References

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The authors of the Letter reply:

To the Editor:

Our response to Pascali and Pezzi- li's three points is as follows:
1. The centrifugal microconcentrator membrane we used was the Centricon 10 unit with a 10,000-Do cutoff.
2. Detection and demonstration of pure proteins is only possible if the sample preparation is rigorous. In these samples, the presence of albumin, and gammaglobulin in urine is clearly demonstrated.
3. The diagnosis of our patients was definitive MS, made by clinical evaluation, chemical tests, and magnetic resonance imaging (MRI). The patients had active disease when they were selected for treatment.

Our study does not rule out the possibility of Bence Jones proteins in urine of some MS patients after a concentration of 300- to 600-fold. Concentrating urine to such a high degree is not a routine procedure in the clinical laboratory. It should also be taken into consideration that Bence Jones proteins—benign, idiopathic, and of undetermined significance—have also been reported in urine (2).

References

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Calibration Problems with the 125I Immunoassay for Measuring Cyclosporin A

To the Editor:

Currently, two radioimmunoassays (RIA) are commercially available for determination of cyclosporine (CsA) in whole blood. In 1987, a monoclonal antibody specific for CsA was introduced into an RIA kit ("Sandimmune" kit; Sandoz, Basel, Switzerland) involving a tritium-labeled tracer. We, along with others, have evaluated the specificity and performance of this assay (1, 2). This assay (y) gave values in whole blood from renal-transplant recipients that averaged 8-10% higher than those measured with HPLC (x) (regression equation: y = 8.63 + 1.04x; r = 0.97; n = 77).

In 1988, a method that included both this monoclonal specific antibody and a 125I tracer was introduced (Cyclo-Trac™ SP-Whole Blood RIA Kit, Incstar Corp., Stillwater, MN). This assay has many advantages over the Sandoz assay with respect to time and ease of use. We compared this Incstar assay (y) with the present Sandoz assay (x) for whole-blood specimens from renal-transplant recipients. The regression equation for the results (y = 25.5 + 0.53x; r = 0.94; n = 157) indicated both constant and proportional error. This agrees with the report by Knepl and McPhillips (3), whose regression equation was y = 38 + 0.85x (r = 0.96; n = 140). In contrast, Wolf et al. (4) found a good correlation between this assay and HPLC and, furthermore, found that the Sandoz RIA gave results higher than those obtained with the Incstar assay.

In February 1989, Incstar restandardized their assay, using a USP standard of CsA. We re-evaluated this modified method to assess its performance. However, in a correlation study with whole-blood specimens from renal-transplant patients, we observed a bias toward higher values than were obtained with the previous version of the kit. This time, the Incstar assay (y) gave values that were on average 35% higher than those obtained with the present Sandoz assay (x): y = 19.8 + 1.26x; n = 25. Similarly, this assay (y) also demonstrated values approximately 40% higher than those obtained by HPLC (x): y = 11.3 + 1.33x; n = 25. However, for the same patients’ specimens, the values obtained with the present Sandoz assay (y) correlated well with those obtained by HPLC (x): y = 4.4 + 0.93x; n = 25.

We further investigated what we believed to be a standardization problem by incorporating the use of Sandoz standards (whole-blood matrix) in the Incstar assay. This restandardization of the latter assay gave values for whole-blood specimens (y) that were closer to those obtained with the Sandoz assay (x): y = 9.7 + 1.14x; r = 0.99. This is in agreement with the results previously reported by Knepl and McPhillips (3).

We believe that the standardization problem may be a result of inappropriate assignment of standard values or a difference in the matrix composition of the standards. The Inestar standards are prepared in stripped serum, whereas the patients’ samples analyzed are whole blood. If laboratories currently using the Incstar method want to report CsA values similar to those obtained by HPLC or by the Sandoz RIA, we recommend that they recalibrate the assay with whole-blood standards prepared in-house.

References
1. Bell PE, Munzer H, Keller HP, Abiah E, Rosenthaler J. Specific 1H radioimmunoass-