Table 1. Day-to-Day Precision [Mean, SD (CV%), n = 12] of Various Control Materials

	Measured		Calculated	
	Magnesium, μmol/L	Protein, mg/L	Mg, μmol/g protein	Mg, fmol/cell
Control 1	8.39 ± 0.22 (2.6%)			
Control 2		130 ± 8 (5.5%)		
Control 3	5.86 ± 0.31 (5.3%)	82 ± 8 (11%)	73 ± 12 (16%)	
Control 4			65 ± 8 (12%)	6.0 ± 1.9 (32%)

Table 2. Concentration of Magnesium in Mononuclear Cells [Mean, SD (CV%)] of Healthy Individuals, as Reported by Different Authors

	Ref. no.	Mg, fmol/cell	Mg, μmol/g protein
Elin and Johnson	6	3.3 ± 0.9 (28%)	59 ± 14 (24%)
Elin and Hosseini	7	2.9 ± 0.6 (20%)	
Ryzen et al.	8		55
Reinhart et al.	9	2.8 ± 0.6 (19%)	
Sjøgren et al.	10	5.4 ± 1.9 (33%)	73 ± 16 (22%)
Here		4.0 ± 0.9 (24%)	63 ± 10 (15%)

individual, and analytical variation all contribute to this variation, but the present results reveal that analytical variation is the main source. An improved method might therefore make the Mg/protein estimate in patients more reproducible and clinically more useful than it appears to be at present.

There is no reason to believe that the Mg/cell estimate is more reproducible than the Mg/protein estimate. On the contrary, the consistent finding that the Mg/cell range is even wider (Table 2) does suggest that, if there is any difference, the Mg/cell estimate is subject to more analytical variation than is the Mg/protein estimate.

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Microalbuminuria in Diabetes Mellitus: More on Urine Storage and Accuracy of Colorimetric Assays

To the Editor:

Diabetics have a high risk of hypertension and renal disease, so assay of urinary albumin excretion (UAE) has recently assumed a central role in the prevention and follow-up of diabetic nephropathy (*1*). Because screening for UAE has become part of routine diabetes care (*1*) and concerns a great number of patients, a suitable method should be used for measuring UAE. Because its concentration in normal urine is low, albumin is usually undetectable by standard laboratory methods (precipitation or "dipstick" techniques). Thus, more-sensitive immunological procedures, such as RIA, EIA, ELISA, immunoturbidimetric assay, fluoroimmunoassay, and laser immunonephelometric assay have been proposed for routine measurement of UAE (*2*). Moreover, because of the great number of samples that may have to be processed, urine specimens are often stored before assay.

Elving et al. (*3*) claimed that freezing of urine samples for determination of UAE by laser immunonephelometry may yield falsely low results, thus suggesting that urines should be stored at 4 °C and assayed within two weeks.

We previously found no decrease in albumin values measured by RIA in several (n = 4) freezing and thawing experiments performed during three weeks on 10 urine samples with various albumin concentrations (*4*).

Moreover, in Figure 1, we have reported the time courses of albumin values measured in three urine pools by two different RIA methods, both used according to the manufacturer's instructions, as reported (*4, 5*). A low- and an intermediate-level pool (pools 1 and 2, part *B* of Figure 1) were assayed by an RIA in which double antibody is used for bound/free separation (H-Albumin kit; Sclavo Diagnostic and Instruments Division, Cinisello Balsamo, Italy). Another intermediate-level pool (pool 3, part *A* of Figure 1) was assayed by an RIA in which a solid phase is used for bound/free separation (Albumin RIA 100 kit; Pharmacia AB, Uppsala, Sweden). Each pool was divided into various 0.5-mL aliquots stored at −20 °C until assay. The assay CVs were 9.76%, 7.03%, and 5.2%, for pools 1, 2, and 3, respectively (Figure 1). A very slight, but significant decrease of albumin concentration was found only in the low-level pool (pool 1, part *B* of Figure 1); the values of the two intermediate-level pools (pools 2 and 3) did not decrease. The rate of decrement of albumin concentration for pool 1, as estimated from the linear-regression analysis, was only 3.5 μg/L per day (about 0.6 mg/L for six months).

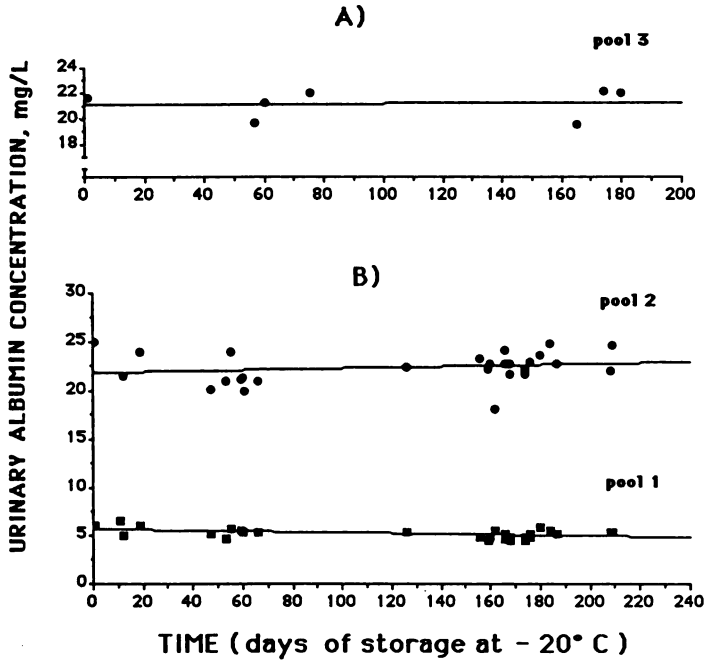

Fig. 1. Time courses of urinary albumin concentration as measured by two different RIAs

Measured albumin values are reported on the vertical axis (*y*-axis); the days of storage at −20 °C until assay are reported on the horizontal axis (*x*-axis). Pool 3 (part *A*) was assayed by an RIA that involves a double-antibody system for separation of bound/free phases. Pools 1 and 2 were assayed by a solid-phase RIA (part *B*)

Therefore, although it was significant ($r = 0.474, P = 0.011$) from the statistical viewpoint, this decrease is of no clinical relevance. Hence, in our experience, storage of urine samples at −20 °C for six months does not affect albumin concentration in the range of albuminuria (15–30 mg/L) that is critical for correctly discriminating between normo- and micro-albuminuric diabetic patients.

How can we explain the discrepancy between our results and those of Elving et al. (*3*)? Differences in methodology (RIA vs laser immunonephelometry) or in the accuracy of the mode of urine collection may play a role. In particular, it could be important to check the sterility of urine specimens. In our laboratory, bacterial growth is currently excluded by negativity of urine cultures (*4*). In the study of Elving et al. (*3*) this precaution has not been reported.

In conclusion, in our hands, long-term (up to six months) storage of urine specimens at −20 °C does not impair the accuracy of UAE evaluation by radioimmunological methods.

Phillipou et al. (*6*) compared the analytical performance of a colorimetric method based on the pyrogallol red–molybdate(IV) reagent with that of a laser nephelometric procedure; they suggest that the colorimetric method is suitable for screening diabetic patients for microalbuminuria.

We have recently compared the chemico-clinical characteristics of an RIA technique with those of a colorimetric assay based on the Coomassie Blue dye-binding method (*7*). Our purpose was to verify if it is really possible to calculate albuminuria from proteinuria (and vice versa) by a simple linear-regression equation, as suggested previously by Pun et al. (*8*).

The colorimetric assay did significantly overestimate albumin concentration as measured by RIA (*7*), especially in the range of albumin excretion rate (15–30 μg/min) known to be predictive of the future development of overt diabetic nephropathy—which needs, therefore, to be reliably unmasked and monitored by an accurate and sensitive procedure. In addition, we found (*7*) that a nonlinear regression fit significantly better the relationship between the values of proteinuria (as measured by colorimetric assay) and those of albuminuria (as measured by RIA) in diabetic patients ($r = 0.8307$, n = 70) than did a linear regression.

Also in the study of Phillipou et al. (*6*) (Figure 1 of their paper) the colorimetric assay greatly (about 2.5 times) overestimated albuminuria as measured by laser immunonephelometric assay. In addition, the correlation between the albumin values obtained by the two methods was significant, but not excellent ($r = 0.74$, for measured protein values <200 mg/L) (*7*), thus confirming that the colorimetric procedure and the laser immunonephelometric assay measure quite different substances.

Findings from our experience (*7*) and from that of Phillipou et al. (*6*) demonstrate that colorimetric techniques are not accurate for UAE evaluation because they probably measure other urinary proteins, in addition to albumin. The ratio between the total proteinuria as measured with the colorimetric methods and the albuminuria as measured by the most specific and sensitive immunological methods (RIA or laser immunonephelometry) varies greatly in diabetic patients with or without glomerular nephropathy (*6, 7*). This finding is not surprising, because of the changing behavior of the urinary protein excretion during the natural course of diabetic nephropathy (*1*). As microalbuminuria increases, the selectivity index—i.e., clearance IgG/clearance albumin—starts to decrease, reaching its lowest values when the albumin excretion rate is around 90 μg/min or more (*1*).

Colorimetric methods appear to be the best procedures for evaluation of total microproteinuria, because they are precise, cheap, and feasible. More specific immunological methods (such as RIA or laser immunonephelometric assay) are needed for the early and accurate detection of microalbuminuria, as well as for the close follow-up of subjects at risk of overt diabetic nephropathy. According to Phillipou et al. (*6*) and our previous study (*7*), however, colorimetric techniques may be useful for the initial screening of diabetic patients and can provide a guide to the dilution required for subsequent immunoassay.

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Serum and Plasma Compared for Use in 19 Common Chemical Tests Performed in the Hitachi 737 Analyzer

To the Editor:

The choice of serum or heparinized plasma as samples for routine chemical analysis and the effects of monoiodoacetate and EDTA, used as anticoagulant–antiglycolytic agents, on common laboratory tests are the subject of this Letter.

The wide use of "random-access" analyzers allows several clinical chemical tests to be done on a single specimen. Is it more nearly accurate to assay simultaneously various constituents, such as substrates and enzymes, in serum rather than in heparinized plasma? Which tests other than glucose can be performed with monoiodoacetate/EDTA on the same sample of blood? To answer these questions we studied healthy controls and patients admitted to our hospital, randomly selected so as to include patients with various diseases. We assayed paired serum/plasma samples after centrifugation for 10 min at 1500 × *g*; the time between the collection of the specimens and their processing was 20 to 60 min at room temperature. All samples were assayed in a "random-access" analyzer (Hitachi 737; Boehringer Mannheim Diagnostics, Mannheim, F.R.G.) in the same run. Table 1 lists mean values for 35 samples for 19 chemical tests commonly done on serum and heparinized plasma. The paired *t*-test was used to determine significant differences between the two means.

We found no significant difference between serum and heparinized plasma concentrations for glucose, urea, creatinine, calcium, magnesium,

Table 1. Results for 19 Common Chemistry Tests Performed on Simultaneously Drawn Serum and Heparinized Plasma

Test	Mean: Plasma	Mean: Serum	Mean difference	*t*-test	*P*
Glucose[a]	1202	1215	−12.7	−1.22	N.S.
Urea	577	589	−11.3	−1.98	N.S.
Creatinine	14.8	14.9	−0.04	−0.37	N.S.
Calcium	88.6	88.2	0.39	0.77	N.S.
Magnesium	18.5	19.1	−0.61	−1.51	N.S.
Phosphorus	34.5	36.0	−1.47	−6.81	<0.0001
Iron (μg/L)	771	832	−61.5	−4.84	<0.0002
Protein, total (g/L)	65.3	62.8	2.49	8.42	<0.0001
Bilirubin	20.9	21.8	−0.85	−1.74	N.S.
Cholesterol	1967	2032	−64.8	−6.12	<0.0001
Triglyceride	1531	1596	−65.2	−3.09	<0.01
Uric acid	34.5	34.7	−0.17	−0.69	N.S.
AST[b]	40.6	41.9	−1.33	−2.35	<0.03
ALT	43.7	45.2	−1.48	−1.38	N.S.
CK	220.1	224.9	−4.76	−1.95	N.S.
LD	478.5	516.5	−38.0	−4.31	<0.0005
Amylase	168.2	169.4	−1.19	−1.07	N.S.
ALP	215.0	225.9	−10.9	−5.59	<0.0001
γ-GT	31.6	34.3	−2.57	−3.79	<0.002

[a] Values in mg/L, except as noted.
[b] Values in U/L.
N.S., not significantly (P <0.05) different.

Table 2. Comparison of EDTA/Monoiodoacetate vs Heparinized Plasma

Test	Mean: Heparin	Mean: EDTA/MIA	Mean difference	*t*-test	*P*
Glucose[a]	1202	1219	−17.2	−1.95	N.S.
Urea	577	576	1.8	0.85	N.S.
Creatinine	14.8	14.7	0.14	1.22	N.S.
Phosphorus	34.5	34.7	−0.15	−0.76	N.S.
Iron (μg/L)	771	636	135	4.4	<0.0007
Protein, total (g/L)	65.3	64.9	0.45	1.13	N.S.
Bilirubin	20.9	20.7	0.2	0.85	N.S.
Cholesterol	1967	1953	13.3	1.44	N.S.
Triglyceride	1531	1550	−18.9	−1.54	N.S.
Uric acid	34.5	29.6	4.8	4.93	<0.0003
AST[b]	40.6	40.1	.50	1.12	N.S.
ALT	43.7	42.7	1.04	1.58	N.S.
CK	220.1	142.2	78.6	5.23	<0.0001
LD	478.5	446.7	31.8	5.00	<0.0001
Amylase	168.2	170.1	−1.9	−0.43	N.S.
ALP	210.0	34.8	174.2	7.21	<0.00001
γ-GT	31.6	31.5	0.1	0.3	N.S.

[a] Values in mg/L, except as noted.
[b] Values in U/L.
All calcium and magnesium values obtained for monoiodoacetate/EDTA plasma <1 mg/L.