AN AUTOMATED ASSAY FOR THE DETERMINATION OF SERUM CERULOPLASMIN FERROXIDASE ACTIVITY

Parant F.[1], Demily C.[2,3] , Bost M.[1,2]

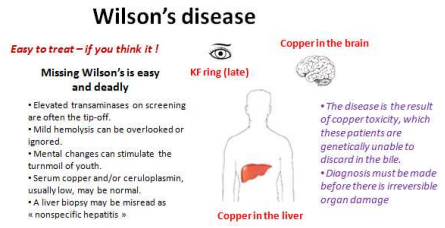
[1] *Service de Biochimie et Biologie moléculaire, Hôpital Édouard Herriot, Hospices Civils de Lyon, Lyon, France*

[2] *Maladie de Wilson – Centre National de Référence, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron, France*

[3] *Centre régional de dépistage et de prise en charge des troubles psychiatriques d'origine génétique, Hôpital le Vinatier & UMR 5229 (CNRS & Université de Lyon), Bron, France*

Introduction

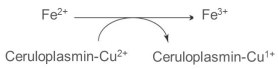
• Studies suggest that enzymatic ceruloplasmin (Cp) assays may be superior to immunologic assays in diagnosing Wilson's disease (WD) (1).



• The aims of our study were 1) to implement and to validate an automated enzymatic assay for the determination of serum Cp ferroxidase activity, and 2) to investigate the correlations between serum Cp ferroxidase activity, serum immunoreactive Cp and serum copper (Cu) in (a) healthy volunteers vs patients affected of WD and in (b) hospitalized patients with psychiatric disorders screened for WD.

Material & Methods

▪ Ceruloplasmin is a ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form.



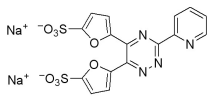
▪ Serum Cp ferroxidase activity was measured by the method of Erel (2) on a PENTRA 400 analyser (ABX).

PENTRA 400 is a compact Clinical Chemistry bench top analyser with throughput of up to 300 tests/hour in colorimetry (a cycle every 12 seconds). The analyser is bidirectionally interfaced to the laboratory information system (LIS).

- The optical system is characterized by a concave reflective grating spectrograph (co-developed with Jobin Yvon).

- The Pentra 400 offers both closed and open channels for customer-specific applications.

Serum Ferroxidase activity by the method of Erel Serum samples were incubated at 37°C with Fe^{2+} in 0.45 mol/L acetate buffer (pH 5.8) and the remaining non-oxidized ferrous ions formed a colored complex with the 3-(2 pyridyl)-5,6-bis(2-[5-furylsulfonic acid])-1,2,4-triazine (**Ferene S®**) chromogen (2).

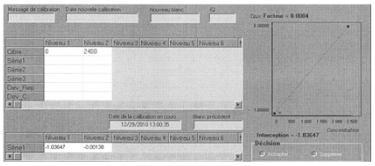


Ferene disodium salt : Ferene is one of the most sensitive colorimetric reagents for iron(II). Both the reagent itself and its iron(II) complex are highly water soluble, making Ferene very suitable for completely automated analyses on a practical scale, The effects of copper interference can be almost entirely eliminated by the addition of thiourea, which preferentially binds the copper in a copper(I) complex.

The reaction is monitored at 600 nm, with 700 nm as reference wavelength.

Deionized water is used as the first calibrator (zero) and EDTA solution as the second (2400 U/L).

A two-point inverse calibration was used



Serum immunoreactive ceruloplasmin and copper determination Serum immunoreactive Cp was determined using an immunoturbidimetric assay (DAKO) and serum Cu was measured using an inductively coupled plasma optical emission spectrometer (Vistapro, Varian).

Patients Serum Ferroxidase activity, immunoreactive Cp and Cu concentrations were determined in 16 normal subjects and in 17 patients with WD. Because 30 % of WD patients may initially present with psychiatric symptoms, 279 patients with psychiatric disorders were screened for WD (MOPSY Study).

Results

Performance characteristics

▪ The **precision** of the enzymatic assay was good with within-run and between-run coefficients of variation lower than 6%.

	Our study			Erel O. 1998 (2)		
	n	Mean (U/L)	CV (%)	n	Mean (U/L)	CV (%)
Within-run precision						
High	20	804	3.7	30	1282	1.6
Medium				30	521	1.5
Low	20	343	3.3	30	353	1.7
Between-run precision						
High	20	825	5.3	30	1320	2.7
Medium				30	512	1.7
Low	20	342	4.5	30	320	1.7

▪ The **limit of detection** (LOD) was below 18 U/L (mean of limit of blank + 3 SD). In accordance with our result, Flemming J.J. reports a LOD of 19 U/L (3).

▪ **Stability of the reagents**: > 6 months at 4°C.

Limitations

▪ **Blood Collection Supplies** EDTA Blood Collection Tube should not be used : EDTA solution completely chelated all ferrous ions and prevented the formation of the colored Fe^{2+} complex. Heparin did not inhibit the assay, but citrate did.

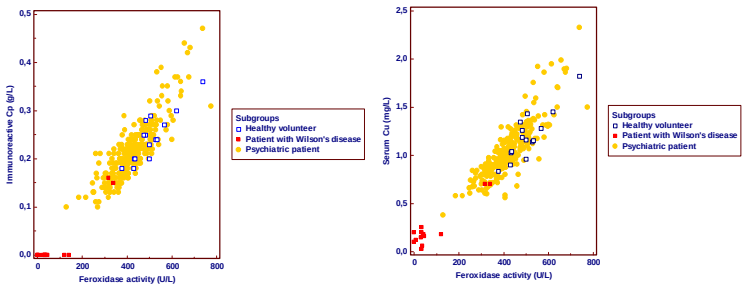
▪ **Inhibition** Sodium azide completely inhibited ferroxidase activity of commercial ceruloplasmin samples (2).

Application In 16 healthy volunteers vs 17 patients with WD, median (min-max) serum Cp ferroxidase activities, immunoreactive Cp and Cu levels were 499 U/L (375-739) vs 35 U/L (<20-339), 0.24 g/L (0.18-0.36) vs <0.03 g/L (<0.03-0.16) and 1.16 mg/L (0.83-1.82 mg/L) vs 0.18 mg/L (0.03-0.7) respectively.

▪ In 279 patients with psychiatric disorders, serum Cp ferroxidase activities, immunoreactive Cp and Cu levels were 419 U/L (130-775), 0.21 g/L (0.1-0.47 g/L) and 1.02 mg/L (0.38-2) respectively.

Ferroxidase activity (U/L)			
	Our study	Erel O., 1998 (2)	Flemming J.J., 2009 (3)
Healthy volunteers	499 ± 88 (n=15)	537 ± 201 (n=250)	571 ± 168 (n=84)
Patients with Wilson's disease	35 ± 106 (range : ND-339) (n=16)	25 (n=1)	76 ± 70 (range : ND-166) (n=17)
Patients with psychiatric disorders	419 ± 93 (range : 130-775) (n=279)		

▪ It appeared a relatively good correlation between serum Cp ferroxidase activities and immunoreactive Cp levels (r=0.857) and between serum Cp ferroxidase activities and Cu levels (r=0.851) (patients with psychiatric disorders).



Methods for measuring ceruloplasmin	
Drawbacks of immunologic methods	Drawbacks of enzymatic methods
• Immunologic methods cross react with apoceruloplasmin • There are method related differences including bias, precision and specificity	• There is no quality control material available for measuring ceruloplasmin activity. Hence only precision of the assays and not accuracy could be assessed.

Conclusion

Measurement of serum ceruloplasmin ferroxidase activity with Ferene S® as chromogen is adaptable to the ABX Pentra 400 analyser and could be applied to large-scale screening of patients for Wilson Disease.

Références **1. Merle U. et al.** Serum ceruloplasmin oxidase activity is a sensitive and highly specific diagnostic marker for Wilson's disease. J Hepatol. 2009 Nov;51(5):925-30

2. Erel O. Automated measurement of serum ferroxidase activity. Clin Chem. 1998 Nov;44(11):2313-9.

3. Fleming J.J. Usefulness of ferroxidase activity of ceruloplasmin in the diagnosis of Wilson's disease. Indian J Clin Biochem. 2009 Jan;24(1):15-22.

Hôpital Édouard Herriot – Lyon *E-mail: francois.parant@chu-lyon.fr*

6th International Symposium FESTEM – 26-28 May 2016 - Catania