SUMMARY

In a first aspect, there is provided a method for the measurement of at least two lipoprotein components in a blood sample from a subject, wherein said at least two lipoprotein components are selected from total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), said method comprising: combining at least a portion of said sample of blood with a first reagent to provide a first combined solution, wherein said first reagent comprises a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer; adding to said first combined solution a second reagent to provide a second combined solution, wherein said second reagent comprises a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant, wherein said time of addition of said second reagent to said first combined solution is termed the initial time; and measuring absorbance of light by said second combined solution within a first time period, within a second time period, and after a third time, wherein said time periods and said third time may be determined with respect to said initial time, and measuring the lipoprotein amounts of at least two lipoprotein components in the blood sample from said absorbance measurements, whereby at least two lipoprotein components in a blood sample from a subject are measured. In the second aspect, there is provided a method for the combined measurement of high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density cholesterol (VLDL-C), total cholesterol (TC), and triglyceride level (TG) in no more than two samples of blood from a subject, said method comprising: combining at least a portion of a first sample or first portion of a sample of blood from said subject with a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer to provide a first combined solution; adding to said first combined solution a second reagent comprising a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant to provide a second combined solution, wherein said time of addition of said second reagent to said first combined solution is termed the initial time; and measuring absorbance of light by said second combined solution within a first time period, within a second time period, and at a third time, wherein said time periods and said third time may be determined with respect to said initial time, wherein HDL-C is measured by the difference in ΔA measured at said initial time and the ΔA measured at the end of said first time period, where ΔA is the difference between absorbance measured at two different wavelengths of light; wherein LDL-C is measured by the difference in ΔA measured at the beginning of said second time period and the ΔA measured at the end of said second time period; wherein TC is measured by ΔA measured at said third time; whereby measurement of HDL-C, LDL-C, and TC is performed on said first sample of blood or said first portion of said sample of blood; measuring TG in a second sample of blood or a second portion of a sample of blood from said subject; and calculating VLDL-C; whereby combined measurement of HDL-C, LDL-C, VLDL-C, TC, and TG in two samples of blood or two portions of a sample of blood is obtained. In the third aspect, there is provided a reagent for use in a cholesterol assay, said reagent comprising a lipoprotein solubilization agent, lipoprotein interactant, and a buffer, wherein said lipoprotein interactant is present at a concentration level that provides for conversion of HDL-cholesterol to a measureable colored product at a substantially different rate than for conversion of LDL-cholesterol to a measureable colored product and at a substantially different rate than for conversion of VLDL-cholesterol to a measureable colored product. In the fourth aspect, there is provided a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, said reagent comprising a lipoprotein solubilization agent, lipoprotein interactant, and a buffer, wherein said lipoprotein interactant is present at a concentration level that provides for conversion of HDL-cholesterol to a measureable colored product at a substantially different rate than for conversion of LDL-cholesterol to a measureable colored product and at a substantially different rate than for conversion of VLDL-cholesterol to a measureable colored product. In the fifth aspect, there is provided a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, said reagent comprising α-cyclodextrin

sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer, wherein the ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02. In the sixth aspect, there is provided a reagent for use in an assay for the measurement of at least two of total cholesterol, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant. In the seventh aspect, there is provided a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant. In the eighth aspect, there is provided a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a peroxidase. In the ninth aspect, there is provided a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent as described herein. In the tenth aspect, there is provided a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer, wherein the ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02. In the eleventh aspect, there is provided a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent as described herein.In the twelve aspect, there is provided a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a sodium phosphate buffer, Triton X-100, pluronic L64, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), cholesterol esterase from *Pseudomonas sp.*, and cholesterol oxidase from *Pseudomonas sp.*In the thirteen aspect, there is provided a kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises a reagent as described herein, and said second reagent comprises a reagent as described herein. In the twelve aspect, there is provided A kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises the reagent as described herein, and said second reagent comprises the reagent as described herein.In the thirteen aspect, there is provided a kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises the reagent as described herein, and said second reagent comprises the reagent as described herein.

[0012] Applicants have found that very accurate and precise values for T-C, LDL-C and HDL-C can be obtained in a single assay for lipoprotein cholesterol using kinetic measurements, without need for precipitation of lipoproteins and without separation steps. Assays as disclosed herein use a lipase (e.g., a cholesterol esterase), an oxidase (e.g., a cholesterol oxidase) and a peroxidase to form a colored product. Assays as disclosed herein may use a lipase (e.g., a cholesterol esterase), a dehydrogenase (e.g., a cholesterol dehydrogenase) and nicotine adenine dinucleotide to form a light-absorbing product (e.g., NADH) or other detectable product. Such products may be measurable by spectrophotometry. In the assays disclosed herein, the amount of product formed from all the cholesterol-containing lipoprotein species is directly proportional to the quantity of cholesterol converted. The assays, methods, reagents, and kits disclosed herein are useful for rapid, efficient, and inexpensive assays of lipoprotein cholesterol and lipoprotein cholesterol sub-fractions, and advantageously provide for measurements of lipoprotein cholesterol and lipoprotein cholesterol sub-fractions in a single assay. Assays disclosed herein advantageously provide for measurements of lipoprotein cholesterol and lipoprotein cholesterol sub-fractions without need for lipoprotein precipitation. Assays disclosed herein advantageously provide for measurements of lipoprotein cholesterol and lipoprotein cholesterol sub-fractions without the need for centrifugation or for electrophoresis.

[0013] Applicants have found that it is possible to provide assay conditions (e.g., reagents, protocol and temperature) so that the kinetics of reactions converting HDL-C, LDL-C and VLDL-C to colored product proceed at significantly different rates, while still allowing essentially complete reaction of all lipoprotein species (e.g., HDL, LDL, and VLDL) by the end of the assay. For example, in assays disclosed herein the time course for HDL-C has a half-time estimated at less than one minute whereas the LDL-C conversion has a lag phase and an overall sigmoid shape centered on about three minutes. In assays disclosed herein, the remaining lipoprotein cholesterol (chylomicrons and VLDL-C) react even more slowly (half-time of about five minutes or longer). These differences in half-time and reaction kinetics between different cholesterol species enable the de-convolution of an assay signal to that attributable to each species using a simple algorithm even though, for example, signal is being produced from more than one species during certain times during the assay. Accordingly, reagent formulations for use in the assays and methods disclosed herein are

designed to achieve the kinetic differentiation of lipoprotein species HDL, LDL, and VLDL in a single assay.

[0014] The novel methods and assays disclosed herein make use of Applicants' surprising finding that in these assays cholesterol sub-species are converted to the measured product at distinct rates. Accordingly, HDL-C is converted very rapidly to product, while LDL-C is converted to product more slowly than HDL-C is converted to product and VLDL-C and chylomicron cholesterol are converted even more slowly. Complete conversion of total cholesterol to product is even slower than the conversion of HDL-C or LDL-C to product. Such differences in rates of conversion to product allow the measurement of cholesterol from different lipoprotein species to be made in a single solution at different times, thus reducing the number of steps needed for such measurements, reducing possible errors and simplifying the procedures.

[0015] Accordingly, Applicants disclose herein methods of determining HDL-C, LDL-C and TC in a single sample, or portion of a sample, of blood. These methods allow determination of HDL-C, LDL-C and TC in a single sample, or portion of a sample, of blood without substantial precipitation of lipoproteins, e.g., without substantial precipitation of HDL or LDL in the sample, or portion of a sample, of blood during the determination. As disclosed herein, the amount of HDL-C in a blood sample may be measured without substantial lipoprotein precipitation by colorimetric determination of the amount of peroxide formed in a first period of time following combination of reagents effective to allow oxidation of cholesterol released from lipoproteins in the sample. The first period of time may be, for example, a period of time that is less than about 3 minutes, and in ~~embodiments~~examples may be a period of time of about 2 minutes. As disclosed herein, the amount of LDL-C in a blood sample may be measured without substantial lipoprotein precipitation by colorimetric determination of the amount of peroxide formed in a second period of time following combination of reagents effective to allow oxidation of cholesterol released from lipoproteins in the sample. In ~~embodiments~~examples, the amount of LDL-C in a blood sample may be measured by the difference between colorimetric determinations made at the beginning of said second period of time and colorimetric determinations made at the end of said second period of time. The second period of time may be, for example, a period of time that is between about 2 minutes and about 6 minutes following combination of reagents effective to allow oxidation of cholesterol released from lipoproteins in the sample. As disclosed herein, the amount of TC in a blood sample may be measured without substantial lipoprotein precipitation by colorimetric determination of the amount of peroxide formed following combination of reagents effective to allow oxidation of cholesterol released from lipoproteins in the sample. This TC measurement (e.g., colorimetric determination) may be made at a time at the beginning of, or at any particular time during, a third period of time. The third period of time comprises a period of time after said second period of time, and may be, for example, a period of time that begins about 6 minutes following combination of

reagents effective to allow oxidation of cholesterol released from lipoproteins in the sample. For consistency of measurement between different samples, where different samples are measured in different determinations, the colorimetric determination for each different sample may be made at the same time after the beginning of said third period of time for each sample.

[0016] Accordingly, HDL-C, LDL-C, and TC measurements may be made in a single sample, or a single portion of a sample, of blood of a subject. In ~~embodiments~~examples, as disclosed herein, HDL-C, LDL-C, and TC measurements may be made sequentially in a single sample, or a single portion of a sample, of blood of a subject. For example, from these measurements, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated by the relation VLDL-C = TC – (HDL-C + LDL-C), or by other relationships. In ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from these measurements, for example, by a relation of the form VLDL-C = αTC + βHDL-C + γLDL-C + λ, where α, β, and γ are constants which multiply TC, HDL-C, and LDL-C, respectively; and where λ is an additive constants to be added to the sum of all other factors, respectively. In yet further ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from other relationships using some or all of the measured values of TC, HDL-C, and LDL-C and multiplicative and additive constants similar in form to the relationships provided above.

[0017] Applicants thus disclose herein novel and useful measurements of HDL-C, LDL-C, and TC, and calculation of VLDL-C, which may be made on a single blood sample, or portion of blood sample, reducing the amount of blood needed for such measurements, reducing the complexity of the methods needed to measure these different lipoprotein fractions in blood, and providing accurate and rapid blood lipoprotein measurements. These measurements may be made without substantial precipitation of lipoproteins in the sample.

[0018] Applicants also disclose herein methods for determining triglyceride levels in blood samples, where the level (equivalently: amount) of triglyceride (TG) may be determined by methods in which a sample of blood is contacted with a lipase, a kinase, and an oxidase to free glycerol from TG in the blood sample, to phosphorylate the glycerol, and then to provide hydrogen peroxide. The hydrogen peroxide, in the presence of a peroxidase forms a colorant, the measurement of which provides a determination of the TG level in the blood sample.

[0019] Applicants disclose herein novel and useful measurements of HDL-C, LDL-C, TC, and TG which provide accurate and rapid blood lipoprotein measurements. The measurements of HDL-C, LDL-C and TC may be made on one sample or portion of sample of blood, while the TG measurements may be made on a different sample or portion of sample of blood. These measurements may be made without substantial precipitation of lipoproteins in the samples. Together, these measurements of four

blood lipid components are made on only two samples, or two portions of a sample or samples, of blood from a subject. Applicants disclose herein further, improved methods for calculating VLDL-C in the blood of a subject, using HDL-C, TC, and TG measurements or using HDL-C, LDL-C, TC, and TG measurements. In ~~embodiments~~examples, VLDL-C may be calculated from HDL-C, TC, and TG measurements, or from HDL-C, LDL-C, TC, and TG measurements, where these measurements are made according to the methods disclosed herein. In ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from these measurements, for example, by a relation of the form VLDL-C = αTC + βHDL-C + γLDL-C + δTG + a_1(TG+ε)(TC+κ) + λ, where α, β, γ, δ, and a_1 are constants which multiply TC, HDL-C, LDL-C, TG, and (TG+ε)(TC+κ), respectively; and where ε, κ, and λ are additive constants to be added to TG, TC, and the sum of all other factors, respectively. In further ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from these measurements, for example, by a relation of the form VLDL-C = αTC + βHDL-C + δTG + a_1(TG+ε)(TC+κ) + λ, where α, β, δ, and a_1 are constants which multiply TC, HDL-C, TG, and (TG+ε)(TC+κ), respectively; and where ε, κ, and λ are additive constants to be added to TG, TC, and the sum of all other factors, respectively. In yet further ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from other relationships using some or all of the measured values of TC, TG, HDL-C, and LDL-C and multiplicative and additive constants similar in form to the relationships provided above.

[0020] In ~~embodiments~~examples, reagent formulations for use in the assays and methods disclosed herein are designed to achieve the kinetic differentiation described above without precipitation of lipoproteins. In reagents and assays disclosed herein, formation of complexes of lipoproteins with cations (optionally divalent cations such as magnesium, calcium, manganese, cobalt, cadmium, or other cations) and a negatively charged polysaccharide (such as, e.g., a dextran ester such as dextran-sulfate, a cyclodextrin ester such as γ-cyclodextrin-sulfate, heparin, or other negatively charged polysaccharide) is achieved in conditions where no precipitation of lipoproteins occurs. For example, in ~~embodiments~~examples, lipoprotein complex formation with magnesium ions and dextran sulfate is achieved in conditions where little or no precipitation of lipoproteins occurs. Amphiphilic compounds, such as surfactants may be included in reagents and assays disclosed herein. For example, a variety of surfactants are suitable for use in keeping the lipoproteins soluble. In ~~embodiments~~examples, the reagents provide ingredients effective to modify the LDL and VLDL lipoprotein particle surfaces in such a way as to restrict but not prevent access (e.g., by cholesterol esterases and/or oxidases) to LDL and VLDL cholesterol and cholesterol esters. The use of reagents and methods suitable for such modification of LDL and VLDL particle surfaces is effective to slow the formation of colored product from these lipoprotein

species as compared to the rate of formation of colored product in similar assays performed without the interactants and surfactants, or with such agents at different concentrations and relative proportions than are found in the assays and reagents disclosed herein. Slowing of the formation of colored product from lipoprotein species may be useful in order to provide separation of reaction kinetics effective to enable the measurement of cholesterol content of different lipoprotein fractions at different times without need for physical separation of the different lipoprotein fractions (e.g., without need for centrifugation or electrophoresis).

[0021] Accordingly, Applicants disclose herein a first reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) in a sample of blood from a subject without substantial precipitation of said blood lipoproteins, comprising a lipoprotein solubilization agent and a lipoprotein interactant. In ~~embodiments~~examples, a first reagent may comprise a buffer. In ~~embodiments~~examples, a lipoprotein solubilization agent for use in a first reagent as disclosed herein may comprise a surfactant. A lipoprotein interactant may comprise, for example, a cyclodextrin, a dextran, or both. In ~~embodiments~~examples, a lipoprotein interactant for use in such a first reagent may comprise a low molecular weight dextran. In ~~embodiments~~examples, a lipoprotein interactant for use in such a first reagent may comprise an α-cyclodextrin. In ~~embodiments~~examples, a first reagent may comprise a colorant. In ~~embodiments~~examples, *a first reagent may include one or both of an aniline-containing compound and an* aminoantipyrene compound, effective that both compounds, in the presence of peroxide and a peroxidase, react to form a detectable product. In ~~embodiments~~examples of the reagents, assays, methods, and kits disclosed herein, such a first reagent may comprise α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4 aminoantipyrene and a buffer, such as a phosphate buffer. In ~~embodiments~~examples of the reagents, assays, methods, and kits disclosed herein, such a first reagent may comprise α-cyclodextrin sulfate (1 mM), dextran sulfate (20 μM), magnesium chloride (4 mM), 4 aminoantipyrene (2.25 mM) and Na_xPO_4 (100 mM) (where Na_xPO_4 is a sodium phosphate salt; e.g., may be NaH_2PO_4, Na_2HPO_4, or Na_3PO_4). In ~~embodiments~~examples, the pH of such a first reagent may be between about pH 5 and about pH 9, or between about pH 6 and about pH 8, optionally between about pH 6.8 and about pH 7.8, e.g., about pH 7.4.

[0022] Applicants also disclose a second reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) in a sample of blood from a subject without substantial precipitation of said blood lipoproteins, said second reagent comprising a lipase, an oxidase, and a colorant. In ~~embodiments~~examples, a second reagent may comprise a buffer. In ~~embodiments~~examples of said second reagent, a lipase may comprise a cholesterol esterase, an oxidase may comprise a cholesterol oxidase, and a colorant may comprise a

peroxidase, a substrate for a peroxidase, or both. In ~~embodiments~~examples, both a peroxidase and a substrate for a peroxidase are present in said second reagent.

[0023] In ~~embodiments~~examples, said second reagent may comprise a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant. In embodiments, an amphiphilic agent for use in such a second reagent may comprise a surfactant. In ~~embodiments~~examples, a colorant for use in such a second reagent may comprise a peroxidase and a substrate for a peroxidase. In ~~embodiments~~examples, a colorant for use in such a second reagent may comprise horseradish peroxidase. In ~~embodiments~~examples, a colorant for use in such a second reagent may comprise an aniline-containing compound, such as N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), may comprise an aminoantipyrene compound, such as 4-aminoantipyrene, or other compounds and combinations of compounds which react with an enzyme such as a peroxidase (e.g., horseradish peroxidase). In ~~embodiments~~examples, a second reagent may include both an aniline-containing compound and an aminoantipyrene compound, effective that both compounds, in the presence of peroxide and a peroxidase, react to form a detectable product. In ~~embodiments~~examples, a lipase may comprise a cholesterol esterase. In ~~embodiments~~examples, a cholesterol esterase for use in such a second reagent may comprise a bacterial cholesterol esterase, such as, for example, a cholesterol esterase from a *Pseudomonas* bacterium. In ~~embodiments~~examples, an oxidase may comprise a cholesterol oxidase. In ~~embodiments~~examples, a cholesterol oxidase for use in a second reagent as disclosed herein may comprise a bacterial cholesterol oxidase, such as a cholesterol oxidase from a *Pseudomonas* bacterium. In ~~embodiments~~examples of the reagents, assays, methods, and kits disclosed herein, such a second reagent may comprise a buffer, such as a phosphate buffer (e.g., Na_xPO_4 (where Na_xPO_4 is a sodium phosphate salt; e.g., may be NaH_2PO_4, Na_2HPO_4, or Na_3PO_4)), Triton X-100, pluronic L64, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), a *Pseudomonas* cholesterol esterase, and a *Pseudomonas* cholesterol oxidase. For example, such a second reagent may comprise Na_xPO_4 (50 mM), Triton X-100 (0.06%), pluronic L64 (3 g/L), N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS) (3 mM), cholesterol esterase from *Pseudomonas sp.* (750 U/L), and cholesterol oxidase from *Pseudomonas sp.* (1.5 kU/L). In ~~embodiments~~examples, the pH of such a second reagent may be between about pH 5 and about pH 9, or between about pH 6 and about pH 8, optionally between about pH 6.8 and about pH 7.8, e.g., about pH 7.4.

[0024] Applicants disclose herein methods and reagents for use in a cholesterol assay, including second reagents comprising a lipase, an oxidase, and a colorant, said second reagents comprising a lipoprotein interactant at a concentration level that, when used in methods disclosed herein, provides for conversion of HDL-C to a measureable colored product (or product measureable, e.g., in the near ultraviolet range of the spectrum) at a substantially different rate than for conversion of LDL-C to a

measureable colored product(or product measureable, e.g., in the near ultraviolet range of the spectrum). Applicants disclose herein methods and reagents for use in a cholesterol assay comprising a lipase, an oxidase, and a colorant, including second reagents comprising a lipoprotein interactant at a concentration level that, when used in methods disclosed herein, provides for conversion of HDL-C to a measureable colored product at a substantially different rate than for conversion of VLDL-C to a measureable colored product. Applicants disclose herein methods and reagents for use in a cholesterol assay comprising a lipase, an oxidase, and a colorant, including second reagents comprising a lipoprotein interactant at a concentration level that, when used in methods disclosed herein, provides for conversion of LDL-C to a measureable colored product at a substantially different rate than for conversion of VLDL-C to a measureable colored product. Applicants disclose herein methods and reagents for use in a cholesterol assay comprising a lipase, an oxidase, and a colorant, including second reagents comprising a lipoprotein interactant at a concentration level that, when used in methods disclosed herein, provides for conversion of HDL-C to a measureable colored product at a substantially different rate than for conversion of LDL-C and/or VLDL-C to a measureable colored product. Applicants disclose herein methods and reagents for use in a cholesterol assay comprising a lipase, an oxidase, and a colorant, including second reagents comprising a lipoprotein interactant at a concentration level that, when used in methods disclosed herein, provide for conversion of LDL-C to a measureable colored product at a substantially different rate than for conversion of HDL-C and VLDL-C to a measureable colored product.

[0025] In ~~embodiments~~examples, a colorant may comprise a peroxidase and a substrate for a peroxidase. For example, ~~in embodiments~~as disclosed herein, a colorant may comprise a peroxidase, an aniline-containing compound, and an aminoantipyrene compound.

[0026] Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, including reagents comprising a lipoprotein interactant at a concentration level that provides for conversion of HDL-C to a measureable colored product at a substantially different rate than for conversion of LDL-C and VLDL-C to a measureable colored product, and provides for conversion of HDL-C to a measureable colored product at a substantially different rate than for conversion of LDL-C and VLDL-C to a measureable colored product.

[0027] Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, including reagents comprising a negatively charged polysaccharide derivative and a divalent cation in concentrations effective that there is no substantial precipitation of LDL, or that there is no substantial VLDL precipitation, or both, when a sample of blood or a portion of a blood sample contacts said reagent. Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said reagents comprising a ratio of negatively charged polysaccharide to divalent cations of between about 0.002 to about 0.02, and in

~~embodiments~~examples of about 0.005, effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said reagent. In ~~embodiments~~examples, said reagent comprises a first reagent as disclosed herein.

[0028] Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said reagents comprising a negatively charged polysaccharide, a divalent cation, and surfactant in concentrations effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said reagent. In ~~embodiments~~examples, said reagent comprises a first reagent as disclosed herein.

[0029] Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said reagents comprising dextran sulfate (or other negatively charged polysaccharide) and magnesium (or other divalent cation) in concentrations effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said reagent. Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said reagents comprising a ratio of dextran sulfate (or other negatively charged polysaccharide) to magnesium ions (or other divalent cations) of between about 0.002 to about 0.02, and in ~~embodiments~~examples of about 0.005, effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said reagent or composition. Applicants disclose herein reagents for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said reagents comprising dextran sulfate, magnesium, and surfactant in concentrations effective that there is no substantial precipitation of LDL, or that there is no substantial VLDL precipitation, or both, when a sample of blood or a portion of a blood sample contacts said reagent or composition. In ~~embodiments~~examples, said reagent comprises a first reagent as disclosed herein.

[0030] Applicants further disclose herein a composition for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) in a sample of blood from a subject without substantial precipitation of said blood lipoproteins, comprising a lipoprotein solubilization agent, a lipoprotein interactant, a lipase, an oxidase, and a colorant. In ~~embodiments~~examples, such a composition may comprise a buffer. In ~~embodiments~~examples, the colorant comprises a peroxidase, an aniline-containing compound, and an aminoantipyrene compound. In ~~embodiments~~examples, the composition includes ingredients from the first reagent and the second reagent as disclosed herein. Such a composition may be provided, for

example, by combination of said first reagent and said second reagent as disclosed herein. In ~~embodiments~~examples, such a composition may comprise a negatively charged polysaccharide and a salt comprising a divalent cation (e.g., a magnesium salt and dextran sulfate), wherein the ratio of negatively charged polysaccharide to divalent cation (e.g., dextran sulfate to magnesium ions) is between about 0.002 to about 0.02, and in ~~embodiments~~examples the ratio is about 0.005, effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said composition. In ~~embodiments~~examples, such a composition comprises said first reagent and said second reagent, or an aliquot of said first reagent and an aliquot of said second reagent. In further ~~embodiments~~examples, such a composition comprises said first reagent, said second reagent, and a sample of blood, or an aliquot or aliquots of one or more of said first reagent, said second reagent, and said sample of blood.

[0031] In ~~embodiments~~examples, a lipoprotein solubilization agent in such a composition may comprise a surfactant. In ~~embodiments~~examples, a lipoprotein interactant in such a composition may comprise a divalent cation (e.g., magnesium ion) and a negatively charged polysaccharide derivative. A negatively charged polysaccharide derivative may comprise a cyclodextrin-ester, a dextran-ester, or both a cyclodextrin-ester and a dextran-ester. For example, a dextran-ester may comprise a low molecular weight dextran-ester, e.g., a low molecular weight dextran sulfate, and a cyclodextrin-ester may comprise an α-cyclodextrin-ester, e.g., α-cyclodextrin-sulfate. In ~~embodiments~~examples, a lipase may comprise a cholesterol esterase. In ~~embodiments~~examples, an oxidase may comprise a cholesterol oxidase. In ~~embodiments~~examples, a colorant may comprise a peroxidase.

[0032] Accordingly, in ~~embodiments~~examples, a composition for use in a cholesterol assay as disclosed herein may comprise a cholesterol esterase; a cholesterol oxidase; a peroxidase; a substrate for a peroxidase; a surfactant; a salt containing a divalent cation; and a negatively charged polysaccharide (e.g., a dextran derivative such as dextran sulfate). In ~~embodiments~~examples, a substrate for a peroxidase in such a composition comprises both an aniline-containing compound and an aminoantipyrene compound, effective that both compounds, in the presence of peroxide and a peroxidase, react to form a detectable product. In ~~embodiments~~examples, a substrate for a peroxidase may comprise other compounds and combinations of compounds which react with a peroxidase (e.g., horseradish peroxidase). An aniline-containing compound may be, for example, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS). An aminoantipyrene compound may be 4-aminoantipyrene.

[0033] In ~~embodiments~~examples, a composition for use in a cholesterol assay as disclosed herein may comprise dextran sulfate (or other negatively charged polysaccharide) and magnesium in concentrations effective that there is no substantial precipitation of HDL, or that there is no substantial

precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said composition. Applicants disclose herein compositions for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said compositions comprising a ratio of dextran sulfate to magnesium ions of between about 0.002 to about 0.02, and in ~~embodiments~~examples of about 0.005, effective that there is no substantial precipitation of HDL, or that there is no substantial precipitation of LDL, or that there is no substantial precipitation of VLDL, when a sample of blood or a portion of a blood sample contacts said reagent. Applicants disclose herein compositions for use in a cholesterol assay comprising a cholesterol esterase and a cholesterol oxidase, said compositions comprising dextran sulfate, magnesium, and surfactant in concentrations effective that there is no substantial precipitation of LDL, or that there is no substantial VLDL precipitation, or both, when a sample of blood or a portion of a blood sample contacts said composition.

[0034] In ~~embodiments~~examples, a composition for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) in a sample of blood from a subject without substantial precipitation of said blood lipoproteins may comprise a cholesterol esterase, a cholesterol oxidase, a peroxidase (such as horseradish peroxidase), 4-aminoantipyrene, ALPS, α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, a surfactant (e.g., Triton X-100 and/or pluronic L64), and a buffer. A suitable buffer may be, for example, a phosphate buffer, and a suitable pH may be in the range of between about pH 5 to about pH 9, or between about pH 6 and about 8, optionally between about pH 6.8 and about pH 7.8, e.g. about pH 7.4. A suitable cholesterol esterase for use in such a composition may comprise a bacterial cholesterol esterase, such as, for example, a cholesterol esterase from a *Pseudomonas* bacterium, and a suitable cholesterol oxidase may comprise a bacterial cholesterol oxidase, such as a cholesterol oxidase from a *Pseudomonas* bacterium.

[0035] In ~~embodiments~~examples, Applicants disclose a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, said reagent comprising a lipoprotein solubilization agent, lipoprotein interactant, and a buffer, wherein said lipoprotein interactant is present at a concentration level that provides for conversion of HDL-cholesterol to a measureable colored product at a substantially different rate than for conversion of LDL-cholesterol to a measureable colored product and at a substantially different rate than for conversion of VLDL-cholesterol to a measureable colored product.

[0036] In ~~embodiments~~examples, Applicants disclose a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said

lipoproteins, said reagent comprising α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer, wherein the ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02.

[0037] In ~~embodiments~~examples, Applicants disclose a reagent for use in an assay for the measurement of at least two of total cholesterol, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant. In ~~embodiments~~examples, the reagent comprises a cholesterol esterase, a cholesterol oxidase, and a peroxidase. In ~~embodiments~~examples of the reagent, the amphiphilic agent comprises a surfactant selected from the group consisting of nonionic surfactants; anionic surfactants; cationic surfactants; zwitterionic surfactants; and derivatives, analogues, and combinations thereof. In ~~embodiments~~examples of the reagent, the colorant comprises one or more of a peroxidase, a peroxidase substrate, an aminoantipyrene compound, and an aniline-containing compound. In ~~embodiments~~examples, the reagent comprises a sodium phosphate buffer, Triton X-100, pluronic L64, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), cholesterol esterase from *Pseudomonas sp.*, and cholesterol oxidase from *Pseudomonas sp.*

[0038] In ~~embodiments~~examples, Applicants disclose a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant. In ~~embodiments~~examples, Applicants disclose a reagent for use in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, comprising a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a peroxidase.

[0039] In ~~embodiments~~examples, reagent may be provided in a kit; a kit may further include instructions for the use of a reagent (e.g., a composition as disclosed herein), or for the use of multiple reagents, e.g., in a cholesterol assay, or in an assay for the simultaneous, rapid measurement of at least two of total cholesterol, LDL-cholesterol and HDL-cholesterol in lipoproteins in a sample of blood from a subject without substantial precipitation of said lipoproteins, or in another assay or assays. A reagent in a kit may be contained in a container, and multiple reagents may be contained in multiple containers.

[0040] Applicants disclose herein a kit comprising reagents for use in an assay for the simultaneous, rapid measurement of at least two of TC, LDL-C and HDL-C in a sample of blood from a subject without substantial precipitation of said lipoproteins. ~~Embodiments~~eExamples of the kits disclosed herein may comprise a first container containing a first reagent comprising a lipoprotein

solubilization agent, a lipoprotein interactant, and a buffer; and a second container containing a second reagent comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant. ~~Embodiments~~Examples of the kits disclosed herein may comprise a first container containing a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer; and a second container containing a second reagent comprising a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant. In ~~embodiments~~examples, a colorant may comprise a peroxidase, a substrate for a peroxidase, or both.

[0041] ~~Embodiments~~Examples of the kits disclosed herein may comprise a first container containing a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer; a second container containing a second reagent comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant; and instructions for their use. In ~~embodiments~~examples, such kits comprise a first container containing a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer; a second container containing a second reagent comprising a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant; and instructions for their use. In ~~embodiments~~examples, a colorant may comprise a peroxidase and a substrate for a peroxidase.

[0042] In ~~embodiments~~examples, Applicants disclose a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent as disclosed herein. In ~~embodiments~~examples, Applicants disclose a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer, wherein the ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02. In ~~embodiments~~examples, Applicants disclose a kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a sodium phosphate buffer, Triton X-100, pluronic L64, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), cholesterol esterase from *Pseudomonas sp.*, and cholesterol oxidase from *Pseudomonas sp.*

[0043] In ~~embodiments~~examples, Applicants disclose a kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises a reagent as disclosed herein (e.g., a reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer), and said second reagent comprises a reagent as disclosed herein (e.g., a reagent comprising a buffer, an amphiphilic agent, a lipase, an oxidant, and a colorant). In ~~embodiments~~examples, Applicants disclose a kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises a lipoprotein solubilization agent selected from a surfactant and a negatively charged α-cyclodetrin

derivative; a lipoprotein interactant comprising a low molecular weight negatively charged dextran derivative, and a buffer; and said second reagent comprises a sodium phosphate buffer, Triton X-100, pluronic L64, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), cholesterol esterase from *Pseudomonas sp.*, and cholesterol oxidase from *Pseudomonas sp.*

[0044] In ~~embodiments~~examples, Applicants disclose a kit comprising at least a first reagent and a second reagent, and instructions for the use of said reagents in a cholesterol assay, wherein said first reagent comprises i) a lipoprotein solubilization agent, lipoprotein interactant, and a buffer, wherein said lipoprotein interactant is present at a concentration level that provides for conversion of HDL-cholesterol to a measureable colored product at a substantially different rate than for conversion of LDL-cholesterol to a measureable colored product and at a substantially different rate than for conversion of VLDL-cholesterol to a measureable colored product, or ii) α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer, wherein the ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02; and said second reagent a) comprises a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant, or b) a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a peroxidase.

[0045] Also disclosed herein are methods for the simultaneous, rapid measurement of at least two of TC, LDL-C and HDL-C in a sample of blood from a subject without substantial precipitation of said lipoproteins. ~~Embodiments~~Examples of methods for the simultaneous, rapid measurement of TC, LDL-C and HDL-C in a sample of blood from a subject without substantial precipitation of said lipoproteins comprise steps of: combining at an initial time at least a portion of said sample of blood with a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer to provide a combined solution; adding to said combined solution a second reagent comprising a buffer, an amphiphilic agent, a lipase, an oxidase, and a colorant to provide a colored solution; and measuring absorbance of light by said colored solution within a first time period, within a second time period, and within a third time period after addition of said second reagent.

[0046] In ~~embodiments~~examples of such methods, said first time period comprises a time period of less than about 3 minutes after said initial time, and said third time period comprises a time period of greater than about 5 minutes after said initial time, where said initial time comprises the time at which said second reagent is added to said combined solution.

[0047] In ~~embodiments~~examples, said methods for the simultaneous, rapid measurement of TC, LDL-C and HDL-C in a sample of blood from a subject without substantial precipitation of said lipoproteins comprise steps of: combining at an initial time at least a portion of said sample of blood with a first reagent comprising a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer to provide a combined solution; adding to said combined solution a second reagent comprising a buffer, an

amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant to provide a colored solution; and measuring absorbance of light by said colored solution within a first time period, within a second time period, and within a third time period.

[0048] In ~~embodiments~~examples of such methods, said first time period comprises a time period of less than about 3 minutes after said initial time, and said third time period comprises a time period of greater than about 5 minutes after said initial time, where said initial time comprises the time at which said second reagent is added to said combined solution.

[0049] In ~~embodiments~~examples of the methods disclosed herein, the first time period may comprise a time period of between about 0 minutes to about 2 minutes after said initial time, the second time period may comprise a time period of between about 2 minutes to about 6 minutes after said initial time, and the third time period may comprise a time period of between about 6 minutes to about 10 minutes, or longer. In ~~embodiments~~examples, said third time period comprises an open-ended time period including any time after about 5 minutes or about 6 minutes after said initial time. In ~~embodiments~~examples, the initial time is the time at which the second reagent is added to the solution.

[0050] It will be understood that other times and time periods may also be used, e.g., longer times and time periods may be used where the amounts of lipase, or oxidase, or both, are decreased. It will also be understood that, e.g., shorter times and time periods may be used where the amounts of lipase, or oxidase, or both, are increased. Similarly, other times and time periods may also be used where the amounts of lipoprotein solubilization agents and/or lipoprotein interactants are altered. Accordingly, in further ~~embodiments~~examples of the methods disclosed herein, the time periods may be longer than the time periods discussed above; for example, reagents and methods may be configured where the first time period may comprise a time period of between about 0 minutes to about 10 minutes after said initial time, the second time period may comprise a time period of between about 10 minutes to about 20 minutes or about 30 minutes after said initial time, and the third time period may comprise a time period of between about 20 minutes to about 40 minutes, or between about 30 minutes to about 60 minutes, or longer. In yet further ~~embodiments~~examples of the methods disclosed herein, the time periods may be shorter than the time periods discussed above; for example, reagents and methods may be configured where the first time period may comprise a time period of between about 0 minutes to about 1 minute after said initial time, the second time period may comprise a time period of between about 1 minutes to about 4 minutes or to about 5 minutes after said initial time, and the third time period may comprise a time period of between about 4 minutes to about 8 minutes, or between about 5 minutes to about 9 minutes.

[0051] In ~~embodiments~~examples, a step of measuring absorbance may comprise measuring absorbance using a spectrophotometer. A step of measuring absorbance will typically comprise measuring absorbance at or near a particular frequency using a spectrophotometer. In

~~embodiments~~examples, a step of measuring absorbance may comprise measuring absorbance at or near a wavelength of about 560 nanometers (nm). In ~~embodiments~~examples, a step of measuring absorbance may further comprise using a spectrophotometer to measure the difference in absorbance at or near to a first wavelength and the absorbance at or near to a second wavelength; such a difference in absorbance measured at two wavelengths may be termed "ΔA." For example, such two wavelengths may be about 560 nm and about 700 nm. In ~~embodiments~~examples, a step of measuring absorbance may further comprise using a spectrophotometer to measure absorbance at a wavelength of about 560 nm and at a wavelength of about 700 nm effective to obtain the difference in absorbance between about 560 nm and about 700 nm. It will be understood that for some colorants, other wavelengths, and other wavelength intervals, may be used; for example, there are chromogenic peroxidase substrates that may be detected at wavelengths between about 400 nm and about 700 nm.

[0052] Such absorbance measurements may be made at a particular time, or at two particular times, or at three, or more, particular times, where a particular time is defined with respect to an initial time. Measurements at a particular time may include measurements made during a range of times, such as between 0 to about 2 minutes after an initial time, or between about 2 minutes and about 6 minutes after an initial time, or between about 6 minutes and about 10 minutes after an initial time, or at other times and/or other time periods.

[0053] In ~~embodiments~~examples, a step of measuring absorbance may comprise measuring absorbance using a spectrophotometer at two times, and recording or calculating the difference between the measurements made at said two times. In ~~embodiments~~examples, absorbance may be ΔA measured as the difference in absorbance measured at two wavelengths, and the difference of the ΔA measured at a first time and the ΔA measured at a second time may be recorded or calculated; such a difference between ΔA measured at two times may be termed "ΔA_t." For example, ΔA_t may be measured by measuring ΔA as the difference between absorbance measured at 560 nm and at 700 nm, where the ΔA measurement is made at about 2 minutes after an initial time and at about 6 minutes after an initial time, where ΔA_t is the difference between the two ΔA measurements. In further ~~embodiments~~examples, ΔA_t may be measured by measuring ΔA as the difference between absorbance measured at 560 nm and at 700 nm, where the ΔA measurement is made at an initial time and at about 2 minutes after an initial time, where ΔA_t is the difference between the two ΔA measurements. In further ~~embodiments~~examples, ΔA_t may be measured by measuring ΔA as the difference between absorbance measured at 560 nm and at 700 nm, where the ΔA measurement is made at about 6 minutes after an initial time and at about 10 minutes after an initial time, where ΔA_t is the difference between the two ΔA measurements. In further ~~embodiments~~examples, where the ΔA measurement is to be made after a particular time, it will be understood that phrases such as, e.g., "after about 5 minutes", "after about 10 minutes", "at a time period greater than about 5 minutes", "at a

time period greater than about 10 minutes", and the like, may be measured between any time after the stated time and a reasonable time thereafter. In ~~embodiments~~examples, such a time period for measurement may not extend in time beyond about 30 minutes, or beyond about 20 minutes, after the initial time.

[0054] In ~~embodiments~~examples, a first reagent may comprise a lipoprotein solubilization agent, a lipoprotein interactant, and a buffer. In ~~embodiments~~examples, a lipoprotein solubilization agent may comprise a surfactant. In ~~embodiments~~examples, a lipoprotein interactant may comprise a dextran (such as a dextran derivative such as a sulfonated or phosphorylated dextran), a cyclodextrin (such as a cyclodextrin derivative such as a sulfonated or methylated cyclodextrin), a negatively charged natural product such as heparin, or combinations thereof. In ~~embodiments~~examples, a lipoprotein interactant may comprise a low molecular weight negatively charged dextran ester, e.g., a low molecular weight dextran sulfate. In ~~embodiments~~examples, a lipoprotein interactant may comprise an α- -cyclodextrin derivative such as an α- -cyclodextrin sulfate ester. In ~~embodiments~~examples, a first reagent may comprise a peroxidase substrate. In ~~embodiments~~examples, a peroxidase substrate may comprise an aminoantipyrene compound, such as 4-aminoantipyrene and an aniline-containing compound, such as ALPS. In further ~~embodiments~~examples, the first reagent may comprise α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene, ALPS, and a buffer, such as, for example, a phosphate buffer (e.g., Na_xPO_4 (where Na_xPO_4 is a sodium phosphate salt; e.g., may be NaH_2PO_4 , Na_2HPO_4 , or Na_3PO_4) for buffering the pH to between about pH 6 to about pH 8. For example, in ~~embodiments~~examples, the first reagent may comprise α-cyclodextrin sulfate (1 mM), dextran sulfate (20 μM), magnesium chloride (4 mM), 4-aminoantipyrene (2.25 mM), 3 mM ALPS, and Na_xPO_4 (100 mM), pH about 7.

[0055] In ~~embodiments~~examples, components of a peroxidase substrate may be separated into different reagents. Separation of peroxidase substrate components into different reagents may be desired for stability of one or both of the reagents, or may be useful to reduce or prevent degradation of one or both of the reagents. Thus, in ~~embodiments~~examples, a first reagent may include an antipyrene compound, and a second reagent may include an aniline-containing compound. In further ~~embodiments~~examples, a first reagent may comprise 4-aminoantipyrene and a second reagent may comprise N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS). In further ~~embodiments~~examples, a first reagent may comprise between about 1 mM and about 5 mM 4-aminoantipyrene and a second reagent may comprise between about 0.5mM and about 20 mM ALPS. For example, a first reagent may comprise 2.25 mM 4-aminoantipyrene and a second reagent may comprise 3 mM ALPS.

[0056] In ~~embodiments~~examples, components of a peroxidase substrate may be contained in a single reagent. Including multiple components in a single reagent may be desired for simplicity of

manufacture, or ease of use, or for other reasons. For example, a reagent containing all components of a peroxidase substrate may comprise an aminoantipyrene compound and an aniline-containing compound. In further ~~embodiments~~examples, a reagent containing all components of a peroxidase substrate may comprise 4-aminoantipyrene and N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS). In further ~~embodiments~~examples, a reagent containing all components of a peroxidase substrate may comprise between about 1 mM and about 5 mM 4-aminoantipyrene and between about 0.5mM and about 20 mM ALPS. For example, a reagent containing all components of a peroxidase substrate may comprise 2.25 mM 4-aminoantipyrene and 3 mM ALPS. In other ~~embodiments~~examples, reagents may be dried so as to increase stability. Reagents may be lyophilized or provided as glassy films, e.g., films which may adhere to a container wall. When such reagents are dried, they may be formulated so as to dissolve rapidly (e.g., within seconds to minutes) upon addition of water or of another aqueous solvent such as a buffer solution.

[0057] In ~~embodiments~~examples of the methods disclosed herein, a second reagent may comprise a lipase, an oxidase, and a colorant. In ~~embodiments~~examples of said second reagent, a lipase may comprise a cholesterol esterase, an oxidase may comprise a cholesterol oxidase, and a colorant may comprise a peroxidase. In ~~embodiments~~examples of the methods disclosed herein, a second reagent may comprise a buffer, an amphiphilic agent, a cholesterol esterase, a cholesterol oxidase, and a colorant. In ~~embodiments~~examples of the methods disclosed herein, an amphiphilic agent may comprise a surfactant. In ~~embodiments~~examples, a colorant may comprise a peroxidase, a peroxidase substrate, and may comprise both a peroxidase and a peroxidase substrate. A peroxidase may comprise, for example, a peroxidase from horseradish (*Armoracia rusticana*) (HRP). A peroxidase substrate may comprise an aniline-containing compound, an aminoantipyrene compound or both. In ~~embodiments~~examples, a colorant may comprise an aminoantipyrene compound, such as 4-aminoantipyrene. In ~~embodiments~~examples, a colorant may comprise N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS). In ~~embodiments~~examples of the methods disclosed herein, a second reagent may comprise a bacterial cholesterol esterase, such as a cholesterol esterase from a *Pseudomonas* bacterium, and in ~~embodiments~~examples may comprise a bacterial cholesterol oxidase, such as a bacterial cholesterol oxidase from a *Pseudomonas* bacterium. In ~~embodiments~~examples of the methods disclosed herein, a second reagent may comprise a buffer, such as a phosphate buffer (e.g., Na_xPO_4 (50 mM)), Triton X-100 (0.06%), pluronic L64 (3 g/L), N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS) (3 mM), cholesterol esterase from *Pseudomonas sp.* (750 U/L), and cholesterol oxidase from *Pseudomonas sp.* (1.5 kU/L).

[0058] In ~~embodiments~~examples, a composition as disclosed herein may comprise α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, a peroxidase substrate (e.g., diaminobenzidine,

or 4-aminoantipyrene and ALPS), cholesterol esterase (e.g., cholesterol esterase from *Pseudomonas sp.*), cholesterol oxidase (e.g., cholesterol oxidase from *Pseudomonas sp.*), a surfactant, and a buffer. In ~~embodiments~~examples, a composition as disclosed herein may comprise a first reagent and a second reagent. In ~~embodiments~~examples, a composition as disclosed herein may comprise a first reagent, a second reagent, and at least a portion of a sample of blood from a subject.

[0059] A buffer, for example, may be a phosphate buffer (e.g., Na_xPO_4 (where Na_xPO_4 is a sodium phosphate salt; e.g., may be NaH_2PO_4, Na_2HPO_4, or Na_3PO_4) for buffering the pH to between about pH 5 to about pH 9, or between about pH 6 to about pH 8, optionally between about pH 6.8 to about pH 7.8, e.g., pH 7.4. In ~~embodiments~~examples, a composition as disclosed herein may comprise between about 100 U/L to about 2000 U/L of cholesterol esterase from *Pseudomonas sp.* (e.g., about 750 U/L), and may comprise between about 100 U/L to about 3000 U/L cholesterol oxidase from *Pseudomonas sp.* (e.g., about 1.5 kU/L).

[0060] In ~~embodiments~~examples, for example, a peroxidase substrate may comprise between about 1 mM and about 5 mM 4-aminoantipyrene and between about 0.5mM and about 20 mM ALPS, e.g., about 2.25 mM 4-aminoantipyrene and about 3 mM ALPS. In ~~embodiments~~examples, a surfactant may be, for example, Triton X-100 and/or pluronic L64, e.g., the composition may comprise about 0.01 to about 1% Triton X-100 and may comprise about 1 to about 10 g/L /or pluronic L64; in particular ~~embodiments~~examples, the composition may comprise about 0.06 % Triton X-100 and about 3 g/L pluronic L64.

[0061] In ~~embodiments~~examples of the methods disclosed herein, a measurement within a first time period may be used to determine HDL-cholesterol in said sample. In ~~embodiments~~examples of the methods disclosed herein, a measurement within a second time period may be used to determine LDL-cholesterol in said sample. In ~~embodiments~~examples of the methods disclosed herein, a measurement within a third time period may be used to determine total cholesterol in said sample. In ~~embodiments~~examples of the methods disclosed herein, a measurement may comprise measuring absorbance using a spectrophotometer. In ~~embodiments~~examples of the methods disclosed herein, a measurement may comprise measuring ΔA (where ΔA is the difference between absorbance measured at the first wavelength and at the second wavelength) using a spectrophotometer. In ~~embodiments~~examples of the methods disclosed herein, a measurement may comprise measuring ΔA_t (where ΔA_t is the difference between absorbance measured at a first time and the absorbance measured at a second time) using a spectrophotometer. Absorbance measurements for measuring ΔA_t may be ΔA measurements.

[0062] In ~~embodiments~~examples of the methods disclosed herein, a method of analyzing cholesterol measurement data obtained from a blood sample of a subject may comprise steps of: measuring product formation from a reaction between cholesterol sub-fractions and a reagent mixture

comprising a lipase and an oxidase within a first time period, within a second time period, and within a third time period to provide cholesterol measurement data; wherein a low density lipoprotein-cholesterol (LDL-C) measurement is obtained from said cholesterol measurement data obtained within said second time period; wherein a total cholesterol (TC) measurement is obtained from said cholesterol measurement data obtained within said third time period; and wherein a high density lipoprotein-cholesterol (HDL-C) measurement is obtained from said cholesterol measurement data obtained within said second time period plus the rate of product formation measured in said first time period; whereby said cholesterol measurement data obtained from a blood sample of a subject is analyzed.

[0063] In particular ~~embodiments~~examples of the methods of analyzing cholesterol measurement data obtained from a blood sample of a subject disclosed herein, said lipase may comprise a cholesterol esterase, and said oxidase may comprise a cholesterol oxidase. For example, ~~in embodiments of the methods~~as disclosed herein, a method of analyzing cholesterol measurement data obtained from a blood sample of a subject may comprise steps of: measuring product formation from a reaction between cholesterol sub-fractions and a reagent mixture comprising a cholesterol esterase and a cholesterol oxidase within a first time period, within a second time period, and within a third time period to provide cholesterol measurement data; wherein a LDL-C measurement is obtained from said cholesterol measurement data obtained within said second time period; wherein a TC measurement is obtained from said cholesterol measurement data obtained within said third time period; and wherein a HDL-C measurement is obtained from said cholesterol measurement data obtained within said second time period plus the rate of product formation measured in said first time period; whereby said cholesterol measurement data obtained from a blood sample of a subject is analyzed.

[0064] ~~Embodiments~~Examples of a method of analyzing cholesterol measurement data obtained from a blood sample of a subject may comprise steps wherein said first time period may comprise less than about 3 minutes after an initial time, wherein said initial time comprises the time at which a second reagent is added to a combined solution, and wherein a combined solution may comprise a first reagent and at least a portion of a blood sample. In ~~embodiments~~examples, a method of analyzing cholesterol measurement data obtained from a blood sample of a subject may comprise a step wherein a third time period comprises a time period of greater than about 5 minutes after said initial time, wherein said initial time comprises the time at which a second reagent is added to a combined solution. In ~~embodiments~~examples, said first time period comprises a time period of between about 0 minutes to about 2 minutes after said initial time, said second time period comprises a time period of between about 2 minutes to about 6 minutes after said initial time, and said third time period comprises a time period of between about 6 minutes to about 10 minutes, or longer. In ~~embodiments~~examples, said third time

period may comprise an open-ended time period, effective to include any time greater than about 5 minutes after said initial time, or greater than about 6 minutes after said initial time.

[0065] In ~~embodiments~~examples, a step of measuring product formation comprises measuring absorbance. In ~~embodiments~~examples, a step of measuring absorbance comprises measuring ΔA using a spectrophotometer. In further ~~embodiments~~examples, a step of measuring absorbance using a spectrophotometer comprises measuring absorbance at a first wavelength and measuring absorbance at a second wavelength to obtain a ΔA measurement, where ΔA is the difference between absorbance measured at the first wavelength and at the second wavelength. In ~~embodiments~~examples, a step of measuring ΔA using a spectrophotometer comprises measuring ΔA at a wavelength of between about 300 nm to about 700 nm, and at a wavelength of between about 600 nm to about 900 nm. In more particular ~~embodiments~~examples, a step of measuring ΔA using a spectrophotometer comprises measuring ΔA at a wavelength of about 560 nm and at a wavelength of about 700 nm.

[0066] In at least some ~~embodiments~~examples, methods disclosed herein include further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, wherein an HDL-C measurement is obtained from said cholesterol measurement data obtained within a second time period plus the rate of product formation measured in a first time period according to the following formula:

$$K_1 - K_2 \times (\text{LDL-C}) + K_3 \times R_1$$

where K_1 is a constant whose value may be between about 0 to about 250;
where K_2 is a constant whose value may be between about 0 to about 100;
where the amount of LDL-C may be determined by the difference in absorbance measured between the beginning of said second time period and the end of said second time period;
where K_3 is a constant whose value may be between about 1 to about 20,000; and
where R_1 may be determined by the difference in absorbance measured between the beginning of said first time period and the end of said first time period, and wherein said time periods may be determined with respect to an initial time, wherein the initial time period comprises the time at which a second reagent is added to a combined solution comprising a first reagent and at least a portion of said blood sample.

In ~~embodiments~~examples, the value of K_1 may be between about 0 to about 100, or between about 0 to about 50. In ~~embodiments~~examples, the value of K_2 may be between about 0 to about 50, or between about 0 to about 25. In ~~embodiments~~examples, the value of K_3 may be between about 0 to about 10,000, or between about 0 to about 5,000. In further ~~embodiments~~examples, the value of K_1 may be between about 0 to about 25, or between about 0 to about 10. In further ~~embodiments~~examples, the value of K_2 may be between about 0 to about 5, or between about 0 to about 2.

[0067] In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, a first time period may comprise a time period of between about 0 minutes to about 2 minutes after an initial time. In ~~embodiments~~examples, a second time period may comprise a time period of between about 2 minutes to about 6 minutes after an initial time. In ~~embodiments~~examples, a third time period may comprise a time period of between about 6 minutes to about 10 minutes after an initial time, or longer. In ~~embodiments~~examples, said third time period may comprise an open-ended time period, effective to include any time greater than about 5 minutes after said initial time, or greater than about 6 minutes after said initial time.

[0068] In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_1 may have a value between about 0 to about 3. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_2 may have a value between about 0 to about 1. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_3 may have a value between about 800 to about 1,800. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_1 may have a value of about 1.30. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_2 may have a value of about 0.446. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, K_3 may have a value of about 1255.

[0069] In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, cholesterol measurement data may comprise data from an absorbance measurement. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, absorbance measurements may comprise absorbance measurements made using a spectrophotometer. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, an absorbance measurement using a spectrophotometer may comprise a ΔA measurement, where a ΔA measurement comprises an absorbance measurement made at a first wavelength and an absorbance measurement made at a second wavelength, effective to obtain the difference in absorbance at said first wavelength and at said second wavelength. In ~~embodiments~~examples of further methods of analyzing cholesterol measurement data obtained from a blood sample of a subject, a measurement of ΔA using a spectrophotometer may comprise absorbance measurements at a wavelength of about 560 nm and at a wavelength of about 700 nm.

[0070] Applicants further disclose herein devices for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject. In ~~embodiments~~examples, a device

for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject may comprise: means for combining a first reagent with at least a portion of a sample of blood from a subject, effective to provide a combined solution; means for combining a second reagent with said combined solution to provide a colored solution; means for measuring an optical property of said colored solution; and means for displaying or reporting the results of said measurement of said optical property of said colored solution. In further ~~embodiments~~examples, a device for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject may comprise: a chamber for combining a first reagent with at least a portion of a sample of blood from a subject, effective to provide a combined solution; a conduit for combining a second reagent with said combined solution to provide a colored solution; an optical detector for measuring an optical property of said colored solution; and a display element or a communication link for reporting the results of said measurement of said optical property of said colored solution. In ~~embodiments~~examples, a display element and/or communication link may be suitable for two-way communication.

[0071] Applicants disclose herein systems for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject. In ~~embodiments~~examples, a system for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject comprises a device as disclosed herein, and a means for communicating information from said device to a computer, a computer network, a telephone, a telephone network, or a device configured to display information communicated from said device. It will be understood that a means for communicating information may include means for one-way communication and may include means for two-way communication, and may include means for communication with multiple locations or entities. In ~~embodiments~~examples, a system for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject comprises a device as disclosed herein, and a channel for communicating information from said device to a computer, said wherein said channel is selected from a computer network, a telephone network, a metal communication link, an optical communication link, and a wireless communication link. It will be understood that a channel for communicating information may be a one-way communication channel and may be a two-way communication channel, and may include channels for communication with multiple locations or entities.

[0072] In ~~embodiments~~examples, triglyceride levels may be determined by methods in which a sample of blood is contacted with a lipase to provide fatty acids and glycerol from TG in the blood sample. This glycerol may be contacted with a kinase, such as glycerol kinase, to provide glycerol phosphate, which may be contacted with an oxidase, such as glycerol 3-phosphate oxidase, to provide dihydroxyacetone phosphate and hydrogen peroxide. Colorants, such as 4-aminoantipyrene and N-Ethyl-N-(sulfopropyl aniline) may be contacted with horseradish peroxidase in the presence of the hydrogen

peroxide effective to form a dye, such as a quinoneimine dye, the measurement of which dye provides a measure of the TG level in the blood sample. Such a dye may be measured by spectrophotometric means; for example, such a dye may be measured, and TG levels determined, by measuring absorbance at a wavelength (e.g., 560 nanometers (nm)), or within a wavelength range (e.g., 555 nm to 565 nm).

[0073] VLDL-C may be estimated from TG measurements by dividing the concentration of TG in a sample by 5:

$$VLDL\text{-}C = TG/5$$

in order to provide a value for the level of VLDL-C in blood sample in which TG has been measured. The level of VLDL-C estimated in this way may further be used to calculate the TC in a blood sample by adding the levels of HDL-C, LDL-C, and VLDL-C (as estimated according to the formula VLDL-C = TG/5).

[0074] However, in place of such an estimate, Applicants disclose herein further methods to calculate VLDL-C based on the novel and useful measurements of HDL-C, LDL-C, TC, and TG disclosed herein. These further methods are believed to provide greater accuracy than prior methods. In ~~embodiments~~examples, VLDL-C may be calculated from HDL-C, TC, and TG measurements, where these measurements are made according to the methods disclosed herein. In ~~embodiments~~examples, VLDL-C may be calculated from HDL-C, LDL-C, TC, and TG measurements, where these measurements are made according to the methods disclosed herein.

[0075] In ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from HDL-C, TC, and TG measurements, for example, by the relation:

$$VLDL\text{-}C = \alpha TC + \beta HDL\text{-}C + \delta TG + a_1(TG+\varepsilon)(TC+\kappa) + \lambda$$

where α, β, δ, and a_1 are constants which multiply TC, HDL-C, TG, and $(TG+\varepsilon)(TC+\kappa)$, respectively; and where ε, κ, and λ are additive constants to be added to TG, TC, and the sum of all other factors, respectively. The term "$(TG+\varepsilon)(TC+\kappa)$" may be called a "cross-term" in which the terms in one parenthesis multiply the terms in the other parenthesis. The multiplicative constants α, β, δ, and a_1 and the additive constants ε, κ, and λ may take any value, whether positive, negative, or zero.

[0076] In ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from HDL-C, LDL-C, TC, and TG measurements, for example, by the relation:

$$VLDL\text{-}C = \alpha TC + \beta HDL\text{-}C + \gamma LDL\text{-}C + \delta TG + a_1(TG+\varepsilon)(TC+\kappa) + \lambda$$

where α, β, γ, δ, and a_1 are constants which multiply TC, HDL-C, LDL-C, TG, and $(TG+\varepsilon)(TC+\kappa)$, respectively; and where ε, κ, and λ are additive constants to be added to TG, TC, and the sum of all other

factors, respectively. The multiplicative constants α, β, γ, δ, and a_1 and the additive constants ε, κ, and λ may take any value, whether positive, negative, or zero. As indicated above, and as used elsewhere herein, the term "(TG+ε)(TC+κ)" and similar terms may be called a "cross-term" in which the terms in one parenthesis multiply the terms in the other parenthesis.

[0077] It will be understood that, in ~~embodiments~~examples, all possible cross-terms, and all possible combinations of cross-terms may be included in relationships used to calculate VLDL-C. Thus, for example, in addition to, and/or in place of, the cross-term (TG+ε)(TC+κ), any or all of the following cross-terms may be included in relationships used to calculate VLDL-C: (TG+ε)(HDL-C+μ); (TG+ε)(LDL-C+ν); (TC+κ)(HDL-C+μ); (TC+κ)(LDL-C+ν); and (LDL-C+ν)(HDL-C+μ), where the additive constants λ, ε, κ, μ, and ν may take any value, whether positive, negative, or zero.

[0078] Accordingly, in ~~embodiments~~examples, VLDL-C may be calculated from the measured quantities as follows:

[0079] $VLDL\text{-}C = \alpha TC + \beta HDL\text{-}C + \gamma LDL\text{-}C + \delta TG + \lambda + a_1(TG+\varepsilon)(TC+\kappa) + a_2(TG+\varepsilon)(HDL\text{-}C+\mu) + a_3(TG+\varepsilon)(LDL\text{-}C+\nu) + a_4(TC+\kappa)(HDL\text{-}C+\mu) + a_5(TC+\kappa)(LDL\text{-}C+\nu) + a_6(LDL\text{-}C+\nu)(HDL\text{-}C+\mu)$

where the multiplicative coefficients α, β, γ, δ, a_1, a_2, a_3, a_4, a_5, and a_6, may take any value, whether positive, negative, or zero; and

where the additive constants λ, ε, κ, μ, and ν may take any value, whether positive, negative, or zero.

[0080] In yet further ~~embodiments~~examples, the level of VLDL-C in a single sample, or a single portion of a sample, of blood of a subject may be calculated from other relationships using some or all of the measured values of TC, TG, HDL-C, and LDL-C and multiplicative and additive constants similar in form to the relationships provided above.

[0081] Applicants further disclose herein methods, devices and systems for measuring, in one device, at least two biologically relevant attributes of a sample. In ~~embodiments~~examples, a biologically relevant attribute of a sample includes, without limitation, a lipoprotein level including TC, HDL-C, LDL-C, and VLDL-C; blood triglyceride (TG) level; blood hematocrit; blood iron content; erythrocyte sedimentation rate (ESR); a cytometric measurement (e.g., determination of the presence and/or amounts, including fractional amounts and/or absolute amounts of a cell type or cell types) including antibody based, dye-based, flow-cytometric, imaging, and other measurements of cells, cell number, cell type, and cellular properties in a sample; a chemical measurement including a measurement of pH, of a salt concentration (e.g., of a cation and/or anion which makes up a salt), presence of and/or concentration of a vitamin, a protein, a metabolite of a protein or small molecule, a marker indicative of a medical condition, and other measurements of blood, urine, tissue, or other biological sample. The measurements of at least two biologically relevant attributes of a sample may be made simultaneously, or may be made in

succession, or, where at least three measurements of biologically relevant attributes of a sample are made, some measurements may be made simultaneously while other measurements of biologically relevant attributes of a sample may be made before or after the measurements that are made simultaneously.

[0082] In ~~embodiments~~examples, one or more of such measurements may be rapid measurements, where a rapid measurement is one that may be made within a time period of about one hour; or may be made within a time period of about one half an hour; or may be made within a time period of about one quarter of an hour; or may be made within a time period of about ten minutes; or may be made within a time period of about 5 minutes; or may be made within a time period of about 4 minutes; or may be made within a time period of about 3 minutes; or may be made within a time period of about 2 minutes; or may be made within a time period of about 1 minute; or may be made within a time period of about 30 seconds; or may be made within a time period of about 15 seconds; or may be made within a time period of about 10 seconds; or may be made within a time period of about 5 seconds; or may be made within a time period of about 1 second.

[0083] In ~~embodiments~~examples, one or more of such measurements may be made using a small volume blood sample, or no more than a small volume portion of a blood sample, where, a small volume blood sample, and a small volume portion of a blood sample, comprises no more than about 5 mL; or comprises no more than about 3 mL; or comprises no more than about 1 mL; or comprises no more than about 250 μL; or comprises no more than about 100 μL; or comprises no more than about 50 μL; or comprises no more than about 25 μL; or comprises no more than about 20 μL; or comprises no more than about 15 μL; or comprises no more than about 10 μL; or comprises no more than about 5 μL; or comprises no more than about 1 μL; or comprises other small volume, e.g., as described herein.

[0084] The assays and methods disclosed herein may be performed on a device, or on a system, for processing a sample. The assays and methods disclosed herein can be readily incorporated into and used in an automated assay device, and in an automated assay system. For example, systems as disclosed herein may include a communication assembly for transmitting or receiving a protocol based on the analyte to be detected (e.g., total cholesterol (TC), a cholesterol sub-fraction (e.g., LDL, HDL, VLDL, etc.), triglycerides (TG), or other analyte) or based on other analytes to be detected by the device or system. In ~~embodiments~~examples, an assay protocol may be changed based on optimal scheduling of a plurality of assays to be performed by a device, or may be changed based on results previously obtained from a sample from a subject, or based on results previously obtained from a different sample from the subject. In ~~embodiments~~examples, a communication assembly may comprise a channel for communicating information from said device to a computer, said wherein said channel is selected from a computer network, a telephone network, a metal communication link, an optical communication link, and a wireless communication link. In ~~embodiments~~examples, systems as disclosed herein may transmit

signals to a central location, or to an end user, and may include a communication assembly for transmitting such signals. Systems as disclosed herein may be configured for updating a protocol as needed or on a regular basis.

[0085] Accordingly, Applicants disclose devices configured to measure TC or a cholesterol sub-fraction, TG, and combinations thereof in a sample of blood according to a method disclosed herein. Devices configured to measure TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes in a sample of blood according to a method disclosed herein may be configured to determine TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes from a sample of blood that comprises no more than about 1000 μL of blood, or no more than about 500 μL of blood, no more than about 250 μL of blood, or no more than about 150 μL of blood, or no more than about 100 μL of blood, or no more than about 50 μL of blood, or, in ~~embodiments~~examples, wherein said sample of blood comprises no more than about 25 μL of blood, or wherein said sample of blood comprises no more than about 10 μL of blood, or wherein said sample of blood comprises less than about 10 μL of blood. Such devices may be configured to measure TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes in a sample of blood in less than about one hour, or, in ~~embodiments~~examples, in less than about 40 minutes, or in less than about 30 minutes.

[0086] Devices disclosed herein may be configured to perform an assay for the measurement of TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes and also to perform an assay for the measurement of another analyte in the blood sample. Devices disclosed herein may be configured to perform an assay for the measurement of TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes and also to perform an assay comprising the measurement of a morphological characteristic of a blood cell in the blood sample. Devices disclosed herein may be configured to perform an assay for the measurement of TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes and also to perform an assay comprising the measurement of another blood analyte, e.g., a vitamin, a hormone, a drug or metabolite of a drug, or other analyte. Such devices may be configured wherein the assays, or the order of performance of assays, that are performed by said device may be altered by communication with another device.

[0087] Applicants also disclose systems comprising a device as disclosed herein. In ~~embodiments~~examples, the system comprises a device that is configured to perform an assay for the measurement of TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes and also to perform an assay for the measurement of another analyte in the blood sample. In ~~embodiments~~examples, the system comprises a device that is configured to perform an assay for the measurement of TC, a cholesterol sub-fraction, TG, and other lipid fractions and analytes and also to perform an assay for the measurement of a morphological characteristic of a blood cell in the blood sample. In

~~embodiments~~examples of such a system, assays, or the order of performance of assays, that are performed by said device may be altered by communication with another device.

[0088] In ~~embodiments~~examples of devices and systems disclosed herein, a device may comprise a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another analyte or attribute of said sample of blood from the subject. In ~~embodiments~~examples, said other analyte or attribute may include TG, and may include TG and a further analyte or attribute of the blood. In ~~embodiments~~examples, said other analyte or attribute may be measured on a different portion of the sample of blood from the subject than the portion used to measure TC or a cholesterol sub-fraction. In ~~embodiments~~examples, said other analyte or attribute may be measured on the same portion of the sample of blood from the subject as is used to measure TC or a cholesterol sub-fraction. In ~~embodiments~~examples, a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another analyte or attribute of said sample of blood from the subject may comprise: means for combining a first reagent with at least a portion of a sample of blood from a subject, effective to provide a combined solution; means for combining a second reagent with said combined solution to provide a colored solution; means for measuring an optical property of said colored solution; means for displaying or reporting the results of said measurement of said optical property of said colored solution; and means for measuring another attribute of said sample of blood from the subject. In ~~embodiments~~examples, said other attribute may include TG, and may include TG and a further attribute of the blood. In ~~embodiments~~examples, a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another attribute of said sample of blood from the subject may be configured for, and able to, make any two or more of a plurality of measurements on a sample, such as a blood sample. In ~~embodiments~~examples of a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another attribute of said sample of blood from the subject, said measurements may be selectable effective that some or all of a plurality of measurements are selected for measurement of a first sample, and a different selection of some or all of said plurality of measurements may be selected for measurements to be performed on a second sample. In ~~embodiments~~examples, said other attribute may include TG, and may include TG and a further attribute of the blood.

[0089] In further ~~embodiments~~examples, a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another attribute of said sample of blood from the subject may comprise: a chamber for combining a first reagent with at least a portion of a sample of blood from a subject, effective to provide a combined solution; a conduit for combining a second reagent with said combined solution to provide a colored solution; an optical detector for

measuring an optical property of said colored solution; a centrifuge; and a display element or a communication link for reporting the results of said measurement of said optical property of said colored solution. In ~~embodiments~~examples, said other analyte or attribute may include TG, and may include TG and a further analyte or attribute of the blood. In ~~embodiments~~examples of such a device, the device may be configured for, and able to, make any two or more of a plurality of measurements on the sample. In ~~embodiments~~examples of such a device, said measurements may be selectable effective that some or all of a plurality of measurements are selected for measurement of a first sample, and a different selection of some or all of said plurality of measurements may be selected for measurements to be performed on a second sample.

[0090] Systems for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a blood sample, and another biologically relevant attribute from said blood sample from a subject may include a device for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another analyte or attribute of said sample of blood from the subject. In ~~embodiments~~examples, said other analyte or attribute may include TG, and may include TG and a further analyte or attribute of the blood. In ~~embodiments~~examples, such a system comprises a device for measuring both a) and b) as disclosed herein, and a means for communicating information from said device to a computer, a computer network, a telephone, a telephone network, or a device configured to display information communicated from said device. In ~~embodiments~~examples, a system for measuring both a) TC or a cholesterol sub-fraction in at least a portion of a sample of blood from a subject, and b) another analyte or attribute of said sample of blood from the subject comprises a device as disclosed herein, and a channel for communicating information from said device to a computer, said wherein said channel is selected from a computer network, a telephone network, a metal communication link, an optical communication link, and a wireless communication link. Systems as disclosed herein may comprise a device configured for, and able to, make any two or more of a plurality of measurements on the sample. In ~~embodiments~~examples of systems comprising such a device, said measurements may be selectable effective that some or all of a plurality of measurements are selected for measurement of a first sample, and a different selection of some or all of said plurality of measurements may be selected for measurements to be performed on a second sample.

[0091] ~~Embodiments~~Examples of systems for measuring total cholesterol or a cholesterol sub-fraction in at least a portion of a blood sample, and another biologically relevant analyte or attribute from said blood sample from a subject may include a device as disclosed in U.S. Patent 8,088,593 or U.S. App. No. 13/244,947 filed Sept. 26, 2011, both fully incorporated herein by reference for all purposes, and may include systems as disclosed therein. For example, systems as disclosed herein may include a communication assembly for transmitting or receiving a protocol based on the analyte to be detected or

based on other analytes to be detected by the device or system. For example, in ~~embodiments~~examples, an assay protocol may be changed based on optimal scheduling of a plurality of assays to be performed by a device, or may be changed based on results previously obtained from a sample from a subject, or based on results previously obtained from a different sample from the subject. In ~~embodiments~~examples, a communication assembly may comprise a channel for communicating information from said device to a computer, said wherein said channel is selected from a computer network, a telephone network, a metal communication link, an optical communication link, and a wireless communication link. In ~~embodiments~~examples, systems as disclosed herein may transmit signals to a central location, or to an end user, and may include a communication assembly for transmitting such signals. Systems as disclosed herein may be configured for updating a protocol as needed or on a regular basis.

[0092] Assays, methods, reagents, kits, devices, and systems as disclosed herein provide advantages over prior assays, methods, reagents, kits, and systems by allowing the rapid and inexpensive measurement of cholesterol and cholesterol sub-fractions in a single assay. The methods and assays disclosed herein allow for measurement of multiple lipoprotein fractions on the same sample of blood, or on the same portion of a sample of blood; for example, HDL-C, LDL-C, and TC may be measured on the same sample of blood, or on the same portion of a sample of blood. These measurements are made without substantial precipitation of lipoproteins in the sample, or sample portion. Providing desired measurements in a single assay simplifies procedures, reduces likelihood of error, reduces variability of results, and allows for more rapid and more inexpensive procedures. The disclosed assays, methods, reagents, kits, and systems are effective to reduce the number of assays required to determine TC, HDL-C, LDL-C, and other cholesterol sub-fractions and blood lipoprotein components (e.g., VLDL-C and TG). Accordingly, the assays, methods, reagents, kits, devices, and systems disclosed herein provide improvements over the art.

[0093] Methods and compositions disclosed herein provide rapid assays which require only small amounts of sample, such as only small amounts of blood. Device and systems disclosed herein are configured to perform such rapid assays which require only small amounts of sample, such as only small amounts of blood. Accordingly, the methods, compositions, devices, and systems provide rapid assays, which require only small biological samples, and thus provide further advantages over other methods, compositions, assays, devices, and systems in requiring only small biological samples and in providing rapid assays.

[0094] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not