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(54) Title: METHODS FOR MEASURING HDL SUBPOPULATIONS

(57) Abstract: This invention provides a capture/detection antibody-based method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample, wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes. This invention also provides related analytical and diagnostic methods, as well as kits for performing same.

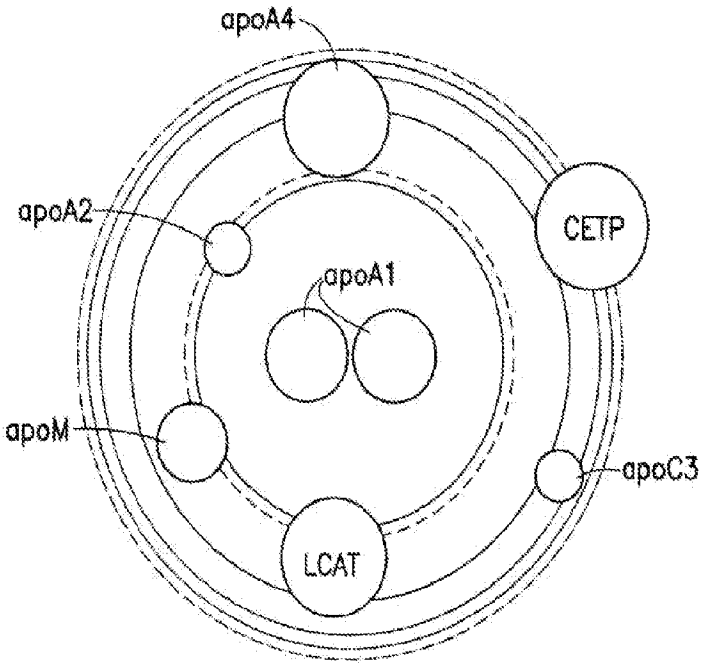

FIG. 1

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METHODS FOR MEASURING HDL SUBPOPULATIONS

This application claims priority of U.S. Provisional Application No. 61/515,101, filed August 4, 2011, the contents of which are incorporated herein by reference.

Throughout this application, various publications are cited. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the state of the art to which this invention pertains.

Field of the Invention

The present invention relates to methods and kits for measuring HDL and diagnosing cardiovascular disease and other HDL-related diseases in a subject. This invention exploits the physical proximity between two protein epitopes to identify and quantify discrete HDL subpopulations present in heterogeneous mixtures, and measure changes in HDL subpopulations as a result of disease or treatment.

Background of the Invention

Cardiovascular Disease and HDL

Cardiovascular disease is a leading cause of morbidity and mortality, particularly in developed nations such as the United States, Western European countries and East Asian countries. The incidence of mortality due to cardiovascular disease in these regions has decreased in last 30 years (Braunwald, E., N. Engl. J. Med. 337:1360, 1997; Hoyert, D. L., et al.,

"Deaths: Preliminary Data for 2003" in National Vital Statistics Reports. Hyattsville: National Center for Health Statistics, 2005; Unal B., et. al., Circulation 109:1101, 2004). Factors contributing to improved patient outcome include improved cardiovascular diagnostics, reduction of major modifiable cardiovascular risk factors and advanced medical technologies to treat acute coronary syndrome. Despite these advances, however, cardiovascular disease remains a leading cause of morbidity and mortality in developed countries (see Hoyert D. L., et al., National Vital Statistics Reports, 2005; Ueshima, H., et. al., Circulation, 118:2702, 2008).

In the end, most cardiovascular deaths result from acute coronary syndromes, including unstable angina pectoris and acute myocardial infarction (see Shah, P. K., Am. J. Cardiol., 79:17, 1997). Coronary syndromes often arise from acute coronary thrombosis, itself typically the result of disruption or rupture of the fibrous cap of a lipid-laden atherosclerotic plaque (see Munger, M.A. and Hawkins, D. W., J. Am. Pharm. Assoc., 44(Suppl 1):S5, 2003). The understanding of the mechanisms mediating atherosclerotic plaque formation, progression and subsequent rupture remains limited. At the cellular level, the pathophysiology of the disease remains in constant evolution, albeit at such a slow pace that it takes years if not decades to reveal itself in the clinical setting. From the moment the genotypic blueprint is set to the environmental inducement brought about through lifestyle choices, the disease has a beginning and an end. The factors that influence this trajectory are numerous and at the molecular level remain mostly undefined. In the absence of detailed molecular knowledge, it is safe to say that the physiological state at any juncture during the progression of this disease is different than at any other point. This can be observed experimentally as wide-ranging biological indicators such as biomarkers, cellular events and functional activities vary over the course of the disease. Particular indicators that precede disease symptoms are often referred to as risk factors that may be predictive of the

pending disease state. Some predictive indicators are closely associated with the disease while others may be intrinsically involved with the disease and its development.

Involvement of plasma cholesterol in the development of atherosclerotic risk and subsequent cardiovascular disease has been validated in both human and animal models alike. Elevated LDL cholesterol and total cholesterol are directly related to an increased risk of cardiovascular disease (Anderson, K. M., et. al., JAMA 257:2176, 1987). The positive relationship between the concentration of low-density lipoprotein cholesterol (LDL-C) and the future risk of cardiovascular events has been observed in many large-scale population studies, and the benefits of reducing LDL-C levels has been proven in numerous intervention studies. The apparent effects of aggressive LDL-lowering are exemplified by various statin treatments leading to risk reductions of over 25-35%, and further declines in LDL-C levels by co-administration of drugs targeting LDL-C levels through independent mechanisms of action (including ezetimibe and resins) could result in plaque regression.

In contrast, it has been established that the risk of cardiovascular disease is inversely proportional to plasma levels of HDL-C and the major HDL apolipoprotein, apoA1 (Gordon, D. J., et al., N. Engl. J. Med 321:1311, 1989). Studies have shown that high HDL-C levels are associated with longevity (Barzilai, N., et al., JAMA 290:2030, 2003). Consistent with these findings, an abnormally low HDL-C level is a well-accepted risk factor for the development of clinically significant atherosclerosis (particularly common in men with premature atherosclerosis) (Gordon, D. J., et al., N. Engl. J. Med. 321:1311, 1989; Wilson, P. W., et al., Arteriosclerosis 8:737, 1988).

Early demonstration of the inverse relationship between HDL-C levels and cardiovascular risk can be found in the Framingham Heart Study, which showed that individuals with HDL-C levels of less than 35 mg/dL at the beginning of the study had a future coronary risk of greater than four times that of individuals with HDL-C levels over 65 mg/dL (Wilson, P. W,, et al., Amer. J. Cardiol., 46:649, 1980). Other prospective population studies including PROCAM, Helsinki Heart Study and Multiple Risk Factors Intervention Trial support the view that risk associated with lower HDL-C is independent of LDL-C levels, and raising levels of HDL-C should be considered as important a therapeutic target as lowering LDL-C. The increased risk associated with a low HDL-C can be seen at all concentrations of LDL-C (Gordon, T., et. al., Am. J. Med., 62:707, 1977). Post hoc analyses of stable CHD and ACS in prospective trials indicate that both HDL-C and triglyceride levels are associated with high risk even at recommended LDL-C goals (Olsson, A. G., et. al., Eur. Heart J., 26:890 2006; Miller, M., et. al., J. Am. Coll. Cardiol., 51:724, 2008; Barter, P., et. al., N. Eng. J. Med., 357:1301, 2007). These studies suggest that for every HDL-C increase of 1 mg/dL, the risk for a CHD event is reduced by 2-5% (Chapman M. J., et al., Curr. Med. Res. Opin. 20:1253, 2004). Thus, a strategy of targeting both high LDL-C and low HDL-C is supported by the results of the INTERHEART Study which showed that the ratio of apoB to apoA1 (reflecting LDL to HDL ratio) demonstrated considerable power for predicting future myocardial infarction in a broad population of differing ethnic origin (Yusuf S., et. al., Lancet 364:973, 2004).

Despite the growing epidemiological evidence indicating that HDL-C is a cardiovascular risk marker and raising HDL-C levels can reduce that risk, ambiguity and debate continue to challenge the concept of HDL as a risk marker or therapeutic target (see Chapman, M. J., et. al., Eur. Heart J., 2011, Apr 29 online). Large failures of HDL-modifying drug trials undermine the

confidence of researchers and clinicians alike (Tall, A. R., Arterioscler. Thromb. Vasc. Biol., 27:257, 2007; Horowitz, J. D., et. al., Cardiovasc. Drug Ther., 25-69, 2011; AIM-HIGH Investigators, Am. Heart J., 161:471, 2011) and have left researchers searching for explanations.

Cholesterol numbers are expressed as different units of measurement in different countries. The United States uses milligrams as the standard for measuring cholesterol, and levels in the blood are expressed as milligrams per deciliter (mg/dL). In Canada, millimoles per liter (mmol/L) are used in measuring cholesterol numbers, and the same goes for many parts of Europe. In the United States, good cholesterol numbers for the average, healthy person are less than 200 mg/dL. Once a person gets to 200 mg/dL, he is considered to have borderline-high levels of cholesterol. At levels of over 240 mg/dL, the person is considered to have high cholesterol. In Canada and many European countries, good cholesterol numbers are those under 5.2 mmol/L. Above 5.2 mmol/L and up to 6.2 mmol/L is considered borderline high. Once a person's levels move above 6.2 mmol/L of blood, his levels of cholesterol are considered high. Sometimes, cholesterol numbers are categorized by the type of cholesterol. In the United States, LDL levels of less than 70 mg/dL are considered best for those at higher risk for developing heart disease, which corresponds to 1.8 mmol/dL in Canada and many parts of Europe. An LDL level of 100 to 129 mg/dL in the United States and 2.6 to 3.3 mmol/L is considered close to optimal for those at lower or average risk of developing heart disease. HDL-C levels are considered good at 60 mg/dL and above in the United States, and more than 1.5 mmol/L in Canada and European countries. The range from 40 to 59 mg/dL (1.3 to 1.5 mmol/L) may be considered acceptable for HDL numbers, depending on gender and other risk factors for heart disease. Anything below 50 mg/dL (1.3 mmol/L) is considered poor for women. Levels of HDL-C below 40 mg/dL (1 mmol/L) are considered poor for men.

The current version of the Framingham Risk Score was published in 2002 (see "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel" Circulation, 106:3143 2002). The publishing body is the Adult Treatment Panel III (ATP III), an expert panel of the National Heart, Lung, and Blood Institute, which is part of the National Institutes of Health (NIH), USA. The Framingham/ATP III criteria were used to estimate CHD risk in the USA. Data from 11,611 patients from a very large study, the NHANES III, were used. The Risk Score is estimated using the 10-year risk for coronary heart disease (CHD). The updated version included age range, gender, total cholesterol, LDL cholesterol, HDL cholesterol, blood pressure, hypertension treatment and smoking, and it excluded diabetes, because diabetes meanwhile was considered to be a CHD Risk Equivalent. Some patients without known CHD have a risk of cardiovascular events comparable to that of patients with established CHD. Cardiology professionals refer to such patients as having a CHD Risk Equivalent. These patients should be managed as patients with known CHD. Diabetes is accepted as a CHD Risk Equivalent.

Guidelines receive regular review and constant revision compelled by ongoing and growing scientific knowledge of the disease. Recent recommendations of the European Atherosclerosis Society (EAS) Consensus Panel (see Chapman, M. J., et. al., Eur. Heart J., 32:1345, 2011) include targeting elevated low HDL-C < 1 mmol/L (40 mg/dL) and/or triglyceride-rich lipoproteins (TRLs) > 1.7 mmol/L (150 mg/dL). These recommendations will facilitate reduction in the substantial cardiovascular risk that persists in patients with cardiometobolic abnormalities at LDL-C goal.

The mechanisms by which HDL prevents cardiovascular disease are the subject of current scientific research. As a predictive risk factor and then as a

functional contributor to atherosclerosis, the role of HDL itself likely varies during the progression of the disease and the associated physiological state of the individual. The biological functions, attributed to the lipoprotein particle population, which are important to the prevention of plaque formation, could in fact be significantly different than those HDL activities critical to reducing inflammation of the arterial wall and unrelated still to the role HDL plays during recruitment of platelets to the growing thrombus. On an individual basis, levels of these various activities likely differ. Preceding the onset of the disease, it is supposed that a state of dyslipidemia has been established which is characterized by an imbalance in favor of circulating levels of proatherogenic, cholesterol-rich apoB-containing particles rather than the antiatherogenic apoA1-containing HDL. Mechanisms related to lipoprotein disequilibrium, such as HDL-mediated protection of LDL from oxidation and lipid exchange between HDL and LDL, may be overwhelmed by such governing principals as mass action. Some believe that HDL protects against LDL oxidative modification that may be a trigger to the initiation and progression of atherosclerosis (Parthasarathy, S., et al., Biochim. Biophys. Acta, 1044:275, 1990; Barter, P. J., et al., Circ. Res. 95: 764, 2004). Others believe that the athero-protective activity of HDL comes from removing cholesterol from artery wall macrophages (Tall, A. R., et al., J. Clin. Invest., 110:899, 2002; Oram, J. F., et. al., Arterioscler. Thromb. Vasc. Biol., 23:720, 2003). Resulting endothelial dysfunction includes arterial stiffness, extracellular matrix signaling, and induced NO-dependent vasorelaxation (Havlik, R. J., et. al., Am. J. Cardiol., 87:104, 2001; Ortiz-Muñoz, G., et. al., FASEB J. 23:3129, 2009; Nofer, J. R., et. al., J. Clin. Invest. 113:569, 2004). Other studies indicate that inflammation is the key process underlying the pathology given that inflammation is a systemic response directed at decreasing toxic effects of harmful agents and repairing vessel endothelial damage (Ross, R., et. al., N. Engl. J. Med., 340:115,1999). A variety of specific functions associated with HDL have been attributed to its anti-

inflammatory activities, including prevention of endothelial inflammation, recruitment of circulating leukocytes resulting in plaque formation followed by recruitment of platelets forming a thrombus (see Toth, P. P., J. Clin. Lipidol., 4:376, 2010; Asztalos, B. F., et. al., Curr. Opin. Lipidol., 22:176, 2011).

The pleiotropic and polygenic nature of cardiovascular disease makes for complex disease etiology, which can obfuscate both prediction and diagnosis. Since the initial studies measuring HDL-C and LDL-C (Eder, H. A., Am. J. Med. 23:269, 1957), methodologies have advanced along with technology, and predictive correlations have improved with ever more complex medical statistical analysis (Modern Medical Statistics: A Practical Guide Brian S. Everitt Wiley 2003). Even so, there continues to be a necessity for improved methods for early assessment of cardiovascular disease and risk.

The Measurement and Properties of HDL

The principal of the surrogate lipid marker cholesterol to classify and quantify lipoprotein particles has been the historical stalwart for over fifty years. Variations include calculating non-HDL-C, which accounts for cholesterol in lipoprotein classes in addition to LDL, including VLDL and intermediate density lipoproteins (IDL). An extension of this methodology uses lipoprotein cholesterol ratios such as LDL-C:HDL-C to improve clinical correlations (Grover, S. A., et. al., Epidemiology 14:315 2002) or total cholesterol:HDL-C. More recently, risk metrics have been employed such as measuring apoA1, a protein surrogate for HDL, or apoB, the surrogate marker for LDL, which may better reflect lipoprotein particle numbers rather than their cholesterol load (Knopp, R. H., Am. J. Med. 83:75 1987; Contois, J. H., et. al., Clin. Chem., 42:507, 1996; Contois, J. H., et. al., Clin. Chem., 42:515, 1996). These approaches rely on immuno-turbidimetric or -nephelometric assays (Marcovina, S. M., et. al., Clin. Chem. 39:773, 1993), provide an alternative

means of measuring those lipoprotein classes, and offer a different perspective given the physiochemical nature of the lipoprotein constituent and the methods used to measure it. Lipoproteins measured using surrogate proteins rather than lipids are reported to be less susceptible to postprandial effects and fluctuations. Similarly, proponents of the apoB:apoA1 ratio believe it to be the single best predictor of coronary risk (Walldius, G., et. al., Clin. Chem. Lab Med. 42:1355, 2004; Holzmann, M. J, et al., Ann Med. 2010 Nov 30 in press). A comprehensive prospective cohort study designed to compare the clinical utility of all said measurements and numerous ratio metric permutations was performed to investigate prediction of coronary heart disease in men and women. The study concluded that the apoB:apoA1 ratio for predicting CHD was comparable with that of traditional lipid ratios, but did not offer incremental utility over total cholesterol:HDL-C (Ingelsson, E., et. al., J. Amer. Med. Assoc., 298:776, 2007).

Other approaches to clinical measures of lipoprotein particle concentration involve sizing and counting using nuclear magnetic resonance (Otvos, J., Clin. Cardiol. 22:II21, 1999). This method offers an additional level of resolution by expanding HDL into three particle subpopulations founded on particle diameter. This method reported discordance between individuals when comparing LDL-C and LDL particle levels which they attributed to disproportionate cholesterol distribution between large and small LDL (Otvos, J. D., et. al., J. Clin. Lipidol., 5:105, 2011). Lastly, both analytical ultracentrifugation and electrophoretic methods used in research settings have led to fractionation of HDL into several subpopulations based on distinct physiochemical property differences (Anderson, D. W., et. al., Biochim Biophys Acta 493:55, 1977, Chapman, M. J., et. al., J. Lipid Res., 22:339, 1981, Kontush, A., et. al., Arterioscler. Thromb. Vasc. Biol. 23:1881, 2003, Asztalos, B. F., et. al., Biochim. Biophys. Acta 1169:291, 1993).

Liquid chromatography-mass spectrometry (LC-MS) is also used in the study of proteomics, where again components of a complex mixture must be detected and identified in some manner. The bottom-up proteomics LC-MS approach is a common method to identify proteins and characterize amino acid sequences and post-translational modifications (Aebersold, R. and Mann, M. Nature 422:198, 2003; Chait, B. T., Science 314:65, 2006). Proteins can be purified first or the crude protein extract digested directly, followed by one or more dimensions of separating the peptides by liquid chromatography coupled to mass spectrometry (a technique known as shotgun proteomics) (Washburn, M. P., et. al., Nat. Biotechnology 19:242, 2001; Wolters, D. A., et. al., Anal. Chem. 73:5683, 2001). By comparing the masses of the proteolytic peptides or their tandem mass spectra with those predicted from a sequence database, peptides can be identified and multiple peptide identifications assembled into a protein identification (Nesvizhskii, A. I., Methods Mol. Biol. 367:87, 2007; Nesvizhskii, A. I., et. al., Nat. Methods 4:787, 2007). Samples of complex biological fluids like human serum may be run in a modern LC-MS/MS system and result in over 1000 proteins being identified, provided that the sample was first separated using physiochemical properties such as density gradient ultracentrifugation, SDS-PAGE or HPLC. Such approaches have been used to identify and quantify proteins associated with lipoprotein particle fractions HDL and LDL.

HDL has unique and measurable physiochemical properties that arise as a direct result of the quantity and relative amounts of its two major constituents, protein and lipid (Rosenson, R. S., et. al., Clin. Chem. 57:392, 2011). Both of these two common constituents can be further divided into specific molecular entities. For lipids, seven classes, including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides, are recognized by the LIPIDS MAPS consortium (Fahy, E., et. al., J. Lipid Res., 50:S9, 2009). At the molecular level, there are ~30,100

distinct lipid entities identified in nature of which ~200 have been detected in fractions of HDL and are referred to as the HDL lipidome. The human plasma proteome has been curated to date to contain 1,175 distinct genes resulting in 7,614 unique protein products (Anderson, N. L., et. al., Mol. Cell. Proteomics, 3:311, 2004). The protein fraction of HDL could consist of ~110 different members, either bound or associating with the lipoprotein particle (Karlsson, H. et. al., Proteomics 5:1431, 2005; Rezaee, F., et. al, Proteomics, 6:721, 2006: Hortin, G. L., et. al., Biochem. Biophys. Res. Commun., 340:909, 2006; Heller, M., et. al., Proteomics 5:2619, 2005; Vasair, T., et. al., J. Clin. Inv., 117:746, 2007; Davidson, W. S., et. al., Arterioscler. Thromb. Vasc. Biol. 29:870, 2009; Davidson, P., et. al., Arterioscler. Thromb. Vasc. Biol., 30:156, 2009). The specific list of proteins associated with HDL is dependent upon the methodology used to separate this lipoprotein subclass away from a serum/plasma sample prior to analysis, given that the separation methodology can result in loss or gain of constituents (Heller, M., et. al., Proteomics 5:2619, 2005; Gordon, S. M., et. al., J. Prot. Res. 9:5239, 2010). The consequence of this observation is that the proteins associated with HDL can vary as a result of the isolation technique.

The totality of all constituents in a single HDL particle combine to generate a physiochemical state. In the physiochemical state reside measurable properties including hydrodynamic radii, volume, charge, and affinity. Such properties influence migration rates used in separation technologies employed, and include, for example, density, size/charge ratio and hydrophobicity. Separation of one particle from another is a direct consequence of differences in their physiochemical states which are defined by the content of their constituents. Typical methods of separating HDL particles from other exogenous contaminants include density ultracentrifugation, gel electrophoresis, gel filtration chromatography and affinity chromatography (Mendez, A. J., et al., J. Biol. Chem. 266:10104,

1991; Guerin, M., et. al., Arterioscler. Thromb. Vasc. Biol. 21:282, 2001; Li, Z., et. al., J. Lipid Res., 35:1698, 1994; Gordon, S. M., et. al., J. Prot. Res. 9:5239, 2010; Krimbou, L., et. al. J. Lipid Res., 44:884, 2003).

HDL particle diversity and heterogeneity is a direct result of the fact that the distribution of both the lipid and protein constituents are in disequilibrium with the HDL particle population as a whole and to each other (Li, Z., et. al., J. Lipid Res., 35:1698, 1994; Kontush, A., et. al., Arterioscler. Thromb. Vasc. Biol. 24:526, 2004; deSouza J. A. et. al., Atherosclerosis 197:84, 2008; Davidson W. S, et. al. Arterioscler. Thromb. Vasc. Biol. 29:870, 2009; Garcia-Sanchez, C., et. al., Clinica Chimica Acta, 412:292, 2011). By definition, this means that any given HDL particle contains only a subset of lipidome and proteome constituents. The molar concentration of individual proteome members in the serum is much lower than that of HDL, suggesting that specific proteome members exist only in subpopulations of HDL (Anderson, L., J. Physiol. 563:23, 2005). Furthermore, it indicates that any two particles can be distinguished from each other by their lipid and protein constituents and by the relative amounts of those molecular entities. Two HDL particles containing the exact same proteome and lipidome, but differing in quantities, can be distinguished from one another by such properties as size or volume. Similarly, two particles could have similar physiochemical properties (such as size, density or migration rate) but contain very different proteome and lipidome constituents.

HDL, when considered as a single entity, is a biologically active complex that contains a plethora of functional activities. In this context, HDL is historically recognized for its antiatherogenic and vasculoprotective activities. Particular focus on its role in cholesterol efflux and reverse-cholesterol transport (RCT), as well as its anti-thrombotic, anti-inflammatory, anti-oxidative, endothelial repair and vasodilation roles, are all believed to be critical activities

contributing to the beneficial and cardio-protective role this lipoprotein class plays (see Kontush, A. and Chapman M. J., Pharmacological Rev., 58:342, 2006; deGoma, E. M., et al., J. Am. Coll. Cardiol. 51:2199; 2008 Navab, M., et al. Nat. Rev. Cardiol. 8:222, 2011). A relationship between HDL and other metabolic-related diseases (including modulation of glucose metabolism, antiapoptotic activity against pancreatic beta cells, platelet function, stem cell maturation and embryogenesis) have been demonstrated. HDL also is involved in innate immunity. HDL demonstrates specific anti-infective activities (Vanhollebeke B. and Pays E., Mol. Microbiol., 76:806, 2010) and a variety of infections modulate HDL (Baker, J., et. al., J. Infect. Dis., 201:285, 2010; Barlage, S., et. al., Intensive Care Med., 35:1877, 2009). This association may be a direct consequence given the number of HDL proteome members involved in innate immunity (Vasair, T., et. al., J. Clin. Inv., 117:746, 2007) and the utilization of HDL metabolic pathways in infection mechanisms (Scarselli, E., EMBO J. 21:5017, 2002; Shi, S. T., et al., Virology 292:198, 2002).

Evidence shows that HDL particles separated from each other based on their physiochemical qualities result in an apportioning of functional activity (Kontush, A., et. al., Atheroscler. Thromb. Vasc. Biol., 24:526, 2004; Shiflett, A. M., et. al., J. Biol. Chem. 280:32578, 2005). In other words, particles of different physiochemical states preferentially contain identifiable and specific measurable functional activities. Such segregation of functional activity with physiochemical properties indicates that bioactivity is particle type-specific. Given that particle physiochemical properties are the direct consequence of the constituent lipidome and proteome associated with the particle, it may be understood that an HDL particle's activity is the direct result of the absolute composition of all constituents. As such, it can be inferred that measuring the particle's constituents can identify a specific biological activity of the particle once it has been defined.

One of the most important aspects of HDL particle analysis is correct collection and storage of the sample set (Dunn, W. B., et. al., Nature Protocols 6:1060, 2011). Beyond this, sample handling may result in various technical complications in a method-dependent manner. As a consequence of HDL particle population heterogeneity and the compositional nature of the particle, analytical methods used to assess HDL that depend on separation by physiochemical properties are susceptible to limitations. The separation process causes the HDL particle to degrade from its natural state in an unpredictable manner. The separation process results in the loss or gain of constituents (Whiteaker, J. R., et. al., J. Proteome Res., 6:828, 2007). The separation process does not resolve the desired end-product from contaminating materials. The separation process does not deliver the necessary precision to resolve HDL subpopulations into distinct groups of particles of identical constituents. Methods designed to limit these issues offer a refined view of HDL, the entity, and provide clearer insights into HDL biology.

Antibodies, Antigens and Immunoassays

An antigen is any substance that the immune system can recognize as foreign. At the molecular level, an antigen is characterized by its ability bind at the antigen-binding site of an antibody. Antigens are usually proteins or polysaccharides. Polypeptides, lipids and nucleic acids can also function as antigens. Small molecules, called haptens, can also act as antigens but typically must be chemically coupled to large carrier proteins such as bovine serum albumin or keyhole limpet hemocyanin (Wu, C. and Cinader, B., J. Exp. Med. 134:693, 1971). Vaccines are examples of immunogenic antigens intentionally administered to induce acquired immunity in the recipient (Immunobiology: The Immune System in Health and Disease, 5th ed., 2001;

Janeway, C.A., Travers, P., Walport, M. and Shlomchik, M. J., Garland Science, NY, 2001). Although antigens are usually thought to be derived from non-self antigens, immunogens derived from host sequences can act as antigens and can induce acquired immunity which produces antibodies capable of binding host proteins.

An epitope is also known as an antigenic determinant. The part of an antibody that recognizes the antigen epitope is called the antigen-binding site of an antibody, or paratope. It is a small region in the antibody's Fv region and is approximately 15–22 amino acids, contributed from both the antibody's heavy and light chains (Immunology, 5th ed., 2003 pp.57-75; Goldsby, R., Kindt, T. J., Osborne, B. A. and Kuby, J., W. H. Freeman and Co., NY). The epitopes of protein antigens are divided into two categories, linear epitopes and conformational epitopes, based on their structure and interaction with the paratope. (Huang, J., and Honda, W., BMC Immunology 7:7, 2006). A linear epitope interacts with the paratope based on primary structure, a continuous sequence of amino acids from the antigen. In contrast, a conformational epitope is typically composed of discontinuous sections of the antigen's amino acid sequence that are brought together upon three-dimensional protein folding. These epitopes interact with the paratope based on tertiary structure and the 3-D surface shape and features of the antigen. In some instances, a conformational epitope can be composed of a continuous sequence of amino acids constrained to a specific tertiary structure. A large number of antibody-antigen interactions have conformational epitopes (Flanagan, N., Genet. Engineer. Biotech. News, 31:x 2011; Banik, S. R. and Doranz, B. J., Genet. Engineer. Biotech. News. 3:25, 2010).

Since antigens are usually proteins that are too large to bind as a whole to any antibody, only a small portion of the protein – a specific epitope – is bound by the paratope. When used to induce an adaptive immune response,

one immunogenic protein results in a polyclonal B cell response producing many different antibodies to that single antigen (Immunology, 5th ed., 2003 pp.57-75; Goldsby, R., Kindt, T. J., Osborne, B. A. and Kuby, J., W. H. Freeman and Co. NY). The protein is recognized by multiple antibodies that interact with different epitopes. These epitopes can reside in distinct regions of the protein found spatially separated from one another while in other instances, multiple, distinguishable and overlapping epitopes can be identified (Mateau, M. J., et. al., J. Gen. Virol., 71:629, 1990).

Epitope mapping is the process of identifying the binding epitope of an antibody to its target antigen (Cunningham B. C. and Wells J. A., Science 244:1081, 1989; Zhou, Y., and Chait, B. T., Anal. Chem., 66:3723, 1994; Komoda, H., et. al., J. Immunological Methods, 183:27, 1995). In some instances, the binding of one antibody to its epitope can prevent the binding of another antibody. Beyond direct overlap of two epitopes, other issues, including steric hindrance caused by neighboring antibody molecules and the distance between an antibody and the support surface, may be at fault (Bin, L., et. al., Analyst, 121:29R, 1996). Identification and characterization of the binding sites of antibodies can aid in the discovery and development of new therapeutics, vaccines, and diagnostics (Gershoni, J. M., et. al., BioDrugs, 21: 145, 2007; Epitope Mapping: a practical approach (A practical approach series), 2001; Westwood, O. M. R. and Hay, F. C., Oxford University Press, Oxford).

An analyte that binds to an antibody is often called an antigen, and assays that use an antibody to measure the analyte are referred to as immunoassays. In addition to binding specificity, the other key feature of all immunoassays is a means to produce a measurable signal in response to a specific binding. One type of assay is a homogeneous immunoassay (or less frequently called non-separation assay). These assays are designed in such

a way that a binding event effects a change in the signal produced by the label. Immunoassays in which the signal is affected by binding can often be run without a separation step. Such immunoassays can frequently be carried out simply by mixing the reagents and sample and making a physical measurement. Assays of this nature may be founded in the principles of time-resolved fluorescence (TRF) and fluorescence resonance energy transfer (FRET) (Mathis, G., Clin. Chem., 39:1953, 1993; Mathis, G., J. Biomol. Screen., 4:309, 1999). The other category of immunoassay is referred to as an enzyme immunoassay (EIA) (van Weeman, B. K. and Schuurs, A. H, FEBS Lett., 15:23 1971), also known as an enzyme-linked immunosorbent assay (ELISA) (Engvall, E. and Perlman, P., Immunochemistry, 8:871, 1971). This type of assay requires that either the antigen or antibody be immobilized on any suitable rigid or semirigid support. Supports may consist of filters, chips, plates, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles or cylinder (Cantarero, L. A., et. al., Anal. Biochemistry, 105:375, 1980; Kellar, K. L., et. al., Cytometry, 45:27, 2001; U.S. Patent No. 7,510,687). The substrate can have a variety of surface forms, such as wells, trenches, pins, channels, and pores to which the polypeptides are bound. For example, a chip, such as a biochip, may be a solid substrate having a generally planar surface to which a detection reagent is attached. Also, for example, a variety of chips are available for the capture and detection of lipoprotein proteome members, from commercial sources such as Ciphergen Biosystems (Fremont, Calif.), Packard BioScience Company (Meriden Conn.), Zyomyx (Hayward, Calif.), and Phylos (Lexington, Mass.). An example of a method for producing such a biochip is described in U.S. Pat. No. 6,225,047. These assays are considered separation assays, given that quantitation of binding events follows the separation of free and bound antibody-antigen complexes. Either the sample can be bound non-specifically by adsorption to the support or specifically by binding a primary (capture) antibody to the support first. Immunoassays of this variety are

called indirect, sandwich and competitive ELISA. They depend on the use of an analytical reagent that is associated with the antibody and acts as a detectable label. A large variety of labels have been successfully used including, for example, radioactive elements; enzymes; fluorescent, phosphorescent, and chemiluminescent dyes; latex and magnetic particles; dye crystalites, gold, silver, and selenium colloidal particles; metal chelates; coenzymes; electroactive groups; oligonucleotides; stable radicals; and others.

Several ELISA immunoassay formats are known (Tijssen, P., Burson, R. H. and van Knippenberg, P. H. 1985, Laboratory Techniques in Biochemistry and Molecular Biology: practice and theory of enzyme immunoassays, Elsevier Scientific Publishing Co., NY). In an indirect immunoassay, the enzyme acts as an amplifier, as only a few bound enzyme-linked antibodies are needed since the linked enzyme molecule produces many signal molecules. Within common sense limitations, the enzyme can go on producing color indefinitely, but the more antigens present, the more secondary (detection) antibody with enzyme will bind, and signal will develop faster. A major disadvantage of the indirect ELISA is that immobilization of the antigen is non-specific. So, proteins in the sample may adhere to the solid support and an antigen must compete with other analytes in the sample for binding. This can result in diminished signal if the proportion of antigen in the sample is small. The direct or sandwich-ELISA provides a solution to this problem, by starting with a capture antibody which is specific for the test antigen and selectively binds a site on the antigen in a sample mixture. This approach preferably immobilizes only the desired antigen and in principle concentrates the analyte. The antigen in the unknown sample is first bound to the antibody site, and then the detection antibody binds to the capture-antibody-antigen complex. The amount of detection antibody bound to capture-antibody-antigen complex generates the measure signal. The resulting measure will be directly

proportional to the concentration of the antigen. As a prerequisite for this assay format, the binding epitope for the capture antibody must be distinct from that of the detection antibody. In a competitive-ELISA, an unlabeled antibody is bound to the antigen. The antibody-antigen complex is added to an antigen coated solid-support and the unbound antibody is washed away. A labeled secondary antibody, which is capable of recognizing the primary antibody is added and generates the signal. The remaining unbound antigen in the unknown sample competes with labeled antigen to bind the antibodies. The amount of labeled antigen bound to the antibody is then measured. In this method, the response will be inversely related to the concentration of antigen in the unknown because the higher the sample antigen concentration, the weaker the signal. The primary advantage of a competitive ELISA over other formats is the ability of the assay to use crude or impure samples and still selectively bind any antigen that may be present. Some competitive ELISA formats rely on enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with the sample antigen. The more antigens in the sample, the less labeled antigen is retained in the well and the weaker the signal. It is common that the antigen is not first positioned in the well.

Immunoassays are used to measure an analyte which is frequently contained in a complex mixture of substances. Analytes in biological liquids (for example, serum or urine) are frequently assayed using immunoassay methods (Voller, A., et. al., Bull. World Health Org., 53:55, 1976). Such assays are based on the unique ability of an antibody to bind with high specificity to one or a very limited group of molecules. Immunoassays can be carried out for either member of an antigen/antibody pair. For antigen analytes, an antibody that specifically binds to that antigen can frequently be prepared for use as an analytical reagent. When the analyte is a specific antibody, its cognate antigen can be used as the analytical reagent. In either

case, the specificity of the assay depends on the degree to which the analytical reagent is able to bind to its specific binding partner to the exclusion of all other substances that might be present in the sample to be analyzed (Boscato, L. M. and Stuart, M. C., Clin. Chem., 32:1491, 1986; Boscato, L. M. and Stuart, M. C., Clin. Chem. 34:27 1988). In addition to the need for specificity, a binding partner must be selected that has a sufficiently high affinity for the analyte to permit an accurate measurement. The affinity requirements depend on the particular assay format that is used (Tijssen, P., Burson, R. H. and van Knippenberg, P. H. 1985, Laboratory Techniques in Biochemistry and Molecular Biology: Practice and Theory of Enzyme Immunoassays, Elsevier Scientific Publishing Co., NY).

Regardless of the method used, interpretation of the signal produced in an immunoassay requires reference to a calibrator that mimics the characteristics of the sample medium. For qualitative assays, the calibrators may consist of a negative sample with no analyte and a positive sample having the lowest concentration of the analyte that is considered detectable. Quantitative assays require additional calibrators with known analyte concentrations. Comparison of the assay response of a real sample to the assay responses produced by the calibrators makes it possible to interpret the signal strength in terms of the presence or concentration of analyte in the sample (Findlay, J. W. A., et. al., J. Pharmaceutical and Biomedical Analysis, 21:1249, 2000).

Summary of the Invention

This invention provides a method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample, wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample.

This invention also provides a method for measuring the amount of each of a plurality of high density lipoprotein (HDL) subpopulations present in an HDL-containing sample, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of each of the HDL subpopulations present in the sample.

This invention further provides a method for determining whether a subject is afflicted with a disorder characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined

protein epitopes, the method comprising (a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the subject's sample; and (b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with the presence and/or absence of the disorder, thereby determining whether the subject is afflicted with the disorder.

This invention provides a method for determining the likelihood of a subject's becoming afflicted with a disorder, wherein the disorder's likelihood of onset is characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, and wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a standard correlative with a known likelihood of the disorder's onset,

thereby determining the likelihood of the subject's becoming afflicted with the disorder.

This invention also provides a method for measuring the success of a high density lipoprotein (HDL)-modifying treatment on a subject, wherein the treatment's success is characterized by a change in the amount of a defined HDL subpopulation, and wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject during or after treatment, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with a successful treatment outcome,

thereby measuring the treatment's success.

This invention further provides a method for characterizing a high density lipoprotein (HDL) particle with respect to the presence of one or more sets of defined protein epitopes, the method comprising performing an antibody-based assay on a population of the HDL particles to determine the presence and/or amount of each set of the defined protein epitopes, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair

is directed to a different set of epitopes than is each other antibody pair, thereby characterizing the HDL particle.

This invention still further provides a method for identifying a subpopulation of high density lipoprotein (HDL) whose abnormal concentration in a subject correlates with a particular disorder, comprising

(a) measuring the amounts of one or more HDL subpopulations present in an HDL-containing sample from a subject afflicted with the disorder, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amounts of the HDL subpopulations present in the subject's sample,

(b) comparing the measured amounts of HDL subpopulations in the subject's sample with a known standard correlative with the amounts of the respective HDL subpopulations present in a healthy subject, and

(c) for each of the measured HDL subpopulations, determining whether the amount of the HDL subpopulation differs from that in the known standard,

whereby any such difference indicates that an abnormal concentration of the HDL subpopulation correlates with the disorder.

Finally, this invention provides kits for performing the instant methods described herein. Each kit comprises (i) a solid substrate suitable for use in

performing an antibody-based assay; (ii) a capture antibody operably affixed to the substrate; and (iii) in a separate compartment, a detection antibody, wherein the capture and detection antibodies are directed to different protein epitopes present on each particle of a predetermined HDL subpopulation.

Brief Description of the Figures

Figure 1: "Solar System" rendering of an HDL particle.

This image is a hypothetical model of an HDL particle. The HDL particle is composed of two major constituents, lipids and proteins. Several major lipid classes are represented as the large shaded concentric rings and each ring reflects the percentage of a lipid which is in proportion to the relative ring area. The overall diameter of the particle can be scaled and is designed to replicate the measured diameter of an HDL particle. Proteome members are denoted by smaller circles layered on top of the lipid rings and are labeled by gene name. Each protein molecule is represented by one circle and the area of the circle is calculated to be proportional to the molecular weight of the post-translation processed mature form of the protein and does not include any mass increase resulting from glycosylation. The distance of the proteome circles from the center of the particle is intended to account for apparent affinity differences proteome members have for the lipoprotein particle. Proteome members exhibiting the lowest affinity for the particle would be arranged furthest from the center. Such proteins would be classified as having higher particle dissociation rates and are likely to exist in both an HDL particle bound and unbound state. Basic positioning of proteome members around the radius of the particle and the relative distances to each other is essentially arbitrary in this modeling view. With exception, apoA1 has been shown to exist as a dimer and is represented by two adjacent circles to reflect this observation.

Both protein and lipid constituents can vary in each particle and the density of the particle is defined by the ratio of lipid to protein. The total amounts of all constituents define the diameter, volume and charge of the particle. Variations in particle physicochemical properties are due to differences in the

mix of constituents and their absolute levels. Each specific combination of constituents and their particle levels serve as a self-contained set of instructions which in turn dictates and directs the particle's physiological activities.

Figure 2: A hypothetical representation of HDL particle subpopulation heterogeneity.

An extrapolation of an HDL particle population displayed using the solar system model and reflecting its heterogeneous nature. This figure provides a hypothetical view that is limited in scope and detail but demonstrates common particle features as well as distinct differences. The HDL particle population exhibits the disequilibrium of the proteome members and lipidome to one another and to the particle population as a whole.

Variations in lipid and protein constituents are reflected in the diameter, shading and protein patterns for each particle. Fractionation techniques can separate particles using physicochemical properties and in doing so result in the apportioning of biological activities. HDL has a large number of measured biological activities many of which associate with cardiovascular health. To reconcile all of these reported observations, a model that depends on particle heterogeneity to account for the variety of physiological activities requires that particle subpopulations, which can be defined by physicochemical characterization or permutations of constituent molecules, perform particular and specific functions. It is the totality of all particle subpopulations that contributes to cardiovascular health, and alterations in subpopulation levels or specific constituents affect particle instructional blueprints that can reflect disease phenotypes.

Figure 3: HDL LipoPrint analysis of plasma sample.

Acrylamide gel electrophoresis of a plasma sample prepared by pooling fifty reportedly healthy individuals was used to separate lipoprotein particle fractions. HDL particles are separated principally based on particle size, with faster migration rates and larger distances from the origin for smaller sizes. Lipoprotein particles are visualized by staining with the dye, Sudan black, which quantitatively binds neutral lipids, primarily cholesteryl esters (CE). Slow migrating VLDL and LDL appear as the last peak to the far left of the chromatograph as these two classes of lipoproteins do not resolve using the HDL LipoPrint gel system. Fast migrating albumin, stained with coomassie blue, to the far right is representative of the free protein fraction in plasma. Sudan black staining of cholesterol provides a distribution profile for that lipid class across the broad HDL fraction. The HDL is subdivided into three fractions identified as large, intermediate and small which can be observed as shading differences delineated by thick black vertical lines according to the analysis software provided by the instrument's manufacturer. Peak fitting (area under the curve; AUC) is calculated by the manufacturer's software provided with the LipoPrint system to estimate relative amounts of the three subpopulations sizes.

LipoPrint gel segments are labeled 1-20 below the chromatograph. Each segment composed of a gel two millimeters in length. The entire gel is 40 millimeters in length starting from the trailing edge of VLDL/LDL peak in fraction 1 to the leading edge of free albumin peak contained primarily in fractions 18-20.

Lipoprotein particles were further isolated from each individual gel segment by buffer extraction and the isolated particles were reduced and denatured and subjected to separation by SDS-PAGE using a 4-12% gradient gel. Following

transfer and immobilization on nitrocellulose, immunoblot analysis is performed to characterize the sub-fraction distribution and relative amounts of the target protein.

The middle panel depicts the immunoblot analysis using an antibody specific for apolipoprotein A-I (ab27630). Staining of apoA1 can be clearly observed in fractions 4-20 and also in fractions 2 and 3 at much lower levels following extended exposures. Each of the apoA1 containing fractions contains varying levels of apoA1 protein. The significant level of apoA1 in fractions 18-20 is indicative of apoA1 protein in very small lipoprotein particles or lipid-free protein, both of which contain undetectable levels of cholesterol. The bottom panel provides a generalized reference for categorizing particle subpopulations into assigned fractions by particle size.

This experiment demonstrates that both apoA1 protein and HDL-cholesterol exist in disequilibrium to each other. Both particle constituents are in disequilibrium to the HDL particle population as a whole. Very large particles contain large ratios of cholesterol to apoA1 and small particles contain larger ratios of apoA1 to cholesterol. Signal levels and distribution patterns for both cholesterol and apoA1 represent profile averaging effects due to pooling of the plasma sample prior to analysis. Individual samples exhibit signal heterogeneity and variations in apoA1 and cholesterol distribution across the HDL fractions.

Figure 4: HDL Lipoprint of a plasma sample characterized by several HDL proteome members.

HDL LipoPrint electrophoresis of a pooled plasma sample divided into 10 segments. LipoPrint gel segments are labeled 1-10 below the chromatograph. Each segment is composed of a gel four millimeters in length. The entire gel

is 40 millimeters in length starting from the trailing edge of the VLDL/LDL peak in fraction 1 to the leading edge of the free albumin peak contained primarily in fraction 10.

Lipoprotein particles were further isolated from each individual gel segment by buffer extraction and the isolated particles were reduced and denatured and subjected to separation by SDS-PAGE using a 4-12% gradient gel. Following transfer and immobilization on nitrocellulose, immunoblot analysis is performed to characterize the sub-fraction distribution and relative amounts of the target protein.

Various commercial antibodies (Tables 3 and 4) targeting several HDL proteome members (Table 1) were used for immunoblot analysis. The following proteome examples: apoA1 (HDL110), apoA2 (H00000336-M03), CLU (mab2937), SerpinF1 (mab1177), SerpinA1 (mab1268), KNG1 (mab15692) and SerpinF2 (mab1470) were tested and demonstrate various distribution patterns for HDL particle sub-fractions separated by particle size. The proteome distribution disequilibrium is observable with these proteome member examples which reflect both broad and restricted distribution patterns across HDL particle sub-fractions and represent profile averaging effects due to the sample consisting of pooled plasma samples from fifty individuals.

This physicochemical separation process does resolve particles into homogeneous sub-populations, and therefore fractions characterized as positive for one or more proteome member do not establish that any two proteome members reside on the same particle. Each sub-fraction still contains multiple particle species that co-migrate under these specific separation conditions, indicating that further resolution of particle sub-populations is possible.

Figure 5: Representation of proteome distribution disequilibrium in lipoprotein particles.

Five HDL particle subpopulations are represented as circles labeled as 2b, 2a, 3a, 3b and 3c (large to small) using standard HDL particle nomenclature. Lipoprotein particles can be fractionated and identified by various physicochemical properties including size and density, but for the purpose of this example, those differences are simply illustrated by circle diameter. Attached to the perimeter of the circle is a variety of unique shapes. Five different proteins are depicted and collectively they represent the HDL proteome. Each proteome member also has two specific epitopes (shaded patches) that are considered unique to the individual protein and different from all other epitopes. The "constellation" of proteome members surrounding each of the five particles (2b, 2a, 3a, 3b, and 3c) is similar but also contains several differences. For example, one proteome member is shared by all particles (circle) while another protein (triangle) is found only on the two largest HDL particle subpopulations (2b and 2a). This drawing exhibits a set of proteome members that are in disequilibrium to the particle population and to each other.

Figure 6: Sandwich ELISA-based measurements of lipoprotein particle proteome.

Historically, and due to the basic principles of sandwich ELISA-based measurement, the technique requires two different antibodies targeting an individual protein, which are indicated as bound to one protein (circle). The antibodies must recognize unique and non-overlapping epitopes and the binding of one antibody must not interfere with the binding of the second. One antibody, bound to a solid support, serves to capture the target protein

while the second detection antibody provides the means of generating a signal. The amount of target protein bound by both antibodies should be proportional to the signal generated, thus providing a means of quantifying the protein. In this drawing, the example proteome member (circle) can exist in HDL particle-bound form or in an unbound state. In some instances, the HDL proteome member may be bound to other classes of lipoproteins such as LDL and VLDL, and displaying the proteome not bound to an HDL particle can also represent such a situation. A comparison of Tables 1 and 2 offers examples of lipoproteins for which this may be true.

Sandwich ELISA measurements such as this are incapable of discerning the bound or unbound state of the target protein unless (1) the lipoprotein particles are first separated into their prospective subpopulations prior to measurement, or (2) either the capture or detection antibody is conformation-dependent and has the capacity to bind the target protein only in instances where the protein adopts the desired conformation in a specific subpopulation-restricted manner. Using routine sandwich-ELISA methods, the quantification of the target protein is aimed at determining the total amount of the protein in the sample.

Figure 7: Method for measuring HDL subpopulations.

This figure exemplifies several fundamental concepts demonstrating the distinct nature of the method to measure HDL subpopulations in this application. (1) This method relies on the fact that HDL proteome member distribution is in disequilibrium to each other and to the particle population as a whole. (2) The distribution of proteome members across the particle population includes individual members that are bound to all particle subpopulations and other proteome members that demonstrate varying degrees of HDL particle subpopulation restriction. (3) This sandwich ELISA

methodology requires, but is not limited to, the use of one antibody to each of the proteome members to be measured.

Using the example presented and the availability of one antibody capable of recognizing each of the five HDL proteome members, a series of sandwich ELISA assays can be devised to identify different HDL subpopulations in a sample composed of a heterogeneous mixture of HDL particles. In the bottom portion of the figure, all possible proteome pairs within each of the five particle subpopulations are represented. Each particle subpopulation can be identified by the proteome pair in which both proteome members exist together on the same particle. The total number of possible pairs is a function of the number of proteome members bound. The capture antibody, which is capable of binding the target protein in the context of any particle, will produce a measurable signal only when the detection antibody is also bound to its target protein held in close proximity on the same particles where both proteome members reside.

Figure 8: Surrogate markers for HDL subpopulations.

Set theory can be used to identify surrogate markers for specific subpopulations. Signals from paired proteome measurements in Figure 7 are rendered using a Venn diagram to demonstrate the use of inclusion and exclusion criteria to identify specific HDL subpopulations. Five groups are labeled as 2b, 2a, 3a, 3b, and 3c. The largest lipid-rich HDL particles, commonly referred to as HDL2, consist of the two subpopulations 2b and 2a and the smaller lipid poor HDL particles, called HDL3, consist of three subpopulations 3a, 3b and 3c. Two proteome pairs can be used to identify larger HDL2 particles (intersection 2b and 2a), while the smaller more dense HDL3 particles include one proteome pair (intersection of 3a, 3b and 3c). In addition to HDL2 and HDL3 specific particles, various other proteome pairs

can serve as surrogate measurements for particle subpopulations of greater homogeneity. Specific to this example are two proteome pairs restricted to the largest HDL 2b particles, and the smallest particle subpopulation contains a single proteome pair that does not exist in any other subpopulation.

This methodology permits the use of restricted proteome particle distribution to identify subpopulations of increasingly defined homogeneity, as combinations of restricted distributions can be overlapped to identify increasingly refined subsets of particles. In a similar fashion, this method offers the means to identify proteome pairs that do not typically exist in normal healthy individuals. Such is the case for one proteome pair which can be observed in the upper left hand corner of the figure. This proteome pair resides outside the boundary of all five particle subsets in the diagram. Such instances, where both proteins and applicable antibodies exist, offer the prospect of identifying surrogate markers for HDL subpopulations that are considered atypical. HDL particles and associated proteome pairs of this nature may occur as a result of underlying genetics or disease states, and this method offers a means for their identification and measurement.

This method provides a means to expand the number of particle subpopulations that can be identified by adding increasing numbers of proteome members from Table 1. Furthermore, this method can utilize the overlapping restricted distribution of two proteome members to measure expanded subsets of particles that cannot be distinguished by a single proteome member.

Figure 9: Method provides for geometric expansion of surrogate markers for HDL subpopulations.

The use of proteome-paired signals to identify HDL subpopulations provides the prospect of geometrically expanding the repertoire of measurements for each new antibody added for use in the proteome pair sandwich ELISA. This example incorporates the drawing from figure 7 (upper panel) for comparison. The lower panel displays a second antibody recognizing an alternative epitope from the first on the protein designated by the circle. The substitution of a different antibody recognizing a second unique epitope on the protein results in additional sandwich ELISAs available from the same proteome pairs, resulting in an increase of the number of possible novel measurements in proportion to the number of proteome members present. Such measurements may result in no observable signal difference and in such instances can only offer independent testing of the first measurement or introducing the second antibody provides an alternative set of measurements depending on the nature of the epitope recognized. This method can increase the number of unique proteome-paired measurements by a factor equivalent to the number of proteome members bound to the particle, thus providing the means to geometrically expand the number of potential surrogate markers for an HDL particle.

Figure 10: Method provides for expansion of surrogate markers for HDL subpopulations.

The top panel displays the components of a sandwich ELISA which include a capture antibody (Ig-C) attached to a solid support (SS). A protein antigen composed of two unique and non-overlapping epitopes and a detection antibody (Ig-D) coupled to an agent capable of producing a measurable signal (*). In some cases the role of the capture and detection antibodies can be

reversed and the resulting signals from both configurations are equivalent. The success of such experimentation is often considered a validation of the assay components and the subsequent measurement they produce. A measurement of this nature is independent of other proteins in the mixture and represents a typical sandwich ELISA.

The middle panel is an illustration of a sandwich ELISA in which the roles of the antibody pair cannot be reversed and doing so will alter the absolute values of the measurement for a given sample. Excluding technical restrictions, such as the inability of the antibody to serve in the capture role due to non-productive coupling to the solid support or to act as a detection antibody as a result of loss or altered binding following labeling signal-generating agent, other molecular explanations are possible. An example is the recognition of post-translational modifications that occurs in only a percentage of the antigen being measured such as a phosphorylation event. In this instance when the Ig-C binds the common epitope to all antigen molecules and the Ig-D binds an epitope of limited distribution, a productive signal is generated only from a subset of the total antigen bound to the Ig-C. Increasing the concentration of the antigen will not alter that ratio, as the amount of non-productive antigen binding increases to the same degree as productive antigen binding until the sandwich ELISA reaches saturation. When the Ig-C and Ig-D are reversed, only the productive antigen is bound and the signal is dependent solely on the concentration of protein containing the epitope of limited distribution. The sandwich ELISA does not saturate at the same concentration of total (productive and non-productive antigen), and the difference in signal between each sandwich ELISA goes to unity as the limited distribution epitope increased to all antigens.

The bottom panel illustrates the unique nature of this method of measuring proteome pairs, and the Ig-C and Ig-D bind epitopes on two different

proteome members. In this situation, the Ig-C and Ig-D cannot be reversed for the same reasons as described for the example above but also accounts for the antigen epitope distribution within the HDL population as well as the bound/unbound considerations described in Figure 6. This specific relational dimension cannot be captured when both the Ig-C and Ig-D interact with unique non-overlapping epitopes on the same antigen. What was a measurement of two independent antigens has been transformed into a relational intramolecular measurement which characterizes two antigens and the four antibodies involved. The eight distinct measurements of HDL subpopulations are a result of both limited epitope distribution associated with the antigen and the distribution disequilibrium of the two proteome members have to each another. Only in instances in which both epitopes exist on all proteome members in the sample and both proteome members maintain identical particle distribution profiles, including HDL particle bound and unbound fractions, does this model not hold true.

Figure 11: A hypothetical array of antibodies in a 96-well format to measure HDL subpopulations.

This rendering displays a collection of antibody pairs organized into ninety-six distinct measurements of HDL subpopulations. This assay construct consists of a labeled network of shaded boxes overlaid on a 96-well (circles) plate template. Plate rows are labeled with letters (A-H) to the left of the plate and columns are labeled above the plate with numbers (1-12). Each well contains two boxes located in diagonal corners. The upper left box identifies a capture antibody by proteome and epitope using a letter and number code. The box in the lower right corner identifies the detection antibody by proteome and epitope using the letter and number code.

Labeling of proteome epitopes is essentially arbitrary, but in this example, the boxes labeled with the letter "Z" represent a non HDL proteome cardiovascular control. Proteome members are designated by a letter (A-J) and unique epitopes by a number. In this illustration, eight antibodies targeting proteome member A contribute to fifty-one sandwich ELISA measurements. Eighteen of these pairs are designed to measure proteome member A to itself using unique and non-overlapping epitopes. Thirty-three measurements utilizing antibodies to proteome member A also involve antibodies to other proteome members. In real terms proteome member A would likely be apoA1 and antibodies selected for their ability to recognize both conformational-dependent and -independent epitopes. Antibodies could serve strictly as Ig-C (A1, A3) or as Ig-D (A2), while others could serve in both roles (A4-A8). Similar design rules would hold true for other HDL proteome members. Some assays (A1/A4, A1/A5, A1/A6, and A1/A7) utilize a single Ig-C and four different Ig-D. Other assays (A4/A2, A5/A2, A6/A2 and A7/A2) utilize different Ig-C and a common Ig-D while other assays (A6/A7, A7/A6) use the same antibody pair with roles reversed. A similar design would be used to measure to proteome members to each other (A9/B2, A9/B3, A4/B2, A4/B3, A5/B1, A5/B3, A6/B4, A7/B4, B1/A9, B3/A9, B1/A2, B3/A2, B2/A5,B3/A5, B4/A6 and B4/A7) where proteome member B would represent apoA2. The remaining wells on the plate depict series of antibody combinations that target various proteome members (Table 1), all of which would have far more limited particle distribution profiles than apoA1 or apoA2.

This method is designed to construct a measurement-matrix for determining the amount of HDL subpopulations in an HDL-containing sample by performing a quantitative assay on a plurality of HDL proteome epitopes. This systematic analysis utilizes the distribution disequilibrium found between two proteome members within the HDL subpopulations and exploits the relationship those epitopes have through the use of common antibodies in

multiple sandwich ELISA assays. This method replaces the typical independent intermolecular measurements of sandwich ELISA, where both antibodies interact with unique epitopes on a single protein, with a series of relational intramolecular measurements based on many antibodies used in various combinations in multiple assays.

Figure 12: Determining whether a subject is afflicted with a disorder characterized by an abnormal amount of an HDL subpopulation.

Subject samples containing HDL are measured using the antibody array matrix to quantify the relative levels of HDL subpopulations defined by the combination of antibody pairs. Ninety-six measurements generate signal intensity levels which are reflected by grayscale shade from least (white) to greatest (black). The subpopulation profile for the subject is the composite view of multiple HDL proteome ELISA signals taken concurrently. The levels and patterns are hypothetical and offer a visual representation of measured differences in samples from individuals afflicted with diseases that affect HDL proteome member levels or their association with HDL particle subpopulations.

Samples from individuals afflicted with disorders characterized by abnormal amounts of an HDL subpopulation or abnormal levels of a proteome member can generate signal levels that are higher or lower than that of a healthy individual. Two diseases, atherosclerosis and type-2 diabetes, are examples of afflictions affecting HDL proteome levels (Kontush, A., et. al., Arterioscler. Thromb. Vasc. Biol. 24:526, 2004; Lyons, T. J., et. al. Invest. Opthomology and Visual Sci. 45:910, 2004; Vaisar, T., et. al., J. Clin. Inv., 117:746, 2008; Green, P. S., et al., Circulation 118:1259, 2008). It is unclear from existing data whether the observed changes in measured protein reflect modulations of protein on the HDL particles or changes in levels of HDL particles

containing those proteins (Corsetti, J. P., et. al., PLOS One 7:e39110, 2012). This subtle difference represents a key feature in assessing HDL and an important dimension that this method brings to correlating HDL to disease states.

Figure 13: Detection of HDL using a sandwich ELISA assay.

Twenty-six antibodies directed at twelve HDL proteome members from Table 1 and one non-immune antibody control (IgG-C) are labeled along the ordinate and abscissa. Cells (shaded) representing a tested sandwich ELISA can be identified by pairing a capture (ordinate) and a detection (abscissa) antibody using HDL-containing samples. Antibodies designated by their catalogue number according to Tables 3 and 4 as well as their clone identification ([clone]) where applicable, are grouped according to the proteome member they target. Sandwich ELISA assays generating a strong signal (dark), weak (intermediate) and no signal ("N" light). Cells (white) indicate antibody pairs not tested. All antibodies were evaluated by immunoblot analysis with HDL samples to confirm their capacity to recognize their cognate proteome member prior to sandwich ELISA testing.

Excluding the IgG-C non immune control, these antibodies comprise a possible 650 unique antibody pairs if a single antibody cannot serve in both the capture and detection role. Of these, 92 sandwich ELISA's were performed, of which 56 generated measurable signals and 36 did not. Some antibodies performed either capture or detection roles. Other antibodies did not work in either position despite pairing with antibodies validated to work in this assay format. Several sandwich ELISA assays utilizing antibody pairs interacting with epitopes on the same protein (apoA1, apoB, apoE) generated signals. Several detection antibodies demonstrated the capacity to work with multiple capture antibodies targeting the same proteome member, and

multiple capture antibodies worked with a common detection antibody. Antibody pairs targeting different proteome members exhibited signals indicating proximity of both proteins on the same particle. Limited testing of antibodies derived from Tables 3 and 4 identified sandwich ELISA assays that place the following proteome members on the same particle: apoA1/apoA2, apoA1/apoB, apoA1/apoE, apoA1/CLU, apoA1/KNG1, apoA1/SerpinA1, apoA1/SerpinC1, apoA1/SerpinF1, and constitute novel ELISA-based measurements of HDL not previously observed.

Antibodies are commercially available and not previously evaluated for use in this sandwich ELISA format. It is expected that not all antibodies or antibody combinations should work. In some instances, technical limitations such as non-productive coupling to the solid support or labeling with a signal generating molecule may be an issue. In other cases, sandwich ELISA-validated antibodies were unable to pair and may reflect epitope availability problems or that both epitopes do not exist on the same particle. Instances of steric hindrance due to overlapping epitopes are also likely.

Detailed Description of the Invention

This invention provides an accurate tool for measuring HDL in a sample. The invention is useful for determining whether a subject is at risk of developing, is suffering from or is shifting between cardiovascular disorders. The methods are based on the physical relationship between two distinct proteins or epitopes held in proximity to one another as part of a single lipoprotein particle. That is, this invention exploits the physical proximity between two protein epitopes to identify and quantify discrete HDL subpopulations present in heterogeneous mixtures, and measure changes in HDL subpopulations as a result of disease or treatment.

Definitions

In this application, certain terms are used which shall have the meanings set forth as follows.

As used herein, the term "antibody" includes, without limitation, (a) an immunoglobulin molecule comprising two heavy chains and two light chains and which recognizes an antigen; (b) polyclonal and monoclonal immunoglobulin molecules; and (c) monovalent and divalent fragments thereof. Immunoglobulin molecules may derive from any of the commonly known classes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include, but are not limited to, human IgG1, IgG2, IgG3 and IgG4. Antibodies can be both naturally occurring and non-naturally occurring. Furthermore, antibodies include chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. Antibodies may be human, humanized or nonhuman.

As used herein, the term "capture antibody" includes, for example, the primary antibody used in a homogeneous immunoassay or an ELISA immunoassay. The capture antibody is immobilized on a solid support, such as a polystyrene microtiter plate, bead or cylinder.

As used herein, the term "cardiovascular disease", also referred to as "cardiovascular disorder" and "CVD", includes, without limitation, heart and blood vessel diseases, such as atherosclerosis, coronary heart disease, cerebrovascular disease, and peripheral vascular disease. Cardiovascular disorders also include, for example, myocardial infarction, stroke, angina pectoris, transient ischemic attacks, and congestive heart failure. Cardiovascular disease, such as atherosclerosis, usually results from the accumulation of fatty material, inflammatory cells, extracellular matrices and plaque. Clinical symptoms and signs indicating the presence of CVD may include one or more of the following: chest pain and other forms of angina, shortness of breath, sweatiness, Q waves or inverted T waves on an EKG, a high calcium score by CT scan, at least one stenotic lesion on coronary angiography, and heart attack.

As used herein, the term "defined protein epitope" includes, without limitation, an epitope defined structurally (e.g., by primary amino acid sequence and/or atomic coordinates) and/or functionally (e.g., able to bind to a defined monoclonal antibody, ideally with a K_d of 10^{-8}M or lower).

As used herein, the term "detection antibody" includes, for example, the secondary antibody used in a homogeneous immunoassay or an ELISA immunoassay. The detection antibody is typically immobile, and contains a label that produces a measurable signal.

As used herein, the term "high density lipoprotein", also referred to as "HDL", includes, without limitation, a particle as exemplified in Figure 1 that is made from protein and lipid, and that (i) has a density of from 1.06 to 1.21 g/mL, (ii) has a diameter from 7.1 nm to 12.6 nm, and (iii) contains at least one of apoA1, apoA2 and apoE (alternatively referred to as ApoA1, ApoA2 and ApoE, respectively). Examples of HDL include HDL3 (having a density of from 1.06 to 1.10 g/mL), and HDL2 (having a density from 1.10 to 1.21 g/mL).

As used herein, the term "HDL subpopulation" means a subset of all HDL. Preferably, the HDL subset differs from all other HDL subsets by the presence or absence of a particular protein or protein epitope.

As used herein, the term "sample", when used with respect to HDL, includes any biological substance present within, or obtainable from, a subject. These substances include, without limitation, blood, bone marrow, urine, saliva, synovial fluid, cerebrospinal fluid or tissue, lesions, ulcers and tumors. Samples may optionally be treated, purified and/or fractionated. For example, when a sample is obtained via fractionation, the fractionation of components may take place in column chromatography by a difference in affinity between a stationary phase and a mobile phase, or by the principals of a gradient. Other fractionation methods include separation by differences in mass, solubility or density that may be induced by methods such as freezing, pH change, organic extraction, precipitation or electrophoretic mobility.

As used herein, the term "subject" includes, without limitation, a mammal such as a human, a non-human primate, a dog, a cat, a horse, a sheep, a goat, a cow, a rabbit, a pig and a rodent.

Embodiments of the Invention

This invention provides a method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample, wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample.

The HDL subpopulation measured by this method can be characterized by any naturally occurring permutation of proteins within the HDL proteome. Members of the HDL proteome are set forth, for example, in Table 1. In a preferred embodiment, the HDL subpopulation being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein. Typically, amounts of HDL in a sample such as blood are measured in mg/dL. Moreover, the "amount" of HDL subpopulation measured by this method can be either the absolute amount (e.g., 10 mg of HDL per dL of blood) or a relative amount (e.g., 1.5 times the concentration of HDL present in normal blood).

Examples of samples containing the HDL subpopulation being measured are set forth above. In a preferred embodiment, the sample is blood, plasma, serum or urine, all preferably from a human.

In this method, the plurality of defined protein epitopes can be present on the same protein. In this scenario, the plurality of defined protein epitopes are

preferably present on one of ApoA1 protein, ApoA2 protein and ApoE protein. Alternatively, the plurality of defined protein epitopes can be present on two or more proteins. In this scenario, the plurality of defined protein epitopes are preferably present on two or more proteins in the HDL proteome set forth in Table 1.

The subject invention employs antibody-based assays to measure HDL subpopulations. Such methods and the antibodies they employ are well known in the art, and are exemplified above. Moreover, the antibodies that can be used in this invention are also well known, and are exemplified in Tables 3 (anti-apoA1 antibodies) and 4 (antibodies directed to various members of the HDL proteome). In one embodiment, the quantitative antibody-based assay is a radioimmunoassay (RIA) or an enzyme immunoassay (EIA). Preferably, the EIA is an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) or an electrochemiluminescence assay (ECL).

This invention also provides a method for measuring the amount of each of a plurality of high density lipoprotein (HDL) subpopulations present in an HDL-containing sample, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of each of the HDL subpopulations present in the sample.

Envisioned is a method wherein a large number of HDL subpopulations is measured concurrently (as are biomolecules using known chip array technology) or in close temporal succession. The number of HDL subpopulations measured by this method can be any number, such as 100, 500, 1,000, 10,000, or more. In one embodiment, the number of HDL subpopulations measured is at least 16. Preferably, the number of HDL subpopulations measured is at least 96.

In this method, the amounts of HDL subpopulations can be measured either sequentially or concurrently. However, the method preferably involves concurrently measuring the amount of each of the plurality of HDL subpopulations present in the HDL-containing sample.

Also, in this method, the HDL subpopulations being measured can constitute any collection of subpopulations (e.g., grouped by disease state or characterizing proteins). In a preferred embodiment, at least one of the HDL subpopulations being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

This invention further provides a method for determining whether a subject is afflicted with a disorder characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising (a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the

subject's sample; and (b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with the presence and/or absence of the disorder (e.g., HDL measurements previously taken from healthy and afflicted subjects), thereby determining whether the subject is afflicted with the disorder.

In this method, the amount of defined HDL in an afflicted subject can be either higher or lower than in a healthy subject. In one embodiment, the amount of the defined HDL subpopulation in an afflicted subject is higher than (e.g., by 5%, 10%, 20%, 50%, 100%, or more) the amount of the defined HDL subpopulation in a healthy subject. In this scenario, the disorder can be, for example, dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD) or coronary heart disease (CHD).

In another embodiment, the amount of the defined HDL subpopulation in an afflicted subject is lower than (e.g., by 5%, 10%, 20%, 50%, or more) the amount of the defined HDL subpopulation in a healthy subject. In this scenario, the disorder can be, for example, dyslipidemia, atherosclerosis, diabetes mellitus, obesity-induced dyslipidemia, coronary artery disease (CAD), coronary heart disease (CHD) or chronic kidney disease (CKD).

This invention still further provides a method for determining the likelihood of a subject's becoming afflicted with a disorder, wherein the disorder's likelihood of onset is characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, and wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection

antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a standard correlative with a known likelihood of the disorder's onset (e.g., HDL measurements previously taken from healthy, at-risk and/or afflicted subjects),

thereby determining the likelihood of the subject's becoming afflicted with the disorder.

In one embodiment, the amount of the defined HDL subpopulation in a subject likely to become afflicted is higher than (e.g., by 5%, 10%, 20%, 50%, 100%, or more) the amount of the defined HDL subpopulation in a subject less likely to become afflicted. In this scenario, the disorder can be, for example, dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD) or coronary heart disease (CHD).

In another embodiment, the amount of the defined HDL subpopulation in a subject likely to become afflicted is lower than (e.g., by 5%, 10%, 20%, 50%, or more) the amount of the defined HDL subpopulation in a subject less likely to become afflicted. In this scenario, the disorder can be, for example, dyslipidemia, atherosclerosis, diabetes mellitus, obesity-induced dyslipidemia, coronary artery disease (CAD), coronary heart disease (CHD) or chronic kidney disease (CKD).

This invention also provides a method for measuring the success of a high density lipoprotein (HDL)-modifying treatment on a subject, wherein the treatment's success is characterized by a change in the amount of a defined

HDL subpopulation, and wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject during or after treatment, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with a successful treatment outcome,

thereby measuring the treatment's success.

The treatment whose success is measured by this method can be any form of treatment, whether pharmaceutical or otherwise (e.g., lifestyle changes and surgery). Pharmaceutical treatments include, for example, cholesterol-lowering medications, antiplatelet agents (e.g., aspirin, ticlopidine, clopidogrel), glycoprotein IIb-IIIa inhibitors (such as abciximab, eptifibatide or tirofiban), antithrombin drugs (blood-thinners such as heparin), beta-blockers, nitrates (e.g., nitroglycerin), calcium-channel blockers, and medications for reducing blood pressure (e.g., ACE inhibitors and diuretics).

In a preferred embodiment, the HDL-modifying treatment is the administration of a statin. Statins are well known in the art, and include, for example, atorvastatin (Lipitor® and Torvast®), fluvastatin (Lescol®), lovastatin (Mevacor®, Altocor®, Altoprev®), pitavastatin (Livalo®, Pitava®), pravastatin

(Pravachol®, Selektine®, Lipostat®), rosuvastatin (Crestor®) and simvastatin (Zocor®, Lipex®), ezetimibe/simvastatin (Vytorin®, Ezetrol®).

By way of example, in a *post hoc* cohort study, statins were shown to raise HDL (measured as HDL-cholesterol (HDL-C) and apoA1), and these elevations were maintained in the long-term (McTaggart, F. and Jones, P., Cardiovasc. Drugs Ther. 22:321, 2008). In patients afflicted with hypercholesterolemia, statins raise HDL-C by approximately 4% to 10%, with the percentage change greatest in patients having low HDL-C baseline levels (including patients having the common combination of high triglycerides (TG) and low HDL-C). Another study compared the effects of five different statins (namely, atorvastatin, simvastatin, pravastatin, lovastatin and fluvastatin) on the lipid, lipoprotein, and apoA1-containing high-density lipoprotein (HDL) subpopulation profiles of 86 coronary heart disease (CHD) patients (Asztalos, B. F., et al., Atherosclerosis 164:361, 2002). This study identified the most effective agents for altering the HDL subpopulation profiles in CHD patients to more closely resemble those found in healthy individuals. Finally, in patients afflicted with coronary artery disease, 12 months of combined atorvastatin and extended-release niacin therapy partially reversed the adverse changes in HDL3 protein composition (Green, P. S., et. al., Circulation 118:1259, 2008).

This invention further provides a method for characterizing a high density lipoprotein (HDL) particle with respect to the presence of one or more sets of defined protein epitopes, the method comprising performing an antibody-based assay on a population of the HDL particles to determine the presence and/or amount of each set of the defined protein epitopes, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair

is directed to a different set of epitopes than is each other antibody pair, thereby characterizing the HDL particle.

This method can be used to characterize any type of HDL particle. In a preferred embodiment, the antibody-based assay is performed on a population of the HDL particles selected from HDL2a, HDL2b, HDL3a, HDL3b, HDL3c, pre-β1 and pre-β2.

This invention still further provides a method for identifying a subpopulation of high density lipoprotein (HDL) whose abnormal concentration in a subject correlates with a particular disorder, comprising

(a) measuring the amounts of one or more HDL subpopulations present in an HDL-containing sample from a subject afflicted with the disorder, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amounts of the HDL subpopulations present in the subject's sample,

(b) comparing the measured amounts of HDL subpopulations in the subject's sample with a known standard correlative with the amounts of the respective HDL subpopulations present in a healthy subject, and

(c) for each of the measured HDL subpopulations, determining whether the amount of the HDL subpopulation differs from that in the known standard,

whereby any such difference indicates that an abnormal concentration of the HDL subpopulation correlates with the disorder.

This method is, in essence, a way to find novel correlations between particular disorders and HDL subpopulations. Each correlation can then form the basis for a diagnostic test for such disorder, whereby an abnormal concentration of the relevant HDL subpopulation indicates an affliction with the disorder. In a preferred embodiment, the disorder is dyslipidemia, obesity-induced dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD), coronary heart disease (CHD), vascular inflammation, atherosclerosis or chronic kidney disease (CKD).

Finally, this invention provides kits for performing the instant methods described herein. Each kit comprises (i) a solid substrate suitable for use in performing an antibody-based assay; (ii) a capture antibody operably affixed to the substrate; and (iii) in a separate compartment, a detection antibody, wherein the capture and detection antibodies are directed to different protein epitopes present on each particle of a predetermined HDL subpopulation.

Antibody-based diagnostic kits of all types and their methods of manufacture and use are well known. In a preferred embodiment, the instant kit is suitable for performing a radioimmunoassay (RIA) or an enzyme immunoassay (EIA). Preferably, the EIA is an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) or an electrochemiluminescence assay (ECL). The inclusion of suitable solvents and instructions for using these kits is envisioned.

In a preferred embodiment of the subject kits, the capture antibody is directed to an epitope present on a protein set forth in Table 1, and the detection antibody is directed to an epitope present on one of ApoA1 protein, ApoA2

protein and ApoE protein, wherein the capture and detection antibodies are directed to different epitopes. In another preferred embodiment, the capture antibody is directed to an epitope present on one of ApoA1 protein, ApoA2 protein and ApoE protein, and the detection antibody is directed to an epitope present on a protein set forth in Table 1, wherein the capture and detection antibodies are directed to different epitopes.

Numerous embodiments (preferred and otherwise) are set forth above in connection with the instant methods and kits. Each embodiment explicitly set forth for any of the instant methods or kits applies, mutatis mutandis, to each of the other instant methods and kits, unless stated otherwise.

This invention will be better understood by reference to the examples which follow, but those skilled in the art will readily appreciate that the specific examples detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Examples

Example 1

This invention provides a method of determining a mammalian test subject's risk of developing CVD by measuring apoA1 with a collection of antibodies, where each paratope is distinct, whose epitopes are distinguishable and interact in both conformation-dependent and -independent manner. The measurements from the subject's sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection of samples representing specific disorders.

Rationale: The lipoprotein apoA1 is the major constituent protein of HDL, accounting for 60-70% of the protein mass. Each particle is wrapped in 2-5 apoA1 proteins depending upon the size of the particle and the lipid composition (McLachlan, A. D., Nature, 267:465, 1977; Wu, Z., et. al., Nat. Struct. Mol. Biol. 14:861, 2007; Huang, R., et. al., Nat. Struct. Mol. Biol., Online 13 March 2011). As the volume of the sphere changes with the gain or loss of lipid molecules, so will the particle diameter and circumference. As a consequence, the apoA1 molecules surrounding the lipid particle are also changing conformation to accommodate varying sphere geometries. Antibodies that recognize conformation-independent epitopes should have a greater probability of binding all apoA1 regardless of particle size. Analyzing serum with a panel of these antibodies should provide a measure of total apoA1 in the sample using a plurality of independent measurements and limits the risk of omission that a single antibody pair will produce. Total apoA1, whose level is predictive of CVD, can be used as a surrogate marker for the entire HDL population. Antibodies that interact with apoA1 in a conformation-dependent manner will recognize only those subsets of particles wherein apoA1 adopts the conformation recognized by that distinct paratope.

Measurements based upon a panel of these antibodies will identify HDL subpopulations based upon selected antibody pair values, whose levels vary between individual samples in a disease-specific manner. In one aspect, the present invention provides a diagnostic test in instances where paratope – epitope interactions are not yet defined.

Methods: A serum sample from an individual and those of a predetermined disease phenotype are subjected to a panel of capture-detection antibody pairs as defined in Table 3. Each of the 37 anti-apoA1 mAbs is evaluated for both its ability to work as a capture antibody and to act as a detection antibody. The total possible number of measurements is 1332 if the same antibody is not used for both capture and detection. Measurements are deemed positive if the positive signal is concentration dependent, saturable, reproducible and exhibits a linear response over a physiologically plausible range of concentration of apoA1.

Results: Antibody pairs demonstrating specific and saturable signals in a dose-dependent manner provide a measure of an existing HDL particle population present in the sample at concentrations that exceed the lowest level of detection that antibody pair affords. For all capture-detection antibody pairs resulting in a signal, analysis can be performed. Each antibody pair signal value can be statistically compared to itself and each other across a library of control samples and samples of known disease conditions. A select set of measurements showing strong correlations to each other across a sample set may represent a plurality of apoA1 epitopes associated with the same or highly similar particle subpopulation. Antibody pair signals that do not correlate with one another may be representative of independent particle subpopulations. Signals demonstrating the least variability across similar samples and the greatest variability between disease states are preferable for establishing predictive biomarkers of CVD. Each antibody pair signal value

can be correlated to the surrogate marker total HDL-C surrogate level of a serum sample. Antibody pair signals having significant correlation to HDL-C are representative of subpopulations associated with large cholesterol-rich particles including the HDL2 particle fraction. Antibody pair signals having the least correlation with HDL-C levels are representative of small dense lipid poor HDL3 particle fraction which remains unaccounted for in the total HDL-C number. The greater the discordance between HDL-C levels and antibody pair signals, the more probable that the antibody pair is measuring an HDL subpopulation whose contributions to the HDL profile are not captured in the surrogate marker HDL-C.

Example 2

This invention provides a method of determining a mammalian test subject's risk of developing CVD by measuring apoA2 with a collection of antibodies, where each paratope is distinct, whose epitopes are distinguishable and interact in both conformation-dependent and -independent manner. The measurements from the subject sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection samples representing specific disorders.

Rationale: The lipoprotein apoA2 is the second most abundant protein on HDL and is found in plasma as a monomer, homodimer, or heterodimer with apolipoprotein D (Schmitz, G., et. al., J. Lipid Res., 24:1021, 1983; Yang, C. Y., et al., Biochem. 33:12451, 1994; Gillard, B. K., et al. Biochem. 44:471, 2005). The differential equilibrium distribution between apoA1 and apoA2 across HDL2 and HDL3 sub-fractions has long been recognized (Cheung, M. C. and Albers, J. J., J. Lip. Res. 23:747, 1982). Most apoA2 is present on apoA1-containing particles and structural studies indicate that apoA2 can cause significant structural changes in apoA1 conformation, affecting both

particle remodeling and activity (Rye, K. A. et. al., J. Biol. Chem., 278:22530, 2003; Boucher, J. et al., J. Lipid. Res. 45:849, 2004). In the plasma, apoA2 is associated predominantly with smaller and less lipid-enriched HDL particles. The denser HDL3 fraction has been shown to contain higher relative amounts of apoA2 than the larger HDL2 particles with apoA1/apoA2 ratios of 3.7 and 4.8, respectively (Brewer, H. B., Jr., et. al., Methods Enzymol. 128:223, 1986). The small dense fraction HDL3 demonstrates superior atheroprotective activities when compared to HDL2 isolated from the same individuals (Zerrad-Saadi, A., et. al., Arterioscler. Thromb. Vasc. Biol., 29:2169, 2009; Kontush, A., et. al., Arterioscler. Thromb. Vasc. Biol. 27:1843, 2007; de Souza, J. A., et. al., J. Cell. Mol. Med. 14:608, 2010), thus opening the possibility that individuals with low levels of HDL3 are thought to be at risk for CVD (see Kontush, A. and Chapman M. J. Nat. Clin Pract. 3:144, 2006).

Methods: A serum sample from an individual and those of a predetermined disease phenotype are subjected a panel of capture-detection antibody pairs directed at apoA2 from the list in Table 4. Each of the anti-apoA2 mAbs is evaluated both for its ability to work as a capture antibody and to act as a detection antibody. Measurements are deemed positive if the positive signal is concentration-dependent, saturable, reproducible and exhibits a linear response over a physiologically plausible range of apoA2 concentrations. Combining the panel of working apoA2 capture-detection antibody pairs and the antibody pairs identified as successfully generating a signal in Example 1 yields a mixed antigen measurement where one apoA1 and one apoA2 are used as capture-detection antibody pairs. As in Example 1, each apoA1|apoA2 mixed antigen antibody pair signal value can be statistically compared to itself and others across a library of control samples and samples of known disease condition.

Results: Antibody pairs demonstrating specific and saturable signals in a dose-dependent manner provide a means of measuring an existing particle population present in the sample at concentrations that exceed the lowest level of detection that antibody pair affords. For all mixed antigen capture-detection pairs resulting in a signal, analysis can be performed. Measurements from apoA1|apoA2 paired antibodies should identify HDL subpopulations containing both proteins. Antibody paired signal demonstrating the least variability across similar samples and the greatest variability between disease states are preferable for establishing predictive biomarkers of CVD. The measurements from the subject sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection of control and disease samples.

Example 3

This invention provides a method of determining a mammalian test subject's risk of developing CVD through measuring a plurality of epitopes from the HDL proteome members defined by Table 1 using a selection of HDL proteome member antibodies from Table 4. Each antibody that binds a distinct proteome member can be used in mixed antigen capture-detection pairs with apoA1 or apoA2 if epitopes are distinguishable and non-overlapping. The measurements from the subject sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection samples representing specific disorders.

Rationale: Both apoA1 and apoA2 are present on the majority of HDL particles. Other proteome members demonstrate degrees of particle restriction and selectivity (Davidson, W. S, et. al. Arterioscler. Thromb. Vasc. Biol. 29:870, 2009; Gordon, S. M., et. al., J. Proteome Res., 9:5239, 2010).

Using a conformational-independent apoA1 capture antibody from Example 1 paired with a detection antibody for another proteome member can provide a measure of the relative concentration of the subpopulation containing both proteins in the sample. Both of the HDL particle populations, those containing the proteome member and those that do not, compete for binding to the same apoA1 capture antibody resulting in diminished signal. In other instances, proteome member capture antibodies will selectively bind only particle subpopulations containing the proteome member, and can preferentially concentrate particles containing the proteome member. Such a combination can increase the lower limits of detection in instances where the subpopulation defined by the proteome member is small. Antibody pair signals generated by measuring apoA1|proteome or proteome|apoA1 are different and can be statistically compared to themselves and others across a library of control samples and samples of known disease condition.

Methods: A serum sample from an individual and those of a predetermined disease phenotype are subjected to a panel of capture-detection antibody pairs derived from pairs successfully generating a signal in Example 1 or 2 with proteome-specific antibodies from Table 4. Each proteome mAb is evaluated both for its ability to work as a capture antibody and to act as a detection antibody. Measurements are deemed positive if the positive signal is concentration-dependent, saturable, reproducible and exhibits a linear response over a range of HDL concentrations. Mixed antigen antibody pair signal values can be statistically compared to themselves and other signals across a library of control samples and samples of known disease condition.

Results: Antibody pairs demonstrating specific and saturable signals in a dose-dependent manner provide a means of measuring an existing particle population present in the sample at concentrations that exceed the lowest level of detection that an antibody pair affords. For all mixed antigen capture-

detection pairs resulting in a signal, analysis can be performed. Measurements from apoA1|proteome and proteome|apoA1 paired antibodies should identify HDL subpopulations containing both proteins. Antibody paired signals demonstrating the least variability across similar samples and the greatest variability between disease states are preferable for establishing predictive biomarkers of CVD.

Example 4

This invention provides a method of determining a mammalian test subject's risk of developing CVD by measuring specific apoA1 conformations associated with levels of functional HDL subpopulations previously identified by physiochemical properties. The measurements from the subject sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection of samples representing specific disorders.

Rationale: Small lipid-poor preβ1-HDL, a minor HDL sub-fraction consisting of a discoidal-shaped particle containing apoA1, PL and unesterified cholesterol, can be identified by 2-D gel electrophoresis (Asztalos, B. F., et al., Arterioscler. Thromb. Vasc. Biol. 20:2670, 2000). Preβ1-HDL is a preferred acceptor of cellular cholesterol, an important first step in the process of reverse-cholesterol transport (Castro, G. R. and Fielding P. E., Biochemistry 27:25, 1988; Kawano, M., et. al., Biochem. 32:5025, 1993; Huang, Y., et. al., Arterioscler. Thromb. Vasc. Biol. 13:445, 1993). Levels of preβ1-HDL are elevated in type 2 diabetes and indicative of patients with hyperlipidemia and CAD (Hirayama, S., et. al., Diabetes Care 30:1289, 2007; Miida, T., et. al., Clin. Chem 42:1992, 1996; Asztalos, B. F., et al., Arterioscler. Thromb. Vasc. Biol. 20:2670, 2000). Rather than characterizing preβ1-HDL using physiochemical separation, the particle population can be

measured using a capture antibody highly specific for an apoA1 conformation found only in preβ1-HDL paired with a conformational-independent apoA1 detection antibody identified from Example 1. Such conformation-dependent antibodies include mAb 55201 (Miyazaki, O, et. al., J. Lipid Res., 41:2083) that recognizes an apoA1 epitope located between residues 140-210 (Sviridov, D., et. al., Arterioscler. Thromb. Vasc. Biol. 22:1482, 2002) or the mAb that recognizes apoA1 residues 137-144 of the mature protein uniquely associated with preβ1-HDL (Fielding, P. E., et. al., Biochemistry 33:6981, 1994). Elevated levels of preβ1-HDL are a predictor of carotid atherosclerosis (Suzuki, M. et. al., Clin. Chem. 51:132, 2005; Hirayama, S., et al., Diabetes Care 30:1289, 2007; Tashiro, J., et. al., Atherosclerosis 204:595, 2009).

Methods: A serum sample from an individual and those of a predetermined disease phenotype are measured using a specific conformation-dependent antibody capable of recognizing apoA1 only when present in the preβ1-HDL subpopulation, paired with a conformation-independent apoA1 detection antibody identified from Example 1. Measurements are deemed positive if the positive signal is concentration-dependent, saturable, reproducible and exhibits a linear response over a range of HDL concentrations. Mixed antigen antibody pair signal values can be statistically compared to themselves and other signals across a library of control samples and samples of known disease condition.

Results: Individuals with normal ranges of total HDL-C and total apoA1 levels can exhibit increased levels of preβ1-HDL subpopulation. Individuals with increased levels of preβ1-HDL are at risk for CAD and may also have compounding factors including dyslipidemia or diabetes.

Example 5

In one embodiment, the present invention provides a method of determining a mammalian test subject's levels of functionally defective HDL particles resulting from specific post-translational modifications of apoA1. The measurements from the subject sample are compared to one or more predetermined values measured in a control population of healthy subjects and to a collection samples representing specific disorders.

Rationale: Post-translation modifications to apoA1 can result in functionally defective HDL (see Kontush, A. and Chapman, M. J., Pharmacol. Rev., 58:342, 2006). Oxidized lipids and proteins associated with lipoprotein particles play a key role in atherogenesis (Barter, P. J., et. al., Circ. Res., 95:764, 2004; Nicholls, S. J., et. al., Trends Card. Med., 15:212, 2005; Deigner, H. P. and Hermetter, A., Curr. Opin. Lipidol., 19:289, 2008). Myeloperoxidase modifies apoA1 at specific susceptible residues (Met86, Met112, Met148, and Tyr192) resulting in functionally defective HDL (Zheng, L. B., et. al., J. Clin. Invest. 114:529, 2004; Shao, B. G., et. al., Proc. Natl. Acad. Sci. USA 105:12224, 2008; Shao, B., et. al., Chem. Res. Toxicol. 23(3):447, 2010). Antibodies developed to detect modification to those residues, MOA-I and mAb17 (Wang, X. S., et. al., J. Lipid Res. 50:586, 2009) can be employed to measure the extent of oxidated apoA1 in HDL when paired with an apoA1 capture antibody with a distinguishable and non-overlapping epitope as identified in Example 1. Another example of post-translational modification affecting HDL particle function is glycation (non-enzymatic glycosylation) which is the result of the bonding of a sugar molecule (fructose, glucose or galactose) with a protein or lipid molecule. Glycation is considered an arbitrary process which differs from glycosylation which involves enzyme-controlled addition of sugars to protein or lipid molecules at defined sites. Glycation can impair the functioning of

biomolecules and this specific modification of apoA1 results in impaired anti-inflammatory activities of HDL (Calvo, C., et. al., Clin. Chim. Acta, 217:193, 1993; Nobécourt, E., et. al., Arterioscler. Thromb. Vasc. Biol., 30:766, 2010; Park, K-H. and Cho, K-H., J. Gerontol., 66A:511, 2011). Methodology devised to generate specific antibodies capable of detecting specific glycation modified proteins can be employed to develop similar measure for glycanated apoA1 (Steward, L. A., et. al., J. Immuno. Method., 140:145, 1991; Cohen, M. P., et. al., Eur. J. Clin. Chem. Clin. Biochem., 31:707, 1993; Qin, X., et. al., Diabetes, 53:2653, 2004). In another example, secreted apoA1 exists as two species in plasma, a pro-protein and mature protein form which differ by six amino acid residues on the N-terminal end of the protein (Zannis, V. I., et. al., Proc. Natl. Acad. Sci. USA, 80:2574, 1983; Stoffel, W., J. Lipid Res., 25:1586, 1984).

Example 6

This invention provides a method of determining the effects of apoC3 levels on a subject's risk for the disorders hypertriglyceridemia and CVD. The measurements from the subject sample are compared to one or more predetermined values measured in a control population of healthy subjects and to a collection samples representing specific disorders.

Rationale: Human apoC3 is a protein constituent of both apoB-containing lipoproteins and HDL (Shin, M. J. and Krauss R. M., Atherosclerosis 211:337, 2010). In addition to rapid transfer between particles, apoC3 redistributes from triglyceride-rich lipoproteins (TRLs) to HDL and transfers back to newly synthesized TRLs (see Jong, M. C., et al., Arterioscler. Thromb. Vasc. Biol., 19:472, 1999; Ooi, E. M. M., Clinical Science, 114:611, 2008). Through a genome-wide association study, APOC3 null mutation carriers were identified who had reduced apoC3 levels and had lower fasting triglycerides and

postprandial serum triglycerides and increased HDL-C. Consistent with the favorable protective lipid profile, APOC3 null mutation carriers were less likely to have detectable coronary artery calcification (Pollin, T. I., et al., Science 322:1702, 2008). To test a subject for risks associated with dyslipidemia due to apoC3 disequilibrium, the combination of capture-detection antibody pairs can be used to measure the levels and disposition of apoC3 in a biological sample. The signal associated with these measurements in the test subject is compared to a predetermined value to determine if the subject is at greater risk of developing or suffering from CAD than subjects with an amount of apoC3 that is at, or higher than, the predetermined value. Moreover, the extent of the difference between the test subject's apoA1|apoC3 and apoA2|apoC3 levels in the biological sample and the predetermined value is also useful for characterizing the extent of the risk, and thereby determining which subjects would most greatly benefit from certain TG-lowering therapies.

Example 7

This invention provides a method of determining the level of one or more lipoprotein proteome members selected from apoJ, PON1, PON3 and PAF-AH, as a method of assessing HDL subpopulations containing anti-oxidative activity. The measurements from the subject sample are compared to one or more predetermined values measured in a control population of healthy subjects and to a collection of samples representing specific disorders.

Example 8

This invention provides a method of determining the level of one or more lipoprotein proteome members associated with HDL selected from AHSG, A1BG, apoF, GC, PLTP, RBP4, serpinA3, serpinA8, serpinF2 and TTR. The detected amount of the lipoprotein proteome member is compared to one or

more predetermined values of the lipoprotein proteome member(s) measured in a control population of healthy subjects to evaluate the level of small dense HDL3. The measurements from the subject sample are then compared to one or more predetermined values measured in a control population of healthy subjects and to a collection samples representing specific disorders.

Example 9

This invention provides methods of screening a human subject who appears healthy, or may be diagnosed as having a low HDL:LDL ratio and/or as being at risk for CVD based on certain known risk factors such as high blood pressure, high cholesterol, obesity, or genetic predisposition for CVD. The methods described herein are especially useful to identify subjects at high risk of developing CVD, in order to determine what type of therapy is most suitable and to avoid potential side effects due to the use of medications in low risk subjects. For example, prophylactic therapy is useful for subjects at some risk for CVD, including a low fat diet and exercise. For those at higher risk, a number of drugs may be prescribed by physicians, such as lipid-lowering medications as well as medications to lower blood pressure in hypertensive patients. For subjects at high risk, more aggressive therapy may be indicated, such as administration of multiple medications.

Envisioned here is a method of detecting arterial disease, atherosclerosis, and fatty lesions formed on the inside of the arterial wall. These lesions promote the loss of arterial flexibility and lead to the formation of blood clots. The lesions may also lead to thrombosis, resulting in most acute coronary syndromes. Thrombosis results from weakening of the fibrous cap, and thrombogenicity of the lipid core. It is well recognized that atherosclerosis is a chronic inflammatory disorder (see Ross, R., N. Engl. J. Med. 340:115, 1999). Chronic inflammation alters the protein composition of HDL, making it

atherogenic (see Barter, P. J., et al., Circ. Res. 95:764, 2004; Chait, A., et al., J. Lipid Res. 46:389, 2005; Navab, M., et al., J. Lipid Res. 45:993, 2004; and Ansell, B. J., et al., Circulation 108:2751, 2003). Accordingly, HDL-associated proteins that serve as lipoprotein proteome member indicators for CVD, and atherosclerotic lesions in particular, may be derived from macrophages, smooth muscle cells, and endothelial cells present in atherosclerotic lesions. Accordingly, HDL-associated lipoprotein proteome members isolated from a blood sample represent a biochemical "biopsy" of the artery wall or endothelium lining the vasculature. It is likely that lesions that are most prone to rupture would increase their output of HDL due to the fact that enhanced proteolytic activity destroys the extracellular matrix and promotes plaque rupture. Indeed, short-term infusion of HDL into humans may promote lesion regression (Nissen, S. E., et al., JAMA 290:2292, 2003), suggesting that HDL can remove components of atherosclerotic tissue. Therefore, the proteins included in the protein cargo associated with HDL, enriched in CVD subjects, and also known to be present in lesion HDL from a population of CVD patients, serve as lipoprotein proteome members that may be used to detect the risk and/or presence of atherosclerotic plaques in an individual subject.

In another aspect, this invention provides assays comprising one or more detection reagents capable of detecting at least the proximity of two lipoprotein proteome members that is indicative of the presence or risk of CVD in a subject. The lipoprotein proteome member is detected by mixing a detection reagent that detects at least one lipoprotein proteome member associated with CVD with a sample containing HDL-associated proteins, and monitoring the mixture for detection of the lipoprotein proteome member with a suitable detection method such as spectrometry, immunoassay, or other method. In one example, the assays are provided as a kit. The kit can have, for example, detection reagents for detecting at least two, three, four, five, ten

or more HDL-associated lipoprotein proteome members in biological samples from a test subject.

The kit also includes written indicia, such as instructions or other printed material for characterizing the risk of CVD based upon the outcome of the assay. The written indicia may include reference information, or a link to information regarding the predetermined signal values for paired proteome measurements of one, two, three, four, five, ten or more HDL-associated lipoprotein proteome members from a reference population of healthy subject samples, and an indication of a correlation between paired proteome measurements of one or more HDL-associated lipoprotein proteome members with samples from subjects having, or at risk of having, CVD.

In one example, the detection reagent comprises one or more antibodies which specifically bind one or more of the lipoprotein proteome members provided in Table 1 or 2 that may be used for the diagnosis and/or prognosis of CVD characterized by the relative abundance of the lipoprotein proteome member in the serum, or an HDL subfraction thereof. Standard values for protein levels of the lipoprotein proteome members are established by combining biological samples taken from healthy subjects. Deviation in the amount of signal produced from an antibody pair between control subjects and CVD subjects establishes the parameters for diagnosing and/or assessing risk levels, or monitoring disease progression.

In another example, this invention provides a method of determining the efficacy of a treatment regimen for treating and/or preventing CVD by monitoring the presence of one or more lipoprotein proteome members in a subject during treatment for CVD. The treatment for CVD varies depending on the symptoms and disease progression. The general treatments include lifestyle changes and medications, and may include surgery. Lifestyle

changes include, for example, weight loss, a low saturated fat, low cholesterol diet, reduction of sodium, regular exercise, and a prohibition on smoking. Medications useful to treat CVD include, for example, cholesterol-lowering medications, antiplatelet agents (e.g., aspirin, ticlopidine and clopidogrel), glycoprotein IIb-IIIa inhibitors (such as abciximab, eptifibatide or tirofiban), or antithrombin drugs (blood-thinners such as heparin) to reduce the risk of blood clots. Beta-blockers may be used to decrease the heart rate and lower oxygen use by the heart. Nitrates, such as nitroglycerin are used to dilate the coronary arteries and improve blood supply to the heart. Calcium-channel blockers are used to relax the coronary arteries and systemic arteries, and, thus, reduce the workload for the heart. Medications suitable for reducing blood pressure are also useful to treat CVD, including ACE inhibitors, diuretics and other medical treatments.

Table 1

EGNM	GeneID	UNIPROT-KB	Protein Name
A1BG	1	P04217	Alpha-1B-glycoprotein
A2M	2	P01023	Alpha-2 macroglobulin
AFM	173	P43652	Afamin
AGT	183	P01019	Angiotensinogen (Serpin Peptidase Inhibitor Clade A Member 8)
AHSG	197	P02765	Alpha-2-HS-glycoprotein
ALB	213	P02768	Serum albumin
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)
APCS	325	P02743	Amyloid P Component Serum (SAP)
APOA1	335	P02647	apolipoprotein A-I
APOA2	336	P02652	apolipoprotein A-II
APOA4	337	P06727	apolipoprotein A-IV
APOA5	116519	Q6Q788	apolipoprotein A-V
APOB	338	P04114	apolipoprotein B-100
APOC1	341	P02654	apolipoprotein C-I
APOC2	344	P02655	apolipoprotein C-II
APOC3	345	P02656	apolipoprotein C-III
APOC4	346	P55056	apolipoprotein C-IV
APOD	347	P05090	apolipoprotein D
APOE	348	P02649	apolipoprotein E
APOF	319	Q13790	apolipoprotein F
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)
APOL1	8542	O14791	apolipoprotein L-I
APOM	55937	PO95445	apolipoprotein M
APOO	79135	Q9BUR5	apolipoprotein O
ATRN	8455	O75882	Attractin
BMP1	649	P13497	Bone morphogenetic protein 1
C1QB	713	P02746	Complement C1q subcomponent subunit B
C1QC	714	P02747	Complement C1q subcomponent subunit C
C1R	715	P00736	Complement C1 r subcomponent
C1S	716	P09871	Complement C1 s subcomponent
C2	717	P06681	Complement C2
C3	718	P01024	Complement C3
C4A	720	P0C0L4	Complement C4-A
C4B	721	P0C0L5	Complement C4-B
C4BPA	722	P04003	Complement C4 binding protein alpha chain
C5	727	P01031	Complement C5
C6	729	P13671	Complement C6
C7	730	P10643	Complement C7

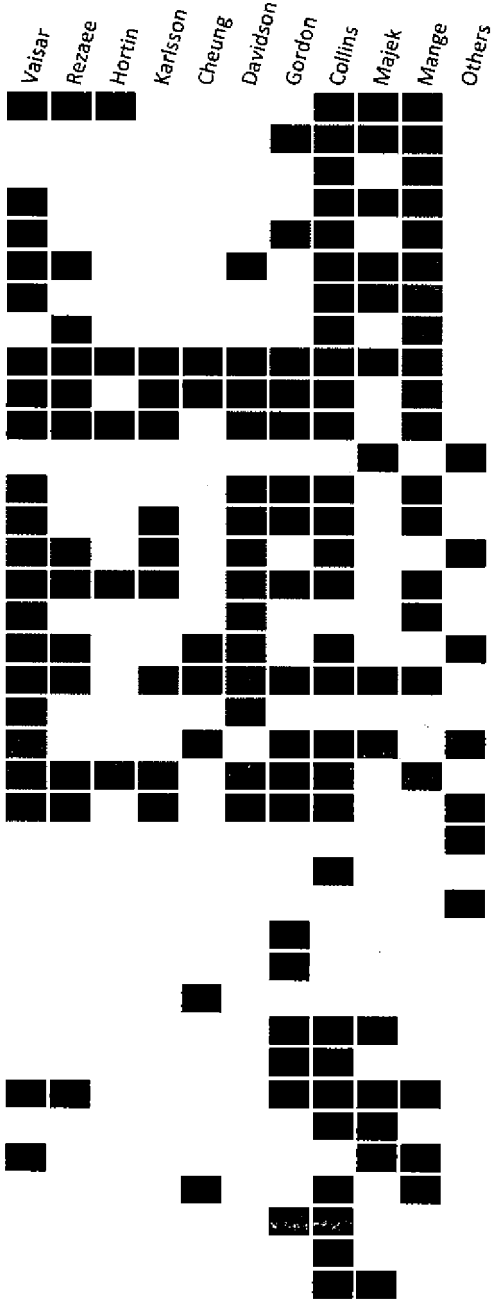

Table 1

C8B	732	P07358	Complement C8 beta chain
C9	735	P02748	Complement C9
CETP	1071	P11597	Cholesteryl ester transfer protein
CFB	629	P00751	Complement factor B
CFH	3075	P08603	Complement factor H
CFI	3426	P05156	Complement factor I
CLEC3B	7123	P05452	Tetranectin
CLU	1191	P10909	Clusterin (apoJ)
CP	1356	P00450	Ceruloplasmin
CPN2	1370	P22792	Caboxypeptidase N polypeptide 2
CRP	1401	P02741	C-Reactive protein
F13B	2165	P05160	Coagulation factor XIII beta subunit
F2	2147	P00734	Prothrombin
F8A	8263	P23610	Coagulation factor VIII intron 22 protein
FCN2	2220	Q15485	Ficolin-2
FCN3	8547	O75636	Ficolin-3
FGA	2243	P02671	Fibrinogen alpha chain
FGB	2244	P02675	Fibrinogen beta chain
FGG	2266	P02679	Fibrinogen gamma chain
FN1	2335	P02751	Fibronectin 1
GC	2638	P02774	Vitamin D-binding protein
GSN	2934	P06396	Gelsolin
HP	3240	P00738	Haptoglobin
HPR	3250	P00739	Haptoglobin-related protein
HPX	3263	P02790	Hemopexin
HRG	3273	P04196	Histidine-rich glycoprotein
IGFALS	3483	P35858	Insulin-like growth factor binding protein acid labile subunit
ITIH1	3697	P19827	Inter-alpha-trypsin inhibitor heavy chain H1
ITIH2	3698	P19823	inter-alpha-trypsin inhibitor heavy chain H2
ITIH3	3699	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3
ITIH4	3700	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4
KLKB1	3818	P03592	Plasma kallikrein B1
KNG1	3827	P01042	Kininogen-1
LCAT	3931	P04180	Lecithin-cholesterol acyltransferase
LPA	4018	P08519	Apolipoprotein(a)
LRG1	116844	P02750	Leucine-rich alpha-2-glycoprotein
LUM	4060	P51884	Lumican
MASP1	5648	P48740	Mannan-binding lectin serine protease 1 precursor
ORM1	5004	P02763	Alpha-1-acid glycoprotein 1 (Orosomucoid 1)
ORM2	5005	P19652	Alpha-1-acid glycoprotein 2 (Orosomucoid 2)
PAFAH1B1	5048	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha
PCYOX1	51449	Q9UHG3	Prenylcysteine oxidase 1

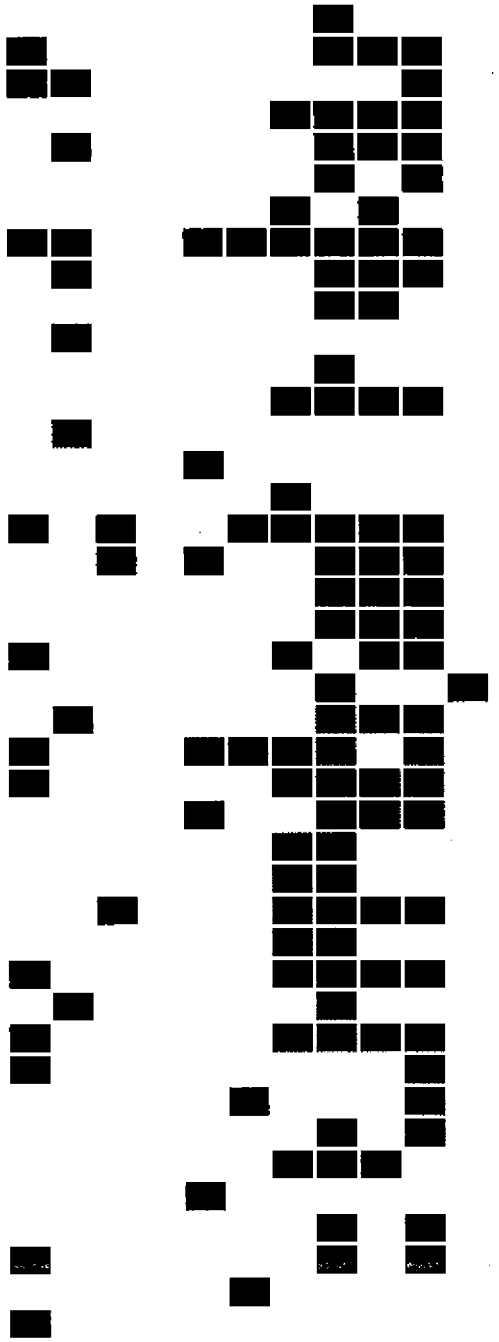

Table 1

PGLYRP2	114770	Q96PD5	Peptidoglycan recognition protein 2
PLA2G7	7941	Q13093	Platelet-activating factor acetylhydrolase (PAFA)
PLG	5340	P00747	Plasminogen
PLTP	5360	P55058	Phospolipid transfer protein
PON1	5444	P27169	Serum paraoxonase/arylesterase 1
PON3	5446	Q15166	Serum paraoxonase/lactonase 3
PPBP	5473	P02775	Platelet basic protein
PROS1	5627	P07225	Vitamin-K-dependent protein S
RBP4	5950	P02753	Retinol-binding protein RBP4
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)
SAA4	6291	P35542	Serum amyloid A-4 protein
SERPINA1	5265	P01009	Alpha-1-antitrypsin (Serpin Peptidase Inhibitor Clade A Member 1)
SERPINA3	12	P01011	Alpha-1-antichymotrypsin (Serpin Peptidase Inhibitor Clade A Member 3)
SERPINA4	5267	P29622	Kallistastin (Serpin Peptidase Inhibitor Clade A Member 4)
SERPINA6	866	P08185	Corticosteroid binding globulin (Serpin Peptidase Inhibitor Clade A Member 6)
SERPINC1	462	P01008	Antithrombin III (Serpin Peptidase Inhibitor Clade C Member 1)
SERPIND1	3053	P05546	Heparin cofactor 2 (Serpin Peptidase Inhibitor Clade D Member 1)
SERPINF1	5176	P36955	Pigment epithelium-derived factor (Serpin peptidase inhibitor Clade F Member 1)
SERPINF2	5345	P08697	Alpha-2-antiplasmin (Serpin peptidase inhibitor Clade F Member 2)
SERPING1	710	P05155	Plasma protease C1 inhibitor (Serpin peptidase inhibitor Clade G Member 1)
SEPP1	6414	P49908	Selenoprotein P
TF	7018	P02787	Serotransferin
TFPI	7035	P10646	Tissue Factor Pathway Inhibitor
TTR	7276	P02766	Transthyretin
VTN	7448	P04004	Vitronectin

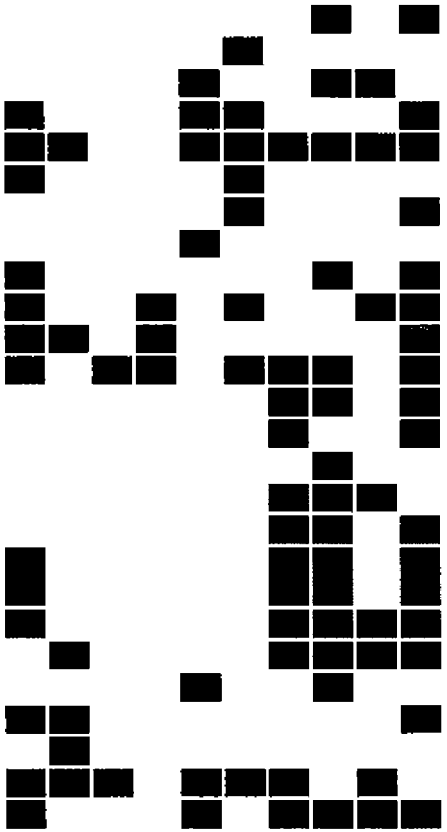

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Table 2

EGNM	GeneID	UNIPROT-KB	Protein Name
A1BG	1	P04217	Alpha-1B-glycoprotein
A2M	2	P01023	Alpha-2 macroglobulin
AHSG	197	P02765	Alpha-2-HS-glycoprotein
ALB	213	P02768	Serum albumin
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)
APCS	325	P02743	Serum amyloid P-component
APOA1	335	P02647	apolipoprotein A-I
APOA2	336	P02652	apolipoprotein A-II
APOA4	337	P06727	apolipoprotein A-IV
APOB	338	P04114	apolipoprotein B-100
APOC1	341	P02654	apolipoprotein C-I
APOC2	344	P02655	apolipoprotein C-II
APOC3	345	P02656	apolipoprotein C-III
APOC4	346	P55056	apolipoprotein C-IV
APOD	347	P05090	apolipoprotein D
APOE	348	P02649	apolipoprotein E
APOF	319	Q13790	apolipoprotein F
APOH	350	P02749	Beta-2-glycoprotein 1 (apolipoprotein H)
APOL1	8542	O14791	apolipoprotein L-I
APOM	55937	P095445	apolipoprotein M
APOO	79135	Q9BUR5	apolipoprotein O
S100A8	6279	P05109	Protein S100-A8
CD5L	922	O43866	CD5 antigen-like
C1QA	712	P02745	Complement component 1 q subunit A
C1QB	713	P02746	Complement component 1 q subunit B
C1QC	714	P02747	Complement component 1 q subunit C
C1R	715	P00736	Complement C1 r subcomponent
C1S	716	P09871	Complement C1 s subcomponent
C3	718	P01024	Complement component C3
C4A	720	P0C0L4	Complement C3
C4B	721	P0C0L5	Complement C4-A
C4BPB	722	P04003	Complement C4-B
C7	730	P10643	Complement C4 binding protein alpha chain
CFH	3075	P08603	Complement factor H
CFHR1	3078	Q03591	Complement factor H-related protein 1
CFHR5	81494	Q9BXR6	Complement factor H-related protein 5
CLU	1191	P10909	Clusterin (apoJ)
CP	1356	P00450	Ceruloplasmin
F13A	2165	P00488	Coagulation factor F XIII alpha subunit
F13B	2165	P05160	Coagulation factor F XIII beta subunit
F2	2147	P00734	Prothrombin
FCN3	8547	O75636	Ficolin-3
FGA	2243	P02671	Fibrinogen alpha chain
FGB	2244	P02675	Fibrinogen beta chain
FGG	2266	P02679	Fibrinogen gamma chain
FN1	2335	P02751	Fibronectin 1
GC	2638	P02774	Vitamin D-binding protein

Table 2

GP1BA	2811	P07359	Platelet glycoprotein Ib alpha chain (Glycocalicin)
HBA1	3039	P69905	Hemoglobin subunit alpha
HBB	3043	P68871	Hemoglobin subunit beta
HP	3240	P00738	Haptoglobin
HPR	3250	P00739	Haptoglobin-related protein
HPX	3263	P02790	Hemopexin
ITIH2	3698	P19823	inter-alpha-trypsin inhibitor heavy chain H2
ITIH3	3699	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3
ITIH4	3700	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4
KNG1	3827	P01042	Kininogen-1
LGALS3BP	3959	Q08380	Galectin-3-binding protein
LPA	4018	P08519	Apolipoprotein (a)
LYSX	38122	P37161	Lysozyme X
LYZ	4069	P61626	Lysozyme C
MASP2	10747	O00187	Mannan-binding lectin serine protease 2 precursor
PCYOX1	51449	Q9UHG3	Prenylcysteine oxidase
PLA2G7	7941	Q13093	Platelet-activating factor acetylhydrolase (PAFA)
PLG	5340	P08519	Plasminogen
PON1	5444	P27169	Serum paraoxonase/arylesterase 1
CFP	5199	P27918	Properdin (complement facctor P)
PROS1	5627	P07225	Vitamin-K-dependent protein S
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)
SAA4	6291	P35542	SAA4
SERPINA1	5265	P01009	Alpha-1-antitrypsin (Serpin Peptidase Inhibitor Clade A Member 1)
SERPINF2	5345	P08697	Alpha-2-antiplasmin (Serpin peptidase inhibitor Clade F Member 2)
TF	7018	P02787	Serotransferin
TGFB	7040	P01137	Transforming growth factor beta-1
TTR	7276	P02766	Transthyretin
VTN	7448	P04004	Vitronectin

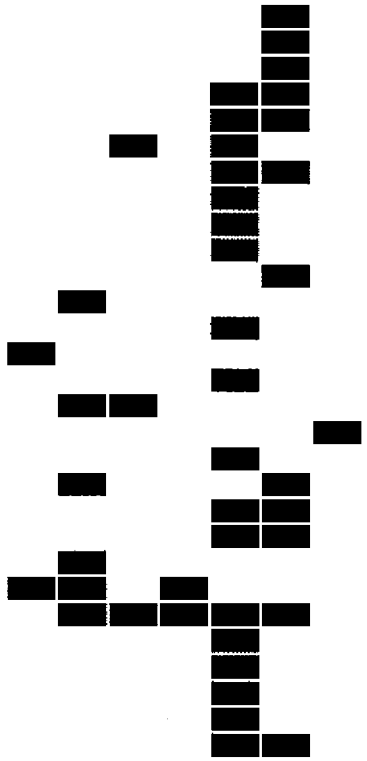

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Table 3

Clone ID	Host	Specificty	Isotype	Vendor	Cat. #
1402	mouse	human	IgG1	Abcam	ab20411
1405	mouse	human	IgG1	Abcam	ab20735
1409	mouse	human	IgG1	Abcam	ab20918
1C5	mouse	human	IgG1	Biodesign Intl.	H61531M
513	mouse	human	IgG1	CalBiochem	178472
6001	mouse	human	IgG2a	CalBiochem	178470
412	mouse	human	IgG1	EMD Millipore	MAB010-A/11
A/13	mouse	human	IgG1	EMD Millipore	MAB011-A/13
EP1368Y	rabbit	human	n.d.	Epitomics	1920-1
LS-B3047	mouse	human	IgG2a,kappa	LifeScience Bio	057-10029
LS-C84251	mouse	human	IgG2a	LifeScience Bio	M55311
LS-C84252	mouse	human	IgG2a	LifeScience Bio	M808121
LS-C35007	mouse	human	IgG1	LifeScience Bio	1402
LS-C35008	mouse	human	IgG1	LifeScience Bio	1404
HDL 110	mouse	human	IgG2b	Mabtech	3710-2-1000
HDL 44	mouse	human	IgG1	Mabtech	3710-3-1000
412	mouse	human	IgG1	Millipore	MAB010-A/11
057-10029	mouse	human	IgG2a,kappa	MYBIOSOURCE	MBS311600
057-16001	mouse	human	IgG2a,kappa	MYBIOSOURCE	MBS311599
G2	mouse	human	IgG1	Novus Biologicals	NB100-65491
12C8	mouse	human	IgG1,kappa	Novus Biologicals	NBP1-05174
2G4	mouse	human	IgG1,kappa	Novus Biologicals	NBP1-41969
6A9G6	mouse	human	IgG1	ProMab	Mab-2008031-1
5F4F5	mouse	human	IgG1	ProMab	Mab-2008031-2
A5.4	mouse	human	IgG1	Sant Cruz Biotechnology	sc-13549
3A11-1A9	mouse	human	IgG2	Sigma	WH0000335M1
2Q2200	mouse	human	IgG1	United States Biological	A2299-08C
6F31	mouse	human	IgG1,kappa	United States Biological	A2299-26A
6F30	mouse	human	IgG1,kappa	United States Biological	A2299-26
2Q2199	mouse	human	IgG1	United States Biological	A2299-08B
5E12	mouse	human	IgG2a,kappa	United States Biological	A2299-25A
8.F.15	mouse	human	IgG2a	United States Biological	A2299-12
2Q2201	mouse	human	IgG1	United States Biological	A2299-08D
7K4	mouse	human	IgG1	United States Biological	A2299-09B
4A89	mouse	human	IgG2a,kappa	United States Biological	A2299-08F
9L39	mouse	human	IgG2a,kappa	United States Biological	A2299-09A1
10H10	mouse	human	IgG1	Yorkshire Bioscience	R1003
7C1	mouse	human	IgG1	Yorkshire Bioscience	R1005

Table 4

EGNM	GeneID	UNIPROT-KB	Protein Name	Clone ID	Host	Specificty	Preparation	Quantity	Vendor
A1BG	1	P04217	Alpha-1B-glycoprotein	4B5	mouse	human	ascites	0.1mL	Novus Biologicals
A1BG	1	P04217	Alpha-1B-glycoprotein	1H1	mouse	human	IgG purified	100µg	Novus Biologicals
A1BG	1	P04217	Alpha-1B-glycoprotein	4F6	mouse	human	IgG purified	100µg	Novus Biologicals
A2M	2	P01023	Alpha-2 macroglobulin	1.B.742	mouse	human	purified by DEAE chromatography.	1mg	United States Biological
A2M	2	P01023	Alpha-2 macroglobulin	9L177	mouse	human	purified by DEAE chromatography.	1mg	United States Biological
A2M	2	P01023	Alpha-2 macroglobulin	9H154	mouse	human	purified by Protein G affinity	500µg	United States Biological
AFM	173	P43652	Afamin	poly	mouse	human	n.d.	50µg	Anitbodies-online.com
AGT	183	P01019	Angiotensinogen (Serpin Peptidase Inhibitor Clade A Member 8)	BGN/KA/22H	mouse	human	affinity purified	200µg	Novus Biologicals
AGT	183	P01019	Angiotensinogen (Serpin Peptidase Inhibitor Clade A Member 8)	7H144	mouse	human	purified by Protein G affinity	100µg	United States Biological
AGT	183	P01019	Angiotensinogen (Serpin Peptidase Inhibitor Clade A Member 8)	7C4	mouse	human	purified	50µg	United States Biological
AGT	183	P01019	Angiotensin I	BGN/KA/4H	mouse	human	Protein A purified	200µg	Novus Biologicals
AGT	183	P01019	Angiotensin II	Ang II E7 (BGN/KA/4L)	mouse	human	affinity purified	200µg	Novus Biologicals
AGT	183	P01019	Angiotensin II	BGN/0856/21	mouse	human	Protein A purified	200µg	Novus Biologicals
AGT	183	P01019	Angiotensin II	1A10	mouse	human	IgG purified	100µg	Novus Biologicals
AGT	183	P01019	Angiotensin II	3C10	mouse	human	IgG purified	100µg	Novus Biologicals
AHSG	197	P02765	Alpha-2-HS-glycoprotein	162919	mouse	human	purified	500µg	R&D Systems
AHSG	197	P02765	Alpha-2-HS-glycoprotein	162922	mouse	human	purified	500µg	R&D Systems
AHSG	197	P02765	Alpha-2-HS-glycoprotein	5D8	mouse	human	IgG purified	100µg	Novus Biologicals
AHSG	197	P02765	Alpha-2-HS-glycoprotein	1D8	mouse	human	IgG purified	100µg	Novus Biologicals
AHSG	197	P02765	Alpha-2-HS-glycoprotein	9H121	mouse	human	purified by Protein G affinity	500µg	United States Biological
ALB	213	P02768	Serum albumin	2F11	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
ALB	213	P02768	Serum albumin	3F1	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
ALB	213	P02768	Serum albumin	4D8	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
ALB	213	P02768	Serum albumin	4G9	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
ALB	213	P02768	Serum albumin	AL-01	mouse	human	purified	50µg	MyBiosource
ALB	213	P02768	Serum albumin	M603208	mouse	human	column chromatography purified	n.d.	LifeSpan Biosciences
ALB	213	P02768	Serum albumin	A1-01	mouse	human	purified	100µg	United States Biological
ALB	213	P02768	Serum albumin	BGN/1328/52	mouse	human	purified IgG - liquid	200µg	MyBiosource
ALB	213	P02768	Serum albumin	15C7	mouse	human	purified, unlabeled	1mg	MyBiosource
ALB	213	P02768	Serum albumin	1A9	mouse	human	purified, unlabeled	1mg	MyBiosource
ALB	213	P02768	Serum albumin	1C8	mouse	human	purified, unlabeled	1mg	MyBiosource
ALB	213	P02768	Serum albumin	6B11	mouse	human	purified, unlabeled	1mg	MyBiosource
ALB	213	P02768	Serum albumin	5B11	mouse	human	n.d.	200µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	15D2	mouse	human	n.d.	200µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	6B7	mouse	human	n.d.	200µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	1G2	mouse	human	n.d.	100µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	AL-01	mouse	human	n.d.	100µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	2B3	mouse	human	n.d.	500µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	2B2	mouse	human	n.d.	500µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	2B6	mouse	human	n.d.	500µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	KT11	mouse	human	n.d.	100µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	KT32	mouse	human	n.d.	100µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	5D5	mouse	human	n.d.	200µg	Thermo Scientific Pierce
ALB	213	P02768	Serum albumin	AL-01	mouse	human	biotin conjugate	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	10000	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	20	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4A7	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4C10	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4F2	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4F4	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4F8	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4G12	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	4G2	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	1F7	mouse	human	IgG purified	100µg	Novus Biologicals
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i21	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i22	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i24	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i25	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i26	mouse	human	purified	100µg	MyBiosource

Table 4

AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i28	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	9i29	mouse	human	purified	100µg	MyBiosource
AMBP	259	P02760	Alpha-1-microglobulin (bikunin)	3F1	mouse	human	IgG purified	100µg	Novus Biologicals
APCS	325	P02743	Amyloid P Component Serum (SAP)	3H48	mouse	human	purified by Protein A affinity	50µg	United States Biological
APCS	325	P02743	Amyloid P Component Serum (SAP)	4F3	mouse	human	IgG purified	100µg	Novus Biologicals
APCS	325	P02743	Amyloid P Component Serum (SAP)	10B161	mouse	human	purified by Protein A affinity	200µg	United States Biological
APCS	325	P02743	Amyloid P Component Serum (SAP)	3H48	mouse	human	purified by Protein A affinity	50µg	United States Biological
APCS	325	P02743	Amyloid P Component Serum (SAP)	5.4A	mouse	human	purified	100µg	EMD Millipore
APCS	325	P02743	Amyloid P Component Serum (SAP)	5.4D.3B	mouse	human	purified	100µg	EMD Millipore
APCS	325	P02743	Amyloid P Component Serum (SAP)	5G6	mouse	human	culture supernatant	500ul	United States Biological
APCS	325	P02743	Amyloid P Component Serum (SAP)	2Q2175	mouse	human	purified by Protein A affinity	100µg	United States Biological
APCS	325	P02743	Amyloid P Component Serum (SAP)	2Q2174	mouse	human	purified by Protein A affinity	100µg	United States Biological
APOA2	336	P02652	apolipoprotein A-II	057-10221	mouse	human	purified, unlabeled	1mg	MyBiosource
APOA2	336	P02652	apolipoprotein A-II	4F3	mouse	human	IgG purified	100µg	Novus Biologicals
APOA2	336	P02652	apolipoprotein A-II	1H6	mouse	human	IgG purified	100µg	Novus Biologicals
APOA2	336	P02652	apolipoprotein A-II	4A90	mouse	human	purified by Protein A affinity	1mg	United States Biological
APOA2	336	P02652	apolipoprotein A-II	10B188	mouse	human	purified by Protein A affinity	100µg	United States Biological
APOA2	336	P02652	apolipoprotein A-II	057-10221	mouse	human	purified by Protein A affinity	1mg	Antibodies-online.com
APOA2	336	P02652	apolipoprotein A-II	7H205	rat	human	purified by Protein G affinity	100µg	United States Biological
APOA2	336	P02652	apolipoprotein A-II	EP2912	rabbit	human	purified, unlabeled	0.1mL	Origene
APOA2	336	P02652	apolipoprotein A-II	EP2913	rabbit	human	purified, unlabeled	0.1mL	Origene
APOA2	336	P02652	apolipoprotein A-II	395906	rat	human	purified by Protein G affinity	100µg	R&D Systems
APOA4	337	P06727	apolipoprotein A-IV	1D6B6,1D4C11	mouse	human	ascitic fluid containing 0.03% sodium az	0.1mL	ProMab Biotechnologies
APOA4	337	P06727	apolipoprotein A-IV	2D1C9	mouse	human	ascitic fluid containing 0.03% sodium az	0.1mL	ProMab Biotechnologies
APOA4	337	P06727	apolipoprotein A-IV	poly	mouse	human	purified	50µg	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	10C457	mouse	human	purified	100µg	MyBiosource
APOA5	116519	Q6Q788	apolipoprotein A-V	2G1H11, 1F1E8	mouse	human	purified antibody in PBS containing 0.03	100µg x 2	ProMab Biotechnologies
APOA5	116519	Q6Q788	apolipoprotein A-V	1G5G9	mouse	human	n.d.	0.1mL	Origene Technologies
APOA5	116519	Q6Q788	apolipoprotein A-V	1F1E8	mouse	human	purified, unlabeled	100µg	MyBiosource
APOA5	116519	Q6Q788	apolipoprotein A-V	2G1H11	mouse	human	purified, unlabeled	100µg	MyBiosource
APOA5	116519	Q6Q788	apolipoprotein A-V	1F1E8	mouse	human	n.d.	100µg	Immuno-Biological Laboratories
APOA5	116519	Q6Q788	apolipoprotein A-V	4H8H8E2	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
APOA5	116519	Q6Q788	apolipoprotein A-V	1G5G9	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
APOA5	116519	Q6Q788	apolipoprotein A-V	8J289	mouse	human	purified by Protein G affinity	0.1mL	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	8J288	mouse	human	purified by Protein G affinity	0.1mL	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	10C456	mouse	human	immunoaffinity purified	100µg	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	10C457	mouse	human	immunoaffinity purified	100µg	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	8A105	mouse	human	ascites	40µg, 5x40µ	United States Biological
APOA5	116519	Q6Q788	apolipoprotein A-V	4H8HBE2	mouse	human	Protein G purified	0.1mL	Novus Biologicals
APOA5	116519	Q6Q788	apolipoprotein A-V	1G5G9	mouse	human	Protein G purified	0.1mL	Novus Biologicals
APOB	338	P04114	apolipoprotein B-100	369717	mouse	human	purified	100µg	R&D Systems
APOB	338	P04114	apolipoprotein B-100	10B190	mouse	human	purified by DEAE chromatography.	1mg	United States Biological
APOB	338	P04114	apolipoprotein B-100	7H145	mouse	human	purified by Protein G affinity	100µg	United States Biological
APOB	338	P04114	apolipoprotein B-100	369717	mouse	human	purified	100µg	R&D Systems
APOB	338	P04114	apolipoprotein B-100	F2C9 (APOB-001)	mouse	human	purified IgG - liquid	1mg	MyBiosource
APOB	338	P04114	apolipoprotein B-100	10B190	mouse	human	purified by DEAE chromatography.	1mg	United States Biological
APOB	338	P04114	apolipoprotein B-100	C1.4	mouse	human	purified	200µg/ml	Santa Cruz Biotechnology
APOB	338	P04114	apolipoprotein B-100	APOB-001	mouse	human	affinity purified	1.0mg	Novus Biologicals
APOB	338	P04114	apolipoprotein B-100	LDL 11	mouse	human	biotinylated, supplied at 1mg/ml	250µg	Mabtech AB
APOB	338	P04114	apolipoprotein B-100	LDL 20/17	mouse	human	purified, supplied at 1mg/ml	250µg	Mabtech AB
APOB	338	P04114	apolipoprotein B-100	5i2	mouse	human	purified	50µg	United States Biological
APOB	338	P04114	apolipoprotein B-100	5F8	mouse	human	n.d.	1mL	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	12G10	mouse	human	n.d.	1mL	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	4C11	mouse	human	n.d.	1mL	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	2000	mouse	human	n.d.	1mL	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	B1	mouse	human	purified	500µg	EMD Millipore
APOB	338	P04114	apolipoprotein B-100	7B8	mouse	human	n.d.	200µg	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	7C2	mouse	human	n.d.	200µg	Thermo Scientific Pierce
APOB	338	P04114	apolipoprotein B-100	9G3	mouse	human	n.d.	200µg	Thermo Scientific Pierce
APOC1	341	P02654	apolipoprotein C-I	2E2-1A3	mouse	human	IgG purified	100µg	Novus Biologicals
APOC1	341	P02654	apolipoprotein C-I	10B191	mouse	human	Purified by Protein A affinity	100µg	United States Biological

Table 4

APOC2	344	P02655	apolipoprotein C-II						
APOC3	345	P02656	apolipoprotein C-III	2Q2198	mouse	human	Ascites	100µg	United States Biological
APOC4	346	P55056	apolipoprotein C-IV	3D10	mouse	human	IgG purified	100µg	Novus Biologicals
APOD	347	P05090	apolipoprotein D	2B15	mouse	human	purified by ammonium sulfate precipita	0.1mL	United States Biological
APOD	347	P05090	apolipoprotein D	36C6	mouse	human	supernatant	0.2mL	BIOLOGO
APOE	348	P02649	apolipoprotein E	WUE-4	mouse	human	protein G purified	0.1mL	Origene Technologies
APOE	348	P02649	apolipoprotein E	A1.4	mouse	human	purified	200µg/ml	Santa Cruz Biotechnology
APOE	348	P02649	apolipoprotein E	40000	mouse	human	Protein G purified	0.1mL	Novus Biologicals
APOE	348	P02649	apolipoprotein E	5B5	mouse	human	purified	200µg	Immuno-Biological Laboratories
APOE	348	P02649	apolipoprotein E	E887	mouse	human	biotinylated, supplied at 0,5 mg/ml	250µg	Mabtech AB
APOE	348	P02649	apolipoprotein E	3D12	mouse	human	purified, unconjugated	100µg	YO Proteins AB
APOE	348	P02649	apolipoprotein E	E3	mouse	human	affinity purified, unconjugated	0.2mL	American Research Products
APOE	348	P02649	apolipoprotein E	IB5-E1	mouse	human	affinity purified, unconjugated	0.2mL	American Research Products
APOE	348	P02649	apolipoprotein E	F48.1	mouse	human	affinity purified, unconjugated	0.2mL	American Research Products
APOE	348	P02649	apolipoprotein E	8B65	mouse	human	purified by Protein G affinity	0.1mL	United States Biological
APOE	348	P02649	apolipoprotein E	7C10	mouse	human	purified by Protein A affinity	50µg	United States Biological
APOE	348	P02649	apolipoprotein E	5F194	mouse	human	purified by Protein G affinity	50µg	United States Biological
APOE	348	P02649	apolipoprotein E	5F211	mouse	human	purified by Protein G affinity	50µg	United States Biological
APOE	348	P02649	apolipoprotein E	E887	mouse	human	biotinylated, supplied at 0,5 mg/ml	250µg	Mabtech AB
APOE	348	P02649	apolipoprotein E	E276	mouse	human	purified, supplied at 0,5 mg/ml	250µg	Mabtech AB
APOE	348	P02649	apolipoprotein E	E6D7	mouse	human	purified	0.1mL	MyBiosource
APOE	348	P02649	apolipoprotein E	2B16	mouse	human	purified by Protein A affinity	0.1mL	United States Biological
APOF	319	Q13790	apolipoprotein F	1D5	mouse	human	IgG purified	100µg	Novus Biologicals
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	5-F7	mouse	human	purified	100µg	EMD Millipore
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	3F10	mouse	human	IgG purified	0.2mL	Novus Biologicals
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	517038	mouse	human	purified	100µg	R&D Systems
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	1D2	mouse	human	tissue culture supernatant - liquid	2mL	MyBiosource
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	9B20	mouse	human	supernatant	2ml	United States Biological
APOH	350	P02749	apolipoprotein H (beta-2-glycoprotein 1)	11C82	mouse	human	purified by immunoaffinity	100µg	United States Biological
APOL1	8542	O14791	apolipoprotein L-I	1D4	mouse	human	ascites	0.1mL	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	3H3	mouse	human	purified	0.1mL	Origene Technologies
APOM	55937	PO95445	apolipoprotein M	10C3G5	mouse	human	Protein A purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	8F12C6B8	mouse	human	Protein A purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	10C3G5	mouse	human	purified antibody in PBS	100µg	ProMab Biotechnologies
APOM	55937	PO95445	apolipoprotein M	8F12C6B8	mouse	human	purified antibody in PBS	100µg	ProMab Biotechnologies
APOM	55937	PO95445	apolipoprotein M	8B63	mouse	human	purified by Protein A affinity	100µg	United States Biological
APOM	55937	PO95445	apolipoprotein M	8B64	mouse	human	purified by Protein A affinity	100µg	United States Biological
APOM	55937	PO95445	apolipoprotein M	8A107	mouse	human	purified by Protein A affinity	40µg	United States Biological
APOM	55937	PO95445	apolipoprotein M	1F10	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	2E5	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	1A2	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	1G9	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	2A8	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	3C7	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	4C7	mouse	human	IgG purified	100µg	Novus Biologicals
APOM	55937	PO95445	apolipoprotein M	6H3	mouse	human	IgG purified	100µg	Novus Biologicals
APOO	79135	Q9BUR5	apolipoprotein O	2F1	mouse	human, mouse	ascitic fluid	0.1mL	ProMab Biotechnologies
ATRN	8455	O75882	Attractin	9H8	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
BMP1	649	P13497	Bone morphogenetic protein 1						
C1Q	712	P02745	Complement C1 q subcomponent subunit ?	3R9/2	mouse	human	Protein A purified	0.1mL	Novus Biologicals
C1QA	712	P02745	Complement C1 q subcomponent subunit A	poly	mouse	human	purified	50µg	United States Biological
C1Q	713	P02746	Complement C1 q subcomponent subunit ?	9G135	mouse	human	purified by Protein A affinity	50µg	United States Biological
C1QB	713	P02746	Complement C1 q subcomponent subunit B	poly	mouse	human	purified	50µg	United States Biological
C1Q	714	P02747	Complement C1 q subcomponent subunit ?	10B372	mouse	human	purified by Protein A affinity	0.1mL	United States Biological
C1Q	714	P02747	Complement C1 q subcomponent subunit ?	10B371	mouse	human	purified	100µg	United States Biological
C1Q	714	P02747	Complement C1 q subcomponent subunit ?	9j110	mouse	human	purified	100µg	United States Biological
C1QC	714	P02747	Complement C1 q subcomponent subunit C	5F91	mouse	human	purified	100µg	United States Biological
C1R	715	P00736	Complement C1 r subcomponent	269104	mouse	human	purified	100µg	R&D Systems
C1S	716	P09871	Complement C1 s subcomponent	306904	mouse	human	purified	100µg	R&D Systems
C1S	716	P09871	Complement C1 s subcomponent	9G173	mouse	human	Protein A purified	100µg	United States Biological
C1S	716	P09871	Complement C1 s subcomponent	9G174	mouse	human	Protein A purified	100µg	United States Biological

Table 4

C1S	716	P09871	Complement C1 s subcomponent	6F44	mouse	human	Protein A purified	100µg	United States Biological
C1S	716	P09871	Complement C1 s subcomponent	6F45	mouse	human	Protein A purified	100µg	United States Biological
C2	717	P06681	Complement C2	269716	mouse	human	purified	100µg	R&D Systems
C2	717	P06681	Complement C2	6F61	mouse	human	purified by Protein A affinity	100µg	United States Biological
C2	717	P06681	Complement C2	6F60	mouse	human	purified by Protein A affinity	100µg	United States Biological
C2	717	P06681	Complement C2	6F62	mouse	human	purified by Protein A affinity	100µg	United States Biological
C3	718	P01024	Complement C3	6F64	mouse	human	purified by Protein A affinity	100µg	United States Biological
C3	718	P01024	Complement C3	8J442	mouse	human	purified by Protein G affinity	50µg	United States Biological
C3	718	P01024	Complement C3	8J444	mouse	human	purified	50µg	United States Biological
C3	718	P01024	Complement C3	8J443	mouse	human	purified	50µg	United States Biological
C3	718	P01024	Complement C3	7C10	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C3	718	P01024	Complement C3	2D8	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C3	718	P01024	Complement C3	6F6	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C3	718	P01024	Complement C3	10A1	mouse	human	affinity purified	100µg	Novus Biologicals
C3	718	P01024	Complement C3	28A1	mouse	human	affinity purified	100µg	Novus Biologicals
C3	718	P01024	Complement C3	474	mouse	human	IgG purified	50µg	Novus Biologicals
C3	718	P01024	Complement C3	755	mouse	human	IgG purified	50µg	Novus Biologicals
C3	718	P01024	Complement C3	5F9	mouse	human	IgG purified	0.2mL	Novus Biologicals
C3	718	P01024	Complement C3	6F65	mouse	human	purified by Protein A affinity	100µg	United States Biological
C3	718	P01024	Complement C3	6F66	mouse	human	purified by Protein A affinity	100µg	United States Biological
C3	718	P01024	Complement C3	6F63	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4A	720	P0C0L4	Complement C4-A	10B390	mouse	human	purified by Protein G affinity	200µg	United States Biological
C4A	720	P0C0L4	Complement component C4	25.3F4	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C4B	721	P0C0L5	Complement C4-B	25300	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C4B	721	P0C0L5	Complement Component 4d	10B396	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4B	721	P0C0L5	Complement Component 4d	10B397	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4B	721	P0C0L5	Complement Component 4c (Complement C4c, C4c)	10B394	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4B	721	P0C0L5	Complement Component 4c (Complement C4c, C4c)	10B395	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4B	721	P0C0L5	complement C4	KT29	mouse	human	affinity purified	100µg	Novus Biologicals
C4B	721	P0C0L5	Complement C4	6F70	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4B	721	P0C0L5	Complement C4	6F71	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4BPA	722	P04003	Complement C4 binding protein alpha chain	40823	mouse	human	Protein A purified	50µg	Novus Biologicals
C4BPA	722	P04003	Complement C4 Binding Protein	40824	mouse	human	purified IgG - liquid	100µg	MyBiosource
C4BPA	722	P04003	Complement C4 Binding Protein	10B393	mouse	human	purified by Protein A affinity	100µg	United States Biological
C4BPA	722	P04003	Complement C4 Binding Protein	1G9	mouse	human	IgG purified	100µg	Novus Biologicals
C5	727	P01031	Complement C5	295009	mouse	human	biotin conjugate	250µg	R&D Systems
C5	727	P01031	Complement C5	295003	mouse	human	purified	500µg	R&D Systems
C5	727	P01031	Complement C5	39725	mouse	human	Protein A purified	100µg	Novus Biologicals
C5	727	P01031	Complement C5	12F3	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C5	727	P01031	Complement C5	11F6	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C5	727	P01031	Complement C5	40821	mouse	human	purified IgG - liquid	100µg	MyBiosource
C5	727	P01031	Complement C5	HCC 5.1	mouse	human	50µg (lyophilized) with 0.5% BSA + 0.09	50µg	MyBiosource
C5	727	P01031	Complement C5	557	mouse	human	Protein G purified	50µg	Novus Biologicals
C5	727	P01031	Complement C5	8J447	mouse	human	purified by Protein G affinity	50µg	United States Biological
C6	729	P13671	Complement C6	056B-214.2.4.2	mouse	human	Protein A purified	100µg	Novus Biologicals
C6	729	P13671	Complement C6	10B400	mouse	human	purified by Protein A affinity	100µg	United States Biological
C6	729	P13671	Complement C6	10B2760	mouse	human	purified by Protein G affinity	1ml	United States Biological
C7	730	P10643	Complement C7	10B2761	mouse	human	purified by Protein G affinity	1ml	United States Biological
C7	730	P10643	Complement C7	10B401	mouse	human	purified by Protein A affinity	100µg	United States Biological
C7	730	P10643	Complement C7	030-113.7.5.4	mouse	human	Protein A purified	100µg	Novus Biologicals
C8B	732	P07358	Complement C8	monoclonal	mouse	human	Protein A column purified	n.d.	LifeSpan Biosciences
C8B	732	P07358	Complement C8	10B402	mouse	human	purified by Protein A affinity	100µg	United States Biological
C8B	732	P07358	Complement C8	056B-373	mouse	human	purified IgG - liquid	100µg	MyBiosource
C9	735	P02748	Complement C9	X197	mouse	human	n.d.	100µg	Thermo Scientific Pierce
C9	735	P02748	Complement C9	22	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C9	735	P02748	Complement C9	53	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
C9	735	P02748	Complement C9	monoclonal	mouse	human	Protein A purified	100µg	Novus Biologicals
CETP	1071	P11597	Cholesteryl Ester Transfer Protein	ATM192	mouse	human	Protein A purified	0.1mL	Novus Biologicals
CETP	1071	P11597	Cholesteryl Ester Transfer Protein	5D2	mouse	human	purified	0.1mL	United States Biological
CETP	1071	P11597	Cholesteryl Ester Transfer Protein	aa143-160	mouse	human	ascites	0.1mL	EMD Millipore
CFB	629	P00751	Complement Factor B	40826	mouse	human	purified IgG - liquid	100µg	MyBiosource

Table 4

CFB	629	P00751	Complement Factor B	014III-33.2.4.3	mouse	human	purified IgG - liquid	100µg	MyBiosource
CFB	629	P00751	Complement Factor B	39730	mouse	human	Protein A purified	100µg	Novus Biologicals
CFB	629	P00751	Complement Factor B	10K167	mouse	human	purified.	100µg	United States Biological
CFB	629	P00751	Complement Factor B	KT21	mouse	human	affinity purified	100µg	Novus Biologicals
CFB	629	P00751	Complement Factor B	10K169	mouse	human	ascites	100µg	United States Biological
CFB	629	P00751	Complement Factor B	10K168	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFB	629	P00751	Complement Factor B	6F80	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFB	629	P00751	Complement Factor B	6F79	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFB	629	P00751	Complement Factor B	6F81	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFB	629	P00751	Complement Factor B	D32B-22.1.X	mouse	human	purified IgG - liquid	100µg	MyBiosource
CFH	3075	P08603	Complement Factor H	6F84	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFH	3075	P08603	Complement Factor H	6F85	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFH	3075	P08603	Complement Factor H	6F86	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFI	3426	P05156	Complement Factor I	271203	mouse	human	purified	100µg	R&D Systems
CFI	3426	P05156	Complement Factor I	6F87	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFI	3426	P05156	Complement Factor I	6F88	mouse	human	purified by Protein A affinity	100µg	United States Biological
CFI	3426	P05156	Complement Factor I	1B3	mouse	human	IgG purified	100µg	Novus Biologicals
CFI	3426	P05156	Complement Factor I	OX-21	mouse	human	purified	50µg	MyBiosource
CLEC3B	7123	P05452	Tetranectin	5B7	mouse	human	n.d.	200µg	Thermo Scientific Pierce
CLEC3B	7123	P05452	Tetranectin	10E3	mouse	human	n.d.	200µg	Thermo Scientific Pierce
CLEC3B	7123	P05452	Tetranectin	11F1	mouse	human	lyophilized supernatant	1mL	Accurate Chemical & Scientific
CLEC3B	7123	P05452	Tetranectin	5B7	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
CLEC3B	7123	P05452	Tetranectin	10E3	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
CLEC3B	7123	P05452	Tetranectin	6F9	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
CLEC3B	7123	P05452	Tetranectin	130-11	mouse	human	purified	200µg	Accurate Chemical & Scientific
CLEC3B	7123	P05452	Tetranectin	130-13	mouse	human	purified	200µg	Accurate Chemical & Scientific
CLEC3B	7123	P05452	Tetranectin	130-14	mouse	human	purified	200µg	Accurate Chemical & Scientific
CLEC3B	7123	P05452	Tetranectin	6F193	mouse	human	purified by Protein A affinity	100µg	United States Biological
CLEC3B	7123	P05452	Tetranectin	6F216	mouse	human	purified by Protein A affinity	100µg	United States Biological
CLEC3B	7123	P05452	Tetranectin	6F192	mouse	human	purified by Protein A affinity	100µg	United States Biological
CLU	1191	P10909	Clusterin	1A11	mouse	human	Supplied in PBS (pH 7.4)	0.1mL	MyBiosource
CLU	1191	P10909	Clusterin	3R3/2	mouse	human	n.d.	100µg	Thermo Scientific Pierce
CLU	1191	P10909	Clusterin	350227	mouse	human	purified	100µg	R&D Systems
CLU	1191	P10909	Clusterin	350270	mouse	human	purified	500µg	R&D Systems
CLU	1191	P10909	Clusterin	350207	mouse	human	biotin conjugate	250µg	R&D Systems
CLU	1191	P10909	Clusterin	10B2265	mouse	human	purified by Protein A affinity	100µg	United States Biological
CLU	1191	P10909	Clusterin	1A11	mouse	human	affinity purified, unconjugated	0.1mL	American Research Products
CLU	1191	P10909	Clusterin	5i231	mouse	human	purified by affinity chromatography	100µg	United States Biological
CLU	1191	P10909	Clusterin	7G24	mouse	human	purified by Protein G affinity	0.1mL	United States Biological
CLU	1191	P10909	Clusterin	6D316	mouse	human	purified	100µg	United States Biological
CLU	1191	P10909	Clusterin	7D1	mouse	human	lyophilized supernatant	0.1mL	Accurate Chemical & Scientific
CLU	1191	P10909	Clusterin	2F12	mouse	human	IgG purified	100µg	Novus Biologicals
CLU	1191	P10909	Clusterin	10K81	mouse	human	biotin conjugate	250µg	United States Biological
CLU	1191	P10909	Clusterin	10K73	mouse	human	purified by Protein G affinity	500µg	United States Biological
CLU	1191	P10909	Clusterin	Hs-3	mouse	human	n.d.	100µg	Thermo Scientific Pierce
CLU	1191	P10909	Clusterin	CLI-9	mouse	human	n.d.	50 µg	Thermo Scientific Pierce
CLU	1191	P10909	Clusterin	41D	mouse	human	purified	100µg	EMD Millipore
CLU	1191	P10909	Clusterin	B-5	mouse	human	purified	200µg/ml	Santa Cruz Biotechnology
CLU	1191	P10909	Clusterin	0.T.18	mouse	human	purified by Protein G affinity	100µg	United States Biological
CP	1356	P00450	Ceruloplasmin	3B11	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
CPN2	1370	P22792	Caboxypeptidase N polypeptide 2						
CRP	1401	P02741	C-Reactive protein	5A9	mouse	human	Protein G purified	0.1mL	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	2611018	mouse	human	purified, protein A	n.d.	AALTO BIO REAGENTS LTD.
CRP	1401	P02741	C-Reactive Protein	2611028	mouse	human	purified, protein A	n.d.	AALTO BIO REAGENTS LTD.
CRP	1401	P02741	C-Reactive Protein	CRP103	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	C7	mouse	human	Protein A purified	200µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	C8	mouse	human	Protein A purified	200µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	C1	mouse	human	Protein A purified	200µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	C2	mouse	human	Protein A purified	200µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	C4	mouse	human	Protein A purified	200µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	C5	mouse	human	Protein A purified	200µg	Novus Biologicals

Table 4

CRP	1401	P02741	C-Reactive Protein	C6	mouse	human	Protein A purified	500µg	Novus Biologicals
CRP	1401	P02741	C-Reactive Protein	CRP169	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	CRP30	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	CRP36	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	CRP11	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	CRP135	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	6402	mouse	human	purified	>1mg	Medix Biochemica
CRP	1401	P02741	C-Reactive Protein	6403	mouse	human	purified	>1mg	Medix Biochemica
CRP	1401	P02741	C-Reactive Protein	6404	mouse	human	purified	>1mg	Medix Biochemica
CRP	1401	P02741	C-Reactive Protein	6405	mouse	human	purified	>1mg	Medix Biochemica
CRP	1401	P02741	C-Reactive Protein	6407	mouse	human	purified	>1mg	Medix Biochemica
CRP	1401	P02741	C-Reactive Protein	232007	mouse	human	purified	500µg	R&D Systems
CRP	1401	P02741	C-Reactive Protein	B893M	mouse	human	purified, unlabeled	1mg	MyBiosource
CRP	1401	P02741	C-Reactive Protein	232026	mouse	human, mouse, porcine	purified	500µg	R&D Systems
CRP	1401	P02741	C-Reactive Protein	5A9	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
CRP	1401	P02741	C-Reactive Protein	10K173	mouse	human	purified by Protein A affinity	100µg	United States Biological
CRP	1401	P02741	C-Reactive Protein	10K174	mouse	human	purified by Protein G affinity	100µg	United States Biological
CRP	1401	P02741	C-Reactive Protein	10K175	mouse	human	purified by Protein G affinity	100µg	United States Biological
CRP	1401	P02741	C-Reactive Protein	SB78c	mouse	human	IgG purified	1.0mg	Novus Biologicals
F13B	2165	P05160	Coagulation factor XIII beta subunit						
F13B	2165	P05160	Factor XIIIa	3F177	mouse	human	purified	0.1mL	MyBiosource
F2	2147	P00734	Prothrombin	AHT-5020	mouse	human	n.d.	100µg	Cell Sciences
F2	2147	P00734	Prothrombin	AHP-5013	mouse	human	n.d.	100µg	Cell Sciences
F2	2147	P00734	Prothrombin	5D10	mouse	human	Protein A purified	250µg	Novus Biologicals
F2	2147	P00734	Prothrombin	5G9	mouse	human	Protein A purified	125µg	Novus Biologicals
F2	2147	P00734	Prothrombin	BDI095	mouse	human	purified	500µg	MyBiosource
F8A	8263	P23610	Coagulation factor VIII intron 22 protein						
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	24-2-C7	mouse	human	purified	0.5mL	Accurate Chemical & Scientific
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	SPM180	mouse	human	n.d.	n.d.	LifeSpan Biosciences
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	RFFVIIIC/10	mouse	human	purified IgG - liquid	500µg	MyBiosource
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	RFFVIIIC/5	mouse	human	purified IgG - liquid	500µg	MyBiosource
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	10C185	mouse	human	purified	500µg	United States Biological
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	10C186	mouse	human	purified	500µg	United States Biological
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	10C187	mouse	human	supernatant	5mL	United States Biological
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	AC-1A1	mouse	human	IgG purified	0.5mL	Novus Biologicals
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	RFF-VII/1	mouse	human	affinity purified	500µg	Novus Biologicals
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	RFF-VII/2	mouse	human	affinity purified	500µg	Novus Biologicals
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	AC-1A1	mouse	human	n.d.	500 µl	Thermo Scientific Pierce
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	4G5	mouse	human	IgG purified	0.2mL	Novus Biologicals
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	M1	mouse	human	IgG purified	100µg	Novus Biologicals
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	8k144	mouse	human	purified	0.5mL	MyBiosource
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	3F177	mouse	human	purified	0.1mL	MyBiosource
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	9L234	mouse	human	purified by Protein G affinity	200µg	United States Biological
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	9L236	mouse	human	purified by Protein G affinity	1mg	United States Biological
F8A	8263	P23610	Coagulation factor VIII intron 22 protein	9L237	mouse	human	purified by Protein G affinity	1mg	United States Biological
F8A	8263	P23610	human Factor VIII, A2 Domain	9L238	mouse	human	Gel filtration and anion exchange chrom	500µg	United States Biological
F8A	8263	P23610	human Factor VIII, A2 Domain	8k143	mouse	human	purified	100µg	United States Biological
FCN2	2220	Q15485	Ficolin-2	297018	mouse	human	purified	100µg	R&D Systems
FCN2	2220	Q15485	Ficolin-2	8H139	mouse	human	purified by Protein G affinity	100µg	United States Biological
FCN2	2220	Q15485	Ficolin-2	9B4	mouse	human	purified by Protein A affinity	100µg	United States Biological
FCN3	8547	O75636	Ficolin-3	296134	mouse	human	purified	100µg	R&D Systems
FCN3	8547	O75636	Ficolin-3	FCN	mouse	human	Protein A purified	100µg	Novus Biologicals
FCN3	8547	O75636	Ficolin-3	4B4	mouse	human	IgG purified	0.2mL	Novus Biologicals
FGA	2243	P02671	Fibrinogen alpha chain	1F7	mouse	human	Protein A purified	200µg	Novus Biologicals
FGA	2243	P02671	Fibrinogen alpha chain	26-E7	mouse	human	Protein A purified	200µg	Novus Biologicals
FGA	2243	P02671	Fibrinogen alpha chain	49D2	mouse	human	Protein A purified	200µg	Novus Biologicals
FGB	2244	P02675	Fibrinogen beta chain	3D1	mouse	human	purified by Protein A affinity	100µg	United States Biological
FGG	2266	P02679	Fibrinogen gamma chain	1F2	mouse	human	IgG purified	100µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	15H12	mouse	human	Protein A purified	100µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	1F9	mouse	human	ascites	0.1mL	Novus Biologicals
FGG	2266	P02679	Fibrinogen	1D6	mouse	human	n.d.	200µg	Thermo Scientific Pierce

Table 4

FGG	2266	P02679	Fibrinogen	2F4	mouse	human	n.d.	200µg	Thermo Scientific Pierce
FGG	2266	P02679	Fibrinogen	5C5	mouse	human	n.d.	100µg	Thermo Scientific Pierce
FGG	2266	P02679	Fibrinogen	27C8	mouse	human	Protein A purified	100µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	15E11	mouse	human	Protein A purified	200µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	1F3	mouse	human	Protein A purified	200µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	41D9	mouse	human	Protein A purified	200µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	6G12	mouse	human	Protein A purified	200µg	Novus Biologicals
FGG	2266	P02679	Fibrinogen	1D12	mouse	human	IgG purified	100µg	Novus Biologicals
FN1	2335	P02751	Fibronectin 1	SG7	mouse	human	alkaline phosphatase (AP) conjugate	1mL	Novus Biologicals
FN1	2335	P02751	Fibronectin 1	2F12	mouse	human	biotin conjugate	100µg	Novus Biologicals
GC	2638	P02774	Vitamin D-binding protein	2B12	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
GC	2638	P02774	Vitamin D-binding protein	6F110	mouse	human	purified by Protein A affinity	100µg	United States Biological
GC	2638	P02774	Vitamin D-binding protein	6F109	mouse	human	purified by Protein A affinity	100µg	United States Biological
GC	2638	P02774	Vitamin D-binding protein	6F111	mouse	human	purified by Protein A affinity	100µg	United States Biological
GC	2638	P02774	Vitamin D-binding protein	6F108	mouse	human	purified by Protein A affinity	100µg	United States Biological
GC	2638	P02774	Vitamin D-binding protein	7H135	mouse	human	purified by Protein G affinity	100µg	United States Biological
GC	2638	P02774	Vitamin D-binding protein	249-01	mouse	human	purified	200µg	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	249-01	mouse	human	purified	50 µg.	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	249-02	mouse	human	purified	200µg	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	249-02	mouse	human	purified	50µg	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	249-05	mouse	human	purified	200µg	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	249-10	mouse	human	purified	200µg	Accurate Chemical & Scientific
GC	2638	P02774	Vitamin D-binding protein	2G2	mouse	human	n.d.	200µg	Thermo Scientific Pierce
GC	2638	P02774	Vitamin D-binding protein	4B9	mouse	human	n.d.	200µg	Thermo Scientific Pierce
GSN	2934	P06396	Gelsolin	8L16	mouse	human, bovine, porcine	ascites	0.1mL	MyBiosource
GSN	2934	P06396	Gelsolin	GEL-42	mouse	human, rabbit, bovine	affinity purified	100µg	Insight Genomics
GSN	2934	P06396	Gelsolin	GS-2C4	mouse	human, cow, pig, rabbit	ascites	0.1mL	Novus Biologicals
GSN	2934	P06396	Gelsolin	20	mouse	human	n.d.	0.2mL	Thermo Scientific Pierce
GSN	2934	P06396	Gelsolin	3SB2	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
GSN	2934	P06396	Gelsolin	3G5	mouse	human	IgG purified	100µg	Novus Biologicals
GSN	2934	P06396	Gelsolin	9J134	mouse	human	purified by Protein A affinity	100µg	United States Biological
GSN	2934	P06396	Gelsolin	10g86	mouse	human, mouse, rabbit,	ascites	100µg	United States Biological
GSN	2934	P06396	Gelsolin	20	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
GSN	2934	P06396	Gelsolin	10C345	mouse	human	purified by Protein A affinity	100µg	United States Biological
HP	3240	P00738	Haptoglobin	9G10	mouse	human	n.d.	200µg	Thermo Scientific Pierce
HP	3240	P00738	Haptoglobin	2SE12	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
HP	3240	P00738	Haptoglobin	170-06	mouse	human	purified	200µg	Accurate Chemical & Scientific
HP	3240	P00738	Haptoglobin	HG-36	mouse	human	n.d.	0.5mL	Accurate Chemical & Scientific
HP	3240	P00738	Haptoglobin	1.C.1	mouse	human	ascites	0.1mL	United States Biological
HP	3240	P00738	Haptoglobin	6F123	mouse	human	purified by Protein A affinity	100µg	United States Biological
HP	3240	P00738	Haptoglobin	9G10	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
HP	3240	P00738	Haptoglobin	2F4	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
HP	3240	P00738	Haptoglobin	1B1	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	1F9	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	2B11	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	2B8	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	2B9	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	4C2	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	4H5	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HP	3240	P00738	Haptoglobin	6H2	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
HPX	3263	P02790	Hemopexin	3A9-1A9	mouse	human	IgG purified	100µg	Novus Biologicals
HPX	3263	P02790	Hemopexin	4	mouse	human	protein A/G purified-biotin conjugate	100µg	Bioporto Diagnostics
HPX	3263	P02790	Hemopexin	32	mouse	human	protein A/G purified-biotin conjugate	100µg	Bioporto Diagnostics
HPX	3263	P02790	Hemopexin	6F124	mouse	human	purified by Protein A affinity	100µg	United States Biological
HPX	3263	P02790	Hemopexin	6F125	mouse	human	purified by Protein A affinity	100µg	United States Biological
HRG	3273	P04196	Histidine-rich glycoprotein	monoclonal	mouse	human	unconjugated, purified	200µg	Sino Biological
HRG	3273	P04196	Histidine-rich glycoprotein	monoclonal	mouse	human	unconjugated, purified	500µg	Sino Biological
HRG	3273	P04196	Histidine-rich glycoprotein	monoclonal	mouse	human	unconjugated, purified	200µg	Sino Biological
HRG	3273	P04196	Histidine-rich glycoprotein	monoclonal	mouse	human	unconjugated, purified	500µg	Sino Biological
HRG	3273	P04196	Histidine-rich glycoprotein	227901	mouse	human	purified	500µg	R&D Systems
IGFALS	3483	P35858	Insulin-like growth factor binding protein acid labile subunit						

Table 4

ITIH1	3697	P19827	Inter-alpha-trypsin inhibitor heavy chain H1	poly	mouse	human	Protein A purified	50µg	Novus Biologicals
ITIH2	3698	P19823	inter-alpha-trypsin inhibitor heavy chain H2						
ITIH3	3699	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3						
ITIH4	3700	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	45A12	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
ITIH4	3700	Q14624	inter-alpha Trypsin Inhibitor	40B10	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
ITIH4	3700	Q14624							
KLKB1	3818	P03592	Plasma kallikrein B1	1.B.715	mouse	human	purified by Protein G affinity	200µg	United States Biological
KLKB1	3818	P03592	Plasma kallikrein B1	13G11	mouse	human	purified	200µg	QED Bioscience
KLKB1	3818	P03592	Plasma kallikrein B1	3G2	mouse	human	IgG purified	100µg	Novus Biologicals
KLKB1	3818	P03592	Plasma kallikrein B1	3D1	mouse	human	IgG purified	100µg	Novus Biologicals
KNG1	3827	P01042	Kininogen-1	236012	mouse	human	purified	500µg	R&D Systems
KNG1	3827	P01042	Kininogen-1	207025	mouse	human	purified	500µg	R&D Systems
KNG1	3827	P01042	Kininogen-1	4A1	mouse	human	IgG purified	100µg	Novus Biologicals
KNG1	3827	P01042	Kininogen-1	24F9	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
KNG1	3827	P01042	Kininogen-1	236012	mouse	human	purified	500µg	R&D Systems
KNG1	3827	P01042	Kininogen-1	236006	mouse	human	purified	500µg	R&D Systems
KNG1	3827	P01042	Kininogen-1	2B5	mouse	human	n.d.	50µg	Thermo Scientific Pierce
KNG1	3827	P01042	Kininogen-1	2B5	mouse	human	n.d.	200µg	Thermo Scientific Pierce
KNG1	3827	P01042	Kininogen-1	C11C1	mouse	human	n.d.	200µg	Thermo Scientific Pierce
KNG1	3827	P01042	Kininogen-1	2B5	mouse	human	purified	200µg	QED Bioscience
KNG1	3827	P01042	Kininogen-1	1.B.708	mouse	human	purified by Protein G affinity	200µg	United States Biological
KNG1	3827	P01042	Kininogen-1	1.B.709	mouse	human	purified by Protein G affinity	200µg	United States Biological
LCAT	3931	P04180	Lecithin-cholesterol acyltransferase						
LGALS3BP	3959	Q08380	Lectin galactoside-binding soluble 3 binding protein	6D67	mouse	human	purified	100µg	MyBiosource
LPA	4018	P08519	Apolipoprotein(a)	8F6A9,8H5C5	mouse	human	ascitic fluid containing 0.03% sodium az	0.1mL	ProMab Biotechnologies
LPA	4018	P08519	Apolipoprotein(a)	2Q2209	mouse	human	ascites	0.1mL	United States Biological
LRG1	116844	P02750	Leucine-rich alpha-2-glycoprotein	2F5.A2	mouse	human	affinity purified	100µg	Novus Biologicals
LRG1	116844	P02750	Leucine-rich alpha-2-glycoprotein	2000	mouse	human	IgG purified	100µg	Novus Biologicals
LRG1	116844	P02750	Leucine-rich alpha-2-glycoprotein	1H1	mouse	human	IgG purified	100µg	Novus Biologicals
LUM	4060	P51884	Lumican	358022	mouse	human, mouse	purified	100µg	R&D Systems
LUM	4060	P51884	Lumican	669401	mouse	human, mouse	purified	100µg	R&D Systems
MASP1	5648	P48740	Mannan-binding lectin serine protease 1 precursor						
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	2F9-1F10	mouse	human	IgG purified	100µg	Novus Biologicals
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	386131	mouse	human	purified	100µg	R&D Systems
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	27A1	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	AGP-47	mouse	human	n.d.	0.2mL	Accurate Chemical & Scientific
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	1.B.732	mouse	human	purified	1mg	MyBiosource
ORM1	5004	P02763	Orosomucoid 1 (Alpha-1-acid glycoprotein 1)	9H10	mouse	human	purified	100µg	MyBiosource
ORM2	5005	P19652	Orosomucoid 2 (alpha-1-acid glycoprotein 2)						
PAFAH1B1	5048	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	G-3	mouse	human	purified	200µg/ml	Santa Cruz Biotechnology
PAFAH1B1	5048	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	5A5	mouse	human	IgG purified	100µg	Novus Biologicals
PAFAH1B1	5048	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	2C12	mouse	human	IgG purified	100µg	Novus Biologicals
PAFAH1B1	5048	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	9G377	mouse	human	purified	100µg	MyBiosource
PCOLCE2	26577	Q9UKZ9	Procollagen C-endopeptidase enhancer 2						
PCYOX1	51449	Q9UHG3	Prenylcysteine oxidase 1	5E151	mouse	human	purified	50µg	United States Biological
PCYOX1	51449	Q9UHG3	Prenylcysteine oxidase 1	aa1-506	mouse	human	IgG purified	50 µg	LifeSpan Biosciences
PCYOX1	51449	Q9UHG3	Prenylcysteine oxidase 1	3D7-D8	mouse	human	IgG purified	100µg	Novus Biologicals
PGLYRP2	114770	Q96PD5	Peptidoglycan recognition protein 2	45G1	mouse	human	affinity purified	100µg	Novus Biologicals
PGLYRP2	114770	Q96PD5	Peptidoglycan recognition protein 2	10K303	mouse	human	ascites	100µg	United States Biological
PLA2G7	7941	Q13093	Platelet-activating factor acetylhydrolase (PAFA)	5B9	mouse	human	IgG purified	100µg	Novus Biologicals
PLA2G7	7941	Q13093	Platelet-activating factor acetylhydrolase (PAFA)	5D1	mouse	human	IgG purified	100µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	4D2	mouse	human	IgG purified	200µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	5H3	mouse	human	IgG purified	200µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	8F11	mouse	human	IgG purified	200µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	9D8	mouse	human	IgG purified	200µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	3C2	mouse	human	IgG purified	500µg	Novus Biologicals
PLG	5340	P08519	Plasminogen	SBF1	mouse	human	azide-free	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	2F6-C6	mouse	human	Protein A column purified	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	11B5-B2	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	13C3-B10	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	1C10-F2	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences

Table 4

PLG	5340	P08519	Plasminogen	4G6-B11	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	9F9-C4	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
PLG	5340	P08519	Plasminogen	270409	mouse	human	purified	100µg	R&D Systems
PLG	5340	P08519	Plasminogen	270412	mouse	human	purified	100µg	R&D Systems
PLG	5340	P08519	Plasminogen	SBF1 C1.21	mouse	human	IgG purified	100µg	Novus Biologicals
PLTP	5360	P55058	Phospolipid transfer protein	2F3-G4	mouse	human	IgG purified	100µg	Novus Biologicals
PLTP	5360	P55058	Phospolipid transfer protein	11C579	mouse	human	IgG purified	100µg	United States Biological
PON1	5444	P27169	Serum paraoxonase/arylesterase 1	2H7	mouse	human	IgG purified	100µg	Novus Biologicals
PON1	5444	P27169	Serum paraoxonase/arylesterase 1	17A12	mouse	human, mouse, rat	n.d.	100µg	Thermo Scientific Pierce
PON3	5446	Q15166	Serum paraoxonase/lactonase 3						
PPBP	5473	P02775	Platelet basic protein	3B9	mouse	human	IgG purified	100µg	Novus Biologicals
PPBP	5473	P02775	Platelet basic protein	59418	mouse	human	purified	500µg	R&D Systems
PPBP	5473	P02775	Platelet basic protein	5C7	mouse	human	purified	200µg	MyBiosource
PPBP	5473	P02775	Platelet basic protein	9L517	mouse	human	purified by Protein A affinity	200µg	United States Biological
PROS1	5627	P07225	Vitamin-K-dependent protein S	391609	mouse	human	purified	100µg	R&D Systems
PROS1	5627	P07225	Vitamin-K-dependent protein S	8D5	mouse	human	n.d.	200µg	Thermo Scientific Pierce
PROS1	5627	P07225	Vitamin-K-dependent protein S	3D7	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	1A2	mouse	human	Protein G purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	5H9	mouse	human	Protein G purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	1E3	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	3D12	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	1A8	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	1E9	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	3B1	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	4D9	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	4E7	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	4H7	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	4B10	mouse	human	IgG purified	100µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB42	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB45	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB48	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB49	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB51	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	RB55	mouse	human	Protein A purified	200µg	Novus Biologicals
RBP4	5950	P02753	Retinol-binding protein RBP4	AT2B4	mouse	human	IgG purified	0.1mL	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	10B160	mouse	human	purified by Protein A affinity	200µg	United States Biological
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	3C11-2C1	mouse	human	IgG purified	100µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA1	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA11	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA12	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA14	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA15	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA6	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	SAA7	mouse	human	Protein A purified	200µg	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	mc1	mouse	human	IgG purified	1.0ml	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	Reu86.5	mouse	human	Protein G purified	0.5ml	Novus Biologicals
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	mc1	mouse	human	n.d.	1mL	Thermo Scientific Pierce
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	mc1	mouse	human	n.d.	1mL	MyBiosource
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	9L409	mouse	human	purified by Protein A affinity	1mg	United States Biological
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	9L410	mouse	human	purified by Protein A affinity	1mg	United States Biological
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	B332A	mouse	human	purified, unlabeled	1mg	MyBiosource
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	B333A	mouse	human	purified, unlabeled	1mg	MyBiosource
SAA1	6288	P02735	Serum amyloid A protein (SAA1 and SAA2)	B336A	mouse	human	purified, unlabeled	1mg	MyBiosource
SAA4	6291	P35542	Serum amyloid A-4 protein	3C11	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	6F4	mouse	human	purified by Protein A affinity	100µg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1.B.737	mouse	human	purified by Protein G affinity	200µg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	8.F.14	mouse	human	purified	500µg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1.B.15	mouse	human	purified by Protein A affinity	1mg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1.B.14	mouse	human	purified by Protein G affinity	500µg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1.B.739	mouse	human	purified by Protein G affinity	1mg	United States Biological
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1C2	mouse	human	affinity purified	0.1mL	Novus Biologicals

Table 4

SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	3C5	mouse	human	affinity purified	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	11G2	mouse	human, canine	affinity purified	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	15H10	mouse	human, primate, canine	affinity purified	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	5B12	mouse	human, primate, canine	affinity purified	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	9A1	mouse	human, primate, canine	affinity purified	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	2B12	mouse	human, mouse	ascites	0.1mL	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	3B7	mouse	human	Protein A purified	125µg	Novus Biologicals
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	6F5	mouse	human	purified	100µg	MyBiosource
SERPINA1	5265	P01009	Serpin Peptidase Inhibitor Clade A Member 1 (alpha-1-antitrypsin)	1AT	mouse	human	purified IgG - liquid	1mg	MyBiosource
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	ACT 14C7	mouse	human	affinity purified	n.d.	LifeSpan Biosciences
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	213907	mouse	human	purified	500µg	R&D Systems
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	3F5	mouse	human	Protein A purified	100µg	Novus Biologicals
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	1E6	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	1C10	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	8J277	mouse	human	purified by Protein G affinity	200µg	United States Biological
SERPINA3	12	P01011	Serpin Peptidase Inhibitor Clade A Member 3 (alpha-1-antichymotrypsin)	10B144	mouse	human	purified	1mg	MyBiosource
SERPINA4	5267	P29622	Serpin Peptidase Inhibitor Clade A Member 4 (kallistatin)	8H119	mouse	human	purified by Protein G affinity	500µg	United States Biological
SERPINA4	5267	P29622	Serpin Peptidase Inhibitor Clade A Member 4 (kallistatin)	209919	mouse	human	purified	500µg	R&D Systems
SERPINA4	5267	P29622	Serpin Peptidase Inhibitor Clade A Member 4 (kallistatin)	209930	mouse	human	purified	500µg	R&D Systems
SERPINA6	866	P08185	Serpin Peptidase Inhibitor Clade A Member 6 (corticosteroid binding globu	1D9	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA6	866	P08185	Serpin Peptidase Inhibitor Clade A Member 6 (corticosteroid binding globu	1F11	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA6	866	P08185	Serpin Peptidase Inhibitor Clade A Member 6 (corticosteroid binding globu	3B12	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINA6	866	P08185	Serpin Peptidase Inhibitor Clade A Member 6 (corticosteroid binding globu	3C12	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	8D5	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	4B3	mouse	human	n.d.	200µg	Thermo Scientific Pierce
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	7E11	mouse	human	n.d.	200µg	Thermo Scientific Pierce
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	8C12	mouse	human	n.d.	100µg	Thermo Scientific Pierce
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	2B12	mouse	human	IgG purified	0.2mL	Novus Biologicals
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	2B12	mouse	human	IgG purified	100µg	Novus Biologicals
SERPINC1	462	P01008	Serpin Peptidase Inhibitor Clade C Member 1 (antithrombin III)	8D5	mouse	human	affinity purified	0.1mL	Novus Biologicals
SERPIND1	3053	P05546	Serpin Peptidase Inhibitor Clade D Member 1 (heparin cofactor II)	373008	mouse	human	purified	100µg	R&D Systems
SERPIND1	3053	P05546	Serpin Peptidase Inhibitor Clade D Member 1 (heparin cofactor II)	74E5	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
SERPINF1	5176	P36955	Serpin peptidase inhibitor Clade F Member 1 (pigment epithelium-derived	1C4	mouse	human	Protein G purified	0.1mL	Novus Biologicals
SERPINF1	5176	P36955	Serpin peptidase inhibitor Clade F Member 1 (pigment epithelium-derived	1C4	mouse	human	Protein G column purified	n.d.	LifeSpan Biosciences
SERPINF1	5176	P36955	Serpin peptidase inhibitor Clade F Member 1 (pigment epithelium-derived	187003	mouse	human	purified	500µg	R&D Systems
SERPINF1	5176	P36955	Serpin peptidase inhibitor Clade F Member 1 (pigment epithelium-derived	1C4	mouse	human	purified	0.1mL	MyBiosource
SERPINF1	5176	P36955	Serpin peptidase inhibitor Clade F Member 1 (pigment epithelium-derived	1C4	mouse	human	purified. Supplied in PBS (pH 7.4)	0.1mL	ATGen Ltd.
SERPINF2	5345	P08697	Serpin peptidase inhibitor Clade F Member 2 (alpha-2-antiplasmin)	236122	mouse	human	purified	500µg	R&D Systems
SERPINF2	5345	P08697	Serpin peptidase inhibitor Clade F Member 2 (alpha-2-antiplasmin)	9L37	mouse	human	purified by Protein A affinity	200µg	United States Biological
SERPING1	710	P05155	Serpin peptidase inhibitor Clade G Member 1 (plasma protease C1 inhibitc	7H118	mouse	human	purified	100µg	MyBiosource
SERPING1	710	P05155	Serpin peptidase inhibitor Clade G Member 1 (plasma protease C1 inhibitc	3F4-1D9	mouse	human	purified.	100µg	United States Biological
SERPING1	710	P05155	Serpin peptidase inhibitor Clade G Member 1 (plasma protease C1 inhibitc	10K343	mouse	human	purified by Protein A affinity	100µg	United States Biological
SERPING1	710	P05155	Serpin peptidase inhibitor Clade G Member 1 (plasma protease C1 inhibitc	10K344	mouse	human	purified	100µg	United States Biological
SERPING1	710	P05155	Serpin peptidase inhibitor Clade G Member 1 (plasma protease C1 inhibitc	7H118	mouse	human	purified by Protein G affinity	100µg	United States Biological
SEPP1	6414	P49908	Selenoprotein P	37A1	mouse	human	n.d.	100µg	Thermo Scientific Pierce
TF	7018	P02787	Serotransferin	5G2	mouse	human	purified by affinity chromatography	0.1mL	Origene Technologies
TF	7018	P02787	Serotransferin	29806	mouse	human	purified	500µg	R&D Systems
TF	7018	P02787	Serotransferin	HTF-14	mouse	human, rabbit	ion exchange chromatography purified	n.d.	LifeSpan Biosciences
TFPI	7035	P10646	Tissue Factor Pathway Inhibitor	374718	mouse	human	purified	500µg	R&D Systems
TFPI	7035	P10646	Tissue Factor Pathway Inhibitor	374720	mouse	human	purified	100µg	R&D Systems
TFPI	7035	P10646	Tissue Factor Pathway Inhibitor	3E8	mouse	human	IgG purified	100µg	Novus Biologicals
TTR	7276	P02766	Transthyretin	9G6	mouse	human	affinity purified	0.05mL	Novus Biologicals
TTR	7276	P02766	Transthyretin	7G8-E11-E7	mouse	human	purified	100µg	EMD Millipore
TTR	7276	P02766	Transthyretin	4D8	mouse	human	IgG purified	100µg	Novus Biologicals
TTR	7276	P02766	Transthyretin	AT7B2	mouse	human	liquid. In PBS (pH 7.4) with 0.1% sodium	0.1mL	MyBiosource
VTN	7448	P04004	Vitronectin	BV2	mouse	human	purified IgG - liquid	100µg	MyBiosource
VTN	7448	P04004	Vitronectin	1E934	mouse	human	purified, unlabeled	1mg	MyBiosource
VTN	7448	P04004	Vitronectin	8E6 (1.110)	mouse	human	IgG purified	0.025mL	Novus Biologicals
VTN	7448	P04004	Vitronectin	BV1	mouse	human	IgG purified	0.2mL	Novus Biologicals
VTN	7448	P04004	Vitronectin	BE10	mouse	human	IgG purified	50µg	Novus Biologicals
VTN	7448	P04004	Vitronectin	NKI-M9	mouse	human	affinity purified	100 tests	Novus Biologicals

Table 4

VTN	7448	P04004	Vitronectin	VN58-1	mouse	human	n.d.	100µg	Thermo Scientific Pierce
VTN	7448	P04004	Vitronectin	ViT-2	mouse	human	n.d.	50 µl	Thermo Scientific Pierce
VTN	7448	P04004	Vitronectin	HV2	mouse	human	n.d.	200µg	Thermo Scientific Pierce
VTN	7448	P04004	Vitronectin	HV23	mouse	human	n.d.	200µg	Thermo Scientific Pierce
VTN	7448	P04004	Vitronectin	10E12	mouse	human	n.d.	0.1mL	Thermo Scientific Pierce
VTN	7448	P04004	Vitronectin	CSI003-02	mouse	human	purified	200µg	Accurate Chemical & Scientific
VTN	7448	P04004	Vitronectin	CSI003-08	mouse	human	purified	200µg	Accurate Chemical & Scientific
VTN	7448	P04004	Vitronectin	CSI003-21	mouse	human	purified	200µg	Accurate Chemical & Scientific
VTN	7448	P04004	Vitronectin	CSI003-23	mouse	human	purified	200µg	Accurate Chemical & Scientific
VTN	7448	P04004	Vitronectin	8E6(LJ8)	mouse	human	purified	100µg	EMD Millipore
VTN	7448	P04004	Vitronectin	BD33M	mouse	human	purified, unlabeled	1mg	MyBiosource
VTN	7448	P04004	Vitronectin	342603	mouse	human	purified	100µg	R&D Systems
VTN	7448	P04004	Vitronectin	2Q557	mouse	human	purified immunoglobulin	100µg	United States Biological
VTN	7448	P04004	Vitronectin	7H101	mouse	human	purified by Protein G affinity	100µg	United States Biological
VTN	7448	P04004	Vitronectin	6F210	mouse	human	purified by Protein A affinity	100µg	United States Biological
VTN	7448	P04004	Vitronectin	9L794	mouse	human	purified by Protein A affinity	1mg	United States Biological
VTN	7448	P04004	Vitronectin	5G26	mouse	human	purified by Protein G affinity	100µg	United States Biological
VTN	7448	P04004	Vitronectin	2Q558	mouse	human	purified by Protein G affinity	100µg	United States Biological
VTN	7448	P04004	Vitronectin	9L765	mouse	human	purified	1mg	United States Biological
VTN	7448	P04004	Vitronectin	9L764	mouse	human	purified by Protein A affinity	200µg	United States Biological
VTN	7448	P04004	Vitronectin	9L795	mouse	human	purified by Protein A affinity	1mg	United States Biological
VTN	7448	P04004	Vitronectin	9L796	mouse	human	purified by Protein A affinity	1mg	United States Biological
VTN	7448	P04004	Vitronectin	1.B.725	mouse	human	purified by Protein G affinity	500µg	United States Biological
VTN	7448	P04004	Vitronectin	2VN	mouse	human	purified	n.d.	Technoclone GmbH
VTN	7448	P04004	Vitronectin	HV2	mouse	human	protein A/G purified, BSA-free	1mg	Bioporto Diagnostics
VTN	7448	P04004	Vitronectin	9F516	mouse	human	ascites	50ul	United States Biological
VTN	7448	P04004	Vitronectin	6A672	mouse	human	purified by Protein G affinity	100µg	United States Biological
VTN	7448	P04004	Vitronectin	5F87	mouse	human	purified IgG.	500µg	United States Biological

What is claimed is:

1. A method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample, wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample.

2. The method of claim 1, wherein the HDL subpopulation being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

3. The method of claim 1, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

4. The method of claim 1, wherein the plurality of defined protein epitopes are present on the same protein.

5. The method of claim 4, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

6. The method of claim 1, wherein the plurality of defined protein epitopes are present on two or more proteins.

7. The method of claim 6, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

8. The method of claim 1, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

9. The method of claim 8, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

10. A method for measuring the amount of each of a plurality of high density lipoprotein (HDL) subpopulations present in an HDL-containing sample, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of each of the HDL subpopulations present in the sample.

11. The method of claim 10, wherein the number of HDL subpopulations measured is at least 16.

12. The method of claim 11, wherein the number of HDL subpopulations measured is at least 96.

13. The method of claim 10, wherein the method comprises concurrently measuring the amount of each of the plurality of HDL subpopulations present in the HDL-containing sample.

14. The method of claim 10, wherein at least one of the HDL subpopulations being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

15. The method of claim 10, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

16. The method of claim 10, wherein for at least one of the HDL subpopulations being measured, the plurality of defined protein epitopes are present on the same protein.

17. The method of claim 16, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

18. The method of claim 10, wherein for at least one of the HDL subpopulations being measured, the plurality of defined protein epitopes are present on two or more proteins.

19. The method of claim 18, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

20. The method of claim 10, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

21. The method of claim 20, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

22. A method for determining whether a subject is afflicted with a disorder characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising (a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the subject's sample; and (b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with the presence and/or absence of the disorder, thereby determining whether the subject is afflicted with the disorder.

23. The method of claim 22, wherein the amount of the defined HDL subpopulation in an afflicted subject is higher than the amount of the defined HDL subpopulation in a healthy subject.

24. The method of claim 23, wherein the disorder is selected from the group consisting of dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD) and coronary heart disease (CHD).

25. The method of claim 22, wherein the amount of the defined HDL subpopulation in an afflicted subject is lower than the amount of the defined HDL subpopulation in a healthy subject.

26. The method of claim 25, wherein the disorder is selected from the group consisting of dyslipidemia, atherosclerosis, diabetes mellitus, obesity-induced dyslipidemia, coronary artery disease (CAD), coronary heart disease (CHD) and chronic kidney disease (CKD).

27. The method of claim 22, wherein the defined HDL subpopulation being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

28. The method of claim 22, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

29. The method of claim 22, wherein the plurality of defined protein epitopes are present on the same protein.

30. The method of claim 29, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

31. The method of claim 22, wherein the plurality of defined protein epitopes are present on two or more proteins.

32. The method of claim 31, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

33. The method of claim 22, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

34. The method of claim 33, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

35. A method for determining the likelihood of a subject's becoming afflicted with a disorder, wherein the disorder's likelihood of onset is characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, and wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby

measuring the amount of the HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a standard correlative with a known likelihood of the disorder's onset,

thereby determining the likelihood of the subject's becoming afflicted with the disorder.

36. The method of claim 35, wherein the amount of the defined HDL subpopulation in a subject likely to become afflicted is higher than the amount of the defined HDL subpopulation in a subject less likely to become afflicted.

37. The method of claim 36, wherein the disorder is selected from the group consisting of dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD) and coronary heart disease (CHD).

38. The method of claim 35, wherein the amount of the defined HDL subpopulation in a subject likely to become afflicted is lower than the amount of the defined HDL subpopulation in a subject less likely to become afflicted.

39. The method of claim 38, wherein the disorder is selected from the group consisting of dyslipidemia, atherosclerosis, diabetes mellitus, obesity-induced dyslipidemia, coronary artery disease (CAD), coronary heart disease (CHD) and chronic kidney disease (CKD).

40. The method of claim 35, wherein the defined HDL subpopulation being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

41. The method of claim 35, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

42. The method of claim 35, wherein the plurality of defined protein epitopes are present on the same protein.

43. The method of claim 42, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

44. The method of claim 35, wherein the plurality of defined protein epitopes are present on two or more proteins.

45. The method of claim 44, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

46. The method of claim 35, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

47. The method of claim 46, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

48. A method for measuring the success of a high density lipoprotein (HDL)-modifying treatment on a subject, wherein the treatment's success is characterized by a change in the amount of a defined HDL subpopulation, and wherein each particle of the HDL subpopulation is

characterized by the presence of a plurality of defined protein epitopes, the method comprising

(a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject during or after treatment, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of HDL subpopulation in the sample; and

(b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with a successful treatment outcome,

thereby measuring the treatment's success.

49. The method of claim 48, wherein the HDL-modifying treatment is the administration of a statin.

50. The method of claim 49, wherein the statin is selected from the group consisting of atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and a combination of ezetimibe and simvastatin.

51. The method of claim 48, wherein the defined HDL subpopulation being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

52. The method of claim 48, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

53. The method of claim 48, wherein the plurality of defined protein epitopes are present on the same protein.

54. The method of claim 53, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

55. The method of claim 48, wherein the plurality of defined protein epitopes are present on two or more proteins.

56. The method of claim 55, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

57. The method of claim 48, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

58. The method of claim 57, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

59. A method for characterizing a high density lipoprotein (HDL) particle with respect to the presence of one or more sets of defined protein epitopes, the method comprising performing an antibody-based assay on a population of the HDL particles to determine the presence and/or amount of each set of the defined protein epitopes, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different

protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby characterizing the HDL particle.

60. The method of claim 59, wherein the antibody-based assay is performed on a population of the HDL particles selected from the group consisting of HDL2a, HDL2b, HDL3a, HDL3b and HDL3c.

61. The method of claim 59, wherein the HDL particle is obtained from blood, plasma, serum or urine.

62. The method of claim 59, wherein at least one set of defined protein epitopes is present on the same protein.

63. The method of claim 59, wherein at least one set of defined protein epitopes is present on two or more proteins.

64. The method of claim 59, wherein the antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

65. The method of claim 64, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

66. A method for identifying a subpopulation of high density lipoprotein (HDL) whose abnormal concentration in a subject correlates with a particular disorder, comprising

(a) measuring the amounts of one or more HDL subpopulations present in an HDL-containing sample from a subject afflicted with the disorder, wherein each particle of each of the HDL subpopulations being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein, for each HDL subpopulation being measured, (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amounts of the HDL subpopulations present in the subject's sample,

(b) comparing the measured amounts of HDL subpopulations in the subject's sample with a known standard correlative with the amounts of the respective HDL subpopulations present in a healthy subject, and

(c) for each of the measured HDL subpopulations, determining whether the amount of the HDL subpopulation differs from that in the known standard,

whereby any such difference indicates that an abnormal concentration of the HDL subpopulation correlates with the disorder.

67. The method of claim 66, wherein the disorder is selected from the group consisting of dyslipidemia, obesity-induced dyslipidemia, hypertension, diabetes mellitus, coronary artery disease (CAD), coronary heart disease (CHD), vascular inflammation, atherosclerosis and chronic kidney disease (CKD).

68. The method of claim 66, wherein at least one of the HDL subpopulations being measured is characterized by the presence of ApoA1 protein, ApoA2 protein and/or ApoE protein.

69. The method of claim 66, wherein the sample is selected from the group consisting of blood, plasma, serum and urine.

70. The method of claim 66, wherein for each HDL subpopulation being measured, the plurality of defined protein epitopes are present on the same protein.

71. The method of claim 70, wherein the plurality of defined protein epitopes are present on a protein selected from the group consisting of ApoA1 protein, ApoA2 protein and ApoE protein.

72. The method of claim 66, wherein for each HDL subpopulation being measured, the plurality of defined protein epitopes are present on two or more proteins.

73. The method of claim 72, wherein the plurality of defined protein epitopes are present on two or more proteins set forth in Table 1.

74. The method of claim 66, wherein the quantitative antibody-based assay is selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

75. The method of claim 74, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

76. A kit for performing the method of any of claims 1, 10, 22, 35, 48, 59 and 66, comprising (i) a solid substrate suitable for use in performing an antibody-based assay; (ii) a capture antibody operably affixed to the substrate; and (iii) in a separate compartment, a detection antibody, wherein the capture and detection antibodies are directed to different protein epitopes present on each particle of a predetermined HDL subpopulation.

77. The kit of claim 76, wherein the kit is suitable for performing an immunoassay selected from the group consisting of a radioimmunoassay (RIA) and an enzyme immunoassay (EIA).

78. The kit of claim 77, wherein the EIA is selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), a homogeneous time resolved fluorescence assay (HTRF) and an electrochemiluminescence assay (ECL).

79. The kit of claim 76, wherein the capture antibody is directed to an epitope present on a protein set forth in Table 1, and the detection antibody is directed to an epitope present on one of ApoA1 protein, ApoA2 protein and ApoE protein, wherein the capture and detection antibodies are directed to different epitopes.

80. The kit of claim 76, wherein the capture antibody is directed to an epitope present on one of ApoA1 protein, ApoA2 protein and ApoE protein, and the detection antibody is directed to an epitope present on a protein set forth in Table 1, wherein the capture and detection antibodies are directed to different epitopes.

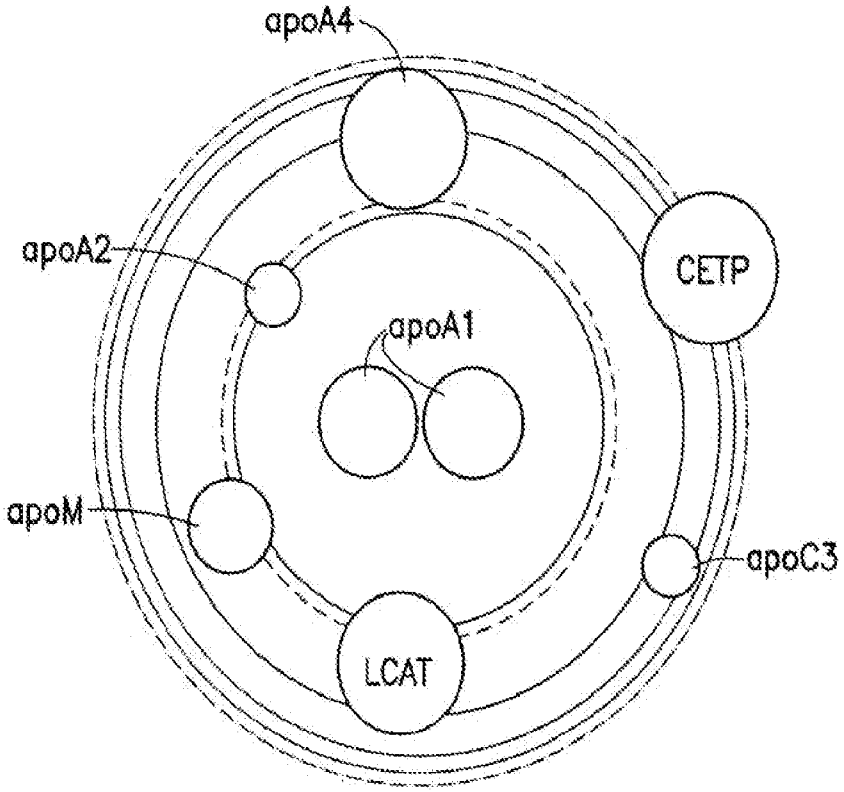


FIG. 1

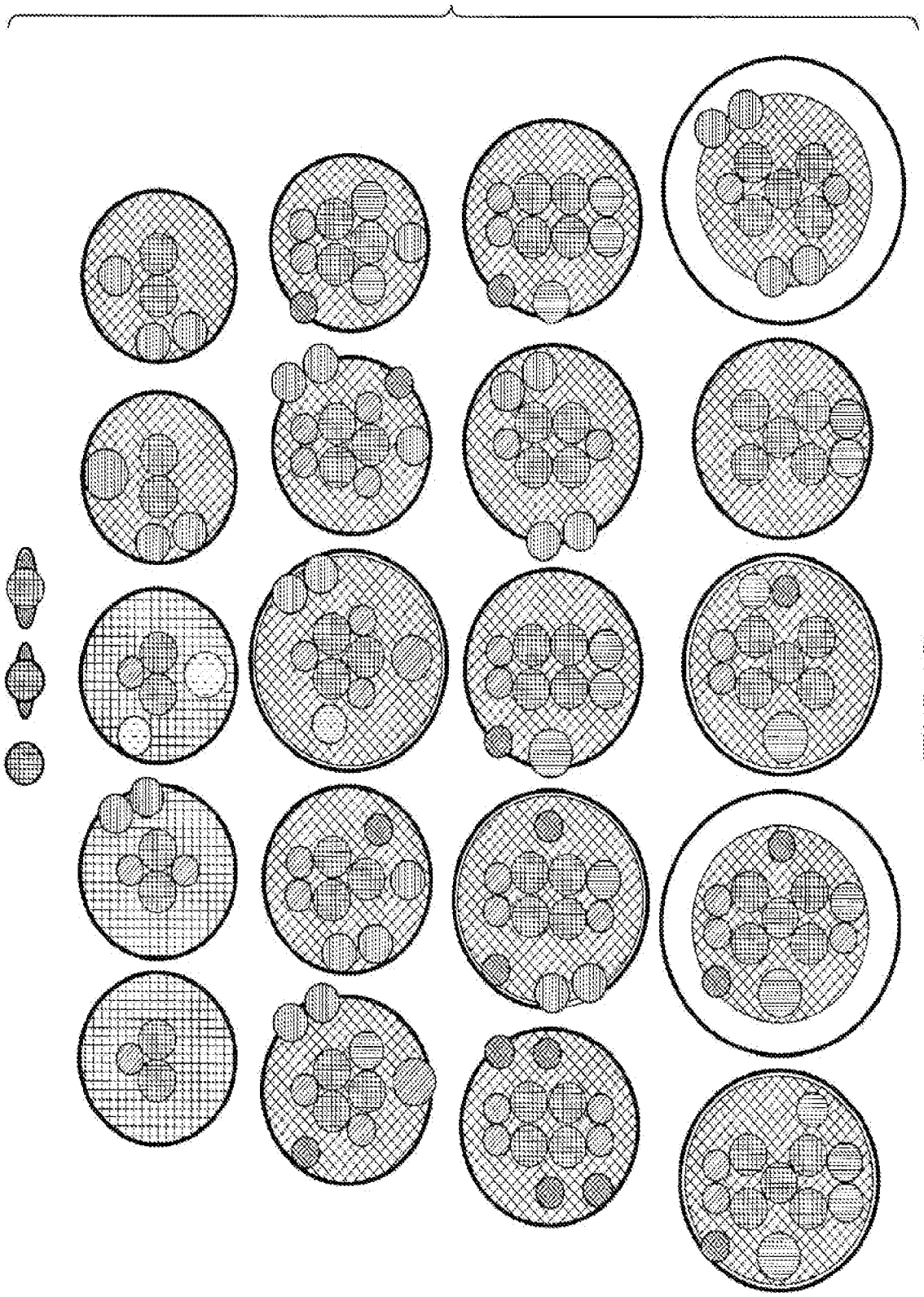
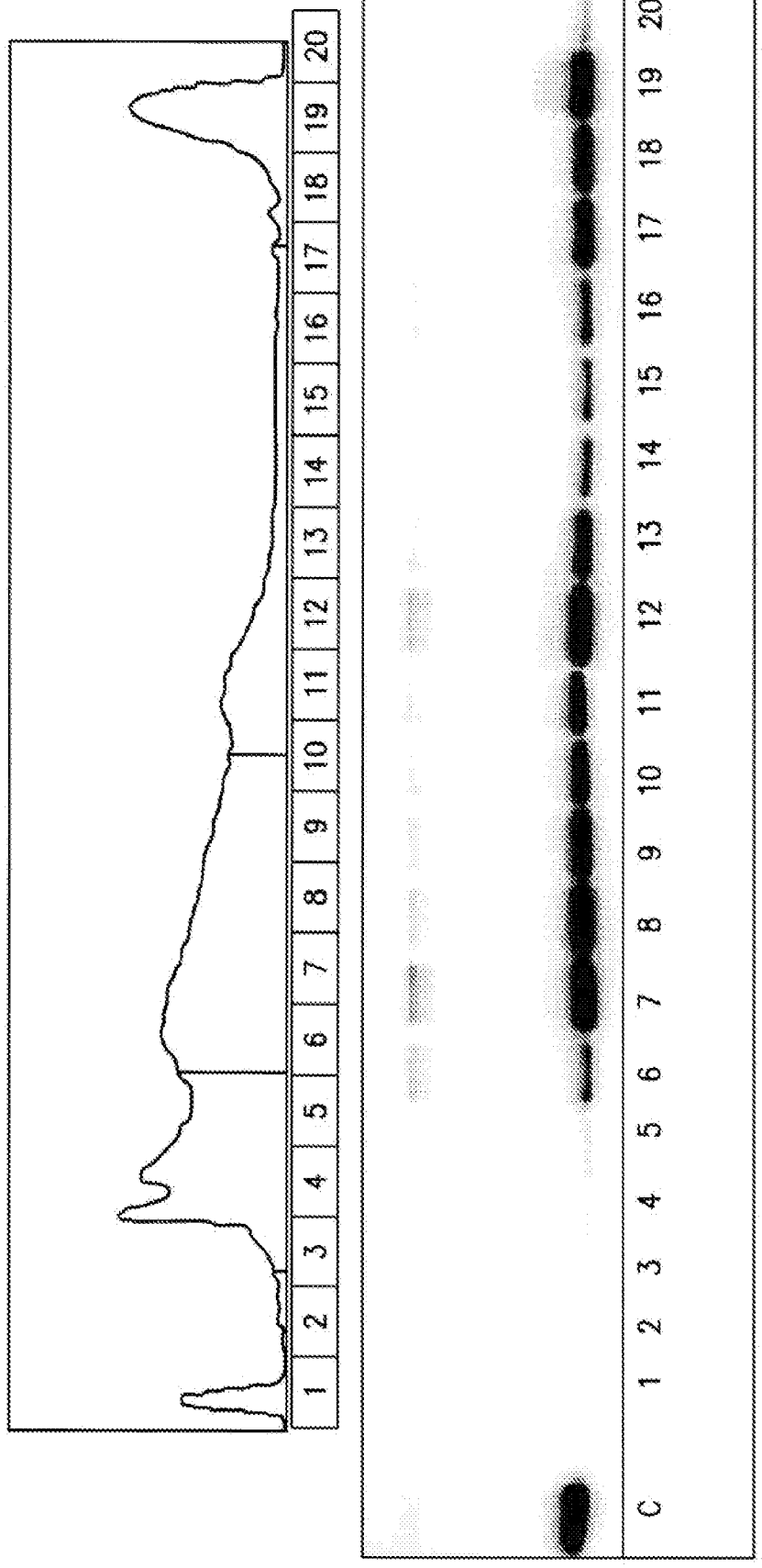


FIG.2



SEGMENT	PROTEIN FRACTION
1	LDL/VLDL
3–5	HDL LARGE
6–10	HDL INTERMEDIATE
11–17	HDL SMALL
18–20	ALBUMIN/UNBOUND

FIG.3

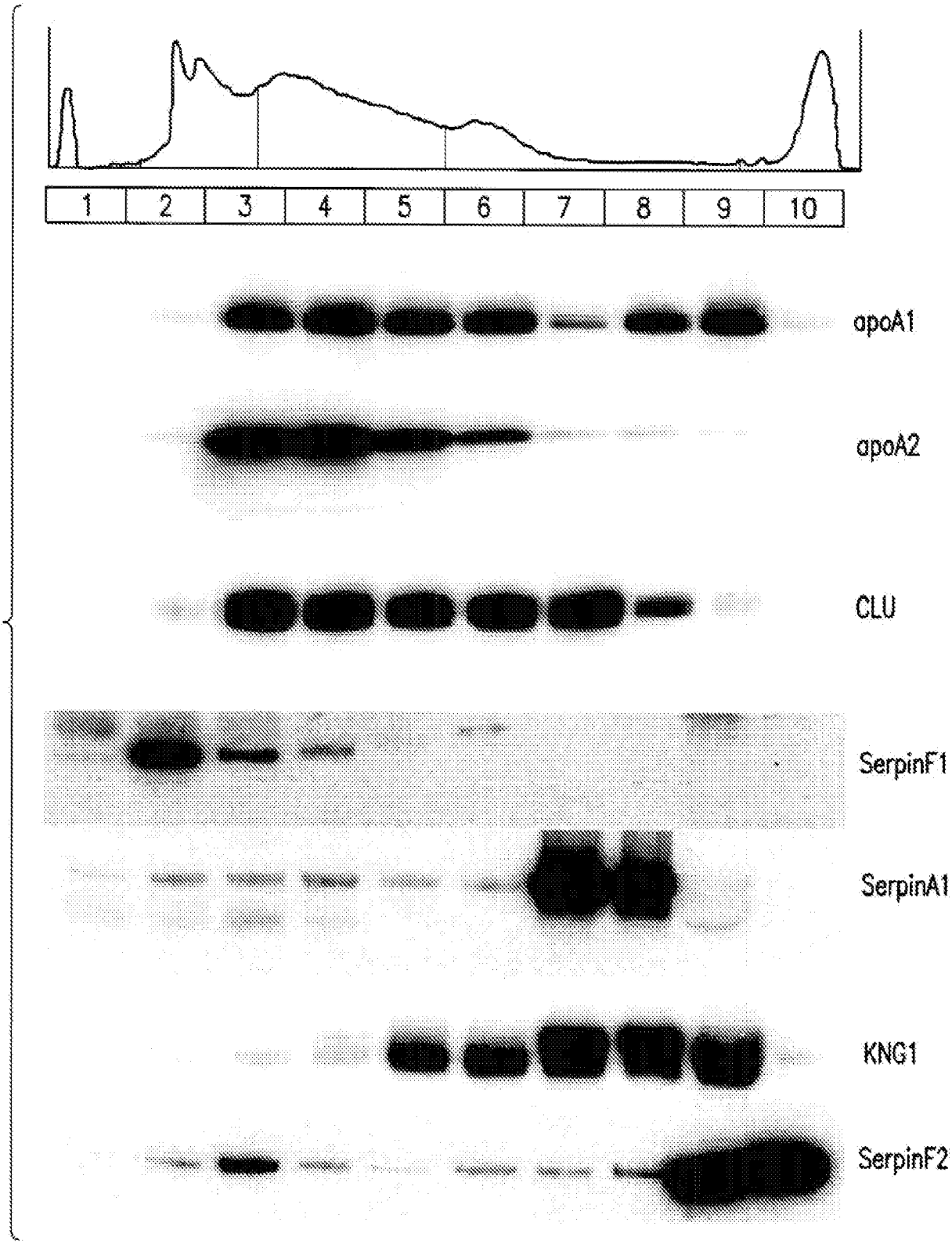

1
2
3
4
5
6
7
8
9
10
apoA1
apoA2
CLU
SerpinF1
SerpinA1
KNG1
SerpinF2

FIG.4

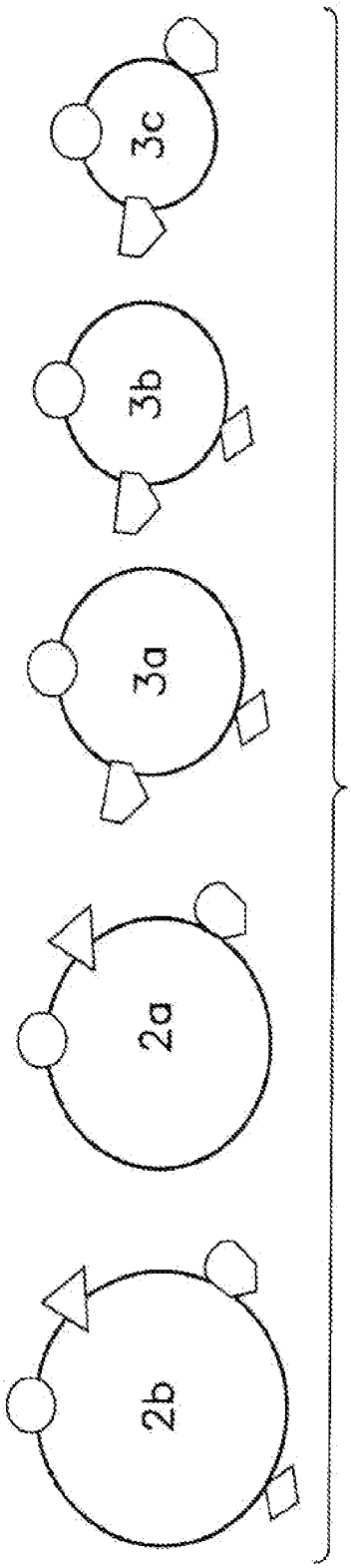


FIG.5

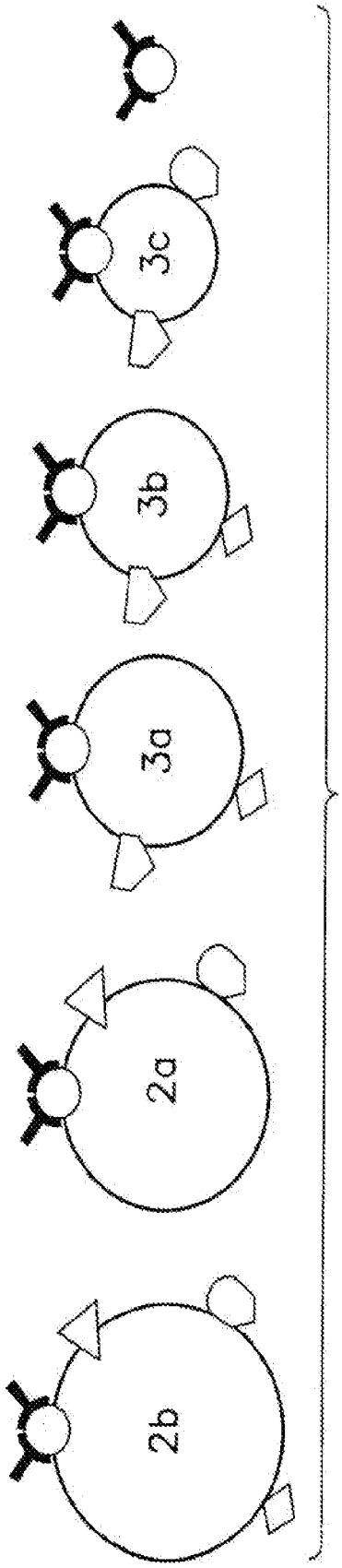


FIG.6

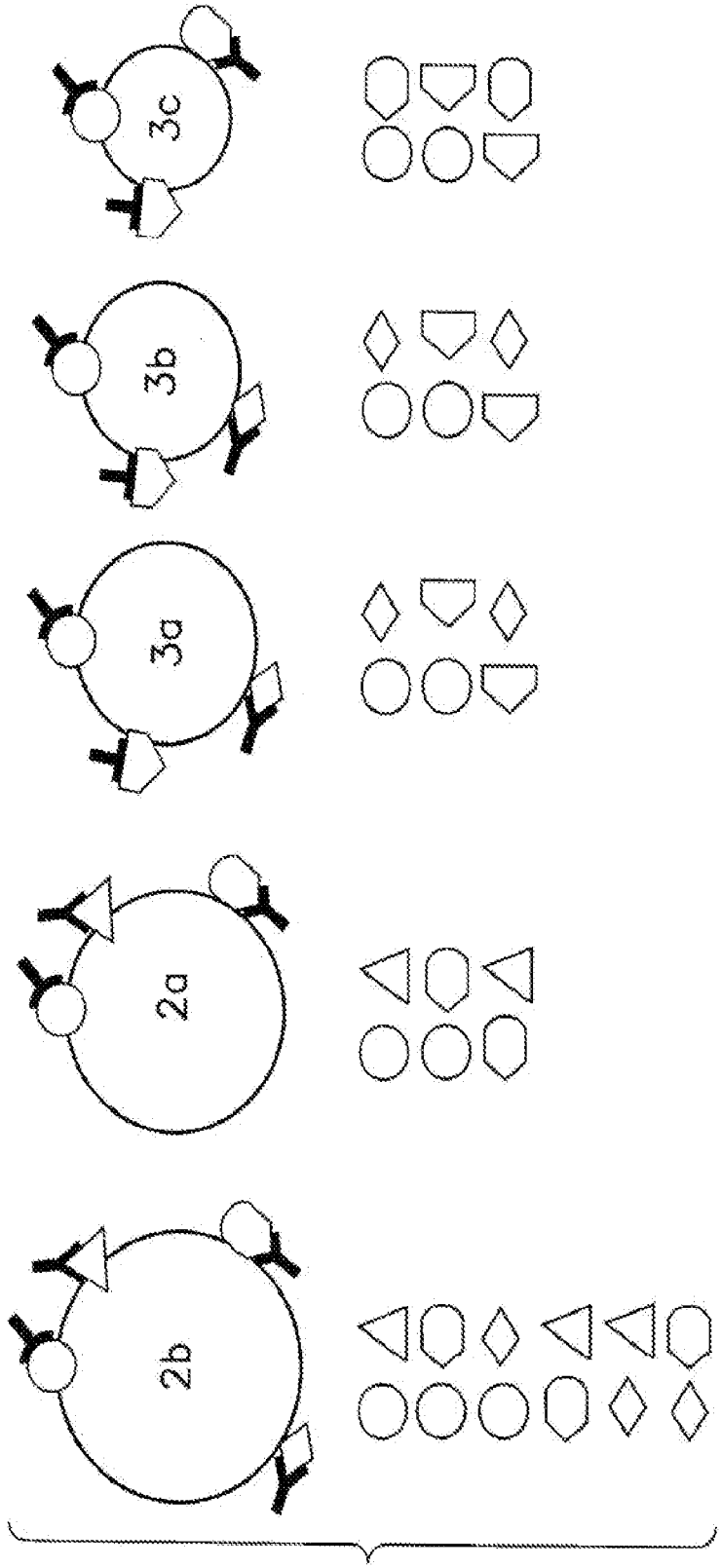


FIG.7

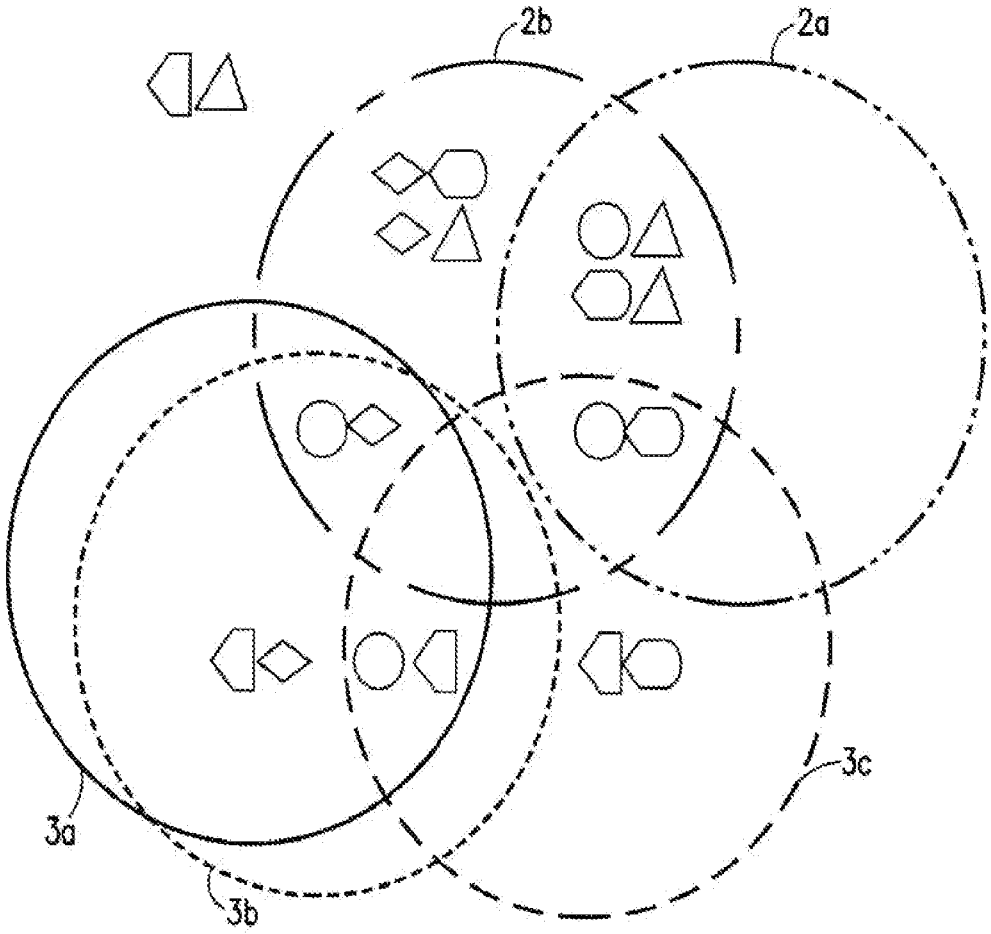


FIG.8

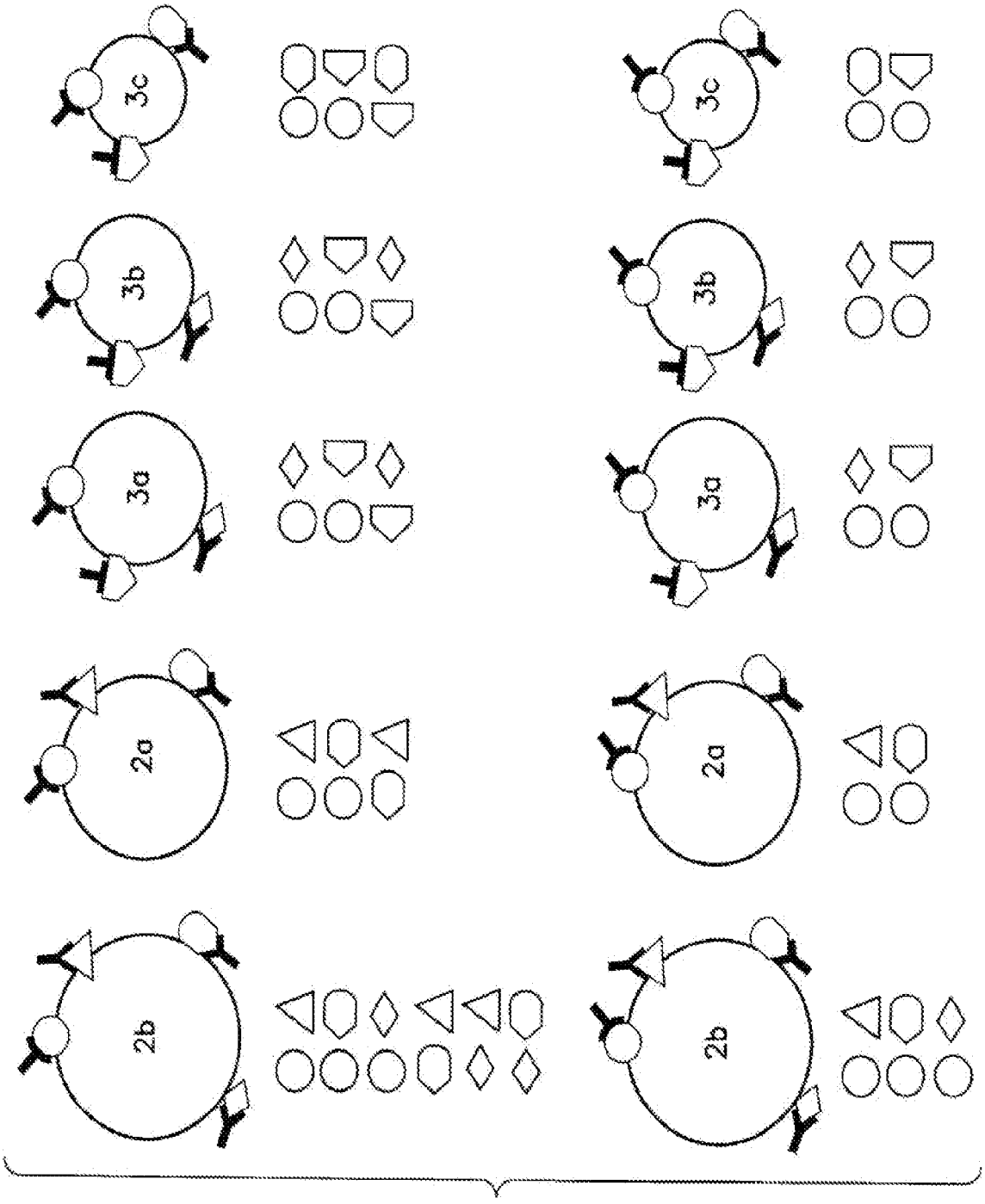


FIG.9

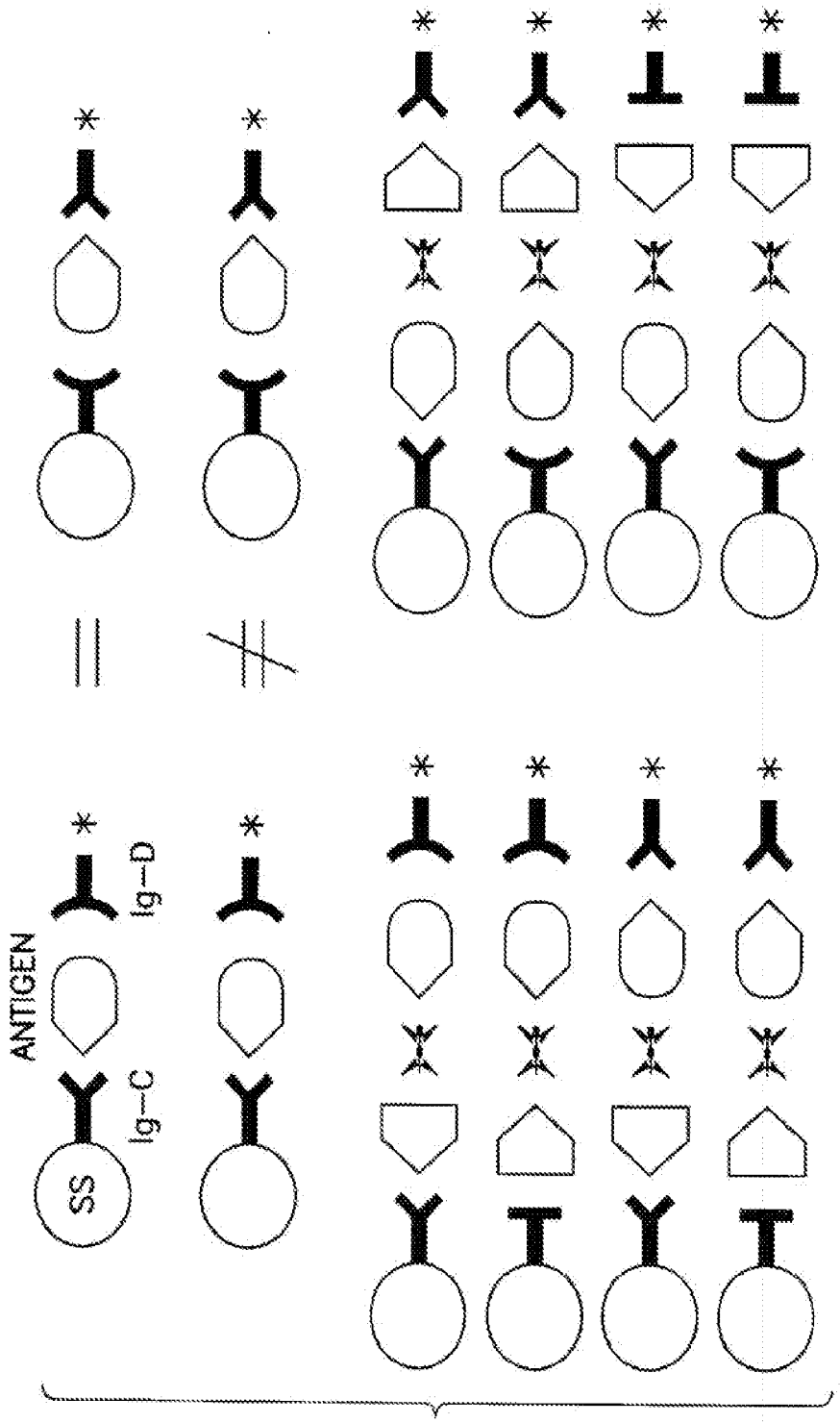


FIG. 10

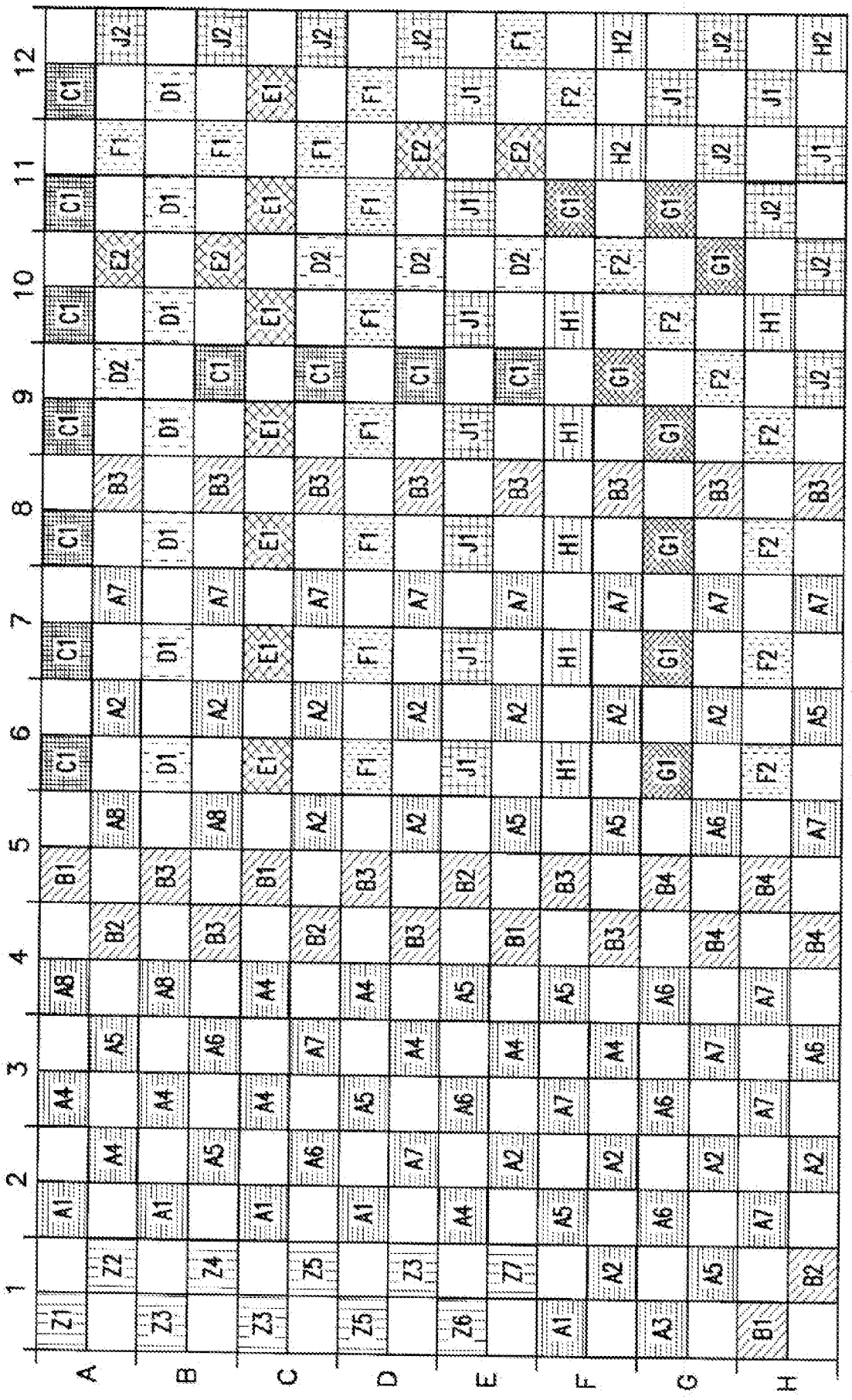


FIG.11

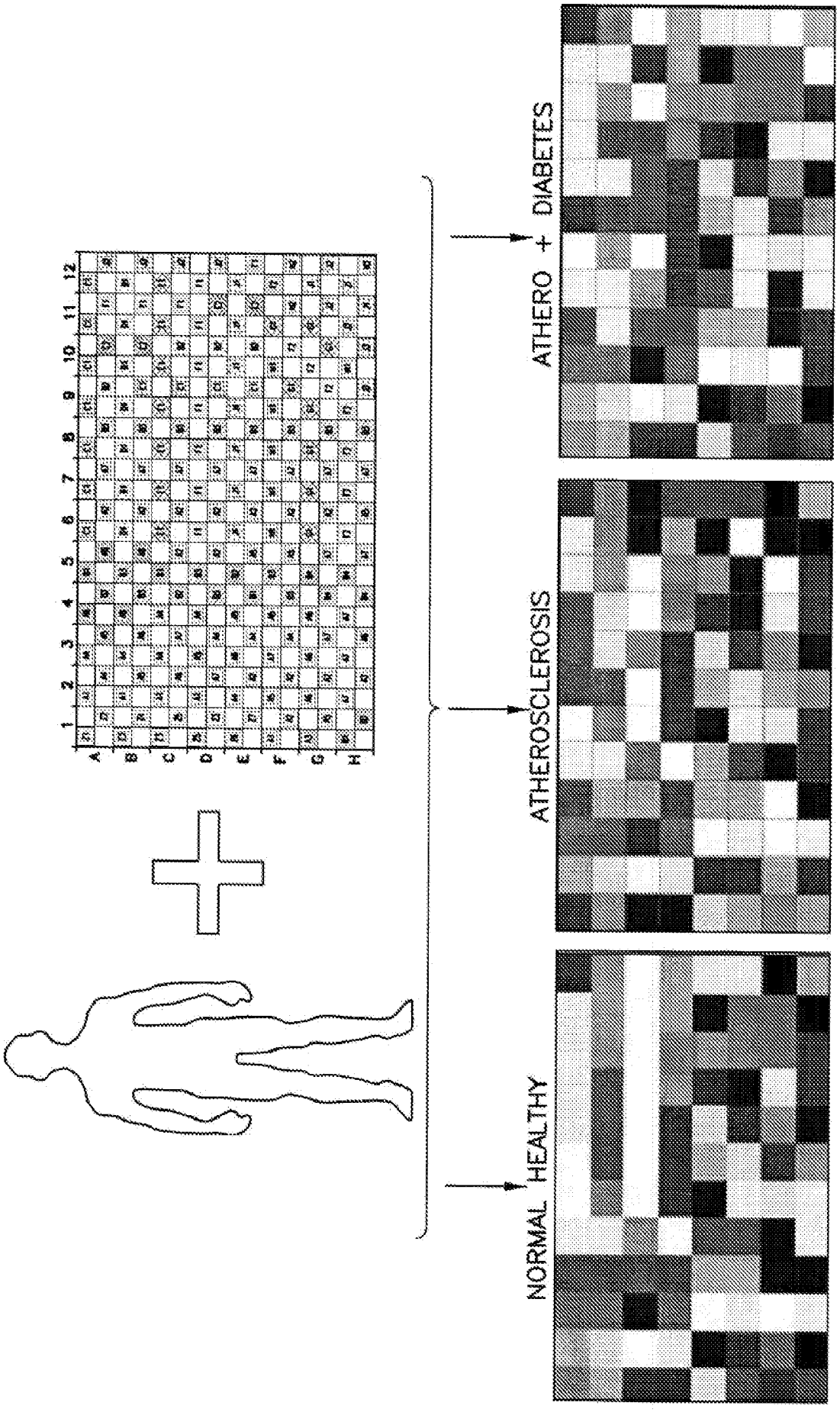


FIG.12

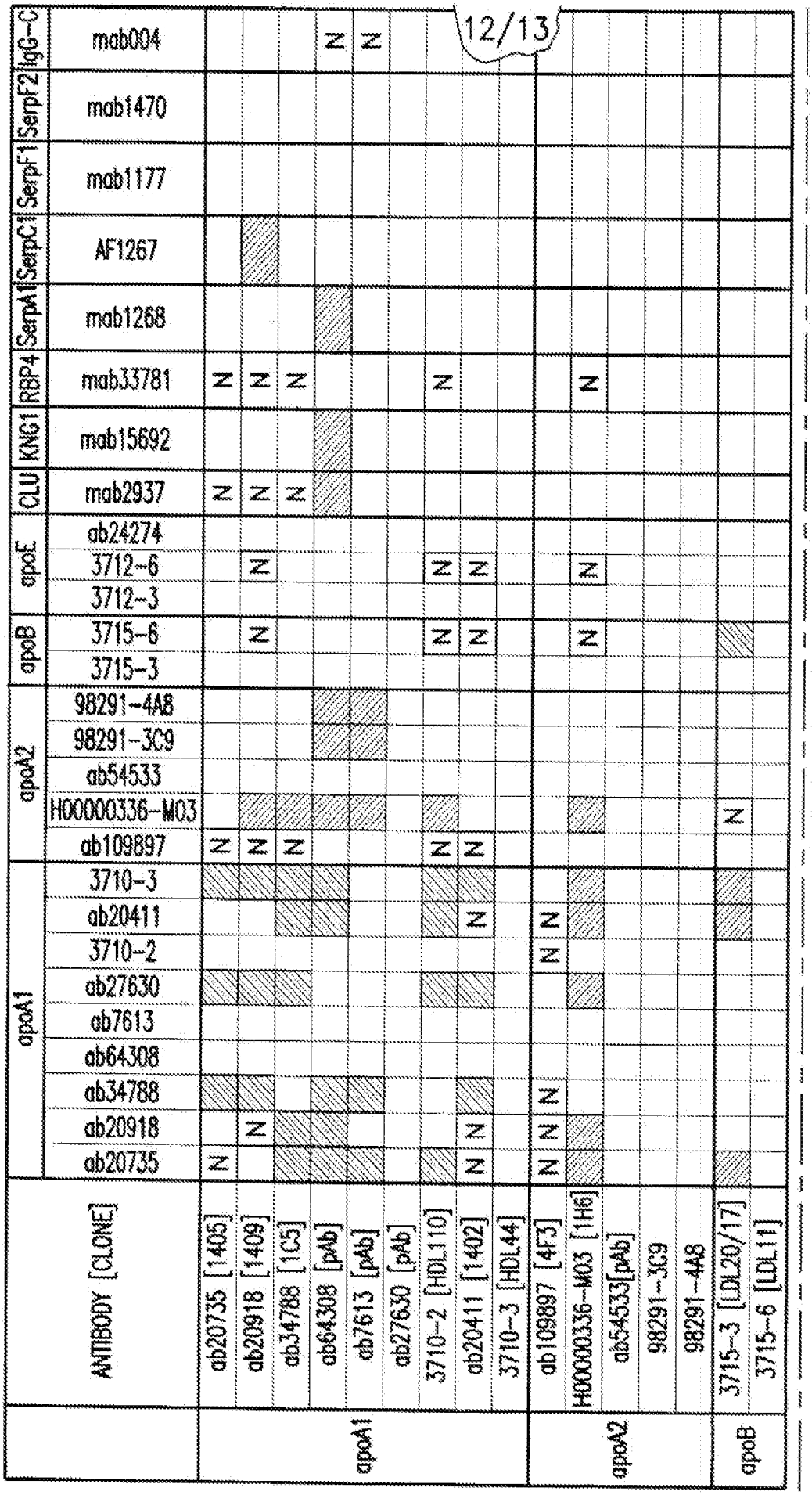


FIG. 13A

FIG. 13A
FIG. 13B FIG. 13

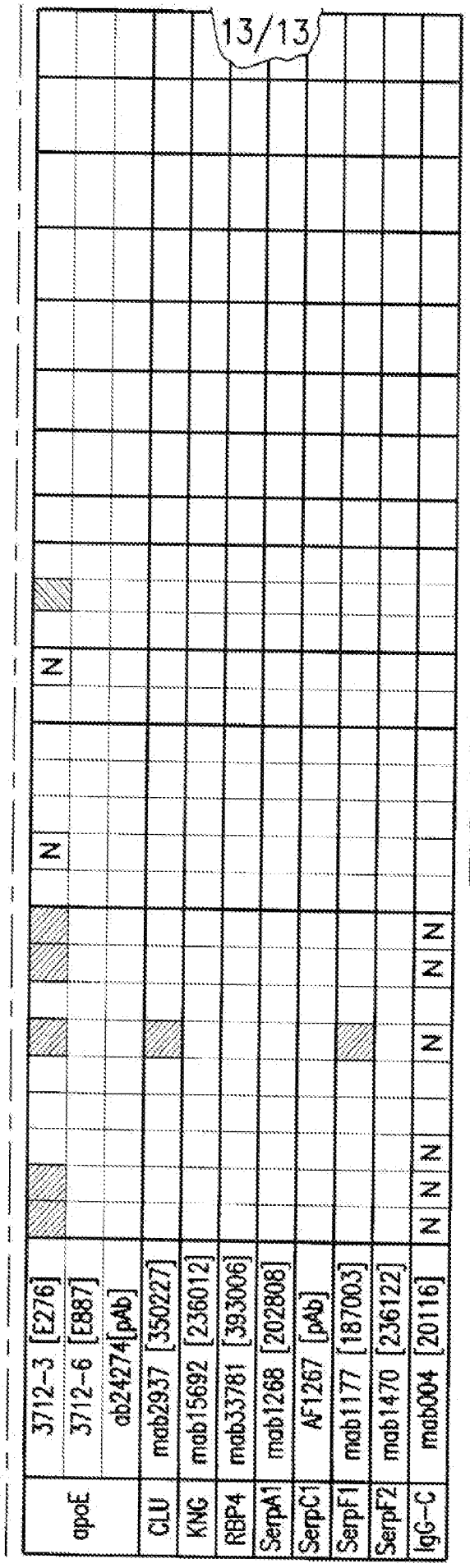

apoE
3712-3 [E276]
3712-6 [E887]
ab24274[pAb]
CLU
mab2937 [350227]
KNG
mab15692 [236012]
RBP4
mab33781 [393006]
SerpA1
mab1268 [202808]
SerpC1
AF1267 [pAb]
SerpF1
mab1177 [187003]
SerpF2
mab1470 [236122]
IgG-C
mab004 [20116]
N
13/13

FIG. 13B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/49317

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53; G01N 33/00; C12Q 1/60(2012.01)
USPC - 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01N 33/53; G01N 33/00; C12Q 1/60(2012.01)
USPC - 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/7.94, 435/11

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Dialog Classic Files -- 2,6,35,64,144,155,315,371,344,347,348,349,654,652,340,345,351; Google Scholar; Google Patents; Search terms -- lipoprotein;HDL;antibody;epitope;sandwich;elisa;lipoprotein;plurality;capture;detect;paratope;subpopulation;epitope;diagnostic;chd;coronary;coronary;apolipoprotein;proteome;risk;treatment;solar system;solar;particle

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0053321 A1 (Koren, et al.) 18 March 2004 (18.03.2004);abstract; para[0088]-[0090];[0101];[0079];[0131];[0151];[0113]; [0040]-[0042];[0115];[0029];[0157]	1-21, 59-65, 76-80 and 76/(1,10,59)
Y	US 2007/0099242 A1 (Heinecke) 3 May 2007 (03.05.2007); [0004]-[0005];[0101];[0103];[0042];Table 5	1-21, 59-65, 76-80 and 76/(1,10,59)
Y	US 2010/0179066 A1 (Chapman et al.) 15 July 2010 (15.07.2010);para[0076]	60

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 December 2012 (05.12.2012)	02 JAN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/49317

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21, 59-65 and 76-80

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/49317

Continuation of:
Box NO III. Observations where unity of invention is lacking

Group I: claims 1-21, 59-65 and 76-80, drawn to a method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample, wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes, the method comprising performing a quantitative antibody-based assay on the sample, wherein (i) the assay employs one or more capture/detection antibody pairs, (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the sample.

Group II: claims 22-58 and 66-80, drawn to a method for determining whether a subject is afflicted with a disorder characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, wherein each particle of the HDL subpopulation is characterized by the presence of a plurality of defined protein epitopes, the method comprising (a) performing a quantitative antibody-based assay on an HDL-containing sample from the subject, wherein (i) the assay employs one or more capture/detection antibody pairs, ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation, and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL subpopulation in the subject's sample; and (b) comparing the measured amount of HDL subpopulation in the subject's sample with a known standard correlative with the presence and/or
absence of the disorder, thereby determining whether the subject is afflicted with the disorder..

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I do not include the inventive concept of a method for determining whether a subject is afflicted with a disorder characterized by an abnormal amount of a defined high density lipoprotein (HDL) subpopulation, as required by Group II.

The inventions of Groups I-II share the technical feature of a method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2007/0099242 A1 to Heinecke et al. (hereinafter 'Heinecke'), in view of US 2004/0053321 A1 to Koren et al. (hereinafter 'Koren'). Heinecke discloses Claim 1, a method for measuring the amount of a high density lipoprotein (HDL) subpopulation present in a sample (para [0004]-[0005], detecting an amount of at least one biomarker protein in a biological sample, or HDL subfraction thereof (including a lipoprotein complex with a density from about 1.06 to about 1.21 g/mL, or from about 1.06 to 1.10 g/mL, or from about 1.10 to about 1.21 g/mL, or a complex containing ApoA-I or ApoA-II), isolated from the subject), wherein each particle of the HDL subpopulation being measured is characterized by the presence of a plurality of defined protein epitopes (para [0101], Table 5), the method comprising performing a quantitative mass spectrometry (para [0050]), but does not specifcally teach applying antibody-based assay on the sample. Koren teaches quantitative antibody-based assay for HDL in the sample (para [0040] and [0113]), wherein (i) the assay employs one or more capture/detection antibody pairs (para [0113]), (ii) the capture and detection antibodies in each pair are directed to different protein epitopes present on each particle of the HDL subpopulation (para [0113]), and (iii) each antibody pair is directed to a different set of epitopes than is each other antibody pair, thereby measuring the amount of the HDL population in the sample (para [0069], mAbs to Apo A-1, AII, B, C-III and E can be used; para [0069], D6 and HB3cB3 mABs bind to sterically distant epitopes on Apo B). It would have been obvious to one of ordinary skill in the art, at the time the invention was made, to have applied the antibody-based assay for HDL of Koren to the method of measuring the amount of a high density lipoprotein (HDL) subpopulation of Heinecke, because it is well known to one of ordinary skilled in the art that the quantitative antibody-based assay such as the sandwich assay of Koren, can be performed in a laboratory setting, without the expensive equipments of mass spectrometry. As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

专利名称(译)	测量HDL亚群的方法		
公开(公告)号	EP2739973A4	公开(公告)日	2015-03-11
申请号	EP2012819804	申请日	2012-08-02
[标]申请(专利权)人(译)	HDL APOMICS		
申请(专利权)人(译)	HDL APOMICS LLC		
当前申请(专利权)人(译)	HDL APOMICS LLC		
[标]发明人	ALTMANN SCOTT W		
发明人	ALTMANN, SCOTT, W.		
IPC分类号	G01N33/53 G01N33/00 C12Q1/60 G01N33/487 G01N33/68 G01N33/92		
CPC分类号	G01N33/92 G01N33/487 G01N33/6842 G01N2333/775 G01N2800/044 G01N2800/50 G01N2800/52		
优先权	61/515101 2011-08-04 US		
其他公开文献	EP2739973A1		
外部链接	Espacenet		

摘要(译)

本发明提供了一种基于捕获/检测抗体的方法，用于测量样品中存在的高密度脂蛋白（HDL）亚群的量，其中所测量的HDL亚群的每个颗粒的特征在于存在多个确定的蛋白质表位。 。本发明还提供了相关的分析和诊断方法，以及用于执行该方法的试剂盒。