Specimen Processing and Renin Activity in Plasma

To the Editor:

Renin activity in plasma (PRA) was the topic of three recent publications from the Cornell University Medical College laboratory (1-3). In the protocol for processing specimens for PRA, Sealey (2) cautioned against collecting samples on ice, refrigerated centrifugation, and thawing frozen samples slowly. Such practices cause an increase in PRA results because of the cryoactivation of prorenin. However, the universal recommendation of reference laboratories and commercial kits, as Sealey so thoroughly reviewed (2), directly contradicts the Sealey-Laragh protocol. Our laboratory compared PRA values from plasma samples processed by the Sealey-Laragh protocol (2) with those processed according to directions from the Incstar Gamma Coat (125I) PRA RIA kit (cat. no. CA 533; Incstar Corp., Stillwater, MN). This kit was the most commonly used PRA procedure in the recent College of American Pathologists' interlaboratory comparison program.

Duplicate blood samples were collected into potassium EDTA-containing Vacutainer Tubes from 25 outpatient patients, selected without conscious bias from those having PRA measured for diagnostic purposes. One of the duplicate samples was placed on ice (designated A), while the other duplicate remained at room temperature (designated B). After 1 h, sample A was centrifuged at 6°C and sample B was centrifuged at room temperature, both for 15 min. Plasma from each was pipetted into identical plastic tubes and capped; plasma A was immediately stored in a standard freezer (−20°C), and plasma B was stored in a colder freezer (−30°C). The colder freezer was used to avoid exposure to the optimum cryoactivation temperature (<6°C) while the plasma was still liquid (2). Specimens remained frozen 1–13 weeks until assayed. On the day of assay, the A samples were thawed on ice for −2 h. The B samples were thawed after ~15 min in front of a fan at room temperature. The A and B duplicates were assayed at the same time according to the Incstar Gamma Coat kit directions. In our hands, the detection limit is 0.2 μg·L⁻¹·h⁻¹ and the intra-assay variation is ~10%.

The PRA was greater in the A samples than in the B samples for 18 of the 25 sample pairs. The mean (SD) PRA was 2.3 (3.2) μg·L⁻¹·h⁻¹ for the A samples and 1.7 (2.2) μg·L⁻¹·h⁻¹ for the B samples. The difference was significant by Student's paired t-test (P < 0.02). The percent difference between the A and B samples was as much as 250%. The greatest actual PRA difference was 5.3 μg·L⁻¹·h⁻¹ for one patient. PRA was the same for five pairs of samples, and two pairs had lower PRA in sample A.

Processing blood specimens by the Sealey-Laragh protocol (at room temperature) resulted in lower PRA values than when samples were processed on ice. Avoiding cryoactivation of prorenin to renin by processing at room temperature could explain the findings. The percentage increases in PRA resulting from cold processing observed here were comparable with those reported by Sealey (2). Moreover, an earlier study found similar percentage increases in PRA of plasma cryoactivated by freezing and thawing (4). The optimum temperature for cryoactivation of prorenin to renin appears to be <6°C, while the plasma is still liquid (2, 4). Once plasma is frozen, cryoactivation is negligible until thawing occurs. After 1 h, the temperature of a freshly drawn blood sample, in a 10-mL Vacutainer Tube standing up and covered with ice, was 4°C on the bottom and 11°C on the top. Thus, some portion of an unmixed tube of blood on ice would be at a temperature facilitating cryoactivation.

The difference in PRA between sample A and B was not clinically relevant in most cases, but two pairs did have substantial differences in PRA that may have affected clinical decisions. The PRA values from these two patients were 6.4 and 7.1 μg·L⁻¹·h⁻¹ in the B samples and 11.7 and 10.8 μg·L⁻¹·h⁻¹ in the A samples, respectively. Research studies investigating subtle changes in PRA may be less tolerant of these subclinical differences. Clearly, it is much easier to process PRA specimens by the Sealey-Laragh protocol than it is by current protocols. It is time for reference laboratories and manufacturers of commercial PRA kits to examine their recommendations for processing specimens for PRA.

References


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Interference with the Astra 8 and Synchro Cox 3 Assays of Urea Nitrogen in Serum; by a High-M, Inhibitor In a Patient with Multiple Myeloma

To the Editor:

We present an unusual case of a patient with multiple myeloma with a circulating large-molecular-mass inhibitor of the Astra/8 assay (Beckman Instruments, Brea, CA) of urea nitrogen in serum. A 70-year-old white woman with a 2.5-year history of multiple myeloma (IgGκ) presented to the Yale–New Haven Hospital for evaluation of progressive renal dysfunction of four months' duration. Chemotherapy with several courses of melphalan and prednisone, and subsequently vincristine, Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea], melphalan, prednisone, and doxorubicin, had been unsuccessful in controlling disease progression. At admission, the patient reported decreased urine volume, increased urinary frequency, and foamy