Chip sensor - Determination of Lead in Human blood by Differential Potentiometric Strpping Analysis

Purposes: Compare the accuracy and reproducibility between two methods in blood lead testing to provide reference data for clinical ,one is hydride generation atomic fluorescence spectrometry ; another one is chip sensor-differential potentiometric stripping .

Methods: Two methods were tested in blood samples and standard substances at the same time, and the results were analysed by statistical methods.

Results: Chip sensor - differential potentiometric stripping method is a quick, accurate and convenient method for clinical blood lead test.

1. Materials and methods

1.1 Instruments and methods

1.1.1 Chip sensor - differential potentiometric stripping method SR-P-100 (special type of blood lead) trace detector, which is provided by Wuxi Shenrui Bio-pharmaceuticals Co., Ltd.

1.1.2 Hydride generate atomic fluorescence AF-610B atomic fluorescence analyzer was manufactured by the Beijing Ruili Analytical Instrument(group) Co.,Ltd.

1.2 Experimental Methods

1.2.1 Specimen collection: Blood samples were collected by the Women and Children Health Center of Tianjin.

1.2.1.1 Venous blood of the adult were collected. Anticoagulant was EDTA-K2.

1.2.1.2 Peripheral blood of children were collected. 0.5% nitric acid cotton balls →75% alcohol cotton balls→ collecting blood after dry cotton balls handling blood collection site.

1.2.2 Detection Operated strictly with instrument rules.

1.3 Statistical analysis Results of blood lead test were statistical analysis by SPSS13.0. The difference between the two methods was using paired t test, and it was considered statistically significant when $P < 0.05$.

2 Results

2.1 Determination of the standard substances (accuracy experiments)

Six standard substances were measured by the chip sensor-differential potentiometric stripping analysis to evaluate the accuracy of this method of measurement, and four parallel determination of each standard substances, whichever is the mean compared with a given target. The comparison results of six standard substances and the target shows that error within the allowable range (±10% of the target was allowable error). It can be seen from Table 1.

(Blood lead standard substances were the testing blood of National External Quality Assessment in 2009, 2010 and 2011).

Table 1 The measurement results of Standard substances (μ g/L)

Number	Mean	Target
1	60.3	61.5
2	127.4	130.0
3	228.9	225
4	231.1	237
5	301.0	319
6	175.7	182

2.2 Comparison of two methods of blood lead measurement

The same 58 samples which selected from adults and children detected blood lead in our Center were used chips-differential electrode dissolution method and atomic fluorescence detection simultaneously.

2.2.1 The results shown in Table 2.

Table 2 The blood lead results of 58 specimens with two methods (μ g/L)

Number	Differential potentiometric stripping	Atomic fluorescence spectrometry
1	66.6	68.6
2	70.9	73.3
3	77.7	78.1
…	…	…
…	…	…
57	67.5	70.2
58	73.2	70.7

2.2.2 Analysis of differences

The data analysis with paired-samples t-test, and the results show in Table 3.

When P=0.867>0.05, the difference was not statistically significant..

Table 3 The results of Paired sample t-test (μ g /L)

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			

Pair VAR00003 1 -VAR00004	-.11379	5.15079	.67633	-1.4681 3	1.2450 4	-.168	57	.867

2.3 Repeatability

Chip sensor-differential potentiometric stripping determined the blood lead selected three level samples with 18 times repeated measurements for each sample, and then calculate the mean ± standard deviation and relative standard deviation (RSD). According to clinical testing rules of blood lead, the RSD of blood lead testing methods should be based on different test concentration ranges. The ranges are RSD≦15% when concentration is 20~100μg/L; and RSD≦10% as concentrations> 100μg/L. Table 4 shows the standard deviation of this method was smaller relatively. Therefore it indicated the reproducibility and precision of the instrument was better.

Table 4 Repeatability

Number	Mean	Relative Standard Deviation (%)
1	51.69±3.61	6,98
2	101.14±4.14	4.09
3	160.26±4.45	2.78

2.4 Recovery experiment

2.4.1 Selected two samples randomly, 475μL blood added to 25μL lead standard solution(1000μg/L) (standard solution is the national standard materials GBW (E) 080129), and chip sensor-differential potentiometric stripping determined the blood lead levels of added before and added. Four times measured repeatedly for each sample, then calculated the mean. The results showed in Table 5.

Table 5 The recovery test of the amount of 50 μ g/L

Number	Add the amount of standard solution（μg/L）	Value of sample（μg/L）	Value of sample added standard solution（μg/L）	Recoveries (%)
1	50	49.4	94.2	89.6
2	50	61.2	121.3	120.2

2.4.2 Selected two samples randomly, 450μL blood added to 50μL lead standard solution(1000μg/L), and chip sensor-differential potentiometric stripping determined the blood lead levels of spiked before and spiked. Four times measured repeatedly for each sample, and then calculated the mean. The results show in Table 6.

Table 6 The recovery test of the amount of 100 μ g/L

Number	Add the amount of standard solution（μg/L）	Value of sample（μg/L）	Value of sample added standard solution（μg/L）	Recoveries (%)
1	100	55.4	152.4	97
2	100	54.8	148.7	93.9

2.4.3 Selected two samples randomly, 400μL blood added to 100μL lead standard solution(1000μg/L), and chip sensor-differential potentiometric stripping determined the blood lead levels of added before and added. Four times measured repeatedly for each sample, and then calculated the mean. The results show in Table 7.

Table7 The recovery test of the amount of 200 μ g/L

Number	Add the amount of standard solution（μg/L）	Value of sample（μg/L）	Value of sample added standard solution（μg/L）	Recoveries (%)
1	200	79.5	263.8	92.15
2	200	57.9	249.8	95.95

Recovery requires 90-120%, in addition to some complicated steps may be required in 90-110%. As can be seen from Table 5-7, recovery rate in the

experiment was calculated and it was in the scope. Therefore this testing method is accurate and reliable.

3 Discussion

The results show that the chip sensor-differential potentiometric stripping method for the determination of blood lead is simple, rapid, high accuracy, moreover repeatability and precision is good. Compared with the hydride generation atomic fluorescence detection in blood lead, it was found no significant difference between the two methods.

(Reported by Women and children health center of Tianjin)

2011 年 7 月