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 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*~~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~~ seventh 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~~ eigth 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~~ neinth 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~~ 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 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~~ eleventh 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~~ twelvth aspect, there is provided ~~A~~ 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 thirteenth 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.

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.

22. The reagent of claim 21, comprising α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer.

23. The reagent of claim 21, said reagent comprising magnesium and dextran sulfate in a ratio of dextran sulfate to magnesium ions effective that there is no substantial precipitation of low-density lipoprotein (LDL), or that there is no substantial precipitation of very low-density lipoprotein (VLDL), or both.

24. The reagent of claim 23, wherein said ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02.

25. The reagent of claim 21, wherein said lipoprotein solubilization agent is selected from a surfactant, and a negatively charged α-cyclodextrin derivative; and said lipoprotein interactant comprises a low molecular weight negatively charged dextran derivative.

26. 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.

27. 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.

28. 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, said 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.*

29. A kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent of any of claims 21 to 27.

30. 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.

31. A kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent of claim 28.

32. 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.*

33. 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 of any of claims 21 to 27, and said second reagent comprises a reagent of claim 28.

34. 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 of claim 25, and said second reagent comprises the reagent of claim 28.

35. 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 of claim 26 or claim 27, and said second reagent comprises either a) 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.

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.

22. The reagent of claim 21, comprising α-cyclodextrin sulfate, dextran sulfate, magnesium chloride, 4-aminoantipyrene and a sodium phosphate buffer.

23. The reagent of claim 21, said reagent comprising magnesium and dextran sulfate in a ratio of dextran sulfate to magnesium ions effective that there is no substantial precipitation of low-density lipoprotein (LDL), or that there is no substantial precipitation of very low-density lipoprotein (VLDL), or both.

24. The reagent of claim 23, wherein said ratio of dextran sulfate to magnesium ions is between about 0.002 to about 0.02.

25. The reagent of claim 21, wherein said lipoprotein solubilization agent is selected from a surfactant, and a negatively charged α-cyclodextrin derivative; and said lipoprotein interactant comprises a low molecular weight negatively charged dextran derivative.

26. 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.

27. 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.

28. 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.

29. The reagent of claim 28, comprising a cholesterol esterase, a cholesterol oxidase, and a peroxidase.

30. The reagent of claim 28, wherein said 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.

31. The reagent of claim 28, wherein said colorant comprises one or more of a peroxidase, a peroxidase substrate, an aminoantipyrene compound, and an aniline-containing compound.

32. The reagent of claim 28, 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.*

33. 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.

34. 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.

35. A kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent of any of claims 21 to 27.

36. 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.

37. A kit comprising a reagent and instructions for the use of said reagent in a cholesterol assay, the reagent comprising a reagent of any of claims 28 to 34.

38. 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.*

39. 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 of any of claims 21 to 27, and said second reagent comprises a reagent of any of claims 28 to 34.

40. 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 of claim 25, and said second reagent comprises the reagent of claim 32.

41. 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 of claim 26 or claim 27, and said second reagent comprises the reagent of claim 33 or claim 34.