ANA BIO CLR CALCIUM ARS (III)

(Arsenazo (III) method)

Intended Use

Calcium Arsenazo (III) is used for the quantitative determination of calcium concentration in serum based on the colorimetric method using Arsenazo III.

Principle

Calcium with Arsenazo III at neutral pH yields a blue coloured complex. The intensity of the colour formed is directly proportional to calcium concentration.

Reagent provided

1. Arsenazo Reagent Ready to use.
2. Standard - Calcium (8 mg / dl).

Working reagent preparation

Ready to use Arsenazo III Reagent.

Reagent storage and stability

The reagents are stable till the expiry date stated on the bottle label; when stored at R.T (≤30℃).
Contamination of the reagent should be strictly avoided. Protect the reagent from direct light. Should the reagent develop turbidity discard the reagent.

Specimen collection and preservation

Blood should be collected in a clean dry container. Avoid the use of plastic or siliconized container which may prolong clotting time. Serum is preferred but heparinized plasma (200 IU/ml of blood) can also be used. EDTA, Citrate, Oxalate and calcium salt of heparin interfere in the assay and should not be used as anticoagulant. Calcium is stable in serum or plasma for 5 days when stored at 2 - 8℃ and 20 days when stored at - 10℃.

Assay guidelines for Analyzers

Reaction Type	End Point with Standard
Reaction slope	Increasing
Incubation time	2 minutes at R.T (25℃ - 37℃)
Wave length	650 nm (620 – 650 nm)
Zero setting with	Reagent Blank
Blank absorbance limit	< 0. 800
Sample Volume	20 µl (0.02ml)
Reagent Volume	1000 µl (1.0ml)
Calcium Standard Concentration	8 mg / dl
Factor Calculation	8 mg / dl ÷ Std. OD
Low normal	8.5 mg / dl
High normal	11 mg / dl
Linearity	Up to 15 mg / dl

Assay guidelines for Manual Procedure

Bring the reagent and standard to room temperature before performing the assay.

Reagents	**Blank**	**Standard**	**Sample**
Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	20 µl (0.02 ml)	-
Sample	-	-	20 µl (0.02 ml)

1. Mix thoroughly and incubate at room temperature for 2 minutes.
2. Read the absorbance against reagent blank at 650 nm.
3. The final colour is stable for 60 minutes (when protected from light). Do not read the test after 60 minutes.

Calculation

Calcium Con. in sample (mg /dl) = $\frac{\text{Sample OD}}{\text{Standard OD}} \times \text{Con. of Std.}$

Note:

- Avoid the contamination of reagent into standard during its repeated use.
- Glassware is the most common source of contamination in the calcium assay. It is strongly recommended that glassware required for assay is rinsed with 0.1 N HCl followed by repeated rinsing with de-mineralized water.
- The specimen and reagent volumes can proportionally be altered without affecting the final results.

Normal Range

Guidance Value - 8.5 – 11 mg / dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation

1. Patients receiving EDTA treatment cannot be assayed for calcium correctly.
2. If calcium value exceeds 15 mg% then dilute the specimen suitably with normal saline. In such case the result obtained should be multiplied with the dilution factor to obtain the correct calcium value.

Quality Control

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, and accuracy of pipetting.

Reference

1. Smith, H. G.Jr.and Bauer, P.J.(1979) *Biochemistry*, 18, 5067-5073.
2. Budesinsky, b. (1969) in chlantes in *Analytical chemistry*.
3. Cadwell P.C. (1970) in calcium An. Cellular function.
4. Cuthbert,A.W.Ed., PP-10-16 macmillan, London.

Symbols

IVD In Vitro Diagnostics. | Caution. | Keep away from sun light. | Date of Manufacture.

LOT Batch No. | Read Instructions. | Fragile. | Product Expiry Date.

CONT Content. | Storage Temperature. | Keep Dry. | Manufactured By.

REF Catalogue No.

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