

To the Editor:

Renin, an aspartic protease, is rate limiting in the generation of angiotensin (Ang) II, which is a key regulator in the maintenance of salt and water balance by regulating aldosterone secretion and blood pressure (1). Renin is synthesized and secreted into the blood exclusively by the juxtaglomerular cells of the kidney from prorenin, an inactive precursor, which is produced not only by the kidney, but by other tissues, including the adrenal glands, gonads, and the uteroplacental unit (2). Whereas the secretion of renin is tightly controlled, prorenin secretion is not and is ;10-fold higher than renin (3).

Although peripheral conversion of prorenin to renin is limited and does not typically occur in vivo, in vitro conversion of prorenin to renin can occur by cryoactivation, so sample handling is critical (4). Historically, samples for renin activity have been collected on ice, frozen, and assayed at 2–8 °C. Cryoactivation occurs when samples are exposed to refrigerated temperatures (4 °C) for ex-

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tended periods of time (4–8). Because of the high concentration of prorenin relative to renin, cryoactivation can lead to a marked increase in renin concentration. Therefore, to avoid cryoactivation, samples should be collected and assayed at room temperature. Recently, IRMA assays have been developed to directly measure renin in the circulation. For many years, plasma renin activity (PRA) has been used to assess the in vivo activity of the renin-angiotensin system. With the PRA assay, plasma is incubated at 37 °C, and renin acts on angiotensinogen to generate Ang I, which is quantified

by RIA. Because plasma angiotensinogen concentrations may vary, PRA is not always a good measure of circulating renin concentrations. We compared renin results from two commercially available kits to determine the influence of sample handling on assay results, using samples collected at room temperature and on ice. The Active Renin kit (Nichols Institute Diagnostics) measures immunoreactive renin. The Rainen kit by DuPont measures PRA by quantification of generated Ang I. EDTA-plasma samples were collected from 41 healthy adults. Two tubes were immediately placed on ice, and two tubes were kept at room temperature. Iced tubes were centrifuged at 4 °C for 20 min, whereas room temperature tubes were centrifuged at 25 °C. Samples were aliquoted and stored at 270 °C until testing. Before testing, iced tubes were allowed to thaw in the refrigerator for ;2 h, whereas room temperature tubes were quick-thawed at room temperature using a fan. Samples were then assayed by both renin kits following the manufacturers' directions. To measure active renin, 200 mL of calibrator, control, or sample was pipetted into plastic tubes, and 100 mL of 125I-labeled renin antibody solution was added to each tube. The tubes were vortex-mixed, and an avidin-coated bead was added to each tube. The tubes were incubated at room temperature for 24 h. Beads were washed three times with 2 mL of working wash solution. Liquid was aspirated, and tubes were counted in a gamma counter for 2 min. The calibration curve was calculated using a four-parameter logistics program. In the assay of PRA, Ang I generation was performed in room temperature

samples at ambient temperature and in iced tubes in an ice bath. Control or sample (0.5 mL) was pipetted into plastic tubes, and 10 mL of dimercaprol solution and 10 mL of 8-hydroxyquinoline solution were added to each tube and mixed. Maleate buffer (1.0 mL) was then added to each tube. After mixing, 0.75 mL of each control or sample mixture was transferred to a second plastic tube. The tubes with the 0.75-mL aliquot were incubated at 37 °C for 1 h. At the end of the 1-h incubation, the 0.75-mL aliquot tubes were transferred to an ice bath.

For the Ang I RIA, 100 mL of calibrator, generated or nongenerated control, or generated or nongenerated sample was pipetted into plastic tubes, and 100 mL of tracer solution was added to all tubes. Blank antiserum (100 mL) was then added to the blank tubes, and 100 mL of antiserum was added to all tubes except the blank. Tubes were vortexmixed and incubated for 2 h. At the end of the first incubation, 0.5 mL of second antibody was added. The tubes were vortex-mixed and incubated for 30 min. At the end of the second incubation, the tubes were centrifuged and then decanted. Tubes were counted for 1 min. The data were calculated using a fourparameter logistics program. Results were multiplied by 3 to correct for dilution factor, and generated was subtracted from nongenerated to give the final result in mg z L²¹ z h²¹. Samples from 41 healthy volunteers were assayed for active renin and PRA. Correlation between the refrigerated and room temperature sample sets was very good for active renin: $y = 0.9681x + 1.05441$; $r^2 = 0.9895$. The average room temperature result was 100% of the average

iced result (Fig. 1A).

For PRA, correlation between the refrigerated and room temperature sample sets was good: $y = 1.1896x + 0.7892$; $r^2 = 0.8838$. The average room temperature result was 75% of the average iced result (Fig. 1B). The correlation (r^2) between PRA and AR was 0.83; $n = 41$; $P < 0.01$. Sample results for active renin showed little variation between re-

Fig. 1. Bland-Altman analysis (9) of PRA (A) and active renin values (B) for both ambient and refrigerated samples. Clinical Chemistry 46, No. 9, 2000 1443 frigerated and ambient samples. This indicates that limited exposure to 4 °C, such as in this evaluation, has minimal or no affect on active renin results. However, the manufacturers' data show a definite effect on samples stored at 4 °C for 24 h or more. These results are consistent with previous observations (2, 3).

The lower values for the ambient sample set for PRA indicates that sample collection and handling does affect assay results. Our findings suggest that the enzyme activity of renin is affected by sample handling, but its immunoassay detection is not influenced by ambient temperature. The apparent increase in renin activity from the refrigerated samples, therefore, does not suggest that 2 h on ice increases renin by cryoactivation of prorenin. Rather, renin enzyme activity appears to be decreased by processing at room temperature.

In addition, our results show a good correlation between PRA and immunoreactive renin. However, immunoreactive renin has advantages of less assay variation than PRA and is not limited by substrate concentrations.

In conclusion, prolonged exposure to refrigerator temperatures should be avoided to prevent cryoactivation of prorenin to renin, which leads to falsely increased renin results. Samples for measurement of immunoreactive renin may be collected at either 4 °C for 2 h or at ambient temperature before assay without affecting assay results. However, collection of samples for assay of PRA should be avoided because it will lead to lower assay values.

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