Magnesium

CPZ

For magnesium determination in serum, plasma and urine

SUMMARY

Magnesium (Mg) is one of the most widely distributed ions of the body. Sixty percent of the body Mg is found in bones and the rest in muscles and other soft tissues.
Mg has a major role in human physiology. It participates in the energetic metabolism through 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 phosphorus, potassium and calcium. 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

The magnesium present in sample binds to chlorophosphonaze III (CPZ III), producing an increase in absorbance at 660 nm. The addition of EDTA removes Mg from Mg-CPZ III complex, releases CPZ III and allows blank reading. The absorbance difference between Mg-CPZ III and CPZ III is equivalent to the absorbance yielded by Mg. The presence of EGTA prevents calcium interference in the reaction.

$$Mg_2^{+} + \text{CPZ III} \xrightarrow{\text{pH 7.5}} \text{Mg-CPZ III}$$

$$\text{Mg-CPZ III} + \text{EDTA} \xrightarrow{\text{pH 7.5}} \text{Mg-EDTA} + \text{CPZ III}$$

PROVIDED REAGENTS

A. Reagent A: 145 mM TES buffer, 0.2 mM CPZ III, 10 mM EGTA, pH 7.5.
B. Reagent B: 100 mM TES buffer, 17 mM EDTA, pH 7.5.

NON-PROVIDED REAGENTS

- Wiener lab's **Calibrador A plus.**

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Patient samples should be handled as if capable of transmitting infection.
Use the reagents according to the working procedures for clinical laboratories.
All reagents and samples should be discarded according to current regulations.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10°C until the expiration date stated on the box.

SAMPLE

Serum, heparinized plasma or urine
a) Collection:
- Serum or plasma: obtain in the usual way.
- Urine: acidify urine with some drops of concentrated hydrochloric acid to obtain a pH lower than 2.

b) Additives: in case plasma is used as sample, heparin must be used as anticoagulant. Anticoagulants such as EDTA, citrate and oxalate build complexes with Mg, causing erroneous results.
c) Known Interfering Substances: hemolyzed, lipemic or contaminated samples should not be used. Precipitated samples should be centrifuged prior to the assay. Contamination with erythrocytes may increase the results because the Mg level is greater that in serum.
No interference has been observed by the following substances: triglycerides up to 1300 mg/dl, hemoglobin up to 800 mg/dl, direct bilirubin up to 21 mg/dl, total bilirubin up to 40 mg/dl, calcium up to 45 mg/dl and heparin up to 50 IU/ml. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: serum or plasma samples may be stored for up to 7 days at 2-10°C or at 15-25°C and for up to 1 year frozen (at -20°C). Avoid repeated freezing and thawing.

REQUIRED MATERIAL (non-provided)

- Micropipettes and pipettes for measuring the stated volumes.
- Automated analyzer.

PROCEDURE

(Automated analyzers)
Below is a general procedure for **Magnesium CPZ** in an automated analyzer. For programming instructions check

the user's manual of the automated analyzer in use.	
Reagent A	160 ul
Sample or Calibrator	4 ul
Incubate during 300 seconds at 37ºC. Measure initial absorbance at 660 nm (OD_1).	
Reagent B	28 ul
Incubate during 180 seconds at 37ºC. Measure absorbance at 660 (OD_2). To obtain the results, the analyzer calculates the absorbance difference ($\Delta A = OD_2 - OD_1$) and multiplies by the obtained calibration factor.	

CALIBRATION
Wiener lab's **Calibrador A plus** is processed as a sample and the corresponding factor is calculated based on it.

QUALITY CONTROL METHOD
If the test sample is serum or plasma, process 2 quality control material levels (**Standatrol S-E 2 niveles**) with known magnesium concentrations. If urine sample is running, a urine-based control should be used. Controls should be tested in the same way as samples.

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.

UNIT 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

PROCEDURE LIMITATIONS
See known interfering substances under SAMPLE.
Recalibration is recommended when changing reagent lot or when established by the quality control.
To preserve the integrity of the reagents all forms of contamination should be avoided, using only perfectly clean and dry micropipettes for measurement.

PERFORMANCE
a) Reproducibility: tested using CLSI protocol EP5-A2. Samples with different Mg levels were tested. Intra-assay and Total Precision were calculated with the obtained data.

Intra-assay precision

Level	S.D.	C.V.
1.8 mg/dl	± 0.016 mg/dl	0.84%
4.0 mg/dl	± 0.028 mg/dl	0.69%

Total precision

Level	S.D.	C.V.
1.8 mg/dl	± 0.046 mg/dl	2.5%
4.0 mg/dl	± 0.065 mg/dl	1.6%

b) Detection limit: 0.17 mg/dl.
c) Quantification limit: 0.35 mg/dl.
d) Linearity: reaction is linear up to 6 mg/dl. The performance data were obtained using Konelab 60i automated analyzer. Therefore, such values may change if a different automated analyzer is used.

The performance results were obtained using Konelab 60i autoanalyzer. Therefore, such values may differ whenever another autoanalyzer or manual technique is used.

PARAMETERS FOR AUTOMATED ANALYZERS
For programming instructions check the user manual of the automated analyzer in use.
For calibration use Wiener lab's **Calibrador A plus**.

WIENER LAB. PROVIDES
105 ml: - 1 x 90 ml Reagent A
- 1 x 15 ml Reagent B
(Cat. Nº 1999803)

118 ml: - 2 x 50 ml Reagent A
- 2 x 9 ml Reagent B
(Cat. Nº 1009289)

140 ml: - 2 x 60 ml Reagent A
- 2 x 10 ml Reagent B
(Cat. Nº 1009367)

140 ml: - 2 x 60 ml Reagent A
- 2 x 10 ml Reagent B
(Cat. Nº 1009629)

REFERENCES

- Tietz Fundamentals of clinical chemistry - Burtis, C., Ashwood, E. (5º Edition) WB Saunders, 2001.
- Clinical Chemistry Principles and Technics, 2nd Edition, Henry, R.J.; Connon, D.C.; Winkelman, J.W., p 670-673, 2001.
- Young, DS. Effects of preanalytical variables on clinical laboratory test. AACC Press. Third Edition, 2007.
- Schmidt-Gayk, H. - Measurement of calcium, phosphate and magnesium - In: Dynamics of bone and cartilage metabolism. Academic Press, p.487-502, 2006.
- EP5-A2 Vol. 24 Nº 25 del CLSI. Evaluation of precision performance of quantitative measurement methods (approved guideline - second edition).
- EP17-A2 Vol. 24 Nº 34 del CLSI. Protocols for determination of limits of detection and limits of quantitation; approved guideline.

Symbols

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

Σ — Conteins 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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