POINTE SCIENTIFIC, INC.
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Magnesium Reagent Set

Intended Use
For the quantitative determination of magnesium in serum.

Clinical Significance
Magnesium in the body is found primarily in bone with some in soft tissue, blood cells, and serum. Decreased levels have been observed in cases of diabetes, alcoholism, diuretics, hyperthyroidism, hypothyroidism, malabsorption, hyperalimenation, myocardial infarction, congestive heart failure and liver cirrhosis. Increased serum magnesium levels have been found in renal failure, diabetic acidosis, Addison's disease, and vitamin D intoxication.

Method History
Serum magnesium measurement was first introduced in the 1920's with the laborious precipitation procedures of Kramer and Tisdall,[1] Briggs,[2] and Denis.[3]
These were followed by a variety of methods including: complexometric EDTA titration procedures,[4] fluorometric procedures involving chelates of magnesium,[5,6] and a dye absorption method based on the reaction of Titan Yellow with magnesium hydroxide to form a red-colored lake.[7] Each of these procedures suffered from numerous technical difficulties that greatly affected the accuracy and precision of their results.
Atomic absorption remains the most accurate method for magnesium determinations. However, this method requires expensive instrumentation and uses large sample volumes that limit its usefulness for pediatric testing.[8]
More recently, colorimetric dye-complexing methods have been developed and are in popular use. These procedures use such dyes as Calmagite, Eriochrome Black T, Magon, and methylthymol blue.[9] The present procedure uses the metallochromic dye Calmagite for a rapid, easy and accurate determination of magnesium in serum.[10,11] Some of the advantages of this method are: protein precipitation is unnecessary, interference from calcium or heavy metals is eliminated, and the method agrees very closely with values obtained by atomic absorption.[11]

Principle
Serum magnesium ions react with Calmagite in alkaline medium to produce a red complex that is measured spectrophotometrically at 530nm. The intensity of color produced is directly proportional to magnesium concentration. Calcium interference is virtually eliminated by use of EGTA.[12] Heavy metal interference is prevented by the presence of cyanide and a surfactant system is included to remove protein interference.

Reagent Preparation
Combine ten volumes of Color with one volume of Buffer reagent in a disposable plastic container, mix.

Combine only the volume of reagent necessary to perform the specific number of tests for that day. Working reagent is stable for 48 hours at 18-25°C.

Disposable plastic container or acid-washed glass containers are recommended to avoid contamination.

Reagent Storage
The magnesium reagent kit should be stored at room temperature, until posted expiration date.
Working reagent is stable for 48 hours at 18-25°C.

Reagents
1. Magnesium Color Reagent: Calmagite 0.006% w/v; stabilizer 1.0% w/v; Surfactant 0.03% w/v. Caution: Do Not Pipette By Mouth.
2. Magnesium Buffer Reagent: 2-Ethylaminoethanol 6.0% w/v; EGTA 1.18mM; Potassium Cyanide 0.10% w/v. Caution: Poison/Caustic Avoid All Contact.

Precautions
This reagent is for *in vitro* diagnostic use only.

Reagent Deterioration
Do not use reagent if:
1. Working reagent fails to achieve established values of fresh control sera.
2. Working reagent becomes visibly turbid.

Specimen Collection and Storage
1. Use fresh, unhemolyzed serum or heparinized plasma.
2. Red cells contain twice the magnesium concentration as serum. A hemolyzed sample would falsely elevate results.[13]
3. Grossly icteric or lipemic specimens should not be used in this method.

Interferences
1. Hemolyzed, grossly icteric or lipemic specimens are unsuitable for this method.
2. A number of drugs and substances affect the concentration of magnesium. See Young, et al.[14]

Materials Provided
1. Magnesium Color Reagent
2. Magnesium Buffer Reagent

Materials Required but not Provided
1. Accurate Pipetting devices
2. Test Tubes (Disposable Plastic)
3. Test Tube Rack
4. Timer
5. Spectrophotometer able to read at 520-530 nm

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Prepare working reagent according to preparation instructions.
2. Label test tubes "Blank", "Standard", "Control", "Patient", etc.
3. Pipette 1.0ml of working reagent to each tube.
4. Pipette 0.01ml (10ul) sample to respective tubes. Mix.
5. Allow tubes to incubate at room temperature (18-25°C) for 10 minutes.
6. After incubation, zero spectrophotometer with the reagent blank at 530 nm (520-530 nm).

Calibration
Use an aqueous Magnesium Standard (2.4mg/dl) or an appropriate serum calibrator.

Quality Control
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known magnesium concentrations.

Magnesium
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Calculations

Abs. = Absorbance

Abs. of Unknown / Abs. of Standard x Conc. Of standard = Value mg/dl

Example: Abs. of Unknown = .140
Abs. of Standard = .120
Concentration of Standard = 2.0 mg/dl

Then: .140 / .120 x 2.0 = 2.3 mg/dl

NOTE: "mg/dl" may be converted to "mEq/L" by dividing the result by 1.21525.

Expected Values

Newborns	1.5-2.2 mg/dl
Children	1.7-2.1 mg/dl
Adults	1.6-2.6 mg/dl

The expected values were taken from literature. Each laboratory should establish their own normal range.

Performance

1. Linearity: 4.86 mg/dl (4.0 mEq/L)
2. Comparison: Studies performed using the present method with a similar Calmagite method yielded a coefficient of correlation of 0.998 with a regression equation of y=1.01x + 0.01. Sample values ranged from 0.9 to 3.9 (N=56).
3. Precision:

Within Run (N=20)			Run to Run (N=20)		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
1.45	0.04	2.8	1.42	0.06	4.2
1.98	0.05	2.5	1.94	0.05	2.6
2.70	0.05	1.9	2.76	0.06	2.2
4.42	0.06	1.4	4.46	0.06	1.3

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