
Hans M. C. Heick
Aziz Mohammed

Children's Hosp. of Eastern Ontario
401 Smyth Road
Ottawa, Ontario
K1H 8L1, Canada

The authors of the Letter in question respond:

To the Editor:

We have seriously considered the comments of Dr. Heick. None of the factors he suggests can account for the relatively large dilutional effects we reported for a Coomassie Blue dye-binding method. We used new plates, test specimens and standards were treated identically (exactly the same timing), and the urines were precisely diluted with water with use of micropipettes before analysis. We also considered possible pH and ionic strength effects, but the contents of the Coomassie Blue reagent suggested that these factors could not account for the observed changes. Molar amounts of phosphoric acid ensured a final pH of approximately 1.3 with high enough ionic strength to be little affected by urine salt content.

Recently, six additional urine specimens were analyzed by a different investigator, using a new lot of the dye solution (the original lot was no longer available). The results are summarized below:

<table>
<thead>
<tr>
<th>Urine no.</th>
<th>Dilution factor</th>
<th>Protein, mg/L (corr. for diln.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>980</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>800 (-18)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>600 (-39)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>920</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>780 (-15)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>600 (-35)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1060</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>800 (-25)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1100 (-21)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2650</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2800 (+5.7)</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>2900</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3300 (+14)</td>
</tr>
</tbody>
</table>

Changes (in %) compared with the smallest diln.

As we reported in our Letter, systematic effects on the measured protein concentrations were caused by dilution of the urines. The effects, in either direction, became more pronounced upon increasing dilution. The dilutional effects observed with this lot of reagent were not as pronounced. Nevertheless, dilution-corrected values for four of the six urinary protein concentrations decreased by more than 20% after fivelfold dilution. A two- to threefold dilution changed the measured protein concentration in both directions by percentages ranging from 8 to 25%

For urines 5 and 6, with higher protein concentrations, dilution probably gives rise to disaggregation of the multimeric proteins expected in more severe proteinurias, thus exposing more binding sites for the dye and resulting in the increase in measured protein. The decrease in measured protein after dilution of most urine specimens needs further investigation. However, those using a Coomassie Blue dye-binding method should be alert to these dilutional effects, which in some cases can significantly alter the clinical interpretation of the results.

Shahram Shahgian
Paul I. Brown
K. Owen Ash

Dept. of Pathol.
Univ. of Utah Med. Center
Salt Lake City, UT 84132

Ed. note: Greg Simpson and Ronald Feld (Univ. of Iowa) have informed us of experiments on dilutions of CAP urine protein reference material and 12 urine specimens. Two- to eightfold dilutions with isotonic saline gave the expected values, within experimental error. They did find that the dye binds to the plastic of the reagent transfer tubing of the instrument they used (Multistat III centrifugal analyzer), and took steps to remove this source of error. Otherwise, the conflicting results reported above are unexplained.

ASTRA Creatinine Reagent Stability

To the Editor:

Bromberg et al. (1), in their instrument evaluation, state that: "The Beckman CRE is stable for one week and can be used on the RA-1000." The following correction is offered to the above statement: "The Beckman Alkaline-Picrate Reagent is a special purpose solution. . . . From date of preparation for use, working reagent is stable for 30 days at ambient temperature." (2).

In light of the documented ASTRA creatinine-reagent stability, the questions now raised are as follows:

Can the Beckman ASTRA creatinine reagent be used in the RA-1000 for 30 days? If not, how long can it be used before it must be replaced?

We wish to dispel any misconceptions that may arise about Beckman's ASTRA creatinine-reagent stability. We hope this letter will encourage other investigators to reporting additional uses for ASTRA reagents.

References
2. "Beckman Creatinine Chemistry Module Operating and Service Instructions." Beckman Instruments, Inc. 200 South Kraemer Blvd., Brea, CA 92621, March 1982.

Alan Posner
Clinical Applications
Automated Chemistry Systems
Beckman Instruments, Inc.
Brea, CA 92621

Changes in Serum Creatine Kinase BB Activity in a Patient with Chest Pain

To the Editor:

After an emergency admission to Glasgow Royal Infirmary with pain in the right upper quadrant, tentatively diagnosed as cholecystitis, a 71-year-old man developed a crushing chest pain. Five years previously he had undergone resection of a colorectal tumor, and micro metastases to the liver were noted. There was also a nine-year history of angina.

An electrocardiogram revealed evidence of an old inferior myocardial infarction, and a cardiac enzyme profile [aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), creatine kinase (CK, EC 2.7.3.2), and lactate dehydrogenase (LDH, EC 1.1.1.27)] showed increased CK and LDH activity. The LDH isoenzyme pattern showed LD2 = 34%, LD3 = 30%, LD1 = 14%, LD5 = 13%, LD4 = 9%. Estimation of CK-B subunit activity (kit from E. Merck, Darmstadt, F.R.G.), at 37 °C, with blank subtraction (1), showed a CK-B/CK ratio of 30%. Electrophoresis of the sample on agarose gel (Corning system), with ultraviolet visualization of the separation showed the presence of CK-MM and CK-BB isoenzymes.

Table 1 shows the serial results for this patient, who was discharged two weeks after admission and lost to follow-up.

The negative electrocardiographic findings and the absence of CK-MB on