CALCIUM AZ III REAGENT SET
For the quantitative determination of total calcium concentration in serum.

INTENDED USE
This reagent is intended for the quantitative determination of total calcium concentration in serum.

CLINICAL SIGNIFICANCE (8)
The normal calcium concentration of serum is maintained by hormones in the parathyroid gland. Decreased levels occur in hypoparathyroidism, vitamin D deficiency, rickets, osteomalacia and renal tubular acidosis. Increased levels are found in hyperparathyroidism, vitamin D intoxication, and are associated with neo-plasms, especially those of bone. A significant fraction of serum calcium is bound to protein. Hyperproteinemia is associated with an increased level of serum calcium and hypoproteinemia is associated with a decreased level of serum calcium.

METHOD AND HISTORY (1, 3)
Historically, serum calcium has been determined by a variety of procedures including flame photometry, atomic absorption spectrophotometry and recently specific dye binding such as o-cresolphthalain complexone. Accurate and precise measurement of calcium in biological fluids has traditionally been difficult. The introduction of Arsenazo III by Michaylova and Ilkova1 provided a highly sensitive and very specific reagent for calcium determination. The King method is based on this dye-binding method.

TEST PRINCIPLE (2, 3)
This method for the determination of calcium presented here uses Arsenazo III (3,6-bis((2-Arylsulphonyl) Azo) -4, 5-dihydroxy-2, 7naphthalenedisulfonic acid)i CAS registry number: 1668-00-4. Arsenazo III is chemically stable and has a very high affinity for calcium in an acid pH range. In this assay system, the Arsenazo III complex with an absorbance maximum at 650nm. The concentration of calcium is proportional to the absorbance of the violet colored Arsenazo III: calcium complex. The color produced by this complex is stable for at least 8 hours at room temperature (18°-26°C).

SPECIMEN COLLECTION AND HANDLING
No special patient preparation is required.

SPECIMEN COLLECTION.
- a. Fresh, clear, unhemolysed serum is the preferred specimen.
- b. Heparinized plasma may also be used.
- c. Plasma prepared using EDTA, oxalate, citrate, which function by removal of calcium, obviously must not be used.
- d. It is recommended that testing be done as soon as possible after sample collection and preparation.
- e. If testing cannot occur immediately, the specimen sample can be stored refrigerated (2°-8°C) for up to 7 days.

MATERIALS
Reagents necessary for the determination of calcium are included in the kit.

REAGENT
Calcium reagent contains: Arsenazo III 0.16 mmol/L, Buffer Solution 91 mmol/L, Preservative and Stabilizer.

WARNINGS AND PRECAUTIONS
- a. For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.
- b. DO NOT pipette by mouth. DO NOT ingest.
- c. Organic arsenic compounds have been classified as potentially carcinogenic; therefore, safe laboratory practices should be carefully observed.
- d. Components of the reagent may be irritating to the skin and mucous membranes; avoid contact.
- e. If contact occurs, wash with copious amounts of water.

REAGENT PREPARATON
The reagent is ready to use as is. If the absorbance of the reagent alone (without sample added) in a 1cm cuvette is greater than 0.600 when measured against distilled water at 650 nm, do not use the reagent.

REAGENT STORAGE AND STABILITY
The reagent is stable at room temperature (18°-26°C) until the expiration date on the label.

ADDITIONAL MATERIALS REQUIRED
1. Spectrophotometer or colorimeter capable of reading absorbance at 650nm.
2. 1 cm cuvettes or a flow cell capable of transmitting light at 650nm.
3. Test tubes capable of holding 2 ml.
4. Pipettes capable of delivering 1 ml and 25 ml.
5. Timer for 1 minute incubation.
6. Distilled or deionized water.
7. Normal and abnormal control for quality control.

PROCEDURE (AUTOMATED)
Refer to specific instrument application instructions.

PROCEDURE (MANUAL)
1. Into separate calcium free test tubes pipette 25 ml of distilled water, calibrator, control, and serum to be assayed.
2. Add 1 ml of calcium reagent and mix, and incubate for 1 minute at room temperature.
3. Read and record the absorbance of the calibrator (C) and of each serum (S) at 650 nm using the distilled water sample as the reagent blank.
4. To calculate results see "Calculation".

PROCEDURE NOTE
a. The color is stable for 8 hours.

b. A major source of difficulty with the assay of calcium is contamination of glassware employed in the performance of the test. Many detergents and water supplies contain calcium and improperly rinsed containers used in this test will lead to inaccurate results.

- c. The calcium assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

- d. Calibration is required with the use of a new lot of reagent, any expiration date on the label.

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CALCULATION AND RESULTS
Absorbance of sample X Concentration of Std. = Calcium (mg/dl)
Absorbance of standard

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

0.81 X 10 = 10.1mg/dl
0.80

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

EXPECTED VALUES (5, 6)
The range of expected values is: 8.5 to 10.5 mg/dl (2.1 to 2.6 mmol/L) These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

MEDICAL ALERT VALUES (9)
Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician.

If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE
Any substance which either chelates calcium or contains calcium will interfere with the assay. Magnesium does not interfere in this assay system. The affinity of Arsenazo III for magnesium is essentially zero at the pH at which the assay is performed. When magnesium concentrations in incremental amounts of 1.2, 4 and 6 mg/dL (magnesium expected range: 1.8 to 2.9 mg/dL) were added to 12 serum samples with calcium values ranging from 7.2 to 9.8mg/dL, there was no increase in the calcium values. There was also no increase due to magnesium in the calcium value (9.8 ± 0.9 mg/dl) in 12 assays of a control serum to which magnesium was added in concentrations ranging from 0.5 to 15mg/dL. Young et al. have published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that for serum calcium. Interference from Ipernia is minimized because of the small amount of sample used.

QUALITY CONTROL
Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

PERFORMANCE CHARACTERISTICS
PRECISION
The estimates of precision shown below were obtained from assays of human control serum.

Within-Run
In this study, 10 replicates of 3 control sera were run.

Between-Run
In this study, 5 runs were made, each run consisting of 5 replicates of 3 control sera.

CORRELATION
A correlation study was done comparing this method (y) with a calcium-o-cresolphthalain method as the reference method (x). 76 samples were run with a range between 6.2 mg/dl to 18.6 mg/dl. A second correlation study was done comparing this method (y) with an atomic absorption method as the reference method (x2). 20 samples were run with a range between 6.2 mg/dl to 19 mg/dl. The results are summarized below.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Regression Equation</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>y = 0.993 x + 0.060</td>
<td>0.994</td>
</tr>
<tr>
<td>20</td>
<td>y = 0.90 x + 0.364</td>
<td>0.997</td>
</tr>
</tbody>
</table>

LINEARITY
The assay is linear to 15 mg/dl calcium. Samples with calcium...
concentrations exceeding 15 mg/dl should be diluted with an equal volume of distilled or deionized water and the assay repeated. Multiply results by 2.

SENSITIVITY
Using a 1:40 sample to reagent ratio and reading at 650nm, a 1 mg/dl calcium sample will produce a net absorbance of approximately 0.051.

REFERENCES
5. Todd, Sanford and Davidson, Clinical Diagnosis and Management by Laboratory Methods, Edited by Henry, J.B., W.B. Saunders Company, Philadelphia, 1979.
8. Todd, Sanford and Davidson, Clinical Diagnosis and Management by Laboratory Methods, Edited by Henry, J.B., W.B. Saunders Company, Philadelphia, 1969, p.575.

WARRANTY
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Protocol Parameters for ATAC 6000

Mode : ENDPOINT.
TYPE : Liquid /S
Filter 1 : 630 nm
Filter 2 : 0
FLOWCELL TEMP. : 37
MAXIMUM NORM. : 10.5
MINIMUM NORM. : 9.5
UNIT : mg/dl
STANDARD : 9.1
FACTOR : 1
NO. OF WASHES : 2
NO of Bead Washes : 0
KIN. READ TIME : 0
GRAPH PRINTOUT : N
TEST LIMIT : 20
MAX. ABS. DELTA : 0

SAMPLER
REAGENT A ul : 500
REAGENT B ul : 0
REAGENT C ul : --
SAMP. VOLUME : 10
INCB. REAG-A : 120
INCB. REAG-B : 0
INCB. REAG-C : --
STABILITY TIME : 20
WASH SOLUTION : Water
REACTION DIR. : Inc.
Dynamic Zero : --

Protocol Parameters for the BT Series

Test methodology : CPC
Method : End Point
Kind of Process : Linear
Filters : 578 / 700
Reaction Direction : Increasing
Sample Starter : Inactive
Delay Time (Sec) : 0
Incubation Time : 0/180
Reading Time (Sec) : 10

Dynamic Blank : Inactive
Reagent Blank : Every Run
Reagent Limit (mABS) : 800
Curve Acceptance (%) : 100
Reagent #1 : 200 ul
Reagent #2 : 200 ul
Number of needle washes : 1/2
Number of cuvette washes : 1
Sample Name : Serum Urine
Sample µl : 8 5
Pre-Dilution : 1.00 3.00
Unit : mg/dl mg/dl

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