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(54) Title: METHOD AND COMPOSITION FOR ELEVATED LIPID QUALITY CONTROL MATERIALS

(57) Abstract

Quality control materials are produced by combining non-primate animal sera or plasma with a non-animal alpha lipoprotein concentrate and lyophilizing. The resulting novel compositions are stable, optically clear upon reconstitution and contain no potentially hepatitis infective human plasma protein fractions.

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METHOD AND COMPOSITION FOR ELEVATED LIPID QUALITY CONTROL MATERIALS

BACKGROUND ART

This invention relates to the preparation of clinical chemistry controls and standards. In particular it relates to the manufacture of controls and standards having cholesterol and triglyceride concentrations above the normal mean for human serum.

Clinical chemistry assays for lipids such as cholesterol and triglycerides in serum are commonly conducted in the laboratory. The accuracy and precision of these assays are continuously evaluated by conducting the test sample assays in tandem with controls or calibrators. The controls and calibrators contain a known amount of lipid and thus provide results against which the continuing performance of the assays can be compared. In addition, quantitative assays depend upon the preparation of a standard curve. Such curves are prepared by assaying a plurality of standards containing

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different predetermined quantities of the various lipids. Hereinafter controls, standards and calibrators will be collectively referred to as quality control materials.

Since the amount of cholesterol and triglycerides in a patient's serum is used for clinical diagnosis and drug therapy monitoring, it is necessary that the quantity be accurately and consistently determined. Thus it is important that day-to-day or test-to-test variation in the assay be carefully controlled.

Lipid-containing control materials for the assay of human test samples are designed to mimic the performance of the test samples as closely as possible. Thus when cholesterol and triglyceride supplements are added to a base plasma or serum they are supplied as complexes with protein, just as they are found in test samples. Such lipoproteins are available from a wide variety of sources such as plasma or eggs, and they have the additional advantage of water solubility.

A disadvantage of using lipoproteins as carrier-solubilizers for cholesterol or triglycerides is the fragility of the protein-lipid complexes. They are sensitive to the pH, ionic strength and temperature of their environment and to abuse such as shear and concentration effects. One particular problem has been the reduction in lipoprotein water solubility brought about by lyophilization of plasma or serum containing quality control materials. This reduction in solubility is evidenced by the development of turbidity in the materials. This is obviously unsatisfactory because the precipitated lipids will perform differently in lipid assays when compared with test

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samples containing native lipoproteins, the control materials will not be homogeneous and the tubidity will interfere with analytical equipment such as spectrophotometers. The preparation of a lipoprotein-containing control material which can be lyophilized and reconstituted to a clear solution has been a particular focus of the art.

Proksch et al, "Clin. Chem." 22/4:456-460 (1976) disclose preparing an optically clear lyophilized human serum as a control. This was done by precipitating pre-beta and beta-lipoproteins from human serum with dextran sulfate, removing the precipitate and lyophilizing the supernatant. The difficulty with this approach was that precipitating these lipoproteins decreased the cholesterol and triglyceride levels to about 25% of the original values. This was understandable when it is considered that 60-80% of the total weight of lipoprotein in human serum consists of very low density (pre-beta) or low density (beta) lipoproteins, and these lipoproteins are known to be the principal plasma carriers for cholesterol and triglycerides. Thus, this control per se was not useful as a control for normal or elevated lipid concentrations in human serum.

U.S. Patents 4,045,176 and 3,955,925 disclose two types of quality control materials which are optically clear upon reconstitution and which contain normal or elevated concentrations of lipids. One control material was made by adding high density human lipoproteins (alpha lipoproteins) to substantially human serum. The high density lipoproteins were stable

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to lyophilization, yet carried sufficient lipid to enable the preparation of an elevated lipid control.

A second control material was made by adding isolated, non-primate lipoproteins to substantially human serum. According to the patentees it was not necessary to first separate the high and low density lipoproteins as was required when using human lipoproteins, and that the low density non-primate lipoproteins could be included without substantial adverse effect. Bovine lipoproteins were most preferred.

While U.S. Patents 4,045,176 and 3,955,925 teach that isolated bovine lipoprotein can be added to human blood serum without adverse effect, the same could not be said for the addition of isolated bovine lipoprotein to serum other than that from humans. U.S. Patent 3,764,556 teaches isolating cholesterol-rich fractions from egg or human plasma and adding them to bovine or equine sera. This was done to enhance the normally low triglyceride and cholesterol concentrations in bovine and equine sera, thereby enabling their use as normal or elevated lipid controls in the assay of human serum test samples. Use of an animal base serum reduced the cost of the controls and, when egg lipoprotein was used, eliminated the hepatitis risk associated with human serum. However, this patent also taught that the cholesterol-rich fraction obtained from bovine or horse sera (as opposed to human) contains low cholesterol levels and, more importantly, the fraction tends to become insoluble after addition to animal sera.

It would be desirable to prepare a control serum that is free of human serum or plasma lipoprotein fractions but which

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contains normal or elevated triglyceride and cholesterol concentrations. The use of an egg lipoprotein concentrate with a non-primate base is not practical; U.S. Patent 4,045,176 discloses that the egg yolk fraction of U.S. Patent 3,764,556 must be used the same day it is prepared since a precipitate forms upon freezing and thawing. In addition, the egg yolk extract does not supply significant amounts of cholesterol.

Accordingly, it is an object of this inveniton to prepare an animal serum or plasma-based lipid quality control material having cholesterol and triglyceride concentrations of at least the levels found in normal human plasma or serum.

It is a further object of this invention to eliminate potentially hepatitis infective human plasma or serum protein components from controls and standards.

It is an additional object to provide an animal serum or plasma-based lipid quality control material which may be lyophilized and reconstituted to a substantially clear solution.

These and other objects will be apparent from consideration of this specification as a whole.

SUMMARY OF THE INVENTION

Applicants have discovered that, contrary to the teachings of the art, a stable quality control material can be prepared by combining a lipoprotein concentrate from animals with an animal sera if the lipoprotein concentrate is enriched in high density or alpha lipoproteins. Applicants have found that it is essential to remove animal chylomicrons as well as low density and very low density lipoproteins from animal lipoprotein

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concentrates before supplementing animal serum with the concentrate.

Accordingly, the objects of this invention are achieved by mixing substantially normal animal plasma or serum with animal high density lipoproteins which are substantially free of animal chylomicrons and low density and very low density lipoproteins. The resulting novel quality control materials should be substantially free of human plasma protein fractions. They can be reconstituted after lyophilization to yield clear solutions.

DETAILED DESCRIPTION OF INVENTION

The animal plasma or serum chosen as raw material for preparation of the lipoprotein concentrate is not critical, although for the purposes herein "animal" is intended to exclude all primates. Suitable sources are cows, horses, chickens, sheep and swine. While the alpha lipoprotein content of animal plasma varies somewhat this should not be a major factor in selecting a suitable source other than from consideration of yield. Bovine and equine serum is most suitable, although bovine is preferred.

The animal high density lipoproteins are obtained by known processes. For example, the lipoprotein II fraction disclosed in Proksch et al., "Clin. Chem." 22/8:1302-1305 (1976) is suitable, as is the bovine high density lipoprotein fraction produced in accordance with Jonas, "J. Biological Chemistry" 247(23):7767-7772 (1972) or Example 1 of U.S. Patent 4,045,176. Animal lipoprotein concentrates useful herein will contain from

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about 80% to 100% alpha lipoproteins by lipid electrophoresis (Sebia electrophoresis cell) and, if purified to a lipoprotein concentrate essentially free of other proteins, serum should have a specific gravity of greater than about 1.065. More preferably the lipoprotein content of the fractions will be about 90% to 95% alpha lipoproteins, preferably about 90%, with the remainder being pre-beta and beta globulins. The less the amount of beta and pre-beta lipoproteins the more suitable the concentrate will be from the standpoint of clarity. However, very high alpha lipoprotein purity raises costs and increases the amount of concentrate needed to supply a given triglyceride value. Such concentrates will generally contain about from 0.30 - 0.50 g triglyceride/liter and about from 10 to 15 g cholesterol/liter. Cholesterol and cholesterol esters will constitute about from 20% to 45% by weight of the lipoprotein, and triglycerides about 15% to 30% by weight. Generally, one will select concentrates having the highest alpha lipoprotein concentration, followed by selection on the basis of the highest cholesterol content. Non-lipoprotein serum proteins which are lyophilization stable, e.g., X_2-globulins, can be included in the lipoprotein concentrates used herein. It is not desirable to have large amounts of gamma globulin, fibrinogen or albumin in the concentrate.

Pooled animal serum will function as the base to which the concentrate is added. It is preferred to use serum from an animal having a low beta and pre-beta lipoprotein content when compared with human plasma. Suitable sources are bovine, equine and sheep serum, with bovine being preferred.

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Commercially available animal plasma is easily converted to serum by clotting with calcium and thrombin and removing the clot by centrifugation, along with any remaining contaminant blood cells. Serum is stored frozen. The serum is a clear, amber fluid from which cellular elements and most fibrinogen have been removed, and it contains components of clinical interest in assays other than those for lipids, e.g., salts, enzymes, glucose, protein and urea. The serum should be supplemented with any of these components which are clinically significant if it is normally deficient in them, as is conventional practice in the art. In accordance with this invention the cholesterol and triglyceride components are supplemented by adding the lipoprotein concentrate described above to the serum. The base serum may also be processed to reduce its beta and pre-beta lipoprotein content, e.g. by selective precipitation with heparin or dextran sulfate in accordance with known processes. All serum starting materials should be free of chylomicrons.

The general process for making quality control materials ordinarily starts with thawing and pooling sera which had been prepared and stored previously. Pooling means combining the sera from a plurality of separate donors into batches that can be economically processed on a commercial scale. The pooled serum is filtered through successively finer filters, terminating in filtration through a 0.3u pore diameter filter.

Then albumin, cholesterol and triglycerides are added to bring the serum up to the desired protein, cholesterol and triglycerides concentrations. The alpha lipoprotein concen-

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trates can provide all cholesterol which is needed and in most cases all the protein and triglycerides as well. If not, then protein and triglycerides can be supplemented from the exogenous sources. Any other constituents that are present in insufficient concentration are then added as required. The pooled, adjusted serum is filled into vials, lyophilized and sealed.

The amount of concentrate to be added to the serum depends upon the amount of triglycerides and cholesterol present in the base serum and in the concentrate. These quantities can be measured conventionally and the proper quantity of concentrate to be added readily calculated. Since the concentrate is more suitable for cholesterol enhancement this will be the controlling component. Generally, the high density lipoprotein concentrate will contain about from 20% to 45% cholesterol by weight of lipoprotein, but this will vary depending upon the species from which the lipoprotein was obtained and the particular lipoprotein fraction obtained by the concentration method employed in that particular instance.

The concentrate is typically added to bring the base serum cholesterol content at least up to the level of normal human serum, i.e. about from 1.2 to 2.5 g cholesterol (free plus ester)/ liter. Triglyceride should be brought up to about from 0.3 to 0.5 g/liter if possible without introducing more cholesterol than is desired. This invention has its principal utility in making hypercholesterolemic sera, i.e., sera in which the cholesterol content is about from 2.5 to 4.5 g/liter. Sera having

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concentrations throughout this range may be lyophilized and reconstituted to optically satisfactory solutions.

The pooled animal sera produced by combining the above-described concentrates with an animal serum base contain a concentration of lipoprotein lipid in excess of that found in pooled serum from animals of the same species. The compositions of this invention include pooled animal sera, particularly that from cattle and horses, which contain lipoprotein-bound cholesterol at a cholesterol concentration of greater than about 1.2g/l, preferably greater than about 2.0 g/l and up to about 4.5g/l.

The novel products of this invention contain an enriched proportion of alpha lipoproteins, i.e., a proportion by weight of total lipoproteins and by weight/volume above that found in normal pooled serum and frequently a level above that in the serum of hypercholesterolemic specimens of the animal in question. Frequently the alpha lipoprotein concentration will be in excess of 25% weight/volume above that of normal pooled plasma or serum. The level of alpha lipoprotein also may be described in terms of the weight ratio of alpha lipoproteins to beta and pre-beta lipoproteins. In the case of bovine sera, the ratio of alpha lipoproteins to beta and pre-beta lipoproteins is at least 3.75:1, usually about 4:1. Sheep sera having a ratio of at least 3.1 to 1, preferably about 3.5 to 1 are also useful but not as desirable as the more conveniently available bovine serum. The lipid, e.g. cholesterol concentration is generally at least about 25% by weight/volume above that of normal pooled plasma or serum.

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It is contemplated that cholesterol concentrates from one animal species can be added to a base serum of another animal. For example, bovine concentrates can be used with normal, pooled horse serum. While the products herein are preferably substantially free of human lipoproteins, contaminant human lipoproteins may accompany supplemental analytes such as human proteins added to adjust analyte concentrations as described above.

EXAMPLE

The pH of a bovine alpha lipoprotein concentrate (Irvine Scientific) having a specific gravity of 1.008 and a cholesterol content of 1,322 mg/dl (DuPont ACA method) was adjusted to 8.0 by 1N N_aOH. The concentrate had a specific gracity of 1.008 because it was not purified alpha lipoproteins; it contained a large proportion of bovine serum non-lipoprotein proteins. This conclusion was confirmed by electrophoresis in known fashion on a Sebia electrophoresis cell using a lipid-active gel and total protein electrophoresis using a Corning system. The pattern observed with the latter assay was consistent with large amounts of non-lipoprotein proteins. The former assay demonstrated only a faint band at the very low density and chylomicron positions, but had a strong, broad stain identified as high density lipoprotein shading faintly into low density lipoprotein. This concentrate was clarified by filtration through a 0.3u pore diameter filter to a final volume of 49,800 ml.

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648.2 liters of filtered bovine serum was mixed gently with the concentrate. The serum before adding the concentrate had a cholesterol content of 118 mg/dl. The concentration afterwards was 2.13 g/l. The product was filled into vials and lyophilized. Upon reconstitution it was of satisfactory clarity and no significant changes in clarity were observed after refrigerated storage for two days.

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WE CLAIM:

1. A method for making quality control materials, comprising combining a non-primate animal plasma or serum with non-primate animal alpha lipoproteins which are substantially free of chylomicrons and low density and very low density non-primate animal lipoproteins.

2. The method of Claim 1 wherein substantially no human lipoproteins are added to the quality control materials.

3. The method of Claim 1 wherein the plasma or serum is bovine.

4. The method of Claim 1 wherein the plasma or serum is equine.

5. The method of Claim 1 wherein animal serum is combined with the alpha lipoproteins.

6. The method of Claims 3 or 4 wherein sufficient alpha lipoproteins are combined with the serum to produce a cholesterol concentration in the quality control material of about from 2.0 to 4.5 g/liter.

7. The method of Claim 6 wherein sufficient alpha lipoproteins are combined to produce a cholesterol concentrate of about from 2.5 to 4.5 g/liter.

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8. The method of Claim 1 wherein the animal plasma or serum contains substantially normal concentrations of constituents for the species from which the plasma or serum was obtained.

9. The method of Claim 5 wherein the serum is bovine and sufficient alpha lipoproteins are combined with the serum to produce at least a 3.75:1 ratio of alpha lipoproteins to the sum of beta and pre-beta lipoproteins.

10. The method of Claim 1 wherein the plasma or serum is a pool of plasma or serum donations from discrete donor individuals.

11. The method of Claim 10 wherein sufficient alpha lipoproteins are combined with the plasma or serum to produce a cholesterol concentration in the quality control material of about from 1.2 to 4.5 g/liter.

12. The method of Claim 1 wherein the serum or plasma is from different animal species than the alpha lipoprotein.

13. The method of Claim 10 wherein sufficient alpha lipoproteins are combined with the serum or plasma to produce quality control material having a cholesterol concentrate greater than that of normal pooled plasma or serum from the same species from which the serum of plasma originated.

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14. The method of Claim 13 wherein the cholesterol concentration is greater than about 25% above that of the normal pooled plasma or serum.

15. The method of Claim 10 wherein the alpha lipoproteins are combined with the serum or plasma to produce a quality control material having a weight of alpha lipoproteins/volume greater than that of normal pooled plasma or serum from the same species.

16. The method of Claim 15 wherein the concentration of alpha lipoproteins is greater than about 25% above that of the normal pooled plasma or serum.

17. The method of Claim 1 wherein after combining the plasma or serum with animal alpha lipoproteins the level of non-lipid, clinically significant serum or plasma constituents is adjusted to within the range found in human serum and the adjusted plasma or serum lyophilized.

18. A quality control material comprising bovine serum and animal alpha lipoprotein, said material having at least a 3.75:1 ratio of alpha lipoproteins to the sum of beta and pre-beta lipoproteins.

19. A quality control material comprising animal serum or plasma, and animal alpha lipoproteins, said material having a

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cholesterol concentration of about from 1.2 to 4.5 g/liter and being substantially free of human lipoproteins.

20. The material of Claim 19 wherein the serum or plasma is from a different animal species than the alpha lipoproteins.

21. A quality control material comprising animal serum or plasma, and animal alpha lipoproteins, said material having a cholesterol concentration greater than that of normal pooled plasma or serum from the same species from which the serum or plasma originated and being substantially free of human lipoproteins.

22. The material of Claim 21 wherein the cholesterol concentration is greater than about 25% above that of the normal pooled plasma or serum.

23. A quality control material comprising animal serum or plasma, and animal lipoproteins said material having a weight of alpha lipoproteins/volume greater than that of normal pooled plasma or serum from the same species from which the serum or plasma originated and being substantially free of human lipoproteins.

24. The material of Claim 23 wherein the concentration of alpha lipoproteins is greater than about 25% above that of the normal pooled plasma or serum.

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25. The material of Claims 18, 19, 21 or 23 which contains levels of non-lipid, clinically significant serum or plasma constituents at a level within the range found in human serum and which is dry.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US83/00571

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL. 436/13,16

INT. CL. 3 G01N 33/92

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	436/13,16,71,175

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 3,764,556, PUBLISHED 09 OCTOBER 1973, M. KUCHMAK, J.H. WILLIAMS, L. TAYLOR AND R.F. WITTER.	
Y	US, A, 4,045,176, PUBLISHED 30 AUGUST 1977, G.J. PROKSCH AND D.P. BONDERMAN.	1-25
Y	US, A, 4,216,117, PUBLISHED 05 AUGUST 1980, G.J. PROKSCH, AND D.P. BONDERMAN.	1-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
22 JUNE 1983	15 JUL 1983
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	ARNOLD TURK