MAGNESIUM (Xylidyl Blue)
MANUAL/AUTOMATED APPLICATIONS
C355-01, V355-12

Intended Use
For **IN VITRO quantitative** determination of Magnesium in serum using manual or automated procedures.

Clinical Significance
Measurements of Magnesium are primarily used for diagnosing Magnesium deficiency, tetany, acute pancreatitis, hypothyroidism, chronic glomerulonephritis, aldosteronism and digitalis intoxication, dehydration, severe diabetic acidosis, uremia, as well as for monitoring the causes and treatments.(1)

Method History
In 1969 Chauhan and Sarkar described a procedure for the determination of traces of Magnesium in biological fluids using calmagite as an indicator. Ratge etal in 1986 described the use of Xylidyl Blue as a superior chromagen indicator. (2) This Catachem Magnesium method is based upon an inverse xylidyl blue reaction where the decrease in absorbance is read either at 630 or 650nm. The choice of these wavelengths improves precision and minimizes interferences from lipemic bilirubin and hemolyzed specimens.

Method Principle
The serum sample is mixed with the Magnesium reagent where the Magnesium ions react with the xylidyl blue indicator to form a Magnesium-chelate complex with concomitant reduction of the reagent absorbance which has a broad absorbance peak between 600 and 660nm. The decrease in absorbance is directly proportional to the Magnesium concentration in the sample. The reaction scheme illustrates the reaction that occurs in this method.

Xylidyl Blue + Mg^{++} ------> Mg^{++} xylidyl blue chelate

Reagent Content
The concentrations of the active ingredients in the reagent are approximately as follows:

Magnesium Reagent
Each liter contains:

Buffer	pH approx. 11.2
Xylidyl Blue	0.14 mmol
EGTA	0.10 mmol
Surfactant	

Precaution
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Catachem Magnesium reagent is packaged in a ready-to-use form. No preparation is required.

Reagent Storage And Stability
Store Catachem Magnesium reagent at 8-26°C. When stored as directed, the reagent is stable until the expiration date stated on the label.

Specimen Collection And Preparation
Test specimens should be fresh, clear, unhemolyzed sera. Serum samples stored for periods longer than eight hours should be refrigerated at 2-8˚C. Under these storage conditions, samples are stable for up to 3 days.

Quality Control
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Several substances have been reported to interfere with the Magnesium method. Environmental contamination of assays will produce erroneous results. Care must be taken to use clean glassware and Magnesium-free distilled or deionized water. Anticoagulants such as oxalates and fluorides will depress Magnesium values. A summary of interferences from drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al (3).

Expected Values
The range of expected values determined for this method for human subjects is 1.6-2.6 mg/dL (0.65-1.05 mmol/L). For other species please consult the appropriate literature. All values however are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located and for the species under test. (1)

Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	2.0 ml for reagent
Pipette	0.02 ml for sample

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Materials Provided
Catachem Magnesium reagent

Calibration
Catachem Calibrator, "Catacal" is recommended for use in the Catachem Magnesium assay. The Calibrator and the unknown should be treated in the same way while performing the Magnesium procedure.

Analytical Parameters

Wavelength	630 or 660nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5.0 minutes
Reagent Volume	3.0 ml
Sample Volume	0.02 ml
Total Volume	3.020 ml
Sample to Rgt Ratio	1:151

Assay Procedures
1. Pipette 3.0ml of Magnesium Reagent into each of three cuvettes marked "Calibrator", "Sample", and "Blank".
2. Pipette 0.020 ml of Calibrator or Sample into their respective cuvettes. Use 0.020ml of distilled water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for 5.0 minutes at 37ºC.
4. Set spectrophotometer wavelength at 630 or 660 nm.
5. Read the "Calibrator", "Sample", and "Blank" absorbances.
6. Calculate the Magnesium concentration (mg/dL) in the Sample(s), as shown in calculations and results below.

Calculations And Results

$$Mg^{++}\ (mg/dL) = \frac{Ba - Sa}{Ba - Ca} \times \text{Calibrator (mg/dL)}$$

Where: Ba = Blank absorbance
Sa = Sample absorbance
Ca = Calibrator

ASSAY OD

Example: Sample 1.46
Calibrator: 1.45
Blank 1.70
Calibrator = 4.0 mg/dL

$$\text{Calcium (mg/dL)} = \frac{0.240}{0.250} \times 4.0 \text{ mg/dL} = 3.84 \text{ mg/dL}$$

NOTE: Samples with Magnesium concentrations greater than 6.2 mg/dL should be diluted with physiological saline and reassayed. Results should be adjusted for dilution.

Method Performance Characteristics

Sensitivity: 0.05-0.07 absorbance units/mg/dL

Linear Range: 0.0 - 6.2 mg/dL (2.5 mmoles/L)

Precision: Within-run and day-to-day precision is summarized below:

Precision Study

Mg^{++}	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
1.7	0.13	7.70	0.12	7.06
3.1	0.17	5.48	0.14	4.52
6.2	0.20	3.22	0.16	2.58

Correlation
A comparison of this method using an automated analyzer resulted in the following regression statistics:

Range	=	0.64 – 4.46 mg/dL
N	=	40
Y	=	0.956 x -0.05
r	=	0.994

References
1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Ratge, D., Kohse, K. P. et Wisser, H., Measurement of Magnesium in Serum and Urine with a Random Access Analyzer by Use of a Modified Xylidyl Blue-1 Procedure, Clin. Chem. Acta, 159 (1986) 197-203.
3. Young D.S, Pestaner LD, Gibberman V. Clin. Chem. 21, 5 (1975).

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