactions by heparin		✓
Interference of ions distributed intra- and extra-cellular space		✓
Inhibition of enzymes by metal binding to EDTA and citrate		✓
Binding of ionised calcium to heparin		✓

Table 2. Advantages (marked) and disadvantages of plasma and serum (according Guder et al., 2016) [26].

available for miniaturised blood plasma extraction within three main formats: the microfluidic chip format; the CD format and the paper format, based on different resources [13].

Remarks for testing:

Time saving. Serum sample must be allowed to clot, this time is variable from 10 min to 30 min and even longer.
Higher specimen volume or yield. Up to 20% more plasma than serum can be yielded after centrifugation.
In general, serum is used widely for the serological diagnosis of infectious diseases. There are some tests, that is, complement fixation or bacterial agglutination tests, where serum must be used only.

2.6. Types of plasma samples. Additives for tubes

The colour codes of anticoagulants are described in ISO/CD 6710: EDTA = lavender/red; citrate 9:1 = light blue/green; citrate 4:1 = black/mauve; heparinate = green/orange; no additives (for serum) = red/white (ISO 6710). To obtain cell-free plasma for laboratory use, the anticoagulated blood should be centrifuged for at least 15 min at 2000–3000 × *g*, temperature should be set from 15 to 24°C [1].

2.6.1. EDTA

A salt of ethylene tetraacetic acid. Dipotassium (K2), tripotassium (K3) and disodium (Na2) are used. EDTAK2 or K3 blood is used in haematology and considerations which one additive to use are still under debates. The cell volume measured after centrifugation, decreases when concentration of this anticoagulant is higher and this is mainly seen with the tripotassium (K3). This fact has been reported by different authors due to erythrocytes dehydration in hypertonic medium. Despite automated analysers work differently, but their MCV is not affected by K3-EDTA concentrations up to 10 times normal, while high concentrations of K2-EDTA, result a slight MCV increase [14, 15].

2.6.2. Citrate

Trisodium citrate with 0.100–0.136 mol/L citric acid. Buffered citrate with 5.5–5.6:84 mmol/L trisodium citrate with 21 mmol/L citric acid. The International Society for Thrombosis and Haemostasis (ISH) recommended to use the Hepes-buffered citrate for all investigations of haemostasis. A mixture of one citrate part with nine parts of blood is recommended for coagulation tests (ISO 6710), see: plasma for coagulation testing. A mixture of one citrate part with four parts of blood for ESR testing.

2.6.3. Heparin

12–30 IU/mL of sodium, lithium or ammonium salt of heparin, with 3–30 kDa of molecular mass. Calcium-treated heparin with concentration of 40–60 IU/mL of blood (dry heparinisation) and 8–12 IU/mL of blood (liquid heparinisation) are in use for ionised calcium determination.

2.6.4. Specificity for glucose samples (grey cap) for blood transporting stability only

Additives: potassium oxalate and sodium fluoride or sodium fluoride/Na2 EDTA or sodium fluoride (no anticoagulant, will result in serum sample).

It is worth to follow CLSI recommended Order of Draw [16].

Anticoagulants are useful to inhibit blood or plasma from clotting ensuring the quantity of additive has no impact for following analysis. Anticoagulation effect is achieved by binding calcium ions or by inhibiting thrombin activity. The very important step is mixing ensuring effective distribution of solid or liquid anticoagulant within the whole blood.

2.7. Plasma for coagulation tests

Coagulation tests (routine and special) in modern laboratory require a lot of knowledge, continuous education, sensitive automatic analytic system, broad spectrum reagents with different purpose, quality manual and high-level communication with clinicians, phlebotomists and nurses with preanalytics algorithms detailed description or specimen collection and handling instructions (**Table 3**).

The sample types for coagulation tests to use in laboratory medicine are: platelet rich plasma (PRP), platelet poor plasma (PPP) and whole blood (**Figure 3**).

PRP is a component of blood (plasma) with concentrations of platelets above normal values. PRP typically contains 3–8 times more platelets concentration than normal plasma. PRP is used for platelet function assays (diagnostics constitutional and acquired thrombopathy, follow-up on anti-platelet treatment) and in a variety of clinical applications, based on the premise that higher content of platelet-derived growth factors should promote better healing. Platelet derivatives represent a promising therapeutic modality, offering opportunities for treatment of wounds, ulcers, soft-tissue injuries, and various other applications in cell therapy [17].

Specimen	Tests	Advantages	Limitations	Centrifugation
PPP	PT, aPTT, fibrinogen, single coagulation factors assays	Representative of circulating blood	Common preanalytics	One step: 1500–2000 × *g* 10–15 min
PRP	Platelet function assays	Two aims possible: (1) Diagnostics (2) Therapy	Sensitive for handling	Two steps: 1st: slow centrifugation: 170 × *g* calculated at the interface blood-plasma for 10 min to eliminate a red blood cells contamination. 2th: fast centrifugation: 2200–2400 × *g* for 20 min. Centrifugation at 4000 rpm
Whole blood	Platelet reactivity tests	Centrifugation is not required, the tests may be performed quickly. This sample type is used for POC analyser often.	Red and white cells can impact on the test results	Without special preparation

POC, point of care.

Table 3. Samples for coagulation.

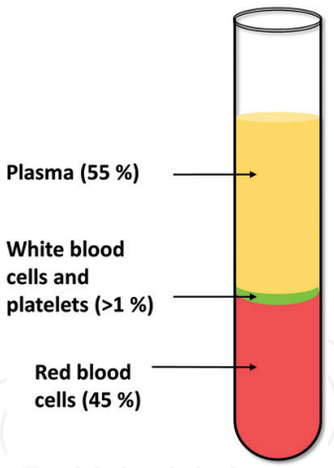

Figure 3. Plasma for coagulation tests.

Trueness of results in modern haemostaziology within modern laboratories depends on three major issues:

- Management preanalytics in the pre-laboratory phase and laboratory phase
- Sensitivity and possibilities of analytical system
- Interpretation of tests together with clinical data (clinical condition, anticoagulants, anti-platelet treatment)

2.7.1. Some preanalytics aspects

Though one mistake in preanalytics may distort the result and clinical interpretation may be wrong.

2.7.1.1. Clot in tube for coagulation tests?

The clot can be formatted *in vitro* in sample via this reasons:

- Slow blood flow into the tube
- Long pressure of tourniquet
- Significant (not acceptable for good practice) manipulative procedures with needle in vein
- Not enough mixing specimen with anticoagulant right away after blood drawing.

2.7.1.2. Serum or clotted sample?

If primary tube is taken inside laboratory, it is not difficult to determine whether blood was taken with proper anticoagulant. But errors may occur when samples get into with secondary tubes. It is not possible to state if proper anticoagulant or serum was taken instead of plasma. A serum does not has fibrinogen or other coagulation factors (FII, FV or FVIII) and high molecular weight of VWF as well. Serum analysis can give high levels of some factors (FVII) due to their activation and coagulation times testing for PT, aPTT and TT are not measured. False diagnosis can be established if plasma factor evaluation is wrong, as it often happens in the case of a certain type of Von Willebrand disease. Problems may occur with Lupus anticoagulant determination.

Modern laboratories try to provide equipment which allows to detect the clots in specimen after the detection of significantly longer clotting times, but a visual check for a clot in a sample should be recommended or use of two wooden applicators/sticks for whole blood. If a clot is found, it is necessary to reject a sample from further investigation [18].

2.7.2. Haemolysis impact for coagulation

Reasons of *In vitro* haemolysis may be due to incorrect blood drawing or inadequate handling of blood after collection. Haemolysis can occur *in vivo* when cell lysis happens inside of vessels by autoimmune haemolysis, severe infections, DIC or post-transfusion reactions.

Analytical systems with mechanical clot detection principle are not affected by the interference factors due to haemolysis, but results of tests may be not accurate because of products of cell lysis, including tissue factor which can activate coagulation. It is called a biological impact of haemolysis. Total effect of haemolysis may decrease the level of fibrinogen, but increase a level of D dimers. Prothrombin time may drop down because of lower concentration of fibrinogen; aPTT may lengthen or shorten depending which process prevails: decrease

of fibrinogen or activation of coagulation. Haemolysis may distort other coagulation tests results, for instance decrease of antithrombin level [19].

If possible, the sample with grossly haemolysis must be rejected. If tests must be performed (e.g., *in vivo* haemolysis, it is not possible to take a new sample), for this case, it is recommended to use mechanical detection systems, not excluding, that potential activation is possible as well.

Samples visually seem haemolysed via haemoglobin substitution, should not be rejected and must be analysed with mechanical or electromechanical clot detection method [20].

CLSI H21-A5:2008 Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays; Approved Guideline—Fifth Edition standard says:

> *When evident, the presence of visible hemolysis, as evidenced by a pink to red tinge to the plasma, should be noted. Lysis of red blood cells and resultant release of intracellular or membrane components may cause clotting factor activation. This activation of coagulation factors may impact clotting time results, whether using an optical or mechanical end-point detection system, although there is discrepancy in the literature about the impact of hemolysis on clot-based assays.[67,68] Until additional studies are published, due to the potential with result interference, grossly hemolyzed samples should not be used. Pink or red-tinged plasma specimens may further impair end-point detection when using an optical system, due to its interference with light transmittance. Plasma may be tinged when there is red cell lysis or when the patient has been administered a hemoglobin substitute. Samples that appear hemolyzed due to the presence of hemoglobin substitutes are not a cause of specimen rejection, and these samples should ideally be tested with an instrument that uses mechanical end-point detection.*

The best way to choose correct method when existing studies which were done with analytical systems and reagents combination and studied how haemolysis impact on routine (PT, APTT, fibrinogen) tests [21].

2.7.3. Specimen handling, storage, transportation

Transportation is a challenge of samples when specimens or samples are taken out to the external laboratories. Tubes should not be subjected to vibration, shaking, vortexing, continuous mixing or agitation. Transport and sorting as bulk goods is not compatible with reliable coagulation diagnostics. Generally, blood samples for coagulation analyses should not be shaken, and dropped samples should be discarded [1].

The transportation often causes a delay of sample analysis, so customer must be sure that specimens will be analysed in time. Blood samples for coagulation diagnostics should be stored at room temperature (20–25°C) until analysis. Storage at lower temperature, or on ice, may strongly influence some of the coagulation assays [1].

A storage of uncentrifuged samples at room temperature up to 6 h may yield acceptable results, although a shorter delay is desirable. Whole blood assays should be performed within 4 h after blood sampling. Data about handling and storage (time and temperature) of samples before transportation are different. Thus a laboratory must take decision how long the analysis may delay [16].

2.7.4. Continuous improvement of preanalytics in coagulation testing

Coagulation laboratory must regular review how to improve preanalytics in medical institution. Bostic et al. [19] studied, how possible to reduce quantity-not-sufficient specimens (QNS) via several methods and to measure effect of expired collection tubes on the amount of blood that can be drawn. During study period the rate of QNS specimens dropped from 0.7 to 0.3%. It was a significant difference in the amount of blood drawn into nonexpired vs. expired (**Image 1**).

The authors of study published a laboratory bulletin about Proper Blood Collection for Coagulation Studies [19].

2.8. Plasma for molecular-miRNR testing

Extracellular RNAs (exRNAs) found in biological samples have a potential to be used as clinical biomarkers for various diseases as well as treatment monitoring. Differences in miRNA expression profiles are associated with tumorigenesis and can be used to classify cancer type, identify the developmental origin, select and monitor treatment [22]. Due to relatively easy acquisition and handling blood plasma is an attractive specimen to be used for miRNA extraction compared to tissue samples. Even though miRNAs have been a major focus for

Image 1. Photograph of sodium citrate tubes containing blood from a single draw performed in a study period. The first three tubes from the left were expired and showed decreasing blood retrieval ability with older expiration dates (from right to left). The black arrow points to the minimum recommended fill level (etched line).

over a decade now, the majority of methodologies used for miRNA extraction and profiling come from a scientific research and are not approved for clinical use yet.

The major points affecting the miRNA extraction and application are choice of coagulant, plasma preparation and biological condition. Among the three most widely used anticoagulants (EDTA, citrate, and heparin), EDTA is shown to be the least interfering chemical in the subsequent miRNA profiling, while heparin and citrate interfere with enzymes used for various PCR [23]. Circulating miRNAs are either encapsulated in vesicles or found in complexes with proteins and lipoproteins, therefore are considered to be relatively stable. However, it is recommended that plasma preparation is done within 2 h of phlebotomy since blood cells start to release miRNAs into the collected sample causing changes in miRNA profile. Even mild haemolysis is also considered as an interfering factor associated with miRNA contamination from blood cells. Visual identification of minor haemolysis is difficult, therefore a simple measurement of absorbance can be an accurate and time saving solution. Prepared plasma samples could be quickly tested for haemolysis by measuring the absorbance peak of free haemoglobin at 414 nm [24]. Plasma samples with $A_{414} > 0.18$ show signs of miRNA released from erythrocytes which might interfere with the overall profile of circulating miRNAs [24]. It is worth noting that lipaemia affects the A_{414} measurement; therefore, it is recommended to perform a second measurement at A_{385} to detect the presence of lipaemia and use it to calculate a lipaemia-independent haemolysis score which could be adopted as a pre-analytical quality control [25]. Circulating miRNAs are often found in association with lipoproteins, therefore biological conditions, such as fasting, might have an impact on miRNA profiling [23]. The use of miRNA biomarkers in clinical setting is only starting therefore to standardise the pre-analytical procedure of sampling and sample preparation it is worth to have as little environmental variables as possible.

2.9. Quality manual for laboratory testing

A medical laboratory or clinical laboratory are laboratories for the examination of materials derived from the human body with a purpose of providing necessary information for diagnosis, prevention and treatment of disease as well as for follow up evaluation of health of a patient during the treatment. The 'International Organization for Standardization' (ISO) is an 'International Authority' for setting up standard guidelines for various organisations and laboratories. Each quality manual is based on internationally accepted standards and provides guidance for public health and clinical laboratories on writing policies and procedures that support a quality management system. It comprises a main document providing information and written procedures for laboratory quality (standard operating procedures, forms, and processes). It is worth to remember always to address quality manual before starting testing, sampling or consulting patients [24].

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Conflict of interest

There is no conflict of interests.

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References

[1] Guder WG, editor. Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and Their Impact on the Quality of Medical Laboratory Results. Walter de Gruyter GmbH & Co KG, Berlin/Boston; 2015

[2] Salive ME, Cornoni-Huntley J, Guralnik JM, Phillips CL, Wallace RB, Ostfeld AM, Cohen HJ. Anemia and hemoglobin levels in older persons: Relationship with age, gender, and health status. Journal of the American Geriatrics Society. 1992;**40**(5):489-96

[3] Alpers JP, Jones LK. Natural history of exertional rhabdomyolysis: A population-based analysis. Muscle & Nerve. 2010;**42**(4):487-491

[4] Tkachenko O, Shchekochikhin D, Schrier RW. Hormones and hemodynamics in pregnancy. International Journal of Endocrinology and Metabolism. 2014;**12**(2):e14098

[5] Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy: Review articles. Cardiovascular Journal of Africa. 2016;**27**(2):89-94

[6] Rodger M, Sheppard D, Gándara E, Tinmouth A. Haematological problems in obstetrics. Best Practice & Research Clinical Obstetrics & Gynaecology. 2015;**29**(5):671-684

[7] Subudhi AW, Jacobs KA, Hagobian TA, Fattor JA, Fulco CS, Cymerman A, Friedlander AL. Changes in Ventilatory Threshold at High Altitude: Effect of Antioxidants. Medicine and Science in Sports and Exercise. 2006;**38**(8):1425-1431

[8] Zakhari S. Overview: How is alcohol metabolized by the body? Alcohol Research & Health. 2006;**29**(4):245-255

[9] Nikolac N. Lipemia: Causes, interference mechanisms, detection and management. Biochemia Medica. 2014;**24**(1):57-67

[10] Gross J, Ungethüm U, Moller R, Priem F, Heldt J, Ziebig R, et al. Preanalytical factors influencing the measurement of NSE levels in blood. Journal of Laboratory Medicine. 1995;**18**:286-289

[11] Sepetiene R, Patamsyte V, Zukovas G, Jariene G, Stanioniene Z, Benetis R, Lesauskaite V. Blood plasma TGF-β1 concentration in sporadic dilatative pathology of ascending aorta: More questions than answers. PLoS One. 2015;**10**(6):e0129353

[12] Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clinical Chemistry. 2002;**48**(12):2242-2247

[13] Kersaudy-Kerhoas M, Sollier E. Micro-scale blood plasma separation: From acoustophoresis to egg-beaters. Lab on a Chip. 2013;**13**(17):3323-3346

[14] Goossens W, Duppen V, Verwilghen RL. K2-or K3-EDTA: The anticoagulant of choice in routine haematology? International Journal of Laboratory Hematology. 1991;**13**(3):291-295

[15] Narayanan S. The preanalytic phase: An important component of laboratory medicine. American Journal of Clinical Pathology. 2000;**113**(3):429-452

[16] CLSI. H3-A5. **23**(32):8.10.2

[17] Dhillon RS, Schwarz EM, Maloney MD. Platelet-rich plasma therapy-future or trend? Arthritis Research & Therapy. 2012;**14**(4):219

[18] Favaloro EJ, Lippi G, Adcock DM. Preanalytical and postanalytical variables: The leading causes of diagnostic error in hemostasis? In: Seminars in Thrombosis and Hemostasis. 2008;**34**(7):612-634

[19] Bostic G, Thompson R, Atanasoski S, Canlas C, Ye H, Kolins M, Smith MD. Quality improvement in the coagulation laboratory: Reducing the number of insufficient blood draw specimens for coagulation testing. Laboratory Medicine. 2015;**46**(4):347-355

[20] Kirschner M, Kao S, Edelman J, Armstrong N, Vallely M, van Zandwijk N et al. Haemolysis during sample preparation alters microRNA content of plasma. PLoS One. 2011;**6**(9):e24145

[21] Dudek MM, Harris LF, Killard AJ. Evaluation of activated partial thromboplastin time (aPTT) reagents for application in biomedical diagnostic device development. International Journal of Laboratory Hematology. 2011;**33**(3):272-280

[22] Gustafson D, Tyryshkin K, Renwick N. microRNA-guided diagnostics in clinical samples. Best Practice & Research Clinical Endocrinology & Metabolism. 2016;**30**(5):563-575

[23] Moldovan L, Batte K, Trgovcich J, Wisler J, Marsh C, Piper M. Methodological challenges in utilizing miRNAs as circulating biomarkers. Journal of Cellular and Molecular Medicine. 2014;**18**(3):371-390

[24] Kirschner M, Edelman J, Kao S, Vallely M, van Zandwijk N, Reid G. The impact of hemolysis on cell-free microRNA biomarkers. Frontiers in Genetics. 2013;**4**:94

[25] Appierto V, Callari M, Cavadini E, Morelli D, Daidone M, Tiberio P. A lipemia-independent NanoDrop®-based score to identify hemolysis in plasma and serum samples. Bioanalysis. 2014;**6**(9):1215-1226

[26] Guder WG, Narayanan S, Wisser H, Zawta B. From the patient to the laboratory: The impact of preanalytical variables on the quality of laboratory results. 4th ed. Weinheim: Wiley-Blackwell; 2009

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