Determining Total Protein Content of Urine: Centrifugation Results in Precipitation of the Protein–Coomassie Brilliant Blue Dye Complex

To the Editor:

The Coomassie Brilliant Blue (CBB) protein dye-binding assay (1) has been favorably assessed for determining the total protein content of urine (2–4), although discrepancies have been noted in its response to different proteins (5) and sample dilution (6). It is commonly accepted that the protein–CBB dye complex is soluble under assay conditions, but our studies reveal loss of color yield after microfugation or filtration of assay tubes containing protein standard or human urine. Precipitation of the complex occurs immediately after dye reagent is added and could cause problems in automated analysis.

Three urine pools, each comprising 8–10 specimens, were prepared by mixing equal volumes of early morning midstream samples. Bovine albumin (protein standard) was freshly prepared at 1 g/L (based on %A_{280} = 6.6) in 0.15 mol/L sodium chloride and microfuged immediately before assay. The CBB dye reagents were prepared as recommended (1, 7, 8) or purchased from Bio-Rad Labs. (Richmond, CA). The increase in absorbance ($A_{295}$ or $A_{300}$) was measured against a reagent blank after 10 min with a Jenway Model 6100 spectrophotometer (Dun- nsw, UK). The assay mixtures were then microfuged ($13,400 \times g$, 5 min; M.E. Micro Centaur Centrifuge, Loughborough, UK) or filtered (0.2 μm; Min-Art filter unit, Sarotius, Bel- mont, UK) and absorbance measurements were repeated on the respective supernatants and filtrates. The assay protocols were as follows:

- Bradford microassay (1): We added 1 mL of Bradford dye reagent (per liter: 0.1 g CBB G-250 (Sigma Chemical Co., St. Louis, MO), 1.6 mol phosphoric acid, and 0.8 mol ethanol) to 5–20 μg samples of the standard protein (0.2 g/L) or 25–100 μL of the urine pool in a sample volume of 0.1 mL.
- Read and Northcote standard assay (7): We added 950 μL of dye reagent (per liter: 0.1 g Blue G (Serva, Heidelberg, FRG), 1.6 mol phosphoric acid, and 0.8 mol ethanol) to 5–20 μg samples of the standard protein (0.4 g/L) or 5–50 μL of the urine pool in a sample volume of 50 μL.
- Bio-Rad microassay: We added 0.2 mL of the commercial dye reagent to 2.5–10 μg samples of the standard protein (0.015 g/L) or 100–400 μL of the urine pool in a sample volume of 0.8 mL.
- Sedmak and Grossberg standard assay (8): We added 500 μL of dye reagent (per liter: 0.6 g Serva Blue G and 0.3 mol perchloric acid) to 25–100 μg of standard protein (1 g/L) or 100–500 μL of the urine pool in a sample volume of 500 μL.

Figure 1 shows the color yield response of the Read and Northcote standard assay to human urine and protein standard. Microfugation resulted in visible precipitation of the blue protein–CBB dye complex with consequent loss of color yield in the respective supernatants (Figure 1). Similar results were obtained with the Bradford microassay and the Sedmak and Grossberg standard assay. In contrast, the Bio-Rad microassay showed incomplete precipitation with an average of 60% recovery in the urine supernatants and 30–90% recovery in the protein-standard supernatants (recovery increased with standard protein amount). Filtration of duplicate portions of the assay mixtures (urine or protein standard) resulted in complete loss of color yield in the recovered filtrates. This was observed with all of the assays tested, including the Bio-Rad microassay.

CBB–protein dye-binding assays are designed to permit interaction between the dye and protein under acidic conditions while maintaining protein solubility (1, 2). The initial dye complex is assumed to be soluble, although it is widely acknowledged that precipitation occurs in the longer term and hence absorbance values should be recorded within 1 h of mixing. The present study indicates that centrifugation or filtration of the assay mix 10 min after adding dye reagent (to urine samples or standard protein) results in complete loss of color yield in the supernatants and filtrates. Thus, the assay measures formation of an insoluble protein–dye complex. This could be important in automated systems incorporating centrifugal analysis and may help explain the variable response of these assays to different proteins (5) and their susceptibility to dilution effects (6) and interference from detergents (1).

References
7. Sedmak JJ, Grossberg SE. A rapid, sen-

Thomas Marshall
Katherine M. Williams
Biochem. Res. Lab.
School of Pharmaceut. & Chem. Sci.
Sunderland Polytechnic
Galen Building
Green Terrace
Sunderland
SR2 7EE
Tyne & Wear
UK

Contaminating DNA in RNA Amplification by Polymerase Chain Reaction

To the Editor:

Some suppliers offer kits to isolate mRNA for amplification without mention of the need for previous DNase treatment of the samples. Many authors of research papers also omit this treatment (and/or negative controls) when using such techniques. We tested various kits and procedures to determine the DNA contamination in the mRNA preparations. For this we prepared human lymphocytes by using Lymphoprep™ (Nycomed as Diagnostica, Oslo, Norway). The methods we assessed were those of (a) Chirgwin et al. (1), (b) Higuchi (2), (c) Quick-prep™ mRNA kit purchased from Pharmacia (Uppsala, Sweden), and (d) RNAGents™ Total RNA Isolation Kit purchased from Promega (Madison, WI). The concentration and purity of the RNAs were determined by spectrophotometry (A260/A280 ratio of 1.8–2.0). To study the possible contamination by DNA, we measured the fluorescence of the samples stained with Hoescht 33258 (Hoefer Scientific Instruments, San Francisco, CA). All showed undetectable DNA (<10 µg/L), except sample d.

RNA amplification was carried out with the commercial primers DM151 (5'-GCTCTGAACTTACGAACTCTTCTATC-3') and DM152 (5'-CATGTCAATTTCTGCAGTCCAC3') which amplify a 420-bp sequence from IL-1α mRNA (3); IL-1α mRNA was used as control template (purchased from Perkin-Elmer Cetus Corp., Norwalk, CT). The negative controls were positive either in the amplification or reamplification assays (negative controls were done as a DNA amplification for RNA samples).

Only when the RNA samples were treated with DNase did negative controls stay negative. These results suggest that even a final oligo(dT) affinity-column purification of mRNA cannot provide mRNA of sufficient purity for direct RNA amplification without a previous DNase treatment.

In conclusion, we believe that RNA amplification procedures must include DNase pretreatment even when DNA is undetectable.

References

A. Reyes
J. L. Dieguez
I. García-Chacón
M. Morell
M. Ruiz
J. C. Montilla

Dept. de Bioquím. y Biol. Mol.
Facultad de Med.
Universidad de Málaga
29080 Málaga
Spain

Comparison of Cyclosporine Blood Concentrations Measured by Radioluminometry and Two Nonisotopic Immunoassays Using Monoclonal Antibodies

To the Editor:

Routine monitoring of cyclosporine (CsA) in whole blood by a selective assay that specifically measures the parent drug and not its metabolites has been recommended (1). Recently, two nonisotopic semiautomated immunoassays—an enzyme-multiplied immuno technique (EMIT; Syva Co., Palo Alto, CA) and a fluorescence polarization immunoassay (FPIA; Abbott Labs., North Chicago, IL)—involved two selective monoclonal antibodies were introduced.

We evaluated the new assays by using trough blood samples, containing EDTA, from 123 transplant recipients—58 renal transplant recipients (RTs) and 74 liver transplant recipients (LTs)—and compared the results with our previously validated method (RIA 125I monoclonal specific INCSStar (Stillwater, MN)). The EMIT method was performed with a Cobas-Mira analyzer (Hoffmann-La Roche Co., Ltd., Basel, Switzerland) and the FPIA with a TDx analyzer (Abbott). All methods were performed according to the manufacturers' recommendations.

To assess the imprecision, we used three commercially prepared controls [Bio-Rad Labs. (Cambridge, MA): B1, B2, and B3] and two blood pools from patients receiving CsA (P1 and P2). During our comparative measurements, the interassay CVs for the EMIT were 12% for P1 (n = 26, x̄ = 68 µg/L), 5% for P2 (n = 26, x̄ = 281 µg/L), 8.9% for B1 (n = 26, x̄ = 82 µg/L), 7.8% for B2 (n = 26, x̄ = 201 µg/L), and 7.1% for B3 (n = 26, x̄ = 364 µg/L) and for the TDx were 9.2% for P1 (n = 11, x̄ = 67 µg/L), 6.4% for P2 (n = 11, x̄ = 340 µg/L), 7.0% for B1 (n = 8, x̄ = 70 µg/L), 4.6% for B2 (n = 8, x̄ = 215 µg/L), and 4.2% for B3 (n = 8, x̄ = 389 µg/L).

Recovery was studied by adding different standard concentrations of CsA to a patient sample and ranged from 93% to 105% for EMIT and from 94% to 108% for TDx. EMIT and TDx correlation to the reference method resulted in the following least-squares regression equations: EMIT = 0.908 RIA + 8.11, r = 0.990, and TDx = 1.118 RIA + 4.169, r = 0.987. When the samples were divided in two groups, RTs and LTs, we obtained the following data for

![Fig. 1. CsA (A) and bilirubin and CsA plus metabolites (B) concentrations in a liver-transplant patient during the immediate post-transplant period.](image-url)

(A) CsA concentrations measured by EMIT, TDx, and RIA; (B) CsA concentration was measured by RIA (non-specific INCStar)