CLINICAL SIGNIFICANCE (1)

Adult human body (70 Kg) contains 21 to 28 g of magnesium. Of this, about 60% is in bone, 20% in skeletal muscle, 19% in other cells, and about 1% in the extracellular fluids. About 30% of magnesium in plasma is associated with proteins (primarily albumin). Consequently, a change in the concentration in albumin can affect the concentration in magnesium.

Hypomagnesaemia may be a secondary effect in hypocalcemic or calcium-deficient tetany. Conditions that have been associated with hypomagnesemia include chronic alcoholism, childhood malnutrition, lactation, malabsorption, acute pancreatitis, hypothyroidism, chronic glomerulonephritis, aldosteronism, digitalis intoxication and prolonged intravenous feeding.

Hypermagnesaemia have been observed in dehydration, severe diabetic acidosis, and immediately following myocardial infarction.

PRINCIPLE (1) (4) (5)

Gindler, Heth and Khayam-Bashi method. Calmagite, a metallochromic indicator (1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid), forms a coloured complex with the magnesium in basic medium. The absorbance, measured at 510-550 nm, is proportional to the concentration of magnesium in the specimen. EGTA reduces Calcium interference, Potassium cyanide (KCN) reduces interference of heavy metals, Polyvinylpyrolidone (PVP) and surfactants reduce the interference of proteins and lipemia.

REAGENTS COMPOSITION

Vial R1 CALMAGITE REAGENT
Calmagite ≥ 223 µmol/L KNC 6.14 mmol/L PVP 40 g/L
KOH 36 mmol/L EGTA 250 µmol/L Surfactants

Vial R2 STANDARD
Magnesium ≥ 2 mg/dL (0.822 mmol/L)

Vial R3 PRECIPITANT
Sodium tungstate 300 mmol/L

Vial R4 SULFURIC ACID
H₂SO₄ 350 mmol/L
Xl R36/38: Irritating to eyes and skin.
S26-S28: After contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, rinse immediately with plenty of water.
S36/37/39: Wear suitable protective clothing, gloves and eyes/face protection

Vial R5 STANDARD
Magnesium ≥ 6 mg/dL (2.47 mmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

MAGNESIUM Calmagite
Reagent for quantitative determination of magnesium in human serum, plasma, red blood cells (RBC) and urines.

TECHNICAL SUPPORT AND ORDERS
Tél : (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

REAGENTS PREPARATION
Reagents are ready for use.

STABILITY AND STORAGE
Store away from air and light, well cap in the original vial at 18-25°C
- Standards (vial R2, R5): Transfer the requested quantity, recap and store at 18-25°C.
- Contents of vial R1: transfer the requested quantity, promptly recap and store at 18-25°C.
- Without contamination, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Discard any reagent if cloudy and reagent (vial R1) if its absorbance measured at 530 nm < 0.600.

SPECIMEN COLLECTION AND HANDLING
Collect in a metal-free container and without preservatives
Unhemolysed serum or heparinised plasma: Collect on fasting. Avoid oxalate, citrate or EDTA. Separate red cells immediately.
Special procedure is required for cloudy or icteric serum (see next page).

Magnesium is stable for several days in serum at 2-8°C. 24h urines (acidified pH 1.0): dilute (1+4) with demineralised water before assay.
RBC: Collect venous blood on heparin and centrifuge. After elimination of plasma, wash RBC 3 times with saline solution, then centrifuge 10 minutes at 4000 RPM and eliminate the supernatant.

INTERFERENCES (3)
- Give a special care to the specimen, calibrators and controls handling, to avoid contamination by the environmental magnesium. The use of disposable tubes or cuvettes and acid washed labware (well rinsed with demineralised water) is suggested.
- Calcium ( < 7.5 mmol/L) does not interfere with this method.
- Plasma, serum: Icterus, lipemia and paraproteins may interfere with the determination. Hemolysis involves an overestimation because of the important intracellular contents in magnesium.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

CALIBRATION (6)
- Standards (vial R2 or R5) enclosed in the kit or BIOLABO Multicalibrator REF 95015 traceable to SRM 909b.
- Or any calibrator traceable to a reference method or material.

It is recommended to calibrate in the following cases:
1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of range, even after using a new vial of fresh serum.
**MANUAL PROCEDURE**

In any case:
- Let stand reagents and specimens at room temperature
- Maintain a constant temperature as the reaction is temperature sensitive.
- Reaction is stable for 60 minutes.

**Procedure n°1: Clear specimen (note 1)**

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent R1</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Demineralised water</td>
<td>10 µL</td>
<td>10 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>Standard R2 (2 mg/dL)</td>
<td></td>
<td>10 µL</td>
<td></td>
</tr>
<tr>
<td>Specimen (Note 1)</td>
<td></td>
<td>10 µL</td>
<td></td>
</tr>
</tbody>
</table>

Mix. Let stand for 5 minutes at constant temperature. Read standard and assays absorbances at 530 nm (510-550) against reagent blank.

**Notes:**
1. Serum, plasma or urines diluted (1+4) with demineralised water.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

**Procedure n°2: RBC (see § SPECIMEN COLLECTION AND HANDLING)**

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demineralised water</td>
<td>3.2 mL</td>
<td>3 mL</td>
<td>3 mL</td>
</tr>
<tr>
<td>Standard R5 (6 mg/dL)</td>
<td>200 µL</td>
<td>200 µL</td>
<td></td>
</tr>
<tr>
<td>Globular clot (RBC)</td>
<td></td>
<td>200 µL</td>
<td></td>
</tr>
</tbody>
</table>

Mix vigorously until complete hemolysis. It is recommended to use a positive moved pipette to dispense globular clot.

Well rinse pipette tip by movement to and fro.

Vial R3 (Precipitant) 400 µL 400 µL 400 µL Mix

Vial R4 (H₂SO₄) 400 µL 400 µL 400 µL Mix well. Let stand for 5 minutes, centrifuge at 4000 RPM for 10 minutes.

Pipette into identified tubes

Reagent R1 1 mL 1 mL 1 mL

Supernatant 100 µL 100 µL 100 µL

Mix. Let stand for 5 minutes at constant temperature. Read standard and assays absorbances at 530 nm (510-550) against reagent blank.

**Procedure n°3: Cloudy or icteric sera**

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard R2 (2 mg/dL)</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demineralised water</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vial R3 (Precipitant)</td>
<td>400 µL</td>
<td>400 µL</td>
<td>400 µL</td>
</tr>
</tbody>
</table>

Mix.

Vial R4 (H₂SO₄) 400 µL 400 µL 400 µL Mix well. Let stand for 5 minutes and centrifuge at 4000 RPM for 10 minutes.

Pipette into well identified test tubes:

Reagent R1 1 mL 1 mL 1 mL

Supernatant 50 µL 50 µL 50 µL

Mix. Let stand for 5 minutes at constant temperature. Read standard and assays absorbances at 530 nm (510-550) against reagent blank.