Evaluation of a New Ferritin Radioimmunoassay Kit

To the Editor:

We have evaluated the new (125I) Ferritin Radioimmunoassay (RIA) Kit (Diagnostic Products Corp., Los Angeles, CA 90045), in which human liver ferritin calibrators in a protein matrix are used over a range of 0–1000 μg/L. Free is separated from bound by a single-step addition of a double antibody–polyethylene glycol–saline solution.

The procedure requires 100 μL of patient’s serum and involves a 3-h incubation at room temperature. Average initial bindings of bound/total (B/T) were about 30%, with nonspecific bindings of 2–4%. Calibrator bound/bound (B/B0) ranged from 96% for the 5 μg/L standard to 12% for the 1000 μg/L standard.

Table 1 shows the within-run precision we found, and comparable results for the Clinical Assays (125I) Ferritin RIA Kit (Cambridge, MA 02139).

Table 2: Linearity Data

Diluent pH and the Stability of the Thiol Group in Monothioglycerol, N-Acetyl-L-Cysteine, and 2-Mercaptoethanol

To the Editor:

Recently (1) we demonstrated that decreasing the pH of the diluent to 6.5 can stabilize human creatine kinase (EC 2.7.3.2) isoenzymes CK-3 and CK-2 for at least seven days at 4 °C in the absence of a protective sulfhydryl agent. How-
however, CK-1 stored at the same pH does require the presence of a sulphydryl compound, preferably monothioglycerol. The role of the protective sulphydryl compound thus seemed minimal and our findings suggested that the reducibility of the SH group on the protective sulphydryl compound did not have a pH dependence. We therefore decided to investigate, in more detail, the influence of diluent pH on thiol agent stability, because previous investigations (2-4) dealt only with diluents at the pH of normal serum (1).

Pooled patients' serum or buffer [50 mmol/L imidazole for pH 6.5 or 50 mmol/L tri(hydroxyethyl)methylamirine for pH 7.5 and 8.5] was used as diluent. Monothioglycerol, 2-mercaptoethanol, or N-acetyl-L-cysteine was added to the two types of diluents and the pH (at 20°C) was set to 6.5, 7.5, and 8.5. The stock N-acetyl-L-cysteine was neutralized to pH 7.0 before it was added to the diluents. The solutions were stored (1) in 1.5-mL conical polypropylene microcentrifuge tubes with caps (Canadian Scientific Products Ltd., London, Ontario, Canada) and kept at room temperature (20°C), 4°C, or −20°C. We assayed for reduced sulphydryl groups (2), with use of 5,5'-dithiobis(2-nitrobenzoic acid), at 0, 5, 24, and 48 h.

The stability of the thiol group is influenced by the storage temperature and the nature of the diluent (2, 3). At any one pH value the thiol agents stored in serum, at a final concentration of 25 mmol/L, are least stable at room temperature and most stable at −20°C. At 4°C, and at room temperature, monothioglycerol is about 30% more stable than N-acetyl-L-cysteine—the least stable of the sulphydryl compounds at these temperatures. Under these conditions the stability of 2-mercaptoethanol is intermediate to that of the other two sulphydryl compounds investigated. At these temperatures the diluent pH does not affect the stability of the sulphydryl compounds. At −20°C the stability of monothioglycerol is not affected by the pH of the diluent, whereas N-acetyl-L-cysteine (the same diluent at pH 8.5) is more stable at pH 7.5; it is less stable at other pH values, particularly at pH 5.5.

Pedersen and Jacobsen (5) showed that the protein content of human albumin increased its reactivity—as measured by exchange with 2,2'-dithiodipipyridine—as the pH changed from 5 to 8, above which the reactivity became independent of the pH value. Because albumin is the major protein in serum, we would have expected to observe a marked change in thiol agent oxidation between pH 6.0 and 8.0. Of course, other serum proteins may react with thiol, with pH profiles that are quite different from that for albumin, so that, as the case appears to be, thiol added to serum may be oxidized at rates almost independent of the serum pH value. It is therefore clear that the response to serum pH of CK-1 (or BB); in the presence of monothioglycerol, that was observed earlier (1) is not ascribable to a pH-dependent variation in its rate of oxidation.

The extent of deterioration of thiol in human serum is also concentration-dependent. Generally, independent of diluent pH, the higher the concentration of the sulphydryl compound in serum the less oxidized and hence the more stable it is. This is particularly evident with N-acetyl-L-cysteine at any pH value; doubling its concentration to 50 mmol/L results in a 30 to 40% increase in the amount of reduced sulphydryl group recovered after 48 h of storage. Our results—the higher the thiol group concentration the greater the recovery of reduced thiol groups—agree with those of Szasz et al. (3). They preferred N-acetyl-L-cysteine over monothioglycerol or 2-mercaptoethanol because the latter two at the suggested 50 mmol/L concentration cause turbidity and gel-ling in the sera, whereas N-acetyl-L-cysteine does not. However, this problem is only evident at temperatures >30°C, temperatures unlikely to be used for the storage of creatine kinase isoenzymes. In addition, Szasz et al. (3) reported that the decomposition products of N-acetyl-L-cysteine diminish the creatine kinase activity less than do 2-mercaptoethanol or monothioglycerol. However, they demonstrated that monothioglycerol is more stable to storage in human serum at 25 and 4°C for 48 h or longer than is either N-acetyl-L-cysteine or 2-mercaptoethanol; our own results confirm this finding. This evidence further strengthens our recommendation that monothioglycerol is currently the thiol agent of choice for stabilizing the human creatine kinase isoenzymes in patients' serum samples stored at 4°C.

Figures illustrating details of these studies are obtainable on request to the authors or the Editorial Office of this journal.

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References


D. A. Nealon
S. M. Pettit

A. R. Henderson

Dept. of Clin. Biochem.
Univ. Hosp.
339 Windermere Rd.
London, Ontario N6A 5A5
Canada

Nafcillin May Cause a Subtle Pseudoproteinuria

To the Editor:

Nafcillin [6-(2-ethoxy-1-naphtha-
dio)penicilloic acid] is a beta-lacta-
mase-resistant, semi-synthetic penicillin that is frequently used to treat serious staphylococcal infection. The manufacturer's (Upjohn, Wyeth Laboratories, Philadelphia, PA 19101) package insert warns of the need for "periodic assessment of organ system function, including renal, . . ." while nafcillin is being administered. Proteinuria in excess of 200 mg/24 h is strongly suggestive of renal dysfunction, so it is essential that urinary protein assay methods be specific for protein. There has been but a single report describing massive pseudoproteinuria caused by nafcillin when either trichloroacetic acid or sulfosalicylic acid methods are used for urinary protein analysis (1). A recent review of drugs associated with pseudoproteinuria included several of the synthetic penicillins, but not nafcillin (2).

We report here our observation that nafcillin may cause a subtle pseudoproteinuria that might go unrecognized as such, especially if the common turbidimetric methods for urinary protein assay are used.

Protein concentrations were estimated in urines obtained from patients receiving nafcillin intravenously, 150–250 mg/kg body wt. per 24 h, for treatment of severe systemic staphylococcal infection. None of the patients had pre-existing renal dysfunction; all had normal results for urinalysis, serum creatinine, and urea nitrogen concentrations, and for creatinine clearance.

After gently mixing 1.5 mL of either trichloroacetic acid, 50 g/L, or sulfosalicylic acid, 30 g/L, with 0.5 mL of urine

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