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DYED ALBUMEN-COHN FRACTION III-LIPID MIXTURES SERUM LIPID ASSAY STANDARD

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ABSTRACT OF THE DISCLOSURE

The present invention relates to a diagnostic aid which comprises a reference standard obtained from a lipid rich fraction of human blood serum which contains predetermined amounts of total lipids, cholesterol, triglycerides, phosphatides, free fatty acids and optionally, glucose.

Recent developments in the study of the blood and its relationship to circulatory and coronary disease has emphasized the desirability of refining certain diagnostic tests and increasing their accuracy. Much work has been directed to determining normal ranges of constituents present in human blood and further determining abnormal variations in such components which have been statistically related to vascular and coronary diseases.

An area which has shown itself to be particularly pertinent in this respect is the lipoprotein balance in human blood. Studies have been underway for several years respecting the cholesterol as a factor in heart and vascular disease. An aid to diagnostic testing for this factor in human blood plasma has been developed by providing a reference standard for cholesterol determination as set forth in Fox, U.S. Pat. 3,260,648 which was issued July 12, 1966. The standard in that case was lyophilized and reconstituted when needed.

The current studies of the relation of cardiovascular disease to blood lipids such as that reported in Geriatrics, December 1970, pp. 64–81, rely on an ability to determine the presence and approximate amounts of several distinct subgroups of lipoproteins. The purpose of this is to make a profile of components of serum which reflects the relationship between present or potential cardiovascular disease and the various amounts of lipoproteins. It is not simply related to cholesterol or to lipid levels in the blood. It has been determined that there are at least five distinguishable syndromes of persons suffering from different types of hyperlipoproteinemia indicated by the varying amounts of four distinct liproprotein subgroups present in a subject's blood.

It is an object of the present invention to provide a reference standard which would comprise knowns for the most important liproprotein groups. If desired, a standard for glucose may be included without interfering with the determination of the other components. The addition of glucose to lipoprotein standard permits a check for diabetes as well as that for hyperlipoproteinemia since it is known that uncontrolled diabetes produces high total cholesterol levels in the blood.

The reference standard of the present invention is not suitable for reduction to the lyophilized state. It has been found that lyophilization greatly reduces the quality of the standard of the present invention which is manifested by reduced recoverable lipoproteins and by increased turbidity of the reconstituted material.

The individual constituents of the reference standards of the present invention may be obtained from commerical sources and blended to give a composition having known amounts of individual constituents within the ranges regarded as normal in human blood. It has been found, however, that greater consistency is obtainable where the

reference standard is obtained from fractions of human blood plasma rich in lipids. Particularly suitable for purposes of the present invention is that portion of the serum which is designated by workers in the art as Cohn Fraction III.

Cohn Fraction III is routinely obtained from the processing of whole blood to plasma where it is obtained as a precipitate formed during an ethanolic extraction. The constituents of Cohn Fraction III vary from batch to batch but fractions having arbitrarily set minimum values for constituents of importance to the establishment of a reference standard have been determined to be satisfactory as starting materials. A description of this type of separation is found in the Journal of the American Chemical Society, March 1946, pp. 459 to 475 in an article by E. J. Cohn et al., "Preparation and Properties of Serum and Plasma Proteins."

It has been empirically determined that batches of Cohn Fraction III suitable for use in the present invention provide an end product which contains the following minimum amounts of constituents when prepared in accordance with the procedure set forth below:

Protein—4.0 g./100 ml.
Total lipids—850 mg./100 ml.
Cholesterol—350 mg./100 ml.
Triglycerides—200 mg./100 ml.
Phospholipids—150 mg./100 ml.
Free fatty acids (measured as palmitic acid)—0.1 meq./100 ml.

Cohn Fraction III filter cakes which are suitable for use in the present invention must be treated further in order to prevent denaturing of the protein which occurs as a result of its prolonged exposure to residual ethanol in the filter cake.

Having generally described the invention examples of the best method for accomplishing it as set forth below by way of exemplification and not by way of limitation.

EXAMPLE I

A 300-gram portion of Cohn Fraction III is suspended in 1000 ml. of 0.85% saline saturated with 0.1% methyl paraben. The temperature is maintained at 7±2° C. The paste suspension is stirred overnight in a cold room while maintaining the reduced temperature. The resulting composition is placed in a cellophane bag and dialyzed against at least 10 times it volume of physiological saline containing a saturated solution of methyl paraben to remove ethanol which is present in the Cohn Fraction III as it is ordinarily obtained. After three hours the initial bath of physiological saline is replaced by a fresh one and dialysis continued for another three hours. When practical the dialysis is run overnight for a period of 13 to 16 hours.

The dialyzed material is then removed from the cellophane bags and centrifuged at 16,000 g. for a half hour in refrigerated centrifuge at a temperature in the range of 4 to 8° C. to remove any undissolved material. The supernatant is the material which will form the basis of the desired serium lipid standard.

A dye system using a combination of 50 mg. percent FD and C Yellow No. 5 and 10 mg. percent Reactone Red 2BF (see Ackermann et al., Melliand Textilberichte, vol. 42, pp. 1167–1172 for the formula of this dye) in 0.85% saline saturated with methyl paraben when added to the dialyzed solution in amounts of 2% improved its appearance.

This change of the color of the stabilized lipid rich fraction to one more closely approximating that of a lipid rich portion of fresh serum was found to be desirable in that it avoided an unfounded doubt on the part

of those making the assay that the serum lipid standard of the present invention might in some way not be the equivalent of the serum lipids tested for.

A serum lipid standard having the additional ability to provide a standard for glucose may be prepared by incorporating a minimum of 95 mg. of glucose/100. ml. of serum lipid standard. This addition is optional but desirable since it does not interfere with assays for serum lipids and lipo proteins while including a standard for glucose. This latter test would often be used in determining a patient's serum lipid profile and its possible underlying causes since it is well known that in cases of uncontrolled diabetes total blood cholesterol tends to be high.

Two percent bovine serum albumin was added to the solution to enhance the stability of the protein constituents which otherwise tend to separate. Any separation which still might occur despite the addition of bovine serum albumin is easily dispersed by swirling the container which readily produces a uniform suspension.

A reference standard for blood serum lipids and subgroups thereof produced in general accordance with the foregoing example are similar in appearance to serum lipid fractions freshly obtained from whole blood and when maintained at about 4° C. remain unchanged for periods in excess of 3 months. The particular assays of the standard starting with raw materials having at least the minimum values set forth above fall within the ranges which would obtain in normal individuals and those having hyperlipoproteinemia.

It is understood that the foregoing description is given by way of illustration and that variations may be made therein without departing from the spirit of the invention.

What is claimed is:

1. A serum lipid assay standard which is a suspension of serum lipids in 0.87% aqueous saline having constituents in at least the following amounts per 100 ml.: protein, 4 g.; total lipids, 850 mg.; cholesterol, 350 mg.; triglycerides, 200 mg.; phospholipids, 150 mg.; free fatty acid (as palmitic acid), 0.1 meq., and obtained by dialyzing a suspension of Cohn Fraction III in cold aqueous 0.85% saline containing methyl paraben against physiological saline until substantially all the ethanol normally present in Cohn Fraction III is removed, centrifuging the dialyzed Cohn Fraction III and separating the supernatant suspension of serum lipids of the above composition, said composition further containing 2% by weight of bovine serum albumen and a dye solution of 50 mg. percent FD and C Yellow #5 and 10 mg. percent Reactone Red 2BF in 0.85% aqueous saline solution.

2. A serum lipid assay standard as set forth in claim **1** wherein there is also present glucose in an amount of about 95 mg. per 100 ml. of the suspension.

3. A serum lipid assay standard as set forth in claim **1** wherein there is also present 2% by weight bovine serum albumin and a dye solution of 50 mg. percent reactone red 2BF in 0.85% aqueous saline.

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