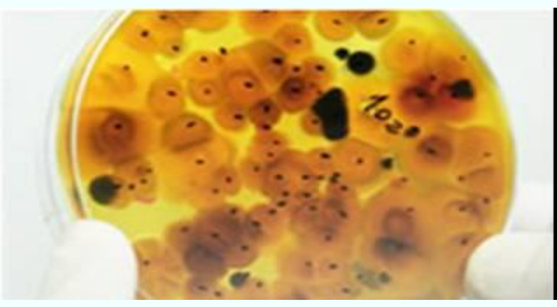


BIOMARKER OF CHRONIC ALCOHOL ABUSE – CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT): Methodology Verification

Filomena Gomes*, Alcina Costa*, Lília Fernandes*, Ana Maria V. da Silva, Miguel Vasconcelos***, Armandina Miranda***

* Department of Health Promotion and NCD Prevention, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

** Lisbon Alchool Unit, Ministery of Health *** Taipas Addiction Center, Lisbon, Ministery of Health

INTRODUCTION

Transferrin is a glycoprotein synthesized in hepatocytes that can appear with different isomorphic forms in the plasma, acquiring different levels of sialization[1,2]. In a healthy person, penta, tetra and trisial isoforms are detectible in plasma, however, in an alcohol abuse and/or dependence, asialo, monosialo and disialotransferrin isoforms are also present called carbohydrate-deficient transferrin (CDT)[3]. This is considered a specific biomarker of alcohol abusive and/or dependence, being useful in the diagnosis and monitoring of this pathology[4]. The CDT gives metabolic information about alcohol consumer retrospectively up six weeks ago[5].

The Minicap system (Sebia) use the principle of capillary electrophoresis (CE) in free solution to quantify the CDT (Figure 1). Separation occurs according to the electrolyte pH and electroosmotic flow. This method entered in IFCC Working-Group Harmonization Measurement Results to CDT[6]). After some randomized trials were compared in different populations and using a Gaussian distribution it was obtained a cut-off of 1.7% [7]. The manufacturer's specifications indicates an uncertainty of 0.3% for the cut-off level of 1.7% (1.7% ± 0.3%).

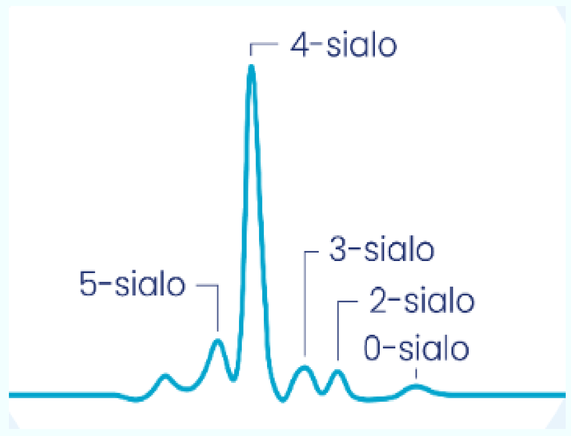


Figure 1- CDT Isoforms by capillary electrophoresis

The aim of this study was verify compliance with the requirements of the manufacturer of the capillary electrophoresis method in laboratory practice and its suitability in determining the CDT.

MATERIALS AND METHODS

The MiniCaps system was used with calibrators traceable to the IFCC international reference procedure and normal and pathological internal control samples.

Repeatability and intermediate precision tests were performed on control samples normal and pathological. 22 replicates of normal control sample and 24 replicates of pathological control were performed in the repeatability test. The intermediate precision tests were performed during 12 different days. From participation in External Quality Assessment (EQA) program (5 rounds - 2 samples each), Bias%, Deviation Index (DI) and Total Laboratory Error (TELab) were obtained. The Measurement Uncertainty (MU) was calculated in an Excel spreadsheet, by the Top Down Method (combined and expanded with a factor of 2), using the results of internal (CV%) and external (Bias%) quality control results.

Formulas

$DI = \frac{Lab\ value - Reference\ value}{SD\ Reference}$ $bias = \frac{Lab\ value - Ref\ value}{Ref\ value} \times 100$

$MU = [\sqrt{\mu(bias)^2 + \mu(CV)^2}] \times 2$ $TE = |bias| + z \times CV$, $z = 2$

RESULTS

In the repeatability tests, for normal control samples (n=22, mean = 1.4%) was obtained a CV = 5.7% and for the pathological sample, (n=24, mean = 5.4%), a CV = 2.2%, (Table1). In intermediate precision tests for the normal control sample, (n = 12, mean = 1.4%) was obtained a CV = 6.7% and for the pathological control sample a CV = 4.9% (Table 2).

Table 1 - Repeatability tests

Normal Control	Pathologic Control
N = 22	N = 24
Mean = 1,4%	Mean = 5,4%
CV = 5,7%	**CV = 2,2%**

Table 2 - Intermediate Precision

Normal Control	Pathologic Control
N = 12	N = 12
Mean = 1,4%	Mean = 5,3%
***CV = 6,7%**	***CV = 4,9%**

* CV% Within laboratory (during 3 months)

In samples from EQA program, were obtained a mean Bias of the -1.0% and TELab = 11.5.(Table 3). DI results obtained were: 1 satisfactory, 7 good and 2 excellent (Table 4.).

Table 3 -Comparison of Laboratory (Lab) results with Westgard Specifications (Westg.)

	Westg.	Lab
Bias (%)	9,8	-1,0
TE (%)	15,7	11,5

Table 4 - CDT – DI Evaluation

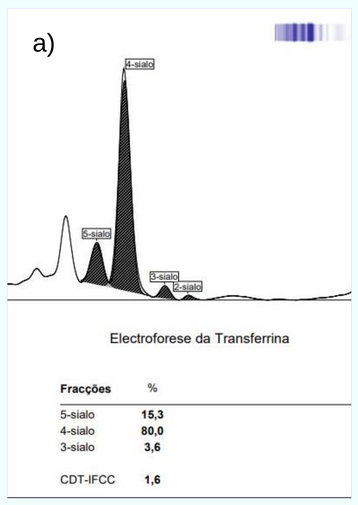
EQC (sample)	1	2	3	4	5	6	7	8	9	10
DI	-1,4	1,0	-1,2	-1,4	0,5	-1,3	2,1	1,7	-0,2	-0,2
Performance Assessement	Good	Good	Good	Good	Good	Good	Sat.	Good	Exc.	Exc.

EQC - External Quality Control DI - Deviation Index Sat. - Satisfactory Exc. - Excellent

DI evaluation criteria : ≤ 0,5 - Excellent; ≤ 2 - Good; ≤ 3 - Satisfactory; >3- Unsatisfactory

MU was calculated from the consensus means of the EQA control samples for normal (1.3%) and pathological (2.6%) levels. For the normal level the uncertainty obtained was 0.3% (1.3% ± 0.3%) which is consistent with the one indicated by the manufacturer. The MU of the pathological level was 0.6% (2.6% ± 0.6%),

The Figure 2 ilustre the CDT normal and pathological electrophoretic migration profiles.



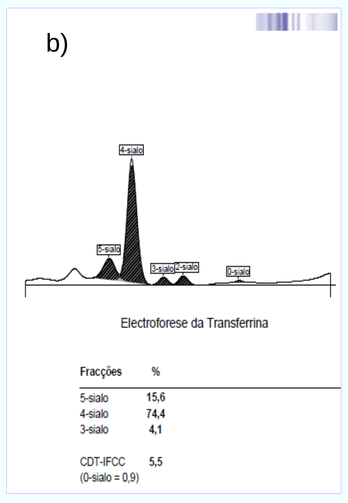


Fig 2- Normal (a) and pathological (b) control samples CDT capillary electrophoresis results

CONCLUSION

The ETLab obtained meets Westgard`s desirable specifications, therefore it is considered the appropriate methodology for use in laboratory diagnosis. However it is considered important to monitor the method with Internal Control samples and participate in AEQ programs, as well as periodic evaluation of Quality Indicators.

REFERENCES

(1) Kohler I, Augsburger M, Rudaz S, et al. - New insights in carbohydrate-deficient transferrin analysis with capillary electrophoresis-mass spectrometry. Forensic Science International. **2014**; 243: (14-22).
(2) Bean, P. et al. - Semiautomated procedures for evaluation of carbohydrate-deficient transferrin in the diagnosis of alcohol abuse. Clinical Chemistry. **1997**; 43, 6: (983-989).
(3) F. Botolotti; D. Sorio, A. Et al. – Analytical and Diagnostic aspects of Carbohydrate deficient Transferrin (CDT): a critical review over years, 2007 – 2017. Journal of Pharmaceutical and Biomedical Analysis, **2018;** 147; (2-12).
(4) I. Jastrzebska, A. Zwolak, M. Szczyrek et al. – Biomarkers of alcohol misure: recent advances and future prospects. Gastroenterology. Rev **2016**;11(2): 78-89
(5) https://insa.min-saude.pt/category/informacao-e-cultura-cientifica/publicacoes/boletim-epidemiológico-observacoes-numero-28/
(6) Helander A, Wielders J, Anton R, and at al. Reprint of standardization and use of the alcohol biomarker carbohydrate deficient transferrin (CDT). Clinica Chimica Acta. **2017;** 467:15-20.
(7) Schellenberg F, Wielders J, Anton R, et al. IFCC approved HPLC reference measurement procedure for the alcohol consumption biomarker carbohydrate-deficient transferrin (CDT): Its validation and use. Clin Chim Acta. 2017 Feb;465:91-100.
(8) Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra-and inter-individual biologic variation. https://www.westgard.com/biodatabase1.htm