

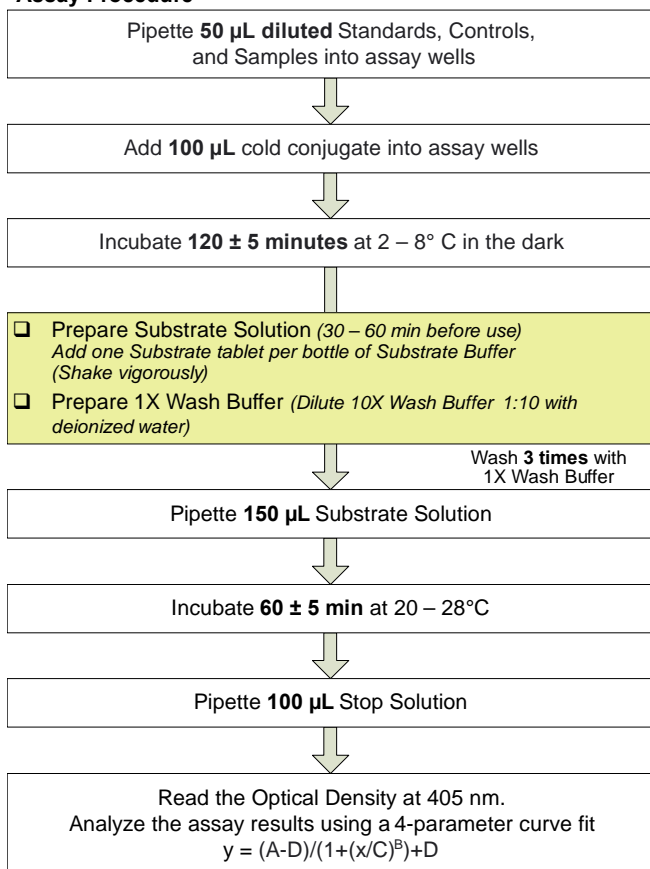
An enzyme immunoassay for the quantitation of deoxypyridinoline (DPD) crosslinks in human urine

MicroVue™ DPD EIA Summary

Reagents and Samples Preparation

- ❑ Prepare Enzyme Conjugate with Assay Buffer Solution, store at 2-8°C (Add 7 mL cold Assay Buffer per vial of Conjugate)
- ❑ Dilute Standards, Controls, urine specimens 1:10 with Assay Buffer Solution (50 µL sample + 450 µL Assay Buffer)

Assay Procedure



INTENDED USE

MicroVue 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.¹ 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 (PYD) and deoxypyridinoline (DPD).^{1,2} DPD is formed by the enzymatic action of lysyl oxidase on the amino acid lysine.³ DPD is released into the circulation during the bone resorption process.^{2,5} DPD is excreted unmetabolized in urine and is unaffected by diet,⁶ making it suitable for assessing resorption.

Bone is constantly undergoing a metabolic process called remodeling.^{2,7} 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.^{2,7} Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is, resorption and formation are in balance.⁷ In abnormal states of bone metabolism this process becomes uncoupled and, when resorption exceeds formation, this results in a net loss of bone.⁷ The measurement of specific degradation products of bone matrix provide analytical data of the rate of bone metabolism.^{2,4,5}

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.⁸ The most common type of osteoporosis occurs in postmenopausal women as a result of the estrogen deficiency produced by the cessation of ovarian function.⁷ Restoration of premenopausal estrogen levels by replacement therapy prevents bone loss and osteoporosis.⁷⁻¹⁰ Estrogens and a class of compounds known as bisphosphonates are antiresorptive therapies which can be used to prevent bone loss or treat osteoporosis.⁷⁻¹² 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.⁷ These include endocrine diseases such as hypo-gonadism, 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.⁷ Other diseases characterized by abnormal bone remodeling include Paget's disease and cancers metastatic to bone.³

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

PRINCIPLE OF THE PROCEDURE

The MicroVue 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. MicroVue DPD results are corrected for urinary concentration by creatinine.

REAGENTS AND MATERIALS PROVIDED

96 Assays for Deoxyypyridinoline Crosslinks

MicroVue DPD EIA Kit contains the following:

A			
B	DPD Standards A – F	Parts 4203–4208	0.3 mL each
C	(A=0, B=3, C=10, D=30, E=100, F=300 nmol/L DPD)		
D	DPD purified from bovine bone in 10 mmol/L phosphoric acid		
E	containing sodium azide (0.05%) as a preservative		
L	Low/High Controls	Parts 4209, 4210	0.3 mL each
H	DPD purified from bovine bone in 10 mmol/L phosphoric acid		
	containing sodium azide (0.05%) as a preservative		
1	Coated Strips	Part 4661	12 each
	Purified murine monoclonal Anti-DPD antibody adsorbed onto stripwells		
2	Stop Solution	Part 4702	15 mL
	0.5N NaOH		
3	10X Wash Buffer	Part 4703	55 mL
	Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative		
4	Assay Buffer	Part 4704	55 mL
	Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative		
5	Substrate Buffer	Part 4705	3 x 10 mL
	A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative		
6	Substrate Tablets	Part 0012	3 x 20 mg
	p-Nitrophenyl phosphate		
7	Enzyme Conjugate	Part 4202	3 each
	Lyophilized DPD, purified from bovine bone, conjugated to alkaline phosphatase containing buffer salts, and stabilizers		
	Plate Tape Cover	Part 0047	3 each

MATERIALS REQUIRED BUT NOT PROVIDED

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

WARNINGS AND PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
6. Store assay reagents as indicated.
7. Do not use Coated Strips if pouch is punctured.
8. Test each sample in duplicate.
9. 0.5N NaOH is considered corrosive 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.
10. Sodium azide is used as a preservative. Incidental contact with or ingestion of buffers containing sodium azide may cause irritation to the skin, eyes, or mouth. Only use buffers for intended purposes and avoid contact with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up.
11. The substrate buffer contains diethanolamine and may cause irritation to the eyes and/or skin with prolonged contact. Wear suitable protective clothing, gloves, and eye/face protection. Contacted areas should be immediately washed with soap and water.
12. Use of multichannel pipets or repeat pipettors is recommended to ensure timely delivery of reagents.
13. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
14. Dilute samples greater than 300 nmol/L in Assay Buffer and retest. Include the dilution factor in the final calculation.
15. This assay may be performed with any validated washing method.
16. 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 optical density reaches 1.2–1.5; then read the strips.
17. Standards and Controls are in 10 mmol/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.
18. The Deoxyypyridinoline 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.

REAGENT PREPARATION

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 20–28°C. Use 1X Wash Buffer within 21 days of preparation.

Special Washing Instructions: Prepare 1X Wash Buffer as above and store at 2–8°C until use.

Enzyme Conjugate

Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of Assay Buffer. Store reconstituted Enzyme Conjugate at 2–8°C until use.

Working Substrate Solution

The Substrate Buffer must be brought to 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 20–28°C Substrate Buffer (see table). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

STORAGE

Store kit at 2–8°C.

Do Not Freeze.

Store unused reagents at 2–8°C.

SPECIMEN COLLECTION AND STORAGE

The MicroVue 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 10:00 am 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.

ASSAY PROCEDURE

Read entire product insert before beginning the assay. See *REAGENT PREPARATION* before proceeding.

PROCEDURAL NOTE: The MicroVue 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 *Washing Step* sections.

Determine the 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. 50 µL sample + 450 µL 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. Place desired number of Coated Strips in the Stripwell Frame. Label strips to prevent mix-up in case of accidental removal from frame.
4. Add 50 µL diluted Standard, Control or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
5. Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of Assay Buffer. Store reconstituted Enzyme Conjugate at 2–8°C until use.
6. Add 100 µL 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.
7. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C Substrate Buffer (see table). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

Washing Step

8. 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 250 µL 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.

Substrate Incubation

9. Add 150 µL of Working Substrate Solution to each well.
10. Incubate for 60 minutes (± 5 minutes) at 20–28°C.

Stop/Read

11. Add 100 µL of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
12. Read the optical density at 405 nm. Assure that no large bubbles are present in the wells and that the bottoms of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.

13. Quantitation software with a 4-parameter calibration curve fitting equation must be used to analyze the MicroVue DPD assay results.

$$\text{Equation: } y = (A-D)/(1 + (x/C)^B) + D$$

14. Determine concentration of samples and Controls from the Standard curve.

15. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

QUALITY CONTROL

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 Quidel Corporation. The optical density 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.

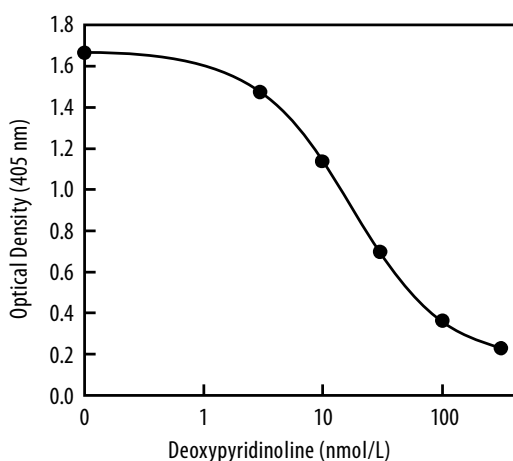
If the optical density of the MicroVue DPD Standard A is less than 0.8, the results should be considered questionable and if possible, the samples should be repeated.

INTERPRETATION OF RESULTS

Results obtained from the MicroVue 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 MicroVue DPD results will be expressed as nmol DPD/mmol creatinine.

Representative Standard Curve

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



LIMITATIONS OF THE PROCEDURE

While MicroVue DPD 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 MicroVue 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. MicroVue 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.

SAMPLE VALUES

MicroVue 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 (nmol DPD/mmol Cr)	SD	Range
Females	25 - 44	5.0	1.4	3.0 - 7.4
Males	25 - 55	3.8	1.0	2.3 - 5.4

The 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).

	% 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 MicroVue DPD Assay is 1.1 nmol/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 precision was determined for ≥ 21 replicates of 3 samples on 2 plates from each of 3 kit lots (6 plates total). Between-run precision was determined for 3 samples run in 9 separate plates from each of 3 kit lots (27 plates total). 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/L DPD)	Within-run ¹ CV%	Between-run ² CV%
1	10.7	8.4	4.8
2	30.0	4.3	4.6
3	174.7	5.5	3.1

¹ n = 21

² n = 9 runs

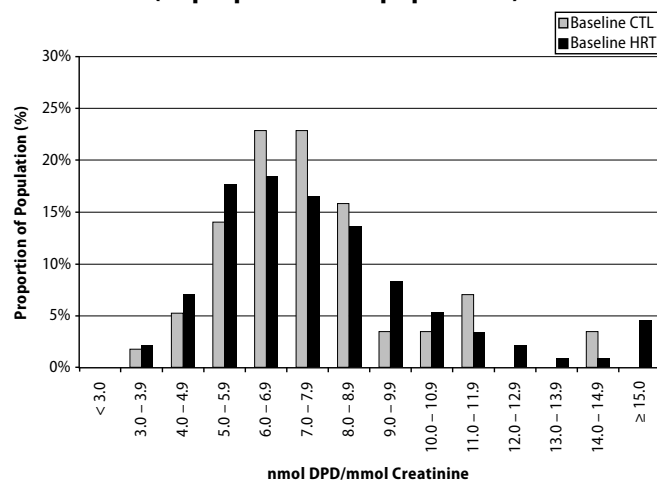
CLINICAL STUDIES

Use of MicroVue 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 MicroVue DPD assay to monitor changes in urinary DPD excretion associated with estrogen/progestin anti-resorptive 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.⁷⁻¹⁰ 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® 2.5 mg/day continuous, Provera 10 mg/day cyclical, or micronized progesterone 200 mg/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. MicroVue DPD results were corrected for creatinine clearance and expressed as nmol DPD/mmol creatinine.

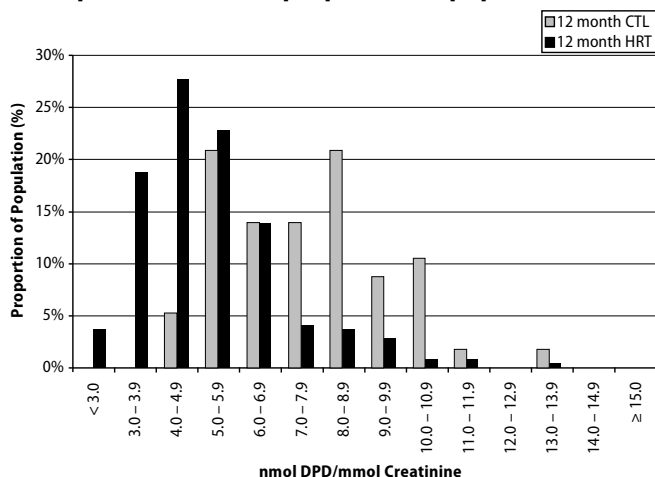
Mean baseline (± 1 SD) DPD concentration (7.56 ± 2.27 vs. 7.94 ± 3.25 nmol/mmol, $p = 0.304$) and lumbar spine BMD (0.97 ± 0.17 vs. 0.97 ± 0.15 g/cm², $p = 0.792$) were similar for CTL and HRT. Distributions of baseline DPD values in HRT and CTL are depicted in Figure 1 by proportion of the study population.

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



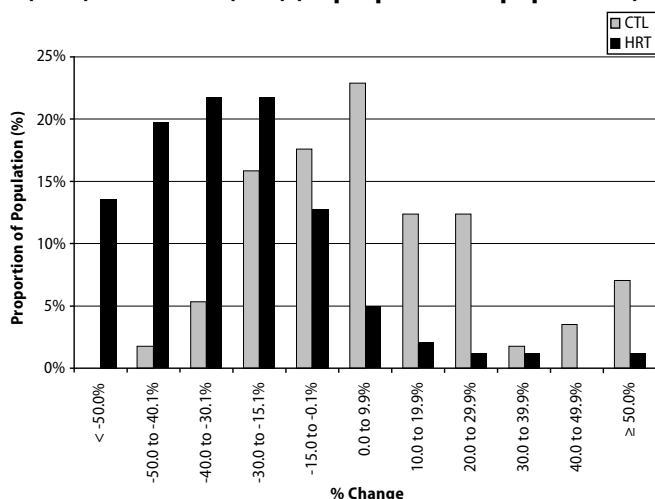
DPD was significantly lower for HRT than CTL at 12 months (5.27 ± 1.78 vs. 8.08 ± 3.63 nmol/mmol, $p < 0.00001$). At 12 months subjects in HRT were more likely than CTL to have a DPD concentration ≤ 7.4 nmol/mmol (89% vs. 51%, $p < 0.00001$) even though baseline proportions were similar for the 2 groups (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 (± 1 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)



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 ²)	12 months (g/cm ²)	Δ (%)
CTL	57	0.97 ± 0.17	0.95 ± 0.17	-1.6 ± 2.7
HRT	244	0.97 ± 0.15	1.01 ± 0.15	$+3.7 \pm 2.7$

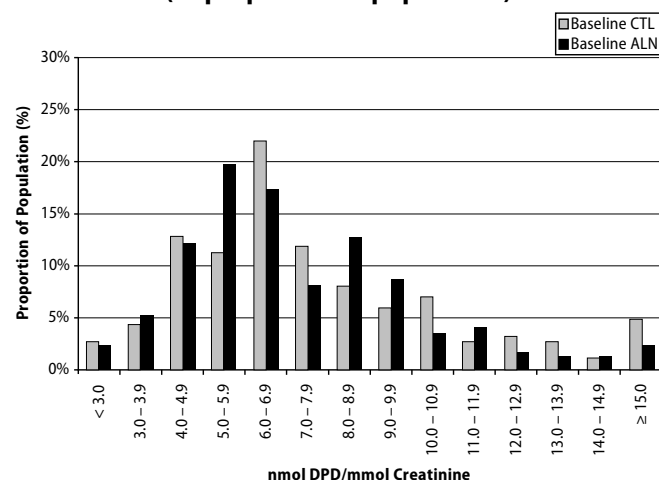
These results indicate that the MicroVue DPD assay is safe and effective for monitoring the antiresorptive effect of hormone replacement therapy in postmenopausal women.

Use of MicroVue DPD for Monitoring Bisphosphonate Antiresorptive Therapy in Osteoporosis

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the MicroVue 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 ± 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 10 mg alendronate and 500 mg calcium per day (ALN) or 500 mg calcium per day (CTL). Second morning urine specimens were obtained at baseline, 3, 6, and 12 months from all subjects. MicroVue DPD results were corrected for creatinine clearance and expressed as nmol DPD/mmol creatinine.

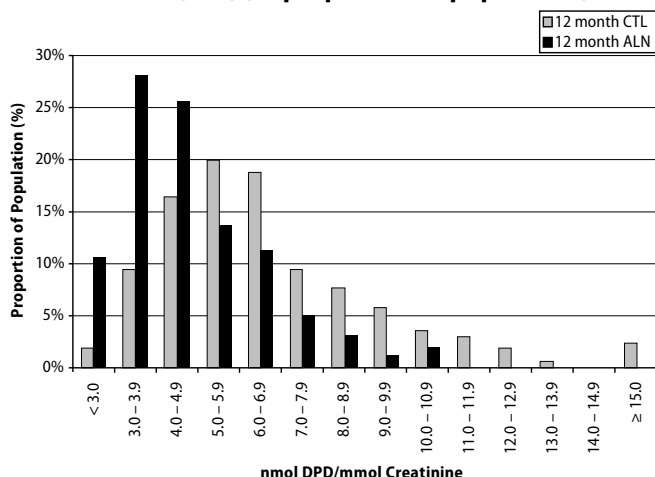
Mean (± 1 SD) baseline DPD concentration (7.35 ± 3.30 vs. 7.74 ± 3.47 nmol/mmol, $p = 0.278$) and lumbar spine BMD (0.75 ± 0.09 vs. 0.74 ± 0.10 g/cm², $p = 0.426$) were similar for ALN and CTL. Distributions of baseline DPD values in ALN and CTL are depicted in Figure 4 by proportion of the study population.

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



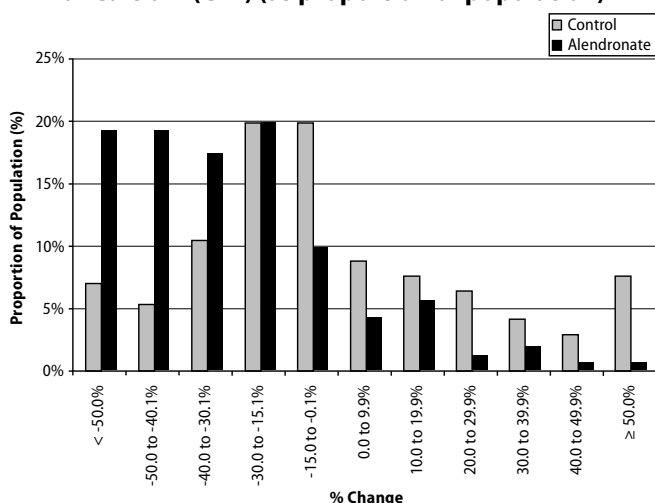
DPD was significantly lower for ALN than CTL at 3 (5.45 ± 2.61 vs. 7.56 ± 3.08 nmol/mmol, $p < 0.00001$), 6 (4.83 ± 1.94 vs. 7.09 ± 3.33 nmol/mmol, $p < 0.00001$), and 12 months (4.78 ± 1.75 vs. 6.73 ± 2.98 nmol/mmol, $p < 0.00001$). At 3, 6, and 12 months, 84, 89, and 91%, respectively, of ALN subjects had a DPD concentration ≤ 7.4 nmol/mmol. Subjects in ALN were more likely than CTL subjects to have a DPD concentration ≤ 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)



The mean (\pm 1 SD) 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.¹⁵ 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 ²)	12 months (g/cm ²)	Δ (%)
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 MicroVue 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 MicroVue DPD assay related to levels obtained by HPLC analysis¹⁴ 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 MicroVue 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 MicroVue DPD assay was compared to a research HPLC method¹⁴ 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

MicroVue DPD	
Relative Sensitivity	69%
Specificity	87%

Figure 7

		HPLC Elevated	PYD Not Elevated
		+	-
MicroVue DPD	+	31	20
	-	14	129

In the second study, the MicroVue 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.

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

MicroVue DPD	
Relative Sensitivity	91%
Specificity	97%

Figure 8

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

ASSISTANCE

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GLOSSARY



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Intended Use

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