The measurement of apoproteins is of growing interest in both the research and the clinical laboratory. Apoprotein levels are of use as markers and as potential predictors of atherosclerotic cardiovascular disease. They aid in the diagnosis of dyslipoproteinemic states and are helpful in monitoring the progress of dietary and drug intervention. However, apoprotein measurements have not realized their full potential because of problems in methodology, inadequate standardization, the lack of suitable common reference materials, and lack of reference methods. This, in turn, has limited the ability to define adequate reference ranges for clinical use.

The great interest in apoproteins has resulted in proliferation of commercial methods for their measurement. Unfortunately, there is significant variation in apoprotein measurements between methods and between laboratories. While some of these differences can be attributed to the values assigned to the calibrators, there remain significant method-dependent differences.

In view of the problems with apoprotein measurements and their standardization, the National Heart, Lung, and Blood Institute initiated the Apoprotein and Antibody Standardization Program (AASP) in October 1985. The overall objective of the program was to collect, analyze, and distribute research data on apoprotein antibodies and immunoassay methods that would be useful for the standardization of apoprotein measurements. Achievements to date include: 1) development of a monoclonal antibody-based enzyme-linked immunoassorbent assay (ELISA) as a candidate reference method for the specific measurement of apoprotein (apo) B-100; 2) evaluation of monoclonal antibody-based ELISAs as potential reference methods for measurement of apo A-I, A-II, and the Lp(a) lipoprotein; 3) new information on the effect of blood collection, handling and storage of apoproteins, and the optimal conditions for preparation of primary apoprotein standards; 4) evaluation of the major commercial apoprotein immunoassay methods; and 5) examination of the suitability of lyophilized material for use as reference material.

Based on the data generated from this program and the collective experience of the AASP Planning Committee, we make the following recommendations: Blood should be drawn under standardized conditions as described for lipids and lipoproteins; serum is collected, the blood should be allowed to clot at room temperature and the clot detached from the tube before centrifugation; for plasma, the blood should be placed on ice and centrifuged at 4°C; after separation, plasma or serum can be stored up to 7 days at 4°C. For longer storage, the plasma or serum should be quick frozen and stored at -70°C in securely sealed vials. If low temperature freezers are not available, it is important that these samples be stored in the frozen state at a low constant temperature.

We recommend that low density lipoprotein (LDL) of a density of 1.030 to 1.050 g/ml obtained from normolipidemic donors be used as a primary standard for apo B-100 to ensure a minimal degree of contamination from non-apo B-100 protein. Purified LDL under appropriate conditions can be stored at 4°C for up to 20 days. Apo A-I and A-II are usually isolated from delipidated high density lipoprotein by column chromatography. Purified A-I and A-II should be stored in solution at -70°C to minimize loss of immunoreactivity.

The AASP Planning Committee has evaluated the Lowry procedure for the measurement of apo A-I, A-II, and B. Given the lack of universally accepted chromogenicity factors and the closeness of the Lowry protein values to those determined by amino acid analysis, we recommend that these apoproteins be expressed in terms of a standard Lowry protein procedure, which includes detergent to minimize potential interference of lipid or apoprotein aggregation. Because the Lowry protein can vary with the source of the albumin standard, we recommend that bovine serum albumin obtained from the National Bureau of Standards (NBS No. SRM 927) be used in all Lowry determinations of apoproteins.

We have examined the more commonly used methods for the measurement of apoproteins and, based upon our collective experience, recommend monoclonal antibody-based ELISAs as reference methods for the apoproteins. Monoclonal antibodies are attractive for use in reference assays because they are highly specific, chemically uniform, and can be produced and purified in large amounts. Furthermore, ELISA procedures can be automated, have convenient incubation times, have long-term reagent stability, and do not require radioisotopes. We have developed a direct-binding ELISA procedure as a candidate reference method for the measurement of apo B-100 in human plasma or serum (Albers JJ, Lodge MS, and Curtiss LK, unpublished observations). This procedure employs the monoclonal antibody MB 47 as the capture antibody and MB 24 conjugated to horseradish peroxidase as the detecting antibody. Candidate reference methods for apo A-I and A-II are still being evaluated.

Currently, the Centers for Disease Control (CDC) has available a lyophilized reference material (pool 1883) with
the apoprotein values assigned by consensus.\textsuperscript{2} The CDC consensus values for apo A-I and B are not accuracy-based target values, but instead represent an average from selected laboratories using diverse methodological approaches. Given the wide range of values obtained on this pool, especially for apo B,\textsuperscript{2} and the potential for "matrix effects" on lyophilized materials, we recommend that the direct binding apo B-100 ELISA be used to assign an apo B value to frozen serum pools. These pools could serve as secondary reference standards for all apo B methods. Furthermore, the standardized Lowry protein procedure should be used to determine the protein content of the purified LDL standard. Adoption of the candidate apo B-100 reference method and its transferability to reference laboratories should significantly reduce the large interlaboratory variability seen with apo B measurements. Establishment of similar reference procedures for apo A-I, A-II, and the Lp(a) lipoprotein is considered a top priority.

Lyophilized materials are generally used as reference materials because of convenience and economy in shipping. However, results on lyophilized materials may not be representative of those on fresh plasma or serum from patients when measured in some assay systems, such as radial immunodiffusion. To ensure that such matrix effects do not occur, we recommend that reference materials give values identical to frozen serum pools in immunoassay procedures. These reference materials should consist of samples containing high, medium, and low concentrations of the apoprotein analyte.

Currently, apoprotein measurements are being used without adequate validation or standardization. Thus, the AASP Planning Committee strongly urges that techniques for apoprotein measurements be standardized and that an apoprotein standardization committee be established to coordinate quality assurance of all large-scale clinical and epidemiological studies involving apoprotein measurements. Adoption of these recommendations and continued support of our efforts in standardization by the National Heart, Lung, and Blood Institute and the scientific community should significantly improve apoprotein standardization and permit apoprotein measurements to reach their full potential in clinical chemistry and biomedical research.

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