

# Serum Chemistries, Cotinine, Blood Alcohol, and Carbohydrate Deficient Transferrin

Robert L. Stout, Ph.D.

## Executive Summary

The purpose of this study was to evaluate the relationships between liver enzyme elevation(s), high density lipoprotein, cotinine, combinations of various serum chemistries and carbohydrate deficient transferrin. The key findings were

- 1) Reflex testing, based on combinations of markers can substantially reduce testing cost;
- 2) Among the sub-population of the group with elevated liver enzymes that are positive with CDT, 90% of the CDT positives are identified by testing only those individuals with HDL of 50 or greater; and
- 3) Samples that screen CDT positive with the Bio-Rad %CDT assay are confirmed positive by more definitive tests only 50% of the time.

Carbohydrate Deficient Transferrin (CDT) is the most commonly ordered reflex marker to detect excess alcohol use in applicants for insurance. The current laboratory model for selection of samples for reflex testing of CDT is a combination of liver enzyme elevations, blood or urine alcohol, and HDL (Cholesterol) above 70mg/dL. The particular reflex protocol varies by company.

CDT results correlate with smoking, blood alcohol and increasing concentrations of GGT, AST, and HDL. Triglycerides and BUN correlate inversely with CDT; ALT and uric acid levels correlate less closely with CDT results. Reflex testing from a single abnormal laboratory result such as AST, HDL or GGT, fails to identify a significant percentage of positive CDT results in the population.

The addition of isolated high HDL (>70mg/dL) as a reflex criterion increases the number of samples testing positive for CDT by 26%. The current data show that for isolated HDL elevations, a cut-off of 70mg/dL should be used for both male and female applicants.

Twenty-three percent (23%) of samples containing alcohol were positive for CDT. There is a linear relationship between the prevalence of CDT and the level of blood alcohol. Adding blood alcohol to the standard profile can increase the percentage of CDT positive by 20-40% in younger applicants.

Reflex testing using combinations of risk factors such as cotinine, high HDL and liver enzyme elevations increases the percentage positive for CDT. Testing only samples with combinations of risk factors decreases the cost of testing, but misses some positives. A reflex protocol using elevations of any liver enzyme and HDL of 50 mg/dL or greater identifies 90% of all CDT positive applicants initially selected for CDT testing due to liver enzyme elevation(s). If, in addition, the protocol also includes samples with HDL above 70 mg/dL and samples with positive alcohol findings, 92.6% of all CDT positive applicants are identified.

Samples selected for CDT testing are screened with the Bio-Rad %CDT assay. Samples that are positive in the Bio-Rad %CDT assay must be con-firmed by quantitative high-pressure liquid chromatography (HPLC) or capillary zone electrophoresis (CZE). Over all, the confirmation rate is about 50%. All positive samples in the Bio-Rad assay are confirmed with CZE at Clinical Reference Laboratory.

## Study

The health risk associated with nicotine and alcohol misuse is well documented. In the insurance industry, nicotine is detected as its chief meta-bolite, cotinine. The clearance of cotinine is relatively slow, allowing identification of smokers after periods of abstinence. With regard to alcohol, only blood alcohol provides an unambiguous marker for the identification of alcohol use. However, blood alcohol is rapidly cleared from circulation and, therefore, is of only limited utility in the detection of alcohol use or abuse. In an effort to identify alcohol misuse, laboratory medicine has relied upon the effect of alcohol on liver tissue, ratio of AST to ALT, mean corpuscular hemoglobin

volume, low BUN, low albumin, and high uric acid to identify individuals at risk. These markers were initially identified in tertiary care populations that are not representative of the general or insurance population.

The reported prevalence of alcohol misuse in members of the general population with at least a high school education, age greater than 25 years and an annual income of more than \$35,000.00, is 2%. This may approximate the upper limit of alcohol misuse in the insurance population.

The consumption of large amounts of alcohol may damage the liver. However, about 50% of the alcoholic population has normal liver enzyme levels. Therefore, liver enzyme elevations are insensitive indicators of alcohol misuse. Carbohydrate deficient transferrin is more sensitive than elevation of liver enzymes in detecting alcohol misuse. Transferrin is a protein produced in the liver. Glycosylation is an enzymatic process which adds carbohydrate to protein. In the liver exposed to large amounts of alcohol, transferrin is incompletely glycosylated.

The relationships between CDT, blood urea nitrogen (BUN), high-density lipoprotein (HDL), triglycerides, uric acid, ALT, AST, GGT, and urine cotinine, were investigated. The study population consisted of 33,082 insurance applicants tested for CDT. In this group, CDT was requested for blood alcohol positive samples, or for samples with liver enzyme elevation(s) or high HDL concentration. The enzymes most commonly used are GGT, ALT and AST. Samples with isolated HDL of 70 mg/dL or greater may also be tested for CDT. Cotinine is routinely assayed for all applicant samples.

## Results

Data for this study are presented in a series of tables. The titles describe each table's content and are accompanied by a summary.

### Prevalence Studies

The population was divided into groups based on the reflex criteria that triggered a CDT test. The data are presented as the number of samples in each group and the number of samples that tested positive. The percentage of CDT positive was calculated for each group.

**Table 1. Reflex Test Criteria and CDT Prevalence.**

Reflex Marker	Samples	CDT Positive	Prevalence %
Enzyme Elevations	29091	493	1.69
HDL>70	3979	127	3.19
Alcohol	132	31	23.50

Elevation of liver enzymes is the most common reflex for CDT. The group also contains the largest number of CDT positive samples. HDL and alcohol are the second and third leading cause for CDT reflex testing. The percentage of positive results is exactly opposite that of group size, with alcohol, HDL>70 and elevated liver enzymes having the highest to lowest percentage positive CDT respectively.

### *Study of the Correlation Between CDT and the Degree of Liver Enzyme Elevation.*

#### Enzyme Elevations and CDT

**Table 2. Prevalence of CDT in Samples with Single Enzyme Elevations.**

Single Enzyme Elevation	GGT+	AST+	ALT+
Number	10517	1052	2862
CDT Positive	151	45	11
%Positive	1.44	4.27	0.38

Elevated GGT is the most prevalent reflex marker and is associated with the highest number of positive CDT's. However, the marker by itself has lower specificity than AST (1.47% compared to 4.89%) for identification of the population positive for CDT. Isolated ALT elevations identify very few CDT positive samples and should be discontinued as a CDT reflex marker.

**Table 3. Prevalence of CDT in Samples by Level of Enzyme Elevation.**

GGT	% CDT	ALT	%CDT	AST	%CDT
66-97	1.34	45-67	1.31	41-61	2.39
98-130	1.66	68-90	1.42	62-82	4.08
130-162	1.83	91-112	1.91	83-102	4.46
163-195	2.09	113-135	2.03	103-123	7.26
196-227	2.81	136-157	2.03	124-143	6.45
228-260	3.74	158-180	0.94	144-164	7.14
>260	4.42	>180	1.06	>164	4.07

In this table, the data are separated by range of elevation for each enzyme, with the level of the other enzymes not considered. For example, for any GGT level, the ALT and AST level may be normal or elevated. Elevated ALT is associated with the lowest percent positive CDT. Even with ALT excluded, 97.8% of CDT positive applicants tested due to liver enzyme elevation(s) were identified. The incidence of positive CDT increases with increasing concentrations of GGT and AST. AST has the best specificity of the three liver enzymes.

### Specific Combinations of Liver Enzyme Elevations

Various combinations of enzyme elevations were compared to CDT results.

**Table 4. Reflex Testing from Combinations of Elevated Liver Enzymes.**

Combination	Number	CDT	%Positive CDT
AST + GGT	535	53	9.9
ALT + AST	3209	56	1.8
ALT + GGT	6001	25	0.41
AST +ALT +GGT	4913	150	3.1

The prevalence of CDT varies by twenty-fold depending on the combination of liver enzymes used to select samples for testing. Among samples with LFT elevations, the combination of AST and GGTP is uncommon, but in that subset almost 10% of the samples are positive for CDT. As with single enzyme elevations, combinations with ALT identify a subset of applicants with a lower prevalence for CDT.

### HDL as a Reflex Marker

Excess alcohol intake has been reported to increase the concentration of HDL. It would therefore seem reasonable to use high HDL levels as a reflex marker for CDT. Various levels for HDL have been suggested. Table 5 presents the data for the relationship between HDL concentration and CDT results.

**Table 5. HDL Levels and CDT.**

HDL mg/dL	Number Tested	Number CDT Positive	% Positive
10.0-20.0	52	0	0.00
21.0-30.0	1060	5	0.47
31.1-40.0	6082	11	0.18
41.0-50.0	9057	42	0.46
Total < 50mg/dL	16251	58	0.36
51.0-60.0	6297	89	1.41
61.0-70.0	3234	104	3.22
71.0-80.0	3227	129	4.00
>80.0	2841	236	8.31
Total 50mg/dL >	15599	558	3.57

The data are subdivided at 50mg/dL HDL. Approximately 50% of the sample falls into each of these two subgroups. While 50% of the samples have a HDL of 50mg/dL or greater, 90% of the CDT positive samples fall into this group.

## Samples with a HDL of 50mg/dL or greater, Liver enzyme elevation(s) and CDT

In this paper, I recommend that HDL of 50mg/ dL or greater be used in combination with liver enzyme elevation(s) to identify samples for CDT testing. The data to support that conclusion are presented in Table 5. The data in Table 6 may be compared to Table 3. The criterion of HDL of 50 mg/dL has been added to Table 3 data to produce the data presented in Table 6.

**Table 6. CDT Reflex for Samples with Liver Enzyme Elevation and HDL of 50mg/dL or Greater.**

GGT	% CDT	ALT	%CDT	AST	%CDT
66-97	2.76	45-67	3.01	41-61	4.88
98-130	3.16	68-90	3.42	62-82	8.33
130-162	3.59	91-112	4.69	83-102	9.18
163-195	3.59	113-135	4.59	103-123	12.58
196-227	5.11	136-157	4.49	124-143	11.36
228-260	6.00	158-180	2.33	144-164	10.53
>260	6.62	>180	2.62	>164	7.89

When the reflex criteria are modified to include HDL of 50mg/dL or greater, the relative prevalence of CDT in the tested population increases significantly. For example, the prevalence of CDT for samples with an AST of 103-123 U/L increases from 7.6% to 12.6% when this HDL criterion is added. While the combination of HDL and liver enzyme elevation increases the specificity, some CDT positives are missed. In the group of CDT positive samples, 445 of 493 (90.3%) have HDL values of 50 or greater. In this same population, the proportion of samples with HDL of 50 or greater is 43.9% (12,775/29,691). Thus, testing cost would be reduced 56.1% by adding the criterion of HDL 50 or greater to the CDT testing protocol on samples with elevated LFT's.

## Serum Alcohol and CDT

The best detector of alcohol abuse is a candid applicant. However, few people that abuse alcohol believe that they have a problem. As a result, self-reported alcohol use is seldom consistent with actual use. After ingestion alcohol is quickly cleared from blood. The rate of elimination is roughly 25mg/dL/hour.

Following collection the amount of alcohol present in a blood sample changes little. Alcohol is stable and not normally metabolized in cell free serum. In isolated cases alcohol may actually be formed if yeast are present in the non-sterile transfer tubes used to transport serum to the laboratory. This problem occurs more often in urine samples that contain glucose. The presence of alcohol, in serum or urine, does not prove alcohol misuse.

The presence of alcohol in a random blood sample suggests a high probability of misuse. At the request of some clients, alcohol is routinely tested in samples obtained from young applicants. A young applicant that misuses alcohol has a higher risk of becoming a fatality and more often has normal liver enzyme levels compared to older applicants. All samples that are positive for alcohol are tested for CDT.

**Table 7. Serum Alcohol and CDT.**

Alcohol (mg/dL)	Number Tested	CDT +	% Positive
10.1 - 50	71	10	14.08
50.1 - 100	36	6	16.67
>100	26	13	50.00
<b>Total</b>	<b>133</b>	<b>31</b>	<b>23.30</b>

Alcohol present in a random blood sample is strongly associated with the probability that the sample will be positive for CDT. The level of serum alcohol correlates with the risk of being CDT positive.

## Nicotine as a Reflex Marker for CDT

Smoking is strongly associated with drinking behavior. While not all drinkers smoke, there is a five-fold increase in CDT positive samples in cotinine positive applicants compared to their cotinine negative peers. As cotinine levels rise, so does the percent positive CDT. However, because non-smokers comprise approximately 80% of the population, the number of CDT positive samples is split almost 50/50 between the smoking and non-smoking population. Therefore, using smoking status as a reflex parameter may not be very beneficial.

**Table 8. Smoking and CDT Test Results.**

Level of Cotinine	Number Tested	CDT	% CDT
Negative (0.0-0.49)	28029	300	1.07
Low (0.5-1.0)	798	30	3.76
Moderate (1.1-2.0)	1099	53	4.82
Heavy (>2.0)	4071	254	6.24

## Additional Reflex Markers

The medical literature has suggested a number of different tests that may be useful for identification of the population that misuses alcohol. The results from our analysis of those markers are included in this paper. However, the data presented are derived for samples that were originally selected for CDT testing due to elevated liver enzymes, blood alcohol or high HDL. Therefore, the result for each additional marker is actually based on a double selection, the initial reflex marker plus the new marker.

## Triglycerides, Uric Acid, BUN and Their Relationship to CDT

As reported in the medical literature, heavy drinking has been associated with elevated triglycerides and uric acid levels. The association is stronger with beer than distilled alcohol. In contrast, the level of blood urea nitrogen (BUN), a marker of protein intake, is reported to decrease during chronic alcohol misuse. For our study population, the concentration for each of these markers is listed in the first column in Table 9 along with the percent of samples that test positive for CDT in the second column of each test. In our study, there was a moderate correlation between low triglyceride levels and positive CDT results and no clear correlation, either positive or negative with uric acid levels. However, there was a very strong correlation between low serum BUN levels and positive CDT results.

**Table 9. Concentration of Triglycerides, Uric Acid, BUN and Their Relationship to CDT.**

Triglycerides	% CDT	Uric Acid	% CDT	BUN	% CDT
10-100	4.25	0-3.0	0.56	0-5	19.72
101-150	2.11	3.1-6.0	2.08	5.1-7.5	10.89
151-200	1.18	6.1-9.0	1.80	7.6-10.0	4.03
201-300	1.02	9.1-12.0	1.43	10.1-15.0	1.75
>300	0.76	>12.0	0.00	15.1-20.0	1.25

## Combinations and Ratios of Reflex Markers

Various combinations and ratios of individual tests have been reported to increase the accuracy for detection of alcohol abuse. For example, a ratio greater than 2 for AST/ALT has been reported to identify all alcohol abusers. Tables 10 and 11 present the data for ratios for AST/ALT, GGT/alkaline phosphatase, HDL/BUN, triglycerides/HDL, and BUN/uric acid. While these are not the only possible ratios of interest, they illustrate how using ratios may affect prevalence in select subgroups of applicants.

**Table 10. Correlation of CDT Results with Ratios of AST/ALT and GGTP/Alkaline Phosphatase.**

Enzyme Ratio	%CDT Positive	
	AST/ALT	GGT/AP
0-0.5	0.25	1.84
0.6-1.0	1.56	1.52
1.1-1.5	4.24	1.58
1.6-2.0	5.58	2.41
2.1-2.5	7.74	2.87
2.6-3.0	8.13	4.23
>3.0	1.75	5.91

As the ratio of GGT to alkaline phosphatase increases, so does the percentage of samples positive for CDT. This pattern is also true for AST to ALT until the ratio exceeds 3.0.

The ratios of other non-enzyme markers were evaluated to determine their relationship to CDT. These include ratios for HDL, triglycerides, BUN, and uric acid. There is a significant relationship between CDT results and the ratios of HDL to BUN, triglycerides to HDL, and BUN to uric acid. These data are presented in Table 11.

**Table 11. Correlation of CDT Results with Ratios of HDL/BUN, Triglyceride/HDL, and BUN/Uric Acid.**

Ratio HDL/BUN	% CDT	Ratio Tri/HDL	%CDT	Ratio BUN/Uric Acid	%CDT
0.0-2.0	0.11	0.0-1.0	7.9	0.0-1.0	16.39
2.1-4.0	0.65	1.1-2.0	2.87	1.1-2.0	2.86
4.1-6.0	2.99	2.1-3.0	1.43	2.1-3.0	1.55
6.1-8.0	5.43	3.1-4.0	1.27	3.1-4.0	1.40
8.1-10.0	10.18	4.1-5.0	0.94	4.1-5.0	1.45
10.1-12.0	13.91	5.1-6.0	0.41	5.1-6.0	1.27
>12.0	24.37	>6.0	0.44	>6.0	1.13

Ratios of various tests may provide a method to select groups of individual samples that have higher than average positive CDT results. By using select ratios, the cost of reflex testing may be decreased.

## Confirmation of Bio-Rad %CDT Screening Test

The Bio-Rad %CDT test is a highly sensitive assay that detects samples with abnormal concentrations of CDT. The manufacturer-listed assay specificity and sensitivity are for the variance of measurement only and do not address the sensitivity or specificity relative to the use of the assay for detection of alcohol misuse. The accuracy of the Bio-Rad %CDT test in comparison to high performance liquid chromatography, a quantitative test, is listed as 98%. Accuracy was defined only as the comparison of data from HPLC and the Bio-Rad %CDT test. This number relates only to the comparison of the two methods and does not address how accurate the semi-quantitative Bio-Rad %CDT is for detecting alcohol misuse. Test accuracy can only be determined if the prevalence of the disease in a population is known and the sensitivity and specificity have been empirically determined for the test. In an effort to obtain an indirect measure of the accuracy of the Bio-Rad assay, test results were compared to companion data from capillary zone electrophoresis, CZE. Quantitative HPLC and CZE have equal specificity and sensitivity, and each demonstrates higher specificity than the qualitative Bio-Rad %CDT screening test.

In order to better understand how relative accuracy can be determined, the following information may be useful. If the prevalence of the disease is 100%, then each positive Bio-Rad %CDT screening test should be confirmed positive by CZE. In contrast, if the prevalence of the disease is 1% and the specificity of the screening test is 99%, then only half (50%) of the samples should be confirmed positive. By analyzing the confirmation rate for CDT, one can obtain a rough measure of both prevalence and specificity. For example, 25.3% of applicant samples with a BUN of 5mg/dL or less screened positive for CDT with the Bio-Rad test, and 77.8% of these cases were confirmed positive by CZE. In contrast, of samples with a BUN greater than 15mg/dL, only 6.4% screened Bio-Rad positive, and only 26.7% of this group was confirmed positive by CZE.

The underwriter may use the following tables to predict how often a sample that screened positive by the Bio-Rad %CDT test would be confirmed positive by the more specific CZE test.

**Table 12. Confirmation Rates for Bio-Rad %CDT assay by Capillary Zone Electrophoresis.**

BUN	% CDT Confirmed by CZE	Triglycerides	%CDT Confirmed by CZE	HDL	%CDT Confirmed by CZE
2.0-7.4	77.78	10-100	58.16	5.0-29.9	23.81
7.5-9.0	73.13	101-150	51.11	30.0-39.9	17.19
10.0-14.0	72.88	151-200	45.16	40.0-49.9	28.08
15.0-19.0	51.21	201-300	41.38	50.0-59.9	46.34
20.0-24.0	41.16	>300	45.00	60.0-69.9	52.34
25.0-30.0	38.30			70.0-79.9	60.91
>30.00	26.67			80.0>	63.90

**Table 13. Confirmation Rates for Liver Enzyme Positive Samples that Screened Positive by the Bio-Rad %CDT Test.**

GGT	% Confirmed	AST	%Confirmed	ALT	%Confirmed
0-65	52.15	0-41	47.02	0-45	52.95
66-97	45.14	42-61	58.37	46-67	47.01
98-130	50.88	62-82	66.28	68-90	50.75
131-163	58.02	83-103	63.83	91-113	54.79
164-195	60.00	104-123	65.38	114-135	57.14
196-228	59.26	124-144	66.67	136-158	57.14
229-260	60.87	145-164	42.86	159-180	60.00
>260	55.42	>164	36.84	>180	28.57

**Table 14. CZE Confirmation Rate for Samples that Screened Positive in the Bio-Rad %CDT Test.**

Cotinine Ug/ml	% Confirmed	Serum Alcohol mg/dL	% Confirmed
0.0-0.49	39.27	10-50	71.4
0.5-1.0	61.22	51-100	60.0
1.1-2.0	56.99	>100	92.9
>2.0	72.16		

CZE confirmation of Bio-Rad % CDT test varies widely. The variance in the confirmation rate is dependent on the prevalence of alcohol abuse in each sub-population. The higher the prevalence of alcohol misuse in a particular group, the higher the confirmation rate. The prevalence of alcohol misuse, as indicated by the CDT confirmation rates, varies by at least ten-fold depending on the marker selected for reflex testing.

## **Cost of CDT Testing**

In the risk selection process, everything bears a cost. CDT was introduced to allow the identification of applicants who misuse alcohol. Liver enzyme elevations result from any insult to liver tissue. In the insurance buying population, most elevations of liver enzymes are the result of insults from sources other than alcohol. These sources would include obesity, over-the-counter and prescription medications, excess coffee, normal aging, heavy metal exposure, viral hepatitis and many others. With the correct selection of reflex protocols, most of the CDT positive applicants can be identified, while controlling the cost of testing.

The underwriter should carefully study the enclosed tables to determine the most cost-effective reflex criteria for his or her company. My current recommendation for CDT testing criteria is: positive blood (urine) alcohol, elevations of GGT, and/or AST with a HDL of 50mg/dL or higher, BUN less than 7.6mg/dL, HDL of 70mg/dL or greater, and for cause.

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