Comparison of Urinary Pyridinolone and Deoxy-
pyridinolone Measurements in 13 US Laboratories, Hu-
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Measurement of biochemical bone markers is commonly
used in the management of various metabolic bone dis-
ease (1, 2). The pyridinium crosslinks pyridinoline (PYD)
and deoxypyridinoline (DPD) are well-characterized
markers for bone resorption that have been available for
several years (3). Assays to measure the sum of free and
peptide-bound urinary PYD or DPD (total PYD or DPD)
or free, non-peptide-bound molecules have been devel-
oped and described (4). Analytical variability of PYD and
DPD measurement is a major problem hampering compar-
ability and interpretation of results. As part of the
CDC program to develop a reference system to standard-
ize the measurements of PYD and DPD, we conducted a
round-robin interlaboratory comparison study to assess
the state of analytical variability.

We invited laboratorians within the US involved in
routine measurement of urinary DPD and/or PYD to
participate in this study. Participants were asked to ana-
lyze five identical double-blinded sets of six unknown
samples for PYD, DPD, and creatinine on 5 days in
duplicate. Information was collected from each partici-
 pant on sample handling and preparation, as well as
 calibrators, including information on sample hydrolysis.

Urine was collected in agreement with CDC Institu-
tional Review Board regulations. We screened individual
urine samples for total PYD and DPD concentrations
using our in-house HPLC assay (5, 6) and then combined
them into three concentrations, from normal to moder-
ately increased pyridinium crosslink concentrations (low-,
medium-, and high-pool). We created a fourth urine pool
by mixing the low and medium pools (1:1 by volume; mixed
pool). One part of the mixed pool was used for the
addition of PYD and DPD calibrators (supplemented
pool; 638 nmol/L PYD and 219 nmol/L DPD). An aque-
ous solution of DPD and PYD calibrators (aqueous sam-
ple; 306 nmol/L PYD and 555 nmol/L DPD) was included
as the sixth sample. We tested immunoreactive, free PYD,
and DPD calibrators (obtained from Metra Biosystems,
Inc.) for identity and purity by mass spectrometry and
spectrophotometry using data described previously (7).
Concentrations were determined spectrophotometrically
with previously described coefficients of absorption (7)
and were confirmed with our in-house HPLC method.
Pools and calibrators were handled under special protec-
tive yellow light. All pools were dispensed into brown
glass vials and shipped frozen on dry ice (overnight
delivery). Bottle-to-bottle variability was tested.

We analyzed data separately for immunoassays
and HPLC assays. We tested for outliers by calculating
the all-laboratory consensus mean ± 3 SD for each sample and
compared each individual result. No result was outside of
this range. All evaluations of imprecision and recoveries
were based on the mean results over 5 days. The follow-
ing measures of imprecision were evaluated: among-
laboratory (within-method), within-laboratory (among-
pools), and among-run (within-laboratory). We expressed
all variations as CV (SD). We calculated the contribution
of within- and among-laboratory variability to the total
variability using a nested random-effects analysis of vari-
ance. We calculated the differences in among-laboratory
and within-pool concentrations of PYD and DPD using
ANOVA. Results with P > 0.01 were considered nonsig-
nificant.

Because there were no available analytical reference
methods that could be used as accuracy checks, we
performed recovery experiments to assess assay accuracy.
Recoveries, reported as mean recoveries (SD), were calcu-
lated individually for each sample with added DPD
and PYD: recovery (%) = [(urine with added PYD and
DPD) — (urine without added PYD and DPD)]/added
concentration of PYD and DPD × 100. Recoveries were
also calculated for the mixed sample: recovery (%) =
measured value/expected value × 100, with the expected
value being the mean of the low pool and medium pool as
determined by each laboratory.

On the basis of assigned PYD and DPD values for the
aqueous sample and the values measured by the labora-
tories for this sample, a factor was calculated (factor =
assigned value/measured value). We multiplied this fac-
tor by the DPD and PYD values of the pools to normalize
the data to the assigned values of the aqueous sample. We
used this procedure to estimate the impact of a common
calibrator on the among-laboratory variability.

Of the 15 laboratories that agreed to participate, 1
laboratory did not report results, and 1 laboratory was
excluded because of problems related to assay processing,
which did not reflect normal laboratory performance. Of
the 13 remaining laboratories, 5 used HPLC assays (4
in-house methods; 1 assay from BIORAD, Inc.), and 8
used immunoassays (Metra Biosystems). The five labora-
tories performing HPLC assays used four different cali-

brators. Two different immunoassays were used to ana-
lyze either DPD (measured by eight laboratories) or PYD
with a cross-reaction for DPD (referred to in the text as
“PYD&DPD”; measured by four laboratories). All immu-
noassays were performed manually with the same cali-
brator. Creatinine was analyzed in all laboratories with a
alkaline picric acid reaction.

The mean within-laboratory and among-pool CVs for
the immunoassays were 8.1% and 10% for PYD&DPD and
DPD, respectively, and for the HPLC assays, 9.0% and
11% for PYD and DPD, respectively. The difference in this
variability between both types of assays was not signifi-
cant (P > 0.6; double-sided t-test). The results for the
supplemented pool and the aqueous sample varied more
than those for the other pools. The mean recoveries for the
mixed pool with the immunoassays were 100.1% and
98.6% for DPD and PYD&DPD, respectively, and 98.9%
and 100.4% with the HPLC assays for DPD and PYD,
respectively. The mean recoveries of the supplemented

pool with the immunoassays were 102.5% and 85.9% for DPD and PYD&DPD, and 108.7% and 104.4% for DPD and PYD, respectively. We found no significant difference in the recoveries between immunoassays and HPLC assays ($P > 0.1$, using double sided $t$-test). The mean within-laboratory, among-pools variability of the creatinine measurement was 4.4%. The mean recovery for creatinine across all laboratories was 99.3% for the mixed urine pool. Creatinine correction increased the within-laboratory, among-pool variability by 14.8% for DPD and 19.8% for PYD.

Within the HPLC assay group, the differences between each laboratory can be considered consistent for all pools (Fig. 1). We did not find such a consistency within the group of immunoassays. The mean among-laboratory CVs for the HPLC group were 28% and 26% for DPD and PYD, respectively, and for the immunoassay group, 12% and 6.6% for DPD and PYD&PYD, respectively (Table 1). As indicated by the high $F$ values, reported concentrations varied substantially and differed significantly among laboratories for most pools. The among-laboratory variability of the immunoassays contributed up to 24% for PYD&DPD and 50% for DPD to the total variability (sum of within- and among-laboratory variability), and up to 88% for DPD and 90% for PYD in the group of HPLC assays (Table 1). For creatinine measurement, this variability accounted for 66% of the total variability. To simulate the effect of a common calibrator, we adjusted the results of the urine pools to the values assigned to the aqueous sample, after which the among-laboratory CVs decreased by 57% for DPD and 74% for PYD within the HPLC group. The changes seen in the immunoassay group are within the assay imprecision.

The high proportion of among-laboratory variability in the total variability in the HPLC group shows that differences among laboratories derived mainly from different mean values and, to a lesser extent, from assay variability, which points to the lack of uniform assay calibration and possible differences in sample handling. Because sample handling was essentially identical across laboratories, the lack of uniform assay calibration may be the reason for the high among-laboratory variability. This may be supported by the consistency in the differences among laboratories. Within the immunoassay group assay, the among-laboratory variability had a less profound impact on total variability. Estimates on the impact of a common calibrator showed that the among-laboratory variability could be substantially reduced within the group of HPLC assays.

Both assay types can be considered similar regarding imprecision and accuracy. The differences in recoveries of the supplemented sample compared with the mixed sam-

![Fig. 1](https://example.com/fig1.png)

Fig. 1. Mean values for PYD&DPD measured (nmol/L) by immunoassay (A) and HPLC (C), respectively, and mean values for DPD measured (nmol/L) by immunoassay (B) and HPLC (D), respectively.
ple, and the highly variable results of the supplemented and aqueous sample point to possible problems in the measurements of samples with high crosslinks concentrations. The reasons for these inconsistencies need to be investigated in further studies. Commutability issues of the aqueous sample and supplemented pool may be less likely because most assays calibrate with aqueous standards and the modifications of the supplemented pool can be considered as minor.

In conclusion, there is an urgent need to improve analytical imprecision and among-laboratory variability. Improvement can be aided by standard reference materials and more external quality-assessment programs.

Tables showing the among-laboratory variability, within-laboratory variability and recoveries, as well as a list of the participating laboratories and a discussion of findings with regard to analytical quality specifications are available as a supplement at Clinical Chemistry Online (http://www.clinchem.org/content/vol47/issue11).

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References

Table 1. Mean, CV, and F and P values for among-laboratory differences.

<table>
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<tr>
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<th>DPD</th>
<th>PYD</th>
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<th>PYD&amp;DPD</th>
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<tr>
<td></td>
<td>Mean,</td>
<td>CV,</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>%</td>
<td>value</td>
<td>value</td>
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<tr>
<td>Low pool</td>
<td>53</td>
<td>34</td>
<td>56.4</td>
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<td>Medium pool</td>
<td>128</td>
<td>71.7</td>
<td>0.0001</td>
<td>487</td>
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<td>22</td>
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<td>Mixed pool</td>
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<td>30</td>
<td>44.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Supplemented pool</td>
<td>322</td>
<td>26</td>
<td>53.9</td>
<td>0.0001</td>
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<tr>
<td>Mean</td>
<td>28</td>
<td>26</td>
<td>44.4</td>
<td>0.0001</td>
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Data adjusted to the aqueous sample

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<th>DPD</th>
<th>PYD&amp;DPD</th>
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<tbody>
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<td>CV,</td>
<td>F</td>
<td>P</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>%</td>
<td>value</td>
<td>value</td>
</tr>
<tr>
<td>Low pool</td>
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<td>14</td>
<td>1.8</td>
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<tr>
<td>Medium pool</td>
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<td>14</td>
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<tr>
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</tr>
<tr>
<td>Supplemented pool</td>
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<td>6.9</td>
<td>8.9</td>
<td>7.1</td>
</tr>
</tbody>
</table>

* Aqu, aqueous.  
* Mean aqueous sample not included.

Cystatin C (cysC) is a 132-amino acid, 13-kDa cysteine protease inhibitor produced by all nucleated cells and whose function is thought to be modulation of the intracellular catabolism of proteins (1). It is formed at a constant rate, freely filtered by the renal glomeruli, and completely reabsorbed and catabolized by the proximal tubular cells (1–5). Plasma cysC values are reported to be unaffected by age, body weight, diet, medications, or pathologies such as inflammation and cancer. On the