

# METRA™ DPD EIA kit

96 Assays for Deoxypyridinoline Crosslinks

For *In Vitro* Diagnostic Use

Store at 2-8°C

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Made in USA

0263J (6/01)

Catalog number 8007

**QUIDEL®**

Read the entire product insert thoroughly before beginning the assay. The Metra™ DPD kit should be stored at 2-8°C until use. Do not subject the kit to freezing.

## QUICK GUIDE TO ASSAY STEPS

1. Add 50µL of 1:10 diluted Standards, Controls and samples
2. Add 100µL cold Enzyme Conjugate
3. Incubate 2 hours ± 5 minutes at 2-8°C in the dark
4. Wash 3 times with 1X Wash Buffer
5. Add 150 µL room temperature Working Substrate Solution
6. Incubate 60 ± 5 minutes at room temperature
7. Add 100µL Stop Solution and read OD at 405nm

## INTENDED USE

Metra™ DPD is a urinary assay that provides a quantitative measure of the excretion of deoxypyridinoline (Dpd) crosslinks as an indicator of bone resorption. Elevated levels of urinary Dpd indicate elevated bone resorption in individuals. Measurement of Dpd is intended for use as an aid in monitoring bone resorption changes in postmenopausal women receiving hormonal or bisphosphonate antiresorptive therapies and in individuals diagnosed with osteoporosis.

## SUMMARY AND EXPLANATION

Approximately 90% of the organic matrix of bone is type I collagen, a triple helical protein<sup>1</sup>. Type I collagen of bone is crosslinked by specific molecules which provide rigidity and strength. Crosslinks of mature type I collagen in bone are the pyridinium crosslinks, pyridinoline (Ppd) and deoxypyridinoline (Dpd)<sup>1,2</sup>. Dpd is formed by the enzymatic action of lysyl oxidase on the amino acid lysine<sup>3</sup>. Dpd is released into the circulation during the bone resorption process<sup>4,5</sup>. Dpd is excreted unmetabolized in urine and is unaffected by diet<sup>6</sup>, making it suitable for assessing resorption.

Bone is constantly undergoing a metabolic process called remodeling<sup>2,7</sup>. This includes a degradation process, bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoblasts<sup>2,7</sup>. Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is, resorption and formation are in balance<sup>7</sup>. In abnormal states of bone metabolism this process becomes uncoupled and, when resorption exceeds formation, this results in a net loss of bone<sup>7</sup>. The measurement of specific degradation products of bone matrix provide analytical data of the rate of bone metabolism<sup>2,4,5</sup>.

## SUMMARY AND EXPLANATION (CONT.)

Osteoporosis is a metabolic bone disease characterized by abnormal bone remodeling. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in susceptibility to fractures<sup>8</sup>. The most common type of osteoporosis occurs in postmenopausal women as a result of the estrogen deficiency produced by the cessation of ovarian function<sup>7</sup>. Restoration of premenopausal estrogen levels by replacement therapy prevents bone loss and osteoporosis<sup>7,10</sup>. Estrogens and a class of compounds known as bisphosphonates are antiresorptive therapies which can be used to prevent bone loss or treat osteoporosis<sup>7,12</sup>. Osteoporosis can also result from attaining an inadequate peak bone mass during the growing years, an age-related imbalance of bone remodeling with a net excess of resorption, and a number of clinical conditions and therapies which induce bone loss or bone remodeling imbalances<sup>7</sup>. These include endocrine diseases such as hypogonadism, hyperthyroidism, hyperparathyroidism, and hypercortisolism; gastrointestinal diseases related to nutrition and mineral metabolism; connective tissue diseases; multiple myeloma; chronic immobilization, alcoholism, or tobacco use; and chronic therapy with heparin or corticosteroids<sup>7</sup>. Other diseases characterized by abnormal bone remodeling include Paget's disease and cancers metastatic to bone<sup>8</sup>.

For the Metra DPD assay, antibody technology was employed to produce a monoclonal antibody that demonstrates specificity for Dpd<sup>13</sup>. The specificity of the monoclonal antibody used in the Metra DPD assay allows for simple, convenient, reproducible and direct quantitation of Dpd in urine.

## PRINCIPLE OF THE PROCEDURE

The Metra DPD assay is a competitive enzyme immunoassay in a microtiter stripwell format utilizing a monoclonal anti-Dpd antibody coated on the strip to capture Dpd. Dpd in the sample competes with conjugated Dpd-alkaline phosphatase for the antibody and the reaction is detected with a pNPP substrate. Metra DPD results are corrected for urinary concentration by creatinine.

## REAGENTS AND MATERIALS

Metra DPD EIA kit part number 8007 contains the following:

- |  |                                |                      |
|--|--------------------------------|----------------------|
| 1. <b>Substrate Tablets</b>  | <b>Part 0012</b>               | <b>3 each</b>        |
| p-Nitrophenyl phosphate (20 mg each).  |                                |                      |
| 2. <b>Enzyme Conjugate</b>   | <b>Part 4202</b>               | <b>3 each</b>        |
| Lyophilized Dpd, purified from bovine bone, conjugated to alkaline phosphatase containing buffer salts, and stabilizers.                                   |                                |                      |
| 3. <b>DPD Standards A - F</b>  | <b>Parts 4203 through 4208</b> | <b>0.3 mL each</b>   |
| <b>(0, 3, 10, 30, 100, 300 nmol/L DPD)</b><br>Dpd purified from bovine bone in 10mmol/L phosphoric acid containing sodium azide (0.05%) as a preservative. |                                |                      |
| 4. <b>Low/High Controls</b>  | <b>Parts 4209, 4210</b>        | <b>0.3 mL each</b>   |
| Dpd purified from bovine bone in 10mmol/L phosphoric acid containing sodium azide (0.05%) as a preservative.   |                                |                      |
| 5. <b>Coated Strips</b>  | <b>Part 4661</b>               | <b>12 each</b>       |
| Purified murine monoclonal Anti-Dpd antibody adsorbed onto stripwells.   |                                |                      |
| 6. <b>Stop Solution</b>  | <b>Part 4702</b>               | <b>15 mL</b>         |
| 1N NaOH  |                                |                      |
| 7. <b>10X Wash Buffer</b>  | <b>Part 4703</b>               | <b>55 mL</b>         |
| Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.   |                                |                      |
| 8. <b>Assay Buffer</b>   | <b>Part 4704</b>               | <b>55 mL</b>         |
| Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.   |                                |                      |
| 9. <b>Substrate Buffer</b>   | <b>Part 4705</b>               | <b>3 each, 10 mL</b> |
| A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative.  |                                |                      |

## WARNINGS

1. For *In Vitro* Diagnostic Use.
2. All urine samples should be treated as potentially biohazardous material.
3. 1N NaOH is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
4. Sodium azide is used as a preservative. It may be fatal if swallowed or absorbed through the skin. Do not mix with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
5. Standards and Controls are in 10mmol/L phosphoric acid. Avoid contact with skin, eyes or clothing. Do not ingest. If contact is made, wash with water. If ingested, call a physician.
6. Test kits and components should be disposed of in a manner consistent with relevant regulations.

## PRECAUTIONS

1. The Deoxy pyridinoline Standards, Controls and Enzyme Conjugate are light sensitive. Avoid prolonged exposure to light, especially direct or indirect sunlight. Store reagents in the dark when not in use. Samples and reagents are not significantly affected by normal, artificial laboratory lighting when handled as directed in the Assay Procedure.
2. All reagents supplied should be used as an integral unit prior to the expiration date indicated on the package label.
3. Assay reagents should be stored as indicated.
4. Do not use Coated Strips if pouch is punctured.
5. Samples greater than 300nmol/L should be further diluted in Assay Buffer and retested. Be sure to include the dilution factor in the final calculation.
6. Each sample should be tested in duplicate.
7. A standard curve must be performed with each assay.
8. A 4-parameter calibration curve fit must be used for accurate results. Equation:  $y = (A-D)/(1+(x/C)^B)+D$
9. This assay may be performed with any validated washing method.
10. The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Metra Biosystems, Inc. The OD values are provided and are to be used as a guideline only. The results obtained by your laboratory may differ.  
Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.
11. If room temperature cannot be maintained between 20-28°C and an absorbance of >2.0 is not compatible with your plate reader, monitor the development of substrate in the Standard A wells; stop the reaction when the OD reaches 1.2-1.5; then read the strips.
12. If the OD of the Dpd Standard A is less than 0.8, the results should be considered questionable and if possible, the samples should be repeated.
13. Use of multichannel pipets or repeat pipetors are recommended to ensure timely delivery of reagents.
14. For accurate measurement of samples, the addition of samples and standards must be precise. Pipet carefully using only calibrated equipment.

## REAGENT PREPARATION AND STORAGE

1. Wash Buffer - See Procedural Note in Assay Procedure Section  
Prepare required amount of 1X Wash Buffer (see table in Assay Procedure Section) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at room temperature (20-28°C). Use 1X Wash Buffer within 24 hours of preparation.  
**Special Washing Instructions:** Prepare 1X Wash Buffer as above and store at 2-8°C until use.
2. Enzyme Conjugate  
Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7mL of Assay Buffer. Store reconstituted Enzyme Conjugate at 2-8°C until use.
3. Working Substrate Solution  
The Substrate Buffer must be brought to room temperature (20-28°C) before beginning the assay. (Two hours to overnight recommended.) Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

## SPECIMEN COLLECTION AND STORAGE

The Metra DPD assay can be carried out using preservative free First Morning Void (FMV) or Second Morning Void (SMV) urine collections. It is recommended collections be made prior to 10:00am to obviate any potential influence of diurnal variation. Keep the urine sample refrigerated (2-8°C) for storage of less than 7 days, or freeze the sample at -20°C for longer storage. Do not subject sample to more than 5 freeze/thaw cycles. Avoid prolonged exposure to light, especially sunlight. During routine processing, samples are not affected by normal, artificial laboratory lighting.

When monitoring therapy, collect baseline samples prior to initiating treatment. For subsequent comparison(s), collect specimen(s) at the same time of day as the baseline specimen.

## METRA DPD ASSAY PROCEDURE

Kit Contents	Qty/Vol	Part
Substrate Tablets	3 each	0012
Tape Cover	3 each	0047
Enzyme Conjugate (lyophilized)	3 each	4202

## METRA DPD ASSAY PROCEDURE (CONT.)

Kit Contents (Cont.)	Qty/Vol	Part
Standard A (Dpd 0nmol/L)	0.3mL	4203
Standard B (Dpd 3nmol/L)	0.3mL	4204
Standard C (Dpd 10nmol/L)	0.3mL	4205
Standard D (Dpd 30nmol/L)	0.3mL	4206
Standard E (Dpd 100nmol/L)	0.3mL	4207
Standard F (Dpd 300nmol/L)	0.3mL	4208
Control, Low	0.3mL	4209
Control, High	0.3mL	4210
Coated Strips (12)	1 each	4661
Stop Solution	10mL	4702
10X Wash Buffer	55mL	4703
Assay Buffer	55mL	4704
Substrate Buffer (3)	10mL	4705

### Materials Required BUT NOT Provided

Micropipettes to deliver 50-300µL  
Items suitable for liquid measurement of 7-300mL  
Container for wash buffer dilution  
Tubes for dilution of samples, standards and controls  
Deionized or distilled water  
Plate reader capable of reading at 405nm  
4-parameter calibration curve fitting software  
Creatinine values (mmol/L) for urine samples

**PROCEDURAL NOTE:** The Metra DPD assay is sensitive to washing conditions. The **entire wash step** should be completed within 2 minutes. If the wash step **CANNOT** be completed within 2 minutes, follow the **Special Washing Instructions** located in the **Reagent Preparation and Substrate Incubation** sections.

Determine amount of each reagent required for the number of strips to be used.

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
Enzyme Conjugate (vial)	1	1	2*	2*
Substrate (bottle)	1	1	2*	2*
1X Wash Buffer (mL)	100	150	200	300

\*When more than one bottle or vial is to be used, combine the contents and mix prior to use

### Sample/Enzyme Conjugate Incubation

1. Dilute samples, Standards and Controls 1:10 with Assay Buffer (e.g. 50µL sample + 450µL Assay Buffer).
2. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table). Ensure that the pouch containing any unused strips is completely resealed.
3. Add 50µL diluted Standard, Control or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
4. Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7mL of Assay Buffer. Store reconstituted Enzyme Conjugate at 2-8°C until use.
5. Add 100µL of reconstituted Enzyme Conjugate to each well. Cover strips with Tape Cover provided. Incubate for 2 hours (±5 minutes) at 2-8°C. This incubation should be carried out in the dark.
6. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

### Substrate Incubation

1. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Manually invert/empty strips. Add at least 250µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat two more times for a total of three washes. Vigorously blot the strips dry on paper towels after the last wash. While strips are inverted, carefully wipe bottom of strips with a lint-free paper towel to ensure that the bottom of the strips are clean.  
**Special Washing Instructions:** Perform wash step as above, using cold (2-8°C) 1X Wash Buffer. After last wash, allow strips to drain for 5-10 minutes on paper towels before adding substrate.
2. Add 150µL of Working Substrate Solution to each well.
3. Incubate for 60 minutes (±5 minutes) at room temperature (20-28°C).

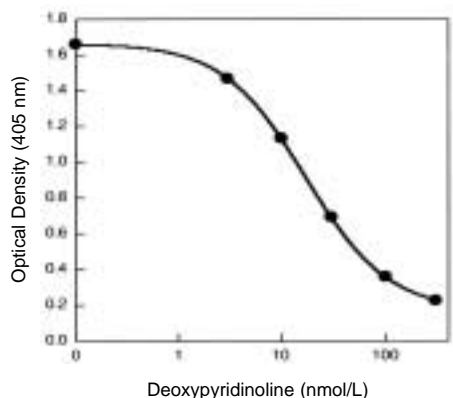
### Stop/Read

1. Add 100µL of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
2. Read the Optical Density (OD) at 405 nm. Assure that no large bubbles are present in the wells and that the bottom of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.
3. Quantitation software with a 4-parameter calibration curve fitting equation must be used to analyze the Metra DPD assay results.
4. Determine concentration of samples and Controls from the Standard curve.
5. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

## METRA DPD ASSAY PROCEDURE (CONT.)

### Representative Standard Curve

Standard Dpd levels: 0, 3, 10, 30, 100, 300 nmol/L



## INTERPRETATION OF RESULTS

Results obtained from the Metra DPD assay must be corrected for variations in urine concentration by dividing the Dpd value (nmol/L) by the creatinine value (mmol/L) of each sample (creatinine mg/dL x 0.088 = mmol/L). The final Metra DPD results will be expressed as nmol Dpd/mmol creatinine.

## LIMITATIONS

While Metra DPD assay is used as an indicator of bone resorption, use of this test has not been established to predict development of osteoporosis or future fracture risk. Use of this test has not been established in hyperparathyroidism or hyperthyroidism. When using Metra DPD to monitor therapy, results may be confounded in patients afflicted with clinical conditions known to affect bone resorption, e.g. bone metastases, in addition to diseases and conditions listed above. Metra DPD results should be interpreted in conjunction with clinical findings and other diagnostic results and should not be used as a sole determinant in initiating or changing therapy.

## DPD EXPECTED VALUES

Metra DPD reference ranges have been established for healthy males (n=121) and healthy premenopausal females (n=312) over 25 years of age. For the purposes of establishing reference ranges, healthy subjects were defined as:

- Basically healthy, no bone, endocrine or chronic disorders
- Regular menstrual cycles (females)
- Not pregnant or breast feeding (females)
- Not currently taking any medication known to influence bone metabolism (e.g. corticosteroids, GnRH analogs, anticonvulsants, heparin, thyroid medication)

Values may be influenced by such factors as low estrogen production, low calcium intake, low physical activity or diseases known to affect bone metabolism, such as osteoporosis, Paget's disease, hyperparathyroidism, hyperthyroidism and bone metastasis. Estrogen deficiency in postmenopausal women can result in elevated bone resorption. It is suggested that the premenopausal reference range be used to interpret results in postmenopausal women. Each laboratory should establish its own normal reference range. The ranges are expressed as nonparametric reference intervals (90% CI).

	Age (yr)	Mean	SD (nmol/mmol)	Range
Females	25 - 44	5.0	1.4	3.0 - 7.4
Males	25 - 55	3.8	1.0	2.3 - 5.4

The expected within-subject variability was determined from urine specimens from 49 healthy subjects collected for five nonconsecutive days over two weeks. The average of the individual within-subject longitudinal variation was 15.5%. Between-subject variability is reflected in the nonparametric reference intervals shown above.

## PERFORMANCE CHARACTERISTICS

### Antibody Specificity

The monoclonal anti-Dpd antibody has selective, high affinity for free Dpd and negligible binding to Dpd peptides and free or peptide bound pyridinoline (Pyd).

## PERFORMANCE CHARACTERISTICS (CONT.)

	% Reactivity
Free Dpd	100%
Free Pyd	< 1%
Pyd/Dpd peptides	
≥ 1000 MW	< 2.5%
≥ 3500 MW	< 2.5%

### Sensitivity

The minimum detection limit of the Metra DPD Assay is 1.1nmol/L, determined by the upper 3 SD limit in a zero standard study.

### Recovery - Spike Recovery

Spike recovery was determined by adding a known quantity of purified Dpd to urine samples with different levels of endogenous Dpd. Typical results are provided below.

Sample	Endogenous (nmol/L)	Added (nmol/L)	Observed (nmol/L)	Recovery (%)
1	3.1	27.3	32.0	106
2	11.2	27.3	38.8	101
3	18.2	27.3	44.9	98

### Recovery - Linearity

Linearity was determined by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed (nmol/L)	Expected (nmol/L)	Recovery (%)
1	neat	65.5	-	-
	1:2	31.8	32.8	97
	1:4	15.4	16.4	94
2	neat	84.6	-	-
	1:2	39.3	42.3	93
	1:4	19.4	21.1	92
3	neat	132.6	-	-
	1:2	65.6	66.3	99
	1:4	30.2	33.2	91
	1:8	16.8	16.6	101

### Precision

Within-run and between-run precision were determined by assaying 3 urine samples in 9 different runs. Samples shown below represent a range of nmol/L values. For a female with a creatinine of 4.5 mmol/L, samples 1 through 3 represent low normal, high normal, and elevated resorption (2.4 nmol/mmol, 6.7 nmol/mmol, and 38.8 nmol/mmol, respectively).

Sample	Dpd (nmol Dpd)	Within-run <sup>1</sup> CV%	Between-run <sup>2</sup> CV%
1	10.7	8.4	4.8
2	30.0	4.3	4.6
3	174.7	5.5	3.1

<sup>1</sup>n=21      <sup>2</sup>n=9 runs

## ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel's Technical Support Number, 1-800-524-6318 (toll free in the USA). If outside the United States, contact your local Quidel office or distributor.

## CLINICAL STUDIES

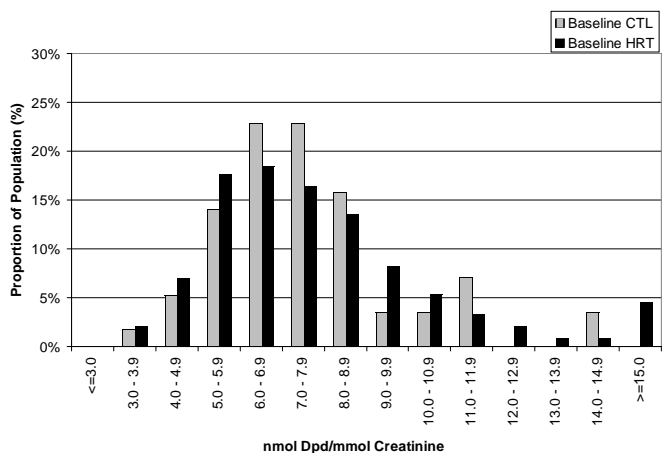
### Use of Metra DPD for Monitoring Hormonal Antiresorptive Therapy in Postmenopausal Women

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Metra DPD assay to monitor changes in urinary Dpd excretion associated with estrogen/progestin antiresorptive therapy. Increased bone resorption and significant loss of bone are often associated with postmenopausal estrogen deficiency. Estrogen replacement has been shown to effectively decrease resorption and protect existing bone mass<sup>7-10</sup>. Subjects were postmenopausal women, aged 45 to 64 years (mean 56±4 years), who had undergone natural or surgical menopause within the last 10 years. At baseline, eligible subjects were randomized to either an active treatment group (HRT): Premarin® (0.625 mg daily) with placebo progestin, Premarin® (0.625 mg daily) and an active progestin (Provera® 2.5mg/day continuous, Provera® 10mg/day cyclical, or micronized progesterone 200mg/day cyclical); or to the control group (CTL): placebo estrogen and placebo progestin. First or second morning urine specimens were obtained at baseline and 12 months from all subjects. Metra DPD results were corrected for creatinine clearance and expressed as nmol Dpd/mmol creatinine.

Mean baseline (±1SD) Dpd concentration (7.56±2.27 vs. 7.94±3.25 nmol/mmol, p=0.304) and lumbar spine BMD (0.97±0.17 vs. 0.97±0.15 g/cm<sup>2</sup>, p=0.792) were similar for CTL and HRT. Distributions of baseline Dpd values in HRT and CTL are depicted in Figure 1 by proportion of the study population.

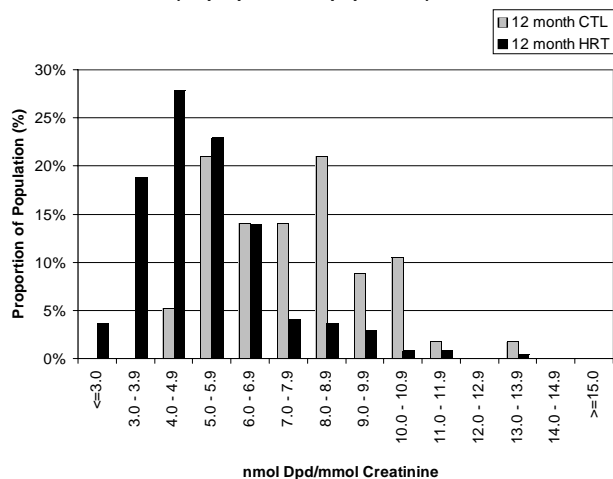
**CLINICAL STUDIES (CONT.)**

**Figure 1: Distribution of Dpd Levels At Baseline (as proportional of population)**



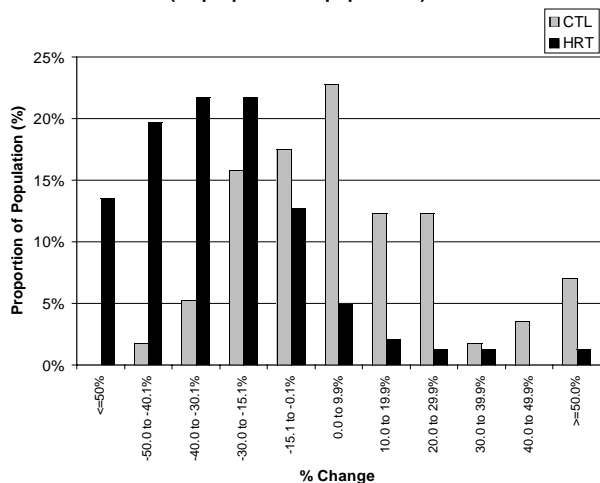
Dpd was significantly lower for HRT than CTL at 12 months ( $5.27 \pm 1.78$  vs.  $8.08 \pm 3.63$  nmol/mmol,  $p < 0.00001$ ). At 12 months subjects in HRT were more likely than CTL to have a Dpd concentration  $\leq 7.4$  nmol/mmol (89% vs. 51%,  $p < 0.00001$ ) even though baseline proportions were similar for the 2 groups (CTL 56%, HRT 53%,  $7.4$  nmol/mmol). Distributions of Dpd values following 12 months in the HRT and CTL groups are depicted in Figure 2.

**Figure 2: Distribution of Dpd Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or placebo (CTL) (as proportion of population)**



The mean ( $\pm$ SD) Dpd concentration in CTL subjects increased slightly from baseline to  $+11.7\%$  ( $\pm 49.7\%$ ) at 12 months ( $p = 0.278$ ) whereas Dpd concentrations in HRT subjects decreased from baseline to  $-29.1 \pm 23.8\%$  at 12 months ( $p < 0.0001$ ). Distributions of the percent change from baseline in Dpd values following 12 months in the HRT and CTL groups are depicted in Figure 3.

**Figure 3: Distribution of Percent Change in Dpd Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or Placebo (CTL) (as proportion of population)**



**CLINICAL STUDIES (CONT.)**

At 12 months, subjects in HRT had gained lumbar spine BMD compared to CTL ( $p < 0.00001$ ) as shown in Table 1.

**Table 1. Changes in Lumbar Spine BMD (Mean $\pm$ SD)**

	n	Baseline (g/cm <sup>2</sup> )	12 months (g/cm <sup>2</sup> )	$\Delta$ (%)
CTL	57	$0.97 \pm 0.17$	$0.95 \pm 0.17$	$-1.6 \pm 2.7$
HRT	244	$0.97 \pm 0.15$	$1.01 \pm 0.15$	$+3.7 \pm 2.7$

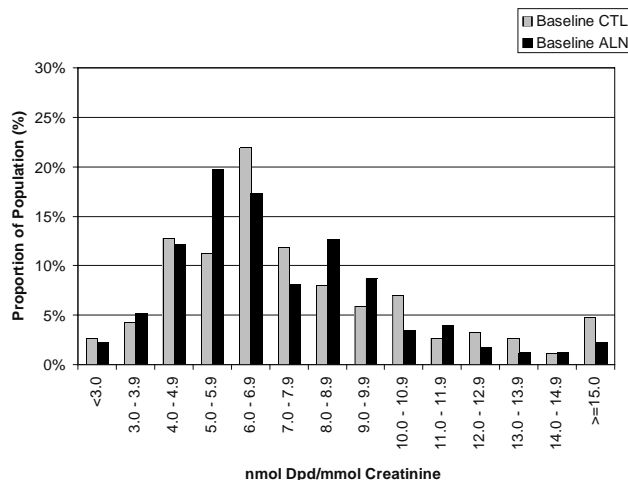
These results indicate that the Metra DPD assay is safe and effective for monitoring the antiresorptive effect of hormone replacement therapy in postmenopausal women.

**Use of Metra DPD for Monitoring Bisphosphonate Antiresorptive Therapy in Osteoporosis**

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Metra DPD assay to monitor changes in urinary Dpd excretion associated with amino-bisphosphonate (alendronate) antiresorptive therapy. Subjects were postmenopausal women, aged 45 to 84 years (mean  $64 \pm 7$  years), diagnosed with osteoporosis (based on clinical presentation or baseline lumbar spine BMD more than 2.5 standard deviations below the mean for mature premenopausal women). At baseline, eligible subjects were randomized to receive either 10mg alendronate and 500mg calcium per day (ALN) or 500mg calcium per day (CTL). Second morning urine specimens were obtained at baseline, 3, 6, and 12 months from all subjects. Metra DPD results were corrected for creatinine clearance and expressed as nmol Dpd/mmol creatinine.

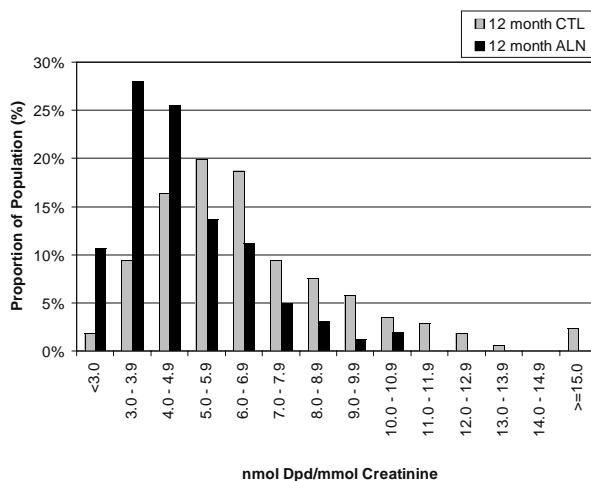
Mean ( $\pm$ SD) baseline Dpd concentration ( $7.35 \pm 3.30$  vs.  $7.74 \pm 3.47$  nmol/mmol,  $p = 0.278$ ) and lumbar spine BMD ( $0.75 \pm 0.09$  vs.  $0.74 \pm 0.10$  g/cm<sup>2</sup>,  $p = 0.426$ ) were similar for ALN and CTL. Distributions of baseline Dpd values in ALN and CTL are depicted in Figure 4 by proportion of the study population.

**Figure 4: Distribution of Dpd Levels At Baseline (as proportion of population)**



Dpd was significantly lower for ALN than CTL at 3 ( $5.45 \pm 2.61$  vs.  $7.56 \pm 3.08$  nmol/mmol,  $p < 0.00001$ ), 6 ( $4.83 \pm 1.94$  vs.  $7.09 \pm 3.33$  nmol/mmol,  $p < 0.00001$ ), and 12 months ( $4.78 \pm 1.75$  vs.  $6.73 \pm 2.98$  nmol/mmol,  $p < 0.00001$ ). At 3, 6, and 12 months, 84, 89, and 91%, respectively, of ALN subjects had a Dpd concentration  $\leq 7.4$  nmol/mmol. Subjects in ALN were more likely than CTL subjects to have a Dpd concentration  $\leq 7.4$  nmol/mmol at all timepoints ( $p = 0.002$ ) even though baseline proportions were similar for the 2 groups (CTL 60.4%, ALN 57.8%  $< 7.4$  nmol/mmol, respectively). Distributions of Dpd values following 12 months in the ALN and CTL groups are depicted in Figure 5.

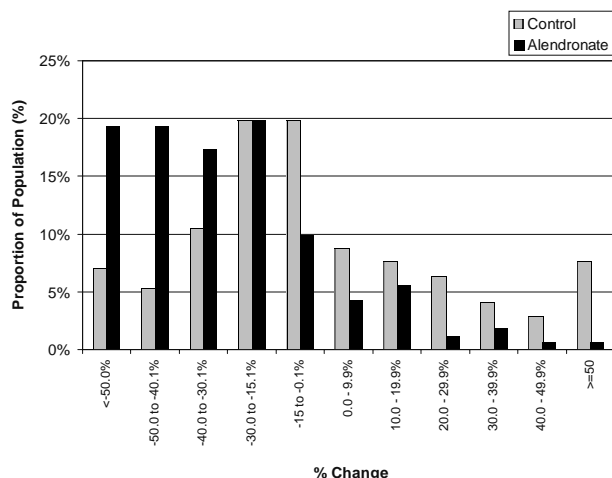
**Figure 5: Distribution of Dpd Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL) (as proportion of population)**



## CLINICAL STUDIES (CONT.)

The mean ( $\pm$ 1SD) Dpd concentration in CTL subjects decreased gradually from baseline to -4.9% ( $\pm$ 34.9%) at 12 months ( $p=0.003$ ) which may reflect the modest bonesparing effect of calcium<sup>15</sup>. Mean Dpd concentrations in ALN subjects decreased 22.9 $\pm$ 37.4% at 3 months, 28.6 $\pm$ 25.8% at 6 months, and 29.5 $\pm$ 26.7% at 12 months. Distributions of the percent change from baseline in Dpd values following 12 months in the ALN and CTL groups are depicted in Figure 6.

**Figure 6: Distribution of Percent Change in Dpd Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL) (as proportion of population)**



At 12 months, subjects in ALN had gained lumbar spine BMD compared to CTL ( $p<0.00001$ ) as shown in Table 2.

**Table 2. Changes in Lumbar Spine BMD (Mean $\pm$ SD)**

	n	Baseline (g/cm <sup>2</sup> )	12 months (g/cm <sup>2</sup> )	$\Delta$ (%)
CTL	167	0.75 $\pm$ 0.09	0.74 $\pm$ 0.09	-0.8 $\pm$ 3.3
ALN	156	0.74 $\pm$ 0.09	0.78 $\pm$ 0.10	+5.7 $\pm$ 4.2

These results indicate that the Metra DPD assay is safe and effective for monitoring the antiresorptive effect of amino-bisphosphonate (alendronate) therapy among subjects diagnosed with osteoporosis.

### Additional Studies

Clinical studies were performed to evaluate urine deoxypyridinoline levels obtained using the Metra DPD assay related to levels obtained by HPLC analysis<sup>14</sup> and clinical diagnosis.

The first of these studies was conducted at clinical investigation sites using 54 samples from healthy volunteers and 140 samples from patients with known bone disorders (including osteoporosis, Paget's disease, hyperparathyroidism and hyperthyroidism). These diseases often involve elevated bone resorption, and this group of subjects was considered a population at risk. However, not all subjects were expected to have elevated bone resorption at the time of sample collection. One hundred and three of the 140 patients diagnosed with a disorder did not have elevated pyridinoline values as measured by HPLC. The Metra DPD deoxypyridinoline values in healthy subjects ranged from 2.3 to 11.2 nmol/mmol and in patients ranged from 1.2 to 37.3 nmol/mmol.

In the study, the Metra DPD assay was compared to a research HPLC method<sup>14</sup> for measuring pyridinoline. The HPLC threshold was determined, in a study of 84 healthy subjects, to be 50 nmol/mmol for males and 60 nmol/mmol for females (95% confidence interval upper limit for each gender). Using elevated pyridinoline determined by HPLC as the classification method, the receiver operating characteristic (ROC) technique was used to define an optimal relative sensitivity and specificity in the described population. Relative sensitivity and specificity are presented in Table 3. A two-by-two contingency table showing the number of subjects in each classification is shown in Figure 7.

**Table 3**

Metra DPD relative sens. = 69%, spec. = 87%

**Figure 7**

Dpd	HPLC Pyridinoline	
	Elevated	Not Elevated
+	31	20
-	14	129

In the second study, the Metra DPD assay results were compared in a mixed population of 39 samples from healthy subjects and 69 samples from Paget's disease patients. Although Paget's disease represents a model for identifying active bone resorption, some of the patients in this study were undergoing treatment or may have been considered in remission and may not have had elevated bone resorption at the time of sample collection. In this study, healthy subjects ranged from 2.3 to 6.4 nmol/mmol. Paget's disease patients ranged from 1.7 to 50.4 nmol/mmol.

## CLINICAL STUDIES (CONT.)

Using the diagnosis of Paget's disease as the classification method, the ROC technique was used to define an optimal relative sensitivity and specificity in this population. Relative sensitivity and specificity are shown in Table 4. A two-by-two contingency table is shown in Figure 8.

**Table 4**

Metra DPD relative sens. = 91%, spec. = 97%

**Figure 8**

Dpd	Paget's Diagnosis	
	Yes	No
+	63	1
-	6	38

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