Mg-color

AA

Direct colorimetric method for the quantitative determination of magnesium in biological fluids

SUMMARY

Magnesium (Mg) is one of the most widely distributed ions of the body. Sixty percent of the body Mg is found in the bones and the rest in the muscles and other soft tissues.
Mg has a major role in human physiology. It participates in the energetic metabolism through the ATP activation, in the transference of high-energy phosphates and is the activating ion for many enzymes involved in the metabolism of lipids, carbohydrates and proteins. Mg is a mediator in the conduction and transport mechanisms through membranes. It is essential in the preservation of macromolecular structures of DNA, RNA and ribosomes and in bone formation and preservation of osmotic pressure. Hypomagnesemia is closely associated to the deficiency of other ions such as P, K and Ca. There are multiple causes for hypomagnesemia: chronic and acute diarrheas, malabsorption syndromes, prolonged nasogastric suction and vomiting, intestinal and biliary fistulas, renal preservation deterioration, diabetes mellitus, hyperthyroidism, primary hyperaldosteronism and chronic alcoholism.
Mg excess may be due to the incorporation or excessive administration of Mg salts and is generally associated with renal failure. Other pathologies associated with hypomagnesemia are: hypocalciuric hypercalcemia, hypothyroidism, mineralocorticoids' deficiency, etc.

PRINCIPLE

In an alkaline solution, magnesium reacts with Xylidyl Blue forming a purple-red complex, being its intensity proportional to the Mg concentration present in the sample. Interference by calcium ions is prevented by the addition of EGTA to the reagent.

PROVIDED REAGENTS

A. Reagent A: 0.1 mM xylidyl blue solution and 0.04 mM EGTA in 0.2 M Tris buffer, pH 11.3.
S. Standard*: 3 mg/dl magnesium solution. See PROCEDURE LIMITATIONS.

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus** for automated technique. It can also be used in the calibration of manual techniques.
- Distilled water.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.
Standard: for use, transfer an excess quantity to a clean tube and pipette the necessary volume discarding the remaining.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Do not ingest. Avoid contact with skin and eyes. If spilt or splash, thoroughly wash affected area with water.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at room temperature (< 25ºC) until the expiration date shown on the box. Tightly cap the Reagent A bottle after use.
Standard: occasionally a light yellowish color may appear, which does not affect the test's performance.

INSTABILITY OR DETERIORATION OF REAGENTS

Discoloration or pH reduction may indicate deterioration of Reagent A. Discard in such case.
Precipitate formation or hazy appearance indicate deterioration of Standard. Discard in such case.

SAMPLE

Serum, heparinized plasma or urine
a) Collection:
- Serum or plasma: obtain in the usual way.
- Urine: it may contain precipitated magnesium which must be dissolved by acidification before the testing. Acidify urine with some drops of concentrated hydrochloric acid to reach a pH ranging between 3 and 4, verifying with pH test strips. Dilute one volume of acidified urine specimen with 4 volumes of distilled or deionized water (dilution 1:5).

b) Additives: in case plasma is used as sample, heparin must be used as anticoagulant.
c) Known Interfering Substances: anticoagulants other than heparin (such as EDTA, citrate or oxalate) form magnesium complexes, producing erroneous results.
Hemolyzed samples should not be used due to the large magnesium concentration present in red blood cells.
No interference has been observed by the following substances: bilirubin up to 200 mg/l (20 mg/dl), calcium up to 16 mg/dl, hemoglobin up to 3.5 g/l (350 mg/dl), triglycerides up to 6 g/l (600 mg/dl) equivalent to mild or moderate lipemia. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. It might be stored for 2 weeks in refrigerator (2-10ºC) or for more than 1 month frozen (-20ºC) without the addition of preservatives.

** Non-provided with all kit sizes*

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometric cuvettes.
- Watch or timer.

ASSAY CONDITIONS
- Wavelength: 510 nm in spectrophotometer or (490-530 nm) in photocolorimeter with green filter.
- Reaction temperature: room temperature (15-25ºC)
- Reaction time: 5 minutes
- Sample volume: 10 ul
- Final reaction volume: 1.01 ml

Sample and Reagent volumes may proportionally vary (e.g. 20 ul Sample + 2 ml Reagent A or 50 ul + 5 ml).

PROCEDURE

In three tubes labeled B (Blank), C (Calibrator or Standard) and U (Unknown), place:

	B	C	U
Sample	-	-	10 ul
Calibrator or **Standard**	-	10 ul	-
Distilled water	10 ul	-	-
Reagent A	1 ml	1 ml	1 ml

Mix and incubate for 5 minutes at room temperature (15-25ºC). Read in spectrophotometer at 510 nm or in photocolorimeter with green filter (490-530 nm) setting the instrument to zero O.D. with Blank.

STABILITY OF FINAL REACTION

Final reaction color is stable for at least 1 hour; therefore absorbance should be read within that period.

CALCULATIONS

1) Magnesium (mg/dl) = U x f

$$f = \frac{\text{Value of Standard (mg/dl)*}}{\text{Absorbance of Standard}}$$

* Magnesium concentration in Wiener lab.'s **Calibrador A plus** or in the Standard

For urine specimens the result must be multiplied by the dilution factor and for 24 hour urines, also by the volume (liters):

2) Urine Magnesium (mg/dl) = Magnesium result x dilution factor

3) Urine Magnesium (mg/24 hours) = Magnesium result x dilution factor x 10 x diuresis (liters)

where:
10 = conversion factor between deciliter to iliter

Example:
Magnesium result = 2.0 mg/dl
Dilution = 1:5
Diuresis = 1.5 liters
Urine Magnesium = 2.0 x 5 x 10 x 1.5 = 150 mg/24 hours

UNITS CONVERSION
Mg (mg/dl) = Mg (mmol/l) x 2.43
Mg (mg/dl) = Mg (mEq/l) x 1.215
Mg (mmol/day) = Mg (mEq/day) x 0.5

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known magnesium concentration. If running urine samples, a urine-based control should be used.

REFERENCE VALUES

Serum or plasma: 1.7 to 2.5 mg/dl (0.70 to 1.05 mmol/l)

Urine: 60 to 210 mg/24 hrs
2.5 to 8.5 mmol/24 hrs
4.10 to 13.80 mg/dl*

* Considering a urine volume of 1.5 L/24 hrs

The literature (Tietz, NW) mentions the following reference range:

Serum or plasma: 1.6 to 2.6 mg/dl (0.66 to 1.07 mmol/l)
Urine: 3.0 to 5.0 mmol/24 hrs.

It is recommended that each laboratory establishes its own reference values.

PROCEDURE LIMITATIONS
- See known interfering substances under SAMPLE.
- The Standard should not be employed in automatic analyzers, it should only be used for calibration of manual techniques.
- To avoid magnesium contamination the use of disposable plastic tubes or cuvettes is recommended, as well as thoroughly clean glassware, free from magnesium and any anticoagulant traces. Thus, it is advisable to wash the glassware with deionized detergents (Wiener lab.'s **Noión**) and rinse with diluted mineral acids, finally rinsing several times with distilled water. The exclusive use of pipettes and tubes for performing this test is recommended.

PERFORMANCE

The assays were performed in an Express Plus analyzer(*). If using by manual procedure, user must validate that similar performance to that stated below is obtained.

a) Reproducibility: precision studies were performed following the guidelines contained in NCCLS (National Committee on Clinical Laboratory Standards) document EP5-A:

Intra-assay precision

	Level	S.D.	C.V.
Serum	2.49 mg/dl	± 0.050 mg/dl	2.01 %
	5.49 mg/dl	± 0.106 mg/dl	1.93 %
Urine	8.83 mg/dl	± 0.132 mg/dl	1.49 %
	22.03 mg/dl	± 0.332 mg/dl	1.51 %

(*) TM Ciba Corning Diagnostics

Inter-assay precision

	Level	S.D.	C.V.
Serum	2.53 mg/dl	± 0.066 mg/dl	2.61 %
	5.23 mg/dl	± 0.170 mg/dl	3.25 %
Urine	8.83 mg/dl	± 0.271 mg/dl	3.07 %
	21.63 mg/dl	± 0.415 mg/dl	1.92 %

b) Linearity: linearity studies were performed following the guidelines contained in NCCLS document EP6-P (Testing for Equality of Variances and Testing for Lack of Fit of the Linear Model).
Results demonstrated that reaction is linear up to 6.0 mg/dl. For higher values repeat testing using diluted sample 1:2 or 1:4 with saline solution, multiplying the obtained result by 2 or 4 respectively.

c) Correlation:
- Serum: magnesium values of 140 specimens were determined using the Wiener lab.'s **Mg-color AA** kit and a commercial kit based on the same principle. The correlation coefficient was:
r = 0.9936, slope b = 0.9437 and intercept a = 0.0844.
- Urine: magnesium values of 55 specimens were determined using the Wiener lab.'s **Mg-color AA** kit and a commercial kit based on the same principle. The correlation coefficient was:
r = 0.9890, slope b = 1.013 and intercept a = 0.4897.

d) Sensitivity studies: minimum detection limit is 0.079 mg/dl and analytical sensitivity is 0.25 mg/dl.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.
For calibration use Wiener lab's **Calibrador A plus**.

WIENER LAB. PROVIDES

- 2 x 50 ml (provided Standard) (Cat. Nº 1580001).
- 6 x 20 ml (non-provided Standard) (Cat. Nº 1009337).
- 6 x 20 ml (non-provided Standard) (Cat. Nº 1009271).

REFERENCES

- Mann, C.K.; Yoe, J.H. - Anal. Chem. 28:202 (1956).
- Duncanson, G. - Clin. Chem. 36/5:756 (1990).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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