A pilot study to investigate interference by the dye brilliant blue G (BBG) on plasma biochemical values in dogs

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Introduction
Following acute spinal cord injury, adenosine triphosphate (ATP) is released into the extracellular space, resulting in activation of a purinergic receptor, P2X7, found in the spinal cord. Activation of P2X7 initiates a cascade of secondary events leading to irreversible cell death. A P2X7 selective antagonist, brilliant blue G (BBG), has potential to mitigate acute spinal cord injury. IV BBG was proven safe and showed to improve histologic and functional neurologic outcome in a rat model. A recent study at our institution showed that IV BBG caused no adverse effects in dogs (Jill Narak, unpublished data). Discoloration of sclera, mucous membranes, skin, and nail beds was observed. Assessment of chemistry data from that study (obtained using a Roche Cobas c 501) revealed apparent interference, with marked discoloration of plasma and markedly increased lipemia index reported for all dogs given BBG 50 mg/kg IV.

Materials and methods
To examine the effect of BBG on analyte measurement and calculation of lipemia, hemolysis, and icterus indices, three levels of quality control material (QCM), chosen to mimic low, normal, and high analyte concentrations, were adulterated with BBG 0.08 µg/mL. A canine biochemical profile was measured in duplicate on both adulterated and non-adulterated samples using a Roche Cobas c 501; ANOVA was used to test for differences.

Results
Statistically significant differences between non-adulterated and BBG-adulterated samples were observed for BUN, cholesterol, chloride, TCO2, and triglyceride for all QCM; however, impact on analyte concentration was small and considered clinically unimportant. Statistically significant differences for lipemia index (increase in BBG-adulterated samples) and icterus index (decrease in BBG-adulterated samples) were observed for all QCM. A statistically significant difference for hemolysis index (decrease in BBG-adulterated samples) was observed only for the QCM having high analyte concentrations/activities.

Discussion & Conclusions
This pilot study involving a small number of data points suggests BBG interferes with calculation of lipemia, icterus, and hemolysis indices on the Cobas c 501. Interference is likely due to overlap of the BBG absorbance curve (peak absorbance 610 nm) with wavelengths used to calculate these indices (Table 1). Similar interference caused by Patent Blue V has been reported for the Roche Modular instrument. No clinically significant direct effect on biochemical values was observed in this study, similar to our results. Inability to identify properly the presence of interferents has potential to adversely affect patient results. Further study using various quality plasma samples is indicated.

Table 1. Wavelengths used by the Cobas c 501 to calculate plasma indices.

<table>
<thead>
<tr>
<th>Index</th>
<th>Wavelength (nm)</th>
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<tbody>
<tr>
<td>Lipemia</td>
<td>660</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>570</td>
</tr>
<tr>
<td>Icterus</td>
<td>480</td>
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Figure 1. Discolored sclera (top), oral mucous membranes (middle), and skin inside an ear pinna (bottom) in a dog given BBG 50 mg/kg IV.

Figure 2. Change in plasma indices following adulteration of QCM with 0.08 µg/mL BBG. The asterisk denotes a statistically significant difference.

Figure 3. Unadulterated QCM (left) and QCM adulterated with 0.08 µg/mL BBG (right).

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Literature Cited