Abstract

Introduction: This study aimed to establish whether an alcoholic antiseptic, wiped or not before venipuncture, may jeopardize alcohol testing with a commercial enzymatic assay and a reference head-space gas chromatography (GC) technique.

Materials and methods: Venous blood was collected from 23 healthy volunteers, with two sequential procedures. In the first blood collection, 2 mL of alcoholic antiseptic (0.5% chlorhexidine, 70% ethanol) were placed on a gauge pad, the venipuncture site of right arm was cleaned but the antiseptic was not let to dry before phlebotomy. In the second blood collection, 2 mL of the same alcoholic antiseptic were placed on another gauge pad, the venipuncture site of left harm was cleaned and the antiseptic was accurately cleansed before phlebotomy. Ethanol was measured with a reference GC technique in whole blood and EDTA plasma, and a commercial enzymatic assay in EDTA plasma.

Results: No subject complained about feeling a particular itchy sensation when the alcohol was not wiped before puncturing the vein. The concentration of alcohol in all EDTA plasma samples was always lower than the limit of detection of the enzymatic assay (i.e., 2.2 mmol/L; 0.1 g/L). Similarly, alcohol concentration was also undetectable using a reference GC technique (i.e., < 0.22 mmol/L; 0.01 g/L) in EDTA plasma and whole blood.

Conclusion: It seems reasonable to conclude that using ethanol-containing antiseptics before venipuncture may not be causes of spurious or false positive results of alcohol measurement at least when ideal venipunctures can be performed.

Key words: laboratory testing; diagnostics; alcohol; errors; pre-analytical phase

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Introduction

The collection of venous or arterial blood specimens is one of the most frequent practices in healthcare (1,2), along with collection of clinical history, medical examination, blood pressure, heart rate and temperature measurement (3). Blood drawing is an essential practice for obtaining a suitable material (i.e., whole blood, serum or plasma), in which the vast majority of laboratory tests can be performed (4). Regardless of which of the many existing blood collection guidelines is followed by the phlebotomist, the need of accurate skin disinfection at venipuncture site is always considered a necessary activity for preventing bacteremia. More specifically, the guidelines of the World Health Organization (WHO) mandates that the venipuncture site should be cleaned with a 70% alcohol swab, and alcohol should then be allowed to dry for not less than 30 seconds before puncturing the vein (5). Alcohol is preferred to povidone iodine due to the fact that blood contamination with this latter antiseptic may generate falsely increased values of potassium, phosphorus or uric acid (5). The Clinical and Laboratory Standards Institute (CLSI) document H3-A6 also man-
dates that skin disinfection with 70% isopropyl alcohol or ethanol is always necessary before venipuncture (6). As in the WHO guidelines, the CLSI also recommends that alcohol should be allowed to dry completely before inserting the needle into the vein (6). The Croatian Society of Medical Biochemistry and Laboratory Medicine has published national guidelines, which recommend that the venipuncture site should be accurately disinfected with 70% isopropyl alcohol or ethanol placed on sterile cotton or gauze pad before drawing blood (7). Notably, when blood alcohol measurement is ordered, the Croatian guidelines also recommend that non-alcoholic disinfectants should be used (i.e., ether or benzene). Finally, the guidelines of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) recommend that the skin at the venipuncture site should be cleansed with an appropriate alcoholic antiseptic, which should then be allowed to dry before puncturing the vein (8).

Besides its use in forensic medicine to provide corroborative evidence of impairment at the wheel, the laboratory measurement of alcohol in whole blood, serum or plasma is crucial for diagnosing alcohol abuse or toxicity (9,10). Despite head-space gas chromatography (GC) remains the gold standard for measuring alcohol for forensic medicine purposes, alcohol assessment in routine clinical laboratories is usually accomplished by enzymatic techniques, most of which based on the alcohol dehydrogenase method (11,12). The use of enzymatic assays in clinical practice is justified by many practical reasons, such as suitability for automation, shorter turnaround time (TAT), lower costs and no need for specialized personnel for running the test compared to GC.

Except for the recommendations of the Croatian Society of Medical Biochemistry and Laboratory Medicine, the indication of using ethanol or other alcoholic antiseptics before drawing blood is one common aspect of all other guidelines. Although two separate studies clearly demonstrated that the practice of avoiding to wipe alcohol is not associated with spurious hemolysis or sample dilution, the issue of potential sample contamination with the alcohol used for cleansing the venipuncture site remains a matter of debate (13,14). The use of alcohol for cleaning the venipuncture site has been recognized as a possible source of contamination of blood specimens since 1976 (15). Moreover, it has also been demonstrated that alcohol could be absorbed through the intact skin of adult humans, thus leading to barely measurable blood alcohol levels, comprised between 0.01 - 0.04 mmol/L (16). The risk of contaminating blood specimens may be magnified when the phlebotomist does not allow the alcohol to dry for at least 30 seconds before puncturing the vein, a practice that is justified for preventing a prolonged placing of tourniquet and the ensuing risk of hemocoagulation and spurious increase of some measurable analytes in blood (17). Notably, this aspect may have substantial forensic implications, since the blood alcohol content (BAC) drink driving limit across Europe is on average 10.8 mmol/L (i.e., 0.5 g/L), but varies between 0 mmol/L in Romania, Slovakia, Hungary and Czech Republic, up to 17.4 mmol/L (i.e., 0.8 g/L) in Malta and in the United Kingdom (18). Even more importantly, the BAC drink driving limit for novice drivers has been set to 0 mmol/L in many European countries including Croatia, Czech Republic, Germany, Hungary, Italy, Lithuania, Romania, Slovakia and Slovenia (18). Although some local forensic regulations currently discourage the use of alcoholic antiseptics for cleansing the skin before collecting blood for alcohol testing (19), the real world practice is often different from theory, with studies reporting that the use of alcohol-based antiseptics is actually commonplace when drawing blood for BAC (20). This is not surprising since the acquisition of blood tubes, integrated blood collection systems, as well as other phlebotomy tools such as (alcoholic) antiseptics, tourniquets, cotton or gauge pads, is now regulated by regional or national tenders in many countries worldwide, so that the local purchase of these materials for particular types of blood collections is no longer allowed by some hospital administrations (21).

Therefore, the aim of this study was to investigate whether the use of an alcoholic antiseptic, as well as the avoidance of wiping the alcohol before venipuncture, may both have an impact on plasma
and whole blood alcohol measurement using a reference head-space GC technique and a routine enzymatic assay.

**Materials and methods**

**Study design and blood collection**

The study population consisted of 23 ostensibly healthy laboratory professionals (18 women and 5 men; mean age, 49 ± 8 years; body mass index, 24.7 ± 4.7 kg/m²), who voluntarily participated to this study. Two sequential procedures were followed for collecting venous blood from each volunteer, who had all abstained from ingesting food or drinking alcoholic beverages for at least 8 hours before the study. A first blood drawn was performed on the right arm, as follows. A pipette (Eppendorf Reference 100 - 1000 μL pipette; Eppendorf, Marburg, Germany) was used to place 2 mL of alcoholic antiseptic (0.5% chlorhexidine digluconate and 70% ethanol; Neoxinal Alcolico; Nuova Farmec, Verona, Italy) on a 30 mm diameter pre-packaged gauge pad (Luigi Salivari SPA, Florence, Italy). This amount of alcohol corresponds to the approximate volume manually placed on the gauge pad by the nurses in our hospital. A tourniquet was applied, the venipuncture site was cleaned, the antiseptic was not let to dry and the needle was inserted into the vein within 5 seconds after the study. A first blood drawn was performed on the right arm, as follows. A pipette (Eppendorf Reference 100 - 1000 μL pipette; Eppendorf, Marburg, Germany) was used to place 2 mL of alcoholic antiseptic (0.5% chlorhexidine digluconate and 70% ethanol; Neoxinal Alcolico; Nuova Farmec, Verona, Italy) on a 30 mm diameter pre-packaged gauge pad (Luigi Salivari SPA, Florence, Italy). This amount of alcohol corresponds to the approximate volume manually placed on the gauge pad by the nurses in our hospital. A tourniquet was applied, the venipuncture site was cleaned, the antiseptic was not let to dry and the needle was inserted into the vein within 5 seconds after the study.

Blood was collected into a 6 mL evacuated blood tube containing K₂EDTA (Vacutest Kima, containing 10.8 mg spray K₂EDTA, Ref. 135400; Kima, Padova, Italy). A second blood drawn was then performed on the left arm, as follows. The same pipette (Eppendorf Reference) was used to place 2 mL of the same alcoholic antiseptic (Neoxinal Alcolico; Nuova Farmec, Verona, Italy) on another 30 mm diameter pre-packaged gauge pad (Luigi Salivari SPA, Florence, Italy). A tourniquet was applied, the venipuncture site was cleansed, but the antiseptic was accurately dried by using another 30 mm diameter cotton ball, using circular motions from centre to periphery, as recommended by the Croatian Society of Medical Biochemistry and Laboratory Medicine (7). The needle was finally inserted into the vein within 5 seconds after drying the antiseptic. Blood was collected into another 6 mL evacuated blood tube containing K₂EDTA (Kima, Padova, Italy).

For each venipuncture blood was collected directly into the evacuated blood tube through a 19 gauge straight needle. All procedures of the venipuncture (i.e., skin cleansing and obtaining blood) were standardized according to national guidelines (8,22) and performed by the same expert phlebotomist. In all circumstances the needle was withdrawn from the vein after the blood tube had been completely filled and had been removed from the holder, as in an optimal venipuncture. Contact between needle and gauge pad was also carefully avoided throughout the venipuncture. Immediately after collection the blood tubes were mixed by 4-time gentle inversion. The blood in each primary tube was then divided in two identical aliquots, the first to be used for performing GC analysis, whereas the second was used for enzymatic testing. Both aliquots were always maintained capped until measurements. Alcohol testing was performed by the reference GC technique using both EDTA blood and EDTA plasma, whereas the enzymatic assessment was performed using EDTA plasma. The EDTA plasma was obtained after centrifugation of whole blood aliquots at 3000 g for 15 min at room temperature.

**Methods**

Ethanol concentration was measured with the reference GC technique, on both blood and plasma EDTA. Briefly, ethanol was first measured in whole blood, by opening the tube, and removing 100 μL of whole blood. The specimen was then immediately recapped to prevent alcohol evaporation, was centrifuged to separate plasma from blood cells and ethanol concentration was finally measured on plasma EDTA. Alcohol measurement with the reference head-space GC technique was carried out using a Young Lin 6100 fully optimized head-space GC analyzer with advanced pneumatic control and flame ionization detector (Young Lin Instrument Co, Anyang, Korea). Both the EDTA plasma and whole blood were mixed 1:5 with an internal standard (tert-butyl alcohol at 0.0975 g/L; Carlo Erba Reagents, Cornaredo, Italy). A total vol-
ume of 1250 µL of sample was injected in the system. The temperature of the syringe was 80 °C and the isotherm separation was carried out at 40 °C. An internal quality standard (Carlo Erba Reagents, Cornaredo, Italy) with an ethanol concentration of 5.4 mmol/L was also measured every 5 test samples. The lower limit of detection of this technique is 0.22 mmol/L (i.e., 0.01 g/L).

The concentration of alcohol in plasma EDTA was also measured using a Roche Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), with an original Roche commercial reagent (Roche Diagnostics GmbH, Mannheim, Germany), based on the alcohol dehydrogenase method. In a previous study, the imprecision of this assay was found to be 2.4% at a plasma ethanol concentration of 31.6 mmol/L (23), whereas the measuring range of the assay has been declared to range between 2.2 - 108 mmol/L by the manufacturer.

All subjects signed a written consent for being recruited to this study, which was performed in accordance with the Declaration of Helsinki, under the terms of relevant local legislation, and was approved by the local Ethical Committee (University Hospital of Verona, Verona, Italy – SOPAV2, protocol number 35747; date of approval: 25 July 2016).

Results

No subject complained about feeling a particular itchy sensation when the alcohol was not wiped before puncturing the vein. The concentration of alcohol in all EDTA plasma samples was found to be always lower than the limit of detection of the enzymatic assay (i.e., < 2.2 mmol/L; < 0.10 g/L). Similarly, the alcohol concentration was also undetectable by the reference GC technique (i.e., < 0.22 mmol/L; < 0.01 g/L) in all EDTA plasma and whole blood specimens.

Discussion

Controversial evidence on alcohol measurement has been previously published when the venipuncture site is cleaned with alcohol-containing antiseptics. The first alert about the possible interference from using alcohol antiseptics for cleansing the venipuncture site was published by Müller and Hundt in 1976 (15). In their elegant study, the authors collected three sequential evacuated blood tubes from each of 10 healthy volunteers, for measuring ethanol concentrations with GC. The skin was cleansed with an antiseptic containing 10% chlorhexidine and 70% ethanol. Before alcohol evaporation had occurred, the needle was inserted into the vein and the first two blood tubes were completely filled, whereas the needle was withdrawn from the vein while the third blood tube was still aspirating. Importantly, the presence of ethanol on the skin did not produce a measurable concentration of blood alcohol in the first two tubes, whereas a measurable concentration of blood alcohol was found in the third tube in eight out of the ten subjects, with blood alcohol values between 0.4 - 743.9 mmol/L.

In a following study, Goldfinger and Schaber measured alcohol concentration with an enzymatic assay in 25 emergency patients, after collecting blood with alcohol prep pad on one arm and non-alcohol-containing germicidal solution on the other (24). No significant difference was appreciated in BAC obtained by either method of skin preparation.

Opposite results were published by Peek et al. (25). Briefly, the authors collected blood from 10 healthy volunteers during heavy drinking by either cleansing the venipuncture site with absolute ethanol on one arm or leaving the venipuncture site unswabbed on the opposite arm. Blood ethanol concentration was found to be significantly increased (up to 3.9 mmol/L) in samples collected from the ethanol-cleansed arm compared to the unswabbed arm. Unlike these findings, the concentration of blood ethanol was unaltered when ethanol was replaced with isopropanol.

McIvor and Cosbey measured BAC by means of head-space GC in 20 subjects, whose blood had been drawn after soaking the venipuncture site with ethanol, isopropyl alcohol or non-alcoholic cetrimide/chlorhexidine swab, and concluded that a modest ethanol interference could be appreciated by using alcohol-based skin cleansing swabs (26). Malingré studied the possible impact of...
清理穿刺部位的酒精棉球，通过测量血乙醇浓度来检测酒精，使用GC和酶法在50名≤12岁的患者和20名健康志愿者中进行了研究（27）。在所有样本中，血乙醇浓度未被检测到（即，<4.3 mmol/L）。与之形成对比的是，Higuchi等人（125）收集了40名健康受试者的血液，将穿刺部位用酒精或生理盐水清洁。虽然用参考GC技术检测到两种液体完全干燥1分钟后没有血液中的乙醇或生理盐水的吸收，但在我们的研究中，40%的受试者在进行皮肤酒精涂抹后5秒钟的穿刺时，观察到微量的酒精摄入（即，约0.2 mmol/L）。这一比率在受试者用酒精浸泡的棉球触摸或擦拭穿刺部位后显著增加至70%。在另一项研究中，Miller等人（65）显示使用含有70%异丙醇溶液的皮肤准备垫不会产生假阳性乙醇水平，因为所有测试结果都低于酶法检测的极限（即，1.1 mmol/L）（28）。Tucker和Trethewy进行了一项有趣的研究，将一只手用70%异丙醇溶液消毒后完全干燥，另一只手用生理盐水消毒后采血（29）。酶法检测到的乙醇浓度没有统计学差异。相反，另一项由Yigit和Arslan进行的研究描述了一名20岁的男患者将头砸在树干上，并被送往急诊室的案件（30）。患者被带入急诊室时的血液乙醇浓度（即，98.3 mmol/L）非常高，尽管患者否认曾摄入任何酒精饮料。负责采血的护士承认曾使用含酒精的消毒剂清理穿刺部位，因此在用碘仿棉球擦拭皮肤后采集了第二份血样。第二份血样的乙醇浓度为0.07 mmol/L。我们的结果表明，不使用含有酒精的消毒剂在血液采血前蒸发，并在EDTA血浆或全血中使用一个传统的酶法（检测限为2.2 mmol/L）和一个参考GC技术（检测限为0.22 mmol/L；0.01 g/L）来测量乙醇是不相关的。这些结果显然适用于理想的采血条件，由熟练的采血人员并严格遵循当地指南。因此，使用含有酒精的消毒剂，以及不使酒精蒸发在采血前，可能被安全且相对无痛地用于酒精测试，无论临床或法医目的，以及采血程序都应严格遵循。

根据我们的数据，使用含酒精的消毒剂可能不被认为是测量酒精的假阳性或假阳性的重要原因。然而，由于它可能是一个不那么理想的采血可能仍然导致含有酒精的消毒剂的血液采集，正如在一些先前的研究（15,20）所示，我们建议在采集血样进行酒精测试时，应优先考虑，在出现表1列出的条件之一或多时，可能更安全地让酒精蒸发后进行采血。在法医酒精测试中。

**Potential conflict of interest**
None declared.

| Conditions potentially leading to contamination of blood tubes by alcohol-containing antiseptics |
|------------------------------------------------------------------------------------------|
| Needle touched by cotton or gauge pad soaked with alcohol                                |
| Needle swabbed by cotton or gauge pad soaked with alcohol                                |
| Venipuncture performed with needle under pressure by cotton or gauge pad soaked with alcohol |
| Needle withdrawn from the vein while the blood tube was still aspirating                  |

**Table 1.** List of conditions potentially leading to contamination of blood tubes by alcohol-containing antiseptics
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