POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

# Magnesium 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, 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 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.

## 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.
3. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

## Reagent Deterioration
Do not use reagent if:
1. Reagent fails to achieve established values of fresh control sera.
2. Reagent is 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.[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. Beckman Coulter AU™ analyzer
2. Instrument application and Operation manuals.
3. Calibrators and controls
4. Water

## Procedure (Beckman Coulter AU™400 application)

**SPECIFIC TEST PARAMETERS**

TEST NUMBER: **#** TEST NAME: **Magnesium** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **3** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **150** DIL. VOL.: **0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **540** ∇ SEC. **800** ∇ FIRST L: **-0.100** FIRST H: **1.500**
METHOD: **END** ∇ LAST L: **-0.100** LAST H: **1.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

**SPECIFIC TEST PARAMETERS**

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

| NORMAL RANGES: | SEX | AGE L YEAR | AGE L MONTH | AGE H YEAR | AGE H MONTH | L | H |
|---|---|---|---|---|---|---|---|
| ○ 1. | # ∇ | # | # | # | # | # | # |
| ○ 2. | # ∇ | # | # | # | # | # | # |
| ○ 3. | # ∇ | # | # | # | # | # | # |
| ○ 4. | # ∇ | # | # | # | # | # | # |
| ○ 5. | # ∇ | # | # | # | # | # | # |
| ○ 6. | # ∇ | # | # | # | # | # | # |
| 7. NONE SELECTED | | | | | | # | # |
| 8. OUT OF RANGE | | L | H | | | # | # |

PANIC VALUE: **#** **#** UNIT: **mg/dl** DECIMAL PLACES: **1**

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**CALIBRATION SPECIFIC PARAMETERS**

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

| | CAL. NO. | OD | CONC. | FAC/OD-L | FAC/OD-H |
|---|---|---|---|---|---|
| POINT 1. | # | | # | **-9999999** | **9999999** |
| POINT 2. | | | | | |
| POINT 3. | | | | | |
| POINT 4. | | | | | |
| POINT 5. | | | | | |
| POINT 6. | | | | | |
| POINT 7. | | | | | |

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

**#: User-Defined**

**For optimal performance a cuvette and probe wash is suggested using the contamination parameter function of analyzer using below settings.**

| Preceding Test | Rgt. Type | Following Test Name | Rgt. Type | Reagent Probe Cleaner | Wash Count | Cancel | Mixer | Cuvette |
|---|---|---|---|---|---|---|---|---|
| (ALL) | R2 | Mg | R2 | Water | 1 | No | Yes | Yes |

**NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853**

## Calibration
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

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of Standard} = \text{Value mg/dl}$$

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

Then: $\frac{.140}{.120} \times 2.4 \text{ mg/dl} = 2.8 \text{ mg/dl}$

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.[13] Each laboratory should establish its own normal range.

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

Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.957 with a regression equation of y = 0.961x - 0.03. (n = 30, range 1.8 – 4.3 mg/dl)

Precision:
Within - day precision study was performed using three levels of material.
Between - day precision study was performed using two levels of control material with 2 runs per day and 2 replicates per run.

| Within Day (N=20) | | | Day to Day | | |
|---|---|---|---|---|---|
| Mean | S.D. | C.V.% | Mean | S.D. | C.V.% |
| 1.6 | 0.09 | 5.6 | 1.8 | 0.08 | 4.6 |
| 2.4 | 0.14 | 5.8 | 3.4 | 0.20 | 5.9 |
| 4.1 | 0.20 | 4.9 | | | |

Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6[14] using an Beckman Coulter AU™400 analyzer

## References

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7. Basinski, D.H., Standard Methods of Clinical Chemistry, 5, New York, Academic Press, pp. 137-142 (1965).
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Manufactured by Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net



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