

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

**A. 510(k) Number:**

k043125

**B. Purpose for Submission:**

New device

**C. Measurand:**

Aminoterminal propeptide of Type 1 procollagen

**D. Type of Test:**

Radioimmunoassay

**E. Applicant:**

Orion Diagnostica

**F. Proprietary and Established Names:**

Orion Diagnostica UniQ™ PINP RIA

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.1050

2. Classification:

Class II

3. Product code:

CIN

4. Panel:

75

**H. Intended Use:**

1. Intended use(s):

See Indications for use section below.

2. Indication(s) for use:

Orion Diagnostica UniQ PINP RIA is a quantitative radioimmunoassay designed for the measurement of intact aminoterminal propeptide of type 1 procollagen, an indicator of osteoblastic activity, in human serum. The test is intended to be used as an aid in the management of postmenopausal osteoporosis. For in vitro diagnostic use.

3. Special conditions for use statement(s):  
For Prescription Use Only
4. Special instrument requirements:  
N/A

**I. Device Description:**

The UniQ PINP RIA kit is a dual-antibody competitive radioimmunoassay that uses  $^{125}\text{I}$  labeled PINP, PINP RIA rabbit antiserum, seven calibrators, two lyophilized controls and a procollagen separation reagent. The calibrators and controls are provided in the UniQ PINP RIA kit and are not available separately. The controls used in the PINP calibrators and controls were tested and found negative for hepatitis B surface antigen, for anti-HIV 1 and 2 antibody and anti-HCV antibodies.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Tandem-R Ostase Immunoradiometric Assay
2. Predicate 510(k) number(s):  
k961573
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	Orion Diagnostica UniQ PINP RIA is a quantitative radioimmunoassay designed for the measurement of intact aminoterminal propeptide of type 1 procollagen, an indicator of osteoblastic activity, in human serum. The test is intended to be used as an aid in the management of postmenopausal osteoporosis. For in vitro diagnostic use.	The Tandem-R Ostase immunoradiometric assay is an invitro device indicated for the quantitative measurement of skeletal alkaline phosphatase (sALP), an indicator of osteoblastic activity, in human serum. This device is intended to be used as a aid in the management of postmenopausal osteoporosis and Paget's disease.
Matrix	Serum	Serum

Differences		
Item	Device	Predicate
Analyte	Intact Aminoterminal Propeptide of type 1 procollagen	Skeletal Alkaline Phosphatase

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

ECCLS Document Vol 3, No. 3: Guidelines for a User Laboratory, Evaluate and Select a Kit for its own use.

NCCLS EP5-A: Evaluation of Precision Performance of Clinical Chemistry devices.

NCCLS EP10-A: Preliminary Evaluation of Quantitative Clinical Laboratory Methods.

NCCLS EP7-P: Interference Testing in Clinical Chemistry.

NCCLS EP6-P: Evaluation of the Linearity of Quantitative Analytical Methods.

**L. Test Principle:**

The UniQ PINP RIA (PINP) is based on the competitive radioimmunoassay technique. A known amount of labeled PINP and an unknown amount of unlabelled PINP in the sample compete for a limited number of high affinity binding sites of the polyclonal rabbit anti-PINP antibody. A second antibody, directed against rabbit IgG and coated to kaolin particles, is used to separate the antibody-bound PINP from free PINP. The radioactivity of the bound tracer antigen is measured using a gamma counter. The amount of labeled PINP in the sample tube is inversely proportional to the amount of PINP in the sampler. The concentrations in unknown samples are obtained from a calibration curve, which is based on the concurrent testing of the UNIQ PINP RIA calibrators that range between 1 and 250 µg/L PINP.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was assessed and calculated according to NCCLS EP5-A. An intra-assay study was conducted by measuring 20 replicates of two sample pools and one control. An inter-assay study was conducted by measuring two sample pools and one control in duplicates with 10 separate assays. The results are summarized in the chart below.

Intra-assay precision of 20 replicates			Inter-assay precision of 10 duplicate determinations		
Sample	Mean (µg/L)	CV (%)	Sample	Mean (µg/L)	CV (%)
1	5	19.8	6	6	10.1
2	12	9.8	7	12	9.8
3	32	6.5	8	32	8.3
4	51	6.5	9	52	7.0
5	173	10.2	10	167	6.0

A recovery study was conducted by adding known amounts of purified PINP antigen to three sample pools and measured with the UniQ PINP RIA assay. The samples were measured in duplicate and the recovery % was calculated. The recovery % equals the increase in concentration divided by the spike concentration multiplied by 100.

Sample 1 (initial concentration of 16.5 µg/L)

Spike (µg/L)	Measured PINP (µg/L)	Increase (µg/L)	Recovery %
-----	16.5		
38.8	50.0	33.4	86
63.9	84.0	67.5	106
95.1	106.4	89.9	95
Mean % recovery			95

Sample 2 (Initial Concentration of 24.5 µg/L)

Spike (µg/L)	Measured PINP (µg/L)	Increase (µg/L)	Recovery %
-----	24.5		
38.8	62.1	37.6	97
63.9	82.9	58.4	91
95.1	103.2	78.7	83
Mean % recovery			90

Sample 3 (Initial Concentration of 35.0 µg/L)

Spike (µg/L)	Measured PINP (µg/L)	Increase (µg/L)	Recovery %
-----	35.0		
38.8	67.1	32.2	83
63.9	88.4	53.4	84
95.1	125.5	90.5	95
Mean % recovery			87

A dilution study was conducted to compare the two possible sample diluents, the zero calibrator or 0.9% NaCl on two controls and one sample pool. The diluted and undiluted samples were measured with the UniQ PINP RIA assay in duplicates and % recovery was calculated from the diluted samples against the undiluted samples. The results supported that samples can be diluted with either the zero calibrator or 0.9% NaCl.

*b. Linearity/assay reportable range:*

Linearity studies were conducted according to NCCLS EP6-P. Linearity studies were conducted on five samples (mini-pools) and two controls. The concentrations of the diluted samples were calculated based on the dilution factors and compared to the result obtained directly from the undiluted sample. The samples were diluted with the 0 µg/L calibrator.

The percent recovery is calculated for each dilution using the equation:

$$\% \text{ Recovery} = \frac{\text{Calculated for undiluted}}{\text{Measured concentration from undiluted sample}} \times 100$$

The calculated for undiluted was obtained using the following equation:

$$\text{Calculated for undiluted} = \frac{\text{Measured concentration from diluted sample}}{\text{Dilution (in \% volume of the original sample)}} \times 100$$

The results are listed in the table below.

Sample	Mean Concentration (µg/L)	Mean % Recovery	SD	%CV
1	74.7	96	5.5	7.4
2	137.2	110	4.3	3.1
3	156.5	102	8.3	5.3
4	163.9	111	9.5	5.8
5	172.9	106	6.5	3.8
6	187.9	112	11.0	5.8
7	240.9	101	20.9	8.7

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* PINP antigen (that is used in controls and calibrators) has been purified from human ascites fluids and characterized by electrophoresis for purity. The value assignment of the first purified antigen stock was done by quantitative analysis of amino acids, after acid hydrolysis of the polypeptide. New preparations of the antigen preparations are conducted by testing the dilutions points are within the calibrator curve of PINP-RIA. The values were calculated as the mean of at least two dilution points. The antigen preparation was found to be negative for hepatitis B surface antigen, for anti-HIV 1 and 2 antibodies and anti-HCV antibodies.

The in-house master calibrators (working calibrators) were prepared gravimetrically using the first calibrator antigen batch to obtain the 6 calibrator concentrations and are stored at -70 °C. PINP working calibrators are in-house master calibrators used to calibrator each lot of manufacturer's products calibrator set, which is produced by diluting a purified PINP-preparation with a high PINP concentration in PINP calibrator buffer (calibrator 0). Product calibrators are stored at -20 °C. Each new PINP product calibrator batch is tested against PINP working calibrators and the previous batch of PINP product calibrators in at least 10 acceptable tests. With each set of the calibrators, the calibrators curve is created and other calibrators are measured as samples. The acceptance criteria are that the deviation of the concentrations between the new product calibrators and

working calibrators is equal or smaller than 8% and that that deviation of the cpm-values between the new product calibrators is equal or smaller than 3%. The validity of the results of each reagent lot is tested using controls (hi and low) and in-house patient sample pool and the measured in four replicates. The controls are lyophilized and stored at 2-8 °C and preserved with sodium azide. The in-house sample pool is stored at -20 °C. The target values for new batches of PINP controls are determined by performing at least twenty tests on both the new and present batch of controls in duplicates. The target values are determined by calculating the means of the results. The stability protocol for the calibrators and controls includes four test points. First test is performed at 0 time point, two tests between the 0 point and the expiry date (e.g., 8 and 16 months) and the last test is performed one month after the expiry date (25 months). Additionally, the reconstituted controls are tested after 7 weeks of storage at +2-+8 °C at each testing point. Bioburden of the calibrators and controls as well as the residual moisture of the lyophilized controls was determined.

The UniQ PINP RIA controls are stable for 24 months in lyophilized state and for 6 weeks after reconstitution (+2 to +8 °C). The UniQ PINP RIA calibrators are stable for 24 months.

*d. Detection limit:*

The detection limit of the UniQ PINP RIA assay was assessed according to the ECCLS Document Vol 3. The detection limit was determined by measuring 20 replicates of the 0 calibrator and the calibration curve four times. The mean of the 4 results was used to establish the detection limit. The minimum detectable concentration of PINP in the assay was approximately 2 µg/l, defined as twice the standard deviation of the 0-binding value. The results are listed in the table below.

Calibrator	Test 1	Test 2	Test 3	Test 4
Mean (AU)	17879	18384	17657	17647
SD	199	204	303	384
CV%	1.1	2.1	1.9	2.8
N=	20	20	20	20
Mean CPM-2xSD	17480	17977	17052	16879
Sensitivity	1.2	2.1	1.9	2.8
Mean of 4 test	2.0 µg/L			

*e. Analytical specificity:*

The effects of hemoglobin, bilirubin and triglycerides were studied. The tested components were added to three sample pools with suitable solvents and samples were assayed in duplicates with the UniQ PINP RIA. The results for all three samples are in the charts below and it was determined that hemoglobin, bilirubin and triglycerides don't interfere with the tested concentrations.

<b>Hemoglobin Effect</b>							
		PINP (µg/L) n=2			% PINP		
Added	Neat	+125 mg/dL	+250 mg/dL	+500 mg/dL	+125 mg/dL	+250 mg/dL	+500 mg/dL
Sample 1	40.4	36.8	36.5	37.1	91	90	92
Sample 2	42.9	40.0	46.1	42.2	93	107	98
Sample 3	50.6	48.5	45.4	44.7	96	90	88
<b>Average % Recovery</b>					<b>93</b>	<b>96</b>	<b>93</b>
<b>Bilirubin Effect</b>							
		PINP (µg/L) n=2			% PINP		
Added	Neat	+5.8 mg/dL	+11.7 mg/dL	+23.4 mg/dL	+5.8 mg/dL	+11.7 mg/dL	+23.4 mg/dL
Sample 1	38.3	36.6	37.7	38.8	95	98	101
Sample 2	38.8	37.7	37.4	37.8	97	96	97
Sample 3	45.6	46.5	45.2	46.3	102	99	102
<b>Average % Recovery</b>					<b>102</b>	<b>99</b>	<b>102</b>
<b>Triglyceride Effect</b>							
		PINP (µg/L) n=2			% PINP		
Added	Neat	+1000 mg/dL	+2000 mg/dL	+3000 mg/dL	+1000 mg/dL	+2000 mg/dL	+3000 mg/dL
Sample 1	34.1	33.1	35.2	31.2	97	103	92
Sample 2	36.6	37.4	36.7	31.7	102	100	87
Sample 3	39.6	39.3	42.9	40.7	99	108	103
<b>Average % Recovery</b>					<b>99</b>	<b>108</b>	<b>103</b>

A cross-reactivity study was conducted of the PINP antiserum towards PIIINP (type III collagen N-terminal propeptide). PIIINP was added to the normal PINP RIA assay reaction to investigate whether if any cross reactivity would occur and would be seen as a reduction in CPM values. The inhibition curve of the PIIINP antigen was compared to PINP inhibition curve. The results showed no cross-reactivity to PIIINP.

A second cross-reactivity study was conducted on the PINP antiserum towards N-terminal Col1-fragment of type I procollagen (Col1), which is a breakdown product of PINP. Col1 was added to the normal PINP RIA assay reaction to investigate any occurring cross reactivity that could be seen as a reduction in CPM values. The inhibition curve of Col1 was compared to the inhibition curve of PINP. The results showed that the cross-reactivity towards Col1 is approximately 1.2%.

*f. Assay cut-off:*

The measurement range of the assay is 5-250 µg/L.

2. Comparison studies:

*a. Method comparison with predicate device:*

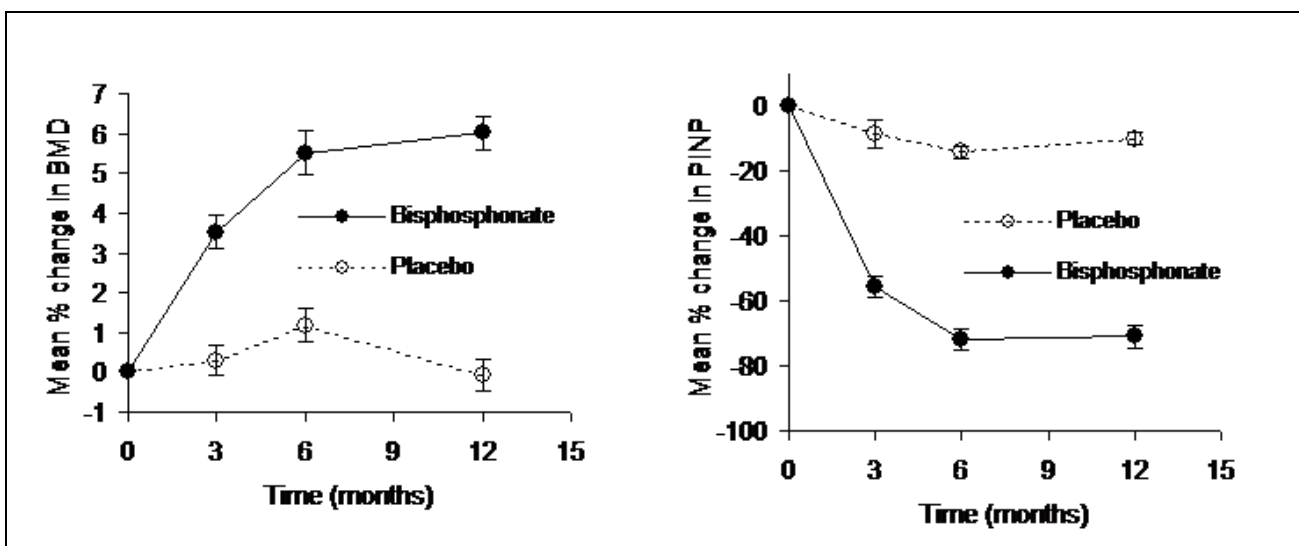
Comparison was conducted with the Beckman Coulter Tandem-R Osteonorm Immunochemical assay (BAP). Since the two assays analyze two different analytes, the predicate and the device are able to be compared through the percent change of bone mineral density (BMD). In order to enable the comparability of the BAP and PINP assays, percent change data are calculated at each time point (3,6 and 12 months) from each individual baseline value. The sponsor states that for comparison purposes, a decrease equal to or greater than 25% is used as a cut-off between biochemical responders and non-responders.

Retrospective analysis of samples from two studies were conducted by the sponsor. Study #1 was a Phase III Osteoporosis treatment study that used bisphosphonate alendronate to treat postmenopausal women with diagnosed osteoporosis. Study #2 analyzed a long term study of hormone replacement therapy in the prevention of postmenopausal bone loss in healthy women. Both studies compare menopausal status, age, lumbar spine bone mineral density (BMD), PINP and BAP measurements. Normality of the data is studied by histogram analysis and by using Shapiro-WILK test. Differences between the means are compared with paired samples t-test (between different time points) or with independent samples t-test (between study groups). For receiver operator characteristics (ROC) analysis, cut-off in BMD for biochemical responder was set to greater than or less than 2% increase in the result. Similarly, for PINP and BAP the cut-off for biochemical responder was set to greater than or less than 25% decrease.

Study #1 retrospectively analyzed a subset of samples from a placebo controlled Phase III Osteoporosis Treatment study. This study was a multi-site, prospective, double blind placebo controlled study in postmenopausal women of the US population with defined osteoporosis, at least 5 years since menopause. A subset of sera has been used to compare BAP to BMD at several sites. Based on a subset of the same serum samples, the sponsor study now compares the response of PINP and BAP to BMD at selected time points within and across the treatment group (TRM) and placebo control group (CTR). Percent change from 0 value at 3, 6, and 12 month time points were calculated and used for analyses. The sponsor stated that the sample size required confirming equivalence of BAP and PINP changes at confidence level of 0.05 and power of 0.80 is a minimum of 36 samples at each time point. 62 patients (248 serum samples) from TRM group and 62 patients (248 serum samples) from CTR group meet this requirement and gave a total of 124 patients and 496 serum samples. Results of this study are shown the chart below for BAP, PINP and BMD in the form of decreases or increases from the baseline values at time 3, 6 and 12 months. The result showed a similar decrease for BAP and PINP while the BMD values show an increase. The results are listed in the chart and figure below.



<b>BAP</b>	<b>3 Months</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↓ 31.8 +/- 29.7%	↓ 45.9 +/-16.2%	↓ 43.4 +/- 19.9%
<b>CTR Group</b>	↓8.0 +/- 15.9%	↓ 10.5 +/- 17.0%	↓ 5.5 +/-20.8%
<b>PINP</b>	<b>3 Months</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↓ 55.4 +/- 34.0%	↓ 71.6 +/- 15.6%	↓ 70.8 +/- 15.5%
<b>CTR Group</b>	↓ 8.6 +/- 25.9%	↓ 14.0 +/- 25.5%	↓ 9.8 +/- 27.4%
<b>BMD</b>	<b>3 Months</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↑ 3.5 +/- 3.2%	↑ 5.1 +/- 4.4%	↑ 6.0 +/- 3.3%
<b>CTR Group</b>	↑ 0.3 +/- 3.1%	↑ 1.2 +/- 3.3 %	↑ 0.1 +/- 3.2%

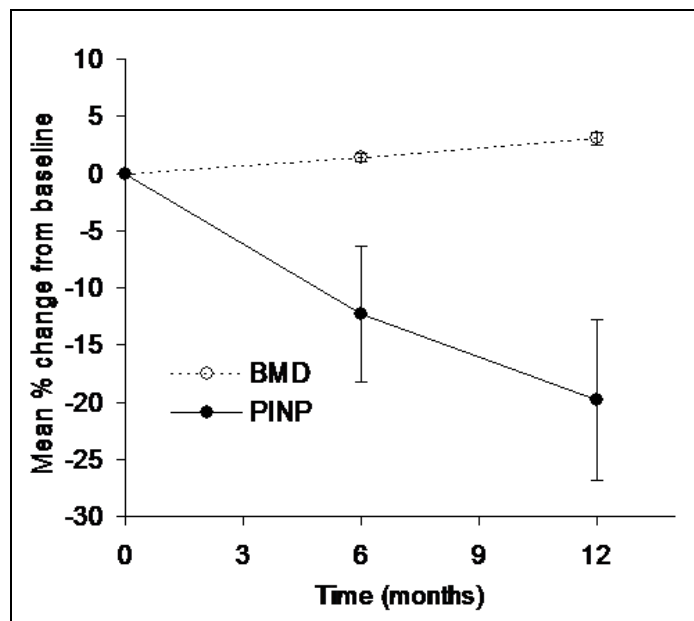


Response as mean percent change ( $\pm$  SE bars) in (A) BMD and (B) PINP concentration in patients receiving bisphosphonate (●) or placebo (○).

Study #2 retrospectively analyzed samples from a postmenopausal osteoporosis hormone replacement therapy. The study analyzed the response of bone to HRT and was followed throughout the study by measuring BMD in the white postmenopausal women from the Finnish population with at least 3 years since the last spontaneous bleeding, aged 45 to 65 years (average age 55.9 years) and with a body mass index less than 30. Retrospectively, PINP and BAP as an aid in monitoring HRT were compared by the sponsor. Based on a subset of the same serum samples, the sponsor study now compares the response of PINP and BAP to BMD at selected time points within and across the treatment group (TRM). Percent change from the baseline (value 0) at 6 and 12 month time points were calculated and used for analyses. The sponsor stated that the sample size required confirming equivalence of BAP and PINP changes at confidence level of 0.05 and power of 0.80 is a minimum of 36 samples at each time point. BAP and PINP were analyzed blind by trained laboratory personnel at Orion Diagnostica by using the Tandem-R Ostase IRMA and the UniQ PINP RIA kits. Only patient samples that obtained the

lowest dose of the estradiol valerate (the treatment used in the study) were used, leaving a total of 44 patients and 132 serum samples. Since this was a dose finding study, placebo controls were not used. Results of this study are shown the chart below for BAP, PINP and BMD in the form of decreases or increases from the baseline values at times 6 and 12 months. The result showed a similar decrease for BAP and PINP while the BMD values show an increase. The results are listed in the chart and figure below.

<b>BAP</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↓ 11.5 +/- 29.4%	↓ 19.1 +/- 29.0%
<b>PINP</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↓ 12.3 +/- 39.6%	↓ 19.8 +/- 46.5%
<b>BMD</b>	<b>6 Months</b>	<b>12 Months</b>
<b>TRM Group</b>	↑ 1.4 +/- 2.3%	↑ 3.1 +/- 3.8%



Response as mean percent change ( $\pm$  SE bars) in BMD ( $\circ$ ) and PINP concentration ( $\bullet$ ) in postmenopausal women receiving low dose combined hormone replacement therapy.

*b. Matrix comparison:*

N/A. This assay is only used with serum.

3. Clinical studies:

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

In order to determine the reference range, serum samples from healthy adults we collected and measured with the UniQ PINP RIA. The mean PINP concentrations for Finnish males, females and postmenopausal females' reference range, .95 CI for upper and lower limits are shown in the following table.

Reference Limits	Male	Female	Female (postmenopausal for 3 years)**
Number of Subjects	323	210	383
Age Distribution	22-65	25-44	52-62
Mean	45 µg/L	42 µg/L	48 µg/L
Reference Interval*	22-87 µg/L	19-83 µg/L	16-96 µg/L
.95Confidence Interval			
Lower reference Limit	20 - 25 µg/L	14 - 23 µg/L	15 - 19 µg/L
Upper reference Limit	77 - 94 µg/L	71 - 96 µg/L	87 - 103 µg/L

\* Reference interval = 0.025 and 0.975 fractiles

\*\* Concentrations are elevated compared to younger women (P=0.0005).

**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.