

## Calcium (Arsenazo) Reagent Set

### Intended Use

For the quantitative determination of Calcium in serum or heparinized plasma using the Mindray BS-200 analyzer.

### Clinical Significance <sup>1,2</sup>

Increased serum calcium may be observed in hyperparathyroidism, vitamin D intoxication, multiple myeloma and some neoplastic diseases of bone. Decreased serum calcium may be observed in hypoparathyroidism, vitamin D deficiency, steatorrhea, nephrosis, and nephritis.

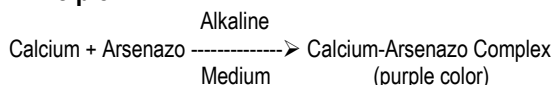
### Method History

Various methodologies have been developed for the determination of calcium including flame photometry, fluorescent, gravimetric and titrimetric procedures, ion selective electrodes, and atomic absorption. Atomic absorption has been recommended as the reference method but it requires expensive instrumentation.<sup>3</sup>

Specific dye binding methodologies have become popular for calcium determination because they are rapid, convenient and inexpensive. Procedures using the dyes alizarin 3-sulfonate and methylthymol blue have been described.<sup>4,5</sup> A method using o-cresolphthalein complexone as the chromagen was developed in 1966 by Connerty and Biggs, and modified by Gitelman in 1967 and Baginski, et al, in 1973.<sup>6,7,8</sup> o-Cresolphthalein complexone procedures have been widely used for the determination of calcium.

The present procedure uses Arsenazo III and has been modified to provide a highly sensitive and stable reagent system. Magnesium interference is prevented by the inclusion of 8-hydroxyquinoline sulfonate. The reagent is provided as a convenient ready to use liquid.

### Principle



Calcium reacts with Arsenazo III in a slightly alkaline medium to form a purple-colored complex which absorbs at 650 nm. The intensity of the color is proportional to the calcium concentration.

### Reagents

Calcium reagent: Arsenazo III  $\geq$  0.15mM, 8-Hydroxyquinoline Sulfonate 5.0mM, Buffer, Surfactant.

### Reagent Preparation

Reagent is ready to use.

### Reagent Storage

Store reagent at room temperature (15-30°C). The reagent is stable until the expiration date appearing on the label when stored as directed.

### Reagent Deterioration

Do not use if the reagent has become noticeably turbid.

### Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Reagent may be irritating to the skin. Avoid contact. Flush with water if contact occurs.

### Specimen Collection and Storage

1. Fresh, unhemolyzed serum is the preferred specimen.
2. Heparinized plasma may also be used.
3. Anticoagulants other than heparin should not be used.<sup>9</sup>
4. Remove serum from clot as soon as possible since red cells can absorb calcium.<sup>10</sup>
5. Older serum specimens containing visible precipitate should not be used.<sup>11,12</sup>
6. Serum calcium is stable for 24 hours at room temperature, one week at 2-8°C, and up to five months frozen (-15 to -25°C) and protected from evaporation.<sup>13</sup> Specimens should not be thawed and refrozen.

### Interferences

1. Substances that contain or complex with calcium cause inaccurate results.<sup>14</sup>
2. Glass tubes often are coated with a residue containing calcium. They should be acid-washed before use. Alternatively, plastic tubes may be used.
3. Bilirubin up to 20 mg/dl and hemoglobin to 500 mg/dl do not interfere.
4. Severe lipemia may cause elevated results. A serum blank should be run for greatest accuracy.
5. For a comprehensive review of interferences see Young, et al.<sup>15</sup>

### Materials Provided

Calcium Reagent

### Materials Required but not Provided

1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

### Mindray BS-200 Test Parameters

Test :	CA	R1 :	250
No.:	010	R2 :	0
Full Name:	Calcium	Sample Volume:	3
Reference No.:		R1 Blank:	
Reac. Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	670nm	Linearity Range:	0.0 – 14.0
Sec. Wave:		Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 8	Factor:	
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	0	<input type="checkbox"/> Prozone check	
Unit:	mg/dl	q1: q2: q3: q4:	
Precision:	0.1	PC: Abs:	

### Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:			

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## Limitations

Samples with calcium values exceeding 14mg/dl<sup>16</sup> should be diluted with an equal volume of saline, the assay repeated, and the result multiplied by two. Severely lipemic samples should be run with a serum blank for greatest accuracy.

## Calibration

Use an NIST-traceable serum calibrator. 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 re-calibrated.

## Calculations (Example)

$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Std.} = \text{Calcium (mg/dl)}$

Example: If the absorbance of sample = 0.81, absorbance of standard = 0.80, concentration of standard = 10mg/dl, then:

$$\frac{0.81}{0.80} \times 10 = 10.1 \text{ mg/dl}$$

NOTE: To correct mg/dl to mEq/L, divide mg/dl value by two.

## Quality Control

The integrity of the reaction should be monitored by use of normal and abnormal control sera with known calcium concentrations. These controls should be run at least with every working shift in which calcium assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

## Expected Value

Adults: 8.5-10.4 mg/dl<sup>17</sup>

Newborns: 7.8-11.2 mg/dl<sup>18</sup>

It is strongly recommended that each laboratory establish its own reference range.

## Performance

1. Linearity: 0-14 mg/dl<sup>16</sup>
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.994 with a regression equation of  $y=0.99x + 0.18$  (n=36).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.<sup>19</sup>

Within Run			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
9.91	0.48	4.8	8.96	0.19	2.12
12.12	0.57	4.7	11.14	0.26	2.33

4. Sensitivity: Recovery studies indicate that this reagent can distinguish calcium concentrations of 0.1 mg/dl throughout the linear range of the assay.<sup>16</sup>

## References

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