Research Article

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Reporting measurement uncertainties with ethanol results

Etanol sonuçlarını ölçüm belirsizliği ile raporlamak

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Abstract

Background: All measurements are affected by errors whose quantitative expressions are called measurement uncertainty. Blood, urine, breath or serum alcohol concentration is measured to assess if an offence has been committed. In this study, we aimed to calculate the measurement uncertainty for ethanol.

Materials and methods: Measurement uncertainty was calculated according to the Nordtest guidelines by tracking six steps. All of the patient ethanol results were scanned and recorded between October 2015 and March 2016 by using electronic patient records.

Results: The measurement uncertainty value was calculated as $\pm 12.25\%$. The results from 1178 patients referred to our hospital for serum ethanol level analyses were scanned. Of the 1178 ethanol results, 597 were driver. Male persons have higher probability of alcohol-impaired

driving than female persons [$p=0.007$; odds ratio was 2.239 (1.211–4.141 with 95% confidence interval)].

Conclusion: The measurement uncertainty for ethanol concentrations measured in our laboratory was lower than the allowable total error (TEa) of the guidelines (20%). Laboratories that analyze ethanol have to be more transparent about measurement procedures when presenting their ethanol result. Measurement uncertainty should be limited with legislative regulations by country-specific scientific committees.

Keywords: Alcohol; Ethanol; Uncertainty; Measurement; Forensic toxicology.

Öz

Amaç: Tüm ölçümler nicel ifadeyle ölçüm belirsizliği olarak ifade edilen hatalardan etkilenir. Adli olay olasılıklarına karşın kan, idrar, nefes ve serumda alkol ölçümleri yapılmaktadır. Bu çalışmada etanol için ölçüm belirsizliği hesaplamayı amaçladık.

Gereç ve Yöntem: Nordtest rehberi kullanılarak altı adımda ölçüm belirsizliği hesaplandı. Elektronik hasta kayıtları üzerinden Ekim 2015-Mart 2016 tarihleri arasındaki hastaların etanol sonuçları tarandı.

Bulgular: Ölçüm belirsizliği $\%12,25$ olarak hesaplandı. Etanol analizi yapılmış olan 1.178 hasta sonucu tarandı. Bu hastaların 597'si sürücü idi. Alkollü araç kullanma olasılığı erkeklerde kadınlardan yüksek bulundu [$p=0,007$; odds oranı 2,239 (1,211–4,141 95% güven aralığında)].

Sonuç: Laboratuvarımızda etanol testi için ölçüm belirsizliği rehberlerde yer alan izin verilen toplam hatadan ($\%20$) düşük bulunmuştur. Laboratuvarların etanol sonuçlarını sunarken ölçüm prosedürleri hakkında daha şeffaf davranmaları ve ölçüm belirsizliği değerlerinin ülkelere özel yasal düzenlemeler ile sınırlandırılması önerilmektedir.

Anahtar kelimeler: Alkol; Etanol; Belirsizlik; Ölçüm; Adli toksikoloji.

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Introduction

Recently it has been established that more than 30,000 people die each year due to motor vehicle accidents in the US [1]. One-third of all traffic-related deaths are directly connected to alcohol-impaired driving. These deaths rank fourth behind heart disease, malignant neoplasm, and chronic lower respiratory diseases as causes of death [2, 3]. There is an urgent need for legislative regulations that enforce blood ethanol concentration measurements, which are analyses of the amount of alcohol in the blood [2]. The first legislation that came forward was in 1936 in Norway and it banned drunk driving. This legislation has gained worldwide recognition in recent years and the legal boundaries have been defined [2, 4]. The legal limit for a driver's blood alcohol concentration (BAC) has been determined using various methods in different countries [5].

Measurement uncertainty is a fundamental concept in the science of measurements, also known as metrology. Being integral to the results, measurement uncertainty is a range within the “true” value of a measurement result [6]. It is strongly recommended that measurement uncertainties be determined for each measurement procedure and reviewed over time [7]. In the International Organization for Standardization (ISO) 15189, which is the basis for accreditation of medical testing laboratories, it is stated that

The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty [8].

Estimation of measurement uncertainty permits laboratories to evaluate the measurement procedures and determine whether the procedures are fit for the purpose to accomplish the aim of the analysis and is a reliable measure [7].

Several variation sources have contributed to measurement uncertainty such as calibration design, calibration frequency, calibrator uncertainty, value assigned to the calibrator, repeatability of the analytical system, stability of reagents, lot to lot variation, maintenance frequency of analytical system, skill of the operators and environmental conditions [9]. Up to date, several international guidelines have explained methods for the estimation of measurement uncertainty. As one of them, Nordtest guideline, developed in Nordic countries for environmental laboratories, has been utilized and advised

routinely in medical laboratories [10]. Also, it is recommended that measurement uncertainty should be lower than total allowable error (TEa), an estimated upper limit of error considering together with random and systematic error components. If total error of a measurement system is smaller than the total allowable error, the analytical method performs acceptably [11].

Alcohol measurements take place in various matrices that most frequently include blood, breath, and urine [12, 13]. BACs have several legal cut-off values that differ according to the country and range from 0.05 to 0.12 g/dL (50–120 mg/100 mL of blood), thus, the reliability of these analyses is very important [2, 14]. We aimed to calculate the measurement uncertainty for our ethanol testing by using Nordtest guideline. Additionally, we aimed to investigate the distributions of patients' ethanol results.

Materials and methods

In this study, internal and external quality assessments were scanned and patient's ethanol results were recorded retrospectively. This study was approved by local ethical committee (date: 2016.06.02, number: 326).

Internal quality assessments were carried out with control sera of a kit manufacturer (Roche Diagnostics, GmbH, Mannheim, Germany) twice a day with one assessment being a low level and the other a high level. All internal quality control samples had same lot number. External quality assessments were performed according to the Randox International Quality Assessment Scheme (RIQAS) from the UK. From October 2015 until March 2016, both internal and external quality control data were recorded. Mean, standard deviation (SD), and coefficient of variations (CVs) of ethanol tests were calculated from the results of internal quality assessments. Biases were calculated from external quality assessment reports and the CVs presented from these assessments were recorded. Uncertainty was calculated according to the Nordtest guidelines by tracking six steps [15].

Serum ethanol levels were determined using the enzymatic method on the Cobas Integra 800 analyzer (Roche Diagnostics, Switzerland) with the original Roche reagent.

All of the serum ethanol results were scanned and recorded between October 2015 and March 2016 by using electronic patient records. Serum ethanol concentrations were classified as level 0, 1 and 2. Undetectable serum ethanol concentration (<10 mg/dL) was named as level 0, a serum ethanol concentration between 10.0 and

49.99 mg/dL was named as level 1 and a serum ethanol concentration equal or higher than 50.00 mg/dL was named as level 2.

Lower limit of detection of the ethanol test was 10.0 mg/dL and upper limit of quantification of ethanol test was 498 mg/dL. Serum samples of over the 498 mg/dL were diluted with saline in 1/3 ratio and results were released by multiplying by 3.

Statistical analysis

Statistical analyses were performed using PASW Statistics 18 software (IBM Corporation, Armonk, NY, USA). Normality of distribution was evaluated using the Kolmogorov–Smirnov test. Chi-square test or Fisher exact test was used to determine whether or not there is a difference between male and female patients in terms of serum ethanol level. Kruskal Wallis test is used to investigate an association between report months and ethanol levels. Also Kruskal Wallis test is used to research a significant difference between patient age and ethanol levels. To determine whether or not there is a correlation between age and serum ethanol concentrations Spearman rank test was used due to abnormality of distribution. Mann Whitney U test was used to determine a difference ages in terms of sex. A p value of <0.05 was considered statistically significant.

Results

Estimation of measurement uncertainty

Step 1. Specify measurand

Serum ethanol levels were determined using the enzymatic method on the Cobas Integra 800 analyzer.

Table 1: Means, SDs and CVs calculated from results of internal quality assessment and relevant calculations.

Internal quality control level (October 2015–March 2016)	Results of internal quality control			
	n	Mean	SD	CV%
Low level internal quality control	172	50.01	2.22	4.43
High level internal quality control	172	149.06	5.41	3.63
Formula of Rw	$((CV\%_{\text{low level}})^2 + (CV\%_{\text{high level}})^2)^{1/2}$			
Result of Rw	5.73			

SD, Standard deviation; CV, coefficient variations; Rw, reproducibility within-laboratory.

Step 2. Quantify reproducibility within the laboratory component

Between-day means, SDs, and CVs calculated from the results of the internal quality assessments at two levels are shown in Table 1. Estimations of reproducibility within-laboratory (Rw) and uncertainty due to Rw ($u[Rw]$) were executed by using internal quality control data. All calculations are shown in Table 1.

Step 3. Quantify the bias component

From external quality assessments over the last 6 months the within-autoanalyzer (peer group) bias results were 2.62, 3.29, -2.15, 2.70, 8.41 and 8.66. In addition to the bias, the mean within-peer group reproducibility of the standard deviation (S_R) of external quality control was 3.40 (Table 2). Executed calculations are also shown in Table 2.

Step 4. Converting components of standard uncertainty values

To reach standard uncertainty, components of $u(Rw)$ and $u(\text{bias})$ were used as shown in Table 3.

Table 2: The data of the last 6 months of the external quality control program and relevant calculations.

Sample no	Our result	Within-Peer group			
		Mean	Bias	n	S_R
1	57	55.55	2.62	21	5.1
2	158.5	153.45	3.29	16	2
3	53.4	54.57	-2.15	16	2.4
4	160	155.80	2.69	15	2.4
5	369.1	340.48	8.41	14	2.1
6	58.5	53.84	8.66	17	6.4

$$RMS_{\text{bias}} = \sqrt{(\sum(\text{bias})^2 / \text{bias number})} \quad u(\text{Cref}) = S_{R(\text{mean})} / \sqrt{n_{\text{mean}}}$$

$$RMS_{\text{bias}} = 5.26 \quad u(\text{Cref}) = 0.84$$

RMS, Root mean square; bias number is 6; n_{mean} , mean of participating laboratory number; $S_{R(\text{mean})}$, mean of reproducibility of the standard deviation associated with a measurement (also called as CV% in external quality control reports).

Table 3: Calculation steps of uncertainty.

Component of uncertainty	Formulas	Results
$u(Rw)$	$\sqrt{(Rw)^2 / 2}$	4.05
$u(\text{bias})$	$\sqrt{(RMS_{\text{bias}})^2 + u(\text{Cref})^2}$	5.33
u_c	$\sqrt{u(Rw)^2 + u(\text{bias})^2}$	6.70
U	$1.96 \times u_c$	13.12

Rw, Reproducibility within-laboratory; $u(Rw)$, uncertainty of reproducibility within-laboratory; u_c , combined standard uncertainty; U, expanded uncertainty.

Step 5. Calculating the combined standard uncertainty (u_c)

Combined standard uncertainty was calculated as shown in Table 3.

Step 6. Calculating the expanded uncertainty value (U)

The expanded uncertainty value was calculated as $\pm 13.12\%$ (Table 3).

Screening for patient results

There were 1178 patients who were referred to the Ataturk Training and Research Hospital in order to have their serum ethanol levels analyzed and have the values scanned. Of the 1178 patients, 18 were below the 18 age and they are excluded the study. Of the 1160 ethanol results, 880 (75.9%) were below the limit of detection (10.0 mg/dL) (level 0). Twenty-five results (2.2%) were between 10.0 and 49.99 mg/dL (level 1). Of the 1160 results, 255 (22.0%) were equal or over the legal limit of 50 mg/dL for our country (level 2).

There was no significant difference patients ages in terms of serum ethanol level ($p=0.505$). Also, there was no significant correlation between age and serum ethanol concentration ($p=0.062$).

In terms of sex, there was no significant difference between level 0 and level 1 patients ($p=0.672$). Also, there was no significant difference between level 1 and level 2 patients ($p=0.249$). Contrarily, there was a significant difference between level 0 and 2 patients ($p<0.001$). Male patients have higher probability of high

serum alcohol level than female patients [$p<0.001$; odds ratio was 1.897 (1.381–2.605 with 95% confidence interval)] (Table 4).

Additionally, there was no significant difference serum ethanol levels in terms of months ($p=0.395$).

In a subset analysis, we screened diagnosis of all ethanol results. By using computer data, medical problems such as gastrointestinal diseases, muscle-skeletal disorders, and nausea; other judicial cases such as gunshot injuries, sharp object injury; and pedestrians involved in traffic accident are excluded. Of the 1160 ethanol results, 597 (51.5%) were accepted as driver involved in traffic accident. Of the 597 ethanol results, 513 (85.9%) were below the limit of detection (10.0 mg/dL) (level 0). Ten results (1.7%) were between 10.0 and 49.99 mg/dL (level 1). Of the 597 results, 74 (12.4%) were equal or over the legal limit of 50 mg/dL for our country (level 2).

Drivers ages were between 18 and 85 years and serum ethanol concentrations were between <10 and 395.2 mg/dL. A significant correlation was found between age and serum ethanol concentration ($r=-0.085$, $p=0.039$). But, there was no significant difference drivers ages in terms of serum ethanol level ($p=0.136$). Similarly, there was no significant difference ages in terms of sex ($p=0.105$).

In terms of sex, there was no significant difference between level 0 and level 1 patients ($p=0.499$). Also, there was no significant difference between level 1 and level 2 patients ($p=0.073$). Contrarily, there was a significant difference between level 0 and 2 patients ($p=0.07$). With respect to statistical analysis, male persons have higher probability of alcohol-impaired driving than female persons [$p=0.007$; odds ratio was

Table 4: Distribution of sex in terms of serum ethanol levels ($n=1160$).

Sex	Level 0 ($n=880$)	Level 1 ($n=25$)	Level 2 ($n=255$)	Total	p-Value
Male (n)	635	19	217	871	–
Female (n)	245	6	38	289	–
Male (%) (within ethanol level)	72.9	2.2	24.9	100	–
Female (%) (within ethanol level)	84.8	2.1	13.1	100	–
Level 0 vs. Level 1					
Male (%) (within sex)	97.1	2.9	–	100	0.672
Female (%) (within sex)	97.6	2.4	–	100	
Level 1 vs. Level 2					
Male (%) (within sex)	–	8.1	91.9	100	0.249
Female (%) (within sex)	–	8.1	91.9	100	
Level 0 vs. Level 2					
Male (%) (within sex)	74.5	–	25.5	100	$<0.001^a$
Female (%) (within sex)	86.6	–	13.4	100	

^aMale patients have higher probability of high serum alcohol level than female patients [$p<0.001$; odds ratio was 1.897 (1.381–2.605 with 95% confidence interval)].

Table 5: Distribution of sex in terms of serum ethanol levels (n = 597).

Sex	Level 0 (n = 513)	Level 1 (n = 10)	Level 2 (n = 74)	Total	p-Value
Male (n)	359	6	63	428	–
Female (n)	154	4	11	169	–
Male (%) (within ethanol level)	83.9	1.4	14.7	100	–
Female (%) (within ethanol level)	91.1	2.4	6.5	100	–
Level 0 vs. Level 1					
Male (%) (within sex)	98.4	1.6	–	100	0.499*
Female (%) (within sex)	97.5	2.5	–	100	
Level 1 vs. Level 2					
Male (%) (within sex)	–	8.7	91.3	100	0.073
Female (%) (within sex)	–	26.7	73.3	100	
Level 0 vs. Level 2					
Male (%) (within sex)	85.1	–	14.9	100	0.007*
Female (%) (within sex)	93.3	–	6.7	100	

*Male persons have higher probability of alcohol-impaired driving than female persons [$p = 0.007$; odds ratio was 2.239 (1.211–4.141 with 95% confidence interval)].

Figure 1: Patient distribution according to serum ethanol level and sex.

2.239 (1.211–4.141 with 95% confidence interval)] (Table 5, Figure 1).

Additionally, there was no significant difference serum ethanol levels in terms of months ($p = 0.158$).

Discussion

Alcoholic beverages include wines, beers, ciders, and several other alcoholic drinks that contain ethanol, which is a legal psychoactive substance that has been widely used in many cultures for centuries [12, 16]. As an important laboratory analysis, blood, breath, or urine ethanol concentrations are generally measured for therapeutic monitoring and diagnostics purposes [17].

Additionally, the measurement of BAC is an important analytical determination required to assess if an offence (e.g. driving under the influence of alcohol) has been committed [13].

Drunk drinking is a leading cause of alcohol-related deaths in many countries [18]. Motor vehicles accidents are considered a major health problem and are associated with driving under the influence of alcohol in approximately 58% of all accidents, and of all alcohol-related traffic deaths, 86% were killed in accidents where at least one driver or occupant had a BAC of 80 mg/dL or higher [1, 19]. The pharmacological effects of alcohol, starting with 20 mg/dL, are responsible for the deterioration of central nervous system functions resulting in increased reaction times, decrease ability to estimate space and distances, increased feelings of self-confidence, and the impairment of other tasks critical to driving including braking, steering, lane changing, and judgment [2, 20]. Methods of reducing alcohol-impaired driving fatalities and injuries include lowering the criminal BAC limit, suspending licenses, and impounding vehicles of drunk drivers [21]. In Canada, legislative regulations punish drunk drivers having BACs ranging from 0.05 to 0.08 mg/dL with punishments including a short-term loss of the offender's drivers' license and vehicle impoundment [14]. In the US, Utah first established an 80 mg/dL BAC limit for breaking the law, regardless of other evidence of ethanol intoxication, and other states soon followed in reducing their BAC limit to 80 mg/dL [19]. In Australia, the government adopted a "zero tolerance" policy for drivers under the age of 25 when driving a motor vehicle under the influence of alcohol, which has been reduced to 50 mg/dL since 1982 [5]. Similarly, in Italy, the legal limit for BAC

was zero for drivers under 21 years of age, for drivers with less than 1 years' experience, and for professional drivers of trucks, buses, and taxis. Additionally, the BAC limit for general drivers is 50–80 mg/dL in terms of administrative fine, whereas the BAC limit for general drivers is 80 mg/dL in terms of criminal offence [20]. The likelihood of having an accident that results in a severe injury to themselves or other drivers when driving under the influence are 15% and 4.6%, respectively [1]. Recently, it has been established that the implementation of lower BAC limits decreases fatal accidents, hospital admissions, and ambulance calls 21%, 8%, and 7.2%, respectively [21]. Mann et al. evaluated the early effects of Ontario's law on alcohol-involved driver fatalities and reported a 14.5% reduction after implementing license suspensions for drunk drivers who had a BAC of 80 mg/dL or more [22]. Contrary to these studies, some researchers have not established a considerable benefit [19, 23]. However, in today's world there is a tendency to establish a BAC limit, high or low.

During the prosecution of inebriated drivers, establishing objective BAC limits instead of subjective definitions that relied on impairment and lack of control was seen as an approach to resolve problems [18]. However, the following questions should be taken into consideration. Are ethanol measurements carried out in laboratories true assessments? Is there any range for ethanol results? Authors have emphasized that the measurement uncertainty results are fundamental concepts in metrology and indicate the range within which the "true" value of a measurement should lie [7]. To estimate the uncertainty of a measurement, all random and systematic sources, which comprise the error, should be elucidated and the contribution of each major component to the measurement uncertainty should be estimated with proper methods [24]. Several methods can be used for measurement uncertainty. In general, two approaches have been proposed for the estimation of measurement uncertainty. The first is a top-down approach based on inter-laboratory studies or validation study results. The second is a bottom-up approach that takes into consideration all error sources of uncertainty and is recommended by the Guide to the Expression of Uncertainty in Measurement [25]. Additionally, there is another approximation that was developed in Nordic countries, namely the Nordtest Report [15]. We calculated ethanol uncertainty using the latter guideline. Which-ever guideline is used, the measurement uncertainty is a very important part of reporting results, especially when interpreting results or passing judgment are part of the decision-making process for adjudicating drunk drivers.

In recent years, besides enzymatic alcohol dehydrogenase (ADH), a large number of new and reliable ethanol determinations have been developed, such as headspace

gas chromatography, headspace solid-phase microextraction, capillary gas chromatography, and proton nuclear magnetic resonance spectroscopy [26–29]. The quantification of ethanol may be accomplished in blood, breath, and urine [13]. In the current study, the measurement uncertainty in our ethanol method was $\pm 13.12\%$. For ethanol concentrations, TEa, an analytical quality requirement that sets a limit for both the random error and systematic error that are tolerable in a single test result, is defined according to the Clinical Laboratory Improvement Amendments (CLIA) as $<20\%$ [30]. The measurement uncertainty of ethanol concentrations measured in our laboratory was lower than the TEa of the guideline. Recently, authors [31] calculated their measurement uncertainty using an Intoxilyzer 8000 as $\pm 3.6\%$ for vaporous ethanol concentrations at or above 0.10 g/210 L.

In this study, it has been demonstrated that male patients have 1.9 times higher probability of high serum alcohol level than female patients. Similarly, male persons have more than 2 times higher probability of alcohol-impaired driving than female persons. Furthermore this result has not interfered with age. Additionally, in subset analysis, we have established that there was a significant correlation between age and serum ethanol concentration. The serum alcohol level decreased as the age got older. Recently, it has been demonstrated that males have presented more risky driving behavior including accidents leading mortality [32, 33].

Our study has some limitations. First, the current study was retrospective in nature. Ethanol results were scanned from patient electronic records. Although we excluded medical problems, other judicial cases, and pedestrians involved in traffic accident, in subset analysis selected ethanol results may be misleading due to erroneous or deficient patient records. Second, BAC determinations were not performed using more reliable methods such as gas chromatography. This limitation may result from higher measurement uncertainty values than in literature [31].

In conclusion, this study has demonstrated that judgments concerning drunk drivers need more evidence on the measurement of ethanol in laboratories that includes pre-analytical, analytical, or postanalytical processes. Laboratories that analyze ethanol have to be more accountable when they present their ethanol results. Also, laboratories should reveal their measurement uncertainty estimations in their test results. This information will have two benefits: shedding light on clinical/legal interpretations of patient results and traceability of analytical processes. Measurement uncertainty should be limited with legislative regulations by country-specific scientific committees. Also, measurement uncertainty needs to be understood and properly presented and explained within the legal community.

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