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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
G01N 31/00	A1	WO 91/01497
		(43) International Publication Date: 7 February 1991 (07.02.91)

(21) International Application Number: PCT/US90/04046

(22) International Filing Date: 18 July 1990 (18.07.90)

(30) Priority data:
383,004 20 July 1989 (20.07.89) US

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE*, DE (European patent)*, DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMPROVED STABLE COAGULATION CONTROLS

(57) Abstract

Improved stable coagulation control plasmas are described which contain blood plasma, and effective amounts of (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate. Preferred control plasmas also contain thimerosal and/or sodium azide. Also described are stable control plasmas having high levels of Factor V and VIII including plasma from blood which has been collected directly from an animal source into a solution containing (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) citrate, and to which, after removal of red blood cells, has been added at least about 2 weight percent of a suitable stabilizing carbohydrate, such as sucrose. Methods for preparing stable control plasmas are also described.

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IMPROVED STABLE COAGULATION CONTROLS

BACKGROUND OF THE INVENTION

The present invention relates generally to blood coagulation, and more particularly to improved blood coagulation controls which demonstrate superior stability, and also sensitivity to variation in reagents used in coagulation tests.

The need for stable and reliable blood coagulation controls is well documented. The continued and increased use of oral anticoagulants for treatment and management of various thrombo-embolytic conditions today, more than ever, is a driving force for their development. As is known, overdosage of anticoagulants, commonly the coumarin derivatives, can lead to serious complications, including hemorrhage from peptic ulcers, as well as other gastrointestinal complications. On the other hand, maintenance of too low a level of anticoagulant reduces or eliminates the efficacy of the prescribed treatment.

It is therefore extremely important that anticoagulant levels be reliably monitored, and, as such, a voluminous body of art has developed documenting attempts of those in the field to produce stable and reliable controls, as well as reagents, to aid in monitoring anticoagulant activity.

For example, U. S. Patent No. 3,947,378 to Babson discloses a process for producing a control plasma deficient in Factors II, VII, IX, and X which involves treating to plasma with 20 to 22% by weight of barium sulfate at ambient temperature and then removing the adsorbent from the adsorbed plasma. The Babson patent reports that abnormal control plasmas produced by mixing the so-adsorbed plasma with normal plasma are more stable after reconstitution and give more uniform results in the activated partial thromboplastin time (APTT) procedure after storage of up to eight hours or more.

S. Zucker, M.H. Cathey, and B. West, *Preparation of Quality Control Specimens for Coagulation*, Amer. J. Clin. Path., June 1970, Vol. 53 pp. 924-927, reports a method for preparing lyophilized plasma specimens for use as quality controls in coagulation testing. Zucker et al. report buffering the plasma specimens with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), which was reported to provide pH and enzyme stability for prothrombin times for eight hours at 25°C. An article by M. Brozovic, D. J. Howarth, L. P. van Halem Visser, and E. A. Loeliger, *Stability of Freeze-Dried Plasma Prepared from Patients on Oral Anticoagulants*, Journal of Clinical Pathology, 1973, Vol. 26, p. 857-963, reported as to the suitability of freeze-dried plasmas from patients on oral anticoagulants to serve as reference material in the calibration of thromboplastins used in the control of oral anticoagulant treatment. The authors studied plasmas from several plasma pools, developed by collecting from each patient 4.5 ml of blood into 0.5 ml of a solution formed by combining 44.62 g of HEPES buffer, 38.00 g trisodium citrate ($2H_2O$), and 0.5 ml aprotinin TRASYLOL (1000 u/ml), and then adding distilled water to 1000 ml. Individual samples were centrifuged, as were the subsequently pooled samples, whereafter 1 ml of 10% sodium azide was added per liter of plasma. Samples of the prepared plasmas were then either frozen or freeze-dried. The authors reported that the freeze-dried plasmas from patients on oral anticoagulants could be used to calibrate thromboplastins provided that they are used immediately after complete reconstitution or kept at 4°C for use within four to six hours of reconstitution. The authors also reported that their plasma samples generally demonstrated varying levels and types of instability after reconstitution and storage at 4°C., 22°C., and 37°C. It was generally reported that this instability resulted after about twenty-four hours at the lower two

temperatures and after about four to six hours at the higher 37°C temperature.

United States Patent No. 4,007,008 to Becker et al. describes a method for reducing enzyme activities in animal serum or plasma including the steps of raising the pH thereof to a level about that of normal serum by adding a base, and thereafter terminating the reaction or reduction of enzyme activities neutralizing the serum or plasma with an acidic medium.

Other general background can be found in United States Patent No. 3,799,885 to Dennis et al. which discloses a calcium chloride test reagent buffered with HEPES buffer which is especially adapted for use in monitoring heparin therapy; in United States Patent No. 4,301,028 to Bartl et al. which reports a control reagent for heparin activity determination; in United States Patent No. 4,116,336 to Sorenson et al. which relates to a package containing a synthetic reference liquid for quality control and/or calibration of blood gas measuring equipment; and in P. S. Roberts, H. N. Hughes, and P. B. Fleming, *The Effects of Hepes Buffer on Clotting Tests, Assay of Factors V and VIII and on the Hydrolysis of Esters by Thrombin and Thrombokinase*, Thrombos, Haemostas, (Stuttg.), 1976, Vol. 35, p. 202, wherein the authors report faster clotting in the presence of 50 mM HEPES buffer.

Another aspect of the prior art control plasmas is that a majority of them, especially those commercially available, consist of or otherwise comprise primate plasma, most commonly human. These plasmas present disadvantages in that they contain unstable human factors, particularly Factors V and VIII, and also present a greater risk to the preparer or user of the controls, since they may harbor active AIDS or hepatitis viruses.

In the face of the voluminous literature and other work relating to plasmas for coagulation controls, there still

remains a need for a coagulation control which exhibits superior stability with respect to one-stage prothrombin times (PT), activated partial thromboplastin times (APTT), and Factor V and VIII activity values, as well as superior sensitivity to variations in clotting test reagents employed. Certain forms of improved controls would also significantly eliminate risk of AIDS or hepatitis contraction to those who prepare and use it. The applicant's invention, in its various aspects, addresses these matters.

SUMMARY OF THE INVENTION

Accordingly, one preferred embodiment of the applicant's invention relates to a stable coagulation control plasma comprising blood plasma, and effective amounts of (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate. In a preferred aspect the control has a total plasma component constituted substantially of non-primate plasma.

Another preferred embodiment of the applicant's invention relates to a coagulation control plasma having high stabilized levels of Factor V and VIII. This control comprises plasma and at least about 2 weight percent of a suitable carbohydrate. The plasma of this embodiment is derived from blood which has been collected directly from an animal source into a solution containing a buffer to maintain a physiological pH, a protease inhibitor, and citrate. The carbohydrate in the control is added after removal of red blood cells from the blood.

Another preferred embodiment of the applicant's invention relates to a process for producing a stable coagulation control plasma. This process includes the sequential steps of collecting the blood from which the plasma is derived directly from an animal source into a solution containing a buffer to maintain physiological pH, a protease inhibitor, and citrate; removing red blood cells from said blood; and adding a suitable stabilizing carbohydrate.

Still another preferred embodiment of this invention relates to a process for producing a stable coagulation control which includes the steps of providing blood plasma; and, adding to said blood plasma at least one purified, stabilized coagulation factor.

DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTIONS

One preferred embodiment of the applicant's invention relates to a stable coagulation control plasma which comprises blood plasma, and effective amounts of (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate. It is contemplated that a carbohydrate as used herein would have ratios of H to O of 2 to 1, as is understood in the art.

As to types of plasmas which are suitable for this embodiment, primate and non-primate plasmas can be used. Preferred plasmas to date have been human, bovine, porcine, equine and rabbit plasmas, although other plasmas including, for instance, goat and sheep plasmas, can be used. Additionally, in this embodiment of the invention, these plasmas may be collected in any suitable manner as known in the art.

As to the composition of the blood plasma in the control, various animal plasmas may be used individually or combined, adsorbed or non-adsorbed. In most cases, however, the applicant has to date preferred combining the plasmas of two or more types of animal. Since different animals have differing levels and differing stabilities of their respective coagulation factors, different animal plasmas can be selected and combined to adjust the ratios of coagulation factors in the controls and thus also adjust stability of the control and clotting times of the PT, APTT, and other tests.

For instance, owing to their particular coagulation constituent levels, rabbit plasma is used to shorten PT values, porcine plasma is used to shorten APTT but not PT values, bovine plasma is used to furnish high levels of factor V, and equine plasma is used to prolong the APTT but not the PT value. By using these principles, the applicant has been able to prepare, as desired, control plasmas

exhibiting particular ranges (e.g.. normal or abnormal) of PT and APTT values. Clotting times can thus be precisely adjusted according to the types and ratios of plasmas used.

Of the preferred control plasmas which have been prepared to date, four have shown to be more preferred. The first exhibits PT and APTT values in the normal range, and has a total plasma component constituted of porcine, bovine and rabbit plasma in ratios of about 1:6:3, respectively. The second has PT and APTT values in the abnormal range and has a total plasma component constituted of porcine plasma adsorbed with aluminum hydroxide gel, and bovine plasma in a ratio of about 35:1, respectively. The third plasma also exhibits PT and APTT times in the abnormal range, and has a total plasma component constituted of porcine plasma adsorbed with aluminum hydroxide gel.

These first three more preferred plasmas provide the advantage of significantly reduced risk of HIV or hepatitis virus infection by those who prepare and/or use them since the pertinent viruses are not present in the non-primate plasmas. Additional advantages have related to high and stable levels of coagulation factors these plasmas have provided. In this regard, it is understood that similar advantages can be derived so long as the non-primate plasma constitutes at least a substantial part of the total plasma component of the control, and thus such control plasmas are also a preferred aspect of this invention, as are controls whose plasma component consists essentially of non-primate plasmas.

The fourth more preferred control has a plasma component constituted of normal human, bovine, and rabbit plasma in respective ratios of about 2:2:1.

With respect to the buffer, to date, HEPES buffer has been preferred, although many other buffers, for instance TRIS, are known and are suitable. The preferred HEPES buffer has been HEPES hemi sodium, which is preferably

present in the control in an amount of at least about 0.08 M, and preferrably above .05 in amount.

As to amounts and types of protease inhibitor and carbohydrate, it has been preferred to date that the inhibitor and carbohydrate be present in the control in amounts of at least about 0.5 U/ml, and 2 weight percent, respectively. The preferred protease inhibitor has been aprotinin, although other such protease inhibitors, for instance soya bean trypsin inhibitors, are known in the art and are suitable. The preferred stabilizing carbohydrate to date has been saccharides, and in particular, sucrose, although others well known in the art can also be used. Additionally, it has been more preferred to date that the sucrose be present in the control in an amount of about 5 weight percent.

As to other components of the control plasma of this preferred embodiment, it has also been preferred that the control plasma include thimerosal in an amount of preferrably at least about 0.02 weight percent and/or sodium azide in an amount of preferrably at least about 0.08 weight percent.

To formulate a batch of control plasma, approximately 1 part (by volume) of the applicant's preferred buffering and preservative solution described in Example 1 below are combined with about 9 parts plasma. More preferred to date has been to combine approximately 1 part of the applicant's preferred buffering preservative solution with about 9 parts of mixed or unmixed non-primate animal plasma.

Further details of the preparation of the control plasmas of this preferred embodiment, as well as details of their stability, can be found in Examples 1-6 and Tables 1-3 below. Collectively, the control plasmas of this embodiment have generally proven stable, meaning that APTT and PT analyses do not change more than about 10% for at least about 3 days at room temperature, or at least about 8 hours at 37°C.

Additionally, as is particularly detailed in Example 6 below, the coagulation controls of this preferred embodiment have demonstrated increased sensitivity to variations in reagents used in clotting time testing. Example 6 details experiments wherein a coagulation control sample prepared in accordance with this embodiment was tested against a commercially available plasma, CITROL I, and fresh normal human plasma as regards sensitivity to dilutions of thromboplastin reagent in the PT test. As the reported results demonstrate, the control prepared in accordance with the applicant's invention herein demonstrated superior sensitivity to the varying dilutions of thromboplastins.

As stated, another preferred embodiment of the present invention involves a coagulation control plasma having high stabilized levels of Factor V and VIII. This control comprises plasma derived from blood which has been collected directly from an animal source into a collecting solution containing a buffer to maintain physiological pH, a protease inhibitor, and citrate, and to which, after removal of red blood cells, has been added at least about 2 weight percent of a suitable stabilizing carbohydrate.

Although others can be used, to date, the preferred buffer into which the blood has been collected has been HEPES hemi-sodium, and the preferred protease inhibitor has been aprotinin. It has also been preferred that the HEPES hemi-sodium and the aprotinin be present in the collecting solution in respective amounts of at least about 0.25 M and 5 U/ml. Citrate can be present in the collecting solution in suitable amount as known in the art. To date, however, it has been preferred that sodium citrate be present in an amount of about 3 weight percent. When these preferred amounts of HEPES hemi-sodium, aprotinin, and citrate are used, about 9 parts (by volume) blood are preferably collected into about 1 part of the collecting solution.

The carbohydrate can be added in solid form or in

solution. Again, the preferred carbohydrate has been sucrose, which has been added after removal of red blood cells from the plasma by centrifuging or another suitable method. Additionally, it has been more preferred to add sucrose to a level of about 5 weight percent in the control. If desired, additional physiological pH buffer can also be added (as a solid or in solution) to effect a desired level in the final control. Preferably, the final control has also contained thimerosal (preferably at least about 0.02 weight percent) and sodium azide (preferably at least about 0.08 weight percent). The thimerosal and/or sodium azide can be present initially in the collecting solution or can be added later as a solid or in solution.

The applicant has discovered that preferred plasmas prepared according to this embodiment are very high in Factor V and VIII content. Additionally, the applicant has found that the Factor V and VIII contents thus produced are highly stable at both room temperature (for at least about 3 days) and at about 37°C (for at least about 8 hours).

Plasmas which are suitable for this embodiment of the invention include primate and non-primate plasmas, with preferred plasmas being beef, pig, rabbit, horse, and human plasmas. Additional details of the preparation of control plasmas of this embodiment can be found in Examples 7-11 below.

Another preferred embodiment of the present invention concerns a method for producing a stable coagulation control. As stated above, this method includes the sequential steps of (a) collecting the blood containing the plasma to be used in the control directly from an animal source into a solution containing a buffer effective to maintain a physiological pH, a protease inhibitor, and citrate, (b) removing red blood cells from said blood, and (c) adding at least about 2 weight percent of a suitable stabilizing carbohydrate. Further details of this preferred

process are analogous to those discussed in the embodiment immediately above, and can further be found in Examples 7-11 below.

Still another preferred embodiment of this invention concerns a method for producing a stable coagulation control plasma, which method involves the steps of providing blood plasma, and adding to said plasma at least one stable purified coagulation factor in order to increase the level of the added factor in the plasma. As is further detailed in Example 12 below, particular factors are purified from various animal plasmas as known in the art. These purified factors are then added to plasma to increase the level of the added factor in the plasma and to adjust the PT and/or APTT values. Preferred methods also involve adding to equine or human plasma stable purified Factor V or VIII from bovines or porcines. Additionally, purified equine Factor II has proven particularly stable and can be added to other plasmas as a source of stable Factor II. Collectively, the plasmas to which the stable purified factors have been added have demonstrated stability superior to similar plasmas without the added factors.

Reference will now be made to specific Examples and Tables for the purposes of further describing and understanding the features of the applicant's preferred embodiments as well as their advantages and improvements over the art. It should be understood that these Examples are representative only, and that such additional embodiments and improvements of the same are within the contemplation and scope of the applicant's invention as would occur to one of ordinary skill in this art.

The Factor V and VIII activity values reported in the following Examples were calculated in the conventional manner against a standard curve prepared from dilutions of normal human plasma. PT intends the one-stage prothrombin time, and APTT intends the activated partial thromboplastin

time. The PT and APTT tests reported were performed in the conventional manner.

EXAMPLE 1

Preparation of Preferred Preservative Solution

100 ml of the applicant's preferred preservative solution were prepared by mixing 500 U aprotinin, about 12.5g HEPES hemi sodium, 0.1g thimerosal, 0.5g NaN_3, and 25g sucrose, and then adding distilled water to 100 ml. This preparation resulted in 100 ml of preservative solution which consisted of:

(a) 0.5 M HEPES hemi sodium;

(b) 0.1% thimerosal;

(c) 25% sucrose;

(d) 0.5% sodium azide; and

(e) 500 U aprotinin.

EXAMPLE 2

Non-Primate Based Normal Range Control (Level 1)

A pilot lot (hereafter designated P1) of non-primate based control was prepared which demonstrated PT and APTT values in the normal range. Particularly, 54 ml beef plasma, 9 ml pig plasma, and 27 ml rabbit plasma were blended with 10 ml of the preferred preservative solution of Example 1. The initial PT and APTT values were 11.0 and 24.1 seconds, respectively. The lot was divided out into 1.0 ml portions which were placed into individual vials and lyophilized in a conventional manner. A vial of the P1 control plasma was reconstituted the next day with distilled water, whereafter it demonstrated a PT value of 11.0 seconds and an APTT value of 24.1 seconds. Similarly reconstituted P1 vials were allowed to stand at room temperature (about 25°C) for up to five days, one being tested each day for PT and APTT values. The results are given in TABLE 1 below, and demonstrate excellent stability.

TABLE 1

Day	2	3	4	5
PT	11.2	10.8	11.7	11.2
APTT	25.7	26.2	27.1	28.3

Equally surprising stabilities for Factors V and VIII were shown in the P1 samples. Immediately after reconstitution, P1 samples exhibited Factor V and VIII values of approximately 1000% and 110%, respectively, of the amounts found in normal human plasma pools. After reconstituted samples were allowed to stand for five days at room temperature, they demonstrated a Factor V value of approximately 1100% and a Factor VIII value of approximately 90%.

EXAMPLE 3

Non-Primate Based Abnormal Range Control (Level 2)

A second pilot lot (P2) of control plasma was prepared by combining 175 ml adsorbed pig plasma, 5 ml beef plasma, and 20 ml of the applicant's preferred preservative solution prepared as in Example 1 above. This lot was prepared so as to have abnormal clotting times, with initial PT and APTT values being 21.1 and 40.8, respectively. The P2 lot was divided and lyophilized in the same manner as the P1 lot described in Example 2 above. After reconstitution with distilled water, a vial of the P2 lot produced a PT value of 20.1 and a APTT value of 41.4 seconds.

Analogous to the reconstituted stability tests of Example 2, several reconstituted P2 vials were allowed to stand at room temperature for up to five days with one being tested each day for PT and APTT values. TABLE 2 below sets forth the results which again demonstrate superior stability.

TABLE 2

Day	2	3	4	5
PT	20.6	22.0	21.6	22.7
APTT	43.8	46.1	44.1	44.4

EXAMPLE 4

Non-Primate Based Abnormal Range Control (Level 3)

A third pilot lot (P3) of control plasma was prepared by combining 90 ml adsorbed (with aluminum hydroxide gel) pig plasma with 10 ml of the applicant's preferred preservative solution prepared in accordance with Example 1 above. Initial PT and APTT values of 41.3 and 69.9 were observed for this P3 material. The P3 material was then divided and lyophilized as were the P1 and P2 lot materials of Examples 2 and 3 above. After reconstitution and storage at room temperature for about one day, a vial of P3 material was analyzed. The PT and APTT values for this reconstituted P3 plasma were 38.1 and 64.1, respectively, showing good stability.

EXAMPLE 5

Human/Non-Primate Normal Range Control

36 ml normal human plasma and 36 ml bovine plasma were combined with 18 ml rabbit plasma and 10 ml of the preservative solution of Example 1 to form a fourth pilot lot (P4) of control material. Initial PT and APTT values were in the normal range at 11.4 and 25.7, respectively. This P4 plasma also demonstrated good stability. A reconstituted sample allowed to stand at room temperature for 7 days registered a PT value of 11.5 and an APTT value of 29.7.

EXAMPLE 6

Sensitivity to Reagent Variations

In this Example, the sensitivity of the applicant's

preferred control plasmas to variations in clotting time reagents was compared to that of fresh, normal plasma, and to that of CITROL I, a commercially available control plasma marketed by American Dade. The PT test was used in this determination. To perform the study, reagents consisting of 100, 80, 60, 40, 20, and 10% thromboplastin were prepared by diluting the thromboplastin using 0.025 M HEPES buffer, Ph 7.35 containing 0.154 M sodium chloride. These reagent dilutions were then used to evaluate the PT values for the three test plasmas. The results are given in TABLE 3 below. As can be seen, the applicant's P1 control plasma material demonstrated significantly greater variation in PT values resulting from a greater sensitivity to the thromboplastin dilutions. Thus this increase in sensitivity allows detection of defective reagents more readily than even fresh plasma.

TABLE 3

% Thromboplastin	*PT/Fresh Normal Plasma*	*PT/ CITROL I*	*PT/ P1 Plasma*
100	11.5	11.4	11.3
80	11.6	11.8	12.1
60	12.4	12.7	13.3
40	13.5	13.7	14.9
20	15.9	16.4	18.3
10	18.8	19.5	22.6

EXAMPLE 7

Preferred Collecting Solution

100 ml of the applicant's preferred collecting solution were prepared by mixing 500 U aprotinin, about 6.25 g HEPES

hemi sodium and 2.94 g sodium citrate, and adding distilled water to 100 ml. This preparation resulted in 100 ml of collecting solution which contained:

(a) 0.25 M HEPES hemi sodium
(b) 2.94 gm sodium citrate
(c) 500 U aprotinin

Preferably, about 9 parts by volume of blood has been collected into about 1 part by volume of this preferred collecting solution.

EXAMPLES 8-11

Direct Collection into Collecting Solution

In order to obtain a control plasma which has high and stable levels of coagulation Factors, particularly Factors V and VIII, Examples 2-6 are repeated except the plasma used is derived from blood which has been collected directly from the animal source into the applicant's preferred collecting solution of Example 7, and, after removal of red blood cells, sucrose, additional HEPES hemi sodium, as well as thiomersal and sodium azide are added in amounts to achieve the same respective levels as in Examples 2-6. The initial Factor V and VIII activity values are tested and are found to be particularly high as compared to plasmas which are collected into simple conventional citrated anticoagulant solutions. Additionally, the Factor V and VIII values are stabilized by the buffering and preservative solution, as is borne out by high Factor V and VIII values obtained after lyophilization and reconstitution, and after reconstituted control plasma samples are allowed to stand at 37°C for eight hours, and at room temperature for about 3 days. PT and APTT values are similarly stable upon testing after reconstituted control plasmas are subjected to these temperature/time conditions.

EXAMPLE 12

Adding Stable Purified Factors

Pooled normal human plasma was assayed and demonstrated respective APTT and PT values of 34 secs and 13 secs. In various experiments, purified factors X and VIII from pig, cow, horse, sheep or rabbit were added to about 1 ml samples of the normal human plasma and in each instance corrected APTT to 26 secs. Similarly, purified Factor II from pig, cow, horse, sheep, or rabbit was added to about 1 ml samples of the normal human plasma and corrected PT to 11 secs. Additionally, in each instance, the lyophilized product showed better stability than the lyophilized normal human plasma.

WHAT IS CLAIMED IS:

1. A stable coagulation control plasma, comprising:
blood plasma; and
effective amounts of:
a buffer to maintain a physiological pH;
a protease inhibitor; and
a suitable stabilizing carbohydrate.

2. The control plasma of claim 1 wherein said protease inhibitor is aprotinin.

3. The control plasma of claim 2 wherein said buffer is HEPES buffer.

4. The control plasma of claim 3 wherein said carbohydrate is sucrose.

5. The control plasma of claim 4 additionally comprising sodium azide.

6. The control plasma of claim 5 additionally comprising thimerosal.

7. The control plasma of claim 6 wherein said HEPES buffer is HEPES hemi sodium, and said HEPES buffer, aprotinin, sucrose, thimerosal, and sodium azide are present in amounts of at least about 0.08 M, 0.5 U/ml, 2 weight percent, 0.02 weight percent, and 0.08 weight percent, respectively.

8. The control plasma of claim 1, 4 or 7 in which said blood plasma comprises porcine, rabbit, bovine, human, equine, sheep or goat plasma.

9. The control plasma of claim 8 in which said plasma comprises equine plasma.

10. The control plasma of claim 8 in which said plasma comprises rabbit plasma.

11. The control plasma of claim 8 in which said plasma comprises human plasma.

12. The control plasma of claim 11 additionally comprising at least one purified factor of a porcine, bovine, rabbit, equine or sheep.

13. The control plasma of claim 8 in which said plasma comprises bovine plasma.

14. The control plasma of claim 13 in which said plasma additionally comprises human plasma.

15. The control plasma of claim 13 in which said plasma additionally comprises rabbit plasma.

16. The control plasma of claim 15 in which said plasma additionally comprises human plasma.

17. The control plasma of claim 16 in which the total blood plasma component of said control is constituted at least substantially of said rabbit, bovine and human plasma.

18. The control plasma of claim 17 in which the total blood plasma component of said control consists of said rabbit, bovine and human plasma.

19. The control plasma of claim 18 in which said

rabbit, bovine and human plasma are present in a volumetric ratio of about 1:2:2, respectively.

20. The control plasma of claim 8 in which said plasma comprises porcine plasma.

21. The control plasma of claim 20 in which said porcine plasma is adsorbed with aluminum hydroxide gel, and in which the total blood plasma component of said control is constituted at least substantially of said porcine plasma.

22. The control plasma of claim 21 in which the total blood plasma component of said control consists of said porcine plasma.

23. The control plasma of claim 20 in which said plasma additionally comprises bovine plasma.

24. The control plasma of claim 23 in which the total blood plasma component of said control is constituted at least substantially of said porcine and bovine plasma.

25. The control plasma of claim 24 in which the total blood plasma component of said control consists of said porcine and bovine plasma.

26. The control plasma of claim 25 in which said porcine plasma is adsorbed with aluminum hydroxide gel, and said porcine and bovine plasma are present in a volumetric ratio of about 35:1, respectively.

27. The control plasma of claim 20 in which said plasma additionally comprises rabbit plasma.

28. The control plasma of claim 27 in which said plasma additionally comprises bovine plasma.

29. The control plasma of claim 28 in which the total blood plasma component of said control is constituted at least substantially of said porcine, bovine and rabbit plasma.

30. The control plasma of claim 29 in which the total blood plasma component of said control consists of said porcine, bovine and rabbit plasma.

31. The control plasma of claim 30 in which said porcine, bovine and rabbit plasma are present in a volumetric ratio of about 1:6:3, respectively.

32. A coagulation control plasma having high stabilized levels of Factor V and VIII, comprising:

blood plasma; and

at least about 2 weight percent of a suitable carbohydrate;

wherein said plasma is derived from blood which has been collected directly from an animal source into a solution containing a buffer to maintain physiological pH, a protease inhibitor, and citrate; and

wherein said carbohydrate has been added after removal of red blood cells from said blood.

33. The control plasma of claim 32 wherein said protease inhibitor is aprotinin.

34. The control plasma of claim 33 wherein said buffer is HEPES buffer.

35. The control plasma of claim 34 wherein said solution contains HEPES hemi sodium, aprotinin, and sodium citrate in respective amounts of at least about 0.25 M, 5 U/ml, and 3 weight percent.

36. The control plasma of claim 35 additionally comprising:

HEPES hemi sodium buffer; and

aprotinin;

wherein said HEPES hemi sodium buffer and aprotinin are present in said control in amounts of at least about 0.08 M and 0.5 U/ml, respectively.

37. The control plasma of claim 36 wherein said carbohydrate is sucrose.

38. The control plasma of claim 37 additionally comprising sodium azide.

39. The control plasma of claim 38 additionally comprising thimerosal.

40. The control plasma of claim 39 in which the total plasma component of the control is constituted substantially of non-primate plasma.

41. The control plasma of claim 40 in which the non-primate plasma comprises porcine plasma.

42. The control plasma of claim 40 in which the non-primate plasma comprises bovine plasma.

43. The control plasma of claim 40 in which the non-primate plasma comprises rabbit plasma.

44. The control plasma of claim 40 in which the non-primate plasma comprises equine plasma.

45. The control plasma of claim 41 in which the non-primate plasma additionally comprises bovine plasma.

46. The control plasma of claim 45 in which the non-primate plasma additionally comprises rabbit plasma.

47. The control plasma of claim 42 in which the non-primate plasma additionally comprises rabbit plasma.

48. The control plasma of claim 41 or 45 in which the porcine plasma is adsorbed with aluminum hydroxide gel.

49. The control plasma of claim 45 in which the porcine plasma is adsorbed with aluminum hydroxide gel, and the porcine and bovine plasma are present in a volumetric ratio of about 35:1, respectively.

50. The control plasma of claim 46 in which the porcine, bovine, and rabbit plasma are present in a volumetric ratio of about 1:6:3, respectively.

51. A process for producing a stable coagulation control plasma which comprises the sequential steps of:

collecting the blood containing the plasma to be used in the control directly from an animal source into a solution containing a buffer effective to maintain a physiological pH, and effective amounts of a protease inhibitor;

removing red blood cells from said blood; and

adding at least about 2 weight percent of a suitable stabilizing carbohydrate.

5 The process of claim 51 in which in said collecting step, said buffer comprises HEPES buffer, said protease inhibitor comprises aprotinin, and a citrate is additionally included.

53. The process of claim 52 in which in said collecting

step, said solution comprises HEPES hemi sodium, aprotinin, and sodium citrate in amounts of at least about 0.25 M, 5 U/ml, and 3 weight percent, respectively.

54. The process of claim 52 or 53 in which in said adding step, said carbohydrate is sucrose.

55. The process of claim 54 additionally comprising the step of:

adding sodium azide in amounts to achieve at least about 0.08 weight percent sodium azide in said control.

56. The process of claim 55 additionally comprising the step of:

adding thimerosal in amounts to achieve at least about 0.02 weight percent thimerosal in said control.

57. The process of claim 51 in which in said collecting step, said blood is non-primate blood, and about 9 parts by volume of said blood are collected into about 1 part by volume of said solution.

58. A process for producing a stable coagulation control comprising the steps of:

providing blood plasma; and

adding to said plasma at least one stable purified coagulation factor.

59. The process of claim 58 wherein said blood plasma is human plasma, and said factor is Factor II, V, VIII or X derived from a non-primate animal.

60. The process of claim 58 wherein said factor is Factor V or VIII derived from a bovine or porcine.

61. The process of claim 60 wherein said plasma is human plasma.

62. The process of claim 60 wherein said plasma is equine plasma.

63. The process of claim 60 wherein said factor is Factor V.

64. The process of claim 58 wherein said factor is Factor II derived from an equine.

65. The process of claim 58 wherein said factor is derived from blood which has been collected directly from an animal source into a solution containing buffer effective to maintain a physiological pH, a protease inhibitor, and citrate.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04046

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

IPC(5): G01N 31/00
U.S. Cl.: 436/16

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	436/8,9,10,11,12,13,14,15,16,17,18; 252/408.1

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]
Y	US, A, 4,409,334 (Lill et al.) 11 October 1983, see the entire document.	1-65
Y	US, A, 4,234,682 (Bartl et al.) 18 November 1980, see the entire document.	1-65
Y	US, A, 4,056,484 (Heimburger et al.) 01 November 1977, see the entire document.	1-65

* Special categories of cited documents: [15]

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
16 October 1990	06 DEC 1990
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	T. J. Wallen