

**DETECTION AND SCREENING
PROCEDURES (ALCOHOL)**

Biochemical Markers as Measures of Return to Drinking

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Keywords

Biomarkers, biochemical tests, laboratory tests, relapse, carbohydrate deficient transferrin

Abstract

This article discusses the role of serum-based laboratory tests in identifying relapse to drinking by alcoholic patients. Particular emphasis is given to carbohydrate deficient transferrin (CDT), a marker of heavy drinking recently approved by the US Food and Drug Administration. After summarizing research on its sensitivity and specificity, several generalizations are offered about its performance and the benefits of its use in conjunction with gamma glutamyl transpeptidase, a commonly employed liver function test. The article concludes by suggesting fruitful directions for future research on biomarkers as aids in identifying return to drinking.

Introduction

Alcoholism has been termed a “chronic relapsing condition” (1). Although treatment for it tends to be at least moderately successful, periodic return to drinking, especially early in the recovery process, is quite common, with perhaps 2/3 of the patients engaging in at least some drinking in the first year (2). From the standpoint of minimizing harm to the patient as well as promoting public health and traffic safety it is important that these events be recognized as soon as possible so that treatment can be intensified or refocused to restore abstinence and help the patient bolster skills to cope with high-risk drinking stimuli. If drinking episodes can be detected quickly, there is also probably less risk of them becoming intractable relapses.

Although self-report measures are often employed to determine drinking status, several studies demonstrate that at least some alcoholics in treatment who have been drinking deny it (3). Hence, objective measures are needed to identify return to drinking. In most instances the measures that have been investigated are biochemical tests. Often these tests are employed as well to initially screen for alcohol problems. Nevertheless, biomarkers of recurrence of drinking must be especially responsive to recent changes in drinking in order to not miscategorize patients early in treatment who are now abstinent. Biomarkers, such as macrocytic volume, must be used with care since they return to reference range values only slowly after drinking has ceased.

Recent Studies on Biomarkers of Relapse to Drinking

Apparently without exception, studies undertaken on biomarkers of relapse over the last several years have included CDT, often with GGT also being considered. Sensitivities and specificities reported by these projects are given in Table 1 (3).

Table 1: Sensitivity (Sens.) and Specificity (Spec.) for CDT, GGT, and Their Combination as Markers of Relapse to Drinking

<u>Study</u>	<u>Sample Size</u>	<u>CDT Sens.</u>	<u>GGT Sens.</u>	<u>CDT Spec.</u>	<u>GGT Spec.</u>	<u>CDT/ GGT Sens.</u>	<u>CDT/ GGT Spec.</u>
(4)-Males Only	905	.56	.53	.81	.74	.77	.65
(4)-Females Only	310	.20	.31	.81	.91	.48	.73
(5)	97	.92		.98			
(6)	144	.55	.50	.97	.93	.80	.90
(7)	58	.43	.57	.88	.63	.70	.57
(8)	57	.74	.66	1.00	.78		
(9)	53	.90	.59	.60	.73		
(10)	101	.33	.34	.96	.77	.51	.79
(11)	86	.76	.79				

Although the formal criterion for relapse in these studies has usually been return to any level of drinking, the actual pattern of consumption by subjects tended to be fairly heavy and sustained. It should be further noted that, with the exception of the study by Allen et al (4) on the Project MATCH data set, all of the investigations were conducted on male-only or almost entirely male samples. Thus, generalizations are, at best, tentative as they would pertain to women.

Across studies for males only or males and females combined the median sensitivity for CDT was .65 and for GGT was .57, with respective specificities of .92 and .78. As indicated by Table 1, four of these studies (4,6,7,10) additionally computed the sensitivity and specificity for the combination of CDT and GGT using the “simple binary rule” (i.e. if elevated on either of the markers, the patient would be labeled as positive). The median sensitivity and specificity for the combination were each .74. Thus, in these projects the gain in sensitivity by adding GGT over CDT alone was .23 with a corresponding loss in specificity of .16. On the other hand, the gain in sensitivity by adding CDT to GGT was .20 with a decrease in specificity of only .04. (Due to the high specificity of CDT, including it with GGT resulted in minimal negative impact.) In the Project MATCH study (4) neither test appeared to do as well with women as with men. Curiously, GGT may actually perform at least marginally better with women than does CDT. The lower performance of both markers among women may be a function of the fact that women who relapsed in MATCH trial consumed only around half the amount of alcohol as did the men who relapsed (4).

Relapse duration appeared slightly related to the likelihood of the markers being above cutoff (4), although for women, only GGT demonstrated this phenomenon in the MATCH sample. Granted their sample was quite small, Rosman et al. (11) failed to detect this effect in male alcoholics at the CDT kit manufacturer’s recommended screening cutoff value of 20mg/l. However, they did find that use of a cutoff of 25 mg/l would have resulted in about half of the minor relapses being missed, while still allowing recognition of the more serious ones. Both markers have also been found to be related to the seriousness of consequences of relapse (9). The association was only slightly less than when drinking itself served as the criterion of relapse.

Importantly, a “heralding” effect has been observed for CDT with relapses often being identifiable by elevation of CDT elevation well in advance of patient self-admission (11). Forty-two percent of the relapses in this study were detected by elevated CDT at least 28 days before the patient’s acknowledgement of drinking. Finally, two of the studies noted that CDT responded to return to drinking more rapidly than did GGT (9,10).

Future Research Directions

Although biomarkers have demonstrated considerable value in identifying relapse in alcohol dependent patients receiving abstinence-oriented treatment, further research is needed on a variety of topics:

1. The capability of the markers to recognize reductions in drinking short of total abstinence remains largely unexplored. One important project in this regard (12), however, found that males accustomed to drinking between 20 to 60 g/day of alcohol who reduced their daily consumption for a month by at least two drinks demonstrated reductions in CDT and GGT of at least 10%. Respective sensitivities for CDT and GGT in indicating this decrease in drinking were .70 and .68, respectively, but the specificities of the two markers differed dramatically, with that of CDT being .80 and of GGT being 0. The markers in combination produced a sensitivity of .96. Studies dealing with this issue should consider lifetime history of alcohol dependence and smoking since a large scale trial by Whitfield et al (13) showed that these two factors mediate the relationship between level of consumption and CDT response.
2. As noted, almost all research to date on biomarkers as indicators of return to drinking has involved samples consisting entirely of males or including such a small number of females that it was impossible to disaggregate possible gender effects. Granted results from Project MATCH cited above (4), the response of CDT, GGT and their combination may well differ between the two sexes. Analyses of a subsample from that study, for example, suggested that for men CDT responded primarily to frequency of drinking and GGT to intensity of drinking; whereas for women both markers responded primarily to intensity of drinking rather than to its frequency (14).
3. Research directly contrasting utility of scoring markers in an ipsative versus normative manner (i.e. within-subject change scores versus evaluating patient drinking status based on a common absolute value) is badly needed. This issue remains hotly contested in the field but could be resolved by projects reporting sensitivities and specificities derived from the two different scoring approaches.
4. Development of algorithms to optimally combine scores from various biomarkers as well as from other indicants of drinking status, such as observational and self-report measures would also be most valuable. Although a few examples have been found in the screening literature of formulas to combine biomarkers in ways other than use of the simple binary inclusion rule, no similar research seems to exist for biomarkers of return to drinking. One would wonder, for example if the drinking status is the same for patients low on all markers as for those with values just slightly below the cutoff on several markers.

5. Investigations are also needed on highly applied topics such as optimal scheduling of follow-up biomarker testing, possible reactive effects of biomarker testing on drinking behavior, and sequencing of biomarker tests.
6. A “gold standard” for recent drinking is needed as a criterion for determining the predictive validity of biomarker as well as other measures that may reflect drinking. Research on this topic has generally been limited to self-report criterion measures. Albeit these measures have often been collected in a more controlled context with special emphasis on getting accurate consumption information (e.g. assurance of confidentiality, intensive questioning, conferral with collaterals, financial incentives for participation in a research protocol, efforts to assure rapport with the interviewer, and use of short-half life, but direct physiological measures, such as 5 hydroxytryptophol and breath analysis) than would typically be possible in a general clinical practice, nevertheless, a reasonably long term objective measure of drinking is needed. With further engineering, the transdermal alcohol sensor (15) may satisfy this critical need or, in a driving context, the interlock device (16) may do so.
7. Although GGT has been most often combined with CDT as a measure of relapse, preliminary studies suggest that aspartate aminotransferase or beta-hexosaminidase might perform at least as well with CDT (17). Research is needed to further examine these alternative combinations as well as to develop new, accurate markers that may prove even more useful independently as well as in combination.
8. Finally, and most importantly, research is needed to develop response curves to drinking patterns for various biomarkers.

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The EDAC Test: A New Biomarker to Monitor Alcohol Abstinence and Relapses

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Keywords

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Abstract

An advanced approach to enhance effective treatment of alcohol dependent patients is to objectively monitor drinking behavior by the use of alcohol abuse biomarkers. One of these biomarkers is the Early Detection of Alcohol Consumption or EDAC test. The EDAC test consists in combining the results of a panel of automated routine blood tests to predict whether an individual is a heavy drinker or a non-heavy drinker. This article describes the diagnostic performance of the EDAC test when used for two applications, 1) to identify alcohol consumption in heavy drinkers and 2) to monitor abstinence and relapses in outpatients.

Introduction

In recent years there has been great progress in the reduction of the rate of drunk driving in the U.S. However, according to a preliminary report of crash data released last month by the U.S. Department of Transportation (DOT), the percentage of traffic deaths that were alcohol-related in 2001 remained unchanged at 40 percent - 16,652 deaths – only one less death than in 2000 (1). The study estimated the number of total highway deaths at 41,730 in 2001, compared to 41,821 in 2000. The number of injuries dropped from 3.2 million in 2000 to 3.0 million in 2001. The death rate per 100 million vehicle miles remained statistically the same: 1.50 in 2001, as compared to the 2000 rate of 1.52.

In addition, preventing alcohol drinking by the use of the drug Disulfiram (Antabuse) has proven challenging due to liability issues. The significant increase in health care costs has resulted in fewer patients being hospitalized for inpatient treatment. A new approach to enhance effective treatment of alcohol dependent patients is to monitor drinking behavior by the use of alcohol abuse biomarkers. One of these biomarkers is the Early Detection of Alcohol Consumption or EDAC test. The EDAC test combines the results of a panel of automated routine blood tests using linear discriminant function (LDF) analysis. LDF analyses the relationship of the routine tests to each other providing a type of biochemical fingerprint. These interrelationships to one another provide a unique statistical profile to identify subjects as heavy drinkers or non-heavy drinkers. This article describes the use of the EDAC in various populations with diverse drinking behaviors (2-5).

Methods

The EDAC is a method of interpreting routine blood profiles to identify individuals who routinely consume large volumes of alcohol. The drinking behavior identified by the EDAC can be either binge drinking or a more steady daily consumption of smaller amounts of alcohol. Most males will be detected at a threshold of 45 gm. of absolute alcohol (4 standard drinks) per day and females at levels above 35 gm. (3 standard drinks) per day. The EDAC uses Linear Discriminant Function (LDF) analysis to combine the results of a routine test panel. The EDAC is calculated using a panel of 12 to 36 routine laboratory tests. The 12 most relevant routine tests used to calculate the EDAC are: sodium, potassium, chloride, total bilirubin, direct bilirubin, aspartate aminotransferase, gamma glutamyltransferase, HDL cholesterol, mean corpuscular volume, platelets, white blood cells and monocytes.

The EDAC reports three pieces of information:

1. Class prediction where a prediction of class 1 is indicative of relapse and/or heavy drinking and a prediction of class 2 is indicative of abstinence or light drinking.
2. The Probability that an individual's profile is that of a heavy drinker or P-Positive
3. The Probability that an individual's profile is that of a light drinker or P-Negative

The Probability that an individual's profile is that of a heavy drinker (P-Positive) is the degree to which the blood profile of the individual being assessed resembles that of other heavy drinkers. Conversely, The Probability that an individual's profile is that of a light drinker (P-Negative) is the degree to which the individual being assessed does not match that of other heavy drinkers. Generally the higher the individuals P-Positive, the greater the risk of alcohol related complications. The sum of P-Positive and P-Negative always adds up to 1.

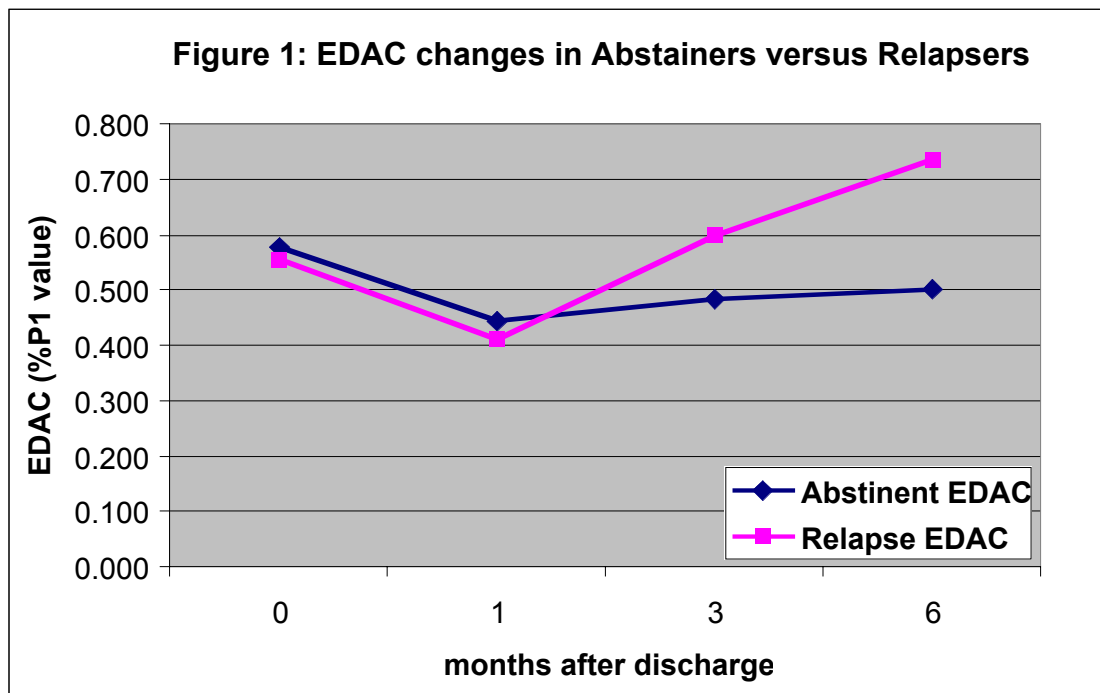
Results

The largest study using the EDAC to identify heavy drinking was done in 807 subjects recruited from 25 different sites in the U.S. (2). Heavy drinking was defined as consuming an average of ≥ 4 standard drinks daily if male and ≥ 3 standard drinks daily if female, the month prior to sample collection. In this population the EDAC sensitivity rates varied from 80% to 87% depending on the level of alcohol consumption, gender and age of the subjects tested. Older chronic drinking males were detected with the highest sensitivity rate (87%) whereas young females were detected with the lowest sensitivity rate (80%). Specificity rates for the corresponding non-abusers groups were 94% for the older males and 87% for young females.

A related study (3) evaluated the performance of the EDAC score in the identification of *at-risk* drinking in young males (mean age = 26.5 years) and young females (mean age = 24.4 years) requesting medical care at facilities in a university setting in the MidWest. At-risk drinking was defined as males drinking at least 14 drinks per week or drinking more than 4 drinks on any occasion in the last 14 days and females drinking at least 7 drinks per week or more than 3 drinks on any occasion in the last 14 days. The EDAC test showed 42% sensitivity and 90% specificity rates in identifying *at-risk* drinking in young females and 30% sensitivity and 96% specificity rates when identifying *at-risk* drinking in young males. In females, the EDAC's sensitivity was higher than traditional laboratory markers previously reported for diagnosis of alcohol abuse such as GGT (6%), MCV (13%) or the combination of GGT, MCV, AST or ALT (14%). In males, sensitivities for GGT, MCV or the combined liver enzyme tests were 8%, 3% and 14%,

respectively. This study shows that as a complement or a substitute to an interview, in subjects who are less candid about their drinking, the EDAC may assist in the assessment of at-risk drinking in young adults, particularly in females

The performance of the EDAC has also been tested in a follow up study (supported by NIAAA grant 1R43AA12366) that monitored abstinence and relapses in 28 males and 21 females for 6 months after discharge from residential treatment (5). Average age was 41 ± 10 years and average length of stay was 56 ± 24 days of residential treatment. A prediction of class 1 or a 30% increase of the probability of heavy drinking (P-Positive or P1 value) from discharge value was indicative of relapse. A cross-sectional analysis at 6 months post-discharge showed that the mean value for the probability of heavy drinking increased by 47% in the relapse group compared to the abstinent group (Figure 1).



Relapsers	1 month	3 months	6 months
Average Drinks	28.22	48.15	184.15

Finally, a case study of a frequent DUI offender with 5 traffic violations is presented to illustrate the application of the EDAC test in clinical practice. This individual denies drinking and his counselor decides to use the EDAC test to support self-report. The counselor requests a panel of 12 laboratory tests and the EDAC is performed with the results of the tests panel. The EDAC test shows a prediction of class 1 and a P-Positive value of 65%; the counselor can now objectively confirm heavy drinking in this frequent DUI offender.

Discussion

The EDAC test is currently being used in the MidWest, a geographic region of the U.S. with a high prevalence of alcohol abuse. Indeed, a big proportion of college students, adults and the elderly regularly consume 6 or more drinks per drinking episode an average of three times a week leading to an elevated EDAC test. When the EDAC test indicates alcohol abuse but the patient reports alcohol abstinence, the medical professional repeats the laboratory tests in a new blood sample after a couple of weeks. Confrontation of the patient by the medical professional usually induces the patient to stop drinking reflected by a subsequent decrease in the probability value (P-Positive) of the second EDAC test. Thus, the EDAC test is a useful tool to support self-report and monitor drinking patterns in DUI offenders and patients in alcohol treatment.

The combined results of the above mentioned studies show that the EDAC test contributes to the patient's treatment program by preventing relapses, enhancing positive outcomes, improving the overall level of care and increasing public safety.

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The Effect of Heat on Blood Samples Containing Alcohol

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Keywords

Alcohol, Blood, Heat

Abstract

In a pilot study the effect of heat on blood samples with and without alcohol present was examined. Blood drawn from donors was exposed to temperatures that ranged from 17 degrees C to 46.8 degrees C over a 78-day period. Temperatures were recorded every 5 minutes for the first 30 days. Tubes were retrieved periodically and the alcohol concentration was determined. The starting alcohol concentrations ranged from 0.062 gm/100ml to 0.123 gm/100ml. An average decrease in the alcohol concentration of 0.018 gm/100ml was observed to occur during the initial 3 days of exposure. After the initial decrease there was minimal decrease. No alcohol was detected in any of the samples that were taken from alcohol free donors. Samples exposed to rather harsh conditions, i.e. high heat, will not cause an elevated alcohol concentration. The loss of alcohol during the first 3 days while unrefrigerated suggests that it would be prudent to refrigerate samples whenever possible.

Introduction

Frequently blood samples taken from Driving While Impaired arrestees are left in patrol vehicles or unrefrigerated storage cabinets for varying lengths of time. Some samples are sent to state laboratories through the postal service. While typically the tubes contain both an anticoagulant and a preservative, the fact that they were not refrigerated becomes a defense issue at trial. There have been some studies conducted by leaving tubes containing blood and alcohol at non-refrigerated temperatures for varying lengths of time in the laboratory (1-9). Nearly all of these studies have relied on either the use of blood from a blood bank or the use of samples submitted for analysis that were obtained from arrestees and for which there is no record of storage conditions. There have been no studies where samples were controlled from the moment of collection until analysis and after being exposed to "real world" conditions.

Methods

Treatment of subjects

Volunteers were given an overview of the study and completed informed consent documents prior to participating. The subjects were dosed with beverage alcohol of their choice. There were 4 adult males dosed with alcohol and 1 male and 1 female non-dosed donors. The target

alcohol concentration was 0.08 gm/100ml of blood. Alcohol breath tests were conducted prior to blood collection and after blood collection.

Sampling of blood

Ten tubes of blood were collected using 7-ml Vacutainer® tubes containing EDTA and Sodium Fluoride. The Collection was made with a Vacutainer® blood collection set via the antecubital vein. All tubes were randomly numbered prior to blood collection and then assigned to a specific subject by letter designation, i.e. A, B, C, D, E, and F by placing a piece of tape with the group designation over the unique number. This was to ensure that the analysts would not know the contents of the tubes as the experiment progressed.

Exposure to heat

Tubes were well mixed after blood collection and either put in a box marked “Trunk” (4 tubes per subject) or “Passenger” (4 tubes per subject) or placed in the refrigerator (2 tubes per subject). Temperature recording devices (Temp100® Ever Ready Thermometer Company, West Patterson N.J.) previously programmed to record the temperature every 5 minutes, were placed in the boxes with the blood samples. The boxes were left at room temperature for 18 hours and then placed in an on duty patrol car. One box was placed in the passenger compartment and one was placed in the trunk. An additional temperature-recording device was mounted under the bumper of the car to record the ambient temperature. The patrol car was used in normal patrol functions including day and night shifts and was not housed in a garage during off-hours. One tube for each subject was retrieved from the boxes at the end of day 3, day 7, day 30 and day 78. The monitoring of the temperature was discontinued at the end of day 30.

The tubes, which were immediately refrigerated after collection, were designated as “Control 0 Days” and “Control 30 Days.” The “Control 0 Days” tubes were analyzed for alcohol concentration the day after collection. The “Control 30 Days” tubes were maintained at 4 degrees C for 30 days and then were analyzed for alcohol concentration.

Blood-alcohol analysis

A Varian 3800 gas chromatograph was used for all blood alcohol measurements. The chromatograph is equipped with dual columns (Restek BAC1 and BAC2) which are manufactured specifically for blood alcohol analyses. An internal standard solution of n-propanol (0.050 gm /100 ml water) was utilized in all runs. The Varian 3800 gas chromatograph was calibrated each time a new internal standard was prepared. The following procedure was used for the calibration: with a Hamilton Microlab 1000 Plus liquid handler, 0.2 ml of each of the calibrator solutions (0.080, 0.200, and 0.500 g/100 ml) and 1.80 ml of the internal standard solution were delivered into separate headspace vials. Each calibrator was prepared in quadruplicate. The calibration standards were chromatographed on the Varian 3800 gas chromatograph to calibrate.

After calibration, duplicate 0.100 g/100 ml verification standards, followed by a single water blank, and duplicate commercial (obtained from Cerilliant Corporation) verification standards, followed by a single water blank were prepared in the same manner that the calibration standards were prepared. These verification standards were chromatographed to check the system for resolution and precision. Any measured component values exhibiting greater than 5% difference

from targeted values were rejected for that component. This step was performed before analysis on each day of analysis.

To prepare the blood samples the following procedure was used: with a Hamilton Microlab 1000 Plus liquid handler, 1.80 ml of internal standard and 0.200 ml of blood were delivered into a headspace vial. Each blood sample was prepared in duplicate. Each blood sample was chromatographed on each column. The results of the 4 analyses of each blood sample were averaged and reported as gm/100ml.

Results

The temperature of the samples was recorded every 5 minutes for 30 days. This resulted in nearly 9,000 temperature measurements being recorded on each recording device. A comparison of the ambient temperature, passenger temperature and trunk temperature (data not presented due to space limitations) showed an expected relationship. The ambient temperatures generally had higher maximums and more rapid changes. The maximum ambient temperature was 46.75° C while the minimum was 17.25° C. The passenger area experienced lower temperatures during vehicle operation (due to air conditioning). The temperature of the trunk area tended to change slower than the passenger area and had an overall narrower range. The length of exposure at various temperature ranges was determined by sorting the captured data on a spreadsheet and then counting the number of recordings within a range and calculating the total time of exposure. That data is presented in Table 1.

Table 1: Hours of exposure at temperature ranges

Temperature Range (Degrees C)	15°-20°	20°-25°	25°-30°	30°-35°	35°-40°	40°-45°	45°-50°
Trunk 3 Days	0 hours	18 hours	20 hours	25 hours	24 hours	4.5 hours	0 hours
Trunk 7 Days	0 hours	37 hours	82 hours	33 hours	31 hours	4.5 hours	0 hours
Trunk 30 Days	0 hours	164 hours	329 hours	175 hours	64 hours	4.5 hours	0 hours
Passenger 3 Days	0 hours	30 hours	29 hours	22 hours	11 hours	0 hours	0 hours
Passenger 7 Days	0 hours	50 hours	62 hours	48 hours	26 hours	2 hours	0 hours
Passenger 30 Days	21 hours	219 hours	265 hours	134 hours	83 hours	11 hours	3 hours

There was an obvious decrease in the alcohol concentration during the first 72 hours of exposure. This was followed by a continuous though slight decrease over the next 75 days. The concentration of the alcohol in the blood samples after various exposures is presented in Table 2 (Passenger area) and Table 3 (Trunk).

Table 2: Alcohol concentration of blood samples stored in the passenger area (gm/100ml)

	Control 0 days	Control 30 days	3 days	7 days	30 days	78 days
Subject	BAC	BAC	BAC	BAC	BAC	BAC
A	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.123	0.123	0.113	0.113	0.107	0.108
D	0.077	0.079	0.070	0.066	0.064	0.064
E	0.079	0.077	0.069	0.067	0.066	0.066
F	0.062	0.060	0.051	0.048	0.047	0.045

Table 3: Alcohol concentration of blood samples stored in the trunk (gm/100ml)

	Control 0 days	Control 30 days	3 days	7 days	30 days	78 days
Subject	BAC	BAC	BAC	BAC	BAC	BAC
A	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.123	0.123	0.115	0.113	0.113	0.108
D	0.077	0.079	0.065	0.064	0.063	0.062
E	0.079	0.077	0.066	0.067	0.066	0.064
F	0.062	0.060	0.047	0.044	0.044	0.042

Discussion

The effect of heat on blood samples containing alcohol continues to be an issue raised in the courtroom. This is in part due to the fact that samples continue to be left both accidentally and intentionally unrefrigerated. This becomes an issue raised by the defense because the situation presents unknowns. How long? How hot? Since those are unknowns their effect on the sample is unknown and therefore could call into question the reliability of the reported analytical result. Previous studies (1-9) have addressed the issue to some extent. Only one study (2) controlled the exposure of the blood from the moment of the blood draw, but the temperature exposure was limited to room temperature (22° C - 29° C) and for a limited time – 14 days. While the effect of sample storage in a vehicle has been examined (9) the samples were of an unknown quality and the actual exposure was not recorded.

The observed decrease in the loss of alcohol between the Day 7 and Day 30 time points, along with the risk of losing captured temperature data, prompted the collection of that data and the discontinuation of temperature capture at Day 30. The initial target for exposure was 30 days. Spare vials of blood were placed in the boxes in case breakage or leakage occurred prior to day 30. At day 30 no vials had been lost due to breakage or leakage so the exposure was extended an additional 48 days on the remaining vials.

It is not felt that the loss of alcohol observed was the result of a pressure event since the repeated heating and cooling during the exposure should have effectively “pumped” the alcohol out over the course of the experiment. The decrease is most likely attributable to a chemical event (oxidation by oxyhaemoglobin (7)) stimulated by the elevated temperatures since no significant change was reported in controlled samples left at room temperature for 14 days (2).

This study is an ongoing effort. We will be further examining a number of unresolved facets. We hope to determine if there is a critical temperature that triggers the alcohol loss and whether samples collected and immediately refrigerated will behave as “fresh” samples when heated. If cold storage doesn’t change the characteristics of the samples it would be easier to conduct larger scaled studies which could then be examined statistically. Finally, since in many jurisdictions samples are sent by postal service, the temperatures experienced under those conditions will be examined.

In this study we controlled the samples from the blood draw to the analysis and were able to record the temperature of exposure, both in duration and in range. The lack of alcohol gain in all of the samples and particularly in those from alcohol free subjects once again dispels a popular defense myth. A comparison of the Day 0 Control and Day 30 Control values show the stability of the samples when stored under refrigeration. The more interesting observation was the decrease in concentration that occurred during the initial 72 hours. While storage without refrigeration may not effect the alcohol concentration, the limitation of this effect is contingent upon the temperature of exposure. Because of this uncertainty it is our conclusion that the continuous storage of a sample under refrigerated conditions is recommended and prudent.

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Breath Alcohol Concentration and Breath Temperature

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Abstract

Breath alcohol concentration, breath temperature, and body temperature are used to extrapolate the breath alcohol concentration to the concentration of alcohol in the deep lung air, which is in equilibrium with the pulmonary blood.

Introduction

The variability that is seen in the breath alcohol test (note: “alcohol” means ethyl alcohol) has always been more or less ignored as inevitable “biological variability”. It has been known from the beginning of the use of breath test for numerical evidence of drunk driving that breath temperature plays a part in the test result.

With the intention of reducing breath test variability, authorities in Germany and Alabama have recently begun extrapolation of the test result from the measured breath temperature to a standard breath temperature, 34°C. The procedure is described by Schoknect and Stock (1) and is based on Henry’s Law.

Extrapolation to a standard temperature does not take into account pulmonary blood temperature, which varies from person to person and with time of day and with it, breath alcohol concentration. Therefore, extrapolation to body temperature, the procedure described below, should further reduce the variability of the breath test as well as to make the BrAC more equitable with the BAC. The procedure assumes equilibrium between pulmonary blood and breath in the deep lung region; but in the upper lung region only ‘near equilibrium’ is assumed between the breath and the water in the mucous surface that lines the airways.

Recent Work

In recent years, several workers have investigated the process leading to a breath alcohol measurement. Lubkin et al (2) have developed a model of the breath alcohol profile, but it does not have a temperature component. Curiously, Lefranc and Montamat (3) concluded that the breath test is not influenced by breath temperature, although they did not measure it. They noticed the effect of hyperventilation on breath alcohol but ascribed it to “a temporary thinning of the arterial blood in the alveoli”. Tsu et al (4) have developed a detailed model of the interaction of alcohol with the lung surfaces that has been tested by George et al (5). It predicts alcohol profiles, but is too complex for police use.

Methods

The inlet end of the breath hose of a National Patent Analytical Systems, Inc. Datamaster infrared type police evidential breath alcohol tester was fitted with a thermostated (at 34°C) fast response gas thermistor temperature probe assembly. The thermistor was calibrated by passing air saturated at 37°C through a 10 foot long 3/8 inch diameter copper tube coiled into a water bath thermostated at temperatures from 30°C to 36°C. Breath alcohol concentration calibration data was obtained immediately before and after each human subject test run using a Repco Marketing simulator operating at 34°C to provide reference alcohol-in-air samples. The flow thermistor was calibrated by use of a Warren Collins, Inc. spirometer connected to the Datamaster sample vents. Compressed air saturated at 34°C was used for this calibration. Oral temperatures to the nearest tenth of a degree Fahrenheit using a digital oral thermometer were obtained from each of 30 drinking human subjects just before being tested with the apparatus. All temperature sensors used were checked with a NIST traceable digital thermometer. The output of the breath temperature thermistor, and the alcohol and flow outputs of the Datamaster, were fed to a data logger, and then to a computer for spreadsheet analysis. The breath temperature probe is shown in Figure 1.

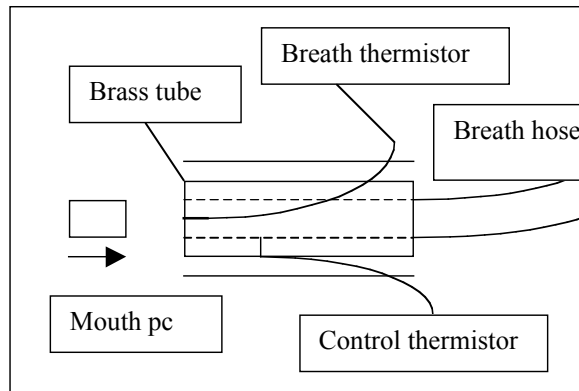


Figure 1. Breath temperature probe

Four types of breath sample were obtained from each subject in the following order:

- A normal police type sample where the subject takes a deep breath before blowing fully into the Datamaster.
- A hyperventilated sample where the subject inhales deeply then fully exhales. This is repeated ten times before finally inhaling and blowing into the Datamaster as above. The purpose of this maneuver is to aggressively cool the airway surfaces of the lung.
- A hypoventilated sample where the subject takes a deep breath and holds it for 20 seconds before blowing into the Datamaster. The purpose of this maneuver is to allow the airway surfaces to warm.
- A re-breathed sample where the subject inhales deeply, then blows his breath fully into an unheated 4 liter plastic bag, then re-inhales the same air from the bag, all the while keeping his nostrils pinched shut to prevent fresh air from entering his lungs. After the 7th inhale, the subject blows into the Datamaster. The purpose of this maneuver is to stabilize the interaction of alcohol vapor in the breath with the airway surfaces.

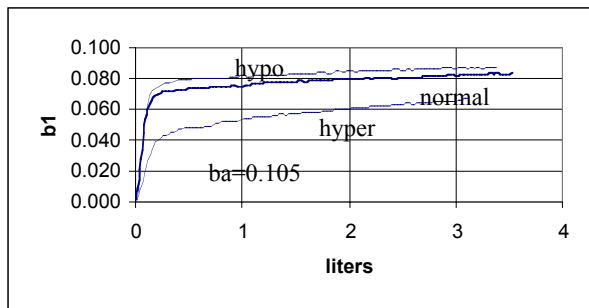


Figure 2a. b1 vs volume (ba=0.105).

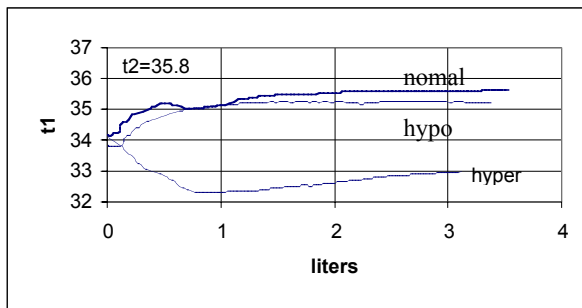


Figure 2b. t1 vs volume (t2=35.8°C)

Results

The relationship of BrAC (g alcohol/210L air) to breath temperature of a breath sample can be seen in Figure 2a and Figure 2b and are typical of the results obtained. Breath flow rate data were used to calculate breath volume. The profiles are from a single subject and were obtained within a period of less than 10 minutes. Comparison of the alcohol and the temperature profiles clearly shows the effect of cooling the lung airway surfaces on the breath alcohol concentration. The sharp rise seen in the alcohol profiles over the first 150 ml or so corresponds to the displacement of air in the Datamaster plus air from the conducting airway volume (from the mouth to the bronchi of the subject). The slower increase that follows corresponds to air from the lungs. The order of the tests had a small influence in the test result, indicating that it takes a few minutes for the lung surfaces to stabilize after the previous blow.

It is surprising that the temperature of hypo samples does not rise to deep lung temperature, assumed to be equal to oral temperature. This may reflect the disturbance of heat balance at the mucous water/air interface due to O₂/CO₂/water condensation/evaporation during breath holding.

Re-breathed samples showed similar behavior. Re-breathed samples were obtained specifically for use as a surrogate for the pulmonary blood alcohol concentration, pBAC (g/100ml blood). Following Harger's work (6) to determine a more stable relationship between BrAC and (venous) BAC, an earlier study in this laboratory (7) involving 30 subjects who had passed into the post-absorption period (at least 2 hours after drinking had stopped) yielded the following empirical relationship between re-breathed BrAC and BAC (at this stage in the post absorption period, BAC=pBAC):

$$\text{Eq. 1} \quad \text{pBAC} = 0.9573\text{BrAC}(\text{re-breathed}) - 0.004$$

for which $R^2 = 0.9844$, and the standard error for predicted pBAC = 0.004.

Since one cannot be certain whether or not the subject is fully post-absorptive unless several hours have elapsed after drinking had stopped, which was not the case in the present study, re-breathed BrAC was used instead of BAC in the calculations below. Fingertip blood, which is equivalent to arterial blood and hence to pulmonary blood, could have been used but this requires specialized equipment and techniques that were not available to this study.

With the assumption that Henry's law holds at the mucous water/air interface in the upper lung to a close enough approximation, an extrapolated deep lung alcohol concentration can be obtained from measurement of BrAC, breath temperature, and deep lung temperature.

The following notation is used:

	Alcohol conc. in lung air (g/210L)	Temperature (°C)	Alcohol conc. in mucous water (g/210L)	Henry's law water/air constant for alcohol
Upper lung/conducting airway	b1 (BrAC) types: Normal, Hyper, Hypo, Re-breathed	t1 (Breath temp)	c1	k1
Deep lung	b2 (BrAC extrapolated from t1 to t2) ba (calculated from pBAC)	t2 (oral temp)	c2	k2

(Oral temperature was used as an estimate of deep lung temperature. No doubt differences the temperature at these two sites in the body exist, and the differences may be variable. In any case, a better measure of deep lung temperature is not available.) By Henry's Law: $b2 = k2c2$ at $t2$, and $b1 \approx k1c1$ at $t1$, so that

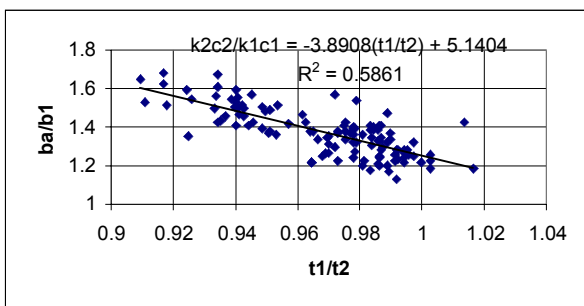
$$\text{Eq. 2} \quad b2 \approx b1(k2/k1)(c2/c1),$$

Or equivalently:

$$\text{Eq. 3} \quad ba/b1 \approx (k2/k1) (c2/c1)$$

where **ba** has been substituted for **b2**. Jones (8) has determined the water content of whole blood (in terms of grams/100 grams) for men (n=20) to be 79.3 ± 0.25 and for women (n=15) 81.1 ± 0.13 by a freeze dry method. Then **c2** equals $1.189pBAC$ for men and $1.163pBAC$ for women in grams per 100ml mucus water. Expressing **c2** in grams/210 liter mucus water leads to:

$$\begin{aligned} \text{Eq. 4: for men} \quad ba &= c2k2 = 2498 pBAC k2 \\ \text{for women} \quad ba &= c2k2 = 2443 pBAC k2 \end{aligned}$$



where **k2** can be obtained from Harger's data (9) and pBAC can be obtained by use of equation 1. Using equation 4 to obtain **ba** (the value of **ba** was raised by 5% to account for the effect of the estimated salt content of mucus), and experimental values of **b1**, the ratio **ba/b1** is plotted against **t1/t2** in Figure 3. Regression of the data yields equation 5.

Figure 3. Graphical evaluation of $(k2/k1)(c2/c1)$

$$(t1/ t2) + 5.1404$$

$$\text{Eq. 5} \quad ba/b1 = (k2/k1) (c2 / c1) = - 3.8908$$

The ratio shows a weak dependence on temperature ($R^2 = 0.5682$). The instability seen is possibly due to instability of t_2 due to heat balance effects during blowing (see below).

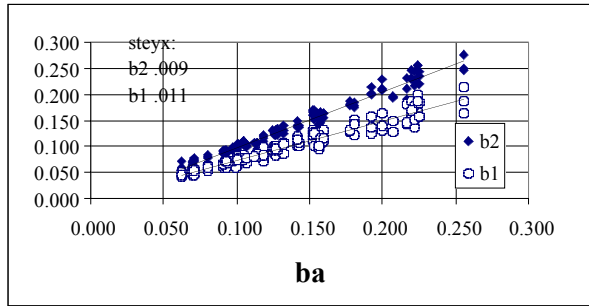


Figure 4. ba vs $b1, b2$.

Summarized in Table 1. $b1$ and $b2$ are plotted against ba in Figure 4.

Substituting the right hand side of equation 5 into equation 2 provides a value of the deep lung alcohol concentration extrapolated from the measured breath temperature for each $b1$ that can be compared with the same quantity calculated from pBAC in equation 4. Consistency of the deep lung concentration obtained by these two methods supports the

validity of the extrapolated value. Results obtained from the 30 human subjects are

Discussion

The data in Table 1 show the increase in accuracy for estimation of deep lung alcohol concentration with reduced variability obtained by extrapolation to deep lung temperature. The procedure seems justifiable on the following grounds:

Table 1. Data summary (n=40).

		$ba-b1$	$ba-b2$
normal	ave	0.030	0.001
	sd	0.013	0.008
hyper	ave	0.040	0.001
	sd	0.015	0.008
hypo	ave	0.021	-0.007
	sd	0.007	0.010

If there were no mucus water/air interaction in the upper lung, alcohol profiles having a distinctly different appearance from what is seen in Figure 2a would be expected. The portion of the profile following the steeply rising conducting airway portion would have a slope closer to zero, and breathing pattern would not produce the effects on concentration and temperature that are seen. It is clear that interaction of alcohol vapors with the surfaces of the upper lung results in loss of alcohol as the breath moves up through the bronchioles.

The final concentration is variable, depending on the temperature of the airway surfaces, surface concentration, and the volume of breath. Hyperventilation prior to blowing causes the greatest cooling and lowest concentrations. Hypoventilation and rebreathing limits cooling, but O_2/CO_2 /water heat balance during breath holding (rebreathing is a form of breath holding) may prevent full warming of the mucous water/air interface to body temperature. (Perhaps measurement of breath humidity and CO_2 would yield a means for a better estimate of deep lung temperature.) Still, highest concentrations are obtained by breath holding and rebreathing, probably because $c1$ approaches $c2$ more closely than would otherwise be the case.

At equilibrium, the alcohol concentration in the lung air is dependent only on the concentration of alcohol in, and the temperature of, the mucous layer. But during the dynamic process of breathing, true equilibrium is not obtained, except presumably in the deep lung region. However, the difference between deep lung temperature and breath temperature is only a few degrees (about $37^\circ C$ vs. about $33^\circ C$ to $36^\circ C$). Furthermore, diffusion of alcohol through the mucus is probably much slower than diffusion through the lung air.

In an experiment to demonstrate how quickly the air-water-alcohol system reaches equilibrium, the bubble tube of a simulator was adjusted from the normal depth of 3 inches below the surface of the solution in inch increments to a final depth of inch. The bubble diameter was kept at about inch by limiting airflow at about 0.1 liters/sec, a flow rate comparable to the lower range of flow rates encountered in police testing. It was found that the alcohol concentration decreased uniformly from a maximum of 0.100 g/210L at 3 inches depth, to 0.096 at inch. This indicates near equilibrium even at the briefest contact times between air inside the bubble and the bubble surface as it passes from the bubble tube to the surface of the solution. Even at the maximum depth, bubbles passed to the surface within less than 1 second, whereas it takes 5 to 10 seconds to deliver the breath sample from the lungs to the breath tester. Calculation shows that, for equivalent volumes, the surface area available for interaction is significantly greater for air in the lungs than air in the inch bubbles.

The above considerations suggest the validity of the estimation of **b₂** via equation 2 above in the case of normal and hyperventilated breath samples, a procedure that does not require individual anatomical data for each subject. The breath alcohol concentration extrapolated to deep lung concentration, **b₂**, would provide a less variable, more direct measure of alcohol load on the brain. The procedure can be performed easily using available and relatively inexpensive technology and without any additional requirement of the test operator.

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Portable Evidential Breath Test (PEBT) Program

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Orange County Sheriff-Coroner Department

Abstract

Orange County Sheriff-Coroner's Department Forensic Science Services (FSS) is committed to providing a countywide portable evidential breath test (PEBT) program for Driving Under the Influence (DUI) enforcement. FSS will initially deploy 75 Alcosensor IV-XL @ Point of Arrest instruments for use in patrol vehicles used by Orange County agencies. In addition to the instruments, the program will include technical support, training, and expert testimony to ensure that the requirements of Title 17 of the California Code of Regulations for forensic breath alcohol testing are met.

The automated design of the PEBT insures accuracy and quick distribution of results to the prosecutor's office and to the Department of Motor Vehicles. The inherent value of this equipment over stationary instruments is the ability to provide evidential results at the time of the DUI stop. With the subject's alcohol level determined in the field, alternate transportation of the subject to the jail can be arranged, thus keeping the arresting officer available for patrol activity. Or, the subject can be released to responsible individuals prior to incarceration, thereby reducing booking fees to the arresting agency. Also, such determination of the subject's alcohol level at the time of the stop will provide more timely evidence for the prosecutor's use in DUI trials.

Traceability and Evidential Breath Analyser

M. Montamat

Laboratoire National d'Essais , Centre de Métrologie et Instrumentation
Chef de la Division Métrologie Chimique .

Since many years, we are using hydroalcoholic solutions for calibration of our checking instruments . The partition coefficient was determined by applying Dubowski's formula without knowledge of uncertainties for coefficients introduced in such a formula . Traceability of such a procedure is not assumed .

A few years ago , BIPM (Bureau International des Poids et Mesures) organized an interlaboratory comparison using dry gases cylinders with very good results . Since , we have built a new generation of checking instruments which are calibrated with dry gas cylinders . Inside the instruments , we introduced a new alcoholic gas manufacturing which allows us to use dry gases for calibration and wet gases for checking EBA .

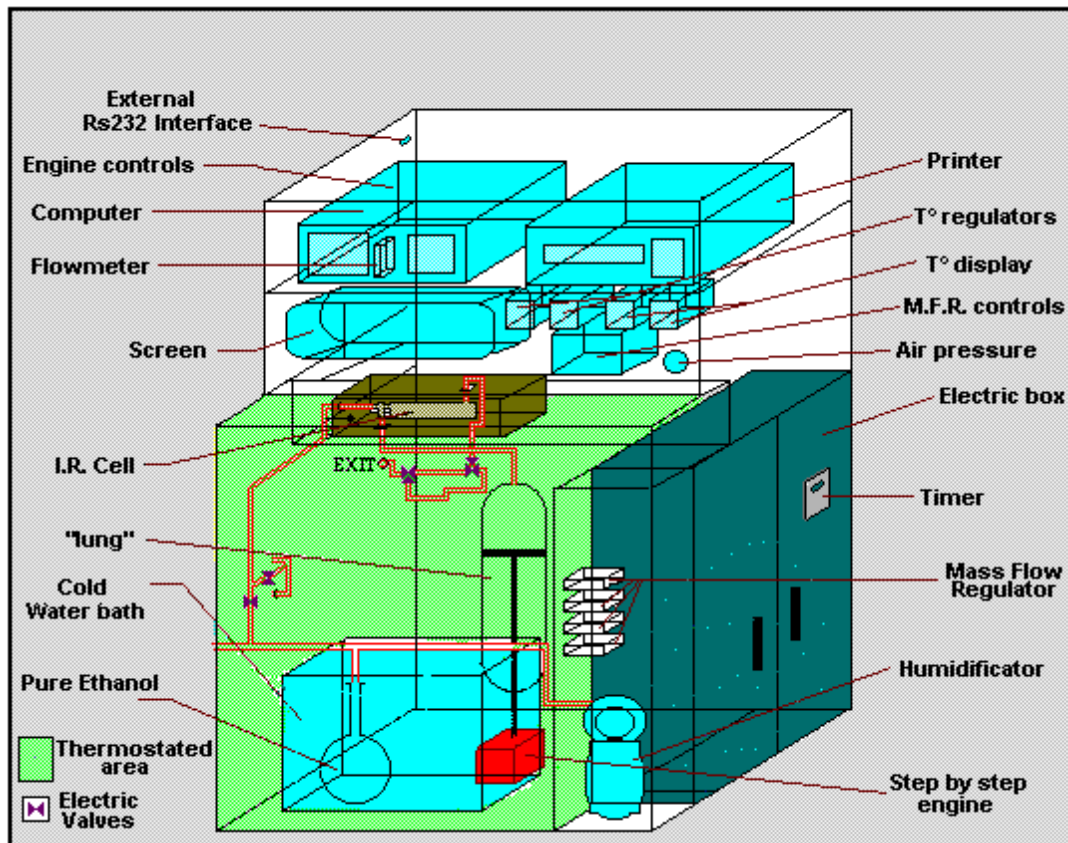
Description

The bench is intended for delivering repeated breathes of which the flow and the ethanol content are accurately known.

These breathes have similar characteristics to that of a human breath (humidity, alveolar volume and dead volume, breath time, CO₂ content and temperature). The bench offers also the possibility to work with dry gases

Principle

The bench is constituted of a temperature regulated enclosure in which a gas mixture is made and restituted at a given flow rate. A piston displaced by a continuous current motor, controlled by a PC, is used for the inspiration/expiration flow rate. The concentration of the gas mixture is obtained with mass flow regulators. The ethylic gas is obtained by a bubbling on a flask containing pure ethanol inside a cold bath which is continuously renewed. An infrared cell and sensors, placed upstream of the flux and connected to the PC, allow to monitor the ethanol content, the flow rate, the pressure, the time of each delivered breath. A pneumatic circuit permits the cell calibration by a dual flask in a regulated enclosure external to the bench for wet gas calibration or ethanol/air bottle for dry gas calibration. An analogic entry allows to do then temperature signal acquiring of ethanol mixture.



Functioning

Temperature

For the temperature regulation, the bench is composed of four distinct zone :

- A not regulated zone : This one at ambient temperature contains all the temperature sensitive elements. Especially the electronic cards (analyser, engine card, analogic/digital card, serial card ...), the piston engine, the flow rate regulators and informatics.
- A 37°C regulated zone : This part is the external enclosure of the calibration flasks and the enclosure of the bench itself. It is in this part where are placed the "lung", the pneumatic circuit, the bathes for the flask. The regulation is performed to 1/10th °C by a regulator following a PID algorithm of adjustable parameters. A display on the front panel gives permanently the enclosure temperature.
- A 34°C regulated zone : This one concerns, the pneumatic circuit outside the regulated enclosure. (entry and exit of infrared cell, exit to the ethylometers).

A 15°C regulated zone : On another part the 15°C regulation concerns the flask system used to obtain the ethanol concentration. As for the enclosure, the regulation is performed by a PID algorithm to 1/10th °C.

Gaz mixture

The mixture is obtained from three constituents : air, CO₂, and ethanol (or/and eventually an interfering component). The air must be, if it comes from the general supply system, of excellent quality (dry, without oil and dust). If it comes from a bottle, the best quality is "N57". For the CO₂, the minimum quality needed is "N45". The purity of ethanol must be of 99,9 %

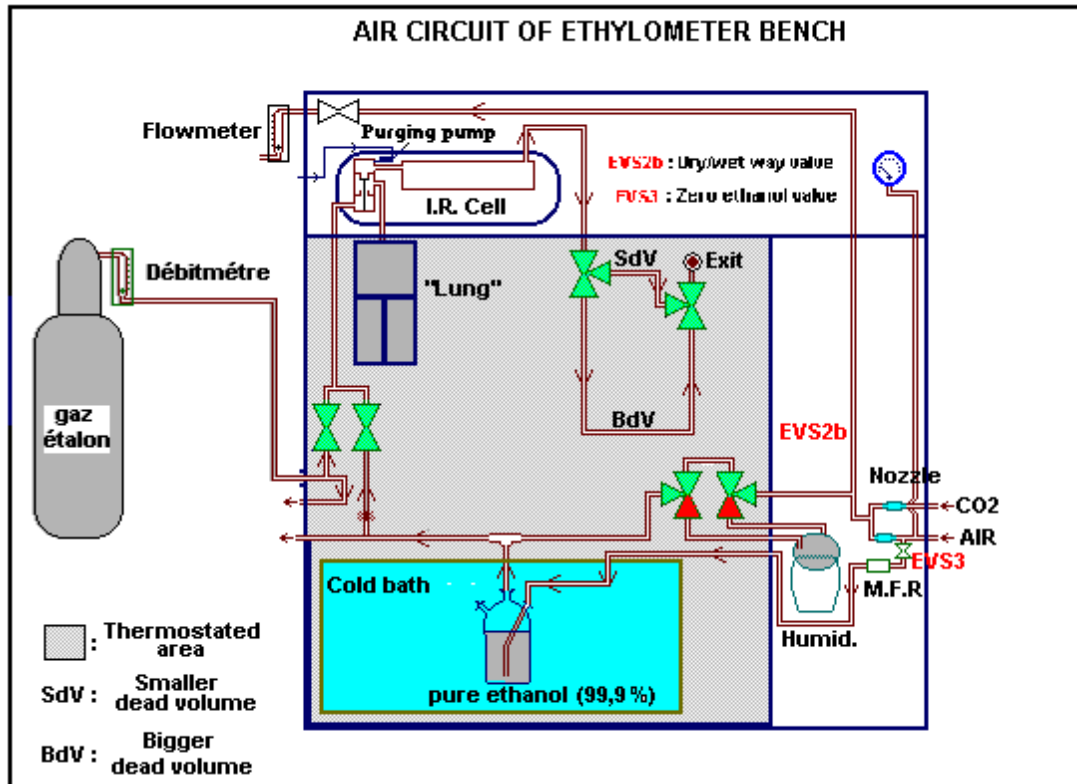
The air, at the entrance, is divided on three parts, one goes outside to supply air zero for dry calibration or the bubbling in dual flask during wet gases calibration, another one sweeps flask inside the bench to undertake ethanol and the last one constitutes the principal flux, the vector gaz in which will be constituted the mixture.

A command box in the front allows to act (automatically with computer or manually) on ethanol flow rate to change the concentration (with a step < 1 µg/l).

For the optimal use of the bench, it is advised to adjust the principal flux rate to 10 l/min (pressure = 1.5 bars) and to vary only the flow rate in the flask. The ratio between this flow rate and the ethanol concentration of the gaz is function of the temperature of the ethanol.

The ethanol saturated air is the last constituent injected in the mixture. The air of the principal flux is mixed with CO₂ and filled with water (humidity > 95 %) in a humidificator (34 °C regulated water reservoir) for work with wet gases, before to be mixed with ethanol. There too the CO₂ flow rate is normally definitely regulated to obtain a 5 % content at 1.5 bars (The pressure can be modified for particular test at 10 % or more).

Flow control and pneumatic circuit



The inspiration/expiration flows are controlled with a piston connected to a three lines electrovalve. This piston, controlled by a continue current motor connected to a PC, allows to obtain very accurate volumes and times of going up or down (precision of 1 ml and 1/10th of second) and then to control the flow of inspiration and breath. When going downwards, the valves are connected at the gaz flux and the "lung" takes the necessary volume (the flow rate must be greater than the inspiration flow). After pressure equilibration, the "lung" is ready to breath. The valve is then connected at the IR cell in the direction of tube for ethylometers connexion. In the cell, the concentration, the flow rate, the pressure and the signal stability, during all the breathes are controlled by the PC.

Two three lines electrovalves allow to change the dead volume modifying then the time of going up in concentration and the during of plate.

The concentration calibration is done by the same process, by inspiring in a flow coming from the external solutions and by expiring afterwards in the infrared cell.

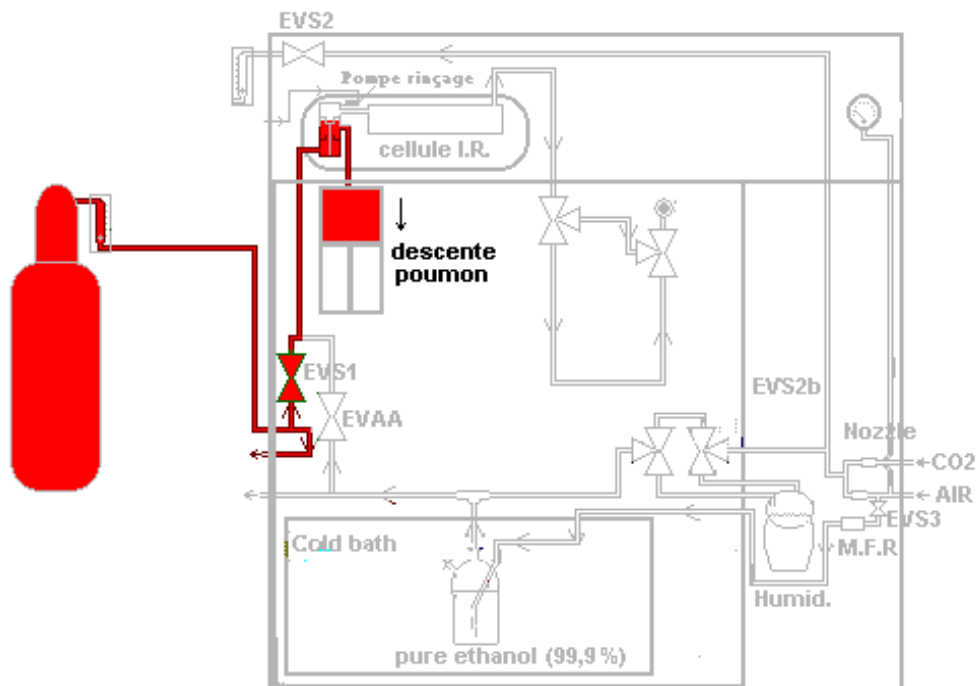
It is to notice as well that pump, beyond the breath periods, ensures permanently the cell and the expiration circuit purging, with zero air, to be sure that there is no residual alcohol between two cycles. The PC verifies the purging and the signal stability.

Software

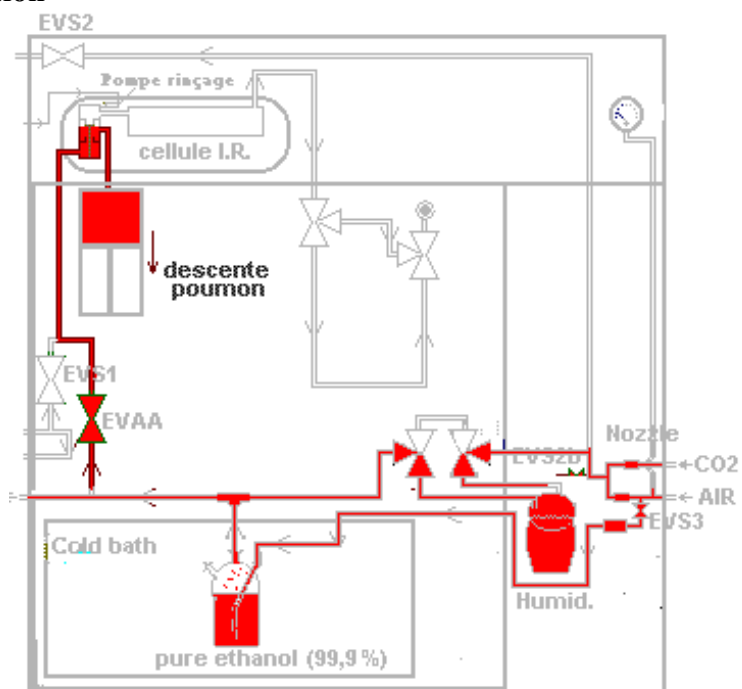
The software allows to parameter and control the bench. It controls the breath cycles, time and volume, the purging and controls the electrovalve. It ensures also the acquisition of the bench parameters (breath flow, concentration, pressure, humidity...) and ensures their graphical and mathematical treatment (regression calculus, standard deviation, results estimation, stability, calibration coefficient evaluation, ...).

Principle of sampling and gas generation

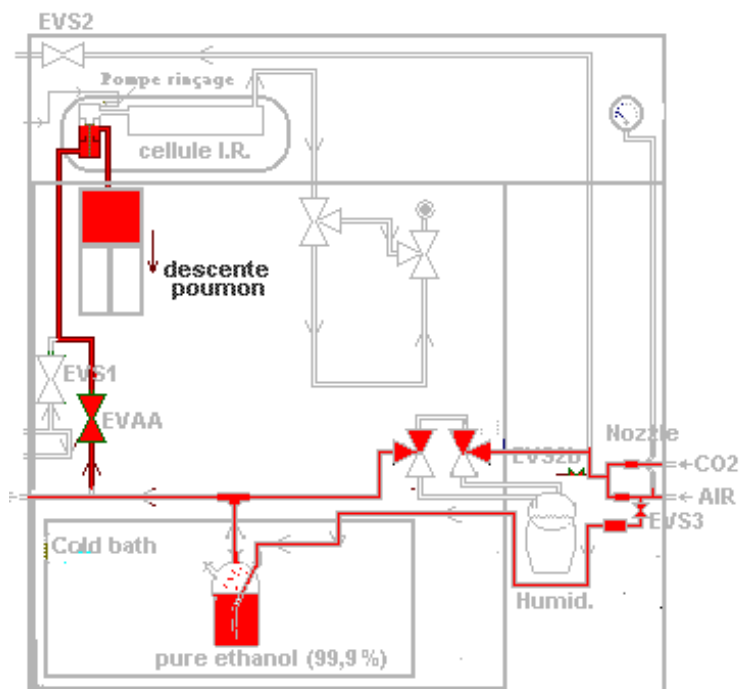
Dry gas calibration



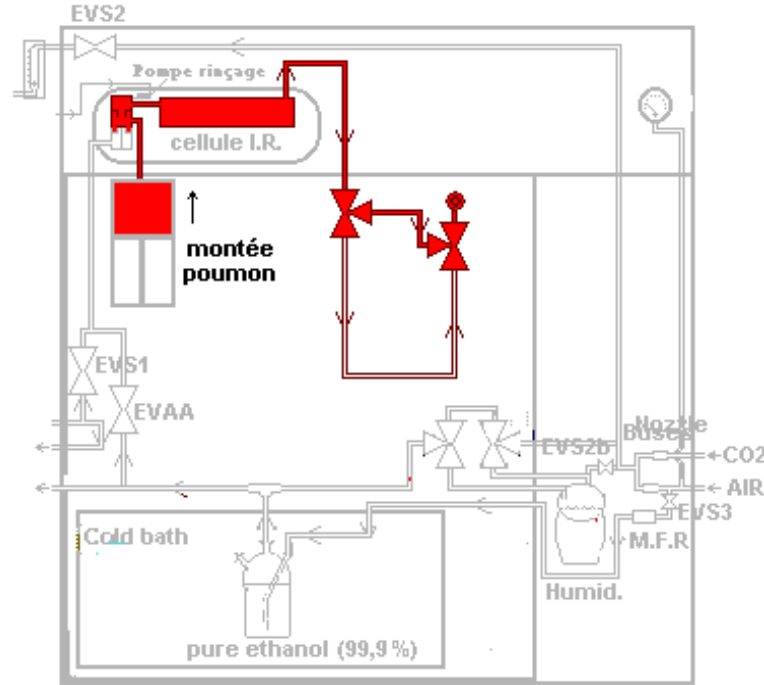
Wet gas generation



Dry gas generation



Breath simulation



Conclusion

Using such an instrument, we studied partition coefficient between hydroalcoholic solutions and air at 34°C.

We found in applying Dubowski's formula we introduced an error of -1% and we are in agreement with the data of Pr Harger.

For example, when we find a concentration equal to 0.394 mg/l, application of Dubowski's formula gives 0.389 mg/l and Harger's data 0.393 mg/l. In another hand, the uncertainty on partition coefficient was +/- 0.66 %

So, we can evaluate uncertainties of our checking instruments.

In using wet calibration, the uncertainty for concentration equal to 0.400 mg/l is +/- 0.0045 mg/l.

In using dry calibration, the uncertainty for the same concentration is +/- 0.0036 mg/l.

The realisation of such an equipment allows an improvement of performances of checking instrument and also to choose the better way for calibrating such a material.

