

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k123171

B. Purpose for Submission:

New device

C. Measurand:

Calcium

D. Type of Test:

Quantitative, colorimetric method

E. Applicant:

HORIBA ABX S.A.S

F. Proprietary and Established Names:

1. ABX Pentra Calcium AS CP
2. ABX Pentra Multical
3. ABX Pentra N Control
4. ABX Pentra P Control
5. ABX Pentra Urine Control L/H

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CJY	II	§862.1145 calcium test system	75-Chemistry
JIX	II	§862.1150 calibrator	75-Chemistry
JJY	I, reserved	§862.1660 quality control material (assayed)	75-Chemistry

H. Intended Use:

1. Intended use(s):

See Indications for use in (2) below.

2. Indication(s) for use:

ABX Pentra Calcium AS CP reagent, with associated calibrator and controls, is a diagnostic reagent for quantitative in vitro determination of calcium in human serum, plasma and urine based on a colourimetric method, using the ABX Pentra 400 Clinical Chemistry analyzer. Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).

The ABX PENTRA Multical is a calibrator for use in the calibration of quantitative Horiba Medical methods on Horiba Medical clinical chemistry analyzers.

The ABX PENTRA N Control is for use in quality control by monitoring accuracy and precision of Horiba Medical methods on Horiba Medical clinical chemistry analyzers.

The ABX PENTRA P Control is for use in quality control by monitoring accuracy and precision of Horiba Medical methods on Horiba Medical clinical chemistry analyzers.

The ABX PENTRA Urine Control L/H is for use in quality control by monitoring accuracy and precision of Horiba Medical methods on Horiba Medical clinical chemistry analyzers.

3. Special conditions for use statement(s):

The device is for prescription use.

4. Special instrument requirements:

For use with ABX Pentra 400 Clinical Chemistry Analyzer

I. Device Description:

The **ABX Pentra Calcium AS CP** is an in vitro diagnostic assay for the quantitative in vitro determination of calcium in human serum, plasma and urine based on colorimetric method. It is composed of a mono-reagent cassette (79 mL). The reagent is a chemical solution with additives, including 100 mmol/L of MES pH 6.50, and 200 µmol/L of Arsenazo III.

The **ABX Pentra Multical** is a single level, lyophilized human serum calibrator with chemical additives and materials of biological origin (extracts from human or animal derived tissue). The assigned values of the calibrator components are given in the

product labeling, ensuring optimal calibration of the appropriate HORIBA ABX SAS methods on the ABX Pentra 400 analyzer. This calibrator is provided in ten vials of lyophilized material sufficient to produce 3 ml when re-suspended in distilled or deionized water.

The **ABX Pentra N Control** and **ABX Pentra P Control** are quality control products consisting of lyophilized human serum with chemical additives and materials of biological origin (extracts from human or animal derived tissue) added as required to obtain given component levels. The assigned values of the control components are given in the product labeling, and reflect normal (N Control) and pathological (P control) calcium levels, ensuring control of the appropriate HORIBA ABX SAS methods on the ABX Pentra 400 analyzer. Each control is provided in ten vials of lyophilized material sufficient to produce 5 ml when re-suspended in distilled or deionized water.

The **ABX Pentra Urine Control L/H** is a two-level (Low and High) quality control consisting of liquid solutions prepared from human urine with chemical additives and materials of biological origin (human and animal origin) added as required to obtain given component levels, including preservatives and stabilizers. The assigned values of the control components are given in the enclosed annex, ensuring control of the appropriate HORIBA ABX SAS methods on the ABX Pentra 400 analyzer. Each control level is provided in one vial of 10 ml, liquid, ready to use.

The sponsor stated in the labeling that each donor unit used in the preparation of the calibrator and controls were tested by FDA accepted methods and found non-reactive for Hepatitis B Surface Antigen (HbsAg), antibodies to Hepatitis C (HCV) and antibodies to HIV-1/HIV-2.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) numbers:

Beckman Olympus Calcium Arsenazo reagent: k061575

Horiba ABX Pentra MULTICAL: k052007

Horiba ABX Pentra N and P Controls: k052007

Horiba ABX Pentra Urine Controls L/H: k070249

2. Device comparison with predicate:

1. Similarities and Differences for ABX Pentra Calcium AS CP		
Item	Candidate Device: ABX Pentra Calcium AS CP (Candidate Device)	Predicate Device: Olympus Calcium Arsenazo Reagent (k061575)
Intended Use/Indications for Use	Same	Reagent for the quantitative determination of calcium in human serum, plasma and urine on automated clinical chemistry analyzers. Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).
Specimen Type	Same	Serum, Plasma, and Urine
Instrument	ABX Pentra 400	Beckman Coulter Clinical Chemistry Analyzers
Method	Same	Colorimetric, quantitative
Expected values	Serum/Plasma: 8.6 -10.3 mg/dL Urine-Male: <300 mg/24 hr Urine-Female: <250 mg/24 hr	Serum/Plasma (Adults): 8.8 -10.6 mg/dL Serum (Children 0 – 10 day): 7.6 – 10.4 mg/dL Serum (Children 2 – 12 year): 8.8 – 10.8 mg/dL Urine-Male: <300 mg/24 hr Urine-Female: <250 mg/24 hr
Measuring range	Serum/Plasma: 4 – 18 mg/dL Urine: 0.64 – 18 mg/dL	Serum/Plasma: 4 - 18 mg/dL Urine: 0.1 - 40 mg/dL
Reagent Configuration	Same	Single part liquid
Reagent Stability	Unopened: 2-8°C until expiration date On board stability: 60 days	Unopened: 2-8°C until expiration date On board stability: 90 days

2. Similarities and Differences for ABX Pentra Multical		
Item	ABX Pentra Multical (Candidate Device)	ABX Pentra Multical (k052007)
Intended Use	Same	The ABX Pentra Multical is a calibrator for use in the calibration of specified quantitative Horiba ABX methods on Horiba ABX clinical chemistry analyzers.
Constituents	Same, with addition of Calcium	Multi-analyte calibrator
Matrix	Same	Lyophilized human serum with chemical additives

3. Similarities and Differences for ABX Pentra N and P Controls		
Item	ABX Pentra N and P Controls (Candidate Device)	ABX Pentra N and P Controls (k052007)
Intended Use	Same	For use in quality control by monitoring accuracy and precision.
Constituents	Same, with addition of Calcium	Multi-analyte control material
Matrix	Same	Lyophilized human serum with chemical additives

4. Comparison of new device (ABX Pentra Urine Controls L/H) with the predicate device (ABX Pentra Urine Controls L/H previously cleared in k070249):

Similarities and Differences for ABX Pentra L/H Urine Controls		
Item	ABX Pentra L/H Urine Controls (Candidate Device)	ABX Pentra L/H Urine Controls (k070249)
Intended Use	Same	For use in quality control by monitoring accuracy and precision.
Constituents	Same, with addition of Calcium	Multi-analyte control material
Matrix	Same	Human urine with chemical additives

K. Standard/Guidance Document Referenced (if applicable):

CLSI, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline –Second Edition (EP05-A2)

CLSI, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision) (EP09-A2-IR)

CLSI, Protocols for Determination of Limits of Detection and Limits of Quantitation (EP17-A)

CLSI, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A)

L. Test Principle:

Calcium ions (Ca²⁺) react with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenearsonic acid) to form an intense purple colored complex. The absorbance of the Ca-Arsenazo III complex is measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision studies for serum samples were conducted on the ABX Pentra 400 following CLSI guidance document EP05-A2. Two levels of control serum and 3 serum-based samples were run 2 times per run, 2 runs per day, for a total of 20 days (n = 80 measurements/sample level). Results are summarized below for within-run and total precision.

	Mean (mg/dL)	Within Run		Total	
		Std Dev	%CV	Std Dev	%CV
N Control	8.72	0.04	0.44	0.13	1.44
P Control	13.0	0.06	0.46	0.19	1.49
Sample 1	7.03	0.03	0.38	0.11	1.56
Sample 2	9.47	0.03	0.31	0.15	1.54
Sample 3	12.9	0.04	0.30	0.20	1.54

Precision studies for urine samples were conducted on the ABX Pentra 400 following CLSI guidance document EP05-A2. Two levels of urine controls

(Level L and Level H) and 3 urine samples were run 2 times per run, 2 runs per day, for a total of 20 days (n = 80 measurements/sample level). Results are summarized below for within-run and total precision.

	Mean (mg/dL)	Within Run		Total	
		Std Dev	%CV	Std Dev	%CV
Level L	7.28	0.07	0.93	0.11	1.45
Level H	10.7	0.07	0.64	0.16	1.50
Sample 1	7.70	0.03	0.40	0.12	1.57
Sample 2	10.3	0.04	0.43	0.16	1.57
Sample 3	14.2	0.05	0.35	0.22	1.56

b. Linearity/assay reportable range:

A serum sample linearity study was performed according to CLSI guidance document EP6-A recommendations. A series of 10 samples was prepared by combining various volumetric proportions of a high concentration calcium human serum pool with a low concentration calcium sample to produce samples with calcium values ranging from 3.39 to 21.0 mg/dL. Each level was measured in quadruplicate using 1 instrument and 1 lot of reagent. Linear regression analysis of a plot of expected values and experimentally determined values produced a curve fit with the following equation: $y = 1.01x - 0.24$, $R^2 = 0.9994$.

A urine sample linearity study was performed according to CLSI guidance document EP6-A recommendations. A series of 12 samples was prepared by combining various volumetric proportions of a high concentration urine pool with saline to produce samples with calcium values ranging from 0.33 to 18.9 mg/dL. Each level was measured in quadruplicate using 1 instrument and 1 lot of reagent. Linear regression analysis of a plot of expected values and experimentally determined values produced a curve fit with the following equation: $y = 0.933x - 0.345$, $R^2 = 0.9996$.

The linearity studies provided by the sponsor support reportable range claims for the ABX Pentra Calcium AS CP device of 4 - 18.05 mg/dL calcium for serum samples, and 0.64 - 18.05 mg/dL calcium for urine samples.

Automatic Dilution

The Pentra 400 performs automatic dilutions (1:3) on calcium samples if the value is > 18.05 mg/dL. A study was performed comparing manual dilutions to the Pentra 400 automated dilutions of 7 pooled serum samples and 7 pooled urine samples spiked with calcium. Samples ranged from 18.4 mg/dL to 54.15 mg/dL, and were measured in duplicate. Manual and automated dilution

factors were the same and each sample was run in duplicate. All results are within +/- 10% recovery. These studies support the sponsor's claim that urine and serum/plasma samples with calcium concentrations up to 54.15 mg/dL can be run on the Pentra 400 system.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The calibrators and controls used in this submission are previously cleared in k052007 for ABX Pentra Multical, ABX Pentra N Control and ABX Pentra P Control and in k070249 for ABX Pentra Urine Control L/H. The calcium material introduced into the calibrators and its corresponding value is traceable to NIST reference material SRM909b. In this submission, calcium values for this assay are being added to the existing calibrator and control materials.

Stability:

Real-time testing was conducted. The stability study protocol and the acceptance criteria have been reviewed and found to be acceptable. The study results support the following stability claims in the labeling:

Item	Storage Conditions	Claimed Stability	
Calibrators	Close-Vial 2-8°C	24 months	
	Open-Vial	-25 °C to -15°C	2 weeks
		2-8°C	2 days
		15°C to 25°C	8 hours
Serum controls	Close-Vial 2-8°C	30 months	
	Open-Vial	-25 °C to -15°C	1 month
		2-8°C	5 days
		15°C to 25°C	12 hours
Urine controls	Close-Vial 2-8°C	2 years	
	Open-Vial 2-8°C	30 days	

Value assignment:

Controls: The target value is determined by the median of 150 results from 6 ABX Pentra 400 analyzers. The lot specific control range is calculated as the target value ± 3 SD. The following is an example of the nominal calcium values which are expected for the ABX Pentra 400 analyzer:

N Control: 8.50 mg/dL

P Control: 13.4 mg/dL
Urine control L: 71.4 mg/dL
Urine control H: 99.4 mg/dL

Calibrator: The target value is determined by the median of 150 results from 6 ABX PENTRA 400 analyzers. The nominal calcium value expected for the ABX Pentra 400 analyzer is 10.55 mg/dL.

Calibration Interval:

ABX Pentra Calcium AS CP assay calibration stability was assessed, and the sponsor claims that calibration is stable for 10 days. The protocol and acceptance criteria have been provided and found to be adequate to support the sponsor's claims. The sponsor also recommends using quality control samples at least once a day, after each calibration.

d. Detection limit:

The sponsor performed a detection limit study for the ABX Pentra Calcium AS CP assay on the ABX Pentra 400 instrument system, following CLSI guidance document EP17-A.

Limit of Blank (LoB) was determined by assaying 30 replicate measurements of a blank sample, performed on each of 3 ABX Pentra 400 instruments over 2 days (N = 180). The LoB was calculated as 0.20 mg/dL.

Limit of Detection (LoD) was determined in serum and urine by assaying 20 replicate measurements of 4 serum and 4 urine samples, respectively, with concentrations ranging between LoB and LoB x 4. The LoDs for serum and urine were calculated as 0.28 mg/dL and 0.23 mg/dL, respectively.

Limit of Quantitation (LoQ) was determined by assaying 10 replicate measurements of 10 low calcium concentration samples for both serum and urine, over 3 days. The LoQ was based on inter-assay precision less than 10% CV. The LoQ in serum and urine were determined as 1.54 mg/dL and 0.64 mg/dL, respectively.

The ABX Pentra Calcium assay has a measuring range of 4 - 18.05 mg/dL for serum sample, and 0.64 - 18.05 mg/dL for urine sample.

e. Analytical specificity:

Interference studies were performed to determine the effects from potential interferents on the ABX PENTRA Calcium AS CP assay. Various concentrations of interferents were spiked into pooled human serum or pooled human urine containing calcium at low (~8.5 mg/dL) and elevated (~15

mg/dL) concentrations. All samples were tested in quadruplicate. Non-significant interference was defined by the sponsor as the highest interferent level tested with bias $\leq 5\%$ of the control for serum samples or $\leq 10\%$ of the control for urine samples. Below is a summary table of the highest concentration of interferent tested and found not to interfere based on the stated acceptance criteria.

Test Interferent	Concentration	
	Serum	Urine
Magnesium	10.7 mg/dL	13.0 mg/dL
Ascorbic Acid (L)	60.0 mg/dL	60.0 mg/dL
Total Bilirubin	46.1 mg/dL	N/A
Direct Bilirubin	26.0 mg/dL	25.3 mg/dL
Intralipid (Turbidity)	500 mg/dL	613 mg/dL
Hemoglobin	500 mg/dL	500 mg/dL
Ibuprofen	50.1 mg/dL	N/A
Acetaminophen	20.0 mg/dL	N/A
Acetylsalicylic acid	65.2 mg/dL	N/A

In addition, the effect of acidification or alkalinization of urine samples on calcium concentration was evaluated. Two urine samples with different calcium concentrations were either acidified (to pH 1.7) or alkalinized (to pH 11.5), and were run in triplicate. In comparison to untreated urine samples no interference was observed for acidification, while significant interference was observed for urine samples with pH greater than pH 7.0.

Since alkaline pH affects the urine calcium results, the sponsor includes the following limitation in their labeling:

“24 hours urine specimens have to be collected with HCl 6N. Non acidified urines which have been refrigerated should be acidified and/or heated at 56°C for 15 minutes to re-dissolve any precipitate.”

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

Serum:

A method comparison study was performed to assess the performance of the ABX Pentra Calcium AS CP assay with the Olympus Calcium Arsenazo Reagent (predicate device) following CLSI guidance document EP9-A2. 145

human serum samples, 16 of which were altered, were assayed using the subject and predicate methods. Samples were measured in singlicate and ranged in calcium concentration from 4.17 to 15.9 mg/dL. Linear regression analysis, resulting from a plot of calcium concentrations obtained on the subject (Y-axis) and predicate (X-axis) devices, resulted in the following line fit equation: $y = 1.007x + 0.028$, $r^2 = 0.9902$.

Urine:

A method comparison study was performed to assess the performance of the ABX Pentra Calcium AS CP assay with the Olympus calcium arsenazo reagent (predicate device), following CLSI guidance document EP9-A2. 143 human urine samples using the subject and predicate methods were analyzed. Samples were measured in singlicate and ranged in calcium concentration from 0.64 to 17.8 mg/dL. Linear regression analysis, resulting from a plot of calcium concentrations obtained on the subject (Y-axis) and predicate (X-axis) devices, resulted in the following line fit equation: $y = 0.977x + 0.097$, $r^2 = 0.9930$.

b. Matrix comparison:

The sponsor performed a matrix comparison study to compare the performance between serum and lithium-heparin plasma samples. The study included 32 paired serum and plasma samples (Lithium-Heparin Plasma) ranging in calcium concentration from 4.0 mg/dL to 17.5 mg/dL; 3 samples were altered. Each sample was tested in singlicate using the ABX Pentra Calcium AS CP assay reagent. Linear regression analysis of the plot of serum (x-axis) and plasma (y-axis) samples showed the following relationships between the matrix types:

$$y = 1.006x - 0.0022, r^2 = 0.996$$

The sponsor concluded that Li-Heparin plasma is an acceptable anti-coagulant to be used with the ABX Pentra Calcium AS CP assay.

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

b. Clinical specificity:

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Reference ranges are provided in the labeling from published literature as follows (Endres D.B., Rude R.K. Mineral and bone metabolism. In: Burtis C.A., Ashwood E.R., editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 1395-1457):

Serum plasma: 8.6 – 10.3 mg/dL

Urine-male: <250 mg/24 h

Urine-Female: <300 mg/24 h

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.