References

Effect of High Creatinine Content on the Kodak Single-Slide Method for Creatinine, S. F. Sena,* D. Syed, and R. B. McComb† (Clin. Chem. Div., Dept. of Pathol. Hartford Hospital, Hartford, CT 06115)

Kodak cautions users of the Ektachem single-slide creatinine method (1) (EKTA/CREA) that high concentrations of creatine in serum may lead to a "Substrate Depletion" flag during the analysis (2). The procedure recommended by Kodak to circumvent this flag is dilution of the specimen and re-analysis. We have encountered this phenomenon on several occasions and herein document these occurrences in two patients.

Case A: This 60-year-old man with a history of angina per se was admitted to the hospital in cardiac arrest. He was found to have an embolic occlusion and thrombosis in the arterial supply to the right lower extremity for which a thrombectomy and a femoral-popliteal bypass were performed. He developed rhabdomyolysis and acute renal failure with anuria that was treated with hemodialysis. The initial EKTA/CREA analysis (Ektachem 700®; Eastman Kodak Co., Rochester, NY 14650) was rejected with a printout of "Out of Range" and "Substrate Depleted". After dilution of the specimen, the creatinine result was 87 mg/L (reference limits 7–17 mg/L) and the serum creatine was 69 mg/L (reference limits 1–4 mg/L). Over the next three weeks the patient's renal function gradually improved, and creatinine concentrations slowly decreased to within adult reference limits (see Figure 1). However, rejection by the Ektachem 700® continued until the creatinine concentration declined to <31 mg/L.

Case B: This 26-year-old man had a history of intravenous drug abuse and chronic renal failure due to membranoproliferative glomerulonephritis. He was admitted with severe anasarca. Lab. studies included anemia, marked hyperbilirubinemia, and antibodies to human immunodeficiency virus (HIV). The initial serum creatinine by EKTA/CREA was rejected as in Case A; however, after dilution of the specimen, a result of 27 mg/L was obtained. The patient's serum creatinine concentration at this time was 136 mg/L. After four days the creatinine concentration had declined to 52 mg/L, a concentration that allowed direct measurement of creatinine on the Ektachem 700 (see Figure 1).

Experimental. To better understand concentration relationships between creatine, creatinine, and final creatinine results, we added various concentrations of creatine and creatinine to our "normal" human serum pool (3), then assayed each sample with the Ektachem 700, using EKTA/CREA (generation 1) slides. Any sample rejected as "Out of Range" by the Ektachem was diluted twofold with a 70 g/L solution of bovine albumin in isotonic saline and re-analyzed, as recommended by Kodak (2). The results are summarized in the following tabulation.

<table>
<thead>
<tr>
<th>Creatinine, mg/L</th>
<th>Creatine concn, mg/L</th>
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<tbody>
<tr>
<td>30</td>
<td>10</td>
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<td>70</td>
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<td>300</td>
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Results at right are from samples diluted twofold with 70 g/L bovine albumin solution. Each value represents a single analysis.

At all concentrations of creatinine investigated, the threshold concentration of creatine initiating a "Substrate Depletion" flag substantially exceeds the upper reference

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limit for creatine, i.e., for men 4 mg/L, and for women 7 mg/L. As expected, samples with a higher creatinine concentration require less creatine for this interference to be manifested.

Evidently creatine interference has no substantive impact on the clinical utility of the Kodak Ektachem single-slide method, because any flagged value is automatically diluted as a part of the routine protocol. Indeed, a "Substrate Depletion" flag should alert one of a possible creatine abnormality and the associated clinical implications, e.g., rhabdomyolysis or other disorders involving destruction or wasting of muscle mass and associated renal failure.

References

An Interleukin 2 Binding Factor in Human Serum,
Vittorio Bellotti, Carla Cavalli, Vittorio Perfetti, Paolo Gobbi, and Giampaolo Merlino (Institute of Clinica Medica II, I.R.C.C.S., Policlinico S. Matteo, 27100 Pavia, Italy)

The role of interleukin 2 (IL2) in immune regulation has been much investigated (1). Theoretically, this lymphokine should have a direct or indirect key role in immune deficiencies in many patients with lymphoproliferative diseases.

Ford et al. (2), using a biological assay to evaluate the IL2 synthesis of T lymphocytes in Hodgkin's disease patients, found their values lower than in normal subjects. These results are encouraging, but that method is complex and not feasible in many laboratories.

Recently, we used a commercial immunoenzymatic assay for IL2 (Intertest 2 Human Interleukin-2 ELISA; Genzyme, Boston, MA 02111) to compare the IL2 concentration in serum from normal subjects and patients with Hodgkin's disease. The test is based on use of a purified mouse monoclonal antibody as first antibody, rabbit polyclonal antibody against IL2 as the second. The standards consist of recombinant IL2 (rIL2) at concentrations from 0.05 to 500 kilo-int. units/L.

We tested the IL2 content in serum from 13 normal subjects and 46 patients with Hodgkin's disease and found values ranging from 0 to 5000 kilo-int. units/L, with no significant differences between the two groups. Nor was IL2 correlated with the disease activity indices, immunoglobulin concentrations, or lymphocyte count.

In eight subjects, four normals and four patients, we found very low concentrations of IL2. Furthermore, when we added a standard solution of rIL2 to these sera, even this IL2 disappeared.

To characterize this phenomenon, we incubated rIL2, 500 kilo-int. units/L, for 1 h with buffer or serum at room temperature, then performed all the assay steps according to the manufacturer's instructions. As shown in Figure 1, IL2 was no longer detectable in the sera. This phenomenon was not due to serum storage: similar results were obtained with fresh samples and with samples stored at -20 °C.

This phenomenon, well-known as the "serum effect," has been described for other proteins (3, 4) and is of particular interest in the case of IL2, because of the biological role of this analyte. The serum effect might be attributable to the presence of increased IL2 receptors in serum of patients with lymphoproliferative disorders (5, 6), but this would not account for the serum effect observed in healthy controls.

We plan to characterize the protein responsible for IL2 "quenching" and to evaluate whether its presence is correlated with the disappearance of biological activity.

References

Clinical Evaluation of a Fluorescence Polarization
Imunoassay for Quantifying C-Reactive Protein, José M. González Buitrago, Fernando Cava, Antonio Gómez del Campo, José M. Moyano, and José A. Navajo (Servicio de Análisis Clínicos, Hospital Virgen de la Vega, 37007 Salamanca, Spain)

The Abbott TDX analyzer (Abbott Laboratories, Chicago, IL) is easy to run and well-suited for urgent analyses. We evaluated a fluorescence polarization immunoassay (FPIA)