Letters to the Editor

The LNSC LC-MS/MS results demonstrated very high cortisol concentrations, confirming the ELISA results (Table 1); however, salivary cortisone concentrations were not increased. Thus, the cortisol-to-cortisone ratios for patients 1–6 were much higher than the reference interval. Patients 7 and 8 had appropriately increased salivary cortisone concentrations and cortisol-to-cortisone ratios within the reference interval, suggesting endogenous hypercortisolism in these 2 patients. The physicians who referred the samples for clinical analyses were subsequently contacted. After intensive interviews of the patients, the physicians confirmed topical hydrocortisone use by 5 of the 6 patients with high cortisol-to-cortisone ratios. Patient 4 was evasive in her response to the physician, and subsequent ELISA data for LNSC obtained after this interview were all <4.2 nmol/L. The physician concluded that the patient had surreptitiously been using exogenous corticosteroids but was unable to confirm this suspicion in interviews with the patient. Patients 7 and 8 were subsequently reported by the referring physician to have endogenous hypercortisolism.

We conclude, as we hypothesized with our previously proposed algorithm (4), that contamination of saliva samples with topical hydrocortisone should be considered when LNSC results obtained by immunoassay are markedly increased, particularly when they are out of proportion with respect to other biochemical test results or clinical findings. In our experience as a reference laboratory, 1 of approximately 200 patients who submit samples has a dramatically increased salivary cortisol value by ELISA, warranting analysis for contamination with topical hydrocortisone. In that scenario, contamination with topical hydrocortisone would be suggested by a normal salivary cortisone concentration as measured by LC-MS/MS and a high cortisol-to-cortisone ratio.

Table 1. LNSC analysis by ELISA and subsequent detection of contamination by analysis of salivary cortisol and cortisone and calculation of the cortisol-to-cortisone ratio.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cortisol (ELISA), nmol/L</th>
<th>Cortisol (LC-MS/MS), nmol/L</th>
<th>Cortisone (LC-MS/MS), nmol/L</th>
<th>Cortisol-to-cortisone ratio</th>
<th>Contamination source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;1600</td>
<td>14,324</td>
<td>16</td>
<td>895</td>
<td>Hydrocortisone ointment</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>245</td>
<td>5</td>
<td>49</td>
<td>Hydrocortisone cream</td>
</tr>
<tr>
<td>3</td>
<td>1256</td>
<td>1110</td>
<td>10</td>
<td>111</td>
<td>Hydrocortisone cream</td>
</tr>
<tr>
<td>4</td>
<td>&gt;1600</td>
<td>208,104</td>
<td>10</td>
<td>20,810</td>
<td>Hydrocortisone suspected</td>
</tr>
<tr>
<td>5</td>
<td>865</td>
<td>800</td>
<td>8</td>
<td>100</td>
<td>Hydrocortisone cream</td>
</tr>
<tr>
<td>6</td>
<td>316</td>
<td>226</td>
<td>6</td>
<td>38</td>
<td>Hydrocortisone ointment</td>
</tr>
<tr>
<td>7</td>
<td>120</td>
<td>92</td>
<td>106</td>
<td>0.9</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>27</td>
<td>66</td>
<td>0.4</td>
<td>None</td>
</tr>
</tbody>
</table>

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Acknowledgments: The authors thank Drs. Batul Valika, Nicholas Tritos, Lisa Nachtingall, Joy Tsai, Pouneh Fazelli, Julia Kharlip, Meng Shumei, Beverly Biller, and Roberto Salvatori. The authors also thank ACL Laboratories, Peter Homar, and Molly Von-Norman for technical assistance.

References


Hershel Raff2* Ravinder J. Singh3

2 Endocrine Research Laboratory
Aurora St. Luke’s Medical Center
Department of Medicine
Medical College of Wisconsin
Milwaukee, WI
1 Department of Laboratory Medicine and Pathology
Mayo Clinic
Rochester, MN

* Address correspondence to this author at:
Endocrinology Research Laboratory
Aurora St. Luke’s Medical Center
2801 W. KK River Pkwy., Suite 245
Milwaukee, WI 53215
Fax 414-649-5747
Email hraff@mcw.edu

Previously published online at
DOI: 10.1373/clinchem.2012.182717

Inaccurate 25-Hydroxyvitamin D Results from a Common Immunoassay

To the Editor:

The great increase in the volume of vitamin D testing over the past several years has created a demand for...
high-throughput vitamin D testing methods (1). Our laboratory instituted 25-hydroxyvitamin D testing on the DiaSorin LIAISON® platform in October 2009. Soon afterward, some samples were noted not to produce the expected result after dilution with 1 volume of the manufacturer’s dilution buffer (i.e., 2-fold dilution). We investigated the frequency of such occurrences, whether the results obtained with diluted or undiluted samples were more consistent with liquid chromatography–tandem mass spectrometry (LC-MS/MS)1 results, and whether the cause of the nonlinear dilution might be due to interfering heterophile antibodies.

25-Hydroxyvitamin D analysis and dilutions were performed on the DiaSorin LIAISON instrument according to the manufacturer’s instructions. 25-Hydroxyvitamin D results for diluted (2-fold) and undiluted samples for 928 serum samples (424 consecutive samples in October 2009 and 504 consecutive samples in September 2011). In addition, between October 7, 2009, and August 5, 2011, we repeated the 25-hydroxyvitamin D analyses for 3475 undiluted samples with results >50 μg/L after diluting these samples 2-fold. Results were determined as discrepant if the results >50 μg/L for undiluted samples differed from the results for the corresponding diluted samples by ≥20%, or if results <50 μg/L for undiluted samples differed from the results for corresponding diluted samples by >20 μg/L. All discrepant samples that we identified during this time were analyzed by LC-MS/MS at Mayo Medical Laboratories without any sample dilution (2). To determine if the discrepant results were caused by heterophile antibody interference, we added 500 μL of patient sample (n = 11) to a Scantibodies Laboratory Heterophilic Blocking Tube (HBT) and incubated the tube for 1 h before measuring 25-hydroxyvitamin D on the LIAISON instrument.

Of 424 consecutive samples analyzed (undiluted and diluted 2-fold) in October 2009, 22 (5.19%) were discrepant. Similarly, we identified 24 discrepant results (4.76%) among 504 consecutive samples from September 2011. During both time periods, the results for these 46 samples showed that the values obtained for the undiluted sample was higher than that for the diluted sample [mean difference in 2009, 88.27% (SD, 45.19%) (P < 0.0001, paired t-test)]; mean difference in 2011, 42.85% (SD 36.9%) (P < 0.001)]. Given that the vast majority of samples showed no difference between the diluted and undiluted samples, we concluded that the differences for these 46 samples were not due to a matrix effect caused by the manufacturer’s dilution buffer.

The results of the October 2009 study prompted us to take all samples from October 2009 to August 2011 that had 25-hydroxyvitamin D results >50 μg/L for undiluted samples and to repeat the analyses of these samples after diluting them 2-fold. Of 3475 samples with results >50 μg/L, 537 (15.57%) of the undiluted and diluted samples showed discrepant results. Undiluted aliquots of all the discrepant samples were sent to Mayo Medical Laboratories for LC-MS/MS analysis. Fig. 1A depicts the results for 100 consecutive discrep-

---

1 Nonstandard abbreviations: LC-MS/MS, liquid chromatography–tandem mass spectrometry; HBT, Heterophilic Blocking Tube.

---

**Fig. 1.** Bland–Altman plots of 100 consecutive discrepant samples comparing 25-hydroxyvitamin D results obtained by LC-MS/MS to results obtained with the DiaSorin LIAISON assay.

Results for undiluted samples (A) and for samples diluted with 1 volume of buffer (2-fold) (B). MS, LC-MS/MS assay.
ant samples for the period between May 1, 2011, and August 5, 2011, demonstrating that the undiluted results were a mean of 27 μg/L (SD, 24.4 μg/L) higher than the LC-MS/MS result [mean difference, 74.3% (SD, 78.9%); P < 0.0001]. Interestingly, the results for the samples diluted 2-fold matched the LC-MS/MS results (Fig. 1B) [mean difference, 0.43 μg/L (SD, 11.0 μg/L); P = 0.6962], confirming that the results for the undiluted samples were falsely increased.

To investigate whether heterophile antibody interference was a cause for the discrepant results, we incubated 11 consecutive discrepant samples (between September 7, 2011, and September 15, 2011) in Scantibodies HBT tubes. This method, which uses a proprietary mix of lyophilized heterophile “blockers,” was chosen because it does not introduce any sample dilution. Results from HBT-treated samples did not differ significantly from the corresponding results for untreated undiluted samples [mean difference, 1.15 μg/L (SD, 10.65 μg/L); P = 0.7427]. Our investigation demonstrates that theDiaSorin LIAISON assay overestimates the 25-hydroxyvitamin D concentration in undiluted samples in approximately 5% of cases, which could lead to an underidentification of vitamin D–deficient individuals. This effect does not appear to be due to heterophile antibody interference, which others have suggested as a cause of some erroneous LIAISON 25-hydroxyvitamin D results (3). A potential cause for the discrepancy between these 2 studies is that samples in the present study were absorbed with a lyophilized blocking reagent that did not dilute the sample. The use of a liquid absorption reagent may have corrected results simply via dilution. A potential weakness of our study is that we had no positive controls for the HBT absorptions. Nevertheless, our data demonstrate that a simple 2-fold dilution provides results comparable to those obtained by LC-MS/MS, but this approach does double reagent costs.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: A.M. Gronowski, Clinical Chemistry, AACC; M.G. Scott, Clinical Chemistry, AACC.

Consultant or Advisory Role: None declared.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: None declared.

Expert Testimony: None declared.

References


Nils Becker2*
Adrain C. McClellan3
Ann M. Gronowski2
Mitchell G. Scott2

2 Department of Pathology and Immunology
Division of Laboratory and Genomic Medicine
Washington University School of Medicine
St. Louis, MO
3 Department of Laboratories
Barnes-Jewish Hospital
St. Louis, MO

* Address correspondence to this author at: Washington University School of Medicine
660 S. Euclid Ave., Campus Box 8118
St. Louis, MO 63144
Fax 314-362-1461
E-mail nbecker@path.wustl.edu

Previously published online at DOI: 10.1373/clinchem.2011.179754