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Original communication

Comparative analysis of hospital and forensic laboratory ethanol concentrations: A 15 month investigation of antemortem specimens

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ABSTRACT

Quantitative serum alcohol concentrations from regional hospitals (from specimens collected at time of hospital admission) were compared to results from whole blood (from specimens collected at the time of hospital admission) concentrations measured at the San Diego County Medical Examiner's Office (SDCMEO). Over a 15 month period (January 2012 to March 2013), the postmortem forensic toxicology laboratory analyzed a total of 2,321 cases. Of these, 280 were hospital cases (antemortem) representing 12% of the overall Medical Examiner toxicology casework. 59 of the 280 hospital cases (or 21%) screened positive for alcohol (ethanol). 39 of these 59 cases were included in the study based on available specimens for quantitative analyses. This investigation indicated that serum hospital ethanol concentrations correlated well ($R^2 = 0.942$) with ethanol values determined at SDCMEO (generally measured in whole blood). There was an observed negative bias with an average of −14.1%. A paired t-test was applied to the data and it was shown that this observed bias is statistically significant. These differences in ethanol concentrations could result from differences in specimen, analytical techniques, and/or calibration. The potential for specimen contamination is also discussed.

1. Introduction

Ethanol (alcohol) is the most common quantitative drug test ordered in both clinical and forensic toxicology.[1,2] The quantitation of ethanol in biological specimens can help determine cause and manner of death.[2] The accuracy of the ethanol quantitation is especially important in forensic toxicology settings. There are multiple analytical methods for ethanol quantitation, which may introduce variation in the measured ethanol value. Within sample variation may also be attributed to the time frame between the two separate analyses. In such instances, the true ethanol concentration may vary since as the sample ages, losses of ethanol due to evaporation can become significant.[3] Other causes for variation in the ethanol values come from systematic biases between the two different analytical methods. This is especially true when comparing an enzymatic assay, commonly used in the hospital setting, with a chromatographic ethanol assay used in forensic settings. Enzymatic assays do not measure the concentration of ethanol directly, instead, they measure an absorbance change caused by the production of NADH which is then related back to the concentration of ethanol.[4] Unfortunately, some small molecules oxidized by their respective enzymes can also produce NADH, therefore possibly increasing the perceived concentration of ethanol.[5,6] Calibration differences between methods may also play a role in the variability of quantitation between different methods.

This study compared the ethanol quantitation values of 39 ethanol positive cases, where samples in the same collection set were analyzed first at the hospital of origin and then again at the San Diego County Medical Examiner's Office (SDCMEO). Ethanol quantitation differences and contributing factors which may have caused them are discussed.

2. Methods

2.1. Inclusion criteria

Specimens included in this ethanol correlation study were collected over 15 months (January 2012 to May 2013). Of the 2,321 cases for which toxicological analysis was performed, 280 were hospital cases (antemortem) representing 12% of the overall Medical Examiner toxicology casework. Ethanol positive cases were

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initially quantified at the hospital of arrival from samples collected before the death of the patient. If the patient death became a medical examiners case, ethanol values were analyzed at the SDCMEO as part of routine casework. Samples were measured at SDCMEO using the in-house ethanol screening and quantitation methods. 59 of the 280 hospital cases screened positive for alcohol (ethanol). From these 59 cases, 39 had sufficient and appropriate material (original whole blood or serum) for ethanol quantitation at SDCMEO. The majority of antemortem whole blood samples received at the medical examiner's office from hospitals were in EDTA lavender top tubes. Other tube types such as sodium citrate and sodium fluoride were also received and tested. All samples, upon arrival at SDCMEO were stored at 4 °C until analyzed. The maximum delay between testing at the hospital and the SDCMEO was ten days. A paired t-test was used to evaluate differences between the concentrations of ethanol determined by the different techniques with a p value <0.05 considered statistically significant.

2.2. Hospital ethanol quantitation

Most hospitals use an enzymatic assay to measure concentration of ethanol in plasma or serum. This enzymatic assay contains a known quantity of the enzyme alcohol dehydrogenase. Oxidation of ethanol by this enzyme generates a stoichiometric amount of NADH, the rate of which is monitored spectrophotometrically at 340 nm.[4]

2.3. Ethanol quantification (SDCMEO)

2.3.1. Chemicals and apparatus

The internal standard n-propanol was reagent grade (Burdick & Jackson) and was purchased from VWR (Radnor, PA). The methanol, isopropanol and acetone standards were OmniSolv grade and were also purchased from VWR. Ultra high purity zero water was obtained from Pall Corporation “Cascada” system. Potassium dichromate was AR Primary standard grade and was purchased from NIST (Gaithersburg, MD). Sodium thiosulfate, potassium iodide, and soluble starch were purchased from Mallinckrodt (St. Louis, MO). Sulfuric acid was purchased from Fisher Scientific (Waltham, MA).

200 proof ethanol was un-denatured, USP grade and was purchased from Spectrum Chemical Corporation (Gardena, CA). Aqueous stock internal standard solutions containing 0.05% n-propanol in deionized water and working internal standards containing 0.00625% n-propanol in deionized water were prepared using calibrated volumetric pipettes. Three calibrators consisting of secondary alcohol standards were created using the following method. Approximate ethanol concentrations of 0.10 g/dL, 0.20 g/dL, and 0.30 g/dL were prepared by volumetric addition of 200 proof ethanol to deionized water. Each of the calibrator's exact concentration was then determined by using a direct oxidimetric method. This method uses a primary standard of potassium dichromate in sulfuric acid. The primary standard of potassium dichromate is traceable to National Institute of Standards and Technology (NIST) potassium dichromate standard. Solutions of exactly 0.1304 N potassium dichromate were made by weighing previously desiccated potassium dichromate using a Mettler AG104 analytical balance followed by dissolution with deionized water in a volumetric flask. Addition of a known quantity of excess of potassium dichromate was used to ensure all ethanol in a sample will be oxidized. After reaction completion, the concentration of the remaining potassium dichromate was determined by addition of potassium iodide and back titration of the produced iodine with sodium thiosulfate. Back calculation of the consumed concentration of sodium thiosulfate yielded the amount of unreacted potassium dichromate. Subtraction of the potassium dichromate's known initial concentration by the unreacted concentration yielded the amount of potassium dichromate consumed which stoichiometrically gives the concentration of ethanol in the solution. The concentration of each secondary alcohol calibrator from the aforementioned method was determined by averaging six replicate measurements. A volatile reference solution (VRS) was prepared by diluting 0.5 mL methanol, 1.0 mL ethanol, 1.0 mL isopropanol and 0.5 mL acetone to 1000 mL with deionized water. This VRS was analyzed with each batch of casework to confirm the accuracy of the alcohol retention times. Two commercial whole-blood toxicology controls containing 0.081 g/dL and 0.202 g/dL of ethanol in whole blood were obtained from Cliniqa Corporation. (San Marcos, CA), and an in-house negative control prepared with only diluent and internal standard (n-propanol) were run with each batch of calibrators and casework.

2.3.2. Specimen preparation

Ethanol and other volatiles were analyzed using a GC-FID-Headspace procedure. 50 μL of calibrator standards, controls or samples (whole blood or serum) were added to individual headspace auto-sampler vials. 2.0 mL of the working internal standard solution containing 0.00625% n-propanol was then added to each vial. Samples were then crimp-capped and placed in the sampler tray for headspace GC analysis which were equilibrated at 40 °C. Samples were run in duplicate and their averages were reported.

2.3.3. Instrumentation

2.3.3.1. Ethanol screen (SDCMEO). Before the described quantification procedure was performed, all cases were initially determined to be positive for ethanol using a screening procedure. The screening method and instrumentation utilized were identical to the quantification procedure described, apart from the analytical column which was a RTX-BAC2 (Restek Technologies) (30 m, 0.32 mm diameter) column, and the method was calibrated using a single alcohol concentration of 0.20 g/dL.

2.3.3.2. Ethanol quantification (SDCMEO). Injections of headspace vapors were made onto a 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a Model G1888 automatic headspace sampler and a flame-ionization detector. The GC column was a RTX-BAC1 (Restek Technologies) (30 m, 0.32 mm diameter) with hydrogen as the carrier gas. The GC oven was held steady at a temperature of 40 °C. The total chromatography time per injection was 3.5 min. Volatile identification was based on retention time and quantitation based on calibrated area ratios of the volatile and the internal standard (n-propanol). Using two columns to confirm the presence of ethanol provides higher confidence that the analysis is not subject to other volatile interferences. A list of common volatiles and their retention times on the columns used for screening and quantitation are shown in Table 1.

2.3.4. Accuracy and Precision

All calibrators were within 5% of the target concentration when they were back calculated. A calibration curve was constructed from all three calibrators. The calibration used a linear regression fit in which $R^2 \geq 0.99$. The limit of detection (LOD) was 0.005 g/dL, and the limit of quantification (LOQ) was 0.02 g/dL. The two positive control samples included in each batch were compared to the prepared values of 0.081 g/dL and 0.202 g/dL which back calculated to be within 5% of the target concentration. Accuracy, assessed over a nine month period, was 0.077 g/dL or 95% of the target (from 128 individual determinations) for the 0.081 g/dL control, and 0.198 g/dL, 98% or the target (from 80 individual determinations) for the 0.202 g/dL control. Precision (% coefficients of variation), over this