(Micro)Albuminuria: Antigen Excess Detection in the Roche Modular Analyzer

To the Editor:
Measurement of urinary albumin is performed both for detection of microalbuminuria (1) and for establishing the selectivity of urinary protein excretion when renal function deteriorates (2); it thus requires assays that can measure albumin concentrations from a few milligrams per liter to several grams per liter. Falsely low concentrations are encountered regularly in immunoturbidimetric assays as a result of antigen excess (3). Procedures to detect antigen excess include additional testing with qualitative measurement of urinary albumin by reagent strip (dipstick) (4–6), quantitative measurement of urinary total protein (7), or albumin analysis with multiple dilutions of the sample (8). Another procedure uses an excessive amount of antibody, which can lengthen the calibration curve considerably (9). All of these procedures are costly. Mathematical procedures based on the kinetic differences between samples with and without antigen excess have been proposed (9,10), but they falsely suggest antigen excess in samples with low antigen concentrations. An efficient procedure involves adding additional albumin after the measurement is completed and interpreting the additional change in turbidity (9). Here we report our experience with such a procedure on the MODULAR® ANALYTICS P-type analyzer (Roche GmbH).

For measurement of urinary albumin [modified for Modular from Ref. (11)], we incubated a sample with 45 g/L PEG-6000 in phosphate-buffered saline (reagent 1, consisting of 10 mmol/L phosphate, 140 mmol/L NaCl, 1 mL/L Triton X-100, pH 7.4) for 1.5 min. We then added an antibody against human serum albumin (reagent 2, consisting of Dako Q328 diluted 10-fold in saline) and monitored the development of turbidity at 18-s intervals for the next 3 min to establish the albumin concentration. We then added more albumin (reagent 3, consisting of an equivalent amount of 200 mg/L albumin prepared by dilution of a human control serum in saline) and monitored the change in turbidity for an additional 1.5 min. This served as the so-called “prozone check”. When the absorbance of the analyzer-generated prozone is less than the lower cutoff limit (0.200), a flag is issued, which initiates an automatic rerun of the 10-fold diluted sample. The detailed settings for the MODULAR ANALYTICS P-type analyzer are given in Table 1 in the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol51/issue6/. The calibrators (2–600 mg/L) consisted of purified human albumin (prod. no. A3782; Sigma) dissolved in phosphate-buffered saline for which the concentrations had been established as described previously (11).

The slope of the calibration curve (sensitivity) was lower at higher concentrations, and the absorbance decreased at concentrations above ~1200 mg/L (Fig. 1A). The additional absorbance change for the prozone check after addition of excess antigen, the so-called prozone value, increased slightly with increasing albumin concentration in all urine samples. (8).

Fig. 1. Absorbance and prozone numbers for diluted control serum (A), and prozone numbers for urine samples (B).

(A), shown is the absorbance (●) for various dilutions of a control serum measured in the assay for urinary albumin as well as the prozone number obtained for each dilution (▲). (B), prozone number (▲) obtained for each albumin concentration in all urine samples.

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fact that the absorbance change produced by the endogenous albumin in the sample may not be complete at 3 min after addition of reagent 2 and therefore contributes to the prozone turbidity. At higher albumin concentrations, the prozone value decreased, and when the curve reached concentrations ≥900–1200 mg/L, the prozone value was reduced to almost 0. Although this system worked with dilutions of a human serum, we also tested the procedure with urine samples from diabetic patients and from patients with renal failure. During the first 2 weeks of the investigation of the prozone measurement, the prozone value calculated by the Modular analyzer was recorded for all samples. Because the frequency of samples with microalbuminuric results was considerably lower than the frequency of samples with normalalbuminuric results, the prozone value was recorded for samples with microalbuminuric results for an additional 2 months and for samples with macroalbuminuric results for another 2 months. In total, 167 samples with normalalbuminuric results, 168 samples with microalbuminuric results, and 140 samples with macroalbuminuric results were assayed. The results for the prozone values, including those obtained in the rerun, are displayed in Fig. 1B.

This procedure with the 0.200 cut-off value for the prozone check effectively detected all samples in which antigen excess caused falsely low microalbuminuric or normal values. The same kind of procedure could be useful for other assays in which antigen excess might cause falsely low values. In our experience, falsely low values attributable to antigen excess occur in daily practice in a limited number of samples, which makes an efficient procedure for antigen excess detection very important.

In conclusion, an effective and efficient procedure for the detection of antigen excess could be included in the assay for urinary albumin on the Roche Modular analyzer and could eliminate the need for additional tests to prevent reporting of falsely low results from antigen excess.

References


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Editor’s note: The manufacturer has promised a reply, but none has been received at press time.

Falsely High Serum Free Triiodothyronine and Free Thyroxine Concentrations Attributable to Anti-Diiodothyronine and Anti-Triiodothyronine Antibodies

To the Editor:

We observed a patient with markedly increased free triiodothyronine (FT₃) and free thyroxine (FT₄) concentrations measured on a Vitros ECI analyzer (Ortho Clinical Diagnostics). The patient was a hospitalized 42-year-old woman with lupus erythematosus who appeared euthyroid and had normal thyroid-stimulating hormone (TSH) concentrations (Table 1 in the Data Supplement that accompanies the online version of this letter at http://wwwclinchem.org/content/vol51/issue6/). Thyroid peroxidase antibody and thyroglobulin antibody were <0.3 kilounits/L, and rheumatoid factor was <6.0 kilounits/L. We suspected interference from heterophilic antibodies (1,2), but our experiments suggested interference from antibodies to diiodothyronine (T₂), T₃₃, or their conjugates, as have been described (3–5). The procedures in this study were in accordance with the Helsinki Declaration of 1975 and the subsequent 1996 amendments.

In contrast to results of the Vitros ECI FT₃ and FT₄ assays, which use solid phases with T₂- and T₃-gelatin, respectively, and labeled sheep antibodies, FT₃ and FT₄ were normal by the Elecsys assays (Roche Diagnostics), which use biotinylated antibodies in a one-step method.

To the Vitros FT3II and FT4 assay wells we added 0.05 mL of serum from the patient or from controls (n = 5) and 0.1 mL of diluent [phosphate-buffered saline (PBS) containing 1 mg/L Tween 20 (Sigma) and 10 g of bovine globulin (Sigma)], and incubated them at 37 °C for 18 min. After washing each well, we added 0.125 mL of horseradish peroxidase-labeled goat anti-human IgG antibody conjugate (Chemicon International, Inc.), diluted 50 000-fold in the same diluent, and incubated the mixtures at 37 °C for 18 min. After each well was