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Forensic analysis of carbohydrate-deficient transferrin (CDT): implementation of a screening and confirmatory analysis concept is hampered by the lack of CDT isoform standards

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Abstract

The aim of the study was to test combinations of commercially available carbohydrate-deficient transferrin (CDT) assays for their usefulness as screening and confirmatory CDT analysis systems. A set of 292 serum samples from routine CDT analysis was analyzed by two assays based on anion-exchanger microcolumn CDT and non-CDT fractionation followed by a turbidimetric immunoassay (ChronAlcoI.D. and %CDT TIA) and a high-performance liquid chromatography with on-line sample preparation (ClinRep CDT on-line). The CDT analysis results were divided into four groups based on the test-specific borderlines of the compared methods: NN with negative CDT results by both tests, PN with positive screening but negative confirmation results, NP with negative screening and positive confirmation results, and PP with positive results by both tests. Regardless of the test combination and whether applying the lower or upper limits of the borderlines, approximately one-third of contradictory (positive screening and negative confirmation or vice versa corresponding to groups PN and NP) were obtained. This was not due to analytical outliers (only 6 of 292 serum samples). Indeed, parametric and non-parametric ANOVA analysis pointed to different calibrations and/or recoveries of the three CDT assays. Our data give again evidence for the urgent need of an international CDT isoform standard material. At this time, we cannot recommend a combination of the three tests for screening and confirmatory analysis in forensic CDT testing.

Keywords: Carbohydrate-deficient transferrin; Anion-exchanger microcolumn; CDT; Turbidimetric immunoassay

1. Introduction

Carbohydrate-deficient transferrin (CDT, asialo- + monosialo- + disialo-Fe_2-transferrin) is currently the most specific laboratory marker of chronic alcohol abuse [1,2]. Due to the high prevalence of chronic alcohol abuse in many countries, CDT plays an important role in the areas of employment, traffic, and forensic medicine. The potentially strong social impact of an increased CDT value justifies the need for a maximally reliable preanalysis, analysis, and interpretation of CDT. There is a highly developed system of quality assurance in toxicologic and forensic drug analysis including standards and calibration material, internal quality control material, external quality control programs, and a system of screening and confirmatory analysis [3–5]. Many state laws regulate medico-legal diagnosis of acute alcohol abuse demanding ethanol analysis by two independent analysis methods: enzymatic NADH-method and headspace-gas chromatography [6]. In contrast to this, the forensic laboratory diagnosis of chronic alcohol abuse by measurement of CDT is essentially not regulated and usually done by only one (immunologic) analytical method. A CDT analysis comprising screening and confirmatory analysis, analogous to forensic drug analysis has not been established so far. The aim of our study was to test combinations of three commercially available CDT assays for their usefulness as screening and confirmatory CDT analysis systems.

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2. Materials and methods

All procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 1986. Only surplus serum volumes from routine CDT analysis were used.

All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). ChronAlcoI.D. assay was purchased from Biodiagnostics (Kiel, Germany). %CDT TIA (trisialotransferrin-free version) was delivered by Bio-Rad (München, Germany). HPLC was done with the ClinRep® CDT HPLC on-line from and by Recipe (München, Germany).

2.1. ChronAlcoI.D. assay

The test is based on anion-exchange chromatography for fractionation of CDT isoforms (asialo-, mono-, and disialo-Fe_2-transferrin) and non-CDT isoforms (trisialo-Fe_2- and higher sialylated transferrin isoforms) followed by a turbidimetric immunoassay. The assay was used in accordance with the test instructions. In short, the test comprises the following steps:

- In-vitro transferrin Fe^{3+}-saturation
  Serum or control sample (100 μL) and 500 μL of ferric saturation reagent were mixed and incubated for 5–10 min at room temperature.
- Anion-exchange microcolumn fractionation of CDT and non-CDT isoforms
  Aliquot (500 μL) of the ferric-treated sample was applied to the microcolumn (adsorption of CDT isoforms) and the effluent with non-CDT isoforms was discarded. After a rinsing step, CDT isoforms were eluted and the eluate was used for the final turbidimetric immunoassay.
- Preparation of a serum aliquot for the total transferrin determination
  This was done while microcolumn separation was taking place. Fe^{3+}-saturated serum or control sample (20 μL) were mixed with 800 μL of elution buffer. This sample was used in the turbidimetric immunoassay for determination of the total serum transferrin concentration.
- Quantification of CDT and total transferrin by a microtiter-plate turbidimetric immunoassay
  Calibrators, microcolumn eluates, and total transferrin dilution samples (200 μL of each) were pipetted directly to the bottom of each well and atypical absorbance (background) was read at 405 nm (Dynatec MR 5000 reader, Dynex Technologies, Denkendorf, Germany). Following this, 100 μL of transferrin antibody solution was added to each well and the final absorbance was read after 15 min at room temperature. The microplate reader was directly connected to a computer. Evaluation of the analysis data was done by use of the Dynex Revelation 3.2 software (Dynex Technologies, Denkendorf, Germany). The results were reported as CDT/transferrin ratios (%CDT).

2.2. %CDT TIA assay

The test is based on anion-exchange chromatography for fractionation of CDT isoforms (asialo-, mono-, and disialo-Fe_2-transferrin) and non-CDT isoforms (trisialo-Fe_2- and higher sialylated transferrin isoforms) followed by a turbidimetric immunoassay. The assay was used in accordance with the test instructions. In short, the test comprises the following steps:

- In-vitro transferrin iron saturation
  Serum or quality control sample (100 μL) and 500 μL ferric saturation reagent were mixed and incubated for 2–15 min at room temperature.
- Anion-exchange microcolumn fractionation of CDT and non-CDT isoforms
  The ferric-treated sample (500 μL) were applied onto the top of the microcolumn (adsorption of CDT isoforms) and the effluent with the non-CDT isoforms was discarded. After a rinsing step, the CDT isoforms were eluted and the eluate was immediately used in the final turbidimetric immunoassay.
- Preparation of a serum aliquot for the total transferrin determination
  The total transferrin solution was prepared while microcolumn non-CDT and CDT fractionation was taking place. The ferric-treated sample (50 μL) was mixed with 2.0 mL of elution buffer. This sample was used in the following turbidimetric immunoassay for the determination of the total serum transferrin concentration.
- Quantification of CDT and total transferrin by a microtiter-plate turbidimetric immunoassay
  Calibrators (in duplicate), microcolumn eluates, and total transferrin dilution samples (200 μL of each) were pipetted directly to the bottom of each well, and atypical absorbance (background) was read at 405 nm (Bio-Rad Microplate Reader-Benchmark, Bio-Rad, München, Germany). After this, 50 μL transferrin antibody solution was added to each well. After gentle agitation and incubation for 15 min at room temperature, the final absorbance was read at 405 nm. The reader was directly connected with a computer system for on-line evaluation of the analysis data (calculation program for %CDT 2.0-97-c international, microtiter Version, Bio-Rad, München, Germany). The results were reported in CDT/total transferrin ratios (%CDT).

2.3. ClinRep® CDT HPLC on-line

The test is based on fractionation of CDT isoforms (asialo-, mono-, and disialo-Fe_2-transferrin) and non-CDT isoforms (trisialo-Fe_2- and higher sialylated transferrin isoforms) by liquid chromatography. The assay was performed by Recipe. In short, the test comprises the following steps:

- In-vitro transferrin iron saturation

Serum or control sample (100 μL) was pipetted into 1.5 mL Eppendorf cups (part of the test kit) containing a ferric-solution and incubated for 1 h at 4 °C in the dark. After centrifugation for 5 min at 3500 rpm, 400 μL supernatant were injected into the HPLC-system.

- HPLC-system

  Sample pretreatment at the precolumn, column switching, and analysis of the transferrin isoforms was computer-controlled. Separation of matrix components from transferrin isoforms was done on a short anion-exchanger pre-column. After automated column switching, the transferrin isoforms were separated on an anion-exchanger analytical column by gradient chromatography. Both columns and solvents were part of the testkit. Transferrin isoforms were detected with a UV/VIS detector at 460 nm. Chromatography time was 11 + 1 min for equilibration of the column. The HPLC-system was from VWR (Darmstadt, Germany).

- Evaluation of the chromatograms

  Quantification of the CDT isoforms was done by the in-system software. Asialo-, monosialo-, and disialo-Fe_2-transferrin (CDT isoforms) were baseline integrated, and trisialo-, tetrasialo-, and pentasialo-Fe_2-transferrin (non-CDT isoforms) were valley-to-valley integrated. The peak areas of CDT isoforms were collectively referred to as CDT and expressed as percentage of the total peak area below all transferrin isoforms (asialo- to pentasialo-Fe_2-transferrin).

2.4. Internal and external quality control

Quality control was done by internal and external quality control material. In each analysis series, two control samples with normal and pathological CDT/transferrin ratios (in-house serum pools), %CDT control set (Bio-Rad, München, Germany), and CDT control set (Biodiagnostics, Kiel, Germany) were placed at the beginning and at the end of the run. The three assays were successfully tested in external quality control programs.

2.5. Test-specific cut-off values (borderlines) for %CDT indicating chronic alcohol abuse

The cut-off for the ChronAlcoI.D. assay was 2.5% corresponding to the 95 percentile of the CDT values obtained from healthy control persons with normal alcohol intake (82 women with <20 g ethanol per day and 45 men with <50 g ethanol per day) [7].

The cut-off for the %CDT TIA assay was 2.6% corresponding to the 95 percentile of the CDT values from 199 apparently healthy women and men (<20 g ethanol per day) [8].

The cut-off for the ClinRep® CDT HPLC on-line was 1.75% corresponding to the 95 percentile of the CDT values from 100 healthy persons with normal alcohol intake (<50 g ethanol per day on at maximum 2 days per week) (personal information and [9]).

Taking into account the intra-individual variance of serum CDT, the analytical imprecision of 10–15% and aiming at an utmost diagnostic specificity, borderlines were suggested for routine CDT analysis [2]. We used 2.5–3.0% for the ChronAlcoI.D. assay [10], 2.6–3.0% for the %CDT TIA assay [11], and 1.75–2.50% for the ClinRep® CDT HPLC [12].

2.6. Statistics

The method of Passing and Bablok [13] has been used for unbiased method comparison. The significance of the differences between means and medians of the CDT results obtained by the three different CDT tests has been tested by (parametric) one-way between subjects ANOVA (means) and non-parametric Kruskal–Wallis ANOVA (medians). All calculations were done with the Analyse-it software for Microsoft Excel (Analyse-it Software Ltd., Leeds, UK). The significance level was set at 95% or $P = 0.05$.

3. Results

A set of 292 serum samples from routine CDT analysis was analyzed by %CDT TIA, ChronAlcoI.D. and ClinRep CDT HPLC assays. The CDT analysis results (CDT in % of total transferrin) were assessed by Passing–Bablok regression analysis and arranged in x–y plots with one test on the x-axis and another on the y-axis (Figs. 1–3). The method on the x-axis was considered as the screening method and the method on the y-axis as the confirmatory analysis method. Applying the test-specific lower or upper limits of the borderlines of serum CDT indicating chronic alcohol abuse, the x–y plot is divided into four quadrants. The quadrant in the left lower corner (NN) contains the data with normal (negative) CDT values by both assays. The quadrant in the right lower corner (PN) shows data pairs with positive CDT results by the x-axis assay and negative results by the y-axis assay. The quadrant in the left upper corner (NP) shows data points with negative CDT values by the x-axis method and positive CDT results by the y-axis method. Finally, the right upper quadrant (PP) shows data pairs with positive CDT results by both assays.

3.1. ChronAlcoI.D. versus ClinRep CDT HPLC

Fig. 1 shows the combination ChronAlcoI.D. assay (considered as the screening method) and ClinRep CDT HPLC (considered as the confirmatory method). Regardless of whether applying the test-specific lower or upper limits of the borderlines, quadrant PN contains many data points corresponding to increased CDT values by the ChronAlcoI.D. assay but normal CDT values by the ClinRep CDT HPLC. If this combination would have been used for screening (ChronAlcoI.D.) and confirmatory (ClinRep CDT HPLC) CDT analysis, about one-third of the positive ChronAlcoI.D. results would not have been confirmed by the HPLC method.

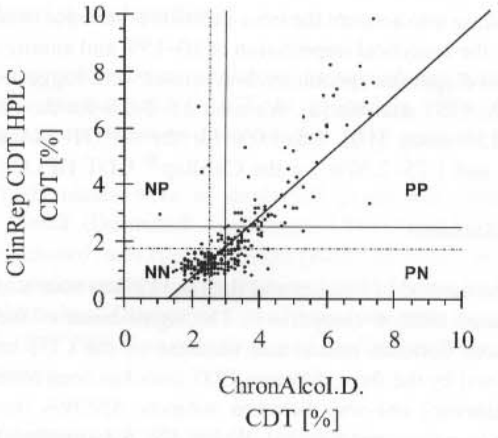

Fig. 1. Simulation of forensic CDT measurement with an immunoassay considered as the screening method (ChronAlcoI.D.) and HPLC considered as the confirmatory method (ClinRep CDT HPLC). Regardless of whether the lower (dashed lines) or upper (solid lines) limits of the test-specific borderlines were used as decision criterions for diagnosis of chronic alcohol abuse, a great number of positive screening results could not be confirmed by HPLC (quadrant PN). The four quadrants formed by the lines of the cut-off values correspond to NN, screening and confirmation with normal (negative) CDT results; PN, screening positive and confirmation negative; NP, screening negative and confirmation positive; and PP, screening and confirmation positive. Borderline for ChronAlcoI.D.: 2.5–3.0% and for ClinRep CDT HPLC: 1.75–2.50%. Even varying the cut-offs to lower or higher values does not improve the discrepancies between screening and confirmatory results. Indeed, the rate of contradictory results in quadrant NP would increase with lower HPLC cut-off values. This is also true for the combination %CDT TIA and HPLC (see Fig. 2).

Otherwise, using the ClinRep CDT HPLC as the screening method and the ChronAlcoI.D. as the confirmatory method, about one-third of theoretically alcohol abuse positive serum samples would not have been detected by the ClinRep CDT HPLC. Table 1 gives the number of each result constellation.

3.2. %CDT TIA versus ClinRep CDT HPLC

Fig. 2 shows the combination %CDT TIA assay (considered as the screening method) and ClinRep CDT HPLC (considered as the confirmatory method). Applying the test-specific lower or upper limits of the borderlines quadrant PN contains many data points corresponding to increased CDT values by the screening method (%CDT TIA) but normal CDT values by the confirmatory method (ClinRep CDT HPLC). If this combination would have been used for forensic CDT testing, about one-third of the positive %CDT TIA assay results would not have been confirmed by the HPLC method. Otherwise, using the ClinRep CDT HPLC as the screening method and the %CDT TIA as the confirmatory method, the same amount of theoretically alcohol abuse positive serum samples would not have been detected by the

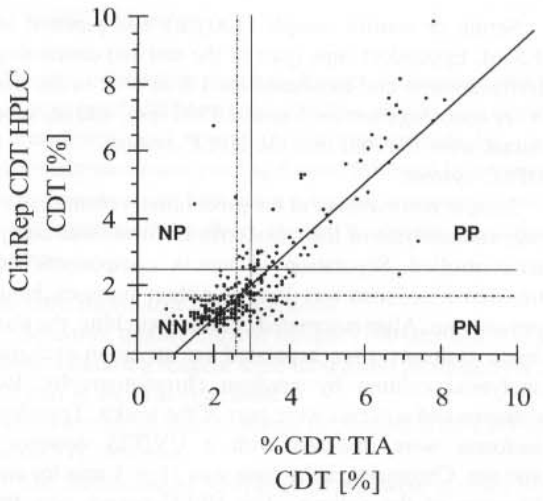

Fig. 2. Simulation of forensic CDT measurement with an immunoassay considered as the screening method (%CDT TIA) and HPLC considered as the confirmatory method (ClinRep CDT HPLC). Regardless of whether the lower (dotted lines) or upper (solid lines) limits of the test-specific borderlines were used as decision criterions for diagnosis of chronic alcohol abuse, a great number of positive screening results could not be confirmed by HPLC (quadrant PN). Borderline for %CDT TIA: 2.6–3.0% and for ClinRep CDT HPLC: 1.75–2.50%. For further explanation, see legend of Fig. 1.

ClinRep CDT HPLC. The number of each result constellation are given in Table 2.

3.3. ChronAlcoI.D. versus %CDT TIA

Although screening and confirmatory analysis method should be based on different separation and detection prin-

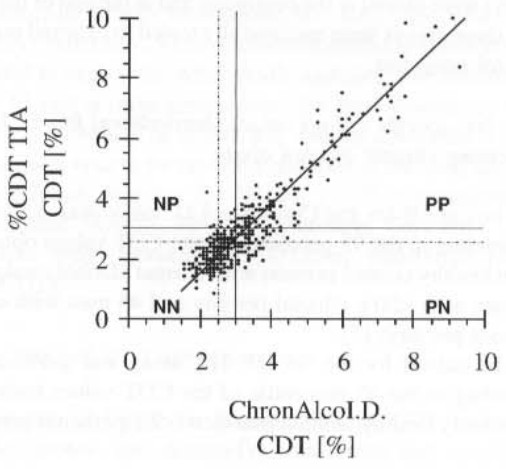

Fig. 3. Simulation of forensic CDT measurement with an immunoassay considered as the screening method (ChronAlcoI.D.) and a second immunoassay as the confirmatory method (%CDT TIA). For borderlines and further information, see legends of Figs. 1 and 2.

Table 1
Forensic CDT testing by ChronAlcoI.D. as the screening method (S) and ClinRep CDT HPLC as the confirmatory method (C) when applying the lower and upper limits of the test-specific borderlines ($n = 301$)

Limit	S positive/C positive	S negative/C negative	S positive/C negative	S negative/C positive
Lower	69	150	78	4
Upper	111	86	100	4

Table 2
Forensic CDT testing by %CDT TIA as the screening method (S) and ClinRep CDT HPLC as the confirmatory method (C) when applying the lower and upper limits of the test-specific borderlines ($n = 292$ of the 301 serum samples from Table 1, the difference is due to exhausted surplus serum volumes for nine serum samples)

Limit	S positive/C positive	S negative/C negative	S positive/C negative	S negative/C positive
Lower	103	117	62	10
Upper	62	180	41	9

ciples, e.g., screening by immunoassay and confirmation by chromatography, we have additionally assessed whether the result of one immunoassay (ChronAlcoI.D.) could be confirmed by the other (%CDT TIA). Altogether, 402 serum samples were analyzed, those from the combinations of immunoassay and HPLC ($n = 301$) and 101 additional serum samples which were not analyzed by HPLC. Regardless of whether using the lower or upper limits of the test-specific CDT borderlines indicating chronic alcohol abuse, a great number of CDT results would have been positive by the ChronAlcoI.D. assay but not by the %CDT TIA assay. There was an increased number of data points in quadrant NP when compared to Figs. 1 and 2. The result constellations were arranged in Table 3.

3.4. Statistical comparison of ChronAlcoI.D., %CDT TIA and ClinRep CDT HPLC

Using parametric ANOVA for testing the significance of the differences between the means of the test results obtained by the different CDT assays, we obtained $3.38 \pm 1.67\%$ (mean $\pm$ S.D.) for the ChronAlcoI.D., $3.29 \pm 2.68\%$ for the %CDT TIA and $2.44 \pm 3.04\%$ for the ClinRep CDT HPLC. The difference between the mean of the ChronAlcoI.D. and %CDT TIA assays was 0.1% and statistically not significantly different from zero (95% confidence interval, 0.310–0.503). The difference between the means of ChronAlcoI.D. and ClinRep CDT HPLC and %CDT TIA and ClinRep CDT HPLC was 0.95% (95% confidence interval, 0.050–1.385) and 0.85% (95% confidence interval, 0.406–1.291), proving both differences to be statistically significantly different from zero. Non-parametric Kruskal–Wallis ANOVA for testing the difference between the medians of the test results of the three CDT assays yielded significant differences for each test combination ($P < 0.001$). Unbiased Passing and Bablok regression analysis yielded the following regression functions: ClinRep CDT HPLC = 1.07 × ChronAlcoI.D. − 1.43. The 95% confidence intervals for the intercept and slope were −1.748 to −1.140 and 0.963–1.178, proving the intercept to be statistically significantly different from zero. ClinRep CDT HPLC = 1.02 × %CDT TIA − 1.01. The 95% confidence intervals were −1.268 to −0.758 for the intercept and 0.925–1.107 for the slope, proving the intercept to be statistically significantly different from zero. %CDT TIA = 1.14 × ChronAlcoI.D. − 0.76. The 95% confidence intervals were −0.960 to −0.595 for the slope and 1.091–1.200 for the intercept, proving the intercept to be significantly different from zero and the slope to be statistically significantly different from 1.

4. Discussion

False-positive CDT reports indicating chronic alcohol abuse can be due to conditions such as transferrin D variants and primary biliary cirrhoses [1,2] or due to analytical

Table 3
Forensic CDT testing by ChronAlcoI.D. as the screening method (S) and %CDT TIA as the confirmatory method (C) when applying the lower and upper limits of the test-specific borderlines ($n = 402$, 301 samples from Fig. 1 + 101 additional serum samples)

Limit	S positive/C positive	S negative/C negative	S positive/C negative	S negative/C positive
Lower	188	100	101	13
Upper	122	210	61	9

problems. The latter can occur by incomplete fractionation of the CDT- and non-CDT isoforms with anion-exchange microcolumns [14]. Since transferrin but not CDT antibodies are used in the subsequent immunologic step for quantification of CDT in the column eluates, coelution of non-CDT isoforms causes false-positive elevated CDT values. This has been previously shown [14] and can be applied to all CDT tests based on microcolumn CDT and non-CDT fractionation followed by immunoassay. Strategies to reduce the analytical non-specificity due to incomplete CDT separation have been discussed in [15]. This paper summarizes also arguments for and against using isoelectric focusing or mass spectrometry as confirmatory CDT analysis methods.

We have no explanation for the very high CDT value obtained by the three assays for sample 1 in Table 4. Genetic transferrin variants were not detected by HPLC and can be excluded as the underlying cause. Information regarding the clinical background of this patient is lacking. Thus, the origin of these CDT values remains unclear. Since ChronAlcoI.D. and ClinRep CDT HPLC produced comparable results for the serum samples 2–6 from Table 4, the outliers obtained by the %CDT TIA assay should be due to invalid anion-exchanger microcolumns causing incomplete CDT- and non-CDT fractionation. Although critical for the single individual, this failure rate corresponds to only 1.5% of the %CDT TIA columns used in this study ($n = 301$). We have not detected such extreme outliers during the last year with several thousand CDT analyses in our routine laboratory by the %CDT TIA assay. This might indicate that the already high quality of the microcolumns has been further improved. Nevertheless, high CDT results by faulty or expired microcolumns have been described earlier [14]. Such findings might be rare. However, determination of CDT by anion-exchanger microcolumn fractionation of CDT and non-CDT isoforms followed by immunoassay (using transferrin and not CDT antibodies) bears the risk of false-positives with strong legal and social consequences due to a single invalid microcolumn. Because of this, a re-analysis of borderline and pathological CDT results in a second independent analysis run using a new column has been suggested [15]. Using the same microcolumn eluate for duplicate immunoassay does not allow detection of invalid microcolumns and does not meet the criteria for good laboratory practise.

Table 4
Extreme outliers of unknown origin (total sample number tested with each of the three assays, $n = 292$)

Sample	ChronAlcoI.D. CDT (%)	%CDT TIA CDT (%)	ClinRep CDT HPLC CDT (%)
1	17.4	29.0	36.4
2	2.3	19.9	1.8
3	2.3	19.5	0.99
4	6.1	17.1	8.2
5	4.8	14.0	3.2
6	3.1	11.8	Not analyzed

Genetic transferrin variants were excluded by HPLC as the underlying cause in each case.

Figs. 1–3 clearly show most of the discrepant analysis results are not due to analytical outliers. Instead, the discrepant result constellations (positive screening/negative confirmation or vice versa) were obtained on the basis of analytically plausible and quality-controlled CDT results. This is also true for a comparison of the CDTect assay (based on anion-exchanger microcolumn CDT and non-CDT fractionation followed by enzyme or radio immunoassay) with HPLC [16] and a comparison of the %CDT TIA with the CDTect assay [17]. Applying the test-specific upper reference limits to the x–y plots (CDTect on the x-axis, HPLC on the y-axis in [16], and CDTect on the x-axis and %CDT TIA on the y-axis in [17]), many data points (approximately one-third in each report) were found in sections corresponding to quadrant PN and NP used in our present report. The fact that many pathological CDT results obtained by the immunoassays were not confirmed by the HPLC method (our data and those in [16]) does not compellingly mean that these immunoassays are non-specific. On the one hand, there are arguments for a higher rate of positive CDT results as it was true for the immunoassays but not for the HPLC assay: the prevalence of alcohol abuse in Germany is 12–16% [18]. We know from 10 years CDT analysis with 100–150 serum samples per day and the corresponding consulting discussions that CDT is increasingly requested only in cases with clinical signs or social indications of chronic alcohol abuse but not as a screening parameter. This should further increase the number of positive CDT results per analysis run, exceeding the 12–16% alcohol abuse prevalence level. Such a high rate was not obtained by the HPLC assay but by the immunoassays. Furthermore, some samples from routine CDT analysis with high immunoassay CDT results but normal HPLC results were analyzed in our laboratory by isoelectric focussing. Increased fractions of CDT isoforms confirmed qualitatively the high CDT values obtained by the immunoassay. On the other hand, Turpeinen et al. [16] found a substantially better separation between moderate and heavy drinkers by the HPLC method when compared with the CDTect assay. Most important, HPLC allows the detection of genetic transferrin D and B variants which can cause a distinct overdetermination (D variants) or underdetermination (B variants) of CDT by immunoassays based on anion-exchanger microcolumn CDT and non-CDT isoform fractionation [2]. Regarding a CDT screening/confirmatory analysis concept, other causes for false-negative CDT results also have to be considered. The lack of information about the prevalence of false-negatives by HPLC or immunoassays has been discussed in [15]. Undoubtedly, such information is essential for forensic CDT testing. We strongly recommend appropriate studies for investigating this issue.

It was not the aim of our present study to assess diagnostic specifities and sensitivities, positive and negative predictive values or receiver–operating–characteristic curves for the three CDT assays. Such studies have been done with varying

results and conclusions on a wide range of control and patient populations and CDT analysis methods [2,17,19]. We wanted to show that test-specific calibration and/or test-specific borderlines cause an unacceptable high number of contradictory CDT results regarding the diagnosis of chronic alcohol abuse. This is true even for combinations of two almost similar immunoassays on the basis of anion-exchanger microcolumn CDT and non-CDT isoform fractionation (our data and those in [17]). Our detailed statistical analysis of the CDT results obtained by each CDT assay clearly points to differences in the calibration and/or recovery of the tests (different mean and median between immunoassays and HPLC; significant differences from zero for the intercept in each case, and in one case, from 1 for the slope of the regression functions). Indeed, even after 10 years of CDT analysis, we have a rather fragmentary documentation of the fundamental analytical criterions like calibration, analytical sensitivity and specificity, linearity, recovery, and interferences for commercial and non-commercial CDT tests. The most important obstacle for investigating these parameters is the lack of standards of the CDT isoforms or at least of an international CDT standard. Who is responsible? At a more general level, several national and international organizations are involved in defining the criteria for Good Laboratory Practice. At a more specific level, professional societies of forensic and toxicologic medicine have established guidelines for drug analysis. Development, validation, and implementation of CDT isoform standards and a CDT analysis reference method like that in [20] would distinctly improve the reliability and (forensic) impact of CDT measurement. We strongly recommend (a more intense) cooperation between industry and science on this subject.

Summing up, differences in the calibration and/or recovery of CDT assays cause an unacceptable high percentage of contradictory analysis results when using one test as the screening and a second test as the confirmatory analysis method. On the basis of our present data and those from the literature, we cannot recommend a certain combination of the commercially available CDT tests for screening and confirmatory analysis in forensic CDT testing. An international standardization of CDT analysis is an urgent prerequisite before further testing diagnostic sensitivities and specificities of CDT as a marker of chronic alcohol abuse. In any case, laboratory (and forensic) diagnosis of chronic alcohol abuse should always be made based on clinical background, questionnaire, CDT and γ-GT activity (measured at two different occasions), and not on a single CDT value alone.

References

[1] H. Stibler, Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed, Clin. Chem. 37 (1991) 2029–2037.

[2] T. Arndt, Carbohydrate-deficient transferrin as a marker of chronic alcohol abuse: a critical review of preanalysis, analysis, and interpretation, Clin. Chem. 47 (2001) 13–27.

[3] D.S. Ferrara, L. Tedeschi, G. Frison, G. Brusini, Quality control in toxicological analysis. Review article, J. Chromatogr. B Biomed. Sci. Appl. 713 (1998) 227–243.

[4] H. Schütz, Screening von Drogen und Arzneimitteln mit Immunoassays, Wissenschaftliche Verlagsabteilung Abbott GmbH, Wiesbaden, 1999.

[5] T.A. Gough (Ed.), The Analysis of Drugs of Abuse, Wiley, Chichester, 1991.

[6] H.J. Gibitz, H. Schütz (Eds.), Bestimmung von Ethanol in Serum, Durchführung und Interpretation im klinisch-chemischen Laboratorium, Mitteilung XX der Senatskommission für klinisch-toxikologische Analytik, VCH Verlagsgesellschaft mbH, Weinheim, 1993.

[7] T. Arndt, L.J. Behnken, B. Martens, R. Hackler, Evaluation of the cut-off for serum carbohydrate-deficient transferrin as a marker of chronic alcohol abuse determined by the Chron-AlcoI.D.™-assay, J. Lab. Med. 23 (1999) 507–510.

[8] Test instructions by the producer of the %CDT TIA assay.

[9] Test instructions by the producer of the ClinRep® CDT HPLC on-line assay.

[10] T. Arndt, J. Kropf, A prolonged time interval between blood sample collection and centrifugation causes an increase in serum carbohydrate-deficient transferrin, Med. Sci. Monit. 8 (2002) BR61–BR64.

[11] A. Helander, M. Fors, B. Zakrisson, Study of Axis–Shield new %CDT immunoassay for quantification of carbohydrate-deficient transferrin (CDT) in serum, Alcohol Alcohol. 36 (2001) 406–412.

[12] U. Bartels, C. Grünert, N. Dirsch, H. Nader, A. Bauer, W. G. Wood, ClinRep® complete kit for the HPLC—determination of transferrin isoforms as a marker of alcohol abuse, in: F. Pragst, R. Aderjan (Eds.), GTFCh Symposium, Nachweis berauschender Mittel im Straßenverkehr, Forensische Aspekte der toxischen Präparation von Lebensmitteln, Verlag, Dr. Dieter Helm, Heppenheim, 1999, pp. 207–212.

[13] H. Passing, W. Bablok, A new biometrical procedure for testing the equality of measurements from different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, J. Clin. Chem. Clin. Biochem. 21 (1983) 709–720.

[14] T. Arndt, R. Hackler, T.O. Kleine, A.M. Gressner, Validation by isoelectric focusing of the anion-exchange isotransferrin fractionation step involved in determination of carbohydrate-defcient transferrin by the CDTect assay, Clin. Chem. 44 (1998) 27–34.

[15] T. Arndt, J. Kropf, Alcohol abuse and carbohydrate-deficient transferrin analysis: are screening and confirmatory analysis required? Clin. Chem. 48 (2002) 2072–2074.

[16] U. Turpeinen, T. Methuen, H. Alfthan, K. Laitinen, M. Salaspuro, U.-H. Stenman, Comparison of HPLC and small column (CDTect) methods for disialotransferrin, Clin. Chem. 47 (2001) 1782–1787.

[17] R.F. Anton, C. Dominick, M. Bigelow, C. Westby, Comparison of Bio-Rad %CDT TIA and CDTect as laboratory markers of heavy alcohol use and their relationships with gamma-glutamyltransferase, Clin. Chem. 47 (2001) 1769–1775.

[18] N. Pörksen, G. Kremer, Screening-Diagnostik-Kurzintervention: Klinische Wirksamkeit und wirtschaftlicher Nutzen, in:

M. Soyka (Ed.), Klinische Alkoholismusdiagnostik, Steinkopff Verlag, Darmstadt, 1999, pp. 80–91.

[19] K. Scouller, K.M. Conigrave, P. Macaskill, L. Irwig, J.B. Whitfield, Should we use carbohydrate-deficient transferrin instead of gamma-glutamyltransferase for detecting problem drinkers? A systematic review and metaanalysis, Clin. Chem. 46 (2000) 1894–1902.

[20] A. Helander, A. Husa, J.-O. Jeppsson, Improved HPLC method for carbohydrate-deficient transferrin in serum, Clin. Chem. 49 (2003) 1881–1890.