

Manual Procedure

Automated procedure on request

MEDICHEM[®]
MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

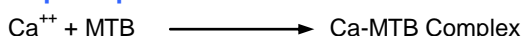
Cat. No.12170	R1	1 x 20	ml
For 50 tests	R2	1 x 20	ml
Cat. No.12171	R1	1 x 50	ml
For 100 tests	R2	1 x 50	ml
Cat. No.12172	R1	3 x 50	ml
For 300 tests	R2	3 x 50	ml

Calcium without deproteinization

Colorimetric, Methylthymol blue method

Liquid Reagents

Test principle



Ca^{2+} forms a colored complex with methylthymol blue in an alkaline medium. The intensity of the color produced is proportional to the concentration of total calcium present in the samples. 8-Hydroxyquinoline prevents Mg^{2+} ions of interfering up to 100 mg/L (4 mmol/L).

Concentrations in the test

Reagent R1		
Monoethanol amine	1.0	mol/L
Reagent R2		
Methylthymol blue	92	$\mu\text{mol/L}$
8-hydroxyquinoline	50	mmol/L
Standard : The concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: liquid, ready to use.

Reagent R2: liquid, ready to use.

All reagents are stable up to expiry date given on the label when stored at $+2 \rightarrow +8$ °C.

Working Reagent: You could mix R1 and R2 in a ratio of (1+1), and use 1 ml of the mixture as a working reagent (Stable for 15 days at 2 - 8 °C in the dark).

Note: The reagent should be clear. Turbidity indicates deterioration and the reagent should be discarded.

Specimen collection and handling

1. Fasting non-hemolyzed serum is the specimen of choice.
2. Anticoagulants other than heparin should not be used.
3. Remove serum from clot as soon as possible since red cells can absorb calcium.
4. Older serum specimen containing visible precipitate should not be used.
5. Serum calcium is stable for 24 hours at 20 - 25°C, 1 week at 2 - 8 °C and 5 months at - 20°C, when protected from evaporation.
6. Urine: collect 24-hour urine specimen in a container containing 10 ml of 6N HCl.
7. Adjust urine pH to 3 - 4 with 0.1 N HCl. Centrifuge and dilute (1+2) with distilled water before testing. Multiply the result by 3.

Calibrator / Standard

MediCal U Cat. No. 15011
Calcium STD. Cat. No. 16041

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 623 nm (600 - 625 nm)
Spectrophotometer	612 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator/ Standard	Sample
Double dist. water	10 μl	--	--
Calibrator / Standard	--	10 μl	--
Sample	--	--	10 μl
Reagent R1	500 μl	500 μl	500 μl
Reagent R2	500 μl	500 μl	500 μl

Mix, incubate for 5 min. at 37°C or 10 min. at 20 - 25 °C. Read the absorbance (A). The final color is stable for at least 30 min.

Procedure notes

1. Lipemic or hemolyzed samples require serum blank. To prepare serum blank add 10 μl of sample to 1 ml distilled water. Mix and read against water. Subtract the absorbance reading from the test reading, then perform the calculations.
2. Use disposable plastic containers or glass equipment cleaned with 1N HCl and rinsed with distilled water to avoid contamination.

Calculation

$$\text{Conc. Calcium (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc. Cal./STD. (mg/dl)}$$

$$\text{mmol/L} \xrightleftharpoons[25 \times 10^{-2}]{\times 4.01} \text{mg/dl}$$

$$\text{mEq/L} \xrightleftharpoons[2 \times]{\times 0.5} \text{mmol/L}$$

Linearity

Up to 15 mg/dl (3.75 mmol/L)

If the result exceeds 15 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Mg²⁺: No significant interference up to 100 mg/L.
2. Specimen of patients receiving bromosulfophthalein (BSP) or EDTA should not be used.
3. Substances affecting the accuracy of calcium values with this procedure is listed by Young.
4. Bilirubin in high concentration introduces a significant error in calcium results.
5. Acetaminophen and the reagent hydralazine cause positive interference

Precautions

1. The reagents should not be pipette by mouth.
2. The reagents may be irritating to the skin. Avoid contact.

Reference range

Serum

1d. - 4 wk.	7.2 - 11.2	mg/dl
2 - 12 mth.	8.4 - 10.8	mg/dl
≥ 1 yr.	8.4 - 10.4	mg/dl
Adults	8.6 - 10.2	mg/dl

Urine

Urine /24 hr.	100 - 320	mg/24 hr.
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References

1. Robertson, G. *et al.* – Clin. Chim. Acta. 1968, 20, 315.
2. Yendt *et al.* – Can. Med. Ass. J. 1968, 98, 331.
3. Elveback, L.R. – J. Am. Med. Ass. 1970, 211, 69.
4. Gindler, E. *et al.* – Am. J. Clin. Path. 1972, 58, 376.
5. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests*, fifth edition 2000, AACC Press, Washington, D.C.