Technical Note

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Effect of changes in laboratory light intensity on biochemistry and haemogram analysis

[Laboratuvar ışık yoğunluğundaki değişikliklerin biyokimya ve hemogram analizi üzerine etkisi]

<https://doi.org/10.1515/tjb-2019-0124>

Received March 26, 2019; accepted December 25, 2019

Abstract

Background: To investigate the effect of changes in laboratory light intensity on chemistry and whole blood analysis.

Materials and Methods: The light intensity of the laboratory environment was measured and chemical and whole blood analysis was performed on 20 patient blood samples. The light intensity was then increased using projectors and re-measured, and the chemical and whole blood analyses were repeated. The values of the tests pre- and post-light increase were compared by statistical analysis using the Wilcoxon test.

Results: Increasing light from 195 to 1,168 lux significantly altered the results of the lipase, alkaline phosphatase, creatinine, and iron chemistry tests, ($p < 0.001$ [11.3%], $p = 0.003$ [2.2%], $p = 0.001$ [2%] and $p < 0.001$ [1.2%], respectively). There was also a significant difference in platelet count ($p < 0.001$ [188%]).

Conclusions: We show that the platelet count is sensitive to changes in laboratory light intensity at clinically unacceptable levels. The lipase, alkaline phosphatase, creatinine and iron tests are also sensitive to changes in laboratory light intensity, but at clinically acceptable levels.

Keywords: alkaline phosphatase; analytical error; creatinine; iron; lipase; thrombocyte.

Öz

Amaç: Laboratuvar ışık yoğunluğundaki değişikliklerin kimyasal ve tam kan analizleri üzerine etkisini araştırmaktır.

Gereç ve Yöntem: Laboratuvar ortamının ışık şiddeti ölçüldü ve yirmi hastanın kan örneklerinde kimyasal ve tam kan analizi yapıldı. Işık şiddeti daha sonra projektörler kullanılarak artırıldı ve yeniden ölçüldü ve kimyasal ve tam kan analizleri tekrarlandı. Işık öncesi ve sonrası testlerin artış değerleri, Wilcoxon testi kullanılarak yapılan istatistiksel analizlerle karşılaştırıldı.

Bulgular: 195 ile 1,168 lux arasında artan ışık, lipaz, alkalın fosfataz, kreatinin ve demir kimyasal testlerinin sonuçlarını önemli ölçüde değiştirdi, ($p < 0.001$ [%11.3], $p = 0.003$ [%2.2], $p = 0.001$ [%2] ve $p < 0.001$ [%1.2]). Trombosit sayısında da anlamlı bir fark vardı ($p < 0.001$ [%188]).

Sonuç: Trombosit sayısının klinik olarak kabul edilemez seviyelerde laboratuvar ışık yoğunluğundaki değişikliklere duyarlı olduğunu gösterdik. Lipaz, alkalın fosfataz, kreatinin ve demir testleri de laboratuvar ışık yoğunluğundaki değişikliklere duyarlıdır, ancak klinik olarak kabul edilebilir seviyelerdedir.

Anahtar Kelimeler: Alkalın fosfataz; Analitik hata; Demir; Kreatinin; Lipaz; Trombosit.

Introduction

Laboratory measurements always contain errors and uncertainties. It is not possible to perform a chemical analysis that is completely error-free and certain [1]. Laboratory errors typically occur in the pre-analytical or post-analytical phases, and less often during the analytical phase [2].

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The level of uncertainty is a range of values given alongside a measurement or test result that are attributable to the measured magnitude [3]. The most important factor limiting the accuracy and sensitivity of an analytical method is the presence of extraneous and unwanted signals called “noise”, which overlap with the analytical signal to be measured. Chemical, instrumental and environmental noise is encountered in any analysis method that makes use of an instrument for measurement [4]. Chemical noise includes undetected variations in temperature or pressure that affect the position of the chemical equilibrium, and changes in light intensity that affects photosensitive materials. Environmental noise covers numerous sources of electromagnetic radiation. These include alternating current power lines, firing systems in gasoline engines, arc switches, brushes in electric motors, radio and TV stations, illumination and ionospheric distortion [4]. Changes in optical radiation can affect both optical and potentiometric sensors. If the optical sensor is based on the use of a spectrophotometer, the ambient light should be kept constant [5]. In this study, we aimed to investigate the effects of changes in laboratory light intensity on chemistry tests and whole blood analyses.

Materials and methods

Study area and ethics approval

In this study, we randomly selected 20 patients who came to Adiyaman University Medical Faculty Medical Chemistry Department from different clinics for analysis. The study was approved by Adiyaman University Ethics Committee and informed consent was obtained from all patients.

Experiment pattern

The light intensity of the laboratory environment was measured as 195 lux with a DT 1301 light metre. Chemistry and full blood analyses were performed on the blood samples of 20 patients. The light intensity of the laboratory environment was then increased using three 1,000 Watts HL102 halogen projectors (Horoz Electric) and the increased light intensity in the laboratory environment was measured as 1,168 lux. The chemistry and full blood analyses were repeated on the blood samples used earlier.

Chemistry and whole blood analyses

The patient blood samples taken for chemical analysis were centrifuged at 3,500 rpm for 5 min at 4 °C and stored at –80 °C. A Roche Cobas C501 automatic photometric analyser (Roche Diagnostics co., Ltd., Mannheim, Germany) was used to test patient plasma for levels of fasting blood sugar (FBS), albumin (ALB), total protein (TP),

amylase (AMYL), lipase (LIP), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), uric acid (UA), creatinine (CREAT), iron, iron binding capacity in the unsaturated blood state (UIBC), total bilirubin (TBIL), direct bilirubin (BILD), magnesium (MG), phosphorus (P), calcium, triglyceride (TG), total cholesterol, and low-density lipoprotein-cholesterol (LDL-C) vs high density lipoprotein-cholesterol (HDL-C). Whole blood samples obtained from patients were immediately analysed using an XT-2000i automatic haematology analyser (Sysmex Corporation of America, Long Grove, Illinois, USA) to measure whole blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), platelet crit (PCT), erythrocyte distribution width-standard deviation (RDW-SD), erythrocyte distribution width-coefficient of variation (RDW-CV), neutrophils absolute neutrophil count (NEUTS, ANC), lymphocytes absolute lymphocyte count (LYMPHS, ALC), and counts for monocytes (MONOS), eosinophils (EOS) and basophils (BAS). Quality control studies for chemistry and whole blood analyses.

In our laboratory, we performed several quality control practices to ensure that our instruments adhered to analytical performance requirements. The Abbott Clinical Chemistry Multiconstituent Calibrator reagent was used to calibrate the photometric analyser for the FBS, ALB, TP, AMYL, LIP, CK, AST, ALT, GGT, ALP, LDH, BUN, UA, CREAT, iron, UIBC, TBIL, direct bilirubin, MG, P, calcium, TG, total cholesterol and LDL-C tests; while the Abbott Clinical Chemistry HDL Cholesterol Calibrator was used for the HDL-C test. For all biochemical parameters, internal quality control was performed with a two-level Lymphochek Assayed Chemistry Control Levels 1, 2 (BIO-RAD) control serum and the EQAS Clinical Chemistry Program (BIO-RAD) was used for external quality control. A daily internal quality control check of the haematology analyser was performed with e-check (XE) control serum (Sysmex) for WBC Count, RBC Count, HB, HCT, MCV, MCH, MCHC, PLT, PDW, MPV, P-LCR, PCT, RDW-SD, RDW-CV, NEUTS (ANC), LYMPHS (ALC), MONOS, EOS, BAS. External quality control was performed with control serum obtained from Bio-Rad external quality assurance services (EQAS).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows, version 15.0 software program was used for statistical analyses. Since the sample size was 20, a nonparametric Wilcoxon test was used to compare the pre- and post-test values. The obtained results were evaluated with a confidence interval of 95% and a significance level of $p < 0.05$.

Results

Chemistry results before and after increase in light intensity. There were no statistically significant differences in the photometric analyser readings for FBS, ALB, TP, AMYL, CK, AST, ALT, GGT, LDH, BUN, UA, UIBC, TBIL,

direct bilirubin, MG, P, calcium, TG, cholesterol and LDL-C vs HDL-C in 195 and 1,168 lux luminous environments. The changes between the values reported in the pre- and post-experimental biochemical tests of LIP, ALP, CREAT, iron, CK, GGT, LDH and direct bilirubin were 11.3, 2.2, 2, 1.2, 2.7, 2.9, 2.2, and 2.5%, respectively. However, a statistically significant difference was found in the analysis results of the LIP, ALP, CREAT and iron chemistry tests ($p < 0.001$, $p = 0.003$, $p = 0.001$ and $p < 0.001$, respectively) (Table 1). The lipase, alkaline phosphatase, creatinine, iron and biochemistry tests were sensitive to changes in laboratory light intensity, but these changes were at clinically acceptable levels. Results for whole blood analyses performed before and after increase in light intensity. There was no statistically significant difference in the haematology analyser results for WBC Count, RBC Count, HB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, NEUTS (ANC), LYMPHS (ALC), MONOS, EOS and BAS when the tests were performed in 195 and 1,168 lux luminous environments. However, a statistically significant difference was found in the PLT parameter of the whole blood analysis ($p < 0.001$) (Table 2). Again, the PDW, MPV, P-LCR and PCT parameters could not be measured in a 1,168 lux luminous environment. Our data reveal that the platelet reading is sensitive

to changes in laboratory light intensity and this change is at clinically unacceptable levels. This result is clinically significant because PLT is one of the determining parameters in the decision to administer platelet suspension treatment to a patient.

Discussion

The ion-sensitive field-effect transistor (ISFET) is one of the most popular electrical biosensors. Traditionally called a pH sensor, an ISFET is used to measure the ion concentrations (H^+ or OH^-) of a solution. In general, enzymes FETs are based on a similar principle, but rely on the binding of an analyte to a sensor such that the concentration of hydrogen ions during an enzymatic reaction is proportional to the substrate level. To date, various enzyme FETs have been developed to detect numerous analytes, such as glucose, urea, creatinine, protein, and TG. It was observed that the long-term stability of ISFETs was strongly influenced by exposure to a fluorescent light. He repeatedly performed deviation tests by switching the light on and off at light intensities ranging from 800 to 1,500 lux and found that the deviation was higher at light intensities

Table 1: Wilcoxon test results for biochemistry analyses.

Test/unit	Before mean + SD (195 lux)	After mean + SD (1,168 lux)	Difference	Asymp. Sig. (2-tailed)	% Variation	1999 Ricos C, TE (%), [7]
FBS/mg/dL	108 ± 33	109 ± 33	0.91	0.211	0.8	10.1
ALB/g/dL	4.7 ± 0.4	4.7 ± 0.4	0.001	0.779	0.02	4.9
TP/g/dL	7.3 ± 0.6	7.3 ± 0.7	0.032	0.794	0.4	4.4
AMY/U/L	73 ± 19	73 ± 20	-0.15	0.341	0.2	18.9
LIP/U/L	28 ± 15	25 ± 14	-3.15	<0.001	11.3	37
CK/U/L	149 ± 169	145 ± 166	-3.95	0.409	2.7	38.1
AST/ U/L	21 ± 6	21 ± 6	-0.24	0.498	1.1	19.2
ALT/U/L	17 ± 7	17 ± 7	-0.26	0.128	1.5	40.4
GGT/U/L	30 ± 31	29 ± 31	-0.88	0.317	2.9	26.9
ALP/U/L	149 ± 98	145 ± 99	-3.31	0.003	2.2	13.9
LDH/U/L	199 ± 56	203 ± 52	4.33	0.007	2.2	14.3
BUN/mg/dL	13.2 ± 4	13.3 ± 4	0.1	0.223	0.7	19.8
UA/mg/dL	4.8 ± 1	4.7 ± 1.1	-0.064	0.033	1.3	14.8
Creat/mg/dL	0.70 ± 0.22	0.72 ± 0.22	0.014	0.001	2	8.4
IRON/mcg/dL	70 ± 31	71 ± 32	0.85	<0.001	1.2	39.7
UIBC/mcg/dL	304 ± 67	306 ± 68	1.72	0.502	0.6	-
TBIL/mg/dL	0.38 ± 0.17	0.38 ± 0.17	0.0051	0.831	1.3	39.8
Direct Bilirubin/mg/dL	0.15 ± 0.06	0.15 ± 0.06	0.0037	0.920	2.5	57.1
MG/mg/dL	2 ± 0.2	1.9 ± 0.2	-0.013	0.255	0.7	6
P/mg/dL	3.9 ± 1.2	3.8 ± 1	-0.052	0.681	1.3	13.1
Calcium/ mg/dL	9.9 ± 0.8	9.8 ± 0.5	-0.028	0.155	0.3	3.1
TG/mg/dL	187 ± 165	186 ± 162	-1.02	0.036	0.5	35.1
Cholesterol/ mg/dL	169 ± 44	170 ± 43	-0.08	0.023	0.05	11.1
HDL-C/mg/dL	42 ± 14	42 ± 14	-0.18	-0.015	0.4	13.5
LDL-C/mg/dL	89 ± 35	90 ± 34	0.25	0.205	0.3	16.4

Bold values represent a statistically significant difference was found in these parameters.

Table 2: Wilcoxon test results for hemogram analyses.

Test	Before mean + SD (195 lux)	After mean + SD (1,168 lux)	Difference	Asymp. Sig. (2-tailed)	% Variation	1999 Ricós C, TE (%), [7]
WBC Count/ $10^3/\mu\text{L}$	8.8 ± 2	8.9 ± 1.9	0.074	0.051	0.84	15.49
RBC Count/ $10^6/\mu\text{L}$	4.9 ± 0.5	4.9 ± 0.5	-0.004	0.570	0.081	16.8
HB/g/dL	13.4 ± 1.7	13.4 ± 1.6	-0.06	0.033	0.44	5.1
HCT/%	41 ± 5	41 ± 5	-0.06	0.431	0.14	5
MCV/fL	83 ± 6	83 ± 6	-0.19	0.109	0.22	2.8
MCH/pg	27 ± 2	27 ± 2	-0.095	0.125	0.35	3.2
MCHC/g/dL	33 ± 1	33 ± 1	0.11	0.175	0.33	2.8
RDWSD/fL	41 ± 3	41 ± 3	-0.08	0.275	0.19	-
RDWCV/%	14 ± 1	14 ± 1	-0.01	0.614	0.07	-
NEUTS, ANC/ $10^3/\mu\text{L}$	4.7 ± 1.6	4.8 ± 1.6	0.058	0.014	1.23	23.35
LYMPHS, ALC/ $10^3/\mu\text{L}$	3.1 ± 1.7	3.1 ± 1.7	-0.013	0.573	0.41	17.6
MONOS/ $10^3/\mu\text{L}$	0.77 ± 0.18	0.77 ± 0.2	0.001	1,000	0.12	27.9
EOS/ $10^3/\mu\text{L}$	0.18 ± 0.15	0.18 ± 0.15	-0.0005	0.875	0.27	37.1
BAS/ $10^3/\mu\text{L}$	0.04 ± 0.02	0.04 ± 0.02	0.0025	0.197	6.25	38.5
PLT/ $10^3/\mu\text{L}$	272 ± 86	784 ± 162	512.05	<0.001	188	16.5
PDW	13.2 ± 2	-	-	-	-	-
MPV	10.9 ± 1	-	-	-	-	-
P-LCR/%	32 ± 8	-	-	-	-	-
PCT/%	0.29 ± 0.8	-	-	-	-	-

Bold values represent a statistically significant difference was found in these parameters.

above 1,000 lux [6]. Ito's study may explain why the LIP, ALP, CREAT and iron readings in our study exhibited a statistically significant difference in 195 and 1,168 lux luminous environments. When the results of pre- and post-experimental chemistry tests were compared using a non-parametric Wilcoxon test, only the LIP, ALP, CREAT and iron tests showed significant differences, while there was no significant difference in the CK, GGT, LDH and BILD tests. It is worth noting that the non-parametric Wilcoxon test does not compare mean values, but yields results by sorting. When we compared the percentage of changes in the LIP, ALP, CREAT and iron test results to the clinically acceptable limit values described by Ricós et al. [7]. We observed that the changes were within the clinically acceptable limits. The Sysmex XT-2000i automated haematology analyser uses the principle of impedance with hydrodynamic focussing to assess erythrocyte and platelet numbers. Large cells, such as erythrocytes and leukocytes, produce a relatively large signal and are counted by a method that uses an aperture of 100 μm in diameter. Since the volumes of the platelets range from 2 to 30 μm , which is between 1/40 and 1/3 of the size of a RBC, the resistance they provide is lower. Therefore, to increase the signal produced by platelets, it is necessary to reduce the size of the aperture (70–50 μm) and to increase the current flowing through the opening. However, in this context, the noise signal produced by blood particles and the natural electronic background noise is very close to the signal produced by platelets [8]. This may explain why there was a

statistically significant difference in PLT, PDW, MPV, P-LCR and PCT parameters in 195 and 1,168 lux environments, while the WBC and RBC parameters were not affected. When we compared the percentage of change in the PLT parameter in our study (188%) to the clinically acceptable limit values of Ricós et al. [7] (16.5%), the percentage of change was found to be unacceptable. This change in the PLT parameter is clinically significant, because the PLT count is one of the determinant parameters in deciding whether to give platelet suspension treatment to a patient [9]. Our study shows that the PLT parameter is sensitive to changes in laboratory light intensity and this change is within the clinically unacceptable limits. This study shows that, similar to temperature and humidity, light intensity may be a significant source of noise affecting the accuracy of biochemical analyses in the laboratory. It is important to note that this study analysed a small number of chemistry and whole blood analysis results, which may not be sufficient to confirm that changes in laboratory light intensity do indeed affect the test results. Future investigations on this topic should ensure that the sample size is large enough to perform parametric statistical analyses. Analytical performance criteria (accuracy, precision, range, linearity, recovery, detection limit, interference, blind reading, specificity and sensitivity) should be determined for analyses performed in 195 and 1,168 lux luminous environments. Further studies are needed to confirm the effect of changes in laboratory light intensity on whole blood and chemistry analyses.

Research funding: Research funding provided by the author himself.

Author contributions: The author has accepted the responsibility of all content of this article and has approved the submission.

Conflict of Interest: No conflict of interest was declared by the author.

Çıkar çatışması: Yazar çıkar çatışması bildirmedi.

Informed consent: Informed consent was obtained from patients before starting this study.

Ethics committee approval: This study was approved by the Adıyaman University Faculty of Medicine Ethics Committee.

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