Determination of Urinary Alanine Aminopeptidase with a Cobas Bio Centrifugal Analyzer

To the Editor:

Assay of urinary alanine aminopeptidase (AAP, EC 3.4.11.2) is used increasingly to assess nephrotoxicity, and I have adapted an optimized method described by Jung and Scholz (1) for use with the Roche Cobas Bio centrifugal analyzer. The substrate, L-alanine-4-nitroanilide hydrochloride (cat. no. A3522), and bovine intestinal alanine aminopeptidase (cat. no. A8779) were purchased from Sigma Chemical Co. Ltd, Poole, U.K.

Working reagent: Mix 10 mL of Tris HCl (59 mmol/L, pH 7.8) and 1 mL of L-alanine-4-nitroanilide hydrochloride solution (26 mmol/L, in 59 mmol/L Tris HCl). This reagent is stable for at least 4 h at 18–22 °C.

Cobas Bio settings:
1 Units/L
2 Calculation factor 1010
3 Standard 1 concn 0
4 Standard 2 concn 0
5 Standard 3 concn 0
6 Limit 1
7 Temp., °C 37.0
8 Type of analysis 2
9 Wavelength, nm 410
10 Sample vol, μL 25
11 Diluent vol, μL 30
12 Reagent vol, μL 250
13 Incubation time, s 0
14 Start reagent vol 0
15 Time of first reading, s 100
16 Time interval, s 10
17 No. readings 21
18 Blanking mode 1
19 Printout mode 1

To measure within-batch precision, I used rat urine, human urine, and three different concentrations of bovine intestinal alanine aminopeptidase dissolved in isotonic sodium chloride. The means (U/L), SDs, and CVs (%) were 31.75, 0.34, 1.08; 8.70, 0.30, 3.45; 463.37, 11.51, 2.48; 96.88, 2.94, 3.03; and 47.25, 1.16, 2.46, respectively, where n = 28. Run-to-run precision was determined with use of intestinal alanine aminopeptidase solutions stored at 4 °C; the means (U/L), SDs, and CVs (%) were 48.10, 2.34, 4.95; 98.70, 4.68, 4.84; and 470.83, 15.27, 3.24, where n = 20. Some control sera appear to contain AAP activity, which can be used to monitor run-to-run precision (2), but the nature of the apparent enzyme activity is uncertain. Undiluted urine cannot be used for run-to-run precision studies because the enzyme is unstable, being particularly influenced by urine pH (3). We omit the preliminary step of gel filtration from the procedures described in the original paper (1) when we test rat urines, because urine volumes are usually small and the use of gel filtration in reducing inhibition of AAP activity is contradictory (1, 2).

Instead, I minimized these effects by increasing the ratio of sample to final volume from 1:6.5 to 1:1.2. I retained the use of Tris HCl, but at a slightly lower concentration than in the original method (1), although Tris reportedly inhibits the enzyme (4).

Using this method, and 5-h urine collections (kept at 4 °C) from food-deprived Wistar rats, I have been able to confirm the reported sex difference for urinary AAP in rats (5, 6). For Wistar rats six to 12 weeks old, the observed means and ranges (mU/5 h) were 449 (50 to 888) for males (n = 16), and 62 (22 to 120) for females (n = 16).

The existence of variant forms of AAP has been confirmed (7); thus it remains to be determined whether this method is optimized for both variants, or whether it can be modified to determine only a single variant, which may have a greater diagnostic significance.

References


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