645172

P/00/001
Section 29

AUSTRALIA
Patents Act 1990

PATENT REQUEST : STANDARD PATENT

We, being the person(s) identified below as the Applicant(s), request the grant of a Standard Patent to the person(s) identified below as the Nominated Person(s), for an invention described in the accompanying complete specification.

Applicant(s) and Nominated Person(s): CENTRE REGIONAL DE TRANSFUSION SANGUINE DE LILLE

Address: 19-21, RUE CAMILLE-GUERIN
F-59012 LILLE
FRANCE

Invention Title: PROCESS FOR AN INDUSTRIAL-SCALE PREPARATION OF A STANDARDIZED HUMAN VON WILLEBRAND FACTOR CONCENTRATE OF VERY HIGH PURITY AND SUITABLE FOR THERAPEUTIC USE.

Name(s) of Actual Inventor(s): Miryana BURNOUF-RADOSEVICH; Thierry BURNOUF

Address for Service: GRIFFITH HACK & CO
509 ST KILDA ROAD
MELBOURNE VIC 3004

Attorney Code: HA

BASIC CONVENTION APPLICATION DETAILS

Application No:	Country:	Application Date:
91 02804	FR	08 March 1991

Drawing number recommended to accompany the abstract: 1

DATED THIS 19TH DAY OF OCTOBER 1993

CENTRE REGIONAL DE TRANSFUSION SANGUINE DE LILLE

GRIFFITH HACK & CO.

Patent Attorney for and
on behalf of the Applicant

AUSTRALIA PATENT OFFICE

P/00/008 28/5/91
Section 29(1)
Regulation 3.1 (2)

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

(To be filed before acceptance)

We, CENTRE REGIONAL DE TRANSFUSION SANGUINE DE LILLE

of 19-21, Rue Camille-Guerin, F-59012 Lille, FRANCE

being the applicant and nominated person in respect of Application No. 11131/92, state the following:-

Part 1 - The actual inventors are Mirjana BURNOUF-RADOSEVICH and Thierry BURNOUF and we would be entitled to have assigned to us any patent granted to any of the said inventors in respect of the invention.

Part 2 - The person nominated for the grant of the patent is the applicant of the basic application FR 9102804 listed on the Patent Request form.

Part 3 - The basic application listed above is the first application made in a Convention country in respect of the invention.

GRIFFITH HACK & CO.

Vincent Lanfe

19 OCT 1993

.....
Patent Attorneys for and
on behalf of the applicants

.....
Date

AU9211131

(12) PATENT ABRIDGMENT (11) Document No. AU-B-11131/92
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 645172

(54) Title
PROCESS FOR AN INDUSTRIAL-SCALE PREPARATION OF A STANDARDIZED HUMAN VON WILLEBRAND FACTOR CONCENTRATE OF VERY HIGH PURITY AND SUITABLE FOR THERAPEUTIC USE

(51)[5] International Patent Classification(s)
C07K 003/28 **A61K 037/02** **C07K 015/06**

(21) Application No. : **11131/92** (22) Application Date : **19.02.92**

(30) Priority Data

(31) Number	(32) Date	(33) Country
91 02804	**08.03.91**	**FR FRANCE**

(43) Publication Date : **10.09.92**

(44) Publication Date of Accepted Application : **06.01.94**

(71) Applicant(s)
CENTRE REGIONAL DE TRANSFUSION SANGUINE DE LILLE

(72) Inventor(s)
MIRYANA BURNOUF-RADOSEVICH; THIERRY BURNOUF

(74) Attorney or Agent
GRIFFITH HACK & CO , GPO Box 1285K, MELBOURNE VIC 3001

(57) Claim

1 - A process for preparing a standardized, highly purified concentrate of human von Willebrand factor enriched in high molecular weight multimers from a cryoprecipitated fraction of plasma by a series of three successive chromatographic steps, the first two being ion exchange chromatography on a large-pore vinyl polymer resin with DEAE groups attached and the third being affinity chromatography on gelatin-sepharose.

2 - The process according to claim 1 wherein the starting material is a cryoprecipitated fraction of plasma, prepurified on aluminium hydroxide.

8 - The von Willebrand factor concentrate of very high purity and high specific activity (measured in RCo and CBA units) and high content in high molecular weight multimers obtained according to any one of claims 1 to 7.

AUSTRALIA

645172

Patents Act 1990

ORIGINAL

COMPLETE SPECIFICATION

STANDARD PATENT

Invention Title:

PROCESS FOR AN INDUSTRIAL-SCALE PREPARATION OF A STANDARDIZED HUMAN VON WILLEBRAND FACTOR CONCENTRATE OF VERY HIGH PURITY AND SUITABLE FOR THERAPEUTIC USE.

The following statement is a full description of this invention, including the best method of performing it known to me:-

ABSTRACT OF THE DISCLOSURE

The invention relates to a process for purifying human von Willebrand factor from a cryoprecipitated plasma fraction.

The process comprises a combination of three chromatographic separation steps. The concentrate obtained has very high specific activity and a high percentage of high molecular weight multimers.

The concentrate is intended, in particular, for therapeutic use.

Field of the Invention

The invention relates to a process for preparing an industrial-scale, standardized human von Willebrand factor concentrate of very high purity, very high specific activity, and high content in high molecular weight multimers, intended in particular for therapeutic use.

Background of Related Art

von Willebrand factor (vWF) is the largest known molecule circulating in plasma. It exists as a series of large disulfide-linked multimers, the basic subunit of which has a molecular weight of about 260 kilodaltons (KDa). The smallest form of vWF in plasma is a dimer of about 440-500 KDa and the largest forms are multimers of the dimer with molecular weights reaching up to 20 million daltons. The assembly of subunits which are linked together may be cell specific, the vWF being synthesized and polymerized in the megacaryocytes and endothelial cells.

This factor plays an essential role in hemostasis through two distinct functions : it transports and stabilizes factor VIII in the blood stream and, as an adhesive protein, it permits the spreading, the attachment and the aggregation of the blood platelets on the vascular subendothelium thus contributing to the swift healing of injured vessels.

A congenital vWF deficiency, or a structural anomaly of this factor, gives rise to von Willebrand disease which initially takes the form of hemorrhages, particularly cutaneous and of the mucous membranes. The clinical forms taken by this disease are very heterogenous and pose major problems in the event of surgery. Treatment of von Willebrand disease is essential in order to correct primary hemostasis (bleeding time) and coagulation (activated cephalin time and F VIII activity) anomalies.

The disease is treated by substitute therapy with vWF-enriched human plasma derivatives (for example, the cryoprecipitated fraction of plasma or the concentrates of Factor VIII containing a sufficient quantity of vWF). However, these products are not standardized for the treatment of von Willebrand disease. In addition, the poorly purified fractions of blood plasma especially cryoprecipitate are not free from the risk of viral contamination because they are often not subjected to any efficient viral inactivation step. Furthermore, they lead to an excess of contaminating proteins which the patient does not need and which can cause immune reactions after multiple injections.

Purified Factor VIII, on the contrary, can be subjected to efficient virus inactivation treatment, but its purification process has been optimized for treating hemophilia A patients and not for vWF deficient-patients. In fact, the recently developed and increasingly effective processes, such as immunoaffinity or ion exchange purification used for preparing Factor VIII, produce concentrates that no longer contain enough vWF to be efficient in the treatment of von Willebrand disease.

It is to meet this need of an efficient way of treating von Willebrand disease that the Applicant has developed a new industrial process for purifying vWF while still obtaining optimum benefit from the isolation of different plasma molecules. In particular, it permits, in one step, to prepare a concentrate of Factor VIII (according to a process described in EP Application 0 359 593) and to recover a separate vWF fraction from the same batch of cryoprecipitate, thus allowing to make an optimal use of human plasma. The vWF fraction thus obtained is purified by two additional

chromatographic steps which provide a vWF concentrate of very high purity.

The complexity of the vWF molecule makes it very difficult to purify. Small-scale methods, i.e., 5 to 2000 ml for the purposes of analytical study, have already been described (Thorell et al., Thromb. Res. 1984, 35: 431-450), but it has not been possible to adapt these methods for vWF preparation on an industrial scale. In addition, the concept of making the best possible use of cryoprecipitate by producing vWF in addition to FVIII was not considered.

vWF has been purified by differential solubilization on sulfated compounds in the presence of glycine (Berntorp et al., Vox Sang. 1989, 56: 212), sulfated compounds (Winkelman et al., Vox Sang. 1989, 57: 97) and by using different methods of chromatographic separation, such as molecular size exclusion (Perret et al., Haemostasis 1984, 14: 289) and ion exchange (Austen et al., Thromb Haemostas. 1982, 48: 295). However, these techniques give either low yields of vWF or have a low gel capacity, or do not make the simultaneous isolation of FVIII and vWF possible, which make them less convenient for an industrial application.

In addition, Berntorp et al. (Vox Sang. 1989, 56: 212) obtain a vWF of low purity : 45 U Ag/mg protein (p. 213) whereas the Applicants obtain 205 U Ag/mg protein. Similarly, Winkelman et al. (Vox Sang. 1989, 57: 97) obtain 10 U Ag/ml protein (p. 101).

Perret et al. (Haemostasis 1984, 14: 289), perform a defibrination step (to eliminate fibrinogen as fibrin molecules) with the use of calcium as well

as enzymes from snake venon. This renders the preparation obviously unsuitable for therapeutic purposes. Moreover, gel filtration systems as the one they used are hardly compatible with industrial scaling up since allowing a flow rate of only 10 cm/h or less and showing a high risk of plugging, especially in the presence of fibrinogen and fibronectin. Also the purification factor is known to be usually low due to the poor resolution of proteins in this chromatographic system.

Austen et al. (Throm. Haemostas. 1982, 48: 46) also obtain a low purity concentrate (8 U Ag/mg protein) and relatively low yield probably due to their drastic chromatographic conditions (pH 5.5).

Harrisson et al. (Thromb. Res. 1983, 50: 295) use dextran sulfate-sepharose as a chromatographic matrix ; this material has a low retention capacity for the vWF. As a result, they obtain a vWF preparation of low specific activity : 2-4 U/mg protein (p. 301).

Finally, most of these products contain a rather large proportion of denatured or inactive forms of vWF as evidenced by the ristocetin cofactor activity (RCo)/antigen ratio ranging from 0.08 to 0.8 (Lawrie et al., Br. J. Haematol. 1989, 73: 100). This makes them less efficient for therapeutic use in von Willebrand disease. On the contrary, the Applicant's procedure allows the recovery of vWF with a RCo/antigen ratio higher than unity which is comparable to that of native vWF from normal pool plasma.

Summary of the Invention

The present invention relates to an industrial process for preparing a vWF concentrate for therapeutic use as a by-product of a high-purity FVIII production process, enabling standardized batches characterized by a high content in high molecular weight multimers, to be produced from very large volumes of plasma (4000 liters or more), and allowing to make an optimal use of cryoprecipitate.

More particularly, the present invention relates to a process for preparing a vWF concentrate that comprises the combination of three successive chromatographic steps allowing an enrichment in high molecular weight multimers which are related to the vWF biological activity. The starting material is the cryoprecipitated fraction of human plasma subjected to a conventional prepurification step involving adsorption on aluminium hydroxide. This material then undergoes viral inactivation, for example using a solvent-detergent treatment, before it is purified.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The purification process according to the present invention comprises a combination of three successive chromatographic steps from a by-product fraction of a FVIII production process, the first two involving ion exchange chromatography, and the third, affinity chromatography.

The two ion exchange chromatography steps are carried out on the same vinyl polymer resin onto which are fixed diethylamino ethyl (DEAE) groups, more particularly on columns of DEAE-Fractogel® TSK 650 (Merck), equilibrated with a buffer solution containing 0.01 M trisodium citrate,

0.11 M sodium chloride, 0.001 M calcium chloride, 0.12 M glycine and 0.016 M lysine, pH 7.

DEAE-Fractogel TSK® 650 is a synthetic hydrophilic gel medium. The support is a copolymer of oligoethyleneglycol, glycidinemethacrylate and pentaerythritol-dimethacrylate to which diethylaminoethyl groups, i.e., $-O-CH_2-CH_2N^+(C_2H_5)_2HCl$, are attached, resulting in a weakly alkaline anion exchanger. DEAE-Fractogel® TSK 650 is available in two particle size ranges (when moistened with water) : Type S (0.025 - 0.050 mm) and Type M (0.045 - 0.090 mm). Both types are useful in carrying out the present invention.

The cryoprecipitated plasma fraction, which has been prepurified and has undergone viral inactivation treatment according to conventional procedures, is applied to the first chromatographic column which retains a large proportion of the vWF. vWF is then eluted by increasing the sodium chloride concentration of the buffer solution to 0.14-0.15 M.

The fraction thus eluted, enriched in vWF, is applied to the second chromatographic column under the same conditions as the first. Since many of the proteins (especially FVIII and fibronectin) which competed for the adsorption sites have already been eliminated from this fraction during the first chromatographic step, the capacity for adsorbing the vWF on the second column is advantageously far greater. After the filtrate has been removed and the column has been rinsed with the equilibration buffer solution, the adsorbed vWF is eluted by increasing the sodium chloride concentration of the buffer solution to 0.15-0.17 M. Due to the excellent capacity and efficiency of the DEAE Fractogel® resin for vWF, vWF can be

eluted from the column at a very high potency (> 150 U RCo/ml). Thus, the mechanical stress of ultrafiltation that would be needed to concentrate the product can be avoided.

The fraction thus eluted is subjected to affinity chromatography on a gelatin-derived gel in the same equilibration buffer solution thus avoiding any dialysis or ultrafiltration step to modify the salt composition ; this column is essentiel to retain the molecules of residual fibronectin that still contaminate the vWF. The choice of gelatin-derived gel is not critical, however : Gelatin-Sepharose, Gelatin-Ultrogel®, Gelatin-Spherodex® and Gelatin-Fractogel® are all suitable for this purpose. Gelatin-Sepharose may be the best choice since it fixes 5 to 10 mg fibronectin/ml gel under the conditions used for the present process.

In the conditions used the highly purified vWF does not bind on the gel and is thus eluted in the filtrate ; as the gelatin affinity step does not induce extensive dilution of the vWF fraction, the product can be directly dispensed without any need for a concentration step by e.g. ultrafiltration. The absence of proteolytic enzymes in the final product makes it very stable during the sterile filtration and freeze-drying steps, without any need for stabilizing agents.

The von Willebrand factor concentrate obtained using the process according to the present invention has an exceptionally high purification factor of > 10,000 fold in relation to the initial plasma, and its specific activity is 345 U CBA/mg protein (units of measurement for the collagen binding activity), and > 100 U RCo/mg protein (units of ristocetin cofactor

activity). The contribution of each chromatographic step to purifying vWF is illustrated in Figure 1.

Quite importantly, improvement in the quality of the product during the successive purification steps was monitored as a function of the proportion of high molecular weight multimers (the molecular forms of vWF having high biological activity) as detected by electrophoretic analysis.

Interestingly, this analysis reveals a progressive enrichment in multimers ≥ 4 (Figure 2), which represent 79 % of the vWF polymers eventhough cryoprecipitation eliminates half of them. Unexpectedly, it is the chromatography on DEAE-Fractogel TSK 650 that favors this selective retention of the very large multimers and eliminates with the filtrate the forms of small size, abnormal structure (having undergone partial proteolysis) and low activity.

The standardized vWF concentrate of high purity, high specific activity and high content in high molecular weight multimers, obtained by the process according to the present invention is thus particularly well suited to therapeutic use in the different forms of von Willebrand disease, as confirmed by preliminary clinical studies.

Preliminary clinical tests have shown that this concentrate led to an efficient shortening of the bleeding time during hemorrhages.

In vitro tests have confirmed that its biochemical and physiologic l properties are identical to those of the native molecule, in particular its ability to fix blood platelets in a perfusion device, and its ability to bind in-vivo endogeneous Factor VIII.

Due to its high purity, the vWF obtained during the process according to the present invention could also be considered for various laboratory applications (fine structural analysis, functionality studies, diagnoses, etc.) and for the production of specific antibodies.

The concentrate according to the present invention can also be used as a stabilizer during the production of Factor VIII by cells transformed by genetic engineering as well as during the purification of the Factor VIII thus produced.

The following example illustrates one form of an embodiment of the present invention without, however, limiting the scope thereof.

EXAMPLE

Starting Material

The cryoprecipitate is prepared from fresh plasma collected in the presence of sodium citrate (4 %) or CPD (citrate, phosphate, dextrose) anticoagulant solution and frozen at the most 6 hours after being obtained. The plasma is deep frozen to -60° C, then preserved at -35° C. The plasma batches contain 1800 to 2000 liters which are pooled into 4000-liters batches for each application of the process. For the purpose of thawing, the plasma is placed in a temperature-regulated chamber at for 12 hours to ensure slow, regular warming to - 7° C, then thawed in a thermostatically controlled enclosure at 0° to 2° C with constant stirring. The cryoprecipitate (which represents about 9 g/l plasma) is recovered by cold centrifugation.

After centrifuging, the cryoprecipitate recovered is resolubilized and adsorbed on aluminum hydroxide to remove some contaminants, i.e. the components of the prothrombin complex (particularly Factor VII) and Factor XII. The supernatant is then cooled to 15° C (which partially removes the fibrinogen and the fibronectin).

This treatment permits the recovery of 80 to 86 % of the Factor VIII/vWF mixture from the cryoprecipitate ; the specific activity of the Factor VIII represents 0.7 IU/mg, and that of the vWF 0.6 U RCo/mg (ristocetin cofactor activity) and 1.2 U CBA/mg (collagen binding activity).

Viral Inactivation Treatment

The solution containing the Factor VIII/vWF mixture is subjected to a solvent-detergent treatment known for its efficiency in destroying lipid

enveloped viruses (Horowitz et al., Transfusion, 1985, 25: 516) and which includes incubation for 8 hours at 25° C in the presence of 0.3 % of tri-n-butyl phosphate (TnBP) and 1 % of Tween 80.

After this treatment, 95 % of the activity of Factor VIII and vWF measured in the preceding step is recovered. Electrophoresis can be used to confirm that the vWF is still in multimeric form.

Chromatographic Separation Process

The purification of the vWF is derived from the Factor VIII purification process disclosed by the Applicant in European patent application No. EP 0,359,593.

The first chromatography is carried out on a column of DEAE-Fractogel® TSK 650 (Type S or M) (Merck). The equilibration buffer solution contains trisodium citrate (0.01 M), calcium chloride (0.001 M), glycine (0.12 M), L-Lysine (0.016 M) and 0.11 M sodium chloride). The vWF, Factor VIII and fibronectin are retained by the column ; the contaminating proteins (chiefly fibrinogen and some IgG) loosely fixed or not fixed by the column and the virus sterilizing agents are eliminated by several successive washings with the same buffer solution.

The column is used at a linear flow rate of 100 cm/h. Under these working conditions, the column used has a vWF retention capacity of approximately 75 % of the amount injected (measured as the antigen, Ag) the remainder being lost in the filtrate. This binding capacity corresponds to 45 U of vWF Ag/ml gel.

The vWF is desorbed from the column by increasing the NaCl concentration of the buffer solution to 0.15 M. The fraction of vWF harvested contains 30 to 35 % of the initial vWF while 40 % of it remains co-adsorbed with the Factor VIII which will be co-eluted by a second increase in the NaCl concentration of the buffer solution to 0.25 M and then co-purified.

The fraction containing the vWF eluted from this first column is reinjected onto a second identical column, after a slight dilution with the equilibration buffer, in order to adjust the ionic strength of the vWF fraction down to an equivalent of 0.11 M sodium chloride.

Since the contaminants and the Factor VIII which competed with the vWF for the adsorption sites of the first column were almost eliminated during the first chromatographic step, binding capacity of the second column is much greater : 320 U of vWF Ag/ml gel.

The vWF is desorbed by increasing the NaCl concentration of the buffer solution to 0.17 M.

This second chromatography permits a concentration rate 8 to 10 times that of the previous one, which eliminates the need for any additional concentration steps by ultrafiltration, for example. Using standardized techniques, the eluate is found to contain the following vWF quantities or activities :

- Antigen (Ag) 88 ± 9 IU/ml
- Ristocetin cofactor (RCo) 97 ± 19 IU/ml
- Collagen binding activity (CBA) 149 ± 13 IU/ml
- High molecular weight multimers (≥ 4 multimers) 79 %

The CBA units (collagen binding activity) are quantified by ELISA as described by Brown and Bosak, (Thromb. Res. 1986, 43: 303). A standard plasma, calibrated against the 2nd British Standard (86/717), was used as a reference to express the values in terms of international units.

The CBA/Ag ratio of 1.69 shows that the activity of the vWF is well preserved. This is in agreement with the high percentage in high molecular weight multimers (79 %) and compares favorably with that of native vWF (70 %) from plasma.

Electrophoretic analysis of this vWF eluate reveals a slight contamination by fibronectin and inter-alpha trypsin inhibitor, a serine-protease inhibitor.

The second vWF eluate is then subjected to a third step of purification on a column of gelatin-Sepharose CL4B (Pharmacia) equilibrated with the elution buffer solution of the preceding column, in order to eliminate fibronectin.

This affinity chromatographic gel has a fibronectin retention capacity of > 5 mg/ml, which enables this contaminant to be reduced to undetectable quantities (< 4 mg/l) in the vWF fraction.

The purified vWF of the present invention is found in the filtrate of this last step and can be directly dispensed and freeze-dried.

Electrophoretic analysis of the final product can no longer detect any contaminants. The vWF content is 205 U Ag/ml protein and its specific activity is 345 U CBA/mg protein and 186-220 U RCO/mg protein.

The total degree of purification in relation to the initial plasma is > 10,000 fold.

Electrophoretic analysis (SDS-agarose and scanning of the bands) demonstrates that the vWF obtained from this purification procedure is composed of 65 to 80 % of high molecular weight multimers, i.e. a proportion comparable with that of the initial plasma, which was 70 %.

The stability of the concentrate was studied in a liquid state at room temperature for 24 hours : no sign of proteolytic activity or any change in specific activity could be detected.

Absence of thrombogenic activity in the concentrate was verified using the conventional tests like the non-activated partial thromboplastin time (NAPTT). Thrombin, PKA and Kallikrein were undetectable.

Therefore, no stabilizing agent needs to be added to the final vWF concentrate.

The possibility of designing a purification process specifically intended for the recovery of vWF as a by-product of a FVIII production process makes thus, for the first time, possible to produce a high-purity, highly effective therapeutic concentrate standardized for the treatment of von Willebrand disease.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1 - A process for preparing a standardized, highly purified concentrate of human von Willebrand factor enriched in high molecular weight multimers from a cryoprecipitated fraction of plasma by a series of three successive chromatographic steps, the first two being ion exchange chromatography on a large-pore vinyl polymer resin with DEAE groups attached and the third being affinity chromatography on gelatin-sepharose.

2 - The process according to claim 1 wherein the starting material is a cryoprecipitated fraction of plasma, prepurified on aluminium hydroxide.

3 - The process according to claim 1 wherein the first and second ion exchange resin is DEAE-Fractogel® TSK 650 equilibrated with a buffer solution containing 0.01 M trisodium citrate, 0.11 M sodium chloride, 0.001 M calcium chloride, 0.12 M glycine and 0.016 M L-lysine.

4 - The process according to claim 1 wherein the prepurified cryoprecipitated fraction of plasma is applied to the first ion exchange chromatography column and the first von Willebrand factor containing fraction is eluted by increasing the sodium chloride concentration of the buffer to 0.14-0.15 M.

5 - The process according to claim 1 wherein the von Willebrand factor containing eluate from the first chromatography is applied to a second ion exchange chromatography column and the von Willebrand factor is eluted by increasing the sodium chloride concentation of the buffer to 0.15-0.17 M.

6 - The process according to claim 1 wherein the eluate from the second chromatography is applied to a gelatin-Sepharose chromatography equilibrated with the elution buffer from the previous chromatographic step which results in the selective adsorption of the residual fibronectin onto the column.

7 - The process according to claim 1 wherein the von Willebrand factor present in the filtrate of the gelatin-Sepharose column is collected, dispensed and freeze-dried.

8 - The von Willebrand factor concentrate of very high purity and high specific activity (measured in RCo and CBA units) and high content in high molecular weight multimers obtained according to any one of claims 1 to 7.

9 - The von Willebrand factor concentrate according to claim 8 characterized by a ratio of activity (measured in CBA units) to the quantity of antigen present of at least 1.5.

10 - The von Willebrand factor concentrate according to claims 8 or 9 wherein the percentage of high molecular weight multimers is at least 65-80 %.

DATED THIS 19TH DAY OF FEBRUARY 1992
CENTRE REGIONAL DE TRANSFUSION SANGUINE DE LILLE
By its Patent Attorneys:
GRIFFITH HACK & CO.
Fellows Institute of Patent
Attorneys of Australia