



STABILITY OF POTASSIUM AND LDH INDICATORS IN HEMOLYTIC AND LIPEMIC BLOOD SERUM

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Abstract: Hemolysis and lipemia disrupt the stability of potassium (K) and lactate dehydrogenase (LDH) indicators in the preanalytical stage, causing falsely high results (pseudo-hyperkalemia). Erythrocyte breakdown (hemolysis) and optical interference from lipids lead to serious diagnostic errors in laboratory analyses. To avoid diagnostic paradoxes, the H-index and L-index should be checked automatically, and samples exceeding the critical limit should be re-analyzed. Studies conducted at the Memorial Hospital clinical laboratory have confirmed that hemolysis and lipemia are serious preanalytical interference factors, especially in the determination of lactate dehydrogenase and potassium. RBC fragmentation leads to pseudo-hyperkalemia, and lipemia reduces the accuracy of the results due to optical interference. These interferences create systematic deviations in laboratory indicators, increasing the risk of making incorrect clinical decisions. Therefore, strengthening sample quality control at the preanalytical stage plays a crucial role in the reliability of diagnostics. The study investigated the effects of hemolysis and lipemia on serum Potassium (K) and Lactate Dehydrogenase (LDH) levels and found that these interferences lead to false hyperkalemia and analytical bias. The results demonstrate the importance of H-index and L-index monitoring for critical diagnostic decisions in laboratory diagnostics. The objective of the experiment is to qualitatively evaluate analytical bias introduced by experimentally induced hemolysis and lipemia in serum K and LDH levels under controlled laboratory conditions and to assess their clinical significance and total allowable error (TAE) limits.

Keywords: preanalytical errors, hemolysis, lipemia, lactate dehydrogenase, potassium, clinical chemistry, HIL index, analytical bias.

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Introduction

The factor that most affects the objectivity of the results in clinical laboratory diagnostics and is considered one of the most critical errors in the preanalytical stage is the breakdown of erythrocytes, that

is, hemolysis (Alkhatib 2019; Atay et al. 2014). The leakage of intracellular elements into the serum is not just a technical defect but a serious threat that can directly lead to incorrect clinical decisions (Lippi et al.

2009; 2018). Statistics show that hemolysis is the cause of more than 60% of rejected samples, and it occurs five times more often than other preanalytical defects (Lippi et al. 2018; Salvagno et al. 2019). Technical errors made during venipuncture, sharp temperature fluctuations, and vibration during transportation are proximal sources that lead to in vitro damage to the erythrocyte membrane (Atay et al. 2014; Urwin et al. 2015; Hébert et al. 2005).

When cellular integrity is compromised, the leakage of internal analytes such as potassium, lactate dehydrogenase (LDH), and aspartate transaminase (AST) into the extracellular medium creates spectrophotometric interference, causing artificial elevations in test results (Atay et al. 2014; Farhana & Lappin 2021). Since the human eye cannot objectively assess these subtle color changes visually, the integration of automated systems and serological index (SII) measurements is inevitable. Automated management eliminates subjectivity, spectrally corrects for analytical bias, and reduces the need for repeated blood transfusions for the patient (Lippi et al. 2009; 2018).

However, there is significant heterogeneity in SII results from different platforms such as Roche, Abbott, Siemens, and Beckman Coulter (Lippi et al. 2018; Nikolac 2014; Tang et al. 2024). This variability necessitates the application of uniform standards between laboratories. In this regard, the study aims to analyze the interference mechanisms of hemolysis and systematize internal quality programs so that each laboratory can individually verify the analytical principles of HIL indices, including coagulation (Atay et al. 2014).

In clinical laboratory practice, one of the most important preanalytical factors that directly affects sample quality and seriously undermines the reliability of results is

lipemia. This condition, characterized by a higher-than-normal lipid concentration in blood plasma or serum, causes samples to appear macroscopically cloudy, sometimes completely milky. Scientific sources note that this macroscopic change is often associated with a sharp increase in triglyceride levels; this condition can occur both due to physiological (postprandial) factors after food intake and due to pathological causes such as various metabolic disorders, hyperlipidemia, and diabetes mellitus. The main danger posed by lipemia is not the visual appearance of the biological material, but the optical chaos it creates in biochemical measuring instruments. Turbidity changes the physical properties of the sample, causing direct interference in spectrophotometric-based analyses and leading to incorrect calculation of results (Lippi et al. 2009; 2018). According to studies, the absorption or scattering of light by lipid particles impairs the measuring mechanism of the device, which leads to artificially increasing or decreasing analyte concentrations (Calmarza & Cordero 2011; McDonough & Youn 2017; Schlüter & Cadamuro 2023). In addition, excessively fatty samples completely block the operation of optical sensors, causing technical failures in the analyzers and complicating the internal quality control of the laboratory. In this regard, the measurement of automated HIL indices is indispensable for timely prediction of potential complications of lipemia. Although the lipemia index (L index) does not always form a complete mathematical correlation with triglyceride concentration, it allows for an objective assessment of the actual degree of turbidity in the sample and the optical obstacle that the device will encounter. This index helps to control the analytical environment more transparently by measuring the contamination caused by large lipoproteins, especially chylomicrons



or VLDL (very low-density lipoproteins), at a wavelength of 660 nm or 700 nm. The interference mechanism of lipemia is not limited to light scattering or absorption; factors such as unequal partitioning of analytes between polar and nonpolar phases, as well as a decrease in the water fraction (volume shift error), also play a role. This situation falsely lowers the electrolyte concentrations measured by indirect potentiometry (Nikolac 2014; Tang et al. 2024). Although it has been proposed to develop standardized lipemic models in laboratories to investigate this interference, models built with artificial substances such as Intralipid cannot fully reflect the lipoprotein heterogeneity in real patient blood. For this reason, the use of natural lipemic sera should be preferred in the evaluation (Berman & Hayes 2024; Nikolac 2014). To prevent misdiagnosis during laboratory tests, appropriate purification methods are needed to overcome this lipid barrier (Farhana & Lappin 2021; Zhou et al. 2022). Although ultracentrifugation, which is considered the reference method, effectively separates lipids, its high cost, non-availability in every laboratory, and prolonged analysis time (TAT) are serious disadvantages. For this reason, many clinical laboratories are turning to high-speed microcentrifugation, a more economical and rapid alternative. Studies have shown that this method demonstrates almost the same efficiency as ultracentrifugation in the determination of routine analytes such as sodium, creatinine, urea, and total protein. However, although high-speed conventional centrifugation easily precipitates the turbidity caused by chylomicrons, it cannot ensure complete separation in samples dominated by smaller VLDL particles; in this regard, ultracentrifugation still maintains its leadership. In some cases of mild lipemia, the use of a serum blank (serum correction) may eliminate the need

for physical purification. In laboratories with limited material and technical resources, chemical precipitation methods using polyethylene glycol 6000 (PEG) or various cyclodextrins (alpha, gamma) are used as an alternative to physical methods. These chemical agents aggregate and precipitate lipid particles and minimize false glucose and albumin changes in photometric analyses. PEG concentrations ranging from 4% to 8% restore the accuracy of enzymatic reactions such as AST and ALT by providing optimal purification in a triglyceride environment of 300–500 mg/dL. Of course, the risk of partial inhibition of enzyme activity by extremely high PEG concentrations such as 20% should not be forgotten, and appropriate dilution corrections should be performed (Wu et al. 2021; Zhou et al. 2022).

Materials and Methods

This controlled experimental laboratory study was conducted at the Clinical Biochemistry Laboratory of Memorial Hospital (Baku, Azerbaijan) to systematically quantify the analytical interference and bias introduced by preanalytical hemolysis and lipemia on serum potassium (K^+) concentrations and lactate dehydrogenase (LDH) activity. A total of 23 non-interfered residual venous blood specimens were prospectively selected from adult patients undergoing routine health screenings. Venipuncture was performed by certified phlebotomists adhering strictly to the Clinical and Laboratory Standards Institute (CLSI GP41-A6) guidelines. Blood was drawn into 5 mL serum separator tubes (SST) containing a clot activator and acrylic polymer gel. To eliminate iatrogenic preanalytical artifacts during collection, standardized protocols were enforced: tourniquet application was restricted to under 60 seconds, gentle tube inversion (5–8 times) was performed, and

mechanical shear stress from forceful aspiration was strictly avoided. Specimens exhibiting micro-clots, inadequate volume (<2.5 mL), or visually detectable baseline hemolysis, lipemia, or icterus were immediately excluded.

Following phlebotomy, specimens were allowed to complete clot retraction at ambient room temperature (20–22°C) for exactly 30 minutes before centrifugation. Centrifugation was executed at 3500 rpm (equivalent to 2000×g) for 15 minutes at 4°C in a temperature-controlled centrifuge. Uncontaminated baseline serum pools were

immediately decanted into secondary polypropylene tubes to prevent cellular leakage. Controlled hemolysis was simulated via Vitruvian-calibrated mechanical disruption of autologous packed red blood cells obtained from the cell pellet, yielding escalating hemoglobin concentration gradients across paired aliquots. Lipemia was experimentally induced through the standardized addition of a 20% intravenous lipid emulsion (Intralipid), creating serial turbidity profiles ranging from mild opalescence to severe milky opacity (**Figure 1**).

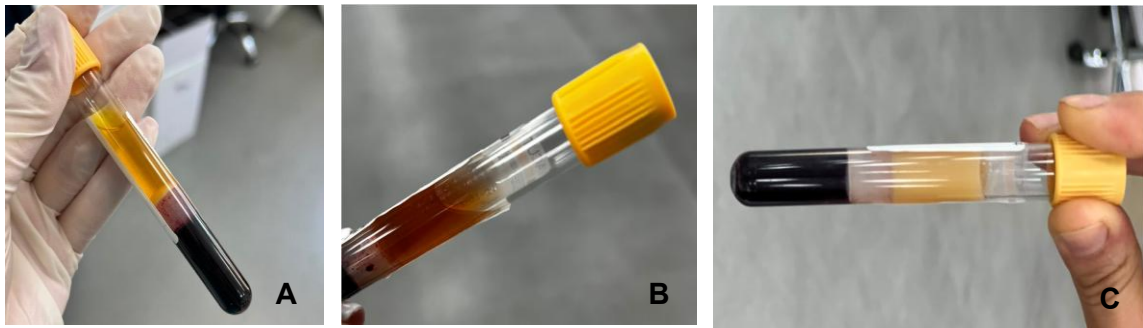


Figure 1. Examined blood samples. **A.** Blood sample after centrifugation; **B.** Hemolytic blood; **C.** Lipemic blood.

Analytical determinations were executed on automated clinical chemistry analyzer platforms under standardized ambient conditions. Serum potassium was measured utilizing an Ion-Selective Electrode (ISE) module operating via potentiometric measurement. LDH catalytic activity was quantified through an IFCC-standardized kinetic enzymatic assay at 340 nm, tracking the rate of nicotinamide adenine dinucleotide (NADH) oxidation during the reversible reduction of pyruvate to lactate. Analytical reliability was guaranteed by processing two levels (normal and pathological) of certified liquid internal quality control (IQC) materials before each run, ensuring total analytical imprecision remained well within target allowable limits. To suppress inter-assay drift and matrix alterations, all experimental aliquots were

analyzed in duplicate within a single operational batch; short-term storage, when necessary, was restricted to 2–8°C for a maximum of 4 hours, strictly avoiding freeze-thaw cycles.

Statistical processing was performed using IBM SPSS Statistics (Version 29.0; IBM Corp., Armonk, NY, USA). Continuous parametric metrics were summarized as mean $\pm$ standard deviation (SD), whereas non-parametric data were presented as median and interquartile range (IQR). The normality of data distributions was tested using the Shapiro–Wilk test alongside visual inspection of Q-Q plots. Paired-sample t-tests or repeated-measures analysis of variance (ANOVA) with Tukey's post hoc correction were utilized to evaluate parametric within-subject concentration shifts across interference tiers. Non-



parametric variables were evaluated using Wilcoxon signed rank or Friedman tests. Linear regression models and Pearson (r) or Spearman (ρ) correlation coefficients were calculated to model interference-bias response curves. Statistical significance was designated at a two-tailed $p < 0.05$. The protocol maintained complete compliance with the ethical directives of the Declaration of Helsinki, utilizing fully anonymized residual diagnostic specimens without patient contact or clinical care disruption.

Results

1. Complex pathophysiological analysis of LDH activity, potassium levels, and specific markers in different age and sex groups of hemolytic blood

To assess cellular integrity, internal homeostasis, and metabolic processes at different ages, a retrospective analysis was performed on the laboratory profiles of three patients of different age and gender groups examined at the Memorial Hospital laboratory. Within the framework of the study, lactate dehydrogenase (LDH) enzyme activity and potassium ion dynamics were examined in parallel with other specific biochemical and endocrine markers in the context of different ages.

2. Pathophysiological evaluation of LDH activity and anabolic markers in the pediatric age group

To evaluate the biochemical dynamics in the pediatric age group, the laboratory parameters of a 3.5-year-old male patient were analyzed. The patient's serum LDH level was determined to be 297 U/L and was within the upper reference limit (314 U/L) set for that age group, i.e., within the physiological normal range (Figure 2). Against the background of intensive growth and tissue development of the general organism in children, it is a normal physiological pattern that the basal level of this enzyme is higher than in adults. The concentration of somatotrophic hormone (STH), which characterizes the patient's anabolic status, was found to be 1.41 ng/mL, which is fully adequate to the reference range set for children (0.067–2.167 ng/mL). At the same time, the Vitamin B12 level was also recorded at 575.8 pg/mL, within the ideal median of the reference range (201–804 pg/mL). This indicator confirms, from a pathophysiological point of view, that there is no hidden hematological dysfunction (e.g., megaloblastic anemia and associated ineffective erythropoiesis in the bone marrow) that could lead to falsely elevated LDH levels in vitro.

Təsdiq	Açar	Xidmət Adı	Flg	Nəticə	Sənəd Status	Nəticə Qrupu	Modul	Min	Max	Ölçə Vəziyyəti	İzəh
☒	☐	LDH*		204		Biokimya	U/L	135	214		
☒	☐	Ca total (qan)*		2,51		Biokimya	mmol/L	2,2	2,55		
☒	☐	Parathormon*		15,28		Hormonal müay.	pg/mL	15	65		
☒	☐	CA 15-3*		26,84		Onkomarker	U/mL	<	30		
☒	☐	CEA*		1,29		Onkomarker	U/mL	<	30		

Təsdiq	Açar	Xidmət Adı	Flg	Nəticə	Sənəd Status	Nəticə Qrupu	Modul	Min	Max	Ölçə Vəziyyəti	İzəh
☒	☐	LDH*		207		Biokimya	U/L	135	214		
☒	☐	CA 19-9*		23,54		Onkomarker	U/mL	<	39		
☒	☐	CEA*		0,751		Onkomarker	U/mL	<	39		

Təsdiq	Açar	Xidmət Adı	Flg	Nəticə	Sənəd Status	Nəticə Qrupu	Modul	Min	Max	Ölçə Vəziyyəti	İzəh
☒	☐	ALT*		24,3		Biokimya	U/L	<	41		
☒	☐	LDH*		158		Biokimya	U/L	135	225		
☒	☐	Kreatin kinaza (CK)	↑	314		Biokimya	U/L	39	308		

Figure 2. LDH analysis result panel

3. Pathophysiological analysis of LDH, ALT and isolated CK activity in young adulthood

The biochemical profile of a 25-year-old male patient in a high stage of biological activity was included in the analysis. In the patient's analysis, the liver-specific transferase enzyme (ALT) was recorded at 24.3 U/L, and the total LDH level was 158 U/L, which were completely within the optimal normative limits (reference: 135–225 U/L). However, in this patient's profile, it was determined that the muscle isoenzyme creatine kinase (CK) was 314 U/L, slightly exceeding the maximum reference norm of 308 U/L. This isolated CK increase, which occurred when the liver and general cellular damage markers were normal, is related to the acute physical activity or heavy sports stress in the patient's anamnesis. The stability of LDH, reflecting general cytoplasmic destruction, and ALT, indicating hepatocellular status, proves that this increase is not due to structural pathology, but is completely transient and functional muscle leakage related to physical exertion.

4. Comprehensive laboratory analysis of electrolyte balance and renal function in

a young female patient

To assess electrolyte regulation in young age, biochemical parameters of a 21-year-old female patient were reviewed. The patient's serum potassium level was determined to be 4.4 mmol/L, which was in the middle of the laboratory reference range (3.5–5.1 mmol/L), indicating an ideal homeostatic level (**Figure 3**). Although the total protein and albumin concentrations in other markers of this patient were normal, the levels of urea, which reflects the excretory function of the kidney, and parathyroid hormone (PTH), which regulates bone-mineral metabolism, sharply exceeded the maximum reference limits. Despite the transient overload of the kidney's filtration function and the tendency for nitrogen residues to accumulate in the blood, the potassium balance is under full control. The clinical picture shows that in a young organism, internal compensatory systems and the internal organization of cell membranes have a high functional reserve. As a result, even during renal filtration stress, the risk of life-threatening hyperkalemia is successfully prevented, and the potassium ion balance is maintained stably.

	Tasdiq	Açar	Xidmat Adı	Fig	Natıca	Sanaad Statusı	Natıca Grupu	Modul	Min	Max	Chaqıyıştıq	Sahı
	☒	☐	K+*		4,75		Biolımya	mmol/L	3,5	5,1	-	-
	☒	☐	TSH*		1,07		Hormonal mda...	uIU/mL	0,27	4,2	-	-
	☒	☐	free T4*		1,47		Hormonal mda...	ng/dL	0,9	1,7	-	-
	☒	☐	Anti-TPO		5,0		Hormonal mda...	IU/mL	<	34	-	-

	Tasdiq	Açar	Xidmat Adı	Fig	Natıca	Sanaad Statusı	Natıca Grupu	Modul	Min	Max	Chaqıyıştıq	Sahı
	☒	☐	Umumi zülal (qan)*		75,9		Biolımya	g/L	66	87	-	-
	☒	☐	Albumin (qan)*		43,8		Biolımya	g/L	35	52	-	-
	☒	☐	Sidik cövhəri*	↑	12,9		Biolımya	mmol/L	1,7	8,3	-	-
	☒	☐	K+*		4,55		Biolımya	mmol/L	3,5	5,1	-	-
	☒	☐	Fosfor*		1,45		Biolımya	mmol/L	0,81	1,45	-	-
	☒	☐	Parathormon*	↑	144,4		Hormonal mda...	pg/mL	15	65	-	-

	Tasdiq	Açar	Xidmat Adı	Fig	Natıca	Sanaad Statusı	Natıca Grupu	Modul	Min	Max	Chaqıyıştıq	Sahı
	☒	☐	Ca total (qan)*		2,25		Biolımya	mmol/L	2,2	2,58	-	-
	☒	☐	K+*		4,57		Biolımya	mmol/L	3,5	5,1	-	-
	☒	☐	Fe*		42,66		Biolımya	ug/dL	27	150	-	-
	☒	☐	TSH*	↑	13,11		Hormonal mda...	uIU/mL	0,27	4,2	-	-
	☒	☐	free T3*		2,84		Hormonal mda...	pg/mL	2	4,4	-	-
	☒	☐	free T4*	↓	0,740		Hormonal mda...	ng/dL	0,9	1,7	-	-
	☒	☐	Anti-TPO	↑	591,4		Hormonal mda...	IU/mL	<	34	-	-

Figure 3. Potassium indicator analysis panel



The results of the conducted experimental study showed that hemolysis and lipemia, as the most important preanalytical interference factors in clinical laboratory diagnostics, significantly affect the determination of biochemical parameters, especially lactate dehydrogenase (LDH) activity and potassium ion concentration. In the study conducted at the Memorial Hospital clinical laboratory center, it was confirmed that these two factors cause systematic and quantitative deviations in laboratory results.

During hemolysis, disruption of the membrane integrity of erythrocytes has been observed to result in the free passage of intracellular LDH and potassium into the serum environment. Due to the significantly higher intracellular concentration compared to the extracellular environment, this leakage has led to an artificial increase in indicators and, in particular, to clinical interpretation errors (pseudo-hyperkalemia).

The kinetic indicators obtained within the framework of the study prove that the absorption rate changes in direct proportion to the increase in the degree of hemolysis and that the analytical bias exceeds critical limits.

Lipemia, on the other hand, increased light scattering in photometric measurements due to the increase in lipid and lipoprotein fractions (mainly chylomicrons and very low-density lipoproteins (VLDL) in plasma. This condition significantly reduced the accuracy of the results by creating optical interference in spectrophotometric analyses. In particular, in kinetic enzymatic measurements conducted at wavelengths of 340 nm and near it, the turbidity caused by lipid particles weakened the transmission of light to the sensors, minimizing the analytical sensitivity of the reactions.

The results of the study show that as the degree of hemolysis and lipemia

increases, the deviations in LDH and potassium indicators also increase in parallel; there is a statistically and pathophysiologically significant correlation ($p < 0.05$) between these changes. These factors directly increase the risk of making incorrect clinical decisions in the interpretation of laboratory results. Thus, strengthening sample quality control at the preanalytical stage, the application of automated serum indices (ASIs), and timely detection of hemolysis/lipemia are crucial in ensuring the reliability of laboratory diagnostics.

Conclusion and Recommendations

- **Preanalytical Standardization:** Laboratory management should fully standardize venipuncture, sample transport, and centrifugation steps.
- **Automated HIL Integration:** Subjective visual assessment should be eliminated, and the device-derived hemolysis (H) and lipemia (L) indices should be quantitatively measured for each sample.
- **Internal Quality Control:** Multi-step control programs based on Westgard rules should be implemented, especially in potassium and LDH assays, which are extremely sensitive to hemolysis.
- **Clinical Correlation:** In isolated marker elevations (e.g., a slight increase in CK in the presence of normal LDH or an increase in urea in the presence of normal potassium), the patient's physical history and compensatory mechanisms should be taken into account.

The factors that most affect the objectivity and reliability of test results in clinical laboratory diagnostics are hemolysis and lipemia, which are critical defects of the preanalytical stage. Statistics show that more

than 60% of the reasons for rejection of unsuitable blood samples are related to these interferences. The main aim of this study is to investigate the quantitative deviations and optical interference mechanisms caused by hemolysis and lipemia in spectrophotometric and potentiometric measurements, to evaluate the purification methods (centrifugation and chemical methods) that eliminate these lipid and hemoglobin barriers, and to analyze the dynamics of lactate dehydrogenase (LDH) and potassium ions in different age and gender groups in parallel with specific markers.

In a 3.5-year-old pediatric patient, LDH levels (297 U/L), somatotrophic hormone (1.41 ng/mL), and Vitamin B12 (575.8 pg/mL) were in physiological harmony with the indicators, reflecting an intensive anabolic process. In a 25-year-old young male patient, an isolated CK elevation (314 U/L) recorded against a background of normal LDH and ALT proved not to be structural damage, but a transient functional muscle leak due to physical exertion. In a 21-year-old female patient, despite the filtration stress caused by high urea and PTH levels, the constant potassium ion level (4.4 mmol/L) confirmed the pathophysiological stabilization of the cell membrane. Experimental analyses have shown that as the degree of hemolysis increases, in vitro false LDH and potassium (pseudo-hyperkalemia) elevations occur due to leakage of intracellular analytes, and optical light scattering and volume shift errors in lipemia lead to systematic deviations. In overcoming this lipid barrier, microcentrifugation and 4%-8% PEG concentrations were found to be effective alternatives in restoring the accuracy of routine analytes, while ultracentrifugation retained its advantage in small VLDL particles.

In conclusion, age-specific physiological adaptations must be taken into

account when interpreting laboratory results in different age groups. To ensure the reliability of laboratory diagnostics, subjective visual inspection should be eliminated, automated serum indices (ASIs) and spectral absorption corrections should be integrated, and each laboratory should validate the analytical working principles of individual analyzer platforms with internal quality control programs.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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