Allelic D Variants of Transferrin in Evaluation of Alcohol Abuse: Differential Diagnosis by Isoelectric Focusing–Immunoblotting–Laser Densitometry

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In the diagnosis of alcohol abuse transferrin (Tf) allelic D variants generate false-positive test results for carbohydrate-deficient transferrin (CDT) as assessed by their electrophoretic migration patterns. The predominant Tf C, allele encodes a protein for which the most prevalent isoform has a pI of 5.4, i.e., four sialic acids and two bound iron molecules. Carriers of allele D encode Tf's with different amino acid sequences, for which the pI is >5.7, despite their identical iron and carbohydrate composition. We used isoelectric focusing, immunoblotting, and laser densitometry (IEF-IB-LD) to distinguish Tf D variants from CDT. Alcohol abusers carrying the D allele tested CDT⁺; D nondrinkers were CDT⁻. Although normal controls (<15 g of alcohol per day for 7–10 consecutive days) carrying variants D₁, D₂, or D₄ exhibited abnormal IEF banding patterns, they did not generate false-positive results for CDT. D₃ variants expressed isoforms that migrate at the same pI as CDT bands. Thus, IEF-IB-LD yields a highly resolved banding pattern to distinguish most Tf D variants from CDT.

Indexing Terms: carbohydrate-deficient transferrin/variation, source of/iron binding/sialic acids/glycosylation

Transferrin (Tf) is a serum glycoprotein that contains a maximum of six sialic acid residues, located at the end of two triantennary branched complex-type carbohydrate structures (1).⁴ Among the isoforms of Tf in blood, the most prevalent contains four sialic acid residues linked to two biantennary oligosaccharides (1, 2). These sialic acid residues influence the electrophoretic mobility of the Tf molecule such that their sequential removal gives rise to six possible charge states. The most acidic isomorph contains six sialic acid residues; the most basic isomorph contains none (1–3). Alterations in the electrophoretic mobility of Tf have been described in association with alcohol abuse (4–6). Disturbances of the Golgi-associated glycosylation pathway, caused by ethanol metabolism, result in increased concentrations of carbohydrate-deficient transferrin (CDT) isoforms containing two, one, and no sialic acid residues. CDT is currently the best available marker for the diagnosis of alcohol abuse because of its high specificity and sensitivity. CDT isoforms have been detected and quantified by charge-based separation procedures, i.e., microanion-exchange chromatography (MAEC) followed by various immunoassays (5), automated HPLC (7), semiautomated isoelectric focusing (IEF) (8), and IEF followed by immunoblotting (IEF-IB) (4); the last named procedure is considered the current gold standard (9, 10).

Methods for CDT quantification must discriminate between Tf isoforms that have deficits in sialic acid residues and normal Tf isoforms that lack iron (Fig. 1). Conditions of total iron saturation (lane C) have been used to separate CDT (bands 3–6) from normal Tf (band 1 and 2), but because these procedures involve incubating sera in buffers lacking synergistic ions, the amount of iron saturation is variable (5, 6). As a result, normal Tf that has not been fully iron-saturated (lane F, band 3, 4, and 6) could give a false-positive result for CDT. We find that partial iron saturation provides excellent conditions for discriminating between the migration patterns of Tf specimens derived from alcohol abusers (bands 8–10) and those from normal consumers or at washers (bands 1–7) (4). Although fully iron-saturated CDT (lane C, bands 3 and 4) may be missed under these conditions, false-positive results are minimized.

The degree of polymorphic variation in Tf is limited most individuals (75–90%) in most ethnic groups express the most common Tf allele, C₁. Indeed, ~29% of the population is heterozygous for the C₁ allele (1). Because Tf gene expression is codominant, C₁ is ex

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³ Nonstandard abbreviations: Tf, transferrin; CDT, carbohydrate-deficient transferrin; IEF-IB-LD, isoelectric focusing, immunoblotting, and laser densitometry; and MAEC, microanion-exchange chromatography.

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Fig. 1. Effects of iron content and sialic acid composition on Tf pl. Tf bands 1–10 and pl markers (5.20–6.55) are indicated. Lane A: Afinity purified Tf from an alcohol abuser analyzed by IEF-Coomassie Blue stain under conditions of partial iron saturation: lane B: a schematic representation of the IEF-CB gel shown in lane A; lane C: fully iron-saturated Tf with various numbers of sialic acid residues (loss of each sialic acid residue results in an increase of 0.1); lane D: apo-Tf with various numbers of sialic acid residue (loss of each sialic acid residue results in a pl increase of 0.1); lane E: Tf with no sialic acid residues under conditions of partial iron saturation (loss of each iron molecule results in a pl increase of ~0.2); lane F: Tf with four sialic acid residues under conditions of partial iron saturation (loss of each iron molecule results in a pl increase of ~0.2). [ , sialic acid residues; , iron molecules; , Tf with two biantennary oligosaccharides (carboxyl terminus at the right end of the protein). ---- indicates faint isoform in our system.
pressed in these individuals in conjunction with another allele product. At least 20 electrophoretic variants are now recognized that differ from C1 with regard to electrophoretic mobilities in starch and acrylamide gels (2, 12). Variants form two main groups: B if the electrophoretic mobility is anodal to C1, and D if it is cathodal. Carriers of the C1 allele encode a Tf protein whose most prevalent isoform, i.e., with four sialic acids, has a pI of 5.4 when it binds two iron molecules. Carriers of the D or B allele encode Tf isoforms whose variation in amino acid sequence give them a pI of 5.7 and 5.2, respectively, even though their iron and carbohydrate composition is the same as that encoded by the C1 allele.

Here we determined whether high-pI (>5.85) Tf isoforms result from a decreased sialic acid content due to alcohol abuse, i.e., true CDT, or represent genetic variants of the D type with a normal carbohydrate content but an altered amino acid sequence. The electrophoretic patterns of serum samples from alcohol abusers and normal controls were compared with each other and with samples of known Tf genotypes (13).

Materials and Methods

Serum specimens. Sera from individuals carrying the C and D variants of Tf were obtained from the Minneapolis War Memorial Blood Bank in Minnesota, where their subtypes had been characterized by IEF techniques (13). Remnant sera were obtained from alcohol abusers admitted to the Emergency Room Hematology Unit, USC-LA County Hospital, Los Angeles, CA; all patients had histories of continuous prolonged alcohol abuse and admitted to having ingested the equivalent of at least 60 g of alcohol per day during the 10–15 days before admission. Sera obtained from normal alcohol consumers were collected at Specialty Laboratories. Upon detailed interview conducted by one of us, we selected healthy donors who reported having consumed alcohol <15 g/day during the 2 weeks before sample collection. All individuals tested agreed to this study at the time of their interviews.

Affinity chromatography. Tf-specific antibodies (Tago Immunologicals, Burlingame, CA) were coupled to Sepharose 4B gel (Pharmacia, Piscataway, NJ) according to the manufacturer’s specifications. To prepare purified Tf from serum, we mixed 3 mL of gel with 1 mL of serum for 30 min at room temperature. The mixture was washed twice with 0.2 mol/L phosphate-buffered saline (pH 7.2), and once with 2 mol/L KI. Purified Tf was eluted with 0.1 mol/L glycine-HCl, pH 2.3; the eluate was neutralized with 1.0 mol/L NaOH. After overnight dialysis against H2O at 4°C, the purified Tf was concentrated by lyophilization.

Neuraminidase digestion. Digestion of serum proteins with neuraminidase (EC 3.2.1.18, from Clostridium perfringens; Sigma Chemical Co., St. Louis, MO) was performed according to the supplier’s specifications. Briefly, reaction mixtures containing 5 μL of serum in 20 mmol/L sodium acetate, pH 5.0 (30 μL final volume) were incubated with 30 mU of neuraminidase at 37°C for 1 h, followed by overnight incubation at room temperature for extensive digestion. Complete neuraminidase digestion was determined by IEF-IB. In the absence of iron, neuraminidase-digested human apo-Tf migrated as a single band corresponding to Tf devoid of iron and sialic acid residues. In the presence of iron, neuraminidase-digested human apo-transferrin migrated as two separate, distinct bands: asialo-Tf with two irons; asialo-Tf with one iron at the amino-terminus; asialo-Tf with one iron at the carboxyl-terminus; and asialo-Tf with no iron residues (4).

IEF–Coomassie Blue staining. IEF analysis of affinity-purified Tf was carried out in 5% polyacrylamide gels containing a pH 4–8 gradient of ampholytes as described previously (4). Briefly, the gel gradient was obtained by mixing 1 part of ampholytes, pI 3–10, with 3 parts of ampholytes, pI 4–6, and pharmalytes, pI 5–8. The electrode solutions were 20 mmol/L NaOH and 20 mmol/L H3PO4. Gels were prefocused for 20–30 min at a constant power of 20 W until 1000 V was reached. After sample loading, the gel was run at the same constant power for 2 h. Staining was done with Coomassie Brilliant Blue.

IEF-IB laser densitometry (LD). Sera and a preparation of human Tf (Sigma) were partially saturated with iron by incubation in a buffered iron-containing solution (per liter: 0.2 mmol of FeCl3, 0.012 mmol of sodium phosphate, and 5 μmol of sodium citrate, pH 7.2) at 37°C for 90 min as described previously (4, 9). IEF analysis of serum (7 μL of a 1:20 dilution) was carried out as specified above for affinity-purified Tf. Proteins separated in IEF gels were electrotransferred to nylon membranes (Millipore Corp., San Francisco, CA) by standard procedures (14). Incubation with rabbit anti-human Tf antibodies (Dako Corp., Carpinteria, CA), diluted 1:1000 with 50 g/L powdered skim milk in phosphate-buffered saline (PBS; 0.015 mol/L phosphate buffer, 0.15 mol/L NaCl), was performed at room temperature for 60 min, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit Ig (Tago Immunologicals) diluted 1:1000 with PBS containing 3 mL of Tween-20 per liter. Color was developed with the Bio-Rad alkaline phosphatase kit (Bio-Rad, Richmond, CA).

In our IEF-IB-LD procedure, CDT is quantified by volume integration of the laser-scanned immunoblot. The absorbances of the bands separated by IEF-IB are determined by a personal densitometer (Molecular Dynamics, Sunnyvale, CA), which has a laser scanner and light-integrated cylinder for quantification. Each lane (specimen) in the test is enclosed by a grid made up of two rows and one column. The upper row of the grid encloses the two CDT diagnostic bands, and the lower row of the grid encloses all other Tf bands. Using volume integration, we determine the sum of absorbance values within the grid. CDT is quantified by a three-step process: (a) For each specimen, the ratio of the absorbance of the criteria bands to that of the other bands is calculated; (b) the absorbance ratio calculated for each specimen is divided by the absorbance ratio obtained for the strongly positive control present in each gel; and (c) the value obtained for each specimen is multiplied by
100 and expressed in units. One densitometry unit (DU) is defined as 1% of the ratio of CDT:Tf exhibited by the strongly positive control run in each gel. The mean value obtained after IEF/IB analysis of 100 normal controls (<15 g of alcohol daily for 7–10 consecutive days) was 4 DU (SD 2).

Results

Banding Pattern of Affinity-Purified Tf from Controls and Alcohol Abusers

Affinity-purified Tf, pretreated with a buffered iron-containing solution as specified in Materials and Methods, was separated on acrylamide gels containing ampholytes, pH 4–8, followed by Coomassie Blue staining (Fig. 2). Sera from the two normal controls (N1, N3) and four alcohol abusers (P1, P2, P3, and P4) displayed the common isoforms (bands 1–6) migrating between the pl 5.2 and 5.85 markers. Sera from the alcohol abusers exhibited additional isoforms migrating between the pl 5.9 and 6.35 markers, i.e., previously described CDT diagnostic isoforms that were faint in the normal controls (4, 5, 9). One of the alcohol abusers (P4) showed, in addition to the seven common bands, three more basic isoforms migrating cathodic to bands 2, 4, and 6, respectively, suggesting that this individual is heterozygous for two codominant Tf alleles. Furthermore, bands 3 and 5 in this patient exhibited the intensity seen for the normal controls rather than the alcohol abusers.

Identification of Tf Variants

In serum of an alcohol abuser. Serum proteins from three alcohol abusers (P2, P3, and P4) and a control (N1) were separated by IEF, transferred to nylon membranes, and incubated with rabbit anti-human Tf-specific antibodies (Fig. 3). The isoforms detected in these immunoblots resemble those obtained for affinity-purified Tf in the Coomassie Blue-stained gels; i.e., seven common isoforms migrating between the pl 5.2 and 5.95 markers were present in the control and in the alcohol abusers. Sera from the three alcohol abusers also exhibited the CDT diagnostic bands migrating between the pl 5.95 and 6.35 markers. The CDT diagnostic bands in P4 were faint, but present. Compared with the common banding pattern of 10 Tf isoforms exhibited by most alcohol abusers, sera from P4 exhibited additional Tf isoforms. These additional isoforms were present in both affinity-purified Tf and serum analyzed by IEF-IB.

After pretreatment of the serum specimens with neuraminidase, the IEF-IB pattern in Fig. 3 showed four bands common to the normal control individual (N1') and the alcohol abusers (P4', P3', and P2') migrating between 5.85 and 6.35 pl markers. These bands represent asialo-Tf with two iron molecules (band A), one iron molecule at the amino terminus (band B), one iron bound at the carboxyl terminus (band C), and no iron molecules (band D)—the same banding pattern as digested human Tf (lane Tf). Four additional bands were present in the sera from alcohol abuser P4 (P4'), each migrating above bands A–D. These additional bands also represent Tf isoforms devoid of sialic acid residues, but they are coded for by a different allele; i.e., they exhibit a distinct charge because of a different amino acid composition.

In serum of a nondrinker. To determine whether a normal nonabuser carrying a cardiac allelic variant of Tf (N2) would give false positives for CDT in our system, we compared the IEF-IB banding pattern for Tf (N2) with that of alcohol abusers exhibiting the common Tf isoforms (Fig. 4). The three control serum specimens run in each immunoblot were a negative control (N1) and two positive controls (from alcohol abusers P2 and P3), which demonstrated weak and strong CDT+ isoform bands, respectively. Quantification of the results by LD showed CDT values of 5 and 12 DU for the negative and weak-positive control, respectively. The intra- and interassay CVs were 10% and 20%, respectively.

Four additional alcohol abusers (P5–P8) displayed a banding pattern that closely resembles that seen in the CDT+ controls. Quantification of CDT by densitometry indicated values of 15, 50, 11, and 8 DU for P5, P6, P7,
indicated.

Sera (1 μL) derived from two normal consumers of alcohol (N1, N2) and six alcohol abusers (P2, P3, P6, P7, P8) were separated by IEF and analyzed by IB. The common Tf isoforms (bands 2, 4, 6) and CDT are indicated.

and P8, respectively. Serum from a nondrinker (N2) showed an abnormal banding pattern, with two prominent isoforms migrating above bands 5 and 6. CDT quantification for this nondrinker gave a value of 1 DU. Thus, although the serum from this nondrinking individual (N2) exhibited additional cathodic Tf isoforms in IEF-IB, CDT quantification by LD yielded a CDT value similar to that found for the control individual (N1) rather than that for the alcohol abusers (P2–P8).

Serum IEF-IB Pattern for Carriers of Tf D Variants

The banding patterns in sera from individuals carrying the Tf allelic variants C1, D1, D2, D3, and D4 were compared with those of normal individuals N1 and N2 and alcohol abusers P3 and P4 (Fig. 5). The most common isoforms (bands 1–7) seen in IEF-IB, as exemplified by the normal control N1 and the alcohol abuser P3, resembled the isoforms displayed by the individual carrying the C1 allele of Tf. As expected, no CDT isoforms were present in the C1 reference serum derived from a nondrinker (C1). The banding pattern exhibited by the alcohol abuser P4 resembled the pattern detected for the individual carrying the D variant of Tf (D3). Additional bands were seen in this patient (P4) within the pH 5.8–6.3 region, indicating Tf allele products deficient in carbohydrates as a consequence of alcohol abuse. The banding pattern exhibited by the normal consumer N2 resembled the bands detected for the individual carrying the TfD3 variant. The most cathodic band in the D1 specimen migrated above band 6, but below the CDT diagnostic isoforms.

The banding patterns derived from all five Tf D variant reference sera were compared with those of a normal individual (N1) and an alcohol abuser (P3) who exhibited the most common C1 allele of Tf (Fig. 5). Tf genetic D variants each showed prominent isoforms cathodic to bands 2, 4, and 6 as reported previously in comparable IEF systems (2, 11–13, 15). In addition to the D allele bands, all seven isoforms displayed by the C1 carrier (bands 1–7) were present, indicating coexpression of the C and D alleles in these reference sera. All D variants exhibited isoforms migrating above the pH 5.9 marker, the migration landmark for the most prominent cathodic isoform in C1 normal controls. However, only genetic D3 variants express isoforms that migrate at the same pH as the CDT diagnostic bands; Tf variants D4, D1, and D3 express allelic products that move faster than the CDT isoforms in this system. CDT quantification by LD indicated values of 3, 5, 6, 74, and 24 DU for Dx, D1, D3, and D3o, respectively (Fig. 5).

Bandung Pattern of Sialic Acid-Free Tf Allelic Variants

To determine the contribution of amino acid composition to the pH of different Tf isoforms, sialic acids were removed by hydrolysis with neuraminidase. Neuraminidase-treated reference sera from individuals carrying the Tf allelic variants C1, D1, D2, D3, and D4 were compared with those of the normal individuals N1 and N2 and the alcohol abusers P3 and P4 (Fig. 6). The bands seen in this gel represent Tf variants with different iron and amino acid content but devoid of sialic acid residues. All specimens showed the four main isoforms (bands A–D) present in the Tf C1 variant control (C1),

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**Fig. 4.** Normal Tf genetic variant, normal control Tf, and alcohol abusers’ Tf compared by IEF-IB analysis of sera.

Sera (1 μL) derived from two normal consumers of alcohol (N1, N2) and six alcohol abusers (P2, P3, P6, P7, P8) were separated by IEF and analyzed by IB. The common Tf isoforms (bands 2, 4, 6) and CDT are indicated.

**Fig. 5.** Tf allelic D variants, normal control Tf, and alcohol abusers’ Tf compared by IEF-IB analysis of sera.

Sera (1 μL) derived from two alcohol abusers (P3, P4) and two normal consumers of alcohol (N1, N2) run in IEF-IB were compared with genetic Tf variant reference sera D1, D2, D3, D3o, and C1. The common Tf isoforms (bands 2, 4, 6) and CDT are indicated.

**Fig. 6.** Tf allelic D variants, normal control Tf, and alcohol abusers’ Tf compared by IEF-IB analysis of neuraminidase-treated sera.

Sera (1 μL) were digested with neuraminidase, run in IEF gel, and blotted onto nylon membranes before incubation with human anti-Tf antibodies. Two normal consumers of alcohol (N1, N2) and two alcohol abusers (P3, P4) were compared with genetic Tf variant reference sera C1, D1, D2, D3, and C1, respectively. The common sialo-Tf isoforms (bands A–D) are indicated.
thus confirming heterozygosity for the D specimens. The D variants exhibited additional isoforms migrating above the bands exhibited by the C1 variant control, with D1 being the fastest and D3 the slowest moving alleles in this system.

The normal drinker N1 and alcohol abuser P3 exhibited only the C1 bands, indicating homozygosity for this allele. The normal drinker N2 and the alcohol abuser P4, exhibiting abnormal IEF-IB patterns in previous gels, showed four additional bands migrating above their C1 allele counterparts (Fig. 6). A comparison of the normal (N2) and the patient’s (P4) samples with the Tf D variant reference sera showed identity with the IEF pattern of bands obtained for D1 and D3, respectively. Thus, neuraminidase treatment of patients’ sera generates a simplified banding pattern that facilitates comparison of abnormal specimens with known Tf allelic reference sera.

**Discussion**

Quantification of CDT by charged-based separation procedures represents the best available laboratory procedure for the detection of alcohol abuse in selected populations (4-6). The CDT isoforms detected by these procedures may lack sialic acids as well as galactose and other sugar residues (16, 17). Two methods, IEF-IB-LD and MAEC-RIA (CDTect; Kabi-Pharmacia, Uppsala, Sweden), provide similar clinical sensitivity and specificity, as documented in a survey of 120 serum specimens from an American population (9). IEF-IB-LD, however, provides a visible pattern of Tf isoforms that has become instrumental in identifying allelic D variants of Tf. These variants, albeit rare in the general population, can give false-positive results for CDT because of amino acid substitutions that raise the pl of these normal Tf isoforms so as to resemble carbohydrate-deficient isoforms.

We first identified a genetic variant in our IEF system while screening controls and patients for alcohol abuse (4). Because the polymorphic variation of Tf is limited, most affinity-purified Tf samples yield a common pattern of bands (i.e., 1-7) in our Coomassie Blue-stained IEF gels. This characteristic banding pattern differs in normal subjects and patients only in the high pl (>5.85) region where CDT migrates (Figs. 1 and 2). The presence of four distinct additional cathodic isoforms in one patient, each migrating above the most prominent bands commonly identified in our gels, indicates genetic diversity. This banding pattern is also observed in immunoblots, when sera are separated by IEF and hybridized with rabbit anti-human Tf antibodies (Fig. 3-5). CDT quantification yields a weakly positive result for this patient.

Genetic diversity of Tf is commonly assessed by treatment of serum with neuraminidase to remove terminal sialic acids from complex-type carbohydrates so that only the contributions of amino acid composition and iron to the pl of the protein are evaluated. Whereas four bands are the hallmark of individuals homozygous for Tf in systems characterized by partial iron saturation, eight bands are seen in the patient bearing the rare Tf pattern (Fig. 3). The presence of additional bands in Tf devoid of sialic acid residues confirms the expression of codominant Tf alleles in this patient. In contrast, the alcohol abuser and normal drinker exhibiting the common Tf banding pattern exhibit only the four bands present in the Tf control sample.

A comparison of this abnormal Tf pattern with a panel of reference sera allows characterization of this individual as a Tf D3 carrier (Fig. 5). Furthermore, a comparison of this CDT* D3 patient with the D4 reference sera from a nondrinker shows that, below the pl 5.85 marker, both D3 carriers exhibit identical isoforms. The patient, however, exhibits additional bands that migrate at the same pl as the CDT bands present in the strongly positive control run in the same gel. Thus, alcohol-abusing carriers of genetic D3 variants of Tf exhibit high-pl isoforms that represent Tf with amino acid substitutions and carbohydrate deficiencies. In contrast, carriers of the Tf D4 variant who consume <15 g of alcohol daily will not exhibit CDT isoforms.

We identified a second Tf allelic D variant while assessing CDT by IEF-IB-LD and MAEC-RIA, in 60 nondrinkers (9). The results obtained for a nondrinking black woman carrying a rare variant of Tf (Fig. 4) demonstrate that expression of this phenotype does not give false-negative CDT results in the IEF-IB-LD system. Characterization of this control individual as a Tf genetic variant D1 correlates with the reported frequency (1%) of this allele in the American black population (15). In fact, we have found this allele in 2 of 200 serum specimens analyzed, both from black, nondrinking women, and both giving CDT- results by IEF-IB-LD.

A comparison of the IEF banding pattern exhibited by a panel of D reference sera with that of a CDT- alcohol abuser exhibiting the common Tf C1 allele shows that Tf genetic variants of the D1, D2, and D3 types exhibit cathodic isoforms. These bands, however, do not migrate at the same pl as the CDT diagnostic bands; only Tf genetic D3 variants, present in blacks (18), Japanese, and New Zealanders (19), generate false-positive CDT results for diagnosis of alcohol abuse in the IEF-IB-LD system.

New alleles of Tf are constantly being identified as a result of improved resolution in novel IEF systems. Of eight Tf D variants reported, four are confirmed to be new variants. Only the old variants, D1, D2, D3, and D4, were analyzed in this study. The new variants, DOku, DAuckland, DSaga, and DShimanyo, were recently identified at low frequencies in a study of 2167 Japanese and 448 New Zealanders (19). The most cathodal variant, Tf DShimanyo, found in a Japanese family, is characterized by having only two sialic acid residues. In all likelihood, DShimanyo lacks an oligosaccharide chain because glycosylation is inhibited by amino acid substitution at one of the two carbohydrate-attachment sites. The identification of an abnormal electrophoretic pattern in these sera, followed by stepwise removal of sialic acids by neuraminidase digestion, allowed its characterization (19). Similarly, our IEF-IB-LD system represents a
valuable tool for ruling out false-positive results for CDT in evaluation of alcohol abuse if patients' sera are analyzed before and after neuraminidase treatment. The demonstration that only genetic variants of the D₃ type cause false-positive results further contributes to the excellent specificity of CDT in alcohol abuse diagnosis.

In conclusion, normal individuals carrying the TfD₁ variant, which is present in 1% of American blacks (15), can be identified as exhibiting an abnormal banding pattern that does not render false-positive results for CDT. Only the rare TfD₃ variants, but not the D₁, D₂, or D₄ types, exhibit isoforms migrating at the same pI as the CDT diagnostic bands in our system. In addition, alcohol abusers carrying the Tf genetic variant Dₓ exhibit a different banding pattern than does a Dₓ non-drinker: The former test as CDT⁺ and the latter as CDT⁻ in our system. Thus, compared with other charge-based separation procedures for CDT quantification (e.g., MAEC-RIA and HPLC), IEF-IB-LD yields highly resolved visible banding patterns, permitting identification of rare Tf allelic polymorphisms that may interfere in the diagnosis of alcohol abuse.

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References