Clinical Chemistry

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REAGENT 90 mL

IVD CE 2797

HORIBA ABX SAS
Parc Euromédecine
Rue du Caducée
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FRANCE

ABX Pentra Calcium AS CP

- **Pentra C200**

Diagnostic reagent for quantitative *in vitro* determination of Calcium in serum, plasma and urine by colorimetry.

Application Release

Serum, plasma: Ca_AS

01.xx

Urine: Ca_AS

01.xx

Intended Use [a] [b]

ABX Pentra Calcium AS CP reagent is intended for the quantitative *in vitro* diagnostic determination of calcium in human serum, plasma and urine based on a colorimetric method.
Clinical laboratories use.
Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases and chronic renal disease and tetany (intermittent muscular contractions or spasms).
Measurement of physiological and pathological variations of Calcium in human serum, plasma and urine is useful for screening or follow-up of these diseases and also in the assessment of electrolyte homeostasis and the acid-base balance of the body.

Clinical Interest (1, 2, 3)

Calcium plays an essential role in many cell functions: intracellularly in muscle contraction and glycogen metabolism, extracellularly, in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium is present in plasma in three forms: free, bound to proteins or complexed with anions as phosphate, citrate and bicarbonate. Under physiological conditions, calcium balance is determined by the relationship between calcium intake and calcium absorption and excretion. Urinary excretion is an important determinant of calcium retention in the body. Decreased total calcium levels can be associated with diseases of the bone apparatus (especially osteoporosis), kidney diseases (especially under dialysis), defective intestinal absorption and hypoparathyroidism. Increased total calcium can be measured in hyperparathyroidism, malignant diseases with metastases and sarcoidosis. Calcium measurements also help in monitoring of calcium supplementation mainly in the prevention of osteoporosis.

Method (4, 5, 6, 7)

Many colourimetric methods for determining calcium have been used in the past. Connerty and Briggs described methods using alizarin 3-sulphonate (4) and cresolphthalein complexone (5) whilst Gindler and King have described a method using thymol blue (6). There have been many subsequent modifications to these methods. The method used here is based on the metallochromogen Arsenazo III. Calcium ions (Ca^{2+}) react with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenearsonic acid) at pH 6.75 to form an intense purple coloured chromophore. The absorbance of the Ca-Arsenazo III complex is measured bichromaticcally at 660/700 nm.
The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample. Arsenazo III has a high affinity ($K° = 1 \times 10^{-7}$) for calcium ions (7) and shows no interference from other cations normally present in serum, plasma or urine.

[a]Modification: modification of CE mark.
[b]Modification: new leaflet form.

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Ca^{++} + Arsenazo III $\xrightarrow{\text{pH 6.50}}$ Ca-Arsenazo III complex (purple)

Reagents

ABX Pentra Calcium AS CP is ready-to-use.

Reagent:

MES pH6.50	100 mmol/L
Arsenazo III	200 µmol/L

ABX Pentra Calcium AS CP should be used according to this notice. The manufacturer cannot guarantee its performance if used otherwise.

Handling

1. Remove the cap of the cassette.
2. If present, remove foam by using a plastic pipette.
3. Place the cassette into the refrigerated reagent compartment.

Calibrator

For calibration, use:
ABX Pentra Multical (A11A01652) (not included)
10 x 3 mL (lyophilisate)

Control

For internal quality control, use:

- **ABX Pentra N MultiControl** (1300054414) (not included)
  10 x 5 mL (lyophilisate)
- **ABX Pentra P MultiControl** (1300054415) (not included)
  10 x 5 mL (lyophilisate)
- **Yumizen C Urine Level 1 Control** (1300023946) (not included)
  6 x 5 mL
- **Yumizen C Urine Level 2 Control** (1300023947) (not included)
  6 x 5 mL

Each control should be assayed daily and/or after a calibration.
The frequency of controls and the confidence intervals should correspond to laboratory guidelines and country-specific directives. You should follow federal, state and local guidelines for testing quality control materials. The results must be within the range of the defined confidence limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.

Materials Required but not Provided

- Automated clinical chemistry analyzer: Pentra C200
- Calibrator: **ABX Pentra Multical** (A11A01652)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
  Yumizen C Urine Level 1 Control (1300023946)
  Yumizen C Urine Level 2 Control (1300023947)
- Standard laboratory equipment.

Specimen (8)

This device intended testing population is general population.

Specimen types

- Serum.
- Plasma in lithium heparin.
- Urine.

Do not use EDTA plasma: EDTA anticoagulant is unsuitable for analysis because this compound chelates calcium, making it unavailable for reaction with the reagent.
Anticoagulants other than those listed have not been tested by HORIBA and are therefore not recommended for use with this assay.
24 hours urine specimens have to be collected with HCl 6N (9). Non acidified urines which have been refrigerated should be acidified and/or heated at 56°C for 15 minutes to redissolve any precipitate.

Stability (8)

Serum, plasma

- At 20-25°C: 7 days
- At 4-8°C: 3 weeks
- At -20°C: 8 months

Urine

- At 20-25°C: 2 days
- At 4-8°C: 4 days
- At -20°C: 3 weeks

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Reference Range (2)

Each laboratory should establish its own reference ranges. The values given here are used as guidelines only.

Serum, plasma

2.15 - 2.57 mmol/L (8.6 - 10.3 mg/dL)

***Urine* (10)**

Women: < 6.24 mmol/24h (< 250 mg/24h)

Men: < 7.49 mmol/24h (< 300 mg/24h)

Clinical sensitivity and specificity, positive predictive value and negative predictive value are not commonly reported for this analyte. This is largely attributed to the fact that this analyte is not sole indicator for the intended purpose and patient treatment decision making. To arrive at a diagnosis and a course of treatment, results from others routine clinical chemistry tests should be used in conjunction with other diagnostic information and the attending health-care professional's evaluation of the patient's condition.

Storage and Stability

Stability before opening:

Stable up to the expiry date on the label if stored at 2-8°C. Store protected from light.

Stability after opening:

Refer to the paragraph "Performance on Pentra C200".

Waste Management

Please refer to local legal requirements.

General Precautions

- This reagent is for professional *in vitro* diagnostic use only.
  For laboratory use.
- For prescription use only.
- This reagent is classified as non-hazardous in compliance with regulation (EC) N°.1272/2008.
- As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.
- Observe the standard laboratory precautions for use.
- The reagent cassettes are disposable and should be disposed of in accordance with the local legal requirements.
- Please refer to the SDS associated with the reagent.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.
- Do not use the product if the recommended storage conditions, including temperature, are not followed.
- User must be trained by a HORIBA representative before attempting to operate the device.
- It is the user's responsibility to verify that this document is applicable to the reagent used.
- For technical assistance, you can call +33 (0)4 67 14 15 16.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.

Performance on Pentra C200

Lot to Lot Variability

The recovery of samples (serum and plasma) done during QC release of three consecutive lots of reagent shows that the lot to lot variability is within specification.

Serum, plasma

The performance data listed below have been obtained on the Pentra C200 analyzer.

Number of tests: approximately 265 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 70 days.

Sample volume: 4.8 µL/test

Detection Limit

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (11) and equals 0.002 mmol/L (0.010 mg/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (11) and equals 0.35 mmol/L (1.40 mg/dL).

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Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (12) with samples tested 20 times:

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	2.26	9.06	1.18
Control specimen 2	3.21	12.85	1.11
Specimen 1	1.68	6.75	0.89
Specimen 2	2.30	9.24	0.79
Specimen 3	3.37	13.50	0.95

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (13) with samples tested in duplicate for 20 days (2 series per day):

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	2.18	8.73	1.3
Control specimen 2	3.27	13.10	1.3
Specimen 1	1.74	6.98	1.3
Specimen 2	2.37	9.52	1.4
Specimen 3	3.25	13.03	1.3

Measuring Range

The assay confirmed a measuring range from 0.35 mmol/L (1.40 mg/dL) to 4.50 mmol/L (18.05 mg/dL).
The measuring range is extended up to 13.50 mmol/L (54.15 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 4.5 mmol/L (18.05 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (14).

Correlation

Patient samples: Serum
Number of patient samples: 183
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (15).
Values ranged from 0.47 mmol/L (1.88 mg/dL) to 4.03 mmol/L (16.16 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (16) is:
Y = 1.011 X - 0.0275 (mmol/L)
Y = 1.011 X - 0.1092 (mg/dL)
with a correlation coefficient $r^2 = 0.992$.

Interferences

Haemoglobin: No significant influence is observed up to 290 µmol/L (500 mg/dL).
Triglycerides: No significant influence is observed up to a triglyceride concentration of 4.56 mmol/L (399 mg/dL).
Total Bilirubin: No significant influence is observed up to 788 µmol/L (46.1 mg/dL).
Direct Bilirubin: No significant influence is observed up to 445 µmol/L (26 mg/dL).
Ascorbic Acid: No significant influence is observed up to 3.4 mmol/L (60 mg/dL).
Magnesium: No significant influence is observed up to 4.40 mmol/L (11.2 mg/dL).
Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (17, 18).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 21 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Conversion Factor

mmol/L x 4.01 = mg/dL

Urine

The performance data listed below have been obtained on the Pentra C200 analyzer.

Number of tests: approximately 265 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 70 days.

Sample volume: 4.8 µL/test

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Detection Limit

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (11) and equals 0.002 mmol/L (0.010 mg/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (11) and equals 0.45 mmol/L (1.80 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (12) with samples tested 20 times:

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	1.73	6.94	1.12
Control specimen 2	2.57	10.32	1.05
Specimen 1	1.83	7.34	0.89
Specimen 2	2.45	9.84	0.97
Specimen 3	3.33	13.36	1.62

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (13) with samples tested in duplicate for 20 days (2 series per day):

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	1.79	7.17	2.2
Control specimen 2	2.60	10.41	2.0
Specimen 1	1.88	7.55	2.0
Specimen 2	2.50	10.03	2.0
Specimen 3	3.49	13.99	1.8

Measuring Range

The assay confirmed a measuring range from 0.45 mmol/L (1.80 mg/dL) to 4.50 mmol/L (18.05 mg/dL).

The measuring range is extended up to 13.50 mmol/L (54.15 mg/dL) with the automatic post-dilution.
The reagent linearity has been assessed up to 4.50 mmol/L (18.05 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (14).

Correlation

Patient samples: urine
Number of patient samples: 141
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (15).
Values ranged from 0.45 mmol/L (1.80 mg/dL) to 4.29 mmol/L (17.20 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (16) is:
Y = 0.9786 X - 0.0314 (mmol/L)
Y = 0.9786 X - 0.1258 (mg/dL)
with a correlation coefficient r^2 = 0.994.

Interferences

Haemoglobin:	No significant influence is observed up to 290 µmol/L (500 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 3.59 mmol/L (314 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 418 µmol/L (24.5 mg/dL).
Ascorbic Acid:	No significant influence is observed up to 3.40 mmol/L (60 mg/dL).
Magnesium:	No significant influence is observed up to 4.95 mmol/L (12.0 mg/dL).
pH:	The urine should not be alkalinized.

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (17, 18).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 21 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Conversion Factor:

mmol/L x 4.01 = mg/dL

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Reference

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