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### **Frozen storage of serum does not affect cholesterol and triglyceride concentration in lipoproteins as separated by gradient ultracentrifugation**

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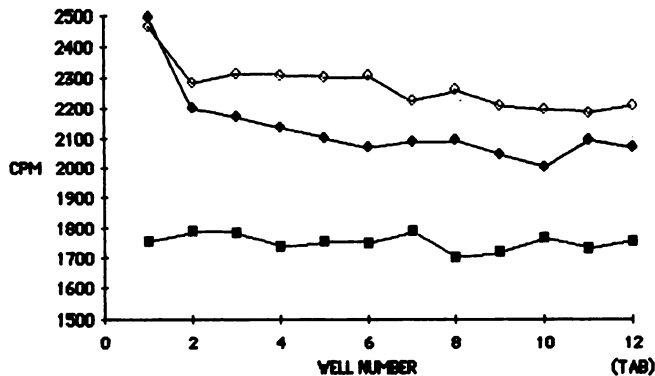
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ity was counted. The figure shows that wells coated with murine (◆—◆) and equine antibody (◇—◇) show a reproducible pattern of reactivity, with an evident increase in counts in the direction away from the identification tab. CVs for counts/min per well within a single strip ranged from 2.1 to 11.9% (murine) and 2.1 to 10.4% (equine), (n = 8).

This phenomenon was not seen in a two-site radioassay for  $\alpha_1$ m. Bound antibody was followed by 100  $\mu$ L of a 1:640 dilution of human serum. After incubation, wells were washed, an  $^{125}$ I-labeled mouse anti- $\alpha_1$ m immunoglobulin of different epitopic specificity was added, and the wells were incubated, washed, and the radioactivity was counted. The increase in counts was absent in the "sandwich" format (■—■); CVs ranged from 2.8 to 5.9% (n = 8).

Kricka et al. (1) report an "edge effect," with variability for competition and two-site immunoassays of  $\pm 11\%$  and  $\pm 18\%$ . Researchers should be aware of these various binding phenomena. These results depend upon as-yet-unidentified variables that are associated with individual proteins.

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### Frozen Storage of Serum Does Not Affect Cholesterol and Triglyceride Concentration in Lipoproteins as Separated by Gradient Ultracentrifugation, Henrica G. M. Tiedink and Martijn B. Katan (Dept. of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands)

We investigated the effect of prior storage of serum at  $-20^\circ\text{C}$  on the concentration of cholesterol and triglycerides measured in lipoprotein fractions separated by density-gradient ultracentrifugation. Fresh, preprandial, normolipemic sera from 14 men and 10 women were divided into five 2.5-mL aliquots (A-E). Aliquots A were stored at  $4^\circ\text{C}$  for  $<24$  h. Aliquots B were stored at  $-20^\circ\text{C}$  for 2.5 h, thawed in air of  $20^\circ\text{C}$ , and stored at  $-20^\circ\text{C}$  for another 18 h. Aliquots C, D, and E were stored at  $-20^\circ\text{C}$  for 2, 11, and 27 weeks, respectively. After storage, serum lipoproteins were separated by density-gradient ultracentrifugation (1) for 22 h in a Beckman Ti 41 SW rotor at  $20^\circ\text{C}$  and 37 000 rpm ( $233\ 000 \times g$ ). Lipoproteins were aspirated and densities checked as described (1), and cholesterol and triglycerides

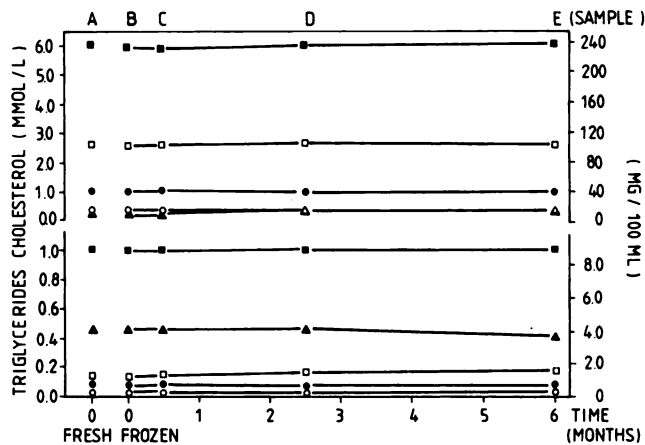


Fig. 1. Cholesterol (upper curves) and triglyceride (lower curves) concentrations measured in lipoprotein fractions after prior storage of sera at  $-20^\circ\text{C}$  for the times indicated

Time 0 FRESH refers to samples stored at  $4^\circ\text{C}$  for less than 24 h; time 0 FROZEN refers to samples that had been stored at  $-20^\circ\text{C}$  for 2.5 h, thawed, and then stored at  $-20^\circ\text{C}$  for another 18 h. Symbols: ■, total serum; □, LDL; ▲, VLDL; ○ HDL<sub>2</sub>; ● HDL<sub>3</sub>.

were assayed enzymatically (2, 3). The CV for cholesterol was 1.3% at 4.80 mmol/L, 0.9% at 8.96 mmol/L, and for triglycerides 1.6% at 0.91 mmol/L, 1.4% at 1.80 mmol/L. Bias for serum pools provided by the Centers for Disease Control, Atlanta, GA, was  $-0.7\%$  for cholesterol and  $+0.9\%$  for triglycerides. The mean percentage recoveries ranged between 97.3% and 101.2% for cholesterol and between 71.1% and 76.0% for triglycerides. The low analytical recovery of triglycerides was probably caused by small amounts of VLDL sticking to the wall of the tubing used in aspirating the fractions. Comparison of aliquots A and B (Figure 1) showed that freezing per se had no effect on the measured concentration of cholesterol and triglycerides in the various lipoproteins; changes averaged 0.00 mmol/L (range,  $-0.02$  to  $0.02$  mmol/L). The measured concentration of total cholesterol in serum was 0.09 mmol/L ( $-1.8\%$ ) lower in aliquots B than in aliquots A.

The concentration of cholesterol in the major cholesterol fractions (LDL, HDL<sub>2</sub>, and HDL<sub>3</sub>) changed on average by less than 4.1% over the full period of 27 weeks (Figure 1). The percentage changes in the minor fractions were sometimes considerably larger, but the absolute changes were similar. There were no systematic up- or downward trend with time in any of the lipoprotein classes. The percentage changes in the triglyceride concentrations were  $<5\%$  (Figure 1) for the major triglyceride fractions (VLDL and LDL) during the first 11 weeks, except for LDL after 11 weeks of storage (change  $+9.4\%$ ). By 27 weeks of storage, triglyceride concentrations had changed by  $-13.0\%$  in VLDL and by  $+13.0\%$  in LDL. The percentage changes in the minor fractions were mostly much larger, but mean absolute changes were again quite small. Although freezing of serum may affect electrophoretic characteristics (4-7) and precipitability (8-9) of lipoproteins with polyanions, cholesterol and triglyceride contents of lipoproteins separated by density-gradient ultracentrifugation appear to be unaffected by prior frozen storage of serum for as long as several months.

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### Effect of High Creatine Content on the Kodak Single-Slide Method for Creatinine, S. F. Sena,<sup>1</sup> D. Syed, and R. B. McComb<sup>2</sup> (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 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<sup>®</sup>; 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

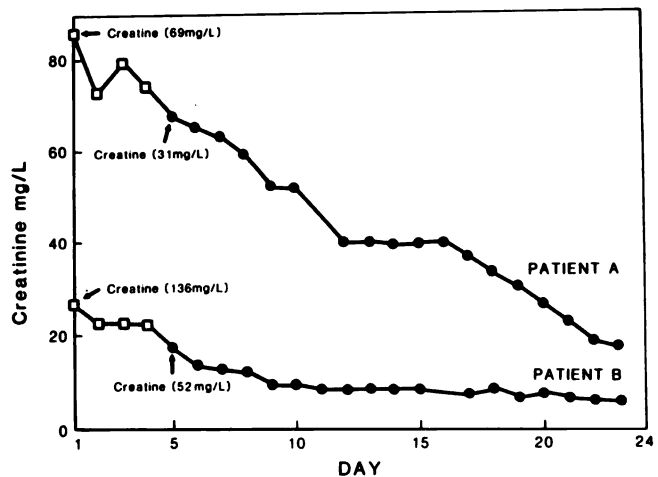


Fig. 1. Summary of the creatinine results for Patients A and B

The arrows indicate the times that creatine values were determined. The open squares, □, indicate the times when the serum samples were rejected by the EKTACHEM because of substrate depletion. In these cases the creatine concentration at the time of sample rejection is substantially increased. Creatine concentration decreases markedly before creatinine results can be obtained without predilution of sample

limits (see Figure 1). However, rejection by the Ektachem 700<sup>®</sup> continued until the creatine 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 ascites. Lab. studies indicated anemia, marked hypoalbuminemia, 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 creatine concentration at this time was 136 mg/L. After four days the creatine 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 creatinine and creatine 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.

| Creatinine, mg/L | Creatine concn, mg/L |     |     |     |     |     |     |     |
|------------------|----------------------|-----|-----|-----|-----|-----|-----|-----|
|                  | 10                   | 20  | 30  | 40  | 50  | 60  | 80  | 100 |
| 30               | 32                   | 31  | 31  | 32  | 32  | 33  | 33  | 34  |
| 70               | 69                   | 68  | 67  | 67  | 68  | 70  | 74  | 75  |
| 90               | 92                   | 90  | 91  | 91  | 94  | 96  | 99  | 98  |
| 120              | 121                  | 121 | 121 | 123 | 125 | 133 | 133 | 137 |
| 150              | 149                  | 148 | 153 | 155 | 166 | 158 | 162 | 165 |

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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