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Reference Materials for the Standardization of the Apolipoproteins A-I and B, and Lipoprotein(a)

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Abstract

Measurement of the apolipoproteins A-I and B, and lipoprotein(a) enable identification of individuals at increased risk of cardiovascular disease. However, the lack of standardized methods to measure these risk markers has resulted for many years in the non-comparability of values and often a conflicting interpretation of clinical studies. Due to the collaborative efforts of the International Federation of Clinical Chemistry and Laboratory Medicine, research organizations, clinical chemistry laboratories and diagnostic companies, secondary reference materials for the apolipoproteins A-I and B, and lipoprotein(a) have been prepared and tested for their ability to harmonize test values. SP1-01 and SP3-07 WHO-IFCC reagents are now available to manufacturers for use in the value transfer of apolipoprotein A-I and B values to master calibrators, and PRM is proposed to be the secondary reference material for Lp(a). By the worldwide use of such reference materials a better traceability and standardization of measurement is being achieved in the clinical laboratories.

Introduction

Apolipoprotein A-I (apo A-I), the major protein in high-density lipoproteins (HDLs), apolipoprotein B (apo B), the major protein in low-density lipoproteins (LDLs), and lipoprotein(a) [Lp(a)], can serve as important predictors of cardiovascular disease risk. Until recently, the lack of internationally accepted standardization impeded the broad application of apolipoproteins in laboratory medicine. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) through its Committee on Apolipoproteins and Working Group on Lp(a), and together with research institutions and several diagnostic companies have succeeded in their effort to achieve a consensus on a practical standardization procedure. This included the preparation of suitable secondary reference preparations needed for calibrating all commercially available immunoassays for measurement of apo A-I and B, and Lp(a).

Lipoproteins and apolipoproteins: Classification and properties

Disorders of lipid metabolism are important causes of atherosclerosis (1). Atherogenic risk is associated with changes in the composition of blood lipids (cholesterol, triglycerides, and phospholipids), and in their transport forms, i.e., the lipoproteins (2). Different atherogenicity is attributed to the different lipoprotein classes with LDLs considered as atherogenic, and HDLs as protective against atherosclerotic vascular changes caused by accumulation of cholesterol in the arteries (3, 4). Lp(a) is considered to act as an atherogenic particle especially when other risk factors are present (5, 6). The functional properties of lipoproteins and their atherogenicity depend mainly on the type and composition of their protein components, i.e., the apolipoproteins (1, 3). The major apoproteins of HDLs are apo A-I and apo A-II, of LDLs is apo B, and of Lp(a) are apolipoprotein (a) [apo(a)] and apo B.

Diagnostic relevance of apolipoprotein measurements

The quantitative measurement of apo A-I and apo B is an alternative to conventional lipid and lipoprotein analysis. Apolipoprotein measurements permit an assessment of cardiovascular risk and response to lipid-lowering therapy, and also enable identification of patients with certain inheritable abnormalities of lipoprotein metabolism not detectable using other methods (7). Prospective studies have confirmed the usefulness of plasma apo B concentration in determining risk (8, 9, 10, 11). The ratio of apo B / apo A-I allows those subjects with and without

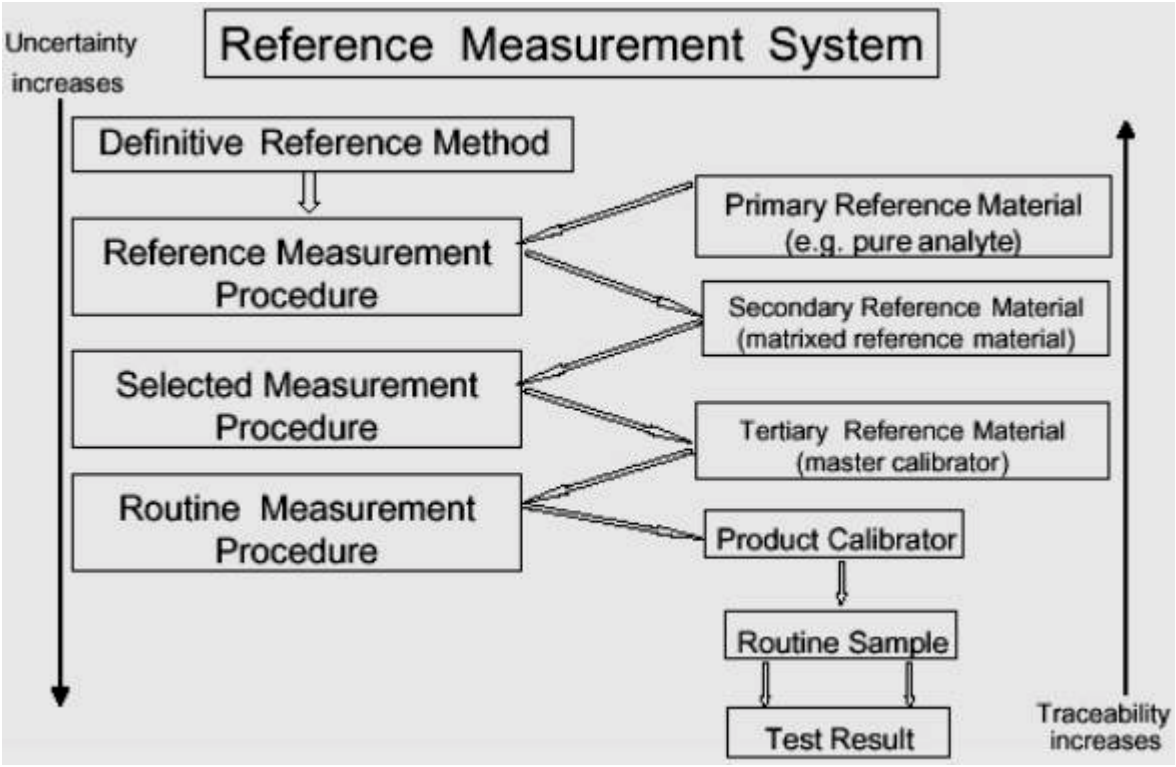

Fig. 1. Hierarchy of international reference materials.

coronary artery disease (CAD) to be distinguished (12, 13) and seems to be useful for assessment of the effectiveness of pharmacological treatment with lipid-lowering drugs (14, 15). Elevated levels of apo B are also found in normolipidemic patients with early CAD even when levels of total and LDL-cholesterol are normal. Apo B measurement may be more relevant to CAD risk assessment because the amount of apo B per LDL particle is relatively constant, whereas that of cholesterol is variable (16, 17). In hypertriglyceridemic patients with impaired glucose tolerance and type II diabetes and where total cholesterol is similar to the non-diabetic population, apo B can differentiate small, dense LDL enriched with cholesterol ester from less atherogenic particles (16).

Numerous case-control and prospective clinical studies have linked elevated levels of plasma Lp(a) to an increased risk of atherosclerotic-related diseases (5, 18, 19, 20, 21). In a meta-analysis of twelve prospective studies and after conversion to the same Lp(a) concentration units for 1617 cases of ischemic heart disease (IHD) and 10,035 controls, it was concluded that Lp(a) is an independent prospective risk factor for IHD, with the effect similar in men and women the populations being 95% Caucasian (22). Lp(a) increases the risk of IHD when LDL-cholesterol is also elevated (6, 18), but is not associated with progression of CAD in subjects without prominent primary or secondary hyperlipidemia (23).

Harmonization of methods for the measurement of apo A-I, apo B and Lp(a)

In routine clinical laboratories the immunochemical measurement of apo A-I, apo B and Lp(a) is a state-of-the-art procedure using commercially available immunoassays based mainly on fully automated immunonephelometric (INA) or immunoturbidimetric (ITA) methods (1, 3, 5, 24). The precision of the tests performed on automated analyzers is high. Nephelometer systems yield a mean interassay variability of ~4% for apo A-I and apo B measurements (1) and a similar precision was obtained for Lp(a) (25, 26). Until recently, the large-scale introduction of apolipoprotein measurement was hampered by the lack of reference materials and methods which resulted in the use of different calibration methods and a lack of comparability of results (27, 28). Other major sources of variability of apolipoprotein results are non-linearity of methods, matrix effects causing non-parallelism between calibrator and samples, and inadequate relative apolipoprotein concentrations in the available reference materials (25, 27). It was soon realized that the preparation, selection, and calibration of appropriate serum-based reference materials were the key to obtaining international standardization and harmonization of immunoassays for measurements of apo A-I and B (29, 30) and Lp(a) (25, 26, 31). Thus, use of common reference materials in a physical state equivalent to that of patient samples is

Analyte	1° Reference Method	1° Reference Material	2° Reference Material	Consensus Method for Value Assignment
Apo A-I	HPLC-MS (candidatea) [CDCb]	BCR – CRM 393, Purified apo A-I	WHO RR SP1-01 (1.50 ± 0.08 g/L)	RIA (CDC)
Apo B	Not available	d=1.03-1.05 kg/L, UC purified LDL	WHO RR SP3-07 (1.22 ± 0.02 g/L)	INA (NWLRLc)
Lp(a)	Not available	Two Lp(a) purified preparationsd	PRM 2B	a-40 ELISAe (NWLRL)
			(107.1 ± 8.6 nmol/L)	a1-1 ELISA (NWLRL)

Table 1. The reference measurement systems for apo A-I, apo B and Lp(a).

a Barr JR, Maggio VL, Patterson DG Jr., Cooper GR, Henderson LO, Turner WE, et al. Isotope dilution-mass spectrometric quantification of specific proteins: model application with apolipoprotein A-I. Clin Chem 1996;42:1676-82.
b Centers for Disease Control and Prevention; c Northwest Lipid Research Laboratories
d Lp(a) isolated by sequential ultracentrifugation and molecular sieve chromatography or by lysine-Sepharose and CsCl density gradient ultracentrifugation
e Marcovina SM, Albers JJ, Gabel B, Koschinsky ML, Gaur VP. Effect of the number of apolipoprotein (a) crinkle 4 domains on immunochemical measurements of lipoprotein(a). Cline Chem 1995;41:246-55.

necessary to reduce the variation in values among laboratories and between different measurement systems. Primary standards for apo A-I and B, and Lp(a), i.e., pure proteins, are not suitable for manufacturers' assay systems because they show instability and/or matrix effects. Pure apo B is particularly unsuited as a primary standard due to self-association, sensitivity to oxidation, and irreversible matrix aggregation. Therefore the use of secondary matrixed (serum) reference materials (SSRMs) is the only possible approach to harmonizing calibration. A hierarchy of reference materials and reference methods constitute the reference measurement system (Fig.1) and form the basis for the establishment of standardized reference values and decision cutoff limits in different ethnic populations.

IFCC standardization programs

In 1989 the IFCC Committee on Apolipoproteins (Chairperson: Professor Santica Marcovina, Seattle, WA, USA), the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, and representatives of 25 diagnostic companies producing test systems for apo A-I and B reached a consensus on the implementation of a collaborative study in 3 phases. Similarly, in 1995 the IFCC Working Group for Lp(a) Assay Standardization (Chairperson: Professor Armin Steinmetz, Andernach, Germany), working in collaboration with manufacturers of diagnostic assays for Lp(a), was formed with a view to selecting a suitable secondary reference material for Lp(a) that would result in closer comparability of Lp(a) values between assay systems.

Preparation of serum reference materials for apo A-I, apo B and Lp(a)

Special effort is needed to prepare suitable SSRMs for apo A-I, apo B, and Lp(a) that can be used to calibrate different test systems uniformly (26, 32, 33). Reference preparations must have the same immunochemical behavior as patient specimens in all systems. The SSRM for Apo A-I was prepared in lyophilized form to ensure long-term stability. In contrast, as the lyophilization process alters apo B structure resulting in changes in immunoreactivity and matrix effects between methods, a stabilized liquid preparation shock-frozen immediately after production and kept at -70 ºC to improve long-term

Table 2. Reference ranges of apo A-I and B for males and females in various populations.

Population	Apo A-I (10th percentile) in g/L		Apo B (75th percentile) in g/L	
	Men	Women	Men	Women
US (Framingham)	1.07	1.22	1.18	1.11
Finland	1.09	1.25	1.41	1.29
Sweden	1.1	1.22	1.53	1.42
Northern Italy	1.16	1.27	1.29	1.31

stability, was used as reference material for apo B. The SSRM for Lp(a) was prepared in lyophilized form from a serum pool and has three predominantly expressed apo(a) polymorphs containing 16, 17 and 18 K4 domains, and three minor polymorphs of 14, 20 and 32 K4 domains.

Apolipoprotein A-I and B Standardization In Phase 1 the harmonization of test results showed that uniform calibration of 26 test systems for apo A-I and 28 for apo B had been accomplished using the same reference materials (32). Apo B levels before the uniform calibration showed a large among-assay variation (>19%) due to different assay standardization. This was significantly reduced to a mean of 6% after uniform calibration. Of the 26 reference materials for apo A-I and apo B proposed by the manufacturers, 15 for apo A-I and 11 for apo B, two lyophilized preparations for apo A-1 and two liquid-stabilized preparations for apo B were selected for further evaluation in Phase 2 of the study (33). Using these materials as the common calibrator, results again showed that by uniform calibration of the 37 test systems harmonization of results could be accomplished. Among-systems variation in apo A-l levels improved from 9% to 5.4% using the common calibrator and from ~20% to 7% for apo B. The linearity and parallelism study showed that the immunoreactivity exhibited by the candidate SSRMs, SP1 and SP3, was closest to that of the fresh and frozen serum pools and there were no significant matrix effects in the tested assays.

In Phase 3, SP1-01 (the batch prepared first) and SP3-07 (a new batch) were examined to determine their ability to transfer assigned values to each calibrator (34, 35). An apo A-I value of 1.50 g/L was assigned to SP1-01 and an apo B value of 1.22 g/L was assigned to SP3-07 using primary reference materials and consensus methods (Table 1). Following a common protocol, the values for apo A-I and apo B were transferred from the two reference materials to the respective calibrators of the test systems of the participating diagnostic companies. Then 50 fresh-frozen serum samples covering a wide range of apo A-I and apo B concentrations were analyzed to test for comparability between various immunoassays. The results obtained by the diagnostic companies were excellent with an interlaboratory variation for results using of 2-6% for apo A-I and 3-7% for apo B. Therefore these SSRMs were proposed as WHO international reference materials.

The materials SP1-01 and SP3-07 were submitted to the WHO Expert Panel on Standardization as proposed reference materials for apo A-I and B, respectively. Based on the results of the reports submitted, the materials were accepted as WHO-IFCC International Reference Reagents (Table 1). The materials are stored at CDC and are available to manufacturers and research institutes for the evaluation and standardization of new assays.

Lipoprotein(a) Standardization

In Phase 1 forty test systems were evaluated for analytical performance by testing serum samples for precision, linearity and parallelism characteristics. Eight manufactured Lp(a) calibrator materials were tested for commutability and method harmonization after reference to an arbitrary calibration (25). In Phase 2, testing was repeated in 27 Lp(a) test systems using four newly prepared materials (26). The Lp(a) material with maximum harmonization achieved a variation of <8% for 18 INA and ITA systems. On the basis of acceptable analytical performance, maximal harmonization effect and documented stability, the lyophilized material PRM 2B was selected as the common calibrator for Lp(a) and assigned a value of 107 nmol/L using a consensus, reference measurement system (Table 1).

In Phase 3, 16 manufacturers and six research laboratories evaluated PRM for its ability to transfer an accuracy-based value to the immunoassay calibrators and for the extent of concordance in results between methods (31). After uniformity of calibration was demonstrated in the 22 evaluated systems, Lp(a) was measured on 30 fresh frozen sera covering a wide range of Lp(a) values and apo(a) sizes. The among-laboratory CV of Lp(a) measurement of the 30 samples ranged from 6% to 31%. In general, these CVs were higher than those obtained for the PRM (2.8%) and higher than those observed in three quality control samples (14%, 12%, and 9% respectively), reflecting the broad range of apo(a) isoforms in the samples and the sensitivity of most of the analytical systems to apo(a) size heterogeneity.

Accuracy of Lp(a) measurement for each system was determined by comparison with target values assigned using the consensus, reference measurement system (31). A Lp(a) latex-enhanced immunoturbidimetric method was the most accurate of the tested systems with an average absolute bias of only 4.4 nmol/L compared with 12.4 to 23.8 nmol/L for other methods. Only two of the tested Lp(a) systems showed minimal bias to apo(a) isoform heterogeneity. From the Phase 3 results it appears that many of the current Lp(a) methods are isoform-biased and hence inaccurate, and that their use has possibly led to incorrect conclusions in clinical studies.

The proposed secondary reference material for Lp(a), PRM, has the characteristics of a suitable reference material with a negligible matrix effect in the tested Lp(a) assays. Through the use of PRM superimposable Lp(a) results were produced for a manufacturer's assay and the reference method indicating that PRM is able to standardize Lp(a) measurement. The incomplete harmonization of values is not a problem of the calibrator but of the apo(a) size-sensitive nature of current assays. No reference material, either a primary or secondary standard will be able to eliminate substantial differences in Lp(a) values measured by analytical systems that are affected by apo(a) size heterogeneity. Based on the results of this study together with the stability data for PRM, the IFCC WG Lp(a) will seek international recognition of PRM as the secondary reference material for Lp(a).

Conclusions The initiative started by the IFCC Committee on Apolipoproteins has contributed to a practical solution to the harmonization of test results of immunoassays for apo A-I and apo B. Therefore through the efforts of the IFCC a worldwide consensus on the calibration of test systems for apo A-I and B has been achieved. The availability of internationally accepted reference materials will eliminate a major factor of variability among test systems. Through the use of IFCC standardized methods traceable to SP1-01 and SP3-07 the value distributions of apo A-I and apo B in various populations have been described (Table 2; 36, 37, 38, 39, 40).

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References

1 Rifai N, Warnick GR, Dominiczak MH. Handbook of lipoprotein testing. Washington, DC: AACC Press, 2000.

2 Rifai N, Chapman JF, Siverman LM, Gwynnes JT. Review of serum lipids and apolipoproteins in risk assessment of coronary hearT disease. Ann Clin Lab Sci 1988:18:429-39.

3 Rossenau M, Widhalm K, Jarausch J. Apolipoproteins in lipid disorders. Vienna, New York: Springer-Verlag; 1991.

4 Brewer HB, Gregg RE, Hoeg JM. Fojo SS. Apolipoproteins and lipoproteins in human plasma: an overview. Clin Chem 1988:34:B4-B8.

5 Marcovina SM, Koschinsky ML. Lipoprotein(a): structure, measurement, and clinical significance. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington, DC: AACC Press, 2000: 345-85.

6 Cantin B, Gagnon F, Moorjani S, DesPres JP, LaMarche B, Lupien PJ, et al. Is lipoprotein(a) an independent risk factor for ischemic heart disease in men? The Quebec Cardiovascular Study. J Am Coll Cardiol 1998;31:519-25.

7 Albers JJ, Brunze11 JD, Knopp RH. Apolipoprotein measurements and their clinical application. Clin Lab Med 1989;9:137-52.

8 Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med 1991;325:373-81.

9 Coleman MP, Key TJA, Wang DY, Hermon C, Fentiman IS, Allen DS, et al. A prospective study of obesity, lipids, apolipoproteins, and ischaemic heart disease in women. Atherosclerosis 1992;92:177-85.

10 Sigurdsson G, Baldursdottir A, Sigvaldason H, Agnarsson U, Thorgeirsson G, Sigfusson N. Predictive value of apolipoproteins in a prospective study of coronary artery disease in men. Am J Cardiol 1992;69:1251-4.

11 Lamarche B, Moorjani S, Lupien PJ, Cantin B, Bernard P-M, Gilles R, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Québec Cardiovascular Study. Circulation 1996;94:273-8.

12 Sniderman AD. Apolipoprotein B and apolipoprotein A-I as predictors of coronary artery disease. Can J Cardiol 1988;4:24A-30A.

13 Reinhart RA, Gani K. Arndt MR, Broste SK. Apolipoproteins A-I and B as predictors of angiographically defined coronary artery disease. Arch Int Med 1990;150:1629-33.

14 Wiklund O, Angelin B, Bergman M, Berglund L, Bondjers G, Carlsson A, Linden T, Miettinen T, Odman B, Olofsson SO, et al. Pravastatin and gemfibrozil alone and in combination for the treatment of hypercholesterolemia. Am J Med 1993;94:13-30.

15 Schaefer EJ, McNamara JR. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH. eds. Handbook of lipoprotein testing. Washington, DC: AACC Press, 2000: 77-101.

16 Bhatnagar D, Durrington PN. Measurement and clinical significance of apolipoproteins A-I and B. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington, DC: AACC Press, 2000: 287-310.

17 Sniderman AD, Pederson T, Kjekshus J. Putting low-density lipoproteins at center stage in atherogenesis. Am J Cardiol 1997;79:64-7.

18 Cremer P, Nagel D, Mann H, Labrot B, Müller-Berninger R, Elster H, et al. Ten-year follow-up results from the Goettingen Risk, Incidence and Prevalence Study (GRIPS). I. Risk factors for myocardial infarction in a cohort of 5790 men. Atherosclerosis 1997;129:221-30.

19 Berg K, Dahlén G, Christopherson B, Cook T, Kjekshus J, Pedersen T. Lp(a) lipoprotein level predicts survival and major coronary events in the Scandinavian Simvastatin Survival Study. Clin Genet 1997;52:254-61.

20 Schaefer EJ, Lamon-Fava S, Jenner JL, McNamara JR, Ordovas JM, Davis CE, et al. Lipoprotein(a) levels and risk of coronary heart disease in men. The Lipid Research Clinics Coronary Primary Prevention Trial. JAMA 1994;271:999-1003.

21 Wild SH, Fortmann SP, Marcovina SM. A prospective case-control study of lipoprotein(a) levels and apo(a) size and risk of coronary heart disease in Stanford Five-City Project Participants. Arterioscler Thromb Vasc Biol 1997;17:239-45.

22 Craig WY, Neveux LM, Palomaki GE, Cleveland MM, Haddow JE. Lipoprotein(a) as a risk factor for ischemic heart disease: metaanalysis of prospective studies. Clin Chem 1998;44:2301-6.

23 Marburger C, Hambrecht R, Niebauer J, Schoeppenthau M, Scheffler E, Hauer K, Schuler G, Schlierf G.Association between lipoprotein(a) and progression of coronary artery disease in middle-aged men. Am J Cardiol 1994;73:742-6.

24 Labeur C, Sheperd J, Rosseneu M. Immunological assays of apolipoproteins in plasma: methods and instrumentations. Clin Chem 1990;36:591-7.

25 Tate JR, Rifai N, Berg K, Couderc R, Dati F, Kostner GM, et al. International Federation of Clinical Chemistry standardization project for the measurement of lipoprotein(a). Phase 1. Evaluation of the analytical performance of lipoprotein(a) assay systems and commercial calibrators. Clin Chem 1998;44:1629-40.

26 Tate JR, Berg K, Couderc R, Dati F, Kostner GM, Marcovina SM, et al. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Standardization Project for the Measurement of Lipoprotein(a). Phase 2. Selection and Properties of a Proposed Secondary Reference Material for Lipoprotein(a). Clin Chem Lab Med 1999;37:949-58.

27 Albers JJ, Marcovina SM. Standardization of apolipoprotein B and A-I measurements. Clin Chem 1989;35:1357-61.

28 Albers JJ, Marcovina SM. Lipoprotein(a) quantification: comparison of methods and strategies for standardization. Curr Opin Lipdol 1994;5:417-21.

29 Marcovina SM, Albers JJ. Apolipoprotein assays: Standardization and quality control. Stand J Clin Lab Invest 1990;50(suppl 198):58-65.

30 Dati F. Standardization of commercial assays for serum Apo A-I and Apo B: A consensus procedure for the calibration of reference material. Stand J Clin Lab Invest 1990;50(suppl 198):73-9.

31 Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). Clin Chem 2000,46:1956-67.

32 Marcovina SM, Albers JJ, Dati F, Ledue TB, Ritchie RF. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. Clin Chem 1991;37:1676-82.

33 Albers JJ, Marcovina SM, Kennedy H. International Federation of Clinical Chemistry standardization project for measurement of apolipoproteins A-I and B. 11. Evaluation and selection of candidate reference materials. Clin Chem 1992;38:658-62.

34 Marcovina SM, Albers JJ, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurement of apolipoproteins A-I and B. III. Comparability of apolipoprotein A-I values by use of international reference material. Clin Chem 1993;39:773-81.

35 Marcovina SM, Albers JJ, Kennedy H, Mei JM, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of international reference material. Clin Chem 1994;40:586-92.

36 Contois JH, McNamara JR, Lammi-Keefe CJ, Wilson PWF, Massov T, Schaefer EJ. Reference intervals for plasma apolipoprotein A-I determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. Clin Chem 1996;42:507-14.

37 Contois JH, McNamara JR, Lammi-Keefe CJ, Wilson PWF, Massov T, Schaefer EJ. Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. Clin Chem 1996;42:515-23.

38 Leino A, Impivaara O, Kaitsaari M, Jãrvisalo J. Serum concentrations of apolipoprotein A-I, apolipoprotein B, and lipoprotein(a) in a population sample. Clin Chem 1995;41:1633-6.

39 Jungner I, Marcovina SM, Walldius G, Holme I, Kolar W, Steiner E. Apolipoprotein B and A-I values in 147576 Swedish males and females, standardized according to the World Health Organization-International Federation of Clinical Chemistry First International Reference Materials. Clin Chem 1998;44:1641-9.

40 Graziani MS, Zanolla L, Righetti G, Marchetti C, Mocarelli P, Marcovina SM. Plasma apolipoproteins A-I and B in survivors of myocardial infarction and in a control group. Clin Chem 1998;44:134-40.