Surfactants in Enzymic Reagents for Determination of HDL-Cholesterol

To the Editor:

The assay method reported by Grillo et al. (1) for HDL-cholesterol, which involves an adaptation of Barham and Trinder's colorimetric reaction (2), does not mention the surfactant used to enable cholesterol esters to be hydrolyzed by cholesterol esterase (EC 3.1.1.13).

A surfactant must be present in such a reagent system, or there will be no hydrolysis of cholesterol esters.

Allain et al. (3) used sodium cholate and Carbowax 6000 (polyethylene glycol) in a similar system for detergent effect. In the enzymic cholesterol reagent marketed by Boehringer Mannheim ("CHOD-PAP") (4), non-ionic surfactants are used, hydroxypolyethoxy-N-alkanes. (Their kit literature does not mention the number of ethoxy groups or the predominant N-alkane.) The concentration of the non-ionic surfactant is given as 0.48% (i.e., 4.8 g/L), which implies that the ethoxy number is a high one.

It would be of great assistance if Grillo et al. revealed the surfactants they used so that their published method may be conveniently reproduced.

References

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Solution to Viscosity Problem in Liquid Control Material Used in the SMAC

To the Editor:

The availability of liquid control material has been a real asset to the quality-assurance programs in clinical chemistry laboratories. Its increased stability has helped lower the costs associated with quality assurance and, because it does not have to be reconstituted daily, it allows the laboratory to evaluate its ability to be accurate and precise rather than the ability of the technologists to accurately reconstitute the controls.

The increased viscosity of the material has led to occasional problems. We encountered one such problem with our Technicon SMAC. We experienced an upward trend with the enzymic cholesterol II, total protein, and total bilirubin. The problem was traced to the "pre-dil" cartridge. It was first rebuilt to specifications and then replaced, but these actions did not resolve the problem. We felt that the problem was probably due to inadequate mixing of the viscous liquid control, because a reconstituted lyophilized control yielded results that were on the mean. Therefore we added two Technicon 178-G196-01 five-turn mixing coils to the pre-dil cartridge, one preceding the 12-turn pre-dil coil and one following it. This promoted better mixing and led to a return of control values to the correct range. We have used the modified pre-dil cartridge for two months and have experienced no additional problems.

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Effect of Storage on HDL-Cholesterol Measurements

To the Editor:

Results of several epidemiological studies now indicate that the HDL fraction of serum lipoproteins slows the development of atherosclerotic lesions in man (1, 2). However, progress in this field is impeded by the uncertainties about optimum conditions for assay of HDL cholesterol, including choice of ultracentrifugation steps, precipitation reagents, or electrophoretic separations. Another critical issue is the effect of freezing and storage, and we would like to comment regarding this.

Some investigators have rejected completely the idea of measuring HDL cholesterol in previously frozen samples, although the negative correlation of this analyte with coronary heart disease was found by assaying samples that had been stored at low temperatures for several years (3). Because samples often must be stored for some time, we studied the effects of freezing and storage on apparent HDL-cholesterol concentrations in plasma, as measured enzymically after precipitation with a poly-anion.

EDTA-treated plasma was obtained after an overnight fast from 49 men and women whose total plasma cholesterol concentrations consistently exceeded 2.5 g/L. None of these samples contained more than 3 g of total plasma triglycerides per liter. Aliquots of the samples were kept at 4°C and at −18°C for 4 h. Just before analysis the frozen samples were thawed at room temperature and brought to 4°C. Each pair was then analyzed within 2 h. Aliquots of 25 of the samples were refrozen, stored at −18°C for five months, thawed, and again analyzed.

For HDL-cholesterol determination, 100 μL of plasma and 10 μL of phosphotungstic acid/MgCl₂ precipitation reagent (48 g/L, 3 mol/L; Boehringer-Mannheim, F.R.G.) were pipetted into microvials (Sarstedt, F.R.G. (4)), sealed with Parafilm, shaken vigorously, and left at room temperature for 10 min. The mixture was then centrifuged (1500 × g, 30 min) and the cholesterol concentration in the supernatant fluid was determined enzymically (CHOD-PAP; Boehringer-Mannheim) within an hour.

Comparison of the apparent HDL-cholesterol values in fresh (y) and briefly frozen (x) samples gave the regression equation y = 0.99 x − 0.49 (Pearson’s r = 0.98). The corresponding comparison of values in fresh samples (y) and in samples stored at −18°C for five months (x) resulted in y = 1.18 x − 0.58 (Pearson’s r = 0.96).

The freezing process per se did not seem to influence the values greatly. There was no discernible bias in the values and the scatter corresponded to that for concurrent measurements in fresh samples. On the other hand, values determined after refreezing and storage for five months at −18°C were considerably lower than the original values, and there was more scatter than was to be expected from measurement error alone. This agrees with previous, more detailed biochemical studies (5), where profound changes of the HDL particles were found under similar conditions. However, the assumption seems to be justified that it is not freezing per se that induces such changes; rather, there are slow conversions in the frozen sample during long-term storage.

Nevertheless, even after five months the correlation of the values for frozen and fresh samples still was fairly good (r = 0.96). We conclude that, with the use of the described method, storage of samples at low temperatures before HDL-cholesterol determination, particularly if only for a limited time, is acceptable.

References