HDL cholesterol has been recognized in recent years as a powerful and independent inverse predictor of coronary heart disease (CHD) risk, established in both case-control and prospective (1,4,7,9,15) studies. The potential for better characterizing CHD risk in patients lead to a rapidly increasing demand for HDL cholesterol measurement by clinical laboratories. As a result, the challenging two-step technique, involving manual separation of HDL followed by analysis of cholesterol, rapidly transferred from a few specialized research laboratories to clinical laboratories and was adapted to a new generation of highly automated instruments using enzymic reagents.

Method accuracy and standardization of results, paramount in the research laboratories, received little attention in this transition, even though accuracy is important for the lipids and especially so for HDL. Since the lipid cutpoints were established by expert opinion from large national population studies (17), standardization to their accuracy base is essential for reliable patient classification. Furthermore, the association of HDL cholesterol with CHD risk is expressed over a narrow concentration range; the U.S. cutpoint of 35 mg/dL differs by only 10 mg/dL from the population mean in males. Also, measured HDL cholesterol is subtracted from total cholesterol in deriving or calculating the atherogenic LDL cholesterol fraction; thus, the errors are reciprocal. Since the risk relationships are opposite, errors in HDL cholesterol substantially compromise the overall estimation of risk.

While HDL is classically defined in terms of

density, as the fraction separating between 1.063 and 1.21 kg/L by ultracentrifugation (12), routine separations generally are made by chemical precipitation or less commonly by electrophoresis. The polyanion heparin with the divalent cation Mn²⁺ was used initially by research laboratories to precipitate VLDL and LDL with HDL quantified as cholesterol in the supernate after low speed centrifugation (5). Because of Mn²⁺ incompatibility with enzymic cholesterol assays, newer methods substituted either phosphotungstate, (6) now the most common reagent, or dextran sulfate of either 500 kDa (6) or 50 kDa (20), with Mg²⁺(FIG. 1). Fewer laboratories used either polyethylene glycol (18) or quantitative lipoprotein electrophoresis (13). Studies have demonstrated systematic differences in specificity among the reagents (21) which can be corrected by appropriate adjustment of reagent concentration (22). Some precipitation reagents and other constituents of the serum matrix may interfere with enzymic assays. Separations are dependent on technique and subject to interference especially from elevated triglycerides (19).


Preanalytical factors including changes during storage either refrigerated or frozen (3), and the dramatic response of HDL cholesterol to metabolic, pharmacological and lifestyle factors (10) contribute to variation in measurements.