Pharmacology & Pharmacy, 2021, 12, 208-218
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

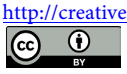
To Investigate the Effect of Using Ethanol Containing Wipes in Collecting Blood for the Measurement of Alcohol Concentration

Takami Nakao[1], Atsushi Nitta[2], Hiroshi Nishioka[2], Munehiro Katagi[2], Noriko Tsuda[3], Yasuhide Kitazawa[1]

[1]Critical Care Medical Center, Kindai University Nara Hospital, Ikoma City, Japan
[2]Forensic Science Laboratory, Osaka Prefectural Police Quarters, Osaka, Japan
[3]Critical Care Medical Center, Kindai University Hospital, Osaka-Sayama City, Japan
Email: t-nakao@med.kindai.ac.jp

How to cite this paper: Nakao, T., Nitta, A., Nishioka, H., Katagi, M., Tsuda, N. and Kitazawa, Y. (2021) To Investigate the Effect of Using Ethanol Containing Wipes in Collecting Blood for the Measurement of Alcohol Concentration. *Pharmacology & Pharmacy*, **12**, 208-218.
https://doi.org/10.4236/pp.2021.1210018

Received: September 4, 2021
Accepted: October 22, 2021
Published: October 25, 2021



Abstract

This study was aimed to establish whether the skin preparation using ethanol-containing skin antiseptics causes ethanol contamination through blood collection. Venous blood was collected from 40 healthy volunteers according to the national guidelines for blood sampling, with four sequential procedures as follows: 1) collecting blood immediately (within 5 seconds) after cleaning the skin with an individually packaged type of ethanol-containing wipe, 2) collecting blood 1 minute after cleaning the skin with an individually packaged type of ethanol-containing wipe, 3) collecting immediately (within 5 seconds) after cleaning the skin with a traditional cleaning method (thoroughly ethanol-impregnated wipe, and 4) collecting 1 minute after cleaning the skin with a traditional cleaning method. Each sequential procedure was performed with and without the ethanol-containing wipe used for skin cleaning on the puncture site on their right and left arms at the time the needle was withdrawn, respectively. The collected specimens were subjected to the determination of ethanol by using headspace gas chromatography-mass spectrometry. In every 80 blood specimens obtained from 40 participants, ethanol was undetectable (<0.001 mg/mL). This study demonstrates that disinfection using ethanol-containing skin antiseptics is unlikely to cause ethanol contamination through blood collection regardless of skin preparation technique according to the guidelines for blood sampling. This may have implications in forensic science.

Keywords

Blood Alcohol Content, Skin Antiseptic, Contamination of Ethanol

1. Introduction

The use of ethanol (ethyl alcohol (EA), C_2H_5OH) has long been deeply involved in our social lives as a favorite drink. Ethanol has the effect of giving people a sense of euphoria and relaxation in human relationships. However, from the scientific point of view, consumption of ethanol causes a decrease in reasoning ability and self-control and may trigger violent crimes such as assault, causing injury to others or even murder. Tragic traffic accidents caused by drunken driving, and acute alcohol poisoning accidents due to overdrinking have also occurred frequently. Drunken driving can lead to major accidents resulting in the death of innocent people, hence, in such cases, the driver is severely punished by applying the Road Traffic Act or the Car Driving Injury Punishment Act in Japan. Despite such strict laws, there are many drunken driving traffic accidents and social issues involved in excessive drinking which has created a big social problem. The mode of action by ethanol is one of inhibitory action on the central nervous system although it may vary from individual to individual and may depend on the ethanol concentration in the blood. At a low-level alcohol consumption, ethanol tends to promote excitement in drinkers by inhibiting the central nervous system. However, as the blood concentration of ethanol increases, the movement and sensory center in the brain is also suppressed, which prolongs the reflex time of movement and reduces the response to nervous stimuli. In addition, elevated blood levels of ethanol can lead to death due to the suppression of brainstem function since there is a close relationship between the blood ethanol concentration and the degree of drunkenness [1]. Therefore, the measurement of blood ethanol concentration is indispensable for judging legal liability. In Japan, the Road Traffic Law defines drunken driving when blood ethanol concentration exceeds 0.3 mg/mL with accurate precise measurements of blood ethanol concentration. This law has played a great role in preventing traffic accidents.

Since the measurement of blood ethanol concentration requires accurate quantification at low concentration regions, highly sensitive and accurate concentration measurement methods such as gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) are widely used. At the same time, great care should also be paid to prevent ethanol contamination during blood sampling. Therefore, when forcibly collecting blood from the persons refusing blood sampling, medical staff is required to collect blood using a disinfectant solution that does not contain ethanol, such as benzalkonium chloride, chlorhexidine gluconate, or isopropyl alcohol as per Japanese standard blood sampling guidelines. Since the above procedure is different from the usual method, it often takes time and effort to collect the blood. In addition, in some cases, blood-collecting has failed and blood sampling from skin areas disinfected with alcoholic skin wipes is used in unwilling people.

People injured in traffic accidents are transported to the hospital for treatment and if they are suspected to be under the influence of alcohol or illegal drug use, the police may request the hospital to measure the patient's alcohol level. These pa-

tients may be under life-threatening conditions that may require prompt life-saving measures in critical care centers, hence, it is nearly impossible to measure the patient's blood-alcohol level. Alternatively, the police may request blood samples collected during treatment where the skin area has been disinfected with alcohol.

This study aims to verify the presence or absence of ethanol contamination during blood collection procedures where the skin area is disinfected usually with ethanol containing wipes. Nowadays, many hospitals use ethanol containing wipes for skin disinfection before blood collection. However, before the small packaging became available, alcoholic cotton had been used to disinfect skin and collect blood. When the alcoholic cotton was not sufficiently squeezed, ethanol contaminated the blood collection which may affect the measurement of ethanol concentration. Therefore, it was often required to retake the blood without using ethanol. However, the ethanol concentration in the recollected blood data may not reflect the patient's condition at the time of the accident since serum ethanol concentration may decrease in proportion to the passage of time after the accident. As anticipated, in most cases, ethanol is not detected in the resampled blood. If ethanol is detected in the collected blood, the backward calculation by Widmark's method is often performed to estimate the ethanol concentration at the time of the accident. But the data have considerable large individual variation caused by alcoholic metabolism taking place mainly in the liver [2] and is also influenced by therapeutic fluid therapy during treatment. Regarding the presence or absence of ethanol contamination in blood collected by skin disinfection using ethanol, there are several reports published on this matter so far [3] [4] [5] [6] [7]. On the other hand, there are also reports that contradict ethanol contamination [8] [9] [10]. Therefore, this study is to verify the presence or absence of ethanol contamination during blood collection.

This study was approved by the Ethics Committee of Kindai University School of Medicine (reception number: 20-019). This study has already been published in the Japanese language in the Japanese Journal of Forensic Science and Technology (25; 123-30, 2020). With the permission of the journal's editorial board, the authors publish this manuscript in English here.

2. Materials and Methods

2.1. Reagents & Materials

The syringes used were Terumo Syringe 5 ml (SS-O5sz), and the injection needles were Terumo Injection Needle 23G x 11/4 (NN-2332R). EA paper is a precision analysis paper made by Fuji Film Wako Pure Chemical Industries, Ltd. EA for disinfection is ethanol for disinfection made by Kenei Pharmaceutical Co., Ltd. For individually wrapped ethanol impregnated wipe, Hakujuji One Shot Plus P EL-2 (containing 1.6 ml of 76.9 - 81.4 vol% ethanol) was used.

2-Methyl-1-Propanol used a special grade reagent manufactured by Fuji Film Wako Pure Chemical Industries, Ltd. as an internal standard. For blank blood containing no ethanol, normal human whole blood (with anticoagulant) manu-

factured by Kojin Bio was used. The ethanol standard solution was used for preparing a 100 μg/mL aqueous solution and diluting it with blank blood in a timely manner.

2.2. Informed Consent

Forty healthy voluntary men and women over the age of 20 (25 men and 15 women) were explained in detail the purpose and content of this study using documents, and we obtained their understanding and consent.

2.3. Pre-Blood Sampling Interview and Measurement of Ethanol Concentration in Exhaled Breath

We asked about the age, gender, presence or absence of alcohol consumption (if consumed, the quantity taken) at the time of blood sampling, and the current medication history of 40 subjects. For subjects who had drunk alcohol the previous day, the ethanol concentration in the exhaled breath was measured using an exhaled alcohol checker (ALC-D1 manufactured by Iris Ohyama) before blood collection to confirm that there is no alcohol in the system.

2.4. Blood Sampling Method

In accordance with Japanese standard blood sampling guidelines, 2 mL of blood was collected from each subject. The skin area was disinfected with an alcoholic wipe and the needle inserted gently in the right arm. After withdrawing 2 mL of blood, the needle was gently pulled out with an ethanol-containing wipe pressed on site. From the left arm, 2 mL of blood was drawn, however, it was not pressed down with a wipe when the needle was pulled out (Figure 1).

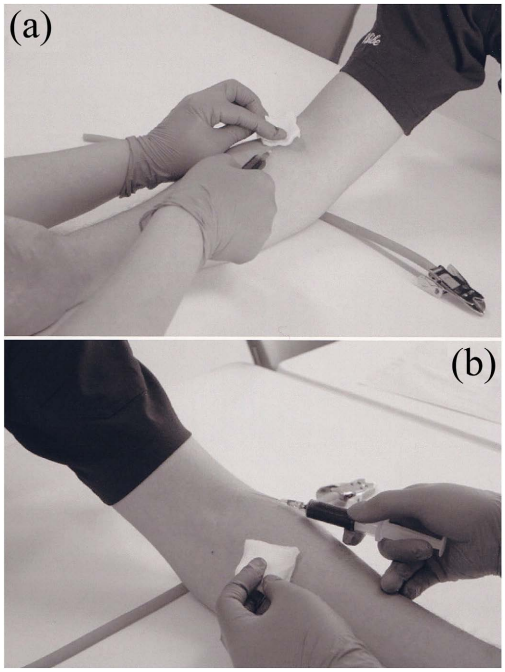


Figure 1. Scenes of blood collecting; withdrawal of the needle.

Forty subjects were divided into four groups of 10 each as follows:

Group I—blood was collected immediately after disinfection with an individually wrapped ethanol-containing wipe.

Group II—blood was collected 1 minute after disinfection with an individually wrapped ethanol-containing wipe.

Group III—blood was collected immediately after disinfection by the conventional method using thoroughly ethanol-impregnated wipe.

Group IV—blood was collected 1 minute after disinfection by the conventional method using thoroughly ethanol-impregnated wipe.

To avoid bias in the blood collection procedure, a total of 15 people (doctors and laboratory technicians) were involved, and they were not informed of the objective of the project. The collected samples were placed in an EDTA-containing blood tube (BD vacuum blood collection tube REF368843) and stored frozen, and later the blood ethanol concentration was measured by gas chromatography-mass spectrometry (GC/MS).

2.5. Ethanol Analysis Method in Blood

Put 0.5 mL of blood sample and 0.5 mL of internal standard (0.25 mg/mL 2-methyl-1-propanol aqueous solution) in a 20 mL glass vial (manufactured by GL Sciences), and seal with a crimp cap with the septum (manufactured by GL Sciences). Ethanol was qualitatively and quantified by headspace gas chromatography-mass spectrometry (HS-GC/MS).

2.6. Equipment and Measurement Conditions

1) Headspace autosampler analysis conditions

Equipment: Perkinelmer Turbomatlix 40 type headspace autosampler

Open temperature: 60˚C

Insulation time: 15 min

Headspace mode: constant

Injection time: 15 min

Needle temperature: 100˚C

Transfer temperature: 150˚C

Pressurization time:1 min

Pull-up time: 0 min

HS carrier gas pressure: 70 kPa

The detection limit was 0.005 mg/mL.

2) GC/MS analysis conditions

Equipment: Shimadzu GCMS-QP2010 Plus type gas chromatograph mass spectrometer

Column: RESTEK Rtx-BAC2 (0.32 mm id × 30 m, Membrane pressure 1.2 μm)

Column temperature: 40˚C (5 min) - 200˚C (1 min), 40˚C/min

Vaporization chamber temperature: 200˚C

Interface temperature: 230˚C
Ion source temperature: 200˚C
Carrier gas: helium
Control mode: linear velocity (60.0 cm/sec)
Ionization method: electron ionization (EI)
Measurement mode: scan mode
The detection limit was 0.001 mg/mL.

3. Result

3.1. Qualitative and Quantitative Analysis of EA by HS-GC/MS

1) Validation

In normal blood ethanol appraisal, qualitative analysis and quantitative analysis was performed by GC/MS scan mode. Validation of EA in HS-GC/MS in scan mode was performed. The ethanol standard aqueous solution was diluted with blank blood to prepare a concentration of 0.03, 0.1, 0.3, 1.0, 2.0 mg/mL. When a calibration curve of blood ethanol was prepared for these samples by HS-GC/MS, good linearity ($Y = 0.511x + 0.00415$, $r^2 = 0.999$) was produced in the range from 0.03 to 2.0 mg/mL. In addition, the intra-day precision and accuracy at each concentration of 0.03, 1.0, 2.0 mg/dL vary by 0.6% and 2.5%, 0.2% and 0.5%, and 0.6% and 0.2% respectively. The measurement accuracy for 6 different days at a concentration of 1.0 mg/mL varied by 1.3%. Furthermore, the detection limit (S/N > 3) of the extracted ion chromatogram of m/z 31 in scan mode was 0.001 mg/mL.

The standard value of ethanol concentration in blood for drunken driving under the Road Traffic Act is 0.3 mg/mL, but the effect of ethanol production due to putrefaction of "from stored blood" has also been reported. Therefore, in the usual BAC (blood alcohol content) test, the detection range of ethanol in blood samples is set to 0.03 to 2.0 mg/mL. But in this study, the lower detection limits were lowered. The detection limits were 0.005 mg/mL for GS, and 0.001 mg/mL for MS. This method has sufficient system sensitivity to verify the effect of ethanol contamination using ethanol disinfection.

2) Analysis of actual specimens

Based on the results in the previous section, HS-GC/MS was performed in scan mode for a total of 80 blood samples collected from 40 people. The result shows (Table 1) ethanol was not detected in any of the 80 samples. The analyzed result of an arbitrary one sample of the actual sample and blank blood to which ethanol was added so that the ethanol concentration was 0.001 mg/mL is shown in Figure 2.

3.2. Examination of the Effect of the Difference between the Ethanol Skin Disinfection Method and the Needle Removal Method on Ethanol Contamination

1) Impact of differences in ethanol skin disinfection methods

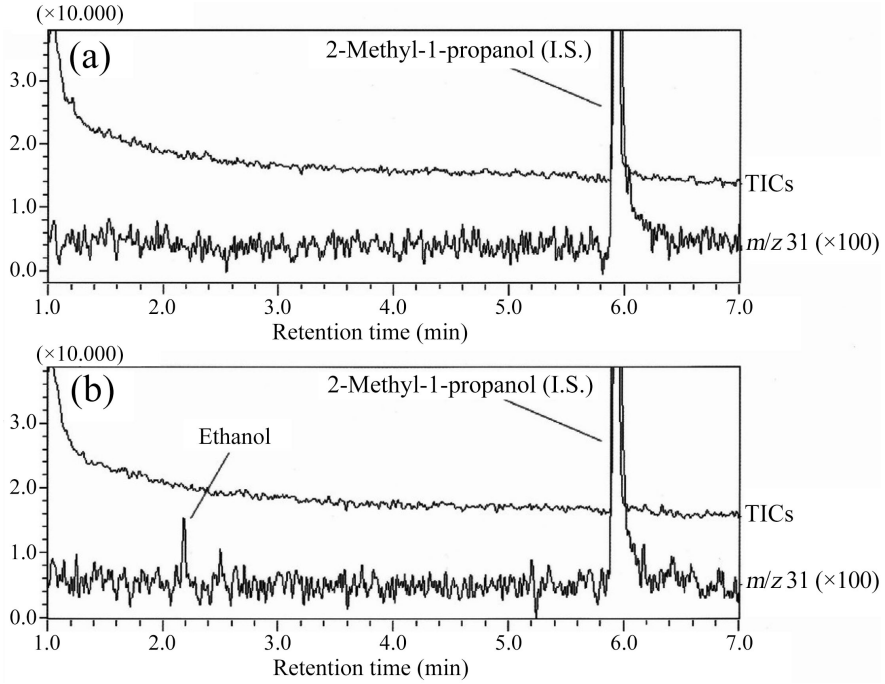


Figure 2. Total ion current chromatograms (TICs) and extracted ion chromatograms (EICs) obtained from (a) one of 80 blood specimens (ethanol was not detected) and (b) the blank blood specimen spiked with ethanol (concentration: 0.001 mg/mL).

Table 1. Results from HS-GC/MS of 80 blood specimens.

Group	Alcohol swab	Waiting time	Method of withdrawal	Number of specimens	Detection of ethanol
I-1	individually packaged type	<5 seconds	With the ethanol-wipe	10	(−)*
I-2			Without the ethanol-wipe	10	(−)*
II-1	individually packaged type	1 minute	With the ethanol-wipe	10	(−)*
II-2			Without the ethanol-wipe	10	(−)*
III-1	Thoroughly ethanol-impregnated wipe	<5 seconds	With the ethanol-wipe	10	(−)*
III-2			Without the ethanol-wipe	10	(−)*
IV-1	Thoroughly ethanol-impregnated wipe	1 minute	With the ethanol-wipe	10	(−)*
IV-2			Without the ethanol-wipe	10	(−)*

* Ethanol was not detected from any specimens.

Japan's standard blood sampling guidelines recommend disinfecting the puncture site and waiting for the disinfectant to dry. However, it is difficult to confirm whether the puncture site is completely dry after the skin disinfection is performed at the time of blood collection. The time from disinfection to blood

collection also varies depending on the blood collector. Particularly, at the scene of a life-saving emergency where time is essential, it is often not possible to wait for drying. Therefore, when ethanol is used for skin disinfection at the time of blood collection, ethanol may remain on the skin surface at the blood collection site, which may cause ethanol contamination to the collected blood depending on the time from disinfection to blood collection.

Therefore, the presence or absence of ethanol contamination was verified in two cases, one in which blood was collected immediately after skin disinfection (groups I and III) and the group where blood was collected 1 minute after disinfection (groups II and IV). The result shows (**Table 1**) ethanol was not detected, and no contamination was found in both methods.

In addition, the ethanol content in the cotton used for skin disinfection may also cause ethanol contamination in the collected blood. Therefore, there are two types of methods: individually wrapped ethanol-containing wipes [1 and 2 groups], which is currently widely used, and a second method where the cotton is sufficiently moistened with a conventionally used disinfectant ethanol (III and IV groups).

The presence or absence of ethanol contamination in the collected blood in the two groups of disinfection methods was verified. The result shows (**Table 1**) ethanol was not detected and no contamination was observed regardless of which ethanol-containing wipe/cotton was used.

2) Effect of difference in needle removal method

In the early guidelines, when removing the needle after blood collection, it is recommended to remove the needle with a lightly applied disinfectant cotton or gauze to the puncture site and press the site. In most cases, the needle insertion site is pressed with skin-sterilized ethanol-containing cotton when removing the needle. There is a possibility of ethanol contamination due to the suction of ethanol with a syringe at the time of needle removal. Therefore, we verified the presence or absence of ethanol contamination in the collected blood in two cases, one (I-1, II-1, III-1, IV-1 group) in which the needle insertion site was pressed with the ethanol-containing cotton used for skin disinfection and the other (I-2, II-2, III-2, IV-2 group) in which the needle insertion site was not pressed.

The result shows (**Table 1**) ethanol was not detected, and no contamination was observed by any of the needle removal methods.

4. Discussion

The effect of ethanol skin disinfection on blood ethanol concentration measurement has been verified under various conditions. Higuchi *et al.* [3] reported that almost no contamination was observed when disinfected with ethanol cotton followed by drying for a minute, but also reported that ethanol contamination occurred when the needle touched the cotton. Müller and Hundt reported that the amount of ethanol contamination was 0.02 - 34 mg/mL when the needle

touched the ethanol cotton during blood collection with a vacuum blood collection tube [3]. Winek and Eastly had an average of 0.026 mg/mL of ethanol contamination when disinfected with ethanol cotton, and an average of 0.006 mg/mL of ethanol contamination when the blood sampler performed the standard method. It is reported that there was an average of 0.89 mg/mL of ethanol contamination when the person intentionally moistened the blood collection site with EA [5]. Peek *et al.* reported that when they were sterilized with ethanol cotton and blood was collected after drying, the amount of ethanol contamination was 0.070 ± 0.067 mg/mL [6]. In addition, Yigit and Arslan recorded an abnormally high blood ethanol concentration of 4.53 mg/mL in emergency patients when blood was collected using ethanol and povidone iodine for disinfection [7]. They additionally reported that the ethanol concentration decreased to 0.003 mg/mL when re-collection of blood was performed without using ethanol for disinfection [7]. These reports indicate that skin disinfection with ethanol causes contamination during blood sampling and affects blood ethanol level measurements. On the other hand, Malingre *et al.* [8] and Lippi *et al.* [9] reported that no contamination was observed and concluded that skin disinfection with ethanol would not affect blood ethanol measurement. Thus, there have been pros and cons whether skin contamination affects ethanol measurement.

Our study shows that when ethanol wipes were used to disinfect the skin area where the venipuncture occurs, there is no ethanol contamination in the ethanol blood assay. Further, this study also shows that no ethanol contamination occurs with any of the needle removal methods used. Our findings appear to support the reports published by Malingré, *et al.* [8], Lippi *et al.* [9], and Tucker, *et al.* [10].

Nonetheless, individual differences occur depending on the time and country, such as the type of disinfectant cotton, how to moisten with ethanol, the time from skin disinfection to blood collection, and how to apply the disinfectant cotton when removing the needle. Inconsistent sampling conditions are the major cause of discrepancies in results. Especially when removing the needle while it is still wet with ethanol, the needle and disinfectant cotton comes in contact with each other. In addition, it has also been reported that ethanol contamination occurs, with a high probability, depending on the operation method at the time of needle withdrawal. In any case, it is a fact that ethanol skin disinfection may cause contamination during blood collection depending on the conditions.

The report by Higuchi *et al.* [3] has been cited as the most credible verification of results in Japan regarding ethanol contamination during blood collection. Therefore, the data of samples taken using ethanol skin disinfection have been considered to have low evidence value in the court in Japan. However, the disinfection method at that time of Higuchi's report was different from the current one. For ethanol skin disinfection, the standard blood sampling method guideline states that individually wrapped products are desirable from the viewpoint of certainty of disinfecting effect and cleanliness. The individually wrapped wipe

is commonly used nowadays. However, in the past, sufficiently moistened cotton wool with disinfectant ethanol was prepared at each facility in the hospital as disinfecting material. When removing the needle after blood collection, it had been common to apply absorbent cotton to the skin. The ethanol contamination observed in the report by Higuchi *et al.* was suggested to be caused by the old method of blood collection. Hence, it is possible that the ethanol remaining in the cotton wool had not been sufficiently squeezed and might have been aspirated at the time of blood sampling. In the current situation where individually wrapped ethanol-containing wipe is wildly used, such a problem does not exist. Therefore, claims by drunken drivers in a Court of Law that high blood ethanol values are caused by faulty methodology becomes questionable.

5. Conclusion

We examined whether skin disinfection by ethanol causes ethanol contamination during blood collection from patients. This study showed that no ethanol contamination occurred by any of the methods employed. Our data from this study proves that if the standard Japanese blood collection guidelines were followed, there will be no ethanol contamination during the process of blood collection from patients. Therefore, this has serious implications that challenge the current forensic blood alcohol sample acquisition in a Court of Law.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Murrell, D. (2018) Alcohol Intoxication: What You Should Know. https://www.healthline.com/health/alcohol-intoxication

[2] Zakhari, S. (2006) Overview: How Is Alcohol Metabolized by the Body? *Alcohol Res Health*, **29**, 245-254.

[3] Higuchi, A., Kurihara, R., Yoshimoto, T., Sano, T., Katsumata, K., *et al.* (2005) Problems in Blood Alcohol Testing of Severely Injured Drivers Brought to Emergency Departments in Japan. *Legal Medicine*, **7**, 299-305. https://doi.org/10.1016/j.legalmed.2005.05.003

[4] Müller, F.O. and Hundt, H.K.L. (1976) Ethyl Alcohol: Contamination of Blood Specimens. *South African Medical Journal*, **50**, 91.

[5] Winek, C.L. and Eastly, T. (1977) Factors Affecting Contamination of Blood Determinations. Appleton & Lange, New York, 147-162.

[6] Peek, G.L., Marsh, A., Keating, J., Ward, R.J. and Peters. T.J. (1990) The Effect of Swabbing the Skin on Apparent Blood Ethanol Concentration. *Alcohol and Alcoholism*, **25**, 639-640. https://doi.org/10.1093/oxfordjournals.alcalc.a045060

[7] Yigit, O. and Arslan, S. (2012) Swabbing Skin with Alcohol Could Change the Blood Alcohol Level Significantly. *The Journal of Emergency Medicine*, **43**, 718-719. https://doi.org/10.1016/j.jemermed.2011.07.025

[8] Malingré, M., Ververs, T., Bos, S., van Kersteren, C. and van Rijn, H. (2005) Alcohol

Swab and Venipuncture in a Routine Hospital Setting: No Effect on Blood Ethanol Measurement. *Therapeutic Drug Monitoring*, **27**, 403-404. https://doi.org/10.1097/01.ftd.0000158868.74618.dd

[9] Lippi, G., Simundic, A.M., Musile, G., Danese, E., Salvagno, G. and Tagliaro, F. (2017) The Alcohol Used for Cleaning the Venipuncture Site Does Not Jeopardize Blood and Plasma Alcohol Measurement with Head-Space Gas Chromatography and Enzymatic Assay. *Biochemia Medica*, **27**, 398-403. https://doi.org/10.11613/BM.2017.041

[10] Tucker, A. and Trethewy, C. (2010) Lack of Effect on Blood Alcohol Level of Swabbing Venepuncture Sites with 70% Is Opropyl Alcohol. *Emergency Medicine Australasia*, **22**, 9-12. https://doi.org/10.1111/j.1742-6723.2009.01248.x