

# Infinity™






## Calcium Arsenazo Liquid Stable Reagent

### PRODUCT SUMMARY

|                     |   |                                       |
|---------------------|---|---------------------------------------|
| Stability           | : | Until Expiry at 2-8°C                 |
| Linear Range        | : | 1.50 - 3.75 mmol/L (6.0 - 15.0 mg/dL) |
| Specimen Type       | : | Serum                                 |
| Method              | : | Endpoint                              |
| Reagent Preparation | : | Supplied ready to use.                |

**IVD**

### SYMBOLS IN PRODUCT LABELLING

|   |                              |   |  |
|---|------------------------------|---|--|
| <b>EC</b> <b>REP</b>  | Authorized Representative    |  | Temperature Limitation                 |
| <b>IVD</b>  | For in vitro diagnostic use  |  | Use by/Expiration Date                 |
| <b>LOT</b>  | Batch code/Lot number        |  | CAUTION. CONSULT INSTRUCTIONS FOR USE. |
| <b>REF</b>  | Catalogue number             |  | Manufactured by                        |
|  | Consult instructions for use |   |  |

#### INTENDED USE

This reagent is intended for the in vitro quantitative determination of calcium in human serum.

#### CLINICAL SIGNIFICANCE

Calcium fulfils a variety of roles in human physiology. In bone, it combines with phosphorus to form hydroxyapatite crystals, giving strength to the bone structure and providing a constant reservoir of calcium for the rest of the body. It is also important in blood coagulation, muscle contraction, and membrane permeability.<sup>1</sup>

Low serum calcium values (hypocalcemia) can be seen in cases of osteomalacia, hypomagnesemia, vitamin D deficiency, hypoparathyroidism, steatorrhea, pregnancy and lactation, nephrosis, nephritis, and hepatocellular or renal parenchymal disease. Elevated serum calcium levels can be seen in hyperparathyroidism, hypervitaminosis D, bone neoplasms, milk-alkali syndrome, sarcoidosis, thyrotoxicosis, multiple myeloma, and polycythemia vera.<sup>1</sup>

#### METHODOLOGY 2,3

A large array of methods is available for the determination of calcium. These include oxalate precipitation, EDTA chelation, flame photometry, atomic absorption, and specific dye binding.

Arsenazo III reacts with calcium to form a bluish-purple colored complex. The amount of color formed is measured by an increase in absorbance of the reaction mixture at 600 - 660 nm.

#### REAGENT COMPOSITION

| Active Ingredient | Concentration |
|-------------------|---------------|
| Arsenazo III      | 0.136 mmol/L  |
| Sodium azide      | 0.05%         |
| Buffer            |               |
| Surfactant        |               |

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity Calcium Arsenazo Liquid Stable Reagent Material Safety Data Sheet.

#### REAGENT PREPARATION

Reagent is ready to use as supplied. Mix the reagent well before using.

#### STABILITY AND STORAGE

##### Prior to use:

When stored at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

##### Once the Reagent is Opened:

When stored capped at 2-8°C, the reagent is stable until expiry.

#### Indications of Reagent Deterioration:

- Calcium Reagent should be a bluish-purple solution.
- Turbidity;
- Reagent Absorbance > 0.800 AU (650 nm, 1 cm light path); and/or
- Failure to obtain control values within the assigned range.

#### SPECIMEN COLLECTION AND HANDLING

##### Collection:

- Specimen collection and storage tubes must be free of calcium.<sup>4</sup>
- Tourniquet use should be avoided or kept to a minimum in collecting blood samples for calcium analysis. Fist clenching should be avoided.<sup>4</sup>
- Prolonged contact of the serum with red blood cells should be avoided.<sup>4</sup>
- It is recommended that specimens be collected and processed as described in NCCLS<sup>5</sup> (H3, H4, H18) or equivalent publications.

**Serum:** Use non-haemolysed serum.<sup>4</sup>

**Storage:** Total calcium is generally considered to be stable in serum for days at 4°C and for months when frozen; evaporation or lyophilization must be avoided.<sup>4</sup>

#### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 600 and 660 nm.
- Analyser specific consumables, eg: sample cups.
- If required, pipettes for accurately dispensing measured volumes.
- 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                 |
| Primary Wavelength           | 600 - 660 nm         |
| Secondary Wavelength         | 700 nm               |
| Assay Type                   | Endpoint             |
| Direction                    | Increase             |
| Sample : Reagent Ratio       | 1:60 – 1:70          |
| eg: Sample Vol               | 5 µL (5 µL)          |
| Reagent Vol                  | 300 µL (350 µL)      |
| Incubation Time              | 60 seconds           |
| Reagent Blank Limits         | Low 0.00 AU          |
| (650 nm, 1 cm lightpath)     | High 0.80 AU         |
| Linearity                    | 1.50 – 3.75 mmol/L   |
| (refer to linearity section) | (6.0 – 15.0 mg/dL)   |
| Analytical Sensitivity       | 0.190 ΔA per mmol/L  |
| (650 nm, 1 cm lightpath)     | (0.047 ΔA 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 | = 0.61                     |
| Absorbance of Unknown    | = 0.54                     |
| Value of Calibrator      | = 3.20 mmol/L (12.8 mg/dL) |

$$\text{Calcium} = \frac{0.54}{0.61} \times 3.20 = 2.83 \text{ mmol/L}$$

$$\text{Calcium} = \frac{0.54}{0.61} \times 12.8 = 11.33 \text{ mg/dL}$$

#### CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with an 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 above the upper limit or below the lower limit of the established ranges 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 are still out of control, contact Technical Services or your local distributor.

## LIMITATIONS

1. It is recommended that disposable plastic labware be used for this procedure. If glassware is used, it must be acid washed.
2. It is recommended that if gloves are worn when performing this procedure, powderless gloves be used.
3. 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 600 mg/dL.  
**Bilirubin:** No interference from Bilirubin up to 479 µmol/L (28 mg/dL).  
**Lipaemia:** No interference from lipaemia up to 365 mg/dL at a calcium level of 7.6 mg/dL, and up to 660 mg/dL at a calcium level of 10.8 mg/dL.
4. Young DS<sup>9</sup> has published a comprehensive list of drugs and substances which may interfere with this assay.

## EXPECTED VALUES

Adult 2.15 - 2.55 mmol/L (8.6 - 10.2 mg/dL)<sup>7</sup>

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population it serves.<sup>8</sup>

## PERFORMANCE DATA

The following data was obtained using a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser.

## PRECISION

Within-run reproducibility was obtained by assaying 3 levels of control sera 20 times.

| WITHIN-RUN               | MEAN        | STD. DEV.    | CV%  |
|--------------------------|-------------|--------------|------|
| Level 1 (mmol/L / mg/dL) | 1.35 / 5.4  | 0.005 / 0.02 | 0.37 |
| Level 2 (mmol/L / mg/dL) | 2.35 / 9.4  | 0.008 / 0.03 | 0.32 |
| Level 3 (mmol/L / mg/dL) | 3.38 / 13.5 | 0.010 / 0.04 | 0.30 |

Run-to-run reproducibility was obtained by assaying 3 levels of control sera for 10 runs.

| RUN-TO-RUN               | MEAN        | STD. DEV.    | CV%  |
|--------------------------|-------------|--------------|------|
| Level 1 (mmol/L / mg/dL) | 1.35 / 5.4  | 0.10 / 0.04  | 0.74 |
| Level 2 (mmol/L / mg/dL) | 2.38 / 9.5  | 0.015 / 0.06 | 0.63 |
| Level 3 (mmol/L / mg/dL) | 3.38 / 13.5 | 0.023 / 0.09 | 0.67 |

## METHOD COMPARISON

Comparison studies were carried out using another commercially available method as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:-

|                         |                                       |
|-------------------------|---------------------------------------|
| Number of samples       | 153                                   |
| Range of results        | 1.78 – 3.40 mmol/L (7.1 – 13.6 mg/dL) |
| Slope                   | 0.927                                 |
| Intercept               | 0.222                                 |
| Correlation Coefficient | 0.996                                 |

## LINEARITY

When run as recommended, the assay is linear between 1.50 and 3.75 mmol/L (6.0 and 15 mg/dL).

## ANALYTICAL SENSITIVITY

When run as recommended, the sensitivity of the assay is 0.190 ΔA per mmol/L or 0.047 ΔA per mg/dL (1cm light path, 650 nm).

## REFERENCES

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5. NCCLS: Standard Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3), Standard Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture (H4), Standard Procedures for Blood Specimen Processing (H18), National Committee for Clinical Laboratory Standards, Villanova, PA.
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7. Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (4th Ed.) Burtis, Ashwood & Bruns (Eds), Elsevier Saunders, 2005; 2258.
8. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.



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REF

## Reorder Information

| Catalogue No. | Configuration         |
|---------------|-----------------------|
| 1265-250      | 2 x 125 mL            |
| 1265-500      | 2 x 250 mL            |
| 1265-400H     | 4 x 100 mL (Hitachi)  |
| TL29401       | 4 x 100 mL (iLab 600) |
| TY29401       | 4 x 50 mL (Hitachi)   |