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A Candidate Reference Method for the Determination of Magnesium in Serum

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Summary: A candidate reference method for the determination of magnesium in serum (analytical range 0.5 to 2.0 mmol/l) by flame atomic absorption spectrometry was commissioned. The relative standard deviation of the 4 replicates of each value ranged from 0.18 to 1.07%, and the standard error of the mean ranged from 0.63 to 8.34 µmol/l. In the analysis of 3 different standard solutions (prepared by weighing the analyte), which was performed in each of three different experiments the values recorded by the candidate reference method deviated by −0.15, 0.44 and 0.24%, respectively. The reference method value did not differ significantly from the definitive value. As the method seems to be as reliable as comparable reference methods for the determination of sodium, potassium or calcium, tests of transferability should now be undertaken.

Introduction

The magnesium concentration in serum is determined by flame atomic absorption spectrometry or various methods using absorption spectrometry (e. g. Magon, Titan Yellow). These field methods are subject to systematic errors of known or unknown magnitude. An unequivocal evaluation of the accuracy of these routine methods is possible only by the use of control sera with reference method assigned values or definitive values. The definitive method for the determination of magnesium in serum – isotope dilution mass spectrometry – is the prerogative of highly specialized laboratories, and therefore not widely accessible. It was decided to set up a reference method based on flame atomic absorption spectrometry, which is more available than the definitive method. As our laboratory had participated in the evaluation of the approved reference method for the determination of calcium (1, 2) we tried to adopt this procedure for the development of a reference method for the determination of magnesium.

Materials and Method Protocol

The operator must be familiar with atomic absorption spectrometry and the relevant analytical techniques, and strictly follow the protocol without any modifications. Usually 4 working days are needed for the determination of 3 reference method values. Room temperature should be between 20 and 25 °C and constant to ± 0.5 °C.

1.1 *Water*: Doubly distilled and deionized water with a specific resistance > 10 kOhm · m at room temperature was used. About 60 l are needed for the determination of 3 reference method values including cleaning of the glassware.

1.2 *Magnesium standard solution*: Magnesium standard solutions were prepared from Standard Reference Material, magnesium digluconate dihydrate $Mg(C_6H_{11}O_7)_2 \cdot 2H_2O$, SRM 929, from the National Bureau of Standards (NBS) (Washington D. C., USA). The SRM was dried before use in a desiccator containing phosphorus(V)oxide for 12 hours.

1.3 *Lanthanum oxide* (La_2O_3) p. a. was from Merck, Darmstadt, F. R. G.; its magnesium content was less than 5 mg per kg.

1.4 *Sodium chloride*, p. a., and *potassium chloride*, p. a., were obtained from Merck, Darmstadt, F. R. G. The materials contained less than 10 mg magnesium per kg. They were dried at 200 °C for 4 hours and stored in a desiccator containing silica gel with moisture indicator at room temperature for at least 4 hours.

1.5 *Hydrochloric acid* (HCl) minimal 30% "Suprapur" from Merck, Darmstadt, F.R.G. Its magnesium content was less than $1 \cdot 10^{-6}$ %.

1.6 *Sodium hydroxide* (NaOH) 1 mol/l p.a. from Merck, Darmstadt, F.R.G.

1.7 The following *control sera* were analysed by the reference method:

(1) Standard Reference Material 909, National Bureau of Standards, Washington D.C., USA,
(2) Control Serum N Roche lot No. P 1039, Hoffmann-La Roche AG, Grenzach-Wyhlen, F.R.G.,
(3) Control Serum P Roche lot No. P 2439, Hoffmann-La Roche AG, Grenzach-Wyhlen, F.R.G.,
(4) Fluinorm N lot No. 621608, Behring Institut, Marburg/Lahn, F.R.G.,
(5) Gilford QCS abnormal lot No. 25501, Ciba Corning Diagnostics Corp., Irvine/CA, USA,
(6) Gilford QCS abnormal lot No. 25505 E, Ciba Corning Diagnostics Corp., Irvine/CA, USA.
(7) Gilford QCS normal lot No. 25501, Ciba Corning Diagnostics Corp., Irvine/CA, USA,
(8) Kontrollogen L lot No. 623125, Behring Institut, Marburg/Lahn, F.R.G.,
(9) Kontrollogen LP lot No. 623210, Behring Institut, Marburg/Lahn, F.R.G.,
(10) Monitrol I lot No. LTD 208, Merz & Dade GmbH, München, F.R.G.,
(11) Monitrol II lot No. LTD 108, Merz & Dade GmbH, München, F.R.G.,
(12) Pathonorm H lot No. 21. Nycomed AS, Oslo, Norway,
(13) Pathonorm L lot No. 20, Nyegaard & Co. AS, Oslo, Norway,
(14) Precinorm U lot No. 153146, Boehringer Mannheim GmbH, Mannheim, F.R.G.,
(15) Seronorm lot No. 166, Nyegaard & Co. AS, Oslo, Norway,
(16) Validate A lot No. 4 × 065, Gödecke AG, Berlin, F.R.G.,
(17) Validate N lot No. OB 924, Gödecke AG, Berlin, F.R.G.,
(18) Validate N lot No. 4 × 023, Gödecke AG, Berlin. F.R.G.

1.8 *Balance*

An analytical balance was used (Sartorius, Göttingen, F.R.G.), and this was officially calibrated and regulary checked with weights. Its readability was 0.01 mg and its standard deviation 0.01 mg.

1.9 *Glassware*

1.9.1 *Required glassware*

All volumetric glassware was of borosilicate material, confirming to class A specifications, and officially calibrated by German authorities.

The determination of 3 reference method values required the following *volumetric stoppered flasks*: three 5 l, six 1 l, twelve 500 ml, two 250 ml, and two 100 ml; and the following *pipettes*: one 50 ml, three 25 ml, three 10 ml, three 5 ml, two 2 ml.

Watch glasses: nine
Cylinder: one 250 ml
Burette: one 25 ml
Conical flasks: four 100 ml
Beaker: four 1 l
Funnel: one large, one small

1.9.2 *Cleaning of the glassware*

1. Glassware was soaked in an aqueous solution of a nonionic phosphate-free detergent (Sodosil RA 15: Riedel de Haën, Hannover, F.R.G.) at 40 °C for 60 min and rinsed 4 times with doubly distilled water (1.1) (1/10 of each container's volume for each rinse). Pipettes were rinsed by drawing water through with a suction pump.

2. All glassware was immersed for 12—16 h in 1 mol/l hydrochloric acid (see 1.5).

3. The glassware was rinsed with doubly distilled water (1.1) 6 times with one tenth of its volume, pipettes with the aid of a suction pump.

4. All glassware was dried at room temperature in a dust-free environment, the pipettes with their tips upwards.

1.10 *Preparation of reagents*
(for the determination of 3 reference method values)

1.10.1 *Hydrochloric acid 7.80 mol/l*
(100 ml required)

Concentrated hydrochloric acid (1.5) was diluted with water (1.1) to approximately 10 mol/l and titrated to 7.80 mol/l ± 0.08 mol/l by addition of aqueous sodium hydroxide (1.6).

1.10.2 *Hydrochloric acid 1.00 mol/l*

Concentrated hydrochloric acid (1.5) was diluted with water (1.1) to approximately 1.5 mol/l, then appropriately further titrated by use of aqueous sodium hydroxide (1.6) to give a final volume of at least 60 ml with an acid concentration of 1.00 mol/l ± 0.01 mol/l.

1.10.3 *Diluent I*

Lanthanum oxide (8.15 g) was washed into a 5 l flask with exactly 50 ml hydrochloric acid (1.10.1), followed by rinsing with 50 ml doubly distilled water (1.1) for complete transfer of salt and acid. When dissolution of lanthanum oxide — promoted by swirling — was complete, the volume was adjusted to the calibration mark by the addition of doubly distilled water (1.1), followed by thorough mixing by inverting and shaking the stoppered flask 30 times.

1.10.4 *Diluent II*

Sodium chloride (0.812 g) (1.4) and potassium chloride (0.037 g) (1.4) were washed into a 5 l flask with 50 ml of diluent I (1.10.3), then rinsed with diluent I for complete transfer. The volume wad adjusted to the calibration mark by addition of diluent I (1.10.3). The solution was mixed thoroughly by inverting and shaking the stoppered flask 30 times.

1.10.5 *Stock blank and stock calibration solutions*

Stock blank (S_0): About 2 ml doubly distilled water (1.1) were pipetted into a 1 l flask and exactly 10 ml hydrochloric acid 1 mol/l (1.10.2) were added. The volume was adjusted to the calibration mark by the addition of doubly distilled water (1.1). The solution was thoroughly mixed (see 1.10.3).

Stock calibration solutions: Stock calibration solution 1 (S_1) was produced by transferring 0.22532 g magnesium digluconate dihydrate (1.2) to a 1 l flask with about 2 ml doubly distilled water (1.1). Exactly 10 ml hydrochloric acid 1 mol/l (1.10.2) were added. After complete dissolution of the salt, the volume was adjusted to the calibration mark by the addition of doubly distilled water (1.1). The solution was mixed thoroughly by inversion (see 1.10.3). Succeeding calibration solutions S_2, S_3, S_4, and S_5 were obtained by a similar procedure; the quantities of magnesium salt added are given in table 1.

Tab. 1. Stock blank and stock calibration solution (1 litre each)

	Magnesium digluconate dihydrate added (mg)		Magnesium concentration (mmol/l)
Stock blank	(S_0)	–	0.00
Stock calibration solution 1	(S_1)	225.32 ± 0.05	0.50
Stock calibration solution 2	(S_2)	225.32 ± 0.05	0.50
Stock calibration solution 3	(S_3)	563.31 ± 0.05	1.25
Stock calibration solution 4	(S_4)	901.30 ± 0.05	2.00
Stock calibration solution 5	(S_5)	901.30 ± 0.05	2.00

1.10.6 *Working blank solution* (W_0) *and working standard solutions* ($W_1 - W_5$)

A single 5 ml run-out pipette was used to dilute the stock blank and the stock calibration solutions (as well as the reference and the unknown sera).

1. To prepare the working blank solution (W_0) about 450 ml diluent II (1.10.4) were transferred to a 500 ml flask.

2. The 5 ml pipette was filled with the stock blank solution (S_0) just above the calibration mark. Then the sucked in volume was discarded. The procedure was repeated two times.

3. The pipette was filled just above the calibration mark with S_0. Its outside was wiped dry with tissue. The volume was adjusted to the calibration mark by running excess solution to waste. The contents of the pipette were delivered into the flask including the remaining fluid that was gently blown out. The outside of the pipette tip was rinsed with 1 ml diluent II and the washings were collected in the flask. The pipette was rinsed by filling it with diluent II three times, each time delivering the solution into the flask.

4. Its volume was adjusted to the mark with diluent II, stoppered and its contents mixed by inverting and shaking 30 times.

5. The pipette was washed out 3 times with doubly distilled water (1.1).

6. The working standard solutions W_1, W_2, W_3, W_4 and W_5 were obtained similarly by substituting stock blank solution (S_0) with S_1, S_2, S_3, S_4, and S_5 respectively. After successful calibration (1.12.5) W_1, W_2, W_3, W_4 W_5 and W_0 were kept at room temperature until the next day.

7. After washing out (1.10.6. (5)) the pipette was dried and put aside for preparing the diluted reference and unknown sera (on the next day).

1.11 *Preparation of reference and unknown sera*

On the following day, provided the instrument check was satisfactory (1.12), reference and unknown sera (1.7) were prepared for determination.

Lyophilized serum specimens were reconstituted as follows:
1. The specimens reached room temperature overnight in a desiccator.

2. The rubber stopper of the vial was carefully removed without loss of freeze-dried material.

3. The appropriate amount of doubly distilled water (1.1) — usually 5 ml — was pipetted into the vial. The volume added was checked by weighing the vial before and after addition of the water. Five ml doubly distilled water weigh 4.991 g at room temperature.

4. The vial was sealed with its stopper, gently inverted and agitated for 20 min at room temperature.

5. Five vials of each serum reconstituted as described were pooled.

6. Each serum pool was diluted in the same way as the stock solutions (see 1.10.6) with minor modifications:
1. Diluent I was used instead of diluent II.
2. The pipette (see 1.10.6.7) was primed with 1.5 ml of the pooled serum 3 times (instead of 5 ml 3 times): About 1.5 ml pooled serum was drawn into the pipette, which was then held in a horizontal position and slowly rotated to wet the internal surface to just above the calibration mark. The "priming" portions of the pooled serum were discarded.
3. Two (instead of one) working dilutions were prepared from each pooled serum.

1.12 *Measurement by flame atomic absorption spectrometry* (FAAS)

The measurements were performed with an atomic absorption spectrometer model 3030 from Perkin-Elmer (Bodenseewerk Perkin-Elmer, Überlingen, F. R. G.) operated at 285.2 nm in the single channel mode. A stoichiometric air-acetylene flame was used and doubly distilled water was aspirated during the 15 min warm-up period of the burner.

1.12.1 While aspirating water (1.1) the instrument was adjusted to zero.

1.12.2 The working standard solution W_4 was nebulized. An absorbance reading between 0.180 and 0.300 was required, and if necessary the instrument settings (aspiration rate, gas flow, burner position etc.) were adjusted to achieve this.

1.12.3 While aspirating W_4 the scale was expanded to give a readout of approximately 1500–1600 units. On changing to doubly distilled water (1.1), the reading was readjusted to zero if necessary. W_0, W_1 and W_2 were measured successively. The reading of W_0 had to be less than 3% of the reading of W_1 or W_2.

1.12.4 The linearity of calibration was checked by measuring successively: doubly distilled water (1.1), W_0, W_1, W_2, W_3, W_4 and W_5. This sequence was repeated 3 times. After the 4th run, water (1.1) was nebulized for 10 min.

1.12.5 With the data obtained in 1.12.4 calculations were performed according to 1.13, using 5 samples ($p = 5$) and 4 runs ($r = 4$). In cases of inadequate precision (criterion 1.14.3), the instrument was checked, readjusted and the measurements (1.12.1 to 1.12.4) and calculations were repeated. In cases of inadequate accuracy (criterion: 1.14.3), one or more of the relevant working dilutions of the stock calibration solutions were prepared again. If this procedure failed, the stock calibration solutions themselves were freshly prepared. Provided that the criteria according to 1.14.3 were met, the instrument was shut down without changing any setting.

1.12.6 The FAAS was continued on the next day, and W_0 to W_5 were measured. If the readings were within 2% of the values

obtained on the previous day, operations proceeded to 1.11. Otherwise the instrument was readjusted or — if this was unsuccessful — the calibration solution was prepared freshly.

1.12.7 After dilution of the reconstituted sera (1.11) the measurement by flame atomic absorption spectrometry was performed:

1. W_0, W_1 to W_5 and the diluted sera were nebulized and afterwards arranged in an ascending order of the (expanded) readings, but care was taken that no two serum dilutions were aspirated successively.

2. In a first run the samples were measured in the prescribed order, beginning with W_0.

3. The second, third and fourth run were performed similarly.

4. The subsequent four runs were performed after adjusting the flame to a slightly fuel-lean condition, but in the same sequence of analyses.

1.13 *Calculations*

The following calculations were performed to obtain the magnesium concentration of the sera (in the example (see tab. 2) one reference (R) and two unknown sera (U) were measured) and evaluate the validity of the values.

1. Each row and each column were summed separately.

2. From the readings x_i of a row S_{xx} was calculated:

$$S_{xx} = \sum_{i=1}^{r} x_i^2 - \frac{\left(\sum_{i=1}^{r} x_i\right)^2}{r}$$

r: number of runs

3. S_{xx} of the p rows (p = 11) were summed. S_{xs} divided by p was subtracted from the sum of S_{xx}. The resulting difference was divided by the number of degrees of freedom (p − 1) (r − 1) in order to obtain the residual standard deviation s.

$$s = \pm \sqrt{\frac{\Sigma S_{xx} - \frac{S_{xs}}{p}}{(p-1)(r-1)}}$$

4. The mean slope (b) of the line y = bx + a (y: reading of the calibration solution; x: magnesium concentration of the relevant calibration solution) was calculated according to the following formula:

$$b = \frac{\Sigma W_4 + \Sigma W_5 - \Sigma W_1 - \Sigma W_2}{r \cdot 2 \cdot 1.5}$$

(W_4, W_5: 2.0 mmol/l magnesium;

W_1, W_2: 0.5 mmol/l magnesium)

The precision index was given by $\frac{s}{b}$ (mmol/l).

5. The effective standard error, s_0, of the mean concentration of a sample was:

$$s_0 = \pm \sqrt{\frac{\left(\frac{s}{b}\right)^2}{r} + 0.015^2} \text{ (mmol/l)}$$

6. The following differences and the corresponding standard errors were calculated:

$$d_1 = \frac{\Sigma W_1 - \Sigma W_2}{r \cdot b};\ s_0\sqrt{2}$$

$$d_2 = \frac{\Sigma W_4 - \Sigma W_5}{r \cdot b};\ s_0\sqrt{2}$$

$$d_3 = \frac{4 \cdot \Sigma W_3 - \Sigma W_1 - \Sigma W_2 - \Sigma W_4 - \Sigma W_5}{r \cdot b};\ s_0 \cdot \sqrt{20}$$

Tab. 2. Determination of the magnesium concentration by the candidate reference method. Example of readings and calculations

Row		Run I	Run II	Run III	Run IV	Σx_i	S_{xx}
1	W_1	401	403	400	404	1608	10.00
2	W_2	406	402	402	404	1614	11.00
3	W_3	991	993	993	1000	3977	46.75
4	W_4	1566	1572	1561	1576	6275	130.75
5	W_5	1570	1579	1578	1584	6311	100.75
6	R_1	737	739	739	746	2961	46.75
7	R_2	737	741	741	746	2965	40.75
8	$U_{1,1}$	1602	1597	1598	1608	6405	74.75
9	$U_{1,2}$	1599	1608	1599	1606	6412	66.00
10	$U_{2,1}$	608	609	608	610	2435	2.75
11	$U_{2,2}$	605	605	603	612	2425	46.75
						total:	577.00 (ΣS_{xx})
Sum of column		10822	10848	10822	10896	43388	3652.00 (S_{xs})

W_1 — W_5: working calibration solution.
R_1 — R_2: working solution of the reference serum.
U_1 — U_2: working solution of the unknown serum.
x_i: readings in one row.

$S_{xx} = \Sigma x_i^2 - (\Sigma x_i)^2 \cdot \frac{1}{r}$

S_{xs}: S_{xx} of sums row

7. The concentration of one serum assay P_{x1} (mmol/l) was estimated to be:

$$P_{x1} = 1.25 + \frac{\Sigma R_{P_{x1}} - \frac{\Sigma W_1 + \Sigma W_2 + \Sigma W_3 + \Sigma W_4 + \Sigma W_5}{5}}{r \cdot b}$$

$R_{P_{x1}}$: expanded reading of the serum

P_{x2} was calculated in the same manner.

8. The same calculations (see 1.13, 1—7) were performed with the data from the measurements with fuel-lean flame conditions.

9. For each serum two pairs of estimates of concentration were thus obtained:

P_{x1}, P_{x2}, P_{x3} and P_{x4}.

The arithmetic mean $\overline{P_x}$ and the corresponding standard deviation were calculated.

10. The reference method value (C_R) ($= \overline{P_x}$) of the reference serum was compared with its definitive value (C_D):

$$\frac{C_D - C_R}{C_D} \cdot 100$$

11. If all the criteria given below (1.14) were fulfilled, $\overline{P_x}$ was a valid estimation of the magnesium concentration of the unknown serum.

1.14 *Criteria of acceptability*

1. The "true" absorbance reading (before expanding the scale) of the working calibration solution (W_4/W_5 see 1.10.6) must be in the range 0.180—0.300 as compared with doubly distilled water.

2. The expanded scale reading of the working blank solution (W_0 see 1.10.6) must be less than 3% of the reading of W_1/W_2.

3.(1) The precision index $\frac{s}{b}$, as calculated from the working solutions W_0—W_5, must not exceed 0.020 mmol/l.

(2) The differences d_1, d_2, d_3 (1.13.6) must be less than twice their corresponding standard errors (1.13.6); otherwise the instrument was adjusted (1) or the calibration solutions were prepared freshly (2).

4. The readings of W_1 to W_5 (1.10.6) must not deviate more than 2% from the readings of these solutions on the previous day.

5. The relative standard deviation (CV) of the 4 replicates of a reference method value must be less than 1.25% (1.13.9).

6. The deviation of the reference method value from the definitive value must be less than 2%.

7. If the criteria 1.14.1 to 1.14.4 and 1.14.6 were fulfilled, only the unknowns with standard deviations > 1.25% had to be excluded.

Tab. 3. Determination of the reference method value of the magnesium concentration of control sera by the candidate reference method. Precision of the contribution values

	I[1]		II[2]				
	X_1	X_2	X_3	X_4	$\bar{X}$[3]	s[4]	CV[5]
Pathonorm L	0.573	0.576	0.574	0.574	0.574	1.26	0.22
Validate A	0.753	0.754	0.750	0.742	0.748	4.93	0.66
Seronorm	0.751	0.760	0.757	0.765	0.758	5.85	0.77
Control Serum N	0.789	0.781	0.799	0.790	0.790	7.37	0.93
Gilford normal	0.877	0.879	0.881	0.876	0.878	2.22	0.25
Monitrol I	0.931	0.932	0.928	0.932	0.931	1.89	0.20
Validate N[6]	0.954	0.951	0.971	0.963	0.960	9.07	0.94
Kontrollogen L	0.987	0.985	0.989	0.983	0.986	2.58	0.26
Precinorm U	1.016	1.004	1.006	0.999	1.006	7.14	0.71
Validate N[7]	1.042	1.041	1.032	1.034	1.037	4.99	0.48
Fluinorm N	1.312	1.309	1.308	1.305	1.309	2.89	0.22
Pathonorm H	1.391	1.391	1.387	1.393	1.391	2.52	0.18
Control Serum P	1.548	1.538	1.571	1.571	1.557	16.67	1.07
Kontrollogen LP	1.601	1.601	1.609	1.606	1.604	3.95	0.25
Gilford abnormal[8]	1.820	1.824	1.828	1.828	1.825	3.83	0.21
Gilford abnormal[9]	1.938	1.940	1.945	1.946	1.942	3.86	0.20
Monitrol II	2.034	2.037	2.245	2.232	2.037	5.72	0.28

[1]) stoichiometric flame
[2]) lean flame
X_1—X_4: magnesium concentration (mmol/l) of an assay
[3]) reference method value (mmol/l)
[4]) 1. standard deviation (µmol/l)
2. standard error of the mean $s_{\bar{x}} \cdot 2$ (i.e. 95% confidence limits of the reference method value)
[5]) relative standard deviation: $\frac{s}{\bar{x}} \cdot 100$
[6]) Validate N lot No. 4 × 023
[7]) Validate N lot No. OB 924
[8]) Gilford QCS abnormal lot No. 25505 E
[9]) Gilford QCS abnormal lot No. 25501

Results

Precision

Seventeen different control sera were analysed by the reference method. The mean relative standard deviation of the results $P_{x1} - P_{x4}$ (1.13.9) contributing to the reference method value was 0.46% (range 0.18 – 1.07%) (tab. 3). The reference method value of one control serum (Seronorm) was determined 8 times. The arithmetic mean of the reference method values was 0.758 mmol/l, its relative standard deviation 0.70%, the range 0.751 – 0.766 mmol/l.

Accuracy

1. An adequate amount of doubly distilled water (1.1) was weighed into a volumetric flask (100 ml) using an analytical balance, and the volume was ajusted to the calibration mark with stock calibration solution S_5 (2.00 mmol/l Mg). The working solutions were prepared as described for sera, but substituting diluent I by diluent II. The reference method values differed by −0.15% (tab. 4) from the concentration given by the pipetting procedure.

2. Magnesium digluconate dihydrate (Standard Reference Material 1.2) was weighed and dissolved in doubly distilled water (1.10.5). The solutions were further processed as sera, but diluent II was used instead of diluent I. The reference method values are given in table 5 (mean deviation 0.44%).

3. Three control sera, which had been previously analysed by the reference method, were spiked with magnesium:
1. The lyophilized control sera were reconstituted and pooled.
2. The pooled serum was diluted with diluent I, and 5 ml S_1 which was washed out into the flask.
3. The recovery of the spiked magnesium was calculated by subtracting the reference method value from the result obtained after spiking (tab. 6) (mean recovery: 100.67%). The mean deviation from the target value was + 0.24%.

4. Only one control serum was available that had been analysed by a definitive method (SRM 909). The reference method values were within the confidence limits of the definitive value (tab. 7).

Transferability

The first preliminary experiments concerning transferability were promising. The reference method values of the two laboratories, which analysed 2 different control sera, agreed well (tab. 8). For the final assessment, however, a large-scale transferability study must still be undertaken.

Tab. 4. Accuracy of the candidate reference method. Determination of the magnesium concentration of primary standard solutions

Magnesium concentration[1]) (y) (mmol/l)	Magnesium concentration determined (x) (mmol/l)	$\frac{x - y}{y} \cdot 100$
0.600	0.599	− 0.17
1.000	0.996	− 0.40
1.600	1.602	+ 0.13

[1]) magnesium concentration obtained by dilution of the stock calibration solution (2.0 mmol/l)

Tab. 5. Accuracy of the candidate reference method. Determination of the magnesium concentration of primary standard solutions

Magnesium concentration[1]) (y) (mmol/l)	Magnesium concentration determined (x) (mmol/l)	$\frac{x - y}{y} \cdot 100$
0.600	0.602	+ 0.33
1.000	1.006	+ 0.60
1.600	1.606	+ 0.38

[1]) magnesium salt weighed (preparation of the solutions as described for stock calibration solutions).

Tab. 6. Accuracy of the candidate reference method. Determination of the magnesium concentration of control sera spiked with magnesium

Control serum	Magnesium concentration[1]) (y) (mmol/l)	Magnesium concentration determined (x) (mmol/l)	$\frac{x - y}{y} \cdot 100$
Kontrollogen L + S_1[2])	1.486	1.483	− 0.20
Validate N + S_1[2])	1.537	1.545	+ 0.52
Seronorm + S_1[2])	1.258	1.263	+ 0.40

[1]) concentration calculated from the reference method value of the control serum containing magnesium added as stock calibration solution (0.50 mmol/l).
[2]) 5 ml stock calibration solution S_1 (0.50 mmol/l) was used instead of diluent I for preparing the working solution of the control serum.

Tab. 7. Accuracy of the candidate reference method.
Comparison of the definitive with the reference method value

Control serum	Magnesium concentration definitive value (y) (mmol/l)	Magnesium concentration reference method value (x) (mmol/l)	$\frac{x - y}{y} \cdot 100$
SRM 909[1])	1.21 (1.18–1.26)[2])	1.190	−1.63
SRM 909[1])	1.21 (1.18–1.26)[2])	1.197	−1.12

[1]) Standard Reference Material 909
[2]) Confidence limits (95%) in brackets

Tab. 8. Transferability of the candidate reference method

Control serum	Magnesium concentration reference method value[1]) (y) (mmol/l)	Magnesium concentration reference method value (x) (mmol/l)	$\frac{x - y}{y} \cdot 100$
Kontrollogen L	0.860	0.859	−0.12
Kontrollogen LP	1.442	1.438	−0.28

[1]) as determined by Prof. *K. Paschen*, Kaiserslautern

Discussion

The concentration of magnesium in serum can be determined by different methods. Encouraged by the results of the calcium reference method, which is based on flame atomic absorption spectrometry, it seemed reasonable to use the same analytical principle for the development of a magnesium reference method. The possible usefulness of other analytical methods for the same purpose is not excluded by this choice. As proposed by *Cali* (3), a detailed and stringent protocol for the method was worked out as a prerequisite of good interlaboratory agreement of results. Detailed instructions concerning the use and handling of the atomic absorption spectrometer, however, were omitted in accordance with other reference methods. It is assumed that other instruments that comply with the requirements (see 1.14) are equally suitable. Measurements by use of a lean flame are performed in order to monitor the initial quality of the flame. Prior to analysis by the reference method, control sera were pooled to minimize vial to vial variability. A control serum, with a magnesium concentration determined by the definitive method, was incorporated to avoid errors from wrongly calibrated solutions; unobserved contamination is a possible cause of erroneous calibration. Imprecision and inaccuracy of the magnesium reference method are comparable to those of the calcium reference method and acceptable for the purpose within the measuring range (0.5–2.0 mmol/l).

Conclusion

The proposed candidate reference method might be used in the future to determine the hitherto lacking, and urgently needed reference method values of magnesium. Reference method values are the basis of the new concept of quality assessment in clinical chemistry (4), which was realized in the new guidelines of the German authorities (5).

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