Letters to the Editor

Interference from Rose Bengal with Total Bilirubin Measurement

To the Editor:

We report an unusual cause for a false-positive interference in the total bilirubin method on the Beckman Coulter DxC 800 analyzer and for the hemolytic index (HI) measured on the Roche Modular D. A total bilirubin result of 53 μmol/L was obtained for a patient who had an earlier result of 7 μmol/L on the DxC 800 analyzer. The direct bilirubin concentration remained at <4 μmol/L, and no other results changed in subsequent multibiochemical profiles. The sample had a red/pink tinge. There was no clinical explanation for the increased bilirubin value. A sample obtained the next day had a total bilirubin result of 7 μmol/L. The patient was a participant in a trial for the treatment of severe melanoma lesions with PV-10 [100 mmol/L rose bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetrachlorofluorescein disodium; MW, 1017.65 Da) in 9 g/L NaCl (i.e., 10% rose bengal disodium in 9 g/L NaCl); Profectus Pharmaceuticals]. PV-10 causes tumor necrosis, possibly owing to the release of cathepsins (1). The trial protocol is to inject PV-10 directly into the lesion and to collect a blood sample within an hour. The sample in question was collected 20 min after the injection. Most likely, the PV-10 entered the bloodstream rapidly because it had been injected deeply into healthy tissue or the lesion was well vascularized.

A scan of the affected sample with a Beckman Coulter DU 640 spectrophotometer (460–610 nm) revealed a peak absorbance at 562 nm. A diluted aliquot (1 part in 1000 with normal saline) of the PV-10 solution showed a color and intensity similar to the colored sample and had a peak absorbance at 549 nm. We prepared 3 different PV-10 dilutions with normal saline and a dilution of 1 part PV-10 in 10 000 with a sample of pooled patient plasma with a bilirubin concentration of 10 μmol/L. We analyzed these dilutions for total bilirubin and serum indices with the Beckman Coulter DxC 800 and Roche Modular D analyzers (Table 1). The apparent total bilirubin con-

Table 1. Results of total bilirubin measurement and hemolytic and icteric indices for samples containing PV-10 (rose bengal).

<table>
<thead>
<tr>
<th>System/assay</th>
<th>PV-10 (1/1000 with saline)</th>
<th>PV-10 (1/2000 with saline)</th>
<th>PV-10 (1/10 000 with saline)</th>
<th>Pooled plasma</th>
<th>PV-10 (1/10 000 with pooled plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter DxC 800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin, μmol/L (reagent)</td>
<td>208</td>
<td>109.5</td>
<td>23.8</td>
<td>9.8</td>
<td>36.2</td>
</tr>
<tr>
<td>HI</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Icteric index</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Roche Modular D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin, μmol/L (reagent)</td>
<td>−9.7</td>
<td>−3.9</td>
<td>−0.5</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>HI, mmol/L</td>
<td>1.35</td>
<td>0.825</td>
<td>0.155</td>
<td>0.037</td>
<td>0.645</td>
</tr>
<tr>
<td>Icteric index, μmol/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

* The Beckman Coulter analyzer measures the HI and icteric index semiquantitatively; an icteric index of 1 is equivalent to 0–25.7 μmol/L total bilirubin, and an HI of 1 is equivalent to 0–0.03 mmol/L free hemoglobin. PV-10 dilutions were prepared by diluting 1 volume PV-10 solution to the indicated total volume.
concentration increased in proportion to the PV-10 concentration on the DxC 800 and in proportion to HI values on the Roche Modular D. From the saline dilutions, we calculated the PV-10 concentration in the affected patient blood to be approximately equal to a dilution of 1 part in 5000, or approximately 20 μmol/L.

To determine the mechanism of the interference, we manually added the bilirubin reagents and the pooled plasma spiked with PV-10 in the same ratios as per the manufacturers’ methods. Absorbances were measured on the Beckman Coulter DU 640 spectrophotometer. The 2 bilirubin methods measure primary/secondary absorbances at 520 nm/580 nm with the Beckman Coulter method and 546 nm/600 nm with the Roche method. Blanking is performed after the addition of reagent A and reagent B and without sample in the Beckman Coulter method, and after reagent 1 and sample are added in the Roche method. Both assays use diazo methods, with azobilirubin being the final product. In both methods, the PV-10 is either chemically altered or compounds are formed with the first reagent in both methods; the compound(s) has a peak absorbance at 562 nm. Blanking with the Beckman Coulter method would not correct for nonspecific chromogens from samples with PV-10, hence the interference. In contrast, blanking with the Roche method is able to correct for nonspecific chromogens(s) from the sample; in fact, the negative values in Table 1 suggest that it may overcorrect. With the Beckman Coulter serum indices method, the sample is added to saline, and absorbances are measured at 570 nm and 600 nm for the HI. The presence of PV-10 causes a substantial absorbance increase at 570 nm.

On the Beckman Coulter analyzers, the icteric index and the direct bilirubin concentration (in which sample blanking is performed with reagent A) remain unchanged in the presence of PV-10. Discordant Beckman Coulter total bilirubin results with no change in the icteric index or the direct bilirubin value and/or an increased HI from the Roche analyzer in the absence of changes in other analytes (e.g., K⁺, lactate dehydrogenase, and so on) may indicate the presence of PV-10. The manufacturer of PV-10 has been informed of these findings. The PV-10 interference illustrates how different method-blanking procedures can help eliminate interferences.

Some details of the success of the PV-10 trial have been published (1), and full details undoubtedly will be published in the near future. In light of the present findings, the trial protocol for the timing of postinjection sample collection should be reviewed. Plans are under way for additional trials of PV-10 for treating other cancers. Therefore, these interferences may become more common with the assays outlined above and potentially with others on different analytical systems.

**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 of Potential Conflicts of Interest:** No authors declared any potential conflicts of interest.

---

**Role of Sponsor:** The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

**Reference**


**Goce Dimeski**

**Brock Jones**

**Jacobus P.J. Ungerer**

Department of Chemical Pathology
Pathology Queensland
Princess Alexandra Hospital
Ipswich Road, Woolloongabba
Queensland, Australia

*Address correspondence to this author at:
Department of Chemical Pathology
Pathology Queensland
Princess Alexandra Hospital
Ipswich Road, Woolloongabba
Queensland, Australia, 4102
Fax 61 7 3240 7070
E-mail goce_dimeski@health.qld.gov.au

Previously published online at DOI: 10.1373/clinchem.2008.116731

---

**Some Notes on Visinin-Like Protein 1 and Alzheimer Disease**

**To the Editor:**

We wish to comment and add some potentially useful information on certain aspects of the report of Lee and collaborators, which was recently published in *Clinical Chemistry* (1). In an earlier study, the authors had proposed visinin-like protein 1 [VILIP-1<sup>1</sup> or VSNL1; VSNL1 gene (visinin-like 1)] as a