

DETERMINING THE NEED FOR REPEAT TESTING OF BLOOD ETHANOL CONCENTRATION: EVALUATION OF THE SYNCHRON BLOOD ETHYL ALCOHOL ASSAY KIT

UTVRĐIVANJE POTREBE ZA PONOVLJENIM ODREĐIVANJEM KONCENTRACIJE ETANOLA: PROCENA SYNCHRON TEST REAGENSA ZA ODREĐIVANJE ETIL ALKOHOLA

Yasemin Ustundağ¹, Kağan Huysal², Sevim Eşmedere Eren³

¹Department of Clinical Biochemistry, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16290, Turkey

²Department of Clinical Biochemistry, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16290, Turkey

³Department of Clinical Biochemistry, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16290, Turkey

Summary

Background: In clinical laboratories, a common practice used to verify tests prior to reporting is repeat testing. Our objective was to evaluate the differences between the results of blood ethanol concentration (BEC) test repetitions and report on the role of repeat testing to prevent reporting of incorrect results.

Methods: We conducted a retrospective study of data retrieved from the Bursa Yuksek Ihtisas Training and Research Hospital's document management system by calculating the percentage change between repeated BEC test runs. To assess for clinical relevance, the bias between two results from the same sample was compared using the 1988 Clinical Laboratory Improvement Amendments' (CLIA) proficiency testing allowable total error (TEa) limits.

Results: From a total of 1,627 BEC tests performed between January 2017 and January 2018, 70% (1,133) were repeat tested. Of these, 830 resulted in BECs between 0–5 mmol/L, of which 237 (28.5%) were above the 25% acceptable TEa. Two hundred seventy-six BEC test results were greater than >14 mmol/L, and there was a

Kratak sadržaj

Uvod: U kliničkim laboratorijama uobičajena je praksa da se izvrši evaluacija testa koji će biti korišćen. Nama je bio cilj da se izvrši evaluacija testa radi utvrđivanja razlika između rezultata pri određivanju koncentracije etanola u krvi (BESC) ponovljenim određivanjem i da iste objavimo kako bi se izbeglo izdavanje netačnih rezultata.

Metode: Izučavanja su rađena u Bursa Yuksek Training i Research Hospital primenom menadžment sistema radi izračunavanja procenta odstupanja u ponovljenim određivanjima primenom BEC testa. Odgovarajuća klinička procena između rezultata dobijenih u istim uzorcima upoređivana je primenom 1988 Clinical Laboratory Improvement Amendments (CLIA) testa koji omogućava izračunavanje granica ukupne greške (TEa).

Rezultati: Od ukupno 1 627 BEC testova izvedenih između januara 2017 i januara 2018, 70% (1 133) su bili ponovljeni testovi. Od ovih, 830 BEC rezultata bili su između 0–5 mmol/L, a samo je 237 (28,5%) bilo iznad 25% prihvatljive TEa. Dvesta sedamdeset šest rezultata BEC testova bilo je veće od > 14 mmol/L, i dobijen je dobar konsenzus između

Address for correspondence:

Yasemin Ustundağ
Bursa Yüksek İhtisas Eğitim ve Araştırma Hastanesi, Kadın Doğum Ek Binası, -1 kat Biyokimya Laboratuvarı, Yıldırım, Bursa 16290, Turkey
Tlf: +905324823692
e-mail: yaseminbudak2000@yahoo.com

good consensus between the initial and repeat test results (99%). In this group, the mean bias was 0.0% (95% CI = -9.8–9.8%). However, three of the repeat test results were considered significantly different. There were two discordant results in the 5–14 mmol/L ethanol level, and the mean bias was 2.1% (95% CI = -15.0–19.1%).

Conclusions: The majority of the repeated BEC test values were the same as the baseline value; therefore, there may be limited benefit in continuing such frequent repeated analyses.

Keywords: blood ethanol concentration, repeat testing

Introduction

Driving under the influence of ethanol, from alcohol consumption, is an important risk factor for serious traffic accidents (1). When an individual is suspected of violating drinking-related laws, blood ethanol concentration (BEC) testing is performed to prove greater than acceptable BECs. BEC is reported as positive or negative according to legislative limits under the Turkish Road Traffic Act (2). All public transport, taxi, commercial, and official vehicle drivers must maintain a zero-blood alcohol concentration, while private vehicle drivers must maintain a 10.85 mmol/L or lower blood alcohol concentration (according to the Turkish Road Traffic Act #2918 dated June 16, 1985) (2).

Measurements in clinical laboratories are always characterized by some uncertainty (3, 4). It is important to determine the reliability of analytical data by using method validation, quality control, and measurement uncertainty when presenting results because individuals driving with blood ethanol values of 10.85 mmol/L or higher are punishable (5). Because the accuracy of testing instruments has greatly increased with current technological developments, analytical errors account for only 8–15% of errors, and 85–92% of these are related to pre- and post-analytical errors (6, 7).

A common practice in clinical laboratories is to perform repeat testing to verify test results before reporting them to clinicians (8–10). In our laboratory, repeat testing of the same blood sample used for the initial result prior to reporting is often performed. The reason for this retesting routine is to ensure the accuracy of the results and avoid reporting false or incorrect data. However, repeat testing is costly and leads to an increase in laboratory service turnaround time (9, 11).

Our objective in this study was to evaluate the differences between the results of BEC test repetitions and to determine whether the role of repeat testing prevents the false or incorrect reporting of results.

inicijalnih i ponovljenih rezultata testova (99%). U ovoj grupi, srednje odstupanje je bilo 0,0% (95%, CI = -9,8–9,8%). Međutim, tri od ponovljenih rezultata su se značajno razlikovala. Postojala su dva neslaganja između rezultata kod nivoa etanola 5–14 mmol/L, pa je srednje odstupanje bilo 2,1% (95%, CI = -15,0–19,1%).

Zaključak: Većina vrednosti ponovljenih BEC testova bila je ista kao i osnovna vrednost, što znači da nema opravdanja da se često vrednosti testova ponavljaju.

Cljučne reči: koncentracija etanola u krvi, ponovljeno testiranje

Materials and Methods

We conducted a retrospective study using data retrieved from the Bursa Yuksek Ihtisas Training and Research Hospital's document management system, which provides services for 1,428 hospital beds. The laboratory data were obtained from electronic records containing patients' demographic data, BEC sample collection, and results and/or report dates and times as part of a routine data management system.

Blood ethanol concentration was measured with an ethanol assay kit (A-E 474947; Synchron Systems Inc.) using an automated analyzer (Beckman-Coulter Olympus AU400; Beckman, Coulter Inc., Melville, NY, USA). The analytical measurements ranged from 1.08 to 130.0 mmol/L, with a lower limit of quantification of 0.8 mmol/L and precision limits between 1.3% and 2.6%. All the tests were carried out according to the manufacturer's instructions. During the study, all reagents, calibrators, and internal quality control materials used were provided by the manufacturer. The repeat testing was always carried out on the sample used for the initial analysis using the same analyzer by qualified medical laboratory staff.

The percentage change between the two test runs was calculated. To assess the clinical relevance, the bias between the two results was compared with the U.S. Federal Register's Clinical Laboratory Improvement Amendments (CLIA) of 1988 allowable total error (TEa) (12). If the percentage of change was higher than the TEa, it was assumed to be an outlier.

We also performed a second analysis with a bias of $\pm 10\%$ or better for ethanol analysis suggested by the Scientific Working Group for Forensic Toxicology (13).

Statistics

Bland-Altman plots were evaluated using Analyse-It, Version 2.04 (Analyse-It Software, Leeds, UK). The results were compared using a paired t-test. Data concordance was evaluated via a linear regression analysis.

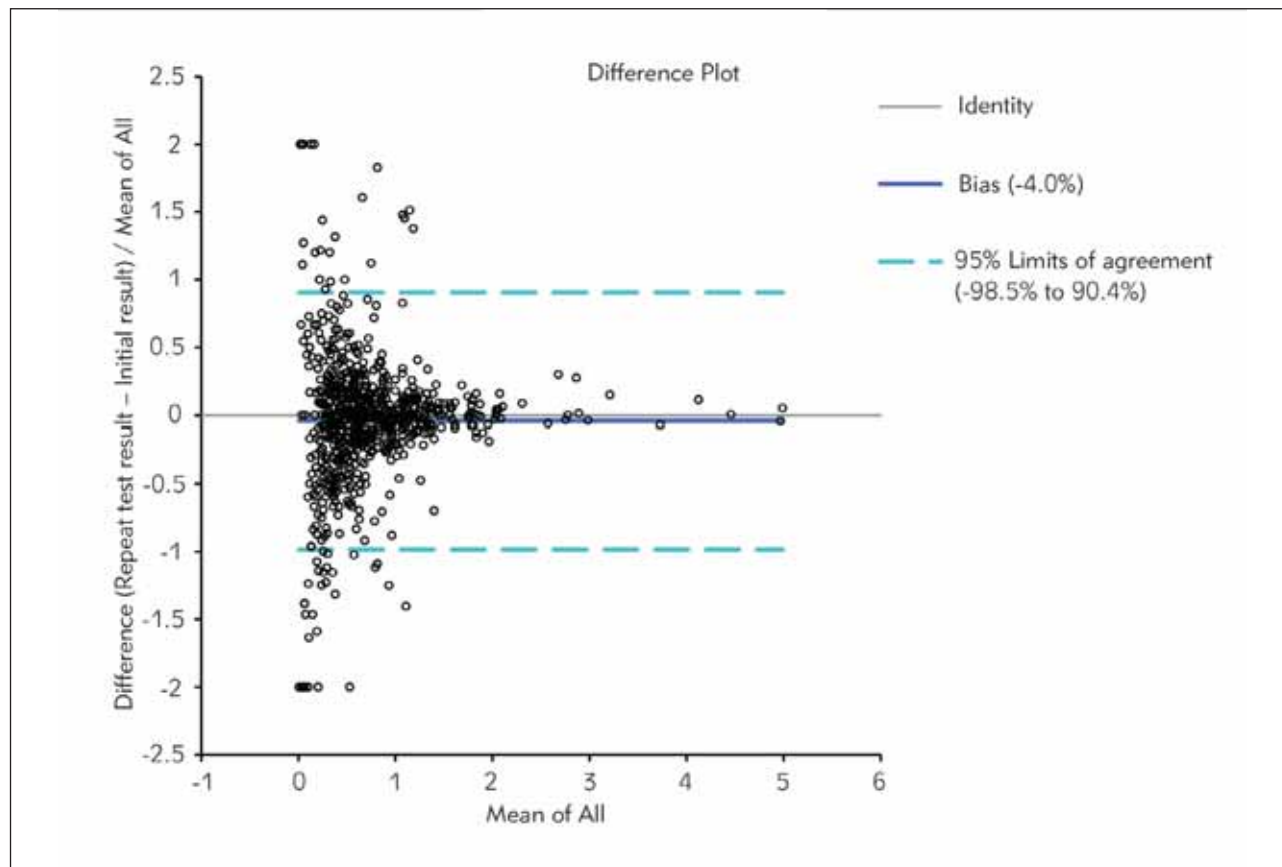


Figure 1 Blood ethanol concentration results between 0–5 mmol/L ($n = 830$): absolute value differences between 2 test runs of individual specimens plotted against the mean results of the 2 test runs.

Results

The CVs 2.8% and 4.0% were calculated using a mean of 1 month's ($n = 60$) of internal QC (Level 1; mean 11.04 mmol/L, Level 2: mean 22.03 mmol/L) data, respectively.

Out of 1,627 blood samples analyzed for ethanol between January 2017 and January 2018, 70% (1,133) were repeat tested. Of these 1,627 samples, 976 (60%) had been collected after a traffic accident and 1,477 (90.7%) had been collected from males. Four hundred and twenty patients with blood ethanol levels between 0 and 5 mmol/L, 2 patients with between 5 and 14 mmol/L, and 32 patients with >14 mmol/L were examined only once, repeat testing was not performed.

The initial and repeat test results were evaluated. The mean initial test result was 10.7 ± 19.1 mmol/L and the mean repeated test result was 10.7 ± 19.3 mmol/L ($p = 0.572$) ($n = 1133$).

We grouped the patients according to their ethanol levels: 237 out of 830 samples (28.5%) with ethanol levels between 0 and 5 mmol/L were above the 25% acceptable TEa and 517 (62.2%) were

above the 10% limit. The Bland–Altman method plotted the mean of the paired ethanol values versus the absolute difference between the paired values over a range of blood ethanol concentrations. The Bland–Altman difference plot between repeated tests revealed a mean of 4.0% below the 5 mmol/L blood ethanol level (Figure 1). The linear regression analysis result of the ethanol levels between 0 and 5 mmol/L was 'Repeat-test result = $0.03 + \text{initial test result} \times 0.994$.

There were two discordant results in the 5–14 mmol/L ethanol level, and the Bland–Altman difference plot between repeated tests revealed a mean of 2.1% (Figure 2).

Of the 1,133 BEC test results, 276 were greater than >14 mmol/L; there was good agreement between the initial and repeated test results (99%). In this group, the mean bias was 0.0% (95% CI = -9.8% to 9.8%) (Figure 3). Three of the repeated test results were considered significantly different, with a 25% TEa. However, 11 of the 276 were beyond the 10% limit.

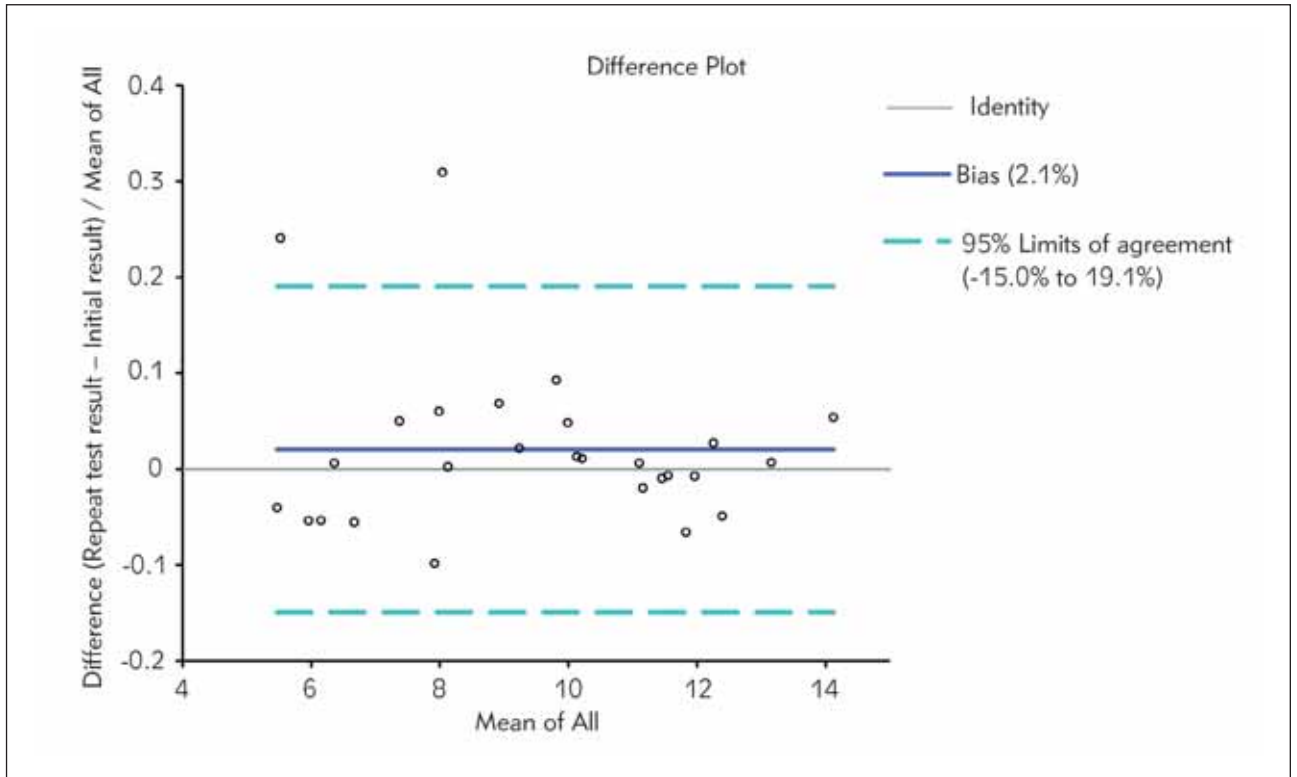


Figure 2 Blood ethanol concentration results between 5–14 mmol/L (n =27): absolute value differences between 2 test runs of individual specimens plotted against the mean results of the 2 test runs.

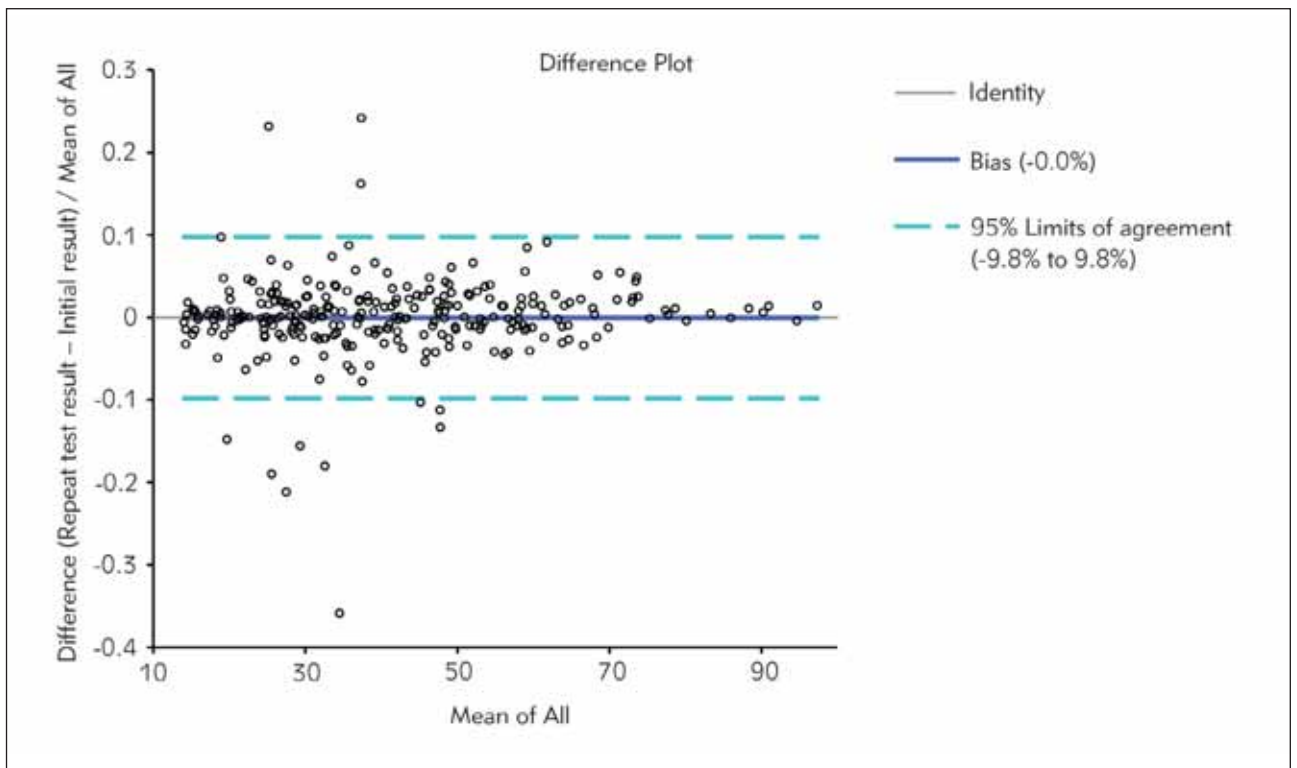


Figure 3 Blood ethanol concentration results >14 mmol/L (n =276): absolute value differences between 2 test runs of individual specimens plotted against the mean results of the 2 test runs.

Discussion

Laboratories perform repeat tests to ensure their accuracy and precision before reporting them to a physician (14). However, the modern technologies used in available chemical analyzers have improved their accuracy to such a degree that they are within perfection of their analytical range (2).

Samples for blood ethanol concentration testing are mostly sent to our laboratory for judicial reporting; the technicians retest 70% of all BEC tests in order to avoid errors. In our hospital, the clinician decides to perform a repeat test on the same patient using an independent sample to confirm the first result or compare the results over time. However, there is no routine rule for the recall of a blood ethanol concentration test; the decision is made by a laboratory technician.

Laboratories generally repeat tests, especially for critical values, before reporting test results (15–17). Recently, the College of American Pathologists (CAP) Q-Probes study (15) found that 61% of laboratories always repeated critical tests. In a 2015 study of 1,589 laboratories by the National Center for Clinical Laboratories in China, 94.8% of the 973 responding laboratories reported repeat testing before searching for a critical value in the laboratory (17). However, the practicality of routinely verifying each critical value as a result of reanalysis has been questioned in recent years (8–10, 14, 17, 18). While we were unable to find a study that analyzed repeat testing of blood ethanol concentrations, our findings are similar to studies of repeat testing practices for common chemistry tests (8, 10).

There are studies that have reported that repeating tests from different test groups leads to inappropriate laboratory use; in particular, routine repetition of tests with critical results has been criticized by some authors from different countries (8–10, 19). There is agreement between these studies that the results of test repetitions do not increase the accuracy, only the cost. It was determined that 97.39–99.3% of tests are unnecessary repetitions because the results are within the total permissible error limits of the CAP and/or the CLIA of 1988 (7–9, 16, 18, 19).

The definition of »meaningful difference« for repeated values, however, varies throughout the literature. Allowable error is a subjective finding that can be defined by biological variability (20, 21). Acceptable total error for quantitative tests is deter-

mined according to an »Acceptable Total Error List« prepared by the CLIA and Guidelines of the German Federal Medical Council (Rilibak) etc. We compared the calculated deviations of the current study with the CLIA of 1988's proficiency testing TEa limit of $\pm 25\%$. This limit is questionable because a TEa of $\pm 25\%$ at the judicial decision-making limit for a BEC of 10.85 mmol/L is high. The Scientific Working Group for Forensic Toxicology has recommended a bias of $\pm 10\%$ or better for ethanol analysis (13).

Health expenditure has increased rapidly in Turkey and globally in recent years. However, limited financial resources are allocated to health services to adopt cost-effective approaches to the efficient use of resources. Consequently, applications to identify and reduce costly practices have increased. Since repeated testing leads to unnecessary costs, defining the criteria to repeat a particular test, rather than depending on the analyst responsible for the test to make the decision, could be a better option (18).

For blood ethanol testing, values close to 10.85 mmol/L are important because they lead to forensic results. It is important to reduce the analytical CV in the 8.7–13.0 mmol/L range. Clinical laboratories detect very rare errors with »repetition rules« and are therefore, arguably, an excessive spending of resources. One study states that 20,844 repeated tests were undertaken to detect 102 errors (11). However, to avoid misclassification, our laboratory suggests repeated testing if the initial test result is between 8.7–13.0 mmol/L.

The weakness of the current study is that it used data from a single laboratory; this data could have been affected by the laboratory's internal quality assurance practices, which could limit the generalization of these findings.

Conclusion

We found that the majority of repeated test values for BEC in our laboratory were the same as the baseline values, meaning there may be limited benefit in continuing frequent repeat analyses.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Martin JL, Gadegbeku B, Wu D, Viallon V, Laumon B. Cannabis, alcohol and fatal road accidents. *PLoS ONE* 2017; 12(11): e0187320.
2. Karakus A, Nuri İdiz N, Dalgiç M, Uluçay T, Sincar Y. Comparison of the effects of two legal blood alcohol limits: The presence of alcohol in traffic accidents according to category of driver in Izmir, Turkey. *Traffic Inj Prev* 2015; 16(5): 440–2.
3. Huysal K, Budak YU. Application of sigma metrics for the assessment of quality assurance using the MQ-2000 PT HbA1c analyzer. *Biochem Med (Zagreb)* 2015; 25(3): 416–20.
4. Panteghini M, Ceriotti F, Jones G, Oosterhuis W, Plebani M, Sandberg S; Task Force on Performance Specifications in Laboratory Medicine of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Strategies to define performance specifications in laboratory medicine: 3 years on from the Milan Strategic Conference. *Clin Chem Lab Med* 2017; 55(12): 1849–56.
5. Ustundag Y, Huysal K. Measurement uncertainty of blood ethanol concentration in drink-driving cases in an emergency laboratory. *Biochem Med (Zagreb)* 2017; 27(3): 03070
6. Restelli V, Taylor A, Cochrane D, Noble MA. Medical laboratory associated errors: the 33-month experience of an on-line volunteer Canadian province wide error reporting system. *Diagnosis (Berl)* 2017; 4(2): 79–86.
7. Goswami B, Singh B, Chawla R, Mallika V. Evaluation of errors in a clinical laboratory: a one-year experience. *Clin Chem Lab Med* 2010; 48: 63–6.
8. Collinson P. Laboratory medicine is faced with the evolution of medical practice. *J Med Biochem* 2017; 36: 211–5.
9. Milinković N, Ignjatović S, Šumarac Z, Majkić-Singh N. Uncertainty of measurement in laboratory medicine. *J Med Biochem* 2018; 37: 279–88.
10. Arslan DF, Karakoyun I, Basok IB, Aksit ZM, Celik E, Dogan K, Duman C. The effects of education and training given to phlebotomists for reducing preanalytical errors. *J Med Biochem* 2018; 37: 172–80.
11. Sun SP, Garcia J, Hayden JA. Repeating critical hematology and coagulation values wastes resources, lengthens turnaround time, and delays clinical action. *Am J Clin Pathol* 2018; 149(3): 247–52.
12. CLIA Requirements for Analytical Quality. Available at: <https://www.westgard.com/clia.htm#tox>
13. Scientific Working Group for Forensic Toxicology (SWG-TOX) Standard practices for method validation in forensic toxicology. *J Anal Toxicol* 2013; 37 (7): 452–74.
14. Baradaran Motie P, Zare-Mirzaie A, Shayanfar N, Kadivar M. Does routine repeat testing of critical laboratory values improve their accuracy? *Med J Islam Repub Iran* 2015; 29: 176.
15. Lehman CM, Howanitz PJ, Karcher DS. Utility of repeat testing of critical values: a Q-probes analysis of 86 clinical laboratories. *Arch Pathol Lab Med* 2014; 138(6): 788–93.
16. Kopicinovic LM, Trifunović J, Pavosevic T, Nikolac N. Croatian survey on critical results reporting. *Biochem Med (Zagreb)* 2015; 25(2): 193–202.
17. Fei Y, Zhao H, Wang W, He F, Zhong K, Yuan S, Wang Z. Current situation of critical value reporting in 973 laboratories in China. *Biochem Med (Zagreb)* 2017; 27(3): 030707.
18. Rodrigues MSP, Souza CL, Oliveira MV. Repetition of biochemistry tests in a laboratory of public hospital in south-west of Bahia, Brazil, and associated cost. *J Bras Patol Med Lab* 2017; 53(4), 233–9.
19. Motie PB, Zare-Mirzaie A, Shayanfar N, Kadivar M. Does routine repeat testing of critical laboratory values improve their accuracy? *Med J Islam Repub Iran* 2015; 29(176).
20. Onyenekwu CP, Hudson CL, Zemlin AE, Erasmus RT. The impact of repeat testing of common chemistry analytes at critical concentrations. *Clin Chem Lab Med* 2014; 52(12): 1739–45.
21. Ricos C, Alvarez V, Cava F, et al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest* 1999; 59: 491–500.
22. Fraser CG. General strategies to set quality specifications for reliability performance characteristics. *Scand J Clin Lab Invest* 1999; 59: 487–90.
23. Monach PA. Repeating tests: different roles in research studies and clinical medicine. *Biomarkers in Medicine* 2012; 6(5): 10.2217/bmm.12.57.

Received: July 12, 2018

Accepted: September 5, 2018