Evaluation of the Analytical Performance of the Olympus AU5421 Applied with Parameters Developed by the Toshiba 200FR for Measurement of Blood Ethanol Concentration

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Background: An accurate determination of blood ethanol concentrations is important. To minimize ethanol degradation in blood samples, sodium fluoride (NaF) collection tubes have been recommended for use. In this study, we attempted to utilize the Olympus AU5421 chemistry analyzer for ethanol analysis based on the parameters established for the Toshiba 200FR. We also evaluated the effect of NaF collection tubes on ethanol concentrations. **Methods:** The precision, linearity, accuracy, and carry-over rate of the AU5421 analyzer were evaluated. The results of analysis using the AU5421 and Abbott AxSYM analyzers were also compared. The effects of NaF collection tubes on ethanol concentrations in stored samples were measured. **Results:** The AU5421 showed a good precision, linearity, accuracy, and carry-over rate. The

ethanol concentrations were well correlated with the results obtained using the AxSYM. There was no statistically significant difference in blood ethanol concentrations between the samples collected in tubes with NaF and those collected in tubes without NaF. **Conclusions:** Since the AU5421 showed excellent analytical performance, the AU5421 could be used as an alternative to AxSYM for the determination of blood ethanol concentrations. Our analysis also indicated that there is no need to use NaF collection tubes if blood ethanol concentrations are analyzed within 3 h after blood collection. We believe that the results obtained in this study will have important implications for the use of the AU5421 system to measure blood ethanol concentrations. J. Clin. Lab. Anal. 29:214–219, 2015. © 2014 Wiley Periodicals, Inc.

Key words: alcohol; ethanol; Olympus AU5421; Toshiba 200FR

INTRODUCTION

The accurate determination of ethanol concentrations in human blood samples is very important for validating the results of clinical analysis and often legal analysis. First, it is necessary to detect alcohol abuse related syndromes and acute alcoholism (1). Measuring blood ethanol concentration is also important for the police officers to detect impaired driving in Korea (2). In addition to minimizing ethanol degradation in blood samples, the use of sodium fluoride (NaF) and potassium oxalate monohydrate collection tubes has also been recommended by the Clinical and Laboratory Standards Institute (CLSI) guideline T/DM 06-A (3).

In Korea, many hospital laboratories measure blood ethanol concentrations using enzymatic oxidation methods, mainly alcohol dehydrogenase (ADH), with chemistry analyzers that have their own parameters. ADH methods are simple, can be performed without sample preparation, and generally meet all analytical require-

ments (4). In our laboratory, the Abbott AxSYM (Abbott Laboratories, Abbott Park, IL) analyzer with the radiative energy attenuation (REA) reagent has been used for automated blood ethanol measurements since the 1990s. However, since 2012, REA reagents are no longer available due to discontinued production; therefore, a new method of measuring the ethanol concentrations was needed. In this study, we attempted to utilize the Olympus AU5421 (Beckman-Coulter, Fullerton, CA) chemistry analyzer to analyze the ethanol concentrations, as a replacement for the Abbott AxSYM, which is the instrument used currently for performing such measurements. However, the

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parameters needed for measuring the ethanol concentrations using the Olympus AU5421 are still unknown. To address this limitation, we attempted to apply the ethanol parameters of the Toshiba 200FR (Toshiba Medical Systems Co., Tokyo, Japan) chemistry analyzer to the Olympus AU5421. We also evaluated the preservation effect of NaF collection tubes on ethanol concentration as a function of storage time.

MATERIALS AND METHODS

Sample Collection

Forty-two blood samples were obtained from the Chung-Ang University Hospital, Seoul, Korea. All samples were collected from patients in whom it was necessary to measure the blood ethanol concentrations, after using a nonalcoholic antiseptic solution to disinfect the venipuncture site (e.g., povidone iodine). Blood samples were collected in 5.4 mg ethylenediaminetetraacetic acid (EDTA) tubes, and 10 mg NaF and 8 mg potassium oxalate-Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). All samples were immediately centrifuged for 5 min at 3,500 rpm; then, the supernatants were pipetted into polystyrene tubes, capped, and processed. This study protocol was reviewed and approved by the Institutional Review Board of Chung-Ang University Hospital, Seoul, Korea.

Analytical Methods

Blood ethanol measurements were carried out with a chemistry analyzer, Olympus AU5421, using MULTIGENT® Ethanol reagent (Abbott Laboratories). The ethanol assay parameters of the Toshiba 200FR chemistry analyzer (sample amount: 12.0 µl, volume of reagent 1: 100.0 µl, volume of reagent 2: 100.0 µl, detection wavelength: 340 and 410 nm, reaction method: rate method) were applied to the Olympus AU5421 chemistry analyzer in order to evaluate the potential of using this system as an alternative to the Abbott AxSYM analyzer. The MULTIGENT® Ethanol reagent utilizes an enzymatic oxidation method with ADH to determine the blood ethanol concentrations. In the presence of ADH and nicotinamide adenine dinucleotide (NAD), ethanol is readily oxidized to acetaldehyde and NADH. The formation of NADH is measured spectrophotometrically at 340 nm/410 nm. The Abbott AxSYM uses a REA technique, which is an ADH method that is linked to diaphorase (4). Their reaction systems use analyte to convert a chromogen to a chromophore. The light-absorbing properties of the chromophore produced cause a decrease of measured fluorescent light intensity (attenuates radiative energy) (5).

Validation Procedures

Precision

According to the CLSI EP 5-A2 guidelines (6), within-run precision and between-run precision were estimated using low-concentration (50.0 mg/dl ethanol) and high-concentration (300.0 mg/dl ethanol) control materials. Within-run precision was determined by repeating the measurements 20 times at each concentration. To determine between-run precision, both the control samples were analyzed in two runs on the same day over a 20-day period, and duplicate measurements were taken for each run. As indicated in the specifications provided by the manufacturer, precision was considered acceptable if the coefficient of variation (CV) was 5% or less.

Linearity

According to the CLSI EP 6-A guidelines (7), two patient samples with a high blood ethanol concentration (336.6 mg/dl) and a low blood ethanol concentration (5.7 mg/dl) were mixed at ratios of 8:0, 7:1, 3:1, 1:1, 1:3, 1:7, and 0:8, and each sample was measured in duplicate. The results were considered to be linear if a straight line that passes within a predefined allowable total error of each specimen point could be drawn. We defined the allowable total error as 25%, as recommended in the allowable total error database (<http://www.dgrhoads.com/db2004/ae2004.php>).

Accuracy

According to the CLSI EP 15-A2 guidelines (8), three nominal concentrations of certified reference materials (CRMs) for ethanol (40.0, 200.0, and 300.0 mg/dl, respectively) were selected from the National Institute of Standards and Technology. The value of each CRM was measured in duplicate for five runs, and then we calculated the verification interval as described in the EP15-A2 guideline. The results were considered to demonstrate accuracy if the calculated verification interval included the assigned value.

Carry-over rate

Two patient samples with a high and a low blood ethanol concentration were assayed in the order H1, H2, H3, H4, L1, L2, L3, and L4 (H for high and L for low), as described in the CLSI EP 10-A3 guideline (9). The carry-over rate between specimens (%) was calculated as $\{L1 - (L3 + L4)/2\} / \{(H3 + H2)/2 - (L3 + L4)/2\} \times 100$, and was considered acceptable if it was less than 1%.

Method comparison

According to the CLSI EP09-A2 guideline (10), 40 blood samples were measured using the Abbott AxSYM analyzer and Olympus AU5421 analyzer. All samples were analyzed in duplicate. For the first measurement, specimens were arranged in an ascending order, and for the second measurement the samples were placed in a descending order.

Effect of NaF collection tubes on blood ethanol concentration

A total of 20 EDTA and NaF/potassium oxalate blood samples were used. Supernatants of each blood samples were pipetted into seven polystyrene tubes. Among them, one EDTA supernatant and one NaF/potassium oxalate supernatant were immediately analyzed (0 min; baseline). Other remaining six supernatants were stored at -70°C . When we have tried to measuring of ethanol concentration, one EDTA supernatant and one NaF/potassium oxalate supernatant were simultaneously thawed and analyzed at the following times: 30, 60, 90, 120, 150, and 180 min, respectively. The blood ethanol concentration in each supernatant from the EDTA tubes and NaF/potassium oxalate tubes was measured using an Olympus AU5421.

Statistical Analysis

To access precision, linearity, accuracy, carry-over rate, and comparability, EP Evaluator Release 10 (David G. Rhoads Associates, Inc., Kennett Square, PA) was used for statistical analysis. To evaluate the effectiveness of the NaF collection tubes, a Wilcoxon signed-rank test was performed using the IBM SPSS Statistics 19 program for Windows. The statistical significance concentration was set at $P < 0.05$.

RESULTS

Precision

The within-run CVs of blood ethanol concentrations in the low- and high-quality control materials were 0.69 and 0.87%, respectively. The between-run CVs of blood ethanol concentrations in the low- and high-quality control materials were 2.07 and 0.65%, respectively. All measurement values were within acceptable ranges.

Linearity

The linear regression equation obtained from the estimated and actual measurements was $y = 0.98x - 0.159$;

Fig. 1. Linearity of the Olympus AU5421 as determined from duplicate measurement, each with seven equally spaced dilutions Line Fit, linear fit Poly. Fit, polynomial fit.

the coefficient of determination (R^2) was 1.000 (Fig. 1). The deviation percent, which was the difference between the best polynomial fit (third-order equation) and the best linear fit, was less than 25%. Thus, this method was found to be acceptably linear over a concentration range of 5.7–336.6 mg/dl (Table 1).

Accuracy

The assigned value for each CRM was 39.0 mg/dl (nominal concentration of 40.0 mg/dl), 170.1 mg/dl (nominal concentration of 200.0 mg/dl), and 298.0 mg/dl (nominal concentration of 300.0 mg/dl), respectively. The calculated verification intervals of ethanol CRM at each concentration were 38.9–42.1, 166.2–174.9, and 283.2–295.2 mg/dl, respectively. For CRMs with a nominal concentration of 40.0 and 200.0 mg/dl, each assigned value was included in the verification interval. Thus, we demonstrated accuracy at the nominal concentration of 40.0 and 200.0 mg/dl. For CRMs with a nominal concentration of 300.0 mg/dl, the assigned value was not included in the verification interval; and hence, we could not demonstrate accuracy at the nominal concentration of 300.0 mg/dl (Table 2).

Carry-Over Rate

For blood ethanol analytes, the carry-over rate between specimens was 0.002%, which was within the acceptable limits of the Olympus AU5421.

TABLE 1. Statistical Analysis for Linearity Evaluation With Olympus AU5421

Specimen	Assigned value (mg/dl)	Mean (mg/dl)	Polynomial fit	Linear fit	Deviation percent (%)
1	5.7	5.3	5.3	5.4	-2.9
2	42.7	50.3	50.4	44.7	12.7
3	85.6	89.6	95.1	90.2	5.4
4	171.2	174.0	170.2	181.1	-6.0
5	256.7	237.2	244.8	271.9	-9.9
6	299.5	289.0	288.9	317.3	-9.0
7	336.6	325.5	333.5	356.7	-6.5

TABLE 2. Statistical Analysis for Accuracy Evaluation With Olympus AU5421

CRMs	Nominal concentration (mg/dl)	Assigned value (mg/dl)	Calculated verification interval (mg/dl)	Demonstration status of accuracy
CRM 1	40.0	39.0	38.9–42.1	Demonstrated
CRM 2	200.0	170.1	166.2–174.9	Demonstrated
CRM 3	300.0	298.0	283.2–295.2	Not demonstrated

CRM, certified reference material.

Method Comparison

A good correlation was observed between the results obtained using the Abbot AxSYM and Olympus AU5421, where the slope, intercept, and correlation coefficient (R) was 1.031, -0.639 ($y = 1.031x - 0.639$), and 0.999, respectively (Fig. 2). At the first medical decision point of 50.0 mg/dl, the 95% confidence interval for the predicted bias range (49.9–51.9 mg/dl) included the defined acceptable bias range (50.9 mg/dl). At the second medical decision point of 200.0 mg/dl, the 95% confidence interval for the predicted bias range (195.8–206.7 mg/dl) included the defined acceptable bias range (205.5 mg/dl). Thus, the bias of this method was not different from the acceptable bias.

Effect of NaF Collection Tubes on Blood Ethanol Concentration

For blood samples that were stored in EDTA collection tubes, the concentrations at baseline, 30, 60, 90, 120, 150, and 180 min, were determined to be 105.5, 106.5, 106.3, 106.3, 106.0, and 106.3 mg/dl, respectively. For blood samples that were stored in NaF collection tubes, the ethanol concentrations at baseline, 30, 60, 90, 120, 150, and 180 min, were found to be 106.9, 106.9, 106.5, 106.5, 106.3, 105.5, and 106.2 mg/dl, respectively. There was no statistically significant difference in blood ethanol concentrations between the samples collected in EDTA tubes and those collected in NaF tubes irrespective of the time point (Table 3).

DISCUSSION

Ethanol is the most widely used chemical substance. Consequently, ethanol measurements are one of the most frequently performed tests in the field of toxicology (11). Accurate measurements of blood ethanol concentrations are very important not only for medical diagnosis but also for an unbiased enforcement of the law. In the present study, we evaluated the potential of using the Olympus AU5421 system to determine the blood ethanol concentration. Since the parameters for determining ethanol concentrations using the AU5421 system are unknown, we applied the known ethanol parameters for the Toshiba 200FR chemistry analyzer. The Olympus AU5421 showed excellent precision and linearity, a low carry-over rate, and was well correlated with the Abbott AxSYM analyzer that is routinely used in our laboratory.

Previous studies have shown that the enzymatic oxidation method is not accurate (4, 12). According to one study carried out in Belgium, the CVs of different analyzers using the enzymatic oxidation method ranged from 2.2 to 2.6% (4). In addition, the CVs of two analyzers using the REA technique were reported to be higher (4.3 and 5.5%, respectively) (4). In this study, however, the total CVs for low- and high-quality control materials were 1.38 and 0.76%, which were better than the CVs obtained using the method described in the study conducted in Belgium.

The principal pharmacologic action of ethanol is to cause depression of the central nervous system (CNS). The effects of ethanol on the CNS vary depending on the blood ethanol concentration. Euphoria begins to appear when the blood ethanol concentration reaches 50.0 mg/dl. At an ethanol concentration of 200.0 mg/dl, mental changes will start to develop; and when the ethanol concentration is above 300.0 mg/dl, death can occur due to various causes, such as suboptimal body temperature and respiratory arrest (11). Blood samples in which the ethanol concentration is higher than 300.0 mg/dl are rarely encountered in the clinical laboratory, and hence samples with higher ethanol concentrations could not be obtained for analysis. Moreover, the medical significance

Fig. 2. Comparability between the Abbott AxSYM and Olympus AU5421 for measurement of blood ethanol levels. Blood ethanol levels were determined by analysis of duplicate samples, first in ascending order, and then in descending order. (A) Scatter plot of individual results and (B) bias plot. Deming Regr, Deming regression Med Dec Pt, medical decision point.

at ethanol concentrations higher than 300.0 mg/dl is negligible. For these reasons, the linearity of the Olympus AU5421 was validated from 5.7 to 336.6 mg/dl, and excellent linearity was observed over this range. We should extend analytical measurable range if blood samples whose ethanol concentrations higher than 300.0 mg/dl were obtained.

The Olympus AU5421 was also shown to be highly correlated with the Abbott AxSYM in terms of blood ethanol analysis at the blood ethanol concentrations of 50.0 and 200.0 mg/dl. In Korea, the maximal acceptable blood alcohol concentration for driving is 50.0 mg/dl; and at a blood ethanol concentration above 200.0 mg/dl, drivers receive maximum legal punishment. Moreover, ethanol-related clinical symptoms begin to appear when the blood alcohol concentration is greater than 50.0 mg/dl, and the symptoms become obvious at concentrations higher than 200.0 mg/dl (11). Therefore, we used the medicolegal decision point of 50.0 and 200.0 mg/dl. Previous studies have shown that the Abbott AxSYM correlates well with head-space gas chromatography, which is the gold standard method for blood ethanol measurement (12, 13). Therefore, it can be assumed that the Olympus AU5421 will be sufficiently accurate to measure the blood alcohol concentrations. To further evaluate the accuracy of this novel approach, we tried to demonstrate accuracy through the recovery of expected values from CRMs with assigned values. At the nominal concentration of 40.0 and 200.0 mg/dl, we confirmed the accuracy of our method in measuring blood ethanol concentrations. Since the accuracy was demonstrated at the medicolegal decision concentrations, we conclude that our method demonstrated a clinically and legally acceptable accuracy. To confirm the accuracy of our method at an ethanol concentration of 300.0 mg/dl, a direct comparison between the Olympus AU5421 and head-space gas chromatography method will be needed in the near future.

Furthermore, according to the CLSI guideline T/DM 06-A for measuring the blood ethanol concentration, the type and amount of anticoagulant present is not important if the specimen is analyzed within 4 h of collection. However, if the analysis is delayed, NaF and potassium oxalate have been recommended as the appropriate

TABLE 3. Comparison of the Mean Differences in Blood Ethanol Levels After Collection With the EDTA Tube and NaF Tube (mg/dl)

Tubes	Measured time points (min)						
	Baseline (0)	30	60	90	120	150	180
EDTA	105.5	106.5	106.3	106.3	106.3	106.0	106.3
NaF	106.9	106.9	106.5	106.5	106.3	105.5	106.2
P value	0.740	0.629	0.614	0.611	0.263	0.629	0.503

anticoagulants (3). In our laboratory, EDTA is the anticoagulant that is used for blood ethanol analysis, and analyses are started as soon as the specimens arrive at the laboratory; typically, this is within 15–20 min after blood collection. In the present study, we compared the preservation effect between EDTA and NaF, and found no significant difference in the ethanol concentration after 3 h of storage. These results are similar to those described in the CLSI guideline (3). For this reason, our hospital laboratory has continued to use EDTA collection tubes for blood ethanol measurements.

In conclusion, the analytical performance of the Olympus AU5421 was excellent when the parameters that are already established for the Toshiba 200FR chemistry analyzer for blood ethanol analytes were used. Therefore, we believe that the Olympus AU5421 can be reliably used in the clinical laboratory for the determination of blood ethanol concentrations, which are stable for up to 3 h irrespective of whether or not the specimens are collected in a NaF tube. We believe the results of this study will be valuable to other researchers who are attempting to measure an analyte using an analyzer for which the analyte parameters are unknown.

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