

## Polyethylene Glycol Method for High-Density Lipoprotein Cholesterol Defended

To the Editor:

While we consider the paper of Warnick, Cheung, and Albers [*Clin. Chem.* 25, 596-604 (1979)] to be an extremely valuable attempt to bring order to the field of high-density-lipoprotein cholesterol measurement, we regret that their summary implies that the polyethylene glycol-6000 (PEG-6000) precipitation method is inherently inaccurate.

As recommended by Viikari (1), they use a final concentration of PEG-6000 of 120 g/L. As they note, Figure 2 in their paper suggests that this concentration is too high, because the supernatant cholesterol becomes nearly constant at 60-80 g of PEG-6000 per liter, decreases by about 2.5% at 100 g of PEG-6000 per liter and further still at 120 g/L, and continues to decline with increasing concentrations of PEG-6000. Our results (2) are similar to theirs, except that we do not find such a large decrease in supernatant cholesterol concentrations at a final PEG-6000 concentration of 120 g/L, a discrepancy that might be caused by variations in PEG-6000. Our supplier was British Drug House Chemicals, Ltd., and their supplier was Sigma Chemical Co. However, the similarity of the portions of the curves between 60 and 100 g/L final concentration of PEG-6000 should be noted. We found that at 60 and 80 g of PEG-6000 per liter there was some material remaining that reacted with  $\beta$ -lipoprotein antiserum. Possibly this is a portion of the ApoB-associated cholesterol with  $d > 1.063$ , as the necessarily high protein content of these molecules might stabilize the lipoprotein structure in comparison with the lower density lipoproteins, low-density lipoprotein and very-low-density lipoprotein, which also contain ApoB. Therefore, it seems to us that the final concentration of 100 g of PEG-6000

per liter is suitable. From inspection of Warnick et al.'s Figure 2, it appears that had they used the value for supernatant cholesterol at 100 g of PEG-6000 per liter the results obtained would have been equal to the values obtained by ultracentrifugation, which they use as a reference method.

Under Warnick et al.'s conditions, as will be seen from their Figure 2, minor variations in final PEG-6000 concentrations will lead to much greater variations in the supernatant cholesterol concentration at 120 g/L than at 80 or 100 g/L final concentration of PEG-6000. In practice, the viscosity of PEG-6000 solutions tends to limit the reproducibility of additions to samples. Indeed, Warnick et al. found a coefficient of variation with 120 g of PEG-6000 per liter that was about twice our coefficient of variation for 100 g of PEG-6000 per liter, although, of course, some of this difference may be due to inter-laboratory variations in methods.

PEG-6000 precipitation is extremely convenient and we would not like to see it discredited if minor changes to the method give acceptable results.

### References

1. Viikari, J., Precipitation of plasma lipoproteins by PEG-6000 and its evaluation with electrophoresis and ultracentrifugation. *Scand. J. Clin. Lab. Invest.* 36, 265-268 (1976).
2. Allen, J. K., Hensley, W. J., Nicholls, A. V., and Whitfield, J. B., An enzymic and centrifugal method for estimating high-density lipoprotein cholesterol. *Clin. Chem.* 25, 325-3257 (1979).

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