Application of the IFCC standardization procedure for CDT measurement to two capillary electrophoresis systems

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Background

Excessive alcohol consumption is at the origin of health, social and economic damages and expenses. Carbohydrate-deficient transferrin (CDT) is considered the most reliable marker in routine conditions. A wide array of method-dependent reference and decision limits have been used until now. This prompted the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to appoint a Working Group on Standardization of CDT (WG-CDT) to develop standardization tools for the manufacturers of commercial kits. An HPLC method for quantification of the serum transferrin glycoforms was selected and validated as the reference measurement procedure (RMP). The ratio disialotransferrin (DiST) on all transferrin glycoforms expressed as percentage was defined as the measurand for CDT. Secondary calibrators with target values given by a network of reference laboratories allow the manufacturers to standardize their procedures against the RMP. To avoid confusion with the non-standardized procedures, CDT results obtained by a standardized procedure are called CDT_{IFCC}. This study was undertaken to check whether the standardization process developed by Sebia (Lisses, France) for the Capillarys® and Minicap® capillary electrophoresis systems was successful.

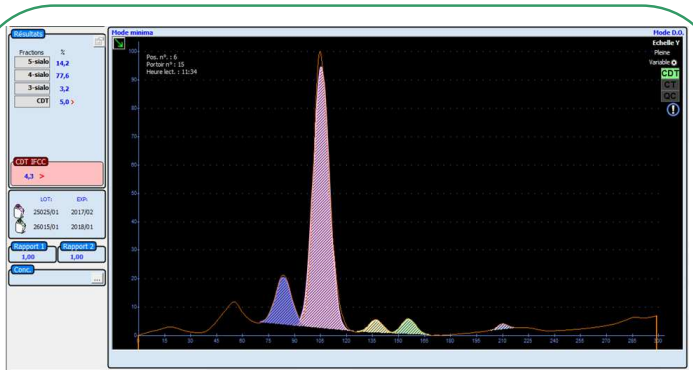

Figure 1: Transferrin glycoform pattern with both expressions of the result: CDT is the result provided by the current non-standardized procedure (sum of disialotransferrin + asialotransferrin ratio) and CDT IFCC the result obtained by the standardized procedure (calibrated disialotransferrin ratio).

Results

Figure 1 shows the modified display including the calculation of the CDT result after IFCC standardization (pink area). The "older" non-standardized results are available to allow the comparison with previous results when a follow-up of CDT values is required. As shown on **figure 2**, standardization dramatically reduced the bias between the current CZE procedures and the RMP, in particular in the low CDT concentrations. The mean gap with the RMP was reduced by standardization from 15% and 17% to more or less 1% (**Table 1**). The concordance coefficient as stratified by Mc Bride increases from "satisfactory" to "almost perfect" for both CZE systems in comparison to the RMP. We calculated the Acceptable Difference Limit (ADL) according to NF EN ISO 5725-6, which is based on the imprecision of the measurement procedures. ADL was evaluated at 18.2% between the HPLC RMP and the studied CZE procedures. **Table 1** shows that standardization reduces the frequency of results with a relative difference higher than ADL to less than 5%, which is the threshold to consider two assays as non significantly different.

Conclusion

This study demonstrates that the tools developed by the IFCC WG-CDT allow the standardization of CDT measurement on the CZE systems Capillarys® and Minicap®, making the results of CDT_{IFCC} given by these systems comparable to those given by the HPLC reference procedure.

Acknowledgements

We thank Sebia for participating to the cost of HPLC RMP measurements and analysis of CDT with the standardized procedure.

Study population

The samples used in this study were leftovers of patients routine samples with a blood alcohol content > 0.5 g/L or CDT prescribed measurement. Samples with abnormal transferrin glycoform pattern or altered baseline on the electropherogram were rejected. The final population comprised 101 men and 25 women aged 17-82 years. After initial CDT measurement (CDT range 0.8 – 18.3%), the samples were anonymously aliquoted and frozen at -20°C.

Study design

CDT was measured in the selected samples by capillary electrophoresis (CZE) using a multicapillary Capillarys® system and by ion exchange chromatography using the RMP to select appropriate samples (CDT value and glycoform pattern). After frozen storage, the samples were analyzed on both CZE systems Capillarys® and Minicap® using the currently marketed procedure and the standardized IFCC procedure. The procedures differ in two points:

-the use of a two level set of calibrators whose target values are assigned to the RMP values via the set of secondary calibrators provided by the WG coordinator (C Weykamp, MCA Laboratory, Winterswijk, The Netherlands). The calibration step requires a triplicate measurement of both calibrators at every change of calibrators lot or at least every three months.

- the calculation of the CDT_{IFCC} as the area ratio between the DiST peak and the sum of all glycoforms as recommended by the IFCC.

Both CDT and CDT_{IFCC} results were compared to those obtained with the HPLC RMP.

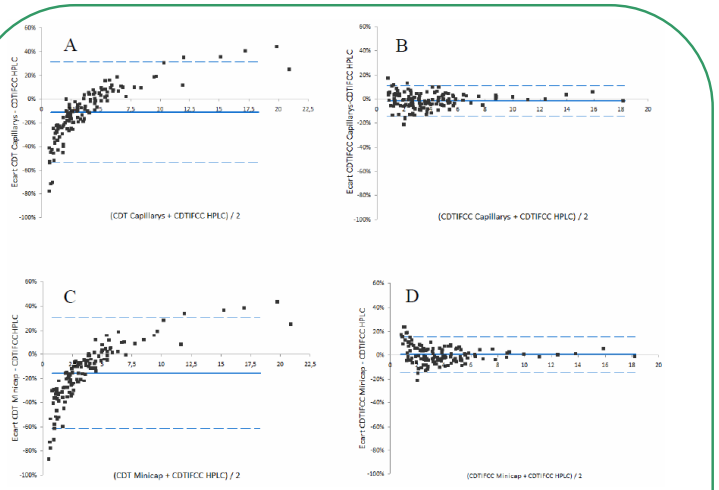

Figure 2: Bland Altman graphs of the difference between the HPLC reference method and the CZE systems without (left) and with standardization (right) for the Capillarys® (A and B) and the Minicap® (C and D) systems. The continuous lines represent the mean bias, the dotted lines the 95th percentile of the confidence interval.

		Capillarys®		Minicap®	
	n	CDT	CDT_{IFCC}	CDT	CDT_{IFCC}
Mean gap with the reference method	126	15,80%	1,40%	17,30%	-0,80%
Number of results with a gap > Acceptable Difference Limit					
CDT ≤ 1,70%	23	23 (100%)	0	23 (100%)	4 (17,4%)
1,7% < CDT < 2,0 %	4	3 (75%)	0	4 (100%)	0
CDT ≥ 2,0%	99	18 (18,2%	1 (1,0%)	30 (30,3%)	1 (1,0%)
All samples	126	44 (34,9%)	1 (0,8%)	53 (42,1%)	5 (4,0%)

Table 1: Differences between the Capillarys® / Minicap® procedures and the HPLC reference method before (CDT) and after (CDT_{IFCC}) standardization: Mean gap and number of results with a gap higher than the Acceptable Difference Limit (18.2%) are shown according to CDT values..