Supplementary materials for manuscript entitled "Development and Analytical Validation of An Enzyme-Linked Immunosorbent assay (ELISA) for the detection of Copper in Human Hair and Serum Samples**"** Manuscripte ID AY-ART-01-2013-000042 for revision to Journal Analytical Methods.

2. Material and Methods Section

Apparatus

Reaction mixtures for Cu^{2+} were prepared in HEPES-KCl buffer (3 mM KCl, 10 mM HEPES, pH 5.0). A Multiskan Ascent reader for microtiter plates was from Thermo Electron Co. (Marietta, OH), this device was controlled by a personal computer. A Perkin-Elmer model AA Analyst 1100B atomic absorption spectrometer was also used (Norwalk, CT). A copper hollow acthode lamp was operated at a spectral width of 1.0 nm, which was selected to isolate the 324.7 nm line.

Collection of hair and serum samples

A total of 4 hair samples were collected from healthy children 5-10 years old, of both genders, without colored or treated hair, living in Nantong, Jiangsu Province, China. Hair samples 1-2 cm long were cut with stainless steel scissors from the nape of the neck close to the occipital region, and stored in plastic bags. Blood was collected into vacutainers tubes without any additives from 5 healthy children 5-10 years old. Immediately after collection, each blood sample was centrifuged at 3000

rpm for 10 min. In order to separate blood cells and suspended particles from serum, the sera were transferred into polyethylene tubes and stored in a freezer at 4 ◦C until the analysis.

AAS procedure

Hair extracts and serum samples were digested completely and analyzed directly with an atomic absorption spectrophotometer (Perkin-Elmer model 1100B). All analyses were performed in peak height mode to calculate absorbance values and analyzed in triplicate. Standard curve was established by using copper atomic absorption standard solution.

Results and Discussion

Linearity (dilutional parallelism)

Dilutional parallelism studies are useful to demonstrate the relative recovery after dilution and thus the linearity of the assay. Linearity is a condition in which test results are directly proportional to the concentration of analyte in the sample within a specified concentration range. The linearity of the assay was evaluated concomitantly with accuracy. Good dilutional linearity ensures that the additional dilution using the assay defined sample diluent won't affect accuracy. The found concentration mean had a linear relationship with the expected concentration. Recovery (found to expected ratios) for two serial dilutions of three serum samples ranged from 87.30%

to 97.40% (mean ± SD: 91.72 ± 3.39%) (Table S1). Recovery for three serial dilutions of two CRM hair extracts ranged from 90.9% to 108.9% (mean ± SD: 97.28 ± 6.7%) (Table S2). 100% is the ideal value, our recoveries were between 80% and 120%, well within the generally acceptable range of ±20%.

Electronic Supplementary Material (ESI) for Analytical Methods

Tables

Table S1 Dilutional parallelism of the Cu^{2+} ELISA in human serum samples (n=3)

Samples	Dilution factor	Found (μg/mL)	Expected (μg/mL)	Recovery (%)
	1		1.024	
Serum 1	2	0.447±0.081	0.512	87.30
	4	0.242±0.032	0.256	94.53
	1		1.198	
Serum 2	2	0.541±0.079	0.599	90.32
	4	0.267±0.043	0.3	89.0
	1		1.409	
Serum 3	2	0.687±0.102	0.705	97.40
	4	0.324±0.061	0.353	91.78

All three serum samples were evaluated at dilutions of 1:2 and 1:4,

[a]Recovery was calculate using (Found concentration/expected concentration)×100

Table S2 Dilutional parallelism of Cu^{2+} ELISA in two certified human hair samples (*n*=3)

Sample	Dilution factor	Found (μg/g)	Expected (μg/g)	Recovery[a] (%)
Human hair (1) NCSDC73347	1		10.6	
	5	1.942±0.293	2.12	91.6
	10	0.995±0.095	1.06	93.9
	20	0.482±0.051	0.53	90.9
Human hair (2) NCSZC 81002b	1		33.6	
	5	7.32±0.877	6.72	108.9
	10	3.40±0.431	3.36	103.8
	20	1.59±0.192	1.68	94.6

CRM hair samples were treated as described in section2 and evaluated at dilutions of 1:5, 1:10, and 1:20.

[a]Recovery was calculate using (Found concentration/expected concentration)×100