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BLOOD-ALCOHOL PROFICIENCY TEST PROGRAM

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INTERIM REPORT

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16. Abstract

A preliminary survey has been performed to ascertain the validity of the blood alcohol analysis performed by a number of laboratories on a voluntary basis. Values of accuracy and precision of the tests are presented.

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PREFACE

Measurements of blood-alcohol concentration by laboratories throughout the country (private, state, municipal, etc.) are used in legal proceedings for the determination of intoxication. These analyses are also used in the statistical assessment of nationwide alcohol-related traffic accidents. The toll of alcohol-related traffic deaths is presently at about 25,000 per year. A survey is being performed to ascertain the accuracy of the blood-alcohol analysis performed by a number of laboratories, on a voluntary basis, the interim results of which are reported herein.

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1. PROFICIENCY TEST PLAN

A meeting was convened by NHTSA in May 1973 in Washington DC to discuss the establishment of a proficiency test program, its goals and other details. Through each of the 10 NHTSA Regional Offices, several laboratories located in each region had been invited to send representatives and, most regions were represented.

It was agreed that such a program was needed, and that the goals of the program would be (a) to provide a means by which any laboratory could compare its performance against that of other laboratories throughout the country, and (b) to determine what, if any, problems exist in the field of blood-alcohol analysis performed by these laboratories. The program would be conducted for NHTSA by TSC.

It was decided that participation in the program should be voluntary, open to all laboratories and be conducted as a service to the participants. It was recognized that sufficient time should be allowed to achieve wide acceptance, perhaps more than a year. Continuance of the program beyond that time would be determined by the needs of the community of laboratories and by NHTSA. Tests would be conducted at four-months intervals. It was agreed that four specimens, two bovine blood samples containing alcohol and specified preservatives and anti-coagulants, and two aqueous samples containing only alcohol, would be analyzed by each participant.

2. PROFICIENCY TESTS

In accordance with the agreed upon schedule, three tests, using samples prepared at TSC, have been conducted to date: in September 1973, in January 1974 and in May 1974. Batches (2 liters) of fresh bovine blood to which the salts sodium flouride and potasium oxalate had been added (5 mg/ml and 4 mg/ml respectively) as preservative-anti-coagulant were spiked with alcohol at selected levels corresponding to those encountered in practice. Aqueous batches(2 liters) were made without the salts. Each batch was first analyzed at TSC for alcohol as a check on the preparation procedure. From each batch, individual specimens were placed in 10 ml serum bottles which were capped and sealed. Sufficient specimens were prepared for all particiapnts and for TSC's needs for later checks.

Until shipments were made, batches or specimens were kept under refrigeration at 4°C. Shipments were made early in the week in order that specimens not be held in the mails unrefrigerated longer than necessary. Consultation with the Center for Disease Control, Atlanta, Georgia had determined that for purposes of this testing, use of sterilized bottles or shipment with a cooling agent was not warranted. Each shipment also contained necessary instructions and reporting forms.

2.1 PARTICIPANTS

Information on the program was disseminated through the NHTSA Regional Offices. Initially, 72 laboratories enrolled in the program. Present enrollment is 96. All U.S. geographic areas are represented including Alaska and Puerto Rico.

2.2 BLOOD-ALCOHOL ANALYSIS TECHNIQUES

It was found, after the first test and receipt of report forms from participants, that three principal analysis techniques were used, namely: gas chromatography, dichromate oxidation and

enzymatic oxidation. The procedures used are briefly described below.

2.2.1 Gas Chromatography

Gas chromatographic techniques employed included direct injection of sample fluids, or head space techniques wherein vapors in equilibrium with the fluids were injected. Also used were a variety of internal standards which compensated for sampling errors. Poropak (polymeric particle packings) type columns were usually used because of their very good resolution of alcohol and other common blood substances. The headspace variation requires attention to the "salting out" effect which can be compensated for by use of properly chosen internal standards. The variation of the headspace method used by TSC is described in Report DOT-TSC-NHTSA-74-4.

2.2.2 Dichromate Oxidation

Dichromate oxidation techniques require separation of alcohol from the specimen to be analyzed, usually by distillation, followed by quantitative determination of reducing substances by oxidation with dichromate solution. Total reducing substances are reported as ethanol. Although the possibility for interference is small, some laboratories screen specimens for possible interferents by gas chromatography.

2.2.3 Enzymatic Oxidation

Enzymatic oxidation involves the conversion of an enzyme from an oxidized form to a reduced form with the consequent oxidation of ethanol to acetaldehyde. Usually the enzyme in reduced form is measured spectrophotometrically for quantification. The reaction is specific for a number of alcohols in additon to ethanol but reacts more slowly with these other alcohols and so is fairly specific for ethanol.

3. RESULTS

Results obtained from the first three tests are listed in Tables 1 and 2. Samples A and B are bovine blood samples and Samples C and D are aqueous samples for all three tests. Table 2 shows that mean BAC* values for the blood samples in all three tests were consistently lower than target values by about 5 percent; whereas, the mean BAC's for the aqueous samples were usually closer to the target BAC being on the average about 2 percent higher. Scatter in the data was about the same for both blood and aqueous samples as evidenced by the standard deviation obtained (about 10% of mean BAC's). As can be seen there is little to indicate any one type of analysis to be superior to any other.

The difference in trends for target vs. mean values for blood and aqueous samples noted above is significant. The reason for the mean blood results being 5 percent lower than target values while the aqueous results are essentially equal to target values was thought to be due to blood sample deterioration occuring sometime between shipment from TSC and analysis at the destination. During this time the samples are unrefrigerated and subject to unknown ambient conditions. Table 3 presents results of analysis of samples from the three proficiency test lots which had been allowed to stand in the laboratory at TSC, at room temperature (about 24°C) for varying numbers of days. The samples were discarded after each analysis so that the BAC's listed do not represent the variation of BAC of a single sample but rather the BAC's of randomly selected samples after a given number of days at room temperature. The values listed represent averages of several aliquots from a given sample. The headspace technique used has an accuracy limit of $\pm$ 2 percent with a standard deviation of about 0.5 percent of the mean obtained. Table 3 shows that the blood samples do deteriorate when not refrigerated. The first and second test lots showed a deterioration of about 10 percent after four days.

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* Blood Alcohol Concentration expressed in grams alcohol per 100 ml blood.

TABLE 1. BAC's REPORTED--AVERAGED VALUES

Test	Technique	A	B	C	D	Number of Laboratories Reporting
1	(TARGET)	(130)	(.080)	(.183)	(.047)	
	All	.123	.076	.185	.049	52
	G.C.	.124	.074	.186	.048	27
	Dichromate Oxidation	.121	.078	.182	.050	17
	Enzymatic	.123	.078	.189	.051	8
2	(TARGET)	(.236)	(.177)	(.077)	(.177)	
	All	.225	.167	.076	.179	82
	G.C. Headspace	.228	.169	.076	.179	23
	G.C. Fluid Injection*	.229	.171	.077	.180	25
	Dichromate Oxidation	.222	.164	.078	.177	23
	Enzymatic	.215	.163	.071	.185	11
3	(TARGET)	(.208)	(.103)	(.194)	(.096)	
	All	.194	.099	.198	.099	86
	G.C. Headspace	.192	.097	.201	.100	26
	G.C. Fluid Injection*	.198	.100	.204	.100	28
	Dichromate Oxidation	.194	.098	.189	.096	22
	Enzymatic	.189	.101	.194	.100	10

* Injection of whole blood or distillate, extract, supernatant, etc., thereof.

TABLE 2. ACCURACY AND PRECISION DATA (PERCENT)

Test	Technique	A		B		C		D	
		$\frac{M}{T}$	$\frac{S}{M}$	$\frac{M}{T}$	$\frac{S}{M}$	$\frac{M}{T}$	$\frac{S}{T}$	$\frac{M}{T}$	$\frac{S}{T}$
1	All	95	9	95	10	101	6	104	16
	G.C.	95	9	93	11	102	7	102	15
	Dichromate Oxidation	93	8	98	11	99	5	106	18
	Enzymatic	95	8	98	6	103	8	109	12
2	All	95	10	94	9	99	14	101	8
	G.C. Headspace	97	10	95	8	99	6	101	5
	G.C. Fluid Injection	97	11	97	10	100	10	102	6
	Dichromate Oxidation	94	7	93	8	101	9	100	5
	Enzymatic	91	10	92	9	92	21	105	16
3	All	93	11	96	10	102	11	103	8
	G.C. Headspace	92	11	94	10	104	8	104	7
	G.C. Direct Injection	97	11	97	10	100	10	102	6
	Dichromate Oxidation	93	13	95	12	97	19	100	11
	Enzymatic	91	10	98	10	100	6	104	8

	A,B		C,D	
	M/T, %	S/M, %	M/T, %	S/M, %
1,2,3 All	95	10	102	11
G.C.	95	10	102	8
Dichromate Oxidation	94	10	101	11
Enzymatic	94	9	102	12

M= mean BAC T= target BAC S= standard deviation

TABLE 3. DETERIORATION OF TEST SAMPLES ON STANDING AT ROOM TEMPERATURE FOR A NUMBER OF DAYS

TEST NUMBER 1	SAMPLE A (TARGET .130)	SAMPLE B (TARGET .080)
Number of Days Unrefrigerated	BAC Obtained,	Percent of Target
0	101	99
4	92	89
TEST NUMBER 2	(TARGET .236)	(TARGET .177)
Number of Days Unrefrigerated	BAC Obtained,	Percent of Target
0	98	98
1	96	96
2	94	95
3	92	95
4	92	90
TEST NUMBER 3	(TARGET .208)	(TARGET .103)
Number of Days Unrefrigerated	BAC Obtained,	Percent of Target
0	100	102
1	99	99
3	95	97
7	98	102
9	100	100
15	96	90
22	93	85

Of the total number of blood analyses reported which were less than 90% of the target value (126 cases out of a possible 460), 48 percent were received by the participating laboratories within 1 day of shipment and 81 percent were received within 2 days of shipment. Assuming that the samples were refrigerated on arrival, one would conclude that deterioration of blood samples en route cannot fully explain the large number of low results reported.

In proficiency test number 3, extra blood samples were sent to selected particiapnts scattered throughout the nation with instructions to return them immediately to TSC. These samples were then analyzed at TSC. Results are listed in Table 4. With the exception of one shipment which apparently had been unrefrigerated for 13 days, there was essentially no deterioration, which reinforces the conclusion that deterioration of samples is not the main source of low results reported for the blood specimens. The blood results more than about 5% higher than target values (37 cases) cannot be ascribed to anything to do with the samples, their preparation or shipment.

The results from these three tests indicate that blood anlysis performed by the typical laboratory will be about 5% lower than the actual value and that the value reported will be associated with a standard deviation of about 10 percent. For example, if a 0.100 BAC sample were delivered to an average laboratory for analysis the chances are that the value reported will be somewhere between 0.114 and 0.076BAC at 95 perecent confidence (2 sigma level).

These results would hold regardless of which of the three major analytical techniques were used as is shown in Table 2. Further, the average standard deviation obtained indicates that differences of about 20 percent ($\pm$1 sigma) between typical laboratories would not be uncommon and that differences beyond 30 percent ($\pm$2 sigma level) would be rare.

TABLE 4. TEST 3 SAMPLES SHIPPED TO PARTICIPANT LABORATORY AND RETURNED TO TSC FOR ANALYSIS

STATE	NUMBER OF DAYS UNREFRIGERATED	SAMPLE A	SAMPLE B
North Carolina	6	.204	.104
Iowa	6	.201	.101
Oklahoma	6	.206	.104
Arizona	6	.201	.100
Alaska	6	.210	.104
Idaho	7	.210	.106
New York	6	.208	.105
Virginia	6	.205	.106
Arizona	6	.198	.102
Ohio	6	.216	.101
Puerto Rico	9	.198	.100
Illinois	6	.206	.094
Minnesota	8	.211	.093
Texas	8	.211	.093
Georgia	13	.180	.084
M/T, percent		98	97
S/M, percent		4	6

M = mean BAC T= target BAC S = standard deviation

TARGET BAC: Sample A .208
Sample B .103

A further test related to possible deterioration effects was conducted in the Boston area and is of some interest. Five laboratories in the Boston area not participating in the program but which perform commercial blood analysis were sent duplicate samples of A and B from test number 3 for analysis on the same day that the blood was prepared. The laboratories were not told that the samples were duplicates. Immediate analysis was requested (and paid for) so that there would be no chance for deterioration. The results are listed in Table 5. Here the mean results were similiar to the results obtained nationally although the scatter was far worse. Discrepancies between these laboratories as high as 40 percent are seen. The point is that the poor results in Table 5 are not due to sample deterioration.

TABLE 5. ANALYSIS OF TEST 3 SAMPLES BY FIVE BOSTON LABORATORIES

LABORATORY (TARGET)	A (.208)	D (.208)	B (.103)	C (.103)
1	.217	.202	.111	.112
2	.235	.238	.139	.139
3	.193	.165	.087	.097
4	.188	.166	.086	.079
5	.143	.163	.055	.061

(Laboratories were not told that sample A was identical to D and that sample B was identical to C.)

	A-D	B-C
M/T, percent	92	94
S/t, percent	17	30

M = mean BAC T = target BAC S = standard deviation

4. COMMENTS RECEIVED FROM PARTICIPANTS

From time to time since the beginning of the program, participants have commented on various aspects of the program either by telephone or by letter. These communications have been helpful in that some feeling for the problems that these laboratories has been obtained, and the program can be (and to a certain extent has been) changed to accommodate needs of the participants that had been previously unknown to TSC. In addition, in May 1974 a letter was sent to all participants requesting comments on any aspect of the program as it is presently run or suggestions on how it can be changed to provide better service to the participants.

From the responses received, the most general one was that the program was of great value in that each laboratory could have an idea of their performance with reference to national test results. Even laboratories participating in statewide proficiency testing expressed appreciation for the NHTSA program because of its national scope. There have been no negative replies. Only one participant has dropped out of the program. The reason given was that the laboratory no longer performs blood alcohol analysis.

4.1 TECHNICAL COMMENTS

Instructions for obtaining advice on analytical problems from the Center for Disease Control (CDC) were included in sample shipments. This was done because of the long experience of CDC in all aspects of blood technology compared to the limited experience of TSC on details of the various analysis procedures developed specifically for blood-alcohol analysis. However, there were a few questions received at TSC concerning procedures. It was decided that only a minimum response to these questions would be made for reasons that will be discussed.

4.2 COMMENTS ON CONCEPT AND FUTURE DIRECTION OF THE PROGRAM

The views of the participants were expressly requested on this subject. However, only five responses addressed directly to the

question were received in addition to several telephonic responses. The responses were that:

a) The program should remain essentially the same.

b) The program should include certification of proficiency for participants.

c) The program should not include proficiency certification unless it were administered in cooperation with a non-government agency, such as the American Academy of Forensic Science, but that the present direction is desirable.

d) Certification, if given should not imply a guarantee of accuracy and precision and that possibly the safest method of influencing satisfactory performance is continued blind testing (i.e., the present program).

5. DISCUSSION OF FUTURE PROGRAM DIRECTIONS

The program as presently conducted remains essentially the same as was originally planned in May 1973. At that time little was known as to what kind of result would be obtained. These three initial tests have served the purpose of providing information for further refinement for the purpose reaching program goals more efficiently. Results indicate that:

a) Scatter obtained (sigma about 10 percent of mean BAC reported) may be high enough to warrant action by NHTSA in cooperation with other interested groups and the participants.

b) Although deterioration of blood samples appears to occur, there are other more serious problems in the blood analysis.

c) Consideration should be given to the needs of the participants (although conflicting) not presently met by the present program.

5.1 VARIABLILITY IN RESULTS

The degree of scatter in the results (sigma about 10% of mean BAC's) may be large enough to warrant some action by DOT. It has been reported that in national tests such as this, it is usual for results to be highly variable. However, it is appropriate to consider that something can be done to reduce it. One logical step is the publication and distribution of a laboratory manual containing a critique of each of the techniques used for blood-alcohol analysis with thorough discussion of sources of error and troubleshooting for each technique.

To better understand the reason for the variability encountered, vists to selected laboratories would be highly beneficial. The number of participants within tolerance of +5 to -10% is shown in Figure 1.

X

Number of blood samples analyzed within tolerance

Y

Number of aqueous samples analyzed within tolerance

	0	1	2	3	4	5	6
0							
1				2	1	1	
2			2			1	
3					4	2	1
4			2	3	2	2	
5			2	2	3	2	5
6		1	1	1		1	4

Figure 1. Number of laboratories analyzing X blood samples and Y aqueous samples within tolerance of +5 to -10 percent of target value. Only laboratories completing 3 (6 blood and 6 aqueous samples) tests (45) tabulated.

5.2 DIFFERENCES NOTED IN RESULTS FOR BLOOD ANALYSIS AND AQUEOUS ANALYSIS

The tendency of the blood analysis to be lower than the aqueous analysis is thought to be due to the increased difficulty in processing blood specimens compared to aqueous specimens. Appropriate action would be similar to that in 5.1 above; i.e., thorough discussion of the problem in a laboratory manual together with vists to selected laboratories to better understand the problem encountered in the field. Future tests using human blood rather than bovine blood may indicate a more serious problem.

5.3 SUGGESTIONS BY PARTICIPANTS

The program has been well received by the participants. Apparently, the major reason for the degree of acceptance is the national scope of the program. The fact that the program is strictly voluntary and that laboratory anonymity is preserved is also appreciated.

A suggestion that certificaton of proficency be granted to paticipants came from a laboratory in a state whcih had no such program. In this case, the possibility of obtaining certification from a national source would appear logical. However, another participant felt strongly that certification, if given, should be in cooperation with a non-government agency such as the American Academy of Science.

5.4 INCREASING PARTICIPATION

The establishment of the program has been disseminated through the NHTSA Regional Offices. From the beginning, requests from new laboratories to enroll have been trickling into TSC and the feeling is that there may be a large number of laboratories which would like to participate in the program but have no knowledge of it. Therefore, information of the program may be more efficiently disseminated through a professional society.