

COMPARISON OF VITROS DRY SLIDE TECHNOLOGY FOR DETERMINATION OF LITHIUM IONS WITH OTHER METHODS

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ABSTRACT

The lithium ions concentration in human serum was determined using Dry-slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic), atomic absorption spectrometry (AAS) method Perkin Elmer 403 and ion-selective electrode (ISE) potentiometry AVL 9181. We compared lithium ions results in sample sera between these methods. Our reference method was AAS. We analyzed lithium ions concentration in 23 sera samples of patients after oral administration of lithium carbonate (3x 300mg) Jadran, Galen Laboratory Rijeka, by dry-slide technology, AAS and ISE methods. The quality control, precision, reproducibility and accuracy for Vitros dry slide technology were assessed. We established that the main difference between AAS method and dry slide technology was not statistically significant at $p < 0.05$ according to Student t-test. Therefore, the dry slide technology may be a useful alternative or it may even replace other methods, such as AAS. The main difference between dry slide technology and ISE methods was statistically significant at $p < 0.05$ using Student t-test. By ISE method, we obtained considerably higher results, which may be explained by the presence of electrolytes or medicaments interfering with lithium ions.

KEY WORDS: dry slide technology, AAS, ISE, and lithium

INTRODUCTION

Lithium carbonate was introduced into psychiatry in 1949 in treatment of mania. In 1970, the US Food and Drug Administration approved the use of lithium compounds in treatment of the manic phase of manic-depressive disorder (bipolar disorder). It is considered that lithium reduces the frequency of manic episodes and, with their repetition, reduces episode intensity. It prevents and stabilizes repetitive bipolar disorders without provoking sedation, amnesia and/or inhibition of mental processes. Around 0.1% of total population in Western Europe and Northern America use lithium preparations. The medical treatment with lithium preparations requires determination of the lithium ions concentration 12 hours after the last dose when the excretion of lithium ions has already started. Therapeutic range for lithium in serum is narrow (0.6-1.2 mmol/L), and patients have to be monitored for levels of the drug constantly because of its potential toxicity. Serious toxic effects appear at concentrations above 2.0 mmol/L although early symptoms may appear between 1.5 and 2.0 mmol/L (1,2). Atomic absorption spectrometry (AAS) and ion-selective electrode (ISE) potentiometry are the techniques used in quantitative determination of serum lithium concentration. Vitros dry slide technology method consists of a colorimetric end-point reaction where the crown-ether chromophore binds lithium in the sample, the resultant dye complex is measured using reflectance spectrophotometry. The advantages of dry slide technology include a shorter turnaround time, easy specimen preparations and less training involved in the operation (3, 4). Using patient samples collected in our laboratory we analyzed lithium concentrations by Vitros dry slide technology, AAS and ISE methods and compared the results.

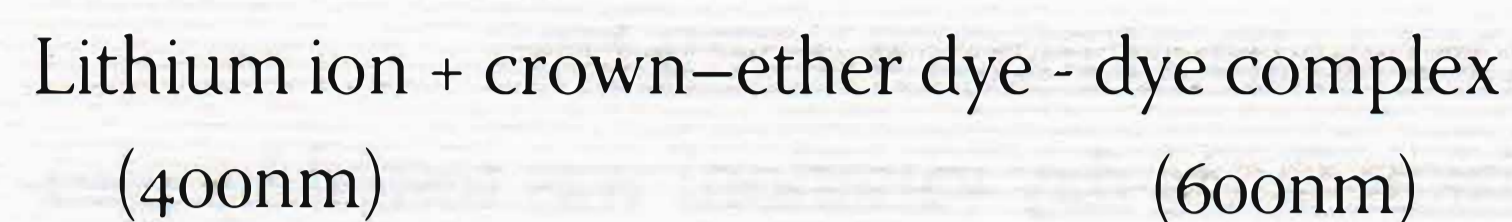
MATERIAL AND METHODS

DRY SLIDE TECHNOLOGY

The dry slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) was used for the determination of lithium ions in serum. In the dry slide technology 10 mL of sample is deposited on the slide and evenly distributed by the spreading layer. It then traverses the buffer and barrier layers to rest on the dye layer where lithium is specifically bound by the crown-ether chromophore conjugate (6 - dodecyl - 6 - (2' hydroxy - 5' - (2", 4" -dinitro phenylazol) benzyl) - 13, 13 dimethyl - 1,4,8,11 - tetraoxacyclotetradecane). Other slide ingredients include pigment, binders, buffer, surfactants and a polymer cross-linking agent. As lithi-

um ion binds to the crown-ether, a shift in the peak absorbance of the chromophore conjugate occurs.

The reaction sequence is:



The increase in absorbance at 600 nm is proportional to the concentration of lithium in the sample. The intensity of the dye is measured by reflectance spectrophotometry for 2.3 minutes at 37°C. The dynamic range for lithium ions determination is 0.20 mmol/L - 4.00 mmol /L (5).

DRUGS AND REAGENTS

Lithium carbonate* 300 mg tablets were obtained from Jadran, Galen Laboratory (Rijeka Croatia). We used control serums Precinorm* from F.Hoffman - La Roche Ltd (Switzerland) and Randox II from Randox laboratories (U.K.). The dry slide technology Vitros 250 Analyser uses clinical chemistry slide for determination of lithium ions.

SAMPLE PREPARATION

Patient samples were collected by serum separator 3.5 ml Vacutainer Tubes (Beckton Dickinson, Rutherford, NJ 07070 USA). After centrifugation at 3000 rpm serum was transferred into capped tubes and usually assayed that same day or stored at -20°C. Sodium heparin or EDTA were used as anticoagulants.

PATIENTS

This study was conducted respecting ethical standards stipulated in Helsinki Declaration. Venous blood was drawn from 10 males and 13 females (age: 20 - 50 years). Samples were collected from patients receiving lithium carbonate tablets (3x300 mg) in our Clinical Chemistry Laboratory over a period of 3 months. Duration of lithium carbonate treatment ranged from four months to thirteen years. The subjects were treated on inpatient basis at Psychiatric Clinic of Sarajevo University Clinics Center and Psychiatric hospital «Jagomir» in Sarajevo. The patients' therapy included lithium carbonate combined with other medications such as: haloperidol, biperiden, tioridazin, amitriptilin, klonazepam, nitrazepam, klomipramin and olanzapin.

QUALITY CONTROL

Clinical chemistry slides (Li) were optimized for use with patients' serum and plasma samples. Therapeutic and an elevated level control are run daily for quality-control. The high and the low range concentrations of commercially available quality-control material such as Precinorm* from F. Hoffman - La Roche Ltd (Switzerland) and Randox II from Randox

laboratories (U.K.) are used. Control samples are analyzed in the same manner as the patients' samples.

PRECISION AND REPRODUCIBILITY

The precision (intra-day variation) was tested by measuring (n = 20) three samples of different concentrations of lithium ions. The reproducibility (inter-day variation) for the same samples of lithium ions (n=20) was tested twice a day over 10 consecutive days.

ACCURACY

The accuracy of measuring was tested in 23 samples of serum collected from patients after oral administration of lithium carbonate (3x 300mg). Measures were obtained by dry-slide technology, AAS and ISE methods. Vitros Analyser 250 (Ortho Clinical Diagnostic) dry slide technology method was calibrated according to the manufactures directions.

STATISTICS

Results were statistically evaluated and expressed by means of standard deviation (SD), mean value (X), and coefficient of variation (CV). Congruency of results was investigated by analyzing linear regression expressed as a coefficient of correlation (r) and Student t test for p< 0.05.

RESULTS AND DISCUSSION

QUALITY CONTROL TESTING

Two control serums Precinorm and Randox II (n=10) were measured for quality control testing. Measurements were taken over 10 days period. Mean value

(X), standard deviation (SD) and coefficient of variation (CV) were calculated for both control serums. Statistical parameters are in Table 1.

The coefficient of variation (CV) for the two control sera using the dry slide technology ranged from 1.93 to 4.03 %.

PRECISION AND REPRODUCIBILITY TESTING

Precision (intra-day variation) and reproducibility (inter-day variation) were determined by running three samples with different concentrations of lithium ions. The mean value, standard deviation (SD) and coefficient of variation (CV) were calculated for all concentrations. Precision and reproducibility results are given in Table 2. Coefficient of variation (CV) for the precision of this method ranged from 3.00 to 4.44 % for serum lithium concentrations. Reproducibility was determined by running samples in the morning and in the afternoon over 10 consecutive days. Coefficient of variation (CV) for the reproducibility of lithium ions concentration in serum varied from 3.60 to 6.12 %.

ACCURACY TESTING

We compared lithium ions concentration measured in 23 blood sera by dry slide technology, AAS, and ISE methods. The results of the comparison between Vitros Analyser 250 and AAS (Perkin Elmer 250) analysis are shown in Figure 1. Sizable correlation was noted between Vitros Analyser 250 and AAS methods in the investigation of 23 blood samples (r = 0.9860). Regression equation revealed a slope of 1.0311 and a y axis intercept of

CONTROL SERUMS	PRECINORM	RANDOX II
Declarative concentration of lithium ions by producer (mmol/L)	1,19-1,49	1,99-2,53
\bar{x}	1,34	2,26
Measured concentration (mmol /L)	1,2-1,3 n=10	2,0-2,1 n=10
\bar{x}	1,24	2,07
CV %	4,03	1,93
SD	±0,0499	±0,0399

TABLE 1. Quality control testing dry slide technology

Concentration spiked (mmol /L)	Concentration found intra – day (mean SD,n = 20) (mmol /L)	Precision intra – day (%)	Concentration found inter-day (mean SD, n = 20) (mmol /L)	Reproducibility (%)
0,4	0,495 ±0,022	4,44	0,49 ± 0,03	6,12
0,6	0,695 ± 0,022	3,16	0,69 ±0,03	4,34
1,0	1,01 ± 0,03	3,0	1,015 ± 0,036	3,60

TABLE 2. Precision and reproducibility using dry slide technology

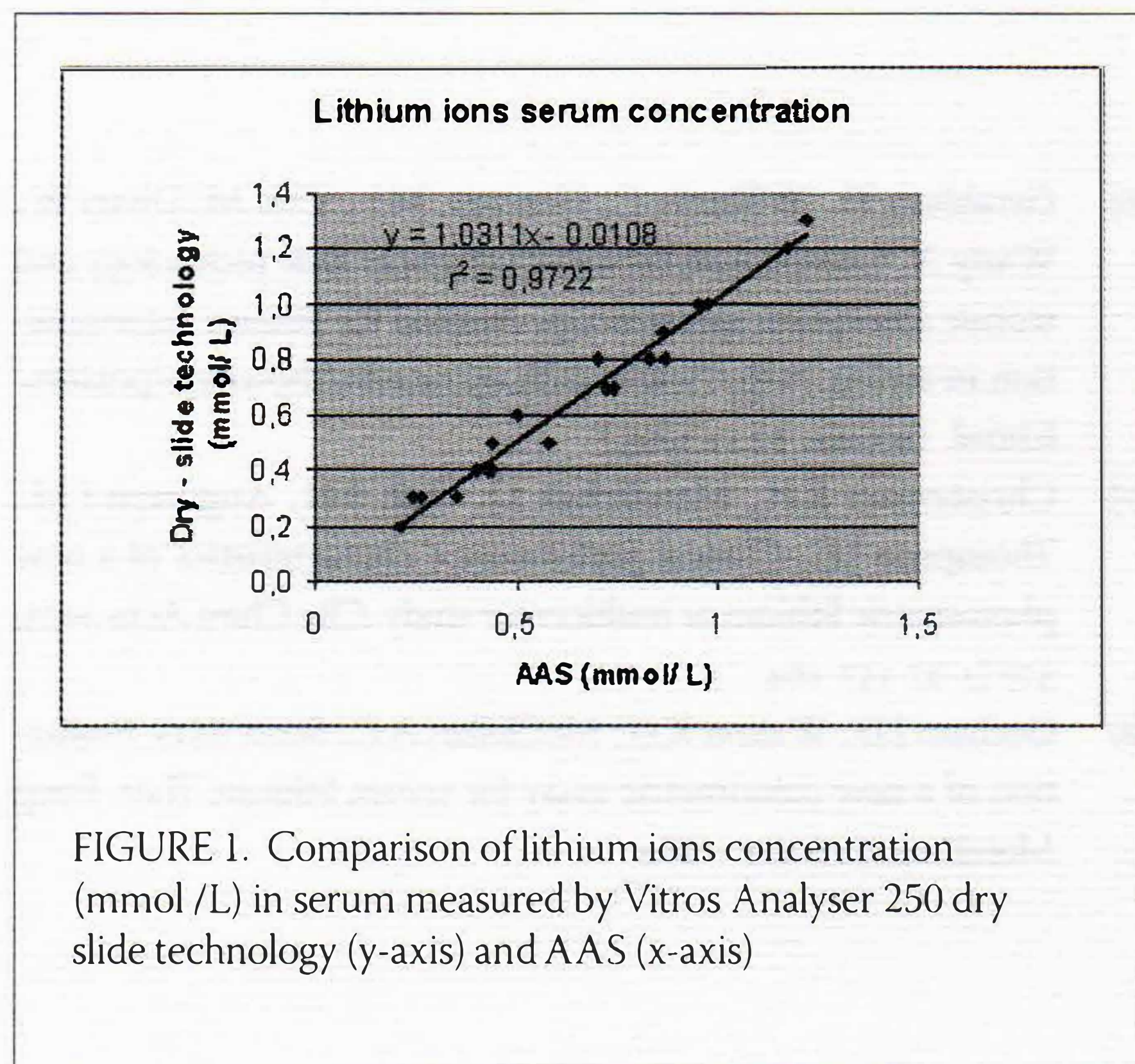


FIGURE 1. Comparison of lithium ions concentration (mmol /L) in serum measured by Vitros Analyser 250 dry slide technology (y-axis) and AAS (x-axis)

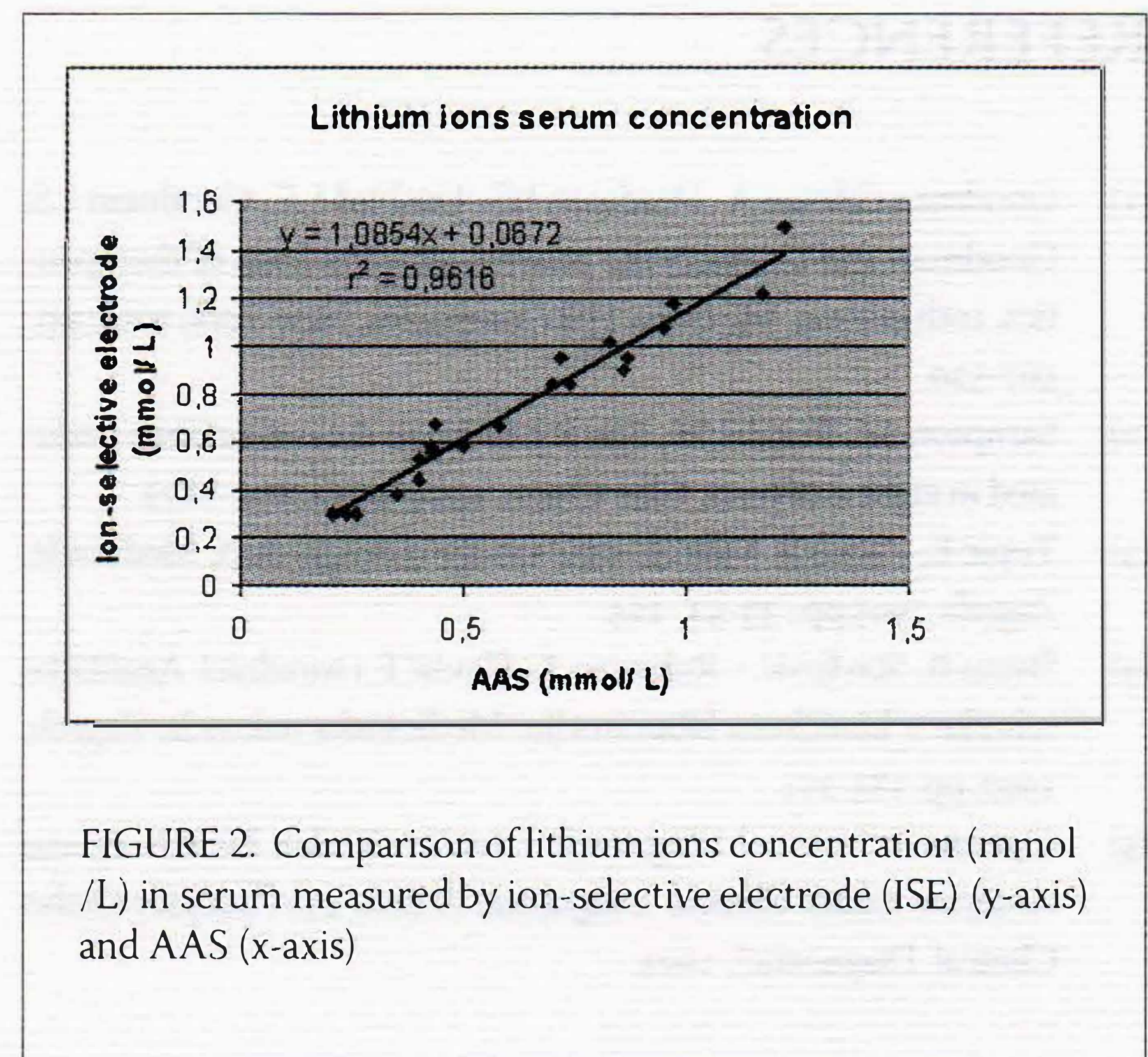


FIGURE 2. Comparison of lithium ions concentration (mmol /L) in serum measured by ion-selective electrode (ISE) (y-axis) and AAS (x-axis)

0.0108. The difference between the methods was statistically not significant for $p < 0.05$ according to Student t-test. The other groups gave similar results with a high correlation coefficient (3,6). The results of the comparison between AAS and ISE methods for lithium determination in serum are shown in Figure 2. Correlation of $r = 0.9806$ exists between AAS and ISE methods. We identified regression line with a slope of 1.0854 and y axis intercept of 0.0672. The difference between the methods was statistically significant for $p < 0.05$ using Student t test. The other authors quote $r = 0.999$ for the correlation between ISE and AAS method (7). Comparison of lithium ions concentration between Vitros Analyser 250 and ISE methods are shown in Figure 3. Correlation coefficient was $r = 0.9708$ and regression line had a slope of 1.0276 and a y axis intercept of 0.0942. The main difference between the methods was statistically significant for $p < 0.05$ using Student t test. In other similar studies, Vitros Analyser dry slide technology gave good results in lithium ions determination in serum that were consistent with those

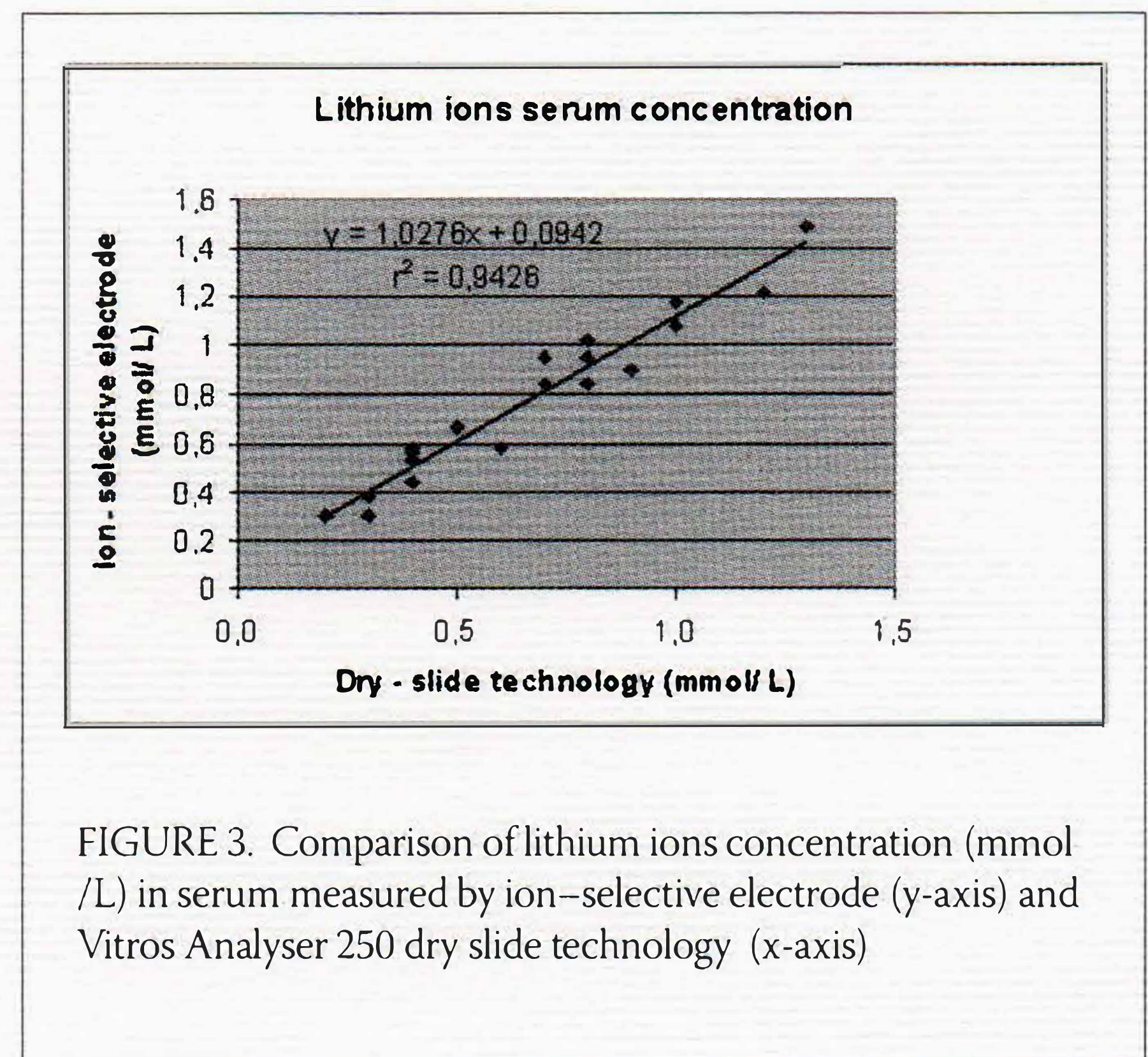


FIGURE 3. Comparison of lithium ions concentration (mmol /L) in serum measured by ion-selective electrode (y-axis) and Vitros Analyser 250 dry slide technology (x-axis)

obtained using AVL method ($r = 0,99$), with correlation coefficient close to one (8). Lower correlation coefficient in our study is probably due to possible interference of other ions such as sodium, potassium, calcium, hydrogen and ammonium in AVL method.

CONCLUSION

Dry slide technology is an applicable method significant in the therapeutic monitoring of patients receiving lithium salts. Also, it may be a useful alternative or it may even replace other methods such as AAS. The main difference between dry-slide technology and AAS methods was not statistically significant for $p < 0.05$ using Student t test, which confirms our assumptions. ISE method showed more variability in the determination of lithium ions than other methods, such as AAS and dry slide technology, which may be attributed to the presence of electrolytes or medicaments interfering with lithium ions. Therefore, lithium ions concentration in serum obtained by ISE method was higher in comparison with the other two methods. We conclude that Vitros dry slide technology method provides reliable lithium concentration results in therapeutic range and is a valid alternative to AAS method.

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