MAGNESIUM

Diagnostic reagent for determination of Magnesium concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic use (IVD). **Do not freeze.**

Ref No	Pack
MH-222	120 mL
MH-223	40 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of magnesium in serum and plasma.

GENERAL INFORMATION

Magnesium is the fourth most abundant cation in the body and the second most common intracellular cation. The total body magnesium content is about 25 g (~1 mol), out of which about 55% is found in the skeleton. One-third of skeletal magnesium is thought to act as a reservoir to maintain extracellular magnesium concentration. About 45% of magnesium is intracellular. The concentration of magnesium in cells ranges from 2.4 to 7.3 mg/dL (1 to 3 mmol/L). In general, the higher the metabolic activity of a cell, the higher its magnesium content. In cells, most magnesium is bound to proteins and negatively charged molecules; 80 to 90% of cytosolic magnesium is bound to ATP, and MgATP is the substrate for numerous enzymes. The nucleus, mitochondria and endoplasmic reticulum contain significant amounts of magnesium. Approximately 0.5 to 5% of total cellular magnesium is free. The transport of magnesium across the cell membrane is regulated by a specific magnesium transport system. Extracellular magnesium accounts for about 1% of the total body magnesium content. In plasma, about 55% of magnesium is free, 30% is associated with proteins (mainly albumin) and 15% is complexed with phosphate, citrate and other anions.[1] Magnesium is a cofactor of more than 300 enzymes in the body.[2] It is required for the formation of substrates of enzymes (e.g. MgATP is a substrate for many enzymes that require ATP). Magnesium is also an allosteric activator of many enzyme systems. Enzymes that require magnesium for their activity include adenylate cyclase, Na+, K+-adenosine triphosphatase (ATPase), ALP, Ca^{+2}-ATPase, phosphofructokinase and creatine kinase. The guanine nucleotide containing the regulatory proteins Gs and Gi requires magnesium for its activity.

Magnesium is important in oxidative phosphorylation, glycolysis, cell replication, nucleotide metabolism and protein biosynthesis. A decrease in plasma magnesium concentration lowers the threshold for axonal stimulation and increases nerve conduction velocity. Magnesium also affects neurotransmitter release at neuromuscular junctions by competitively inhibiting the entry of calcium into presynaptic nerve terminals. Decreased plasma magnesium concentration results in increased neuromuscular excitability. Magnesium deficiency can therefore cause various metabolic abnormalities and clinical consequences.[1]

Daily magnesium intakes of 400 to 420 and 310 to 320 mg/day are recommended for adult men and women, respectively.[3] Between 20 and 80% of dietary magnesium is absorbed through the small intestine and absorption is proportional to the amount present in the diet. The kidneys play an important role in regulating magnesium balance. 70 to 80% of magnesium is ultra-filterable, where 15 to 25% is then passively reabsorbed in the proximal tubules. Then 65 to 75% is reabsorbed paracellularly with facilitated transport by the tight junction protein claudin-16 (also known as paracellin-1) and claudin-19 through the thick ascending arm of the henle handle. Between 5 and 10% of the filtered magnesium is reabsorbed intercellularly via the transient receptor potential melastatin 6 (TMRP6) in the distal convoluted tubules. Magnesium serves as an activator in various physiochemical processes, including phosphorylation, protein synthesis and DNA metabolism. It is also involved in neuromuscular transmission and in the excitability of skeletal and cardiac muscles.[1]

It has been reported that 10 percent of patients applying to hospitals and 65 percent of patients in intensive care units are hypomagnesemic.[4-7]
Moderate or severe magnesium deficiency is usually due to magnesium loss from the gastrointestinal tract or kidneys. Vomiting and nasogastric suction can deplete magnesium stores in the body, as upper gastrointestinal fluids contain about 1.2 mg/dL (<0.5 mmol/L) magnesium. More commonly, magnesium deficiency is associated with losses in the lower intestine. Diarrhea can cause significant magnesium losses; therefore, acute diarrheal conditions, regional enteritis and ulcerative colitis are often complicated by magnesium deficiency. Excessive urinary magnesium loss from the kidneys is an important cause of magnesium deficiency. Clinically important causes include alcohol, diabetes (osmotic diuresis), and the use of convulsive diuretics (e.g. furosemide), aminoglycoside antibiotics and proton pump inhibitors (e.g. omeprazole, lansoprazole). Increased sodium excretion (parenteral fluid therapy) and increased calcium excretion (hypercalcemic states) also cause renal magnesium loss. Familial hypomagnesemia

has been reported in patients with loss-of-function mutations in TRPM6 and missense mutations in claudin genes. Since magnesium deficiency is often secondary to another disease process or a therapeutic agent, features of the primary disease process may complicate or mask magnesium deficiency. Neuromuscular overstimulation with tetany and seizures may be present. These signs and symptoms may also be due to hypocalcemia, and magnesium deficiency is a common cause of hypocalcemia.[1] Hypomagnesemia impairs PTH secretion and causes resistance to PTH in the kidneys and bone; it has been associated with osteoporosis in epidemiologic studies and animal experiments.[1,8]

One of the most serious complications of magnesium deficiency is cardiac arrhythmia. Premature atrial complexes, atrial tachycardia and fibrillation, premature ventricular complexes, ventricular tachycardia, torsades de pointes and ventricular fibrillation may occur in magnesium deficiency. These effects may be caused in part by hypokalemia, renal loss and intracellular potassium depletion caused by hypomagnesemia. Hypomagnesemia is often temporary and is not an indication of magnesium deficiency. Conversely, intracellular magnesium depletion and magnesium deficiency may be present despite a normal plasma magnesium concentration. Consequently, hypocalcemia, hypokalemia, neuromuscular hyperirritability and cardiac arrhythmias should be a warning against the possible presence of magnesium deficiency.

Although up to 12% of hospitalized patients may have a mild to moderate increase in serum magnesium concentration, magnesium intoxication is not a common clinical problem.[9,10] Symptomatic hypermagnesemia is almost always caused by excessive intake from administration of antacids, enemas and parenteral fluids containing magnesium. Many of these patients have coexisting renal insufficiency, which limits the ability of the kidneys to excrete excess magnesium. Magnesium used to treat pre-eclampsia and eclampsia can cause magnesium intoxication in mothers and newborns.
Depression of the neuromuscular system is the most common manifestation of magnesium intoxication. Hypermagnesemia causes a decrease in plasma calcium concentration, probably due to inhibition of both PTH secretion and the end-organ action of PTH by magnesium. The possibility of magnesium intoxication should be considered, especially in patients with renal impairment who are taking magnesium. Replacement therapy should be ceased in patients with a mild to moderate increase in serum magnesium.[1]

TEST PRINCIPLE

Colorimetric Method

Xylidyl blue (1–azo–2–hydroxy–3-[2,4–di-methylcarboxanilido] naphthalene–19-[2-hydroxybenzene]) binds magnesium in alkaline solution, causing a spectral shift and forming a red complex. The absorbance value measured at 546 nm is directly proportional to the Mg concentration in the sample.

REAGENT COMPONENTS

Xylidyl blue	: ≤ 0.12 mmol
NaCl	: ≤ 0.90 mol
EGTA	: ≤ 0.26 mmol
Triethanolamine	: ≤ 0.8 mmol
Good's buffer	:
Surfactant	
Preservative	

Note: Ethyleneglycol Bis(2-Aminoethyl Ether)-N,N,N',N' Tetraacetic Acid (EGTA), a calcium chelating agent, is added to reduce the interference of calcium. The reagents also contain some compounds like surfactants to reduce interference from protein and lipemia.

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Opened vials are stable for 21 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.[5]

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[11]

SAMPLE REQUIREMENTS

Serum and plasma heparinate can be used and are collected according to the standard procedures. Anticoagulants such as citrate, oxalate or EDTA should not be used as they form a complex with magnesium. Samples of patients on EDTA therapy should not be used.
Collect urine samples in metal-free containers. Urine samples should be acidified to pH 1 with concentrated HCl to prevent precipitation of magnesium ammonium phosphate.[23]

Stability of the serum and plasma samples[24]:

8 hours at +20/+25°C,
3 days at +2/+8°C,
3 months at -20°C.

Stability of the urine sample[25]:

3 days +20/+25°C,
3 days +2/+8°C,
1 year -20°C.

Urine samples should be stored after acidification to pH <2.

Unit Conversion:

The magnesium concentration in erythrocytes is about three times that of plasma. The conversion factors for the units used to express magnesium concentration are as follows:

mmol/L × 2.43 = mg/dL
mEq/L × 0.5 = mmol/L
mEq/L × 1.22 = mg/dL

Note 1: Serum or plasma should be separated from the clot or red blood cells as soon as possible to avoid an increase in serum/plasma magnesium due to cell leakage.

Note 2: Factors that change the concentration of free calcium by altering the distribution of calcium between free, protein-bound and complex pools may also change the concentration of free magnesium. For this reason, samples should be processed anaerobically to avoid loss of carbon dioxide and analyzed without delay to avoid pH changes caused by metabolism.

Note 3: Similar to free calcium, high concentrations of heparin should also be avoided. Some silicones or other tube additives and thiocyanate (smokers and dieters) interfere with free magnesium determinations.[1]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Iron-Magnesium Standard or Arcal Auto Calibrator.

Iron-Magnesium Standard
Ref.No: VT-031

Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability is 3 days.Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.
Traceability is provided with NIST SRM 956d material.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum/Plasma[26]

Newborn	: 1.5 - 2.2 mg/dL
5 month-6 year old	: 1.7 - 2.3 mg/dL
6 - 12 year old	: 1.7 - 2.1 mg/dL
12 - 20 year old	: 1.7 - 2.2 mg/dL
Adult	: 1.6 - 2.6 mg/dL

Urine[26]

24 hour	: 72.9 - 121.5

For 24-hour urine excretion, to convert results from mg/dL to mg/day;

24 h urine = [(V x c) / 100] mg/day

V = 24 hour urine volume
c = analyte concentration (mg/dL)

There are some important medical decision levels regarding serum/plasma magnesium concentrations:

- 1 mg/dL (0.41 mmol/L): This is the concentration where clinical magnesium deficiency is seen.
- Deep tendon reflexes are lost at plasma magnesium concentrations above 5 to 9 mg/dL (2.06 to 3.7 mmol/L), at concentrations above 10 to 12 mg/dL (4.11 to 4.94 mmol/L), decreased breathing caused by voluntary muscle paralysis and apnea may occur.
- Higher concentrations may cause cardiac arrest. Somnolence, hypotension, nausea, vomiting and flushing of the skin may also occur.

Note: The adequate reference interval is somewhat controversial, as reference concentrations at the lower end may be associated with cardiovascular risk.[12]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using Clinical and Laboratory Standards Institute (CLSI) EP28-A3c protocol.[13]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, “Measuring Interval” refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte’s lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[14]
The determined analytic measuring interval for Magnesium is 0.58 – 6 mg/dL

Detection Capability

Limit of Detection (LoD): 0.15 mg/dL

Limit of Quantitation (LoQ): 0.58 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[15]

Linearity

This method shows measurement linearity in the activities up to 6 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[16]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[17]
Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Magnesium have been given in the table 1 and 2 respectively.

Table 1. Magnesium Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
1.92 mg/dL	0.03	2.01	80
3.27 mg/dL	0.08	2.45	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[18]

Table 2. Magnesium Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
1.92 mg/dL	0.09	4.69	80
3.27 mg/dL	0.13	3.98	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[18]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[19]
y= 1.00 – 0.10 mg/dL
r=0.976

Interference

Endogenous interferant and analyte concentrations that have been used in the Iron scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[20,21]
The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Iron interference scanning test is appropriate, is determined as ±10%.[22]

In Magnesium test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interfering Substance and Concentration	Magnesium Target (mg/dL)	N*	Observed Recovery %
Bilirubin 7.11 mg/dL	1.62	3	110
Lipemia 433 mg/dL	1.89	3	106

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[21]

Note 1: Non-haemolysed samples must be used. Haemolysed samples are unacceptable because erythrocytes contain higher concentrations of magnesium than serum or plasma.

Note 2: Interference due to icterus or lipemia is method dependent and can be reduced by performing blind reading with bichromatic analysis. Lipemic samples should be subjected to ultracentrifugation.[1]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[29]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.

Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practice
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests