Irreproducible Results

To the Editor:

I regret to inform you that after re-analysis of the original data and review by colleagues, the experiments upon which were based the report of anti-viral activity of rIl-6(serine) in the article entitled "Development of a Vector System of the Expression of Bioengineered Proteins" [Clin Chem 1989;35(Suppl):B7–12] appear to be inconclusive. I raised this matter with Stuart Bondurant, M.D., the Dean of the School of Medicine of the University of North Carolina at Chapel Hill, who appointed a Committee to review the data. The Committee found that "re-analysis of the original data, and a failed attempt to replicate the experiment, suggests that the published experiments reporting demonstration of anti-viral activity for rIL-6 are inconclusive."

Thus, I wish to notify you that the conclusions based on the data shown in Figure 7 are not supported by the primary, non-reduced data. After carrying out a re-examination of the primary data and by repeating the experiments, no other aspects of that report have been found to be inaccurate.

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Ed. note: In a separate communication, Dr. Fowlkes informed this journal that the above-named article "has been in part published in PNAS [Proc Natl Acad Sci] USA 85:9426–9430 (1988) in an article entitled 'High-level Expression of a Bioengineered, Cysteine-free Hepatocyte-stimulating Factor (Interleukin 6)-like Protein.'"

Microtiter Plate Techniques for Estimating Urinary Calcium, Protein, and Creatinine

To the Editor:

As part of a study aimed at relating concentrations of urinary calcium and protein during pregnancy to risk of preeclampsia, assay procedures were required that were not only reliable and economical but also capable of high sample throughput. We previously reported a method that meets these criteria for urinary protein (*1*), in which a microtiter plate technique replaced the standard spectrophotometric method, and here describe a similar approach for assays of calcium and creatinine.

Table 1. Assay Precision for Determinations of Urinary Calcium, Creatinine, and Protein

	Calcium	Creatinine	Protein
Quality-Control 1			
Mean	1.44[a]	2.08[a]	33[b]
No. of samples	38	26	26
Total assay CV, %	5.3	2.6	5.6
Quality-Control 2			
Mean	4.93	8.94	174
No. of samples	38	26	26
Total assay CV, %	2.6	2.1	3.1

[a] mmol/L. [b] mg/L.

The procedure for calcium is based on the colorimetric method of Baginski et al. (*2*), in which cresolphthalein complexone (CPC) reagent is used. Urine samples are diluted 10-fold with aqueous hydrochloric acid (10 mmol/L); 20 μL of diluted sample is dispensed into the well of a microtiter plate, followed by 300 μL of freshly prepared CPC reagent. The plate is left at room temperature for 5 min before the absorbance at 570 nm is measured (we used a Bio-Tek EL311 plate reader). Calcium standards of 0, 1.25, 2.5, and 5.0 mmol/L are made up in aqueous hydrochloric acid (10 mmol/L) and diluted 10-fold before use. The relationship between absorbance and concentration of standards is linear for calcium, creatinine, and protein. Samples for which the initial value exceeds 7.2 mmol/L are reassayed after 20-fold dilution, whereas those >8.4 mmol/L are diluted 40-fold.

Urinary creatinine is measured by adapting the alkaline picrate method reported by Yatzidis (*3*). In our procedure, only the alkaline picrate reagent, buffered at pH 11.5, is used because interference by noncreatinine chromogens is found to be negligible. Urine samples are diluted 10-fold with aqueous hydrochloric acid (10 mmol/L), and 50 μL of this is then added to the microtiter plate well, followed by 250 μL of the picric acid reagent. After 120 min at room temperature, the absorbance at 490 nm is recorded. Working creatinine standards, in aqueous hydrochloric acid (10 mmol/L), are 0, 2.8, 5.7, and 14.2 mmol/L and are diluted 10-fold before use.

Determination of urinary protein is as previously reported (*1*) except that urine and reagent sample sizes are increased to 50 and 250 μL, respectively. This modification allows increased assay reproducibility at the lower urinary protein concentrations observed for nondiabetic subjects. All urine samples for which protein exceeds 200 mg/L are reassayed after fivefold dilution.

Assay bias and proportional error with respect to established methods (*4*) for calcium (n = 59) and creatinine (n = 65) are as follows: $y = 1.00(0.97–1.03)x + 0.12(0.05–0.22)$, and $y = 0.98(0.97–0.99)x + 0.26(0.21–0.34)$, where the comparison ($x$) methods are atomic absorption spectrometry and the Technicon SMAC II, respectively. Assay reproducibility is summarized in Table 1.

References

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4. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytic methods. J Clin Chem Clin Biochem 1983;21:709–20.

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A Longitudinal Study of Thyroid Function in the Last Eight Weeks of Pregnancy

To the Editor:

We have previously reported a reduction in free thyroxin associated with increased thyrotropin during pregnancy (*1*), but this association has not been observed by all investigators (*2*). The changes we observed were