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LIQUID BLOOD SERUM CONTROL STANDARD

Allan L. Louderback, Temple City, Calif., assignor to Baxter Laboratories, Inc., Morton Grove, Ill.

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ABSTRACT OF THE DISCLOSURE

A liquid control standard for use in the analysis of blood serum, which standard is prepared from anticoagulant-stored blood plasma by treating the defibrinated plasma with ion exchange resin to reduce the alkali and alkaline-earth metal cation level followed by drying, removing the lipoprotein component by extraction with a fat solvent and then reconstituting the product with water.

This invention relates to a liquid blood serum control standard and method of preparation thereof.

Blood serum is a complex biological fluid containing numerous components of substantial physiological importance. In the normal or average healthy person the concentrations of these components fall within certain reasonably well-defined limits. When one or more of these components is determined upon analysis to fall outside of these acceptable limits, various diseases or pathological conditions of the body systems are indicated.

In recent years various automated procedures have been developed for conveniently analyzing multiple components of blood serum. Illustrative of the analytical equipment for these purposes are the Technicon "AutoAnalyzer," the Warner Chillcott "Robot Chemist" and the Beckman "Discrete Analyzer." These instruments are capable of rapidly and sequentially determining the concentrations of a host of blood serum components in a single sample, for example, up to a dozen or more values.

In the performance of the analytical tests made by the above and similar equipment on blood serum and other biological samples, it is necessary to use laboratory standard materials or so-called "reference or control standards" for purposes of calibration and control of the instrument. Accurate results in the use of these instruments, particularly in the case of multi-automated procedures, are dependent upon rigid and constant standardization of the biochemical determinations.

Illustrative of the control standards used in actual practice is the freeze-dried serum which is reconstituted with aqueous ammonium bicarbonate prior to use as described in U.S. Pat. 3,466,249. For certain purposes, a complete liquid control standard which does not require such reconstitution would be convenient and find much use in practice.

Accordingly, it is an object of this invention to provide a liquid blood serum control standard.

It is another object of this invention to provide a stable blood serum reference standard which can be used for the direct calibration and control of multi-automated analytical procedures.

Other objects and advantages of the invention will be apparent to those skilled in the art after reading the present disclosure.

In accordance with the present invention, liquid blood serum is treated with a strong acid cation exchange resin to reduce the sodium, potassium and calcium level in the serum, and the pH of the serum is maintained at, or adjusted to, about its original level by sufficient alkaline reagent which does not contain any sodium, potassium, calcium or ammonium ions. The resin-treated and pH-adjusted serum is then dried and the lipoprotein component

is extracted with a fat solvent. The serum which is thereby essentially free of lipoprotein is reconstituted with water to provide a base liquid blood serum. Depending upon the analysis of the starting material and the desired concentration of various components in the final product, materials can be added to the base liquid blood serum to provide the final control standard.

The method of preparing the liquid blood serum control standard of this invention is particularly adapted for use with defibrinated blood plasma containing high concentrations of the inorganic ions: sodium, potassium and calcium, for example, stored blood plasma containing the anticoagulants sodium citrate, potassium oxalate, heparin sodium or sodium ethylene diamine tetraacetate, citrated blood plasma (containing sodium citrate or sodium citrate and dextrose), ACD blood plasma (containing citric acid, sodium citrate and dextrose), and CPD blood plasma (containing citrate, phosphate and dextrose).

Defibrination can be carried out by the addition of, for example, calcium ions to citrated blood or Polybrene® (hexadimethrene bromide) to heparinized blood.

Examples of strong acid cation exchange resins which can be employed in the practice of this invention are the sulfonic acid cation exchange resins. These resins can be, for example, sulfonated phenol, sulfonated polystyrene, sulfonated phenolformaldehyde, sulfonated copolymers of monoalkenyl aromatic hydrocarbons with from about 0.5 to about 25 weight percent of a dialkenyl cross-linking compound, e.g., divinylbenzene, divenyltoluene, divinylxylene and the like resins. Resins of this type are commercially available under the trademarks "Dowex 50," (Dow Chemical Co.), "Amberlite IR–120" (Rohm & Haas Co., Inc.), "Driolite C–3" (Chemical Process Co., Inc.), and "Diaion SK #1" (Mitsubishi Chemical Industries Ltd.). A preferred resin is "Dowex 50" which is a sulfonated polystyrene cross-linked with divinylbenzene such as described in U.S. Pat. 2,366,007.

Other suitable strong acid cation exchange resins which can be used in this invention will be apparent to those skilled in the art and can be prepared by reference to a text such as Kunin, "Ion Exchange Resins," John Wiley & Sons, Inc., New York, 1950.

A preferred resin is a polystyrene nuclear sulfonic acid ion exchange resin on the hydrogen cycle such as "Dowex 50," 50 to 100 mesh.

The blood serum is mixed with the ion exchange resin preferably in proportions of from about 25 to about 100 grams of resin per liter of serum. Thus, mixing about 40 grams of "Dowex 50" resin with one liter of serum has been found to provide excellent results whereby the sodium ion level is reduced from a range of about 165 to 205 milliequivalents per liter of serum to a range of about 90 to 95 milliequivalents per liter of serum.

A preferred alkaline reagent for maintaining or adjusting the pH of the serum to about its original level is a tris buffer [tri(hydroxy methyl)aminomethane]. The tris buffer preferably is an aqueous solution of from about 30 to about 50 grams of tris per liter of distilled water and has a pH of from about 5.8 to about 6.8 prior to drying.

According to one aspect of this invention, an important blood component to be contained in the control standard is urea nitrogen or blood urea nitrogen (BUN). The BUN preferably is determined by the Bertholet test or by a modified-Bertholet test such as described, for example, in U.S. Pats. 3,119,751, 3,467,499 and Re. 26,125. It has been found, however, that the use of tris buffer interferes with the determination of BUN by these Bertholet or modified-Bertholet tests. Unexpectedly, the use of lithium hydroxide or strontium hydroxide does not interfere with these BUN tests and, at the same time, retains the unique advantages of the tris buffer in being free from potassium, sodium, calcium and ammonium ions. The pre-

ferred lithium hydroxide employed in this invention preferably is an aqueous solution of from about 50 to about 70 grams of lithium hydroxide per liter of distilled water.

The drying of the resin-treated and pH-adjusted serum preferably is carried out by conventional freeze drying or lyophilization methods whereby the serium is dried from the frozen state under a high vacuum and ice or other frozen solvent rapidly sublimes to yield a porous solid.

The lipoprotein content of the dried serum is then removed by extraction with a fat solvent. Examples of fat solvents which can be employed in this invention are organic solvents such as esters, ethers, ketones, hydrocarbons and chlorinated hydrocarbons. Examples of these organic solvents are hydrocarbons such as light paraffinic petroleum fractions or naphthas, particularly of the hexane or heptane types, cyclic hydrocarbons such as cyclohexane, chlorinated solvents such as chloroform and carbon tetrachloride, ethers such as diethyl ether, and ketones such as acetone and butanone. A preferred fat solvent is a fluorocarbon or "Freon" such as monofluorotrichloromethane, dichlorodifluoromethane, monochlorodifluoromethane, monofluorodichloromethane and various mixtures thereof.

The solvent extraction is carried out by thoroughly admixing the dried serum with the fat solvent and then separating the undissolved material from the fat solvent. The dried serum is mixed with the solvent preferably in proportions of from about 30 to about 90 grams of serum per liter of solvent for about 10 to about 60 minutes with stirring. The fat solvent can be removed by filtering and the like separation procedures, with any residual solvent being allowed to evaporate from the retained dry serum particles.

The serum which is the essentially free from lipoprotein is reconstituted to about its original volume or in other suitable proportions with water, preferably distilled water, to provide the base liquid blood serum control standard.

Illustrative of the blood serum components which can be included in the control standard of this invention are substances selected from the group consisting of albumin, acid phosphatase, alkaline phosphatase, amylase, bilirubin, calcium, carbon dioxide, chloride, cholesterol, creatinine, glucose, haemoglobin, lactic dehydrogenase, phosphorous, potassium, sodium, total protein, transaminases such as serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase, triglycerides, urea nitrogen, uric acid and other such substances found in blood serum.

A preferred control standard of this invention contains predetermined concentrations of the following blood serum components in admixture: sodium, potassium, chloride, phosphorous, calcium, BUN, glucose, uric acid, creatinine, total protein, protein bound iodine (PBI) and carbon dioxide (HCO_3^-). The base liquid serum can be brought to the desired predetermined levels of these components by adding suitable amounts of appropriate materials containing the components. For the twelve foregoing blood serum components, the following materials can be added, respectively: sodium acetate, potassium hydroxide, sodium chloride, potassium dihydrogen phosphate, calcium chloride, urea, glucose, uric acid, creatinine, dried blood serum, thyroxine and sodium bicarbonate. This control standard is storage stable at customary refrigerated temperatures (4° C.) for three months and provides suitable assay values for these components.

The following examples will further illustrate the present invention although the invention is not limited to these specific examples.

EXAMPLE 1

Citrated human blood serum is defibrinated by the addition of calcium ions followed by filtration. The resulting serum is then mixed with a polystyrene nuclear sulfonic acid ion exchange resin on the hydrogen cycle ("Dowex 50," 50 to 100 mesh) in a ratio of 40 grams of resin per liter of serum to reduce the level of the sodium and potassium ion (and calcium ion when present) in the serum. As the hydrogen ion is released into the serum by the exchange for sodium and potassium ions, the pH of the serum is maintained at or adjusted to a level of about 6.2±0.2 with an aqueous solution of lithium hydroxide. The sodium ion level is reduced by this ion exchange treatment from a range of about 165 to 205 milliequivalents per liter of serum to a range of about 90 to 95 milliequivalents per liter of serum. The pH of the final treated serum is checked and adjusted to 6.2±0.2 with lithium hydroxide. The resin-treated and pH-adjusted serum is lyophilized and then extracted with "Freon TF" (trichlorotrifluoroethane) by thoroughly admixing 75 grams of the dried serum with 1500 ml. of the "Freon." The mixture is filtered through Whatman #1 filter paper and the residual solid material is spread to dry. The dry material is then reconstituted with distilled water and filtered to remove any remaining particulate matter. This product is retained as the base liquid blood serum control standard and assayed for desired components.

In this example, three control standards were prepared having predetermined low, medium and high concentrations as follows:

Component	Control standard concentration [1]		
	Nc 1, low concentration	No 1, medium concentration	No 3, high concentration
Sodium (meq./l)	125	140	155
Potassium (meq./l.)	3	5	7
Calcium (mg., percent)	6	10	14
Chloride (meq./l.)	80	100	120
BUN (mg., percent)	10	40	80
Creatinine (mg., percent)	1.0	4.0	7.0
Uric acid (mg., percent)	3.5	7.5	11.5
Glucose (mg., percent)	80	160	400
Total protein (gm., percent)	4.5	6.0	7.2
Albumin (mg., percent)	2.7	3.6	4.3
$CO_2(HCO_3^-)$	18	25	35
PBI (μg., percent)	4.3	6.0	9.0
P (mg., percent)	3	8	13

[1] Each concentration is within ±10% of the stated value.

In order to prepare these three levels, the above prepared, essentially lipoprotein-free dried serum is divided into three parts and reconstituted to three different levels with distilled water using the below-stated protein levels as the base. The reconstituted samples were assayed as follows:

Component	Reconstituted sample concentration		
	No. 1	No. 2	No. 3
Sodium (meq./l.)	72.8	96.9	102
Potassium (meq./l.)	1.14	1.52	1.6
Calcium (mg., percent)	3.93	5.23	5.5
Chloride (meq./l.)	72.11	95.95	101
BUN (mg., percent)	7.14	9.5	10
Creatinine (mg., percent)	0.57	0.76	0.8
Uric acid (mg., percent)	2.14	2.7	3
Glucose (mg., percent)	61.4	81.7	86
Total protein (mg., percent)	4.5	6.0	6.3
Albumin (mg., percent)	2.7	3.6	3.8
$CO_2(HCO_3^-)$	1.76	2.35	2.47
PBI (μg., percent)	3.07	4.09	4.3
P (mg., percent)	2.43	3.23	3.4

Reconstituted Samples 1, 2 and 3 were then used to make the foregoing control standards of low, medium

and high concentrations by addition of suitable amounts of the appropriate materials as follows:

Material added	Amount added to samples		
	No. 1	No. 2	No. 3
Sodium acetate (grams)	2.38	1.53	0.467
Potassium hydroxide (ml.)	1.01	1.16	1.382
Calcium chloride (grams)	3.45	7.97	0.142
Sodium chloride (grams)	0.239	0.058	0.513
Urea (grams)	0.034	0.366	0.84
Creatinine (mg.)	2.58	19.44	37.2
Uric acid (grams)	0.008	0.029	0.051
Glucose (grams)	0.112	0.470	1.884
Dried serum (grams)	0	0	9
Sodium bicarbonate (mg.)	818.5	1,141.6	1,639.5
Thyroxine (μl.)	20.2	31.3	77.05
Potassium dihydrogen phosphate (grams)	1.500	0.126	0.253

The adjusted control standard material is sterile filtered through a "Millipore" filter (0.22 micron) and placed in vials for use as the final control standards which can then be made available to clinical laboratories and other such analytical and testing laboratories having a need for blood serum control standards.

Various other examples and modifications of the foregoing examples will be apparent to those skilled in the art after reading the foregoing specification and the appended claims without departing from the spirit and scope of the invention. All such further examples and modifications are included within the scope of the invention as defined in the following claims.

What is claimed is:

1. The method of making a liquid blood serum control standard from anticoagulant-stored blood plasma comprising thoroughly admixing the defibrinated plasma with a strong cation exchange resin to substantially reduce the sodium, potassium and calcium ion level in the serum followed by drying, removing the lipoprotein component by extraction with a fat-solvent and then reconstituting the serum with water the pH of said resin-treated serum being adjusted to or maintained at a pH of from about 5.8 to about 6.8 with a reagent selected from the group consisting of aqueous lithium hydroxide, strontium hydroxide and tris(hydroxymethyl)-aminomethane.

2. The method of claim 1 in which the reagent for pH adjustment or maintenance is aqueous lithium hydroxide.

3. The method of claim 1 in which the ion exchange resin is a polystyrene nuclear sulfonic acid ion exchange resin on the hydrogen cycle.

4. The method of claim 1 in which the cation exchange resin is a polystyrene nuclear sulfonic acid ion exchange resin on the hydrogen cycle and is mixed with the defibrinated plasma in proportions of from about 25 to about 100 grams of resin per liter of said plasma, in which the fat-solvent is a chlorinated hydrocarbon and is mixed with the dried serum in proportions of from about 30 to about 90 grams of resin per liter of solvent.

5. A liquid blood serum control standard comprising anticoagulant-stored blood plasma which is defibrinated, resin-treated with strong cation exchange resin to substantially reduce the sodium, potassium and calcium ion level followed by drying, removing the lipoprotein component by extraction with a fat-solvent and then reconstituting the serum with water, the pH of said resin-treated serum being adjusted to or maintained at a pH of from about 5.8 to about 6.8 with a reagent selected from the group consisting of aqueous lithium hydroxide, strontium hydroxide and tris(hydroxymethyl)-aminomethane.

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ROBERT F. BURNETT, Primary Examiner

M. E. McCAMISH, Assistant Examiner

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