EXPERIENCES IN C.D.T. TESTING

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ABSTRACT

The value of carbohydrate deficient transferrin (CDT) as the most direct and most accurate indicator of alcohol abuse has been amply demonstrated in the scientific literature. The CDT assay used by GIB Laboratories to assess serum samples for significant levels of alcohol abuse was validated using 184 clinically defined positive and 203 clinically defined negative specimens. Using a defined cutoff of significance and a special testing algorithm, GIB Laboratories has determined, in this study, that the sensitivity of the commercial CDT enzyme immunoassay (CDT EIA) is 88 percent, the specificity is greater than 99 percent, and the intra- and inter-run reproducibility is less than seven percent, about three times more precise than most confirmatory tests for CDT. Given the very low expected frequency of true physiological causes of non-specificity (less than one percent), the excellent specificity of the assay (in GIB Laboratories experience), and the technical problems, time delays, and costs associated with all "confirmatory" technologies, it cannot be recommended that CDT confirmatory testing be performed, unless an individual laboratory experiences unacceptable specificity in its screening tests. It would appear that, performing CDT "confirmatory" testing when the specificity of the screening test is as high as this study has found it to be, can result in three probabilities: 1) an increase in the number of false negative results; 2) few corrections in the very small number of false positive results; and 3) in greater cost and delay for the laboratory's clients.

INTRODUCTION

Currently, there is no universally accepted definition of alcohol abuse. According to many clinicians, the accepted clinical definition of an alcohol abuser is someone for whom alcohol poses a significant health risk and/or contributes to personal, occupational and or legal dysfunction. This generally correlates to someone who routinely ingests 40 to 60 or more grams of alcohol (four to six "standard drinks") per day. This corresponds to the approximate equivalent of at least four 1.5-oz. shots of hard liquor, or four 12-oz. beers (depending upon the percentage of alcohol in the specific type of beer), or five nine-oz. glasses of wine.

The prevalence of alcohol abuse in the United States varies from seven to 10 percent, or even higher (depending upon the source of the information). The American Psychiatric Association estimates it to be 7.41 percent in their diagnostic and statistical manual, fourth edition, whereas in the third edition it was estimated to be 8.63 percent.

The excess use of alcohol is involved in large percentages of many causes of death. This is illustrated by the following chart of "alcohol-attributable fractions for mortality."

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal cancer</td>
<td>75</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>75</td>
</tr>
<tr>
<td>Homicide</td>
<td>46</td>
</tr>
<tr>
<td>Deaths in fires</td>
<td>45</td>
</tr>
<tr>
<td>Mouth cancer</td>
<td>40-50</td>
</tr>
<tr>
<td>Motor vehicle accidents</td>
<td>42</td>
</tr>
<tr>
<td>Drownings</td>
<td>38</td>
</tr>
<tr>
<td>Accidental falls</td>
<td>35</td>
</tr>
<tr>
<td>Suicides</td>
<td>28</td>
</tr>
<tr>
<td>All other injuries</td>
<td>25</td>
</tr>
<tr>
<td>GI Tract diseases</td>
<td>10</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5</td>
</tr>
</tbody>
</table>

The accurate and economical differentiation between individuals who are abusers of alcohol and non-abusers is a substantial benefit for insurance underwriters. In addition to providing them with information to appropriately rate applicants for health and life insurance, based on historically established risks, evidence has shown that there is a strong association between alcohol use and cancers of the esophagus, pharynx, and mouth, as well as a possible link with cancer of the liver, breast, colon and rectum. In addition to the other types of injury and mortality normally associated with alcohol, the above cancers account for more than 125,000 deaths annually.

Chronic alcoholism can also result in nervous system and psychiatric disorders, pancreatic and liver ailments, including cirrhosis, and ranks as the sixth leading cause of death.
ALTERNATIVES TO EVALUATE ALCOHOL ABUSE

Historically, questionnaires, batteries of diagnostic tests, and liver status tests (LSTs) have been used to identify alcohol abusers. Denial, an almost universal characteristic of the alcohol abuser, renders questionnaires of dubious value. A lack of agreement in defining a uniform, reliable battery of tests which can give a valid indication of alcohol abuse, has caused underwriters until recently, to be guided almost exclusively by LST levels.

The LSTs – GGT, SGPT/ALT, and SGOT/AST especially – have historically been looked upon as markers, either alone or as part of a compound marker, for identifying alcohol abusers. The use of these enzymes and other semi-specific markers for this purpose, however, is contra-indicated for the following reasons:

1) LST levels can be elevated by any number of liver (viral and other types of hepatitis, drugs, etc.), heart (myocardial infarction), muscle (strenuous exertion) and other tissue traumas and/or other physiological traumas (autoimmune disease, drug use, infection, etc.), etc. LSTs are very indirect and non-specific indicators of liver trauma.

2) If LSTs are relied upon, many instances of alcohol abuse may be missed since prolonged (sometimes for years) biochemical insult by alcohol abuse is frequently necessary to result in consistent elevation of LSTs (due to alcohol abuse alone and/or combined with other causes).

3) Hemoglobin-associated aldehyde (HAA) is too insensitive (requiring six to eight standard drinks for about two weeks to result in a positive determination) and if made more sensitive, it becomes too non-specific. It is too insensitive, especially in individuals of American Indian and Asian extraction to be used as a reliable, sensitive, accurate marker for alcohol abuser identification.

The CDT molecule is a variety of transferrin (which is a normal serum protein having a normal concentration range = 200 to 400 mg./dl.). Its function is to serve as a safe, accessible repository for soluble iron, until it is needed for hemoglobin synthesis or some other physiological function. CDT formation is caused by the interference in the production of normal transferrin in the liver by the first metabolic product of alcohol degradation in the body, acetaldehyde. Acetaldehyde interferes in sialic acid incorporation into transferrin during its synthesis.

In the research and clinical arenas, CDT has long been proven to be the most reliable indicator of alcohol abuse, being validated in over 100 scientific publications. It has also been shown to be accurate and specific in different ethnic populations.

EVALUATION OF C.D.T. ASSAY

Assay background

The absence of sialic acid in CDT causes it to have different physico-chemical properties (isoelectric point, pI, of greater than or equal to 5.7) from normal transferrin (isoelectric point, pI, of less than or equal to 5.4). The column pretreatment (micro anion exchange chromatography) of the sample in the commercial CDT Enzyme Immunoassay (CDT EIA) assay makes use of this difference to separate CDT from normal transferrin prior to assaying for CDT.

With the adoption of the same commercial CDT assay, the CDT EIA, by almost all the major insurance laboratories, the value of CDT as a valid marker for identifying alcohol abusers has finally been technically acknowledged for risk assessment purposes. Prior to the availability of the CDT EIA assay, many laboratories used proprietary technologies to screen for CDT. These have been generally abandoned in favor of the commercially available test.

GIB Laboratories was one of the last risk assessment laboratories to recommend actively CDT testing to its clients. This step was taken only after we were certain of the diagnostic validity of CDT as a marker for alcohol abuse. About three-plus years ago, because GIB Laboratories was dissatisfied with the sensitivity and specificity of currently available CDT assay technologies, we developed and validated our own enzyme immunoaasay-based CDT assay which met our requirements for sensitivity, specificity, and economy. Shortly after this was accomplished, STC Diagnostics released a commercial enzyme immunoadsay-based CDT assay. Since this assay was produced in an FDA-approved manufacturing facility, and since use of their CDT EIA kit permitted a greater economy (which GIB Laboratories could pass on to its clients), we evaluated the sensitivity, specificity, accuracy and reproducibility of the STC kit.

Samples

Many studies which are focused upon evaluating a kit or a technology have a deficiency. This deficiency is associated with sample selection. Most of these studies rely upon one test to define the status of a sample, and the “standard” test is usually validated statistically, not experimentally. The analysis of the “test” method/technology compares the agreement of results from the “standard” method with those of the “test” method. Lack of agreement then assumes an imperfection of the “test” method. This is, of course, not valid at all. The only conclusion that can be drawn from such a study is concordance or discordance between the two assays’ results.

In evaluating the CDT EIA assay prior to offering it to our insurance company clients, we used clinically defined samples from
alcohol abusers and non-abusers, not simply samples that another technology had defined as positive. Serum samples which were defined as positive were obtained from individuals entering one of three alcohol detoxification programs (with which we collaborated) within 24 hours of the patient regularly (for at least 10 days to two weeks previously) consuming at least four to six standard drinks per day (by the donor's own admission). This is particularly important, since denial is one of the hallmarks of alcoholism. Clinicians who participated in supplying samples for this study warned that the ingestion of alcohol as claimed by donors was almost always less than the amount actually consumed.

Negative samples were obtained from detoxification clinics; from a complete spectrum of subjects who were total abstainers to those who claimed to ingest less than four standard drinks per day. Several potential negative “non-abusing” donors were rejected because their claims of alcohol ingestion were in question.

To ensure accurate results, it is crucial that the first step of the assay achieves the almost perfect separation of normal transferrin from CDT. Because of the technology employed (anion exchange chromatography in this step) it is crucial to ensure that all the transferrin molecules (CDT and normal isoforms) in the sample are saturated with iron. If they are not so saturated, erroneous results will be obtained due to imperfect separation, prior to assaying for CDT. All samples in this study, and in normal CDT evaluation at GIB Laboratories are subjected to this step to ensure that such iron saturation is accomplished.

**Analysis procedure**

For a CDT assay to return a significant result, the individual must have ingested at least 40 to 60 grams of alcohol per day for at least 10 days to two weeks prior to the taking of the blood sample.

GIB Laboratories analyzed 164 positive and 203 negative blood samples in order to determine sensitivity, specificity, overall accuracy, and the predictive values of positive and negative results of the STC CDT assay. The reproducibility of the assay was determined by repetitiously testing a uniform pool of samples.

Additionally, 1,016 random risk assessment specimens were tested to determine if there was a correlation of CDT level, liver enzyme test results, and to define certain demographics related to the frequency of alcohol abuse. It should be noted that the alcohol ingestion habits of the 1,016 anonymous applicants are unknown. This evaluation was performed only after the assay credibility (i.e. sensitivity, specificity, overall accuracy, and the predictive values) had been defined.*

The purpose of risk assessment testing is to confirm that individuals are good risks, and not to identify poor risks per se. Therefore, GIB Laboratories developed a custom testing algorithm and defined and validated a cutoff of significance to maximize accuracy, favoring specificity (the absence of false positive/ elevated results) over sensitivity.

**Results**

In order to achieve the very high specificity we knowingly sacrificed a small degree of sensitivity in defining our cutoff of significance. In doing so, any “false negative” results so obtained would be from marginal abusers only. If we set the cutoff of significance to a more sensitive level, we could have achieved a greater degree of sensitivity, but at the unacceptable cost of poor specificity.

Testing a pool of samples ten times on three separate days permitted us to determine the coefficient of variation of the CDT EIA assay from the raw test data.

With the battery of clinically defined test samples, GIB Laboratories obtained the following tabulated calculations which define the CDT assay (using our testing algorithm and cutoff of significance):

**CDT EIA assay validation**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>88.4</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.5</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>94.6</td>
</tr>
<tr>
<td>Predictive value of an “elevated” CDT result</td>
<td>99.3</td>
</tr>
<tr>
<td>Predictive value of “normal” CDT result</td>
<td>91.4</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>&lt; or = 7</td>
</tr>
</tbody>
</table>

The following tables show the results and correlations obtained on 1,016 random, risk assessment specimens received by GIB Laboratories. All samples were tested for CDT. Each sample defined as “elevated” for CDT gave repeatedly “elevated” levels above our designated cutoff of significance. The LST results from our standard insurance risk profile testing were retrieved for each sample and correlated with CDT results:

**Frequency of elevated LST results**

<table>
<thead>
<tr>
<th>Number</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,016</td>
<td>Number of random specimens</td>
<td>795</td>
</tr>
<tr>
<td>221</td>
<td>Total “elevated” LSTs</td>
<td>21.76</td>
</tr>
</tbody>
</table>

The frequency of elevated LST results (21.76 percent) is very similar to that which GIB Laboratories routinely obtains. This gives credence that the random 1,016 samples are representative, and probably predictive, of our overall sample population.

* No confirmatory testing was performed on any sample. The reasons for this are explained in the “discussion.”
Frequency of elevated CDT results

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of random specimens</td>
<td>1,016</td>
</tr>
<tr>
<td>Total &quot;normal&quot; CDT</td>
<td>959</td>
</tr>
<tr>
<td>Total &quot;elevated&quot; CDT</td>
<td>57</td>
</tr>
</tbody>
</table>

So 5.61 percent of the 1,016 random specimens tested gave repeatedly elevated CDT results.

LST results in normal CDT samples

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Normal&quot; CDT results</td>
<td>959</td>
</tr>
<tr>
<td>&quot;Normal&quot; CDT and no elevated LSTs</td>
<td>751</td>
</tr>
<tr>
<td>&quot;Normal&quot; CDT and any elevated LST</td>
<td>208</td>
</tr>
</tbody>
</table>

Only slightly fewer samples with "normal" CDT levels also had elevated LST levels (20.5 percent), than was the case overall (21.76 percent).

LST Results in elevated CDT samples

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Elevated&quot; CDT of total samples</td>
<td>57</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and no elevated LSTs</td>
<td>44</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and any elevated LST</td>
<td>13</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and all elevated LSTs</td>
<td>1</td>
</tr>
</tbody>
</table>

Only 22.8 percent of alcohol abusers (as represented by those samples with "elevated" CDT results) would have been identified if LST reflexing were the only criterion for ordering CDT tests to be performed.

Correlation of elevated CDT, elevated LST results

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Elevated&quot; CDT of total samples</td>
<td>57</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and any elevated LSTs</td>
<td>13</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and elevated GGT</td>
<td>9*</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and elevated AST</td>
<td>4*</td>
</tr>
<tr>
<td>&quot;Elevated&quot; CDT and elevated ALT</td>
<td>6*</td>
</tr>
</tbody>
</table>

* Some samples were elevated for more than one LST.

Elevated and normal CDT results in elevated LST

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total elevated LSTs</td>
<td>221</td>
</tr>
<tr>
<td>Elevated LST / &quot;normal&quot; CDT</td>
<td>208</td>
</tr>
<tr>
<td>Elevated LST / &quot;elevated&quot; CDT</td>
<td>13</td>
</tr>
</tbody>
</table>

The diagnostic efficiency of reflexing orders for CDT testing from elevated LST results is 5.9 percent.

Frequency of elevated CDT results by age

<table>
<thead>
<tr>
<th>Age</th>
<th>Distribution of &quot;elevated CDTs&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>5.3%</td>
</tr>
<tr>
<td>26 - 30</td>
<td>10.5%</td>
</tr>
<tr>
<td>31 - 40</td>
<td>45.6%</td>
</tr>
<tr>
<td>41 - 50</td>
<td>22.8%</td>
</tr>
<tr>
<td>51 - 60</td>
<td>14.0%</td>
</tr>
<tr>
<td>60 - 70</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

Total number of "elevated" CDTs = 57. Most (68.4 percent) of the "elevated" CDT samples were from applicants from 31 to 50 years of age.

Elevated CDT results by policy face amount

<table>
<thead>
<tr>
<th>Policy face amount *</th>
<th>Distribution of &quot;elevated CDTs&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 100</td>
<td>42.9%</td>
</tr>
<tr>
<td>101 - 150</td>
<td>7.1%</td>
</tr>
<tr>
<td>151 - 200</td>
<td>3.6%</td>
</tr>
<tr>
<td>201 - 250</td>
<td>16.1%</td>
</tr>
<tr>
<td>251 - 300</td>
<td>10.7%</td>
</tr>
<tr>
<td>301 - 500</td>
<td>5.4%</td>
</tr>
<tr>
<td>501 - 1,000</td>
<td>7.1%</td>
</tr>
<tr>
<td>1,001 - 10,000</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

* (x $1,000)

Total number of "elevated" CDTs = 57

DISCUSSION AND CONCLUSIONS

GIB Laboratories has found that the CDT EIA test is both sensitive and specific enough to differentiate between alcohol abusers and non-abusers, based upon the testing data using the specimens evaluated. Both the size and the type of the serum sample population (clinically defined) used to evaluate the CDT EIA assay lend credence to the findings.

In examining the test results of the random specimens, the frequency of elevated LST results (21.76 percent) is very similar to that which GIB Laboratories routinely obtains. This allows the assumption that the random 1,016 samples are representative, and probably predictive, of our overall sample population.

The DSM IV incidence of alcohol abuse, 7.41 percent, is higher than was observed (5.61 percent) in this study. This was expected. Taking into account the assay's 88 percent defined sensitivity in this study, and the expectation that the prevalence of alcohol abuse in the insurance buying population is lower than that in the general population, it would have been surprising if the study's incidence was higher than that in the general population. That would have indicated a high degree of non-specificity. This data
is confirmed by the excellent specificity (>99 percent) using defined clinical specimens.

**LST reflexing**

The data shows that ordering the CDT assay, based only upon reflexing off one or more elevated liver status tests, is not supported fully. A strong positive correlation did not exist between CDT assay results and elevated liver status tests (GGT, AST, and/or ALT) and vice versa. Only 13 of 221 (5.9 percent) specimens with elevated LSTs also had "elevated" CDT result. Liver enzymes in the remaining 208 specimens were elevated for undefined reasons other than current alcohol abuse. Among the other cause(s) of the LST elevation might have been infectious hepatitis, therapeutic and/or recreational pharmaceuticals, pathological degenerative liver disease, etc.

With elevated LST-only reflexing, only 22.8 percent of the alcohol abusers (as defined by "elevated" CDT results) would have been reported. The 77.2 percent of all "elevated" CDT samples (alcohol abusers) would not have been detected.

Given the validated sensitivity of the CDT assay, as presented here, it is possible that about 12 percent of the LST positive/CDT "normal" specimens might be from marginal alcohol abusers, not detected as "elevated" by the assay. As was stated previously, the 88 percent sensitivity was found to be acceptable in order to achieve the excellent level of specificity (99 percent) obtained in this study.

**Demographic information**

By far, the group of applicants showing the highest frequency of alcohol abuse (in the study of 1,016 random risk assessment samples) was the group applying for less than $100,000 of insurance. Those applying for more than $100,000, collectively however, did account for the majority of "elevated" CDT results.

Secondly, individuals in the 31 to 40 year and 41 to 50 year age brackets accounted for 68.4 percent of detected alcohol abusers in this study, as indicated by "elevated" CDT results.

**Evaluating CDT test results**

In assessing CDT results, these points should be considered:

- Non-alcohol abusers may have levels (below the cutoff of significance level) of CDT in their serum. This may be due to non-abusing consumption or, very rarely, pathological, genetic errors in the structure of transferrin.
- It is possible that an abuser who has been abstinent for about 10 days to two weeks may be reported as a non-abuser. Since the half-life of CDT (the rate of disappearance of the CDT from the body) is only about 10 to 14 days, abstinence for that period (or even less) can result in the drop to a CDT level below the cutoff for significance. This is a physiological and biochemical fact of life.
- The sensitivity of proper transferrin synthesis to disturbance by alcohol's initial metabolic product (acetaldehyde), which is the substance which causes CDT formation) can vary within the population. A greater sensitivity of transferrin synthesis to disruption by alcohol/acetaldehyde results in higher than typical CDT levels in the serum than might be indicated by amount of alcohol ingestion. The converse is also true.
- Each person's ability to metabolize alcohol can vary greatly. For instance, someone who converts alcohol to acetaldehyde slowly will have a lower than expected level of CDT in their serum, and vice versa.
- One CDT value which is double another does not necessarily mean that the first person consumes twice as much alcohol as does the second person. Although elevated CDT is found in the vast majority of all alcohol abusers, the consumption of alcohol is generally, but not precisely and directly proportional to the production of CDT from person to person for the above reasons.

For all these reasons it is not recommended to report quantititative levels of CDT as measured in an applicant's serum sample. "Negative/positive" or "normal/elevated" reporting format conveys the best, most useful information to the underwriter.

LST values are not the direct result of alcohol upon the liver. Any effect of alcohol upon the liver which results in the elevation of LSTs is due to many intermediary biochemical and physiological steps. Alcohol's effect upon CDT generation within the liver is direct: alcohol's first metabolite (acetaldehyde) has been shown to interfere with sialic acid incorporation into transferrin during its synthesis in the liver. CDT and LST results may or may not coincide. The following table can serve as a guide in understanding the physiology of CDT and LST results.

<table>
<thead>
<tr>
<th>CDT</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated</td>
<td>Very probably alcohol induced.</td>
</tr>
<tr>
<td></td>
<td>LSTs may also be elevated by other causes</td>
</tr>
<tr>
<td>LST</td>
<td>Alcohol abuser not long enough for liver to be damaged</td>
</tr>
<tr>
<td>Normal</td>
<td>Liver insensitive to alcoholic insult.</td>
</tr>
<tr>
<td></td>
<td>&lt; 1 percent of the time, D-variant</td>
</tr>
</tbody>
</table>
It is obvious that there are instances of “disagreement” between CDT results and LST levels. Such results can be seen as in disagreement or as “discrepancies” only when one forgets the production of elevated amounts of CDT in an individual is a direct connection to alcohol abuse and the development of LST elevations by prolonged alcohol abuse are brought about by a very indirect series of biochemical mechanisms which are independent of CDT formation. These very indirect biochemical mechanisms result in the release of LST enzymes, usually (but not always) from the liver. One cannot expect a one-to-one relationship between different independent physiological mechanisms!

Inborn errors in glycoprotein metabolism and genetic D-variants of transferrin sometimes result in elevated levels of CDT to be reported. This situation is very rare, with a frequency estimated between 100 cases worldwide to far less than one percent of any given population. Is a transferrin D-variant merely a genetic and biochemical quirk, a peculiarity, an anomaly, or does it cause clinically significant difficulties (whether or not it is statistically important)? One argument given for performing confirmatory CDT tests is that a person who is a D-variant will be unfairly rejected for insurance. Individuals who are true D-variants are likely to have a variety of significant pathological difficulties. Another article, by Dr. Stibler describes the variety of symptoms of individuals so afflicted (actual phenotypic expression, not merely carrying a masked, mutated, non-expressed gene) with the transferrin D-variant mutation. Here are some of the maladies/abnormalities listed in her article:

- severe neurological impairment
- abnormal enzymes
- abnormal lipid levels
- cerebellar hypoplasia
- skeletal abnormalities
- low serum cholesterol,
- psychomotor retardation
- lipodystrophy
- low albumin level
- various neuropathies
- hepatomegaly

Clearly, these unfortunate individuals are not normal, healthy people. Given all of these physical and/or pathological difficulties, an elevated CDT would probably not be the only indicator that would cause an underwriter to be concerned with the risk of insuring the applicant. Even if it was the only adverse indicator, there seems to be ample reason to rate such an individual more stringently. It would seem that identifying D-variants as well as alcohol abusers is not a bad idea.

**Confirmatory testing**

For a confirmatory test to be useful and valid for any diagnostic purpose, all of the following essential criteria must be met:

- It is needed only if the screening test is insufficiently specific.
- Its returned values must be more precise than those of the screening test (less than ~7 percent for CDT), and minimize or eliminate subjectivity in interpreting the test results.
- It uses a truly different technology, so that errors associated with the screening technology are not perpetuated.
- It must be at least as sensitive as the screening test it is supposed to confirm; otherwise, false negative results will be returned. If this is not achieved, the result will be lower overall accuracy (due to a higher frequency of false negatives than corrected number false positives identified by the confirmatory test).
- It must be 100 percent specific and be able to discriminate between target and non-target substances.

Several major technologies are used or have been used in the past by those laboratories that choose to confirm elevated CDT test results. These technologies are listed and discussed below:

- Isoelectric focusing (IEF): This is a qualitative procedure which is costly, demonstrates a lack of sensitivity, requires subjective interpretation, and is unsuitable for mass evaluation (very labor intensive). Since its interpretation is so subjective and non-numerical, a CV cannot be determined.

- Combination of IEF, immunoblotting (IB), and laser densitometry (LD): This entails transferring (literally by blotting) the separated proteins from the IEF gel to a membrane. This immunological procedure amplifies the visibility of transferred proteins. Analyzing the results on a laser densitometer improves on the IEF's lack of sensitivity and irreproducibility. Although this technique appears promising, it is uncertain if it has been validated with a significant enough number of clinically defined samples. In order truly to differentiate between CDT (contains an error in its carbohydrate component) and transferrin D-variant (contains an error(s) in the protein component), an original IEF, IB and LD, followed by an enzymic digestion, and a repeat IEF, IB and LD (on suspect samples) is needed. Every diagnostic procedure has its own inherent degree of imprecision. The more steps there are, the more imprecision is added to the result. This confirmatory scheme has a reported CV of between 15 and 20 percent. Which means that a given reported result could be anywhere from 15 to 20 percent below to 15 to 20 percent above the reported value (an accuracy range of 30 to 40 percent) The CDT FIA assay has a reported CV of less than or equal to seven percent. This means an accuracy range of only 14 percent, or about three to four times more reliable!

- Capillary zone electrophoresis (CZE): This technique is an improvement over IEF. Laboratories we have contacted in-
dicate it may suffer from imprecision (a functional CV of ~15 to 20 percent). Originally it was used as a screening technology. Now some labs use it for CDT confirmation.

- Western blot (WB). Western blot technology is immuno-blotting, as described above, but without electrophoresis to separate actively the proteins. This use of a membrane as the solid phase of an immunoassay confirmatory test can only correct non-specific, random attachment of extraneous proteins (such as the assay’s amplifier molecules) to the solid phase plastic of the screening test. This is especially true if the nature of the identifying assay’s amplifier molecule of the screening and the WB assays are the same. Its only valid use can be to correct deficiencies in the architecture and/or biochemistry of the screening test which can cause falsely elevated CDT results. It cannot be used by itself to confirm truly an elevated CDT result.

Based upon the data obtained in this study, additional confirmatory testing cannot be recommended to validate elevated CDT EIA test results. There are several reasons for this recommendation. They are:

- The specificity of the CDT assay in this study exceeded 99 percent (i.e. there are very few, if any false positive results).
- Only two very rare pathologies are believed to cause significantly elevated levels of CDT. The incidence of these is not significant enough to justify the financial cost and time delay in performing confirmatory testing.
  - Genetic D-variant syndrome’s prevalence is estimated to be significantly less than one percent confirmed cases worldwide. Only about 50 percent of genetic D-variant individuals tested for CDT have been shown to have elevated CDT assays.
  - Primary biliary cirrhosis, a rare liver disease, has an extremely low prevalence and is usually encountered in women over 50 years of age almost exclusively. Women so afflicted will demonstrate other markers of liver function abnormality.

- Technologies available for confirmation are too insensitive, too irreproducible, too subjective in interpretation, too costly and/or too time consuming. The insensitivity of the confirmatory assays is expected to result in more false negatives than “corrected” false positives.

CDT has long been proven to be an accurate and specific marker to differentiate between alcohol abusers and non-abusers. The CDT assay in use by most of the major insurance risk assessment testing laboratories has been shown in this study to have excellent sensitivity and specificity, clinical accuracy, precision, and a very high predictive value. Given the test results, the very low probability of encountering true physiological causes of falsely elevated CDT results presented here, the cost, which is typically amortized in whole or in part over the total number of CDT tests performed, and the time delay associated with confirmatory testing, confirmatory testing can not be justified.

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