



# Comparison of magnesium by HEDTA and EGTA as chelating agent

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## ABSTRACT

Introduction-Magnesium estimation in serum is done by various methods like Arsenazo III, Ortho Vitros Microslide System, Atomicabsorption, Calmagite, Xylidyl blue, Methylthymol blue. One of the methods is Xylidyl blue in which chelating agents HEDTA or EGTA can be used. In this study, Comparison is done between HEDTA and EGTA as chelating agent. EGTA is a chelating agent with a much higher affinity for  $Ca^{2+}$  at pH 11.00. HEDTA is a chelating agent for di- and trivalent metal ions. HEDTA binds to metals via four carboxylate and two amine groups. Magnesium is abundant cation in the human body. Majority is present in bone and muscle, Extra Cellular Fluid (ECF). Magnesium is a responsible for energy metabolism and the synthesis of proteins and nucleic acids. Methods- Measurement of magnesium is done by Xylidyl blue method using EGTA and HEDTA as Chelating agent. Random Patient's left over 50-serum samples are selected from indoor as well as outdoor of New Civil Hospital, Government Medical College Surat. Magnesium ions react with xylidyl blue in an alkaline medium to form a water soluble purple-red chelate, the colour intensity of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with EGTA and HEDTA. Results and Statistical Analysis -Linear regression analysis using spreadsheet functions shows slope of 0.96 and  $r^2$  of 0.91. Slope of 0.96 indicate that Magnesium with HEDTA, on an average gives about 4% higher result as compared to Magnesium with EGTA. Bland altman plot of magnesium (EGTA) vs. difference (HEDTA-EGTA) shows positive bias in Magnesium(HEDTA) in comparison with Magnesium(EGTA).Conclusion-Results in patient's sample as well as in quality control sera are comparable with acceptable accuracy and precision. As adequate research study are not available for magnesium with HEDTA method till date so further study is required for validation and verification of this method.

**Keywords:** Magnesium; EGTA; HEDTA; Xylidyl blue

## INTRODUCTION

### Magnesium

Magnesium is the fourth most abundant cation in the human body. Majority is present in bone and muscle, only approximately 1% being present in the Extra Cellular Fluid (ECF). Magnesium is a cofactor in more than 300 enzyme-catalyzed reactions .It is responsible for energy metabolism and the synthesis of proteins and nucleic acids. It controls various trans membrane ion channels and membrane excitability. Magnesium is widely distributed in foodstuffs, green vegetables being a particular rich source because it is a component of chlorophyll. The recommended daily intake is 420 mg (18

mmol) in men and 320 mg (10 mmol) in women (Bazydlo, 2014). It acts as a competitive inhibitor to calcium, preventing calcium entry into the presynaptic nerve and thus inhibiting the release of neurotransmitters. Deficiency of magnesium can lead to numerous metabolic and nervous-system pathology.

### Deficiency

Hypomagnesemia can cause hypokalemia (through adverse effects on  $Na^+$ ,  $K^+$  -ATPase) and hypocalcemia (para-thyroid hormone is dependent on magnesium), and these may be responsible for some of its clinical manifestations.

## Hypermagnesemia

Increased serum magnesium levels are associated with dehydration, severe diabetic acidosis and Addison's disease. Symptomatic hyper magnesemia is caused by excessive intake, resulting from administration of antacids, enemas, and parenteral fluids containing magnesium.

Magnesium estimation in serum is done by Arsenazo III, Ortho Vitros Microslide System, Atomicabsorption, Calmagite, Xylidyl blue, Methylthymol blue, chlorophosphonazo III, ENZYMATI methods. One of the methods is xylidyl blue in which chelating agents is used. In this study, Comparison is done between HEDTA and EGTA as chelation agent.

## EGTA-(ethylene glycol-bis ( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid)

EGTA is a chelating agent with a much higher affinity for  $\text{Ca}^{2+}$  at pH 11.00

## HEDTA-ethylenediaminetetraacetic acid

HEDTA is a chelating agent for di- and trivalent metal ions. HEDTA binds to metals via four carboxylate and two amine groups. Ethylenediaminetetraacetic acid is a chelating agent of divalent cations such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  that is particularly useful for hematologic examinations including transfusion medicine applications, measurement of intracellular drugs such as cyclosporine or tacrolimus, HbA 1c analysis isolation of genomicDNA (Nguyen, 2020).

## METHODOLOGY

This study is planned for patients of New Civil Hospital, Government Medical College Surat; Random Patient's left over serum samples are selected from indoor as well as outdoor Opd.

Inclusion Criteria: All the age group patient's left over serum sample are randomly selected from inpatients and outpatients department (Rifai, 2017).

Exclusion Criteria: We have made criteria for rejection sample with following condition.

- Haemolysed sample
- Sample collected in other than plain vacuttee
- Serum less than 2 ml
- Lipamic sample

## Sample Preparation

Sample were identified and sorted for study. Samples were given unique Lab ID number and registration was done in Laboratory Information System (LIS). All samples were centrifuged for 10 minutes at 3000 rotation per minute speed.

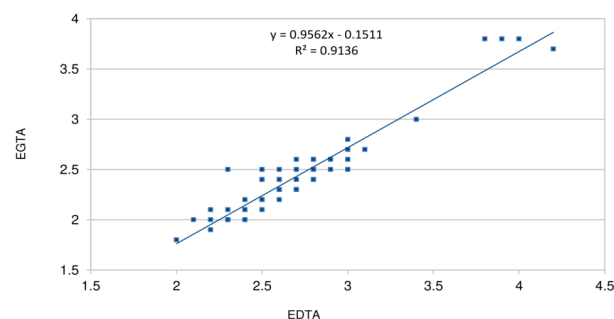
Calibration and Quality Control: calibration of magnesium reagent with HEDTA AND EGTA was done first. Calibration was done by Randox Quality Control (RQC). 1 Cup of DI water, 1 cup of half diluted normal level quality control, 1 cup of normal level quality control ( Lot No-1502UN), 1 cup of 1:1 mixture of Normal level and high level Quality control (100ul Normal QC +100ul high QC ),1 cup of ( Lot No-1195UE) high level quality control are used for calibration. Each level is run 5 times and average OD is used and factor is calculated. All samples are analyzed after calibration with EGTA AND HEDTA in single batch of 50 samples.

**Principle:** Magnesium ions react with xylidyl blue in an alkaline medium to form a water soluble purple-red chelate, the colour intensity of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with EGTA and HEDTA Reagent Content: Magnesium reagent and contain Tris base,  $\text{Na}_2\text{CO}_3$ , Xylidyle blue, Triton X-100 ,Magnesium reagent contain HEDTA Chelating agent and contain EGTA Chelating agent. Reagent preparation- Above mentioned reagents is in-house reagents. All Glassware which used are being washed by 10% HCL or 10% acetic acid. Purpose of washing is minimizing the contamination of reagents from various unmeasured ions present on surface of the glassware. Fixed amount of chemicals were added in particular sequence. pH of reagent is most important matter to be maintained. All above reagents are stable on board for long time. Around 10 ml of R1 is being kept in sterile plastic container and placed in ERBA XL-1000 reagent tray for use (Ryan, 1998).

Magnesium test parameter in XL-1000: magnesium measured by 2 point method, with sample-reagent ratio is 1:100 and reading will be taken by 505 nm wavelength.

## RESULT AND DISCUSSION

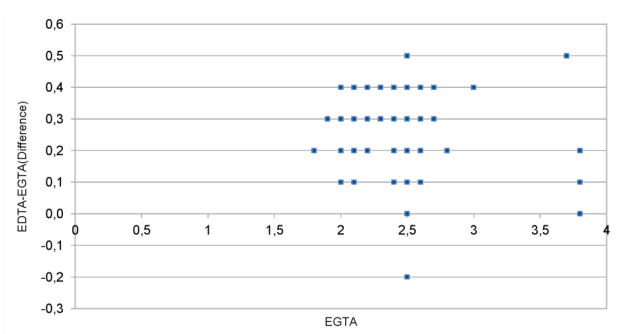
Results of Magnesium obtained by both the method were entered in a spreadsheet. Following is the scatter plot of magnesium (HEDTA) vs. magnesium (EGTA).



**Figure 1:** The scatter plot of magnesium (HEDTA) vs. magnesium (EGTA).

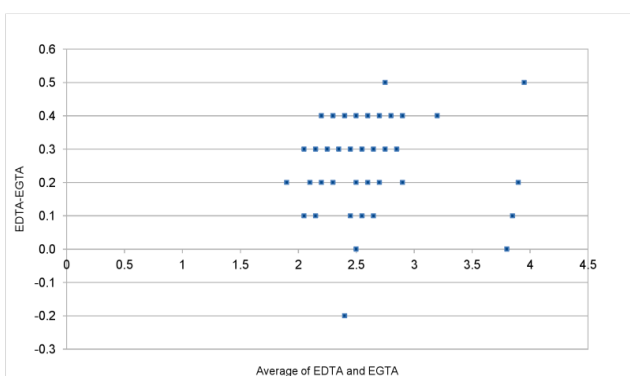
Linear regression analysis using spreadsheet functions shows slope of 0.96 and R2 of 0.91 Slope of 0.96

indicate that magnesium with HEDTA, on an average gives about 4% higher result as compared to magnesium with EGTA. Bland altman plot of magnesium (EGTA) vs difference (HEDTA-EGTA) is shown below. It also shows positive bias in magnesium (HEDTA) in comparison with magnesium (EGTA).



**Figure 2:** Bland altman plot of magnesium (EGTA) vs difference (HEDTA-EGTA)

Bland Altman plot of magnesium average of HEDTA and EGTA vs difference (HEDTA-EGTA) is shown below. It also shows positive bias in magnesium (HEDTA) in comparison with magnesium (EGTA).



**Figure 3:** Bland Altman plot of Magnesium average of HEDTA and EGTA vs difference (HEDTA-EGTA).

Comparison of both Bland Altman Plot shows that, there is relatively constant positive bias in magnesium (HEDTA) with respect to magnesium (EGTA).

Magnesium estimation by xylydyl blue is well known method. Magnesium can be quantified through either an increase in absorption of chelate at 505 nm or a decrease in absorption of xylydyl blue at 600 nrm. In the xylydyl blue

method, calcium in serum or urine is generally chelated selectively by GEDTA or EGTA (included in the reagent mix) prior to analysis of magnesium in the sample. Currently commercially available magnesium estimation kit by xylydyl blue method is contained EGTA as chelating agent. In this study, HEDTA is used as chelating agent instead of EGTA and comparison is done between two methods. Results are comparable between two methods. Intra-run average CV% of two level (normal and abnormal range) quality control for HEDTA is 6.39% and for EGTA is 8.71%. No adequate research data and study are available for magnesium estimation with HEDTA. More detailed study with larger sample size is needed to do to find out linearity, interfering and influencing factors.

## CONCLUSION

Comparison of serum Magnesium level in patients and two level quality control sera by HEDTA and EGTA as chelating agent is studied. It was found that Magnesium with HEDTA gives on an average 4% higher result as compared to Magnesium with EGTA. According to CLIA, total acceptable performance for Magnesium is upto 15%. Our bias is 4% so at 95% confidence interval 8 % variations are found which is less than CLIA acceptable performance criteria. Patient's sample as well as in quality control sera are comparable with acceptable accuracy and precision. As adequate research study are not available for magnesium with HEDTA method till date so further study is required for validation and verification of this method.

## REFERENCES

- Bazydlo LA, Needham M, Harris NS (2014) Calcium, magnesium, and phosphate. *Lab Med.* 45(1):e44-50. Life science interchim product information.
- Nguyen HT, Morgan N, Roberts JR, Swick RA, Toghiani M (2020). Copper hydroxychloride is more efficacious than copper sulfate in improving broiler chicken's growth performance, both at nutritional and growth-promoting levels. *Poult Sci.* 99(12):6964-73.
- Rifai N (2017) Tietz textbook of clinical chemistry and molecular diagnostics.
- Ryan MF, Barbour H (1998) Magnesium measurement in routine clinical practice. *Ann Clin Biochem.* 35(4): 449-459.