



PRINCIPLE

The method¹ is based on the specific binding of calmagite, a metallochromic indicator², and magnesium at alkaline pH with the resulting shift in the absorbtion wavelength of the complex. The intensity of the cromophore formed is proportional to the concentration of magnesium in the sample.

Calmagite + Magnesium — Calmagite-Magnesium complex

REAGENT COMPOSITION

- R1 Chromogen. Calmagite 75 mmol/L, EGTA 60 mmol/L, amin methyl-propanol 0.2 mol/L, KCl 0.2 mol/L, surfactant 0.05 % (w/v).
- **CAL** Magnesium standard. Magnesium 2 mg/dL (0.82 mmol/L) Organic matrix based primary standard.

STORAGE AND STABILITY

Store at 2-8°C. The Reagent and the Standard are stable until the expiry date stated on the label.

REAGENT PREPARATION

The Monoreagent and the Standard are ready to use. Discard the mixture if the blank presents an absorbance above 0.575 at 520 nm against distilled water (see Notes).

SAMPLES

Serum or heparinized plasma free of hemolysis. Other anticoagulants (EDTA, oxalate and citrate) must not be used. Magnesium in serum or plasma is stable for 10 days at 2-8°C. Frezze for longer storage.

MAGNESIUM MR

CALMAGITE Colorimetric method ENDPOINT

INTERFERENCES³

- Any substance which either chelates magnesium or contain magnesium will interfere with the assay.
- Many detergents and water supplies are a major source of contamination of the glassware used for this test.
- The interference by calcium is prevented by the use of EGTA into the buffer.
- Lipemic samples (>0,5 g/dL) interfere.
- Bilirubin (>20 mg/dL) does not interfere.

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 520 \pm 20 nm.
- Pipettes with disposable plastic tips to measure reagents and samples.
- Disposable plastic tubes for the tests.

PROCEDURE

- 1. Bring reagents and samples to room temperature.
- 2. Pipette into labelled test tubes:

TUBES	Blank	Sample	Standard
Reagent	_	10 μL	-
Sample	_	_	10 µL
Standard	1.0 mL	1.0 mL	1.0 mL

3. Mix and let stand the tubes 2 minutes at room temperature.

 Read the absorbance (A) of the samples and the standard at 520 nm against the reagent blank at 37°C.

The color is stable for at least 1 hour.

CALCULATIONS

Serum, plasma

A_{Muestra}

— x C_{Patrón} = mg/dL magnesium

A_{Patrón}

Samples with concentrations higher than 5 mg/dL (2.06 mmol/L) should be diluted 1:2 with saline and assayed again. Multiply the results by 2.





If results are to be expressed as SI units apply: mg/dL x 0.412 = mmol/L

REFERENCE VALUES⁴

Serum, plasma

cromatest*

Children (2-12 years)	1.7-2.3 mg/dL (0.70-0.94 mmol/L)		
Adults (12-60 years)	1.6-3.0 mg/dL (0.66-1.23 mmol/L)		

It is recommended that each laboratory establishes its own reference range. $% \label{eq:linear}$

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used. To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.



1980005 HUMAN MULTISERA NORMAL Borderline level of magnesium. Assayed.

REF 1985005 HUMAN MULTISERA ABNORMAL Elevated level of magnesium. Assayed.

CLINICAL SIGNIFICANCE

Magnesium is considered an essential nutrient and a major intracellular cation. More than 50% of the total magnesium found in the body is complexed with calcium in the skeleton; however, only 1% of total body magnesium is found in the circulation. Between 60% and 70% of serum magnesium is free; the remaining percentage is bound to albumin, phosphate, citrate, and other ions.

Magnesium is also used as an *activator* (inorganic ion that is needed as a cofactor for an enzyme reaction) for more 300 enzymes, especially those involved in oxidative phosphorylation, glycolysis, cell replication, and protein synthesis.

The absorption of magnesium in the intestinal tract and its reabsorption in the renal tubules are directly associated with the body's need, balance and health.

Hypomagnesemia (an abnormal decrease in the level of serum magnesium) is usually associated with severe prolonged diarrea, impairment of neuromuscular function, gastrointestinal malabsorption and alcoholism.

Hypermagnesemia (an abnormal elevation in the level of serum magnesium) is usually associated with renal glomerular failure, dehydration, sever diabetic acidosis, and Addison's disease.

NOTES

 Most of the detergents and water softening products used in the labs contain quelating agents. A defective rinsing will invalidate the procedure. Keep the glassware acid washed and thoroughly rinsed at all times.

ANALYTICAL PERFORMANCE

- Linearity. Up to 5 mg/dL

- Precision

mg/dL	Within-run			Between-run		
Mean	1.12	2.25	4.5	1.12	2.25	4.5
SD	0.03	0.04	0.04	0.038	0.038	0.051
CV%	2.7	1.8	0.9	3.4	1.7	1.2
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Replicates: 10 for each level.Replicates: 10 for each levelInstrument: CECIL CE 2021for 8 days.

- Sensitivity. Using a 1:100 sample/reagent at 520 nm, 1 mg of magnesium will produce a net absorbance of approximately 0.075.
- **Correlation.** This assay (y) was compared with a similar commercia method (x). The results were:

N = 20 r = 0.985 y =0.972x + 0.006

REFERENCES

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- 3. Chauhan, U.P.S., and Sarkar, B.C.R. Anal. Biochem. 32:70 (1969).
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