

# Calcium Reagent

## Arsenazo III

### PRODUCT SUMMARY

|                     |   |                                 |
|---------------------|---|---------------------------------|
| Stability           | : | Until Expiry at 2 - 25°C        |
| Linear Range        | : | 0.0 - 4.0 mmol/L (0 - 16 mg/dL) |
| Specimen Type       | : | Serum, plasma or urine          |
| Method              | : | Endpoint                        |
| Reagent Preparation | : | Reagent supplied ready to use.  |

**IVD**

### INTENDED USE

This reagent is intended for the in vitro quantitative determination of calcium in serum, plasma or urine.

### CLINICAL SIGNIFICANCE

Calcium has numerous functions within the body, not only as a structural factor in bones and teeth, but also in normal neuromuscular function and the clotting of blood.

The level of serum calcium may be affected by intestinal malabsorption, by alterations in PTH secretion, by changes in bone metabolism and by alterations in plasma protein levels, especially albumin, which should be measured concurrently with calcium. For a more detailed discussion of calcium metabolism the user should refer to the excellent monograph by Beeler and Catrou<sup>1</sup> or a standard textbook such as Kaplan.<sup>2</sup>

### METHODOLOGY

Many colourimetric methods for determining calcium have been used in the past. Connerty and Briggs described methods using alizarin 3-sulphonate<sup>3</sup> and cresolphthalein complexone<sup>4</sup> whilst Gindler and King have described a method using thymol blue.<sup>5</sup>

There have been many subsequent modifications to these methods. The method used here is based on the metallochromogen Arsenazo III. Arsenazo III combines with calcium ions at pH 6.75 to form a highly coloured chromophore, the absorbance of which is measured at 650 nm. Arsenazo III has a high affinity ( $K^c = 1 \times 10^{-7}$ ) for calcium ions<sup>6</sup> and shows no interference from other cations normally present in serum, plasma or urine.

For bichromatic analysers the blank reading should be made at 700 nm.

### REAGENT COMPOSITION

#### Active Ingredients

|                  | Concentration |
|------------------|---------------|
| Arsenazo III     | 0.2 mmol/L    |
| Imidazole buffer | 100 mmol/L    |

pH 6.75 ± 0.1

Reagent also contains surfactant and stabilizers necessary for optimum reagent performance.

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the Calcium Arsenazo III Reagent Material Safety Data Sheet.

### REAGENT PREPARATION

The reagent is supplied ready to use.

### STABILITY AND STORAGE

When stored at 2-25°C reagent is stable until the expiration date stated on the bottle and kit box label

Indications of reagent Deterioration:

- Turbidity; and/or
- Failure to recover control values within the assigned range.

### SPECIMEN COLLECTION AND HANDLING









**Collection:** Ideally the specimen should be drawn with the patient in a resting position and without venous stasis if possible. The use of a tourniquet and erect posture can elevate calcium levels by up to 0.15 mmol/L (0.6 mg/dL)<sup>2</sup>.

**Serum:** Use non-haemolysed serum. Serum from patients receiving EDTA treatment for hyperparathyroidism should not be used. Analysis by atomic absorption spectrometry is recommended in these patients.

**Plasma:** Use heparin (Refer to Limitations Section). Do not use EDTA, Oxalate or Citrate

**Urine:** 24 hour specimens acidified with 15 mL of concentrated HCl.<sup>7</sup> Non acidified urines which have been refrigerated should be acidified and/or heated at 56°C for 15 minutes to redissolve any precipitate. Acidified specimens are usually

### SYMBOLS IN PRODUCT LABELLING

|   |                              |   |  |
|---|------------------------------|---|--|
|  | For in vitro diagnostic use  |  | Temperature Limitation                 |
|  | Batch code/Lot number        |  | Use by/Expiration Date                 |
|  | Catalogue number             |  | CAUTION. CONSULT INSTRUCTIONS FOR USE. |
|  | Consult instructions for use |  | Manufactured by                        |

unsuitable for urate or creatinine estimations. The urine specimen should be diluted 1 in 2 with an equal volume of deionised water before analysis.

**Storage:** Serum or plasma samples are stable at 4°C for days or months when frozen<sup>8</sup>. Urine specimens are stable for 7 days when stored at 4°C.<sup>7</sup>

### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 650 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or a suitable aqueous calcium standard.

### ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

#### System Parameters

|                              |                        |
|------------------------------|------------------------|
| Temperature                  | 37°C                   |
| Wavelength                   | 650 nm(630 - 660 nm)   |
| Assay Type                   | Endpoint               |
| Direction                    | Increase               |
| Sample : Reagent Ratio       | 1:50                   |
| eg: Sample Vol               | 6 µL                   |
| Reagent Vol                  | 300 µL                 |
| Incubation Time              | 60 seconds             |
| Reagent Blank Limits         | Low 0.0 AU             |
| (650 nm, 1 cm lightpath)     | High 2.0 AU            |
| Linearity                    | 0 - 4.0 mmol/L         |
| (refer to linearity section) | (0 - 16.0 mg/dL)       |
| Sensitivity                  | 0.25 ΔAbs per mmol/L   |
| (650 nm, 1 cm lightpath)     | (0.065 ΔAbs per mg/dL) |

### CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Calcium} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

|          |                          |   |                         |
|----------|--------------------------|---|-------------------------|
| Example: | Absorbance of calibrator | = | 0.80                    |
|          | Absorbance of unknown    | = | 0.71                    |
|          | Value of calibrator      | = | 3.20 mmol/L(12.8 mg/dL) |

$$\text{Calcium} = \frac{0.71}{0.80} \times 3.20 = 2.84 \text{ mmol/L}$$

$$\text{Calcium} = \frac{0.71}{0.80} \times 12.8 = 11.36 \text{ mg/dL}$$

For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in litres.

$$\text{Urine Calcium (mmol/24 hours)} = \frac{\text{Calcium Result (mmol/L)}}{\text{Dilution Factor}} \times \text{Volume at 24hr Collection (L)}$$

|          |                 |   |            |
|----------|-----------------|---|------------|
| Example: | Calcium         | = | 1.0 mmol/L |
|          | Dilution        | = | 1 : 2      |
|          | Volume of urine | = | 1.25 L     |

$$\text{Urine Calcium} = 1.0 \times 2.0 \times 1.25 = 2.5 \text{ mmol/24Hours}$$

### NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The temperature of the reaction is not critical, however the temperature of the spectrophotometer should be held constant as the absorbance of the dye is temperature sensitive.

- The color developed is stable for at least 2 hours.
- Because of the need to obtain highly accurate calcium results, it is recommended that the manual assay should be performed in duplicate.
- S.I. conversion factor: mmol/L x 4 = mg/dL.

#### CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

#### QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the established limits indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control, contact Technical Services or your local distributor.

#### LIMITATIONS

- Care must be taken to avoid calcium contamination. The use of disposable plastic tubes or cuvettes is strongly recommended. The user should assure themselves that such disposables are free from calcium contamination. If glassware is used, it should be soaked in dilute HCl or a strong laboratory cleanser and thoroughly rinsed with distilled or deionized water.
- Gross haemolysis and lipaemia may cause falsely elevated results unless a sample blank is used.
- Grossly elevated citrate levels may cause falsely low results.
- If bichromatic analysis is available it is recommended that plasma samples be run using a bichromatic wavelength of 700 nm.
- Copper may interfere with this method.<sup>9</sup> Therefore, care should be taken to avoid contamination with reagents that contain high concentrations of copper such as Biuret reagent (Total Protein reagent).
- For a more comprehensive review of factors affecting calcium assays refer to the publications of Young, et al.<sup>10</sup> and Friedman, et al.<sup>11</sup>
- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:  
**Haemoglobin:** No interference from haemoglobin up to 540 mg/dL.  
**Bilirubin:** No interference from bilirubin up to 340 µmol/L (20 mg/dL).  
**Lipaemia:** No interference from lipaemia, measured as triglycerides up to 20.2 mmol/L (1770 mg/dL).

#### EXPECTED VALUES

It is generally agreed that with current methods for serum calcium the reference range is approximately 2.10-2.55 mmol/L (8.4-10.2 mg/dL) with a mean value of approximately 2.30-2.40 mmol/L (9.3 mg/dL).<sup>2,12</sup> This is somewhat lower than older published normal ranges.

As the reference range depends to some extent on collection techniques and laboratory procedures, the user should verify the reference range in their own laboratory. For 24 hour urine specimens with patients on a normal diet, the reference range is 2.5-7.5 mmol/day (100-300 mg/day).<sup>12</sup>

#### PERFORMANCE DATA

The following data was obtained with the Calcium Reagent on an automated clinical chemistry analyser. Users should establish product performance on the specific analyser used.

#### IMPRECISION

Imprecision was evaluated using two levels of commercial controls and following the NCCLS EP5-T procedure.<sup>13</sup>

#### SERUM

| Within Run            | LEVEL I                  | LEVEL II                 |
|-----------------------|--------------------------|--------------------------|
| Number of data points | 80                       | 80                       |
| Mean                  | 1.45 mmol/L (5.8 mg/dL)  | 2.85 mmol/L (11.4 mg/dL) |
| SD                    | 0.03 mmol/L (0.11 mg/dL) | 0.04 mmol/L (0.16 mg/dL) |
| CV (%)                | 1.8                      | 1.4                      |

| Total                 | LEVEL I                 | LEVEL II                 |
|-----------------------|-------------------------|--------------------------|
| Number of data points | 80                      | 80                       |
| Mean                  | 1.45 mmol/L (5.8 mg/dL) | 2.85 mmol/L (11.4 mg/dL) |
| SD                    | 0.05 mmol/L (0.2 mg/dL) | 0.07 mmol/L (0.28 mg/dL) |
| CV (%)                | 3.5                     | 2.4                      |

The following precision data was obtained for urine samples:

#### URINE

| Within Run            | LEVEL I                  | LEVEL II                 |
|-----------------------|--------------------------|--------------------------|
| Number of data points | 20                       | 20                       |
| Mean                  | 1.01 mmol/L (4.0 mg/dL)  | 3.84 mmol/L (15.4 mg/dL) |
| SD                    | 0.01 mmol/L (0.04 mg/dL) | 0.07mmol/L (0.28 mg/dL)  |
| CV (%)                | 1.5                      | 1.7                      |

| Between Run           | LEVEL I                  | LEVEL II                 |
|-----------------------|--------------------------|--------------------------|
| Number of data points | 16                       | 16                       |
| Mean                  | 1.10 mmol/L(4.4 mg/dL)   | 3.84 mmol/L (15.4 mg/dL) |
| SD                    | 0.04 mmol/L (0.16 mg/dL) | 0.10 mmol/L (0.40 mg/dL) |
| CV (%)                | 3.2                      | 2.6                      |

#### ACCURACY

Comparison studies were done using another commercially available colourimetric calcium reagent. Normal and abnormal patient serum, plasma and urine samples were assayed in parallel. The results were compared by least squares regression and the following statistics were obtained:

|                           | Plasma/Serum | Urine     |
|---------------------------|--------------|-----------|
| Number of Pairs           | 70           | 15        |
| Range of Results (mmol/L) | 1.20-4.08    | 0.86-7.92 |
| Range of Results (mg/L)   | 4.8-16.3     | 3.4-31.7  |
| Reference Method (mmol/L) | 2.45         | 3.52      |
| Reference Method (mg/dL)  | 9.8          | 14.1      |
| Calcium Results (mmol/L)  | 2.47         | 3.53      |
| Calcium Results (mg/dL)   | 9.9          | 14.1      |
| Slope                     | 0.99         | 1.02      |
| Intercept                 | 0.05         | -0.05     |
| Correlation Coefficient   | 0.99         | 0.99      |

#### LINEARITY

When run as recommended the assay is linear to 4.0 mmol/L (16.0 mg/dL).

#### SENSITIVITY

When run as recommended the sensitivity of the assay is 0.26 ΔA per mmol/L (0.065 ΔA per mg/dL).

#### REFERENCES

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Fisher Diagnostics  
a division of Fisher Scientific Company, LLC  
a part of Thermo Fisher Scientific Inc.  
Middletown, VA 22645-1905 USA  
Phone: 800-528-0494  
540-869-3200  
Fax: 540-869-8132

840298 (R1)

REF

#### Reorder Information

| Catalogue No. | Configuration |
|---------------|---------------|
| TR29221       | 2 x 125 mL    |
| TR29226       | 2 x 250 mL    |
| TR29298       | 2 x 500 mL    |