Magnesium - XB
Reagent Set

Intended Use
For the quantitative determination of magnesium in serum. For in vitro diagnostic use only.

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, hyperalimentation, 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 procedures4, fluorometric procedures involving chelates of magnesium5,6, and a dye complexing 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 which 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 which limit its usefulness for pediatric testing.8 Most recently, colorimetric dye-complexing methods have been developed and are in popular use. These procedures use such dyes as Calmagite, Eriochrome Black T, Xylidyl Blue (Magon), and methylthymol blue.9 The present procedure uses the metallochromic dye Xylidyl Blue for a rapid, easy and accurate determination of magnesium in serum.

Principle
Serum magnesium ions react with Xylidyl Blue in an alkaline solution to produce a red complex that is measured spectrophotometrically. The intensity of color produced is directly proportional to magnesium concentration. Calcium interference is virtually eliminated by use of EGTA and a surfactant system is included to remove protein interference.

Reagent Composition
When combined the reagent contains: xylidyl blue 0.1mM, EGTA 0.13mM, DMSO 1.4M, Buffer, surfactant, non-reactive stabilizers including potassium cyanide at 0.02% w/v. CAUTION: Poison/caustic. Avoid all contact.

Reagent Preparation
The reagents are supplied as ready to use liquids. Some applications may require the preparation of a single working reagent. To prepare a single working reagent mix equal parts R1 and R2. Refer to the instrument application for specific instructions.

Reagent Storage and Stability
The magnesium reagent kit should be stored at room temperature, until the posted expiration date. Working reagent is stable for 48 hours at 18-25°C. Do not use if:
1. The reagent fails to achieve established values of fresh control sera.
2. The reagent becomes visibly turbid.

Precautions
1. This reagent is for in vitro diagnostic use only.
2. Reagents are poison/caustic. Avoid all contact.

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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.10
3. Grossly icteric or lipemic specimens should not be used in this method.
4. Specimen collection should be carried out in accordance with NCCLS M29-T2.11 No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

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.12

Materials Provided
Magnesium (xylidyl blue) reagent R1 and R2

Materials Required but not Provided
1. Automated analyzer or Spectrophotometer
2. Test tubes and rack
3. Calibrators and controls
4. Timer

Procedure - Manual
1. Prepare working reagent according to preparation instructions.
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 37°C for 3 minutes.
6. After incubation, zero spectrophotometer with the reagent blank at 550 nm.
7. Read and record absorbances of all tubes.
8. See “Calculation” section to determine values.

Procedure - Automated (Hitachi 717)
TEST NAME: [MAG]
ASSAY CODE: [2-POINT];[24]-[50]
SAMPLE VOLUME: [4][2]
R1 VOLUME: [250][50][NO]
R2 VOLUME: [250][50][NO]
WAVELENGTH: [700][546]
CALIBRATION: [LINEAR][0][0]
STD (1) CONC.-POS: [0][1]
STD (2) CONC.-POS: [1][1]
STD (3) CONC.-POS: [NO]
STD (4) CONC.-POS: [NO]
STD (5) CONC.-POS: [NO]
STD (6) CONC.-POS: [NO]
SD LIMIT: [0.1]
DUPLICATE LIMIT: [250]
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SENSITIVITY LIMIT: [2000]
ABS. LIMIT (INC/DEC) [0][INCREASE]
PROZONE LIMIT: [-32000][LOWER]
EXPECTED VALUE: ['*']
PANIC VALUE: ['*']
INSTRUMENT FACTOR: [1.0]

* Indicates user defined parameter.

For other instrument applications, including a manual procedure, please contact the manufacturer’s Technical Service Department.

Calibration
Use an NIST-traceable magnesium standard or serum based calibrator. The procedure should be calibrated according to the instrument manufacturer’s calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Quality Control
The validity of the reaction should be monitored by use of control sera with known normal and abnormal magnesium values. These controls should be run at least with every working shift in which magnesium assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Calculation (Ratiometric Calculation)
Abs. = Absorbance
Abs. of Unknown x Conc. of = Value mg/dl
Abs. of Standard Standard

Example:
Abs. of Unknown = .140
Abs. of Standard = .120
Conc. of Standard = 2.4 mg/dl

Then: .140 x 2.4 mg/dl = 2.8 mg/dl
.120

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

Expected Values
Newborns 1.8 - 2.8 mg/dl
Children 1.7 – 2.3 mg/dl
Adults 1.6 – 3.0 mg/dl

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

Performance
Linearity: 4.86 mg/dl (4.0 mEq/L)

Comparison: Studies performed using the present method with a similar method yielded a coefficient of correlation of 0.0.825 with a regression equation of y=0.984x-0.031. Sample values ranged from 1.8-2.6 (N=20).

Precision:

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<tr>
<th>Within Day (N=22)</th>
<th>Day to Day (N=22)</th>
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<tbody>
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<td><strong>Mean</strong></td>
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<td>C.V.%</td>
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<td>1.27</td>
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</table>

References

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