

JVRM/SC

14th January 1975

Department of Health and Social Security,  
Finsbury Square House,  
33/37A Finsbury Square,  
London,  
EC2A 1PP.

For the attention of Dr. M. E. Duncan

Dear Dr. Duncan,

Anti-haemophilic Factor PL/0037/0071

Herewith please find an application for a variation covering new stages in the manufacturing process and consequent other changes. It may be of interest to know that the new method has been accepted by the F.D.A.

During our telephone conversation in December, you raised the matter of the unit used. I have gone into this very carefully. The unit used by Abbott is the F.D.A. standard which is identical with the W.H.O. standard. Although we do not mention the International Unit on our labels at present, I shall be proposing that this should eventually be done.

I take this opportunity of bringing to your notice some minor amendments to the submission of 22nd August 1974:-

page 4 2nd line. Between "of kilogram" and "activity" please insert  
"of body weight, a 2% rise in Factor VIII"

Please delete the "of" immediately preceding "activity"

page 14. Sub-section 11.5.1.2. Second line:

should be part 273 instead of 373

page 20. Sub-section 11.5.1.9:

the missing page numbers are "42 ff"

/Cont.....

(2)

Dr. M. E. Duncan

9th January 1975

Anti-haemophilic factor PL/0037/0071/Cont.....

I would also bring to your notice that we have added the following to the list of Donor Centres (p.6 of submission):-

United Biologics,  
1220 National City Avenue,  
National City, California 92050.

American Blood Components,  
623 Jefferson,  
Memphis, Tennessee 38103.

American Blood Components,  
615 Main Street,  
Little Rock, Arkansas 72201.

American Blood Components,  
511 8th Avenue South,  
Nashville, Tennessee 37203.

American Blood Components,  
1101 W. Broadway,  
Louisville, Kentucky 40203.

American Blood Components,  
1800 Chester Avenue,  
Cleveland, Ohio 44114.

American Blood Components,  
844 S. Patterson Boulevard,  
Dayton, Ohio 45402.

American Blood Components,  
916 E. McMillan,  
Cincinnati, Ohio 45206.

American Blood Components,  
1124 Washington,  
St. Louis, Missouri 63101.

I shall be happy to answer any questions you may have on the enclosed application for a variation, or any other relevant matter.

Yours sincerely,

GRO-C

J. V. R. Marriott, B.Sc., Ph.D.  
Manager - Regulatory Affairs

Encl:-

APPLICATION FOR VARIATION OF PRODUCT LICENCE  
NOTIFICATION OF CHANGE IN PRODUCT LICENCE

1. Licence Number: 0037/0071 Your reference: JVRM/SC

	<u>At present</u>	<u>Any proposed change</u>
2. Name and Address of Licence Holder:	Abbott Laboratories Ltd., Queenborough, Kent, ME11 5EL.	

Name of Product: ANTIHAEMOPHILIC FACTOR (HUMAN)

3. Please indicate if you have changed or propose to change any of the following:

- |   |   |
|---|---|
| <input type="checkbox"/> Pharmaceutical Form              | <input type="checkbox"/> Activities covered by Licence              |
| <input type="checkbox"/> Active Ingredients               | <input type="checkbox"/> Assembler                                  |
| <input type="checkbox"/> Indications                      | <input type="checkbox"/> Arrangements for Storage                   |
| <input type="checkbox"/> Dosage                           | <input type="checkbox"/> Container                                  |
| <input type="checkbox"/> Contra-Indications and Warnings  | <input type="checkbox"/> Shelf Life or Storage Precautions          |
| <input type="checkbox"/> Method of Retail Sale and Supply | <input checked="" type="checkbox"/> Method of Manufacture           |
| <input type="checkbox"/> Manufacturer                     | <input checked="" type="checkbox"/> Quality Control Procedures      |
| <input type="checkbox"/> Date of Expiry of Licence        | <input checked="" type="checkbox"/> Finished Product Specifications |
|   | <input type="checkbox"/> Excipients                                 |
|   | <input type="checkbox"/> Supplier of Active Ingredients             |
|   | <input checked="" type="checkbox"/> Other (specify) Name of Product |

4. On the attached sheet, give the present particulars, the change or proposed change and the reason. Supporting evidence should be attached to the application. Please indicate the number of volumes and number of copies sent:--

5. If you need approval urgently, please give the date by which an answer is required: 5th February 1975

For Licensing Authority use only

F N M P C A NF U

Pharm:  
Med:

Received .....

Acknowledged .....

Approved:

Date:

Licence Number: 0037/0071

Your reference: JVRM/SC

Name of Product: ANTIHAEMOPHILIC FACTOR (HUMAN)

Give the present particulars and the change or proposed change. If the particulars appear on the licence document itself, you should give them exactly as they are given on the licence, or as you propose they should be given. (The items in the lefthand column of (3) are usually specified on product licences.)

<u>Present</u>	<u>Proposed</u>
Method of Manufacture	
1) Flow sheet as per Section 13, p.15	1) Flow sheet as per attached (page 1 attachment)
2) As per sub-sections 3.3.0 to 3.3.7 and in particular 3.3.2 to 3.3.4	2) As per sub-sections 3.3.2-3.3.4 of attached page 2
Use of "Sodium Chloride for Injection" for reconstitution	Use of "Sterile Water for Injection USP" for reconstitution. Deletions and additions detailed on attached page 3
In-process controls for AHF. As per sub-section 11.3.1, p.12	As per p.4
Finished product specifications: As per sub-section 11.4.1, p.13	Introduction of limits for pH, Na, Al; increase in total protein maximum; increase of activity. As per page 5
Analytical controls on Finished products: as per sub-section 11.5.1, p.14	Addition of methods for pH, Na and Al, as per p.6
Stability. As per Section 15, p.23 ff (continue on a separate sheet if necessary)	Stability data for new product

Reason for the change

The new process of manufacture (Method C) gives a better yield of AHF which is also improved in the speed with which it can be reconstituted and in its activity.

The use of Water for Injection with this product gives a solution nearer to isotonicity than the use of Sodium Chloride solution with the old product.

I hereby make application for the above licence to be varied in accordance with the proposals given above.

Signed:

GRO-C

J. V. R. Marriott, B.Sc., Ph.D.  
Manager - Regulatory Affairs

Date:

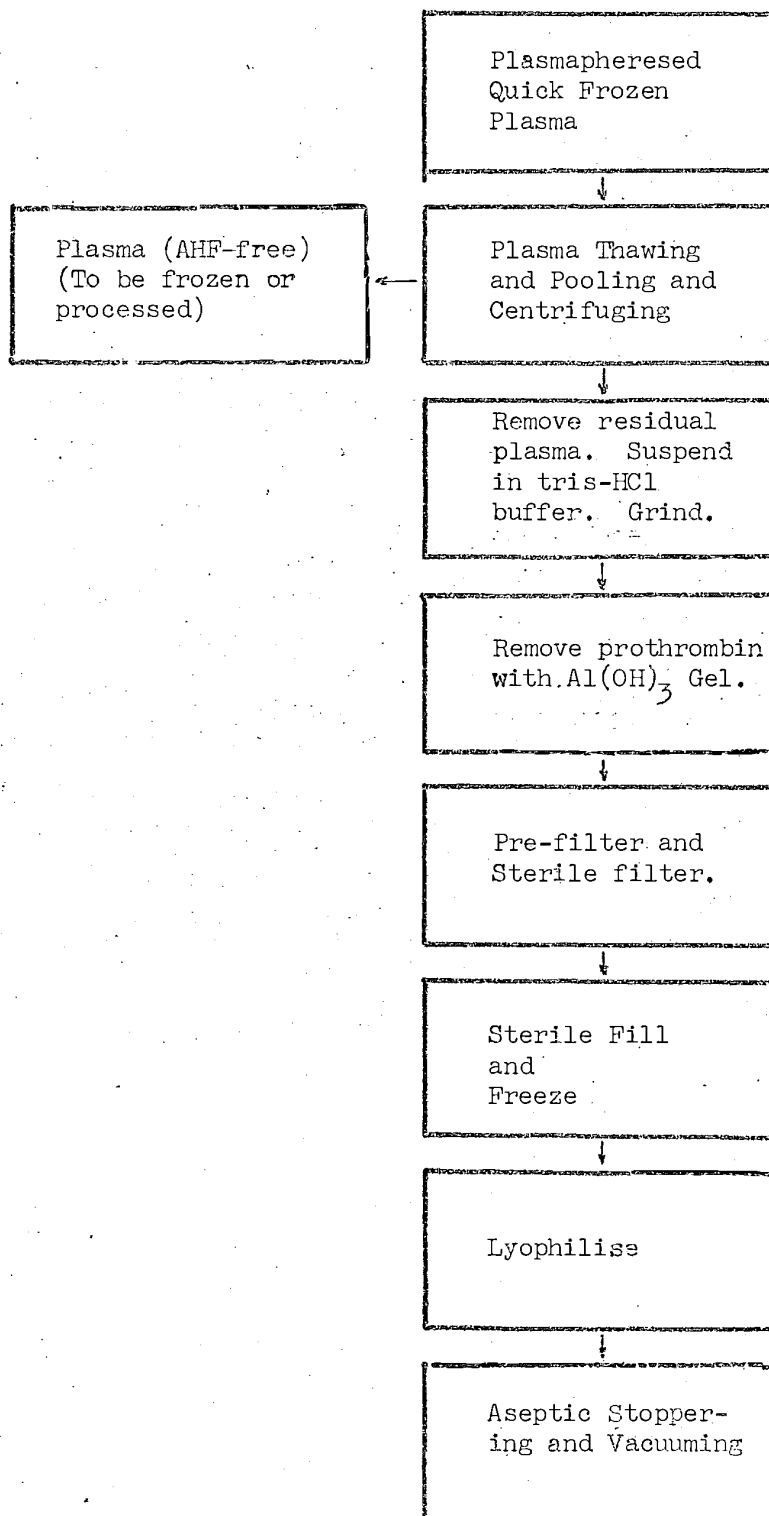
9th January 1975

FLOW SHEET (METHOD C)

To replace flow-sheet on page 5 of submission of 22nd August 1974:-

13. Manufacture and Assembly:

a) The manufacturing process is summarised in the following flow-sheet:-



/Cont.....

"NEW STAGES IN MANUFACTURING PROCESS" (METHOD C)

To replace stages of same numbers of pages 9 and 10 of submission of 22nd August 1974:-

3.3.2. Removal of residual plasma:

The paste is carefully transferred to a sterilised nylon cloth stretched over a perforated stainless steel tray and rapidly washed with water for irrigation to remove superficial residual plasma.

The paste is then added to a calculated amount of 0.02 M tris-hydroxymethyl aminoethane at 4°C (which is not more than 24 hours old) and fed into a grinder. Paste remaining in the grinder at the end of the operation is removed by hand (a new pair of sterile gloves having been donned), broken up and added to the ground suspension in the receiving jacketed vessel.

3.3.3. Removal of prothrombin:

The suspension is gently stirred with a non-aerating mixer for 30 minutes at  $20 \pm 1^\circ\text{C}$ . The pH is then adjusted to  $7.0 \pm 0.1$  with 0.1 N HCl.

Quality Assurance approved  $\text{Al}(\text{OH})_3$  gel in calculated amount is added and stirred in for 10 minutes at  $20 \pm 1^\circ\text{C}$ . The suspension is pumped, using a peristaltic pump, through a centrifuge, the operation taking place in a room maintained at a temperature of  $4 \pm 1^\circ\text{C}$ . It is further clarified by filtration under nitrogen pressure through an assembly of membranes of 8.0, 3.0, 1.2, 0.45 and 0.22  $\mu\text{m}$  pore size.

3.3.4. Stabilisation and sterile filtration:

A calculated amount of 0.05 M sodium citrate is added to the AHF solution followed by an amount of 0.1 N HCl calculated to neutralise the basic solution. The pH is then adjusted to  $7.0 \pm 0.05$  by addition of small increments of HCl solution.

The solution is sterile filtered first through a 0.8  $\mu\text{m}$  filter (Sartorius membrane), then through a 0.2  $\mu\text{m}$  filter.

N.B. Stages 3.3.1, 3.3.5, 3.3.6, 3.3.7 as described in the original application remained unchanged. The general remarks of 3.3.0. still obtain.

REPLACEMENT OF RECONSTITUTION MEDIUM, INITIALLY "SODIUM CHLORIDE FOR INJECTION", BY  
"STERILE WATER FOR INJECTION U.S.P.".

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Material relevant to "Sodium Chloride for Injection" in the submission of  
22nd August 1974 to be deleted:-

Page 11 Sub-Sections 9.2.2.  
Section 10  
Sub-Section 11.1.2.

Page 12 Sub-Section 11.2.2.

Page 13 Sub-Section 11.3.2.

Page 14 Sub-Section 11.4.2.

Page 21 Sub-Section 11.5.2.

The following is relevant to "Sterile Water for Injection U.S.P." and has been given  
the decimal notation numbers indicating their relation to the original submission:

In-Process controls:

11.3.2 On bulk "Sterile Water for Injection

Before sterilisation: pH: this should be within the range  
5.0-7.0 after the addition of 0.3 ml  
of saturated KCl solution to a 100 ml  
sample of water (potentiometric  
determination).

Chloride: No opalescence produced on addition  
of 5 drops of nitric acid and 1 ml of  
AgNO<sub>3</sub> TS to a 100 ml sample

Finished Product Specification:

11.4.2 "Sterile Water for Injection U.S.P.

Passes all tests as detailed in U.S.P. XVIII

and Identification: Absence of opalescence on addition of  
AgNO<sub>3</sub>

Volume: Between 24.5 and 26.0 ml

Analytical Control in Final Products:

11.5.2 "Sterile Water for Injection"

/Cont.....

- 11.5.2.1. Sterility: 10 bottles chosen at random from autoclaved lot are tested
- 11.5.2.2. Pyrogen: 3 random bottles from each autoclaved lot are tested. The contents are pooled, made isotonic with 0.9 g pyrogen-free NaCl for each 100 ml water and tested by the U.S.P. method.
- 11.5.2.3. Identification: A bottle is checked for lot number and identity. To the water is added a few drops of 0.1 N  $\text{AgNO}_3$ . There should be no change of appearance.
- 11.5.2.4. Volume: 3 bottles are tested by transferring to a measuring cylinder. The volume should be between 24.5 and 26.0 ml for each bottle.



~~IN-PROCESS CONTROLS~~

As a result of the introduction of the new stages, the in-process controls become:-

Bottles of thawed plasma are examined for haemolysis and fat and those positive rejected.

During centrifuging the temperature and flow-rate are checked and recorded every 20 minutes.

The tris-HCl buffer, made from ingredients which have been approved by Quality Assurance, is maintained at a temperature of  $4^{\circ}$  and is never more than 24 hours old.

Temperature of the cryoprecipitate mixture is controlled to the  $20 \pm 1^{\circ}\text{C}$  and the time of mixing is not allowed to exceed 30 minutes.

The  $\text{Al}(\text{OH})_3$  gel is purchased against specification (as well as the other materials).

During the centrifuging to remove the  $\text{Al}(\text{OH})_3$ , the temperature is maintained at less than  $4^{\circ}\text{C}$  and the pump flow is controlled.

The 0.5 M sodium citrate solution is prepared from U.S.P. Sodium Citrate and Abbott Water for Irrigation and is maintained at  $4^{\circ}\text{C}$  before use.

The pH of the stabilised solution is measured and adjusted to  $7.0 \pm 0.05$ .

The protein and activity are determined in the bulk solution and tests performed for sterility.

At all stages, starting and finishing times are recorded. At the beginning and end of most stages, counts, weights or volumes, as appropriate, are recorded.

FINISHED PRODUCT SPECIFICATION

The specification for the product manufactured by Method C is unchanged as regards the following:-

Sterility  
Safety  
Identity  
Pyrogen  
Moisture  
Isoagglutinins  
Identity labelled Final Container

The following items are deleted:

Thrombin (which is completely removed by the  $\text{Al}(\text{OH})_3$  treatment).

Clottable protein (as all tests have shown that the clottable protein values are less than the total protein values)

The following items are changed to:

Total protein: Does not exceed 4.4 g % upon reconstitution

Activity: Minimum 7.0 AHF units/ml

The following items are new:

pH: 6.5 to 8.5

Aluminium: Not to exceed 1  $\mu\text{g}$ /unit AHF.

Sodium: 200 mEq/litre maximum

## ANALYTICAL CONTROLS IN FINAL PRODUCTS

The following methods should be added to those described in sub-section 11.5.1. of the original submission:-

### 11.5.1.11. pH:

Reconstitute product with Sterile Water for Injection.

With pH-meter in stand-by mode, rinse electrodes with distilled water and dry with soft tissue. Adjust mid-range value selector to 7, slope knob to the room temperature and mode selection knob to pH.

Immerse electrodes in buffer solution of pH 6.85 and calibrate to that pH in the expanded scale mode.

Return to stand-by mode, rinse and dry electrodes.

Immerse electrodes in sample and read pH in the expanded scale mode.

### 11.5.1.12. Sodium:

Dilute all final container or final bulk samples 1:100 in 0.2% Sterox solution. Turn on Perkin Elmer flame photometer's power control. Depress the ignitor button. Turn on the gas supply. Once the flame appears, release the ignitor button. Open the oxygen supply and adjust the oxygen pressure to 13 pounds per square inch. Observe the colour of the flame. The flame should be dark blue in colour. If the flame has a yellow appearance, adjust the flame using the mixer control. Set the filter selection knob to the element being determined. When determining potassium, use the serum potassium setting. Fill one blue sample cup, each, with low standard, 0.2% Sterox solution and high standard. Place the cups in the sample tray in the order mentioned. Fill two red sample cups with sample and place them in the sample tray. Move the low standard into the feed position. If sodium is being determined, set the value to read 75 meq/l on the sodium scale, using the zero adjust knob. If potassium is being determined, set the reading to zero on the potassium scale, using the zero adjust knob. Move the 0.2% Sterox solution into the feed position and rinse the aspirator for a few seconds. Move the high standard into position. When determining sodium, adjust the reading to 140 meq/l on the sodium scale, using the standardise knob. If potassium is being determined, adjust the reading to 5 meq/l on the potassium scale. Rinse the aspirator with 0.2% Sterox solution, then check the zero with the low standard. Readjust if necessary. Check the calibration of the high standard and adjust if necessary. Repeat these steps until the high and low standards read the proper values. Move the sample into the feed position and record the values. Report the average reading to the nearest whole unit for sodium. Report the average reading for potassium to the nearest tenth of a unit for values greater than 1.0 meq/l. For values less than 1.0 meq/l, report the potassium concentration as "less than 1.0 meq/l".

/Cont.....

## Stock Solutions:

## 1) 0.2% Sterox solution

Dilute 10 ml of 2% Sterox solution, available from Perkin Elmer Corporation, to 1.0 litre with distilled water

## 2) High standard - Dilute 10 ml of commercially available stock standard containing 140 meq/l sodium and 5 meq/l potassium to one litre with 0.2% Sterox solution.

## 3) Low standard - Dilute 10 ml of commercially available stock standard containing 75 meq/l sodium and 0.0 meq/l potassium to one litre with 0.2% Sterox solution.

## Equipment:

## 1) Perkin-Elmer Flame Photometer

## 2) Oxygen cylinder and regulator

## 3) Natural gas source

11.5.1.13. Aluminium:

By the method of Gentry and Sherrington, "The direct photometric determination of Aluminium with 8-hydroquinoline". The Analyst, 1946, 71, p.432.

STABILITY:

Activity contained in the reconstituted contents (25 ml) of containers, as marketed, was determined by the thromboplastin generation test. The containers were stored at a temperature of 2-8°C.

Lot No. 008-3 Date of initial assay: 7 December 1972 Value initial assay: 241 units

<u>Weeks storage</u>	<u>Units/container</u>
6	241
13	247
18	253
26	263
34	245
66	246
86	233
97	253

Lot No. 008-4 Date of initial assay: 21 December 1972 Value initial assay: 229 units

<u>Weeks storage</u>	<u>Units/container</u>
5	222
11	236
16	265
17	247
23	268
31	247
64	283
84	215
95	277

Lot No. 008-5 Date of initial assay: 9 January 1973 Value initial assay: 212 units

<u>Weeks storage</u>	<u>Units/container</u>
8	247
13	244
21	222
29	259
61	321
92	249

XXXX  
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Dr. J.V.R. Marriott, B.Sc., Ph.D.,  
Abbott Laboratories Ltd,  
Queenborough,  
Kent.  
MEL1 5EL.

JVRM/SC

MED/AP

16 December 1974

Dear Dr. Marriott,

Anti-haemophilic Factor - PL 0037/0071

Thank you for your letter of 13th December 1974, accepting the conditions stipulated by the CSM and detailed in my letter of 10th December 1974.

In the context of the batch release procedure, our understanding of the term "full protocols" is full details of assay and test conditions and the methods which have been employed, together with the results which have been obtained.

I hope that this answers your query.

Yours sincerely,

M.E. Duncan

JVRM/SC

13th December 1974

Department of Health and Social Security,  
Finsbury Square House,  
33/37A Finsbury Square,  
London,  
EC2A 1PP.

For the attention of Dr. M. E. Duncan

Dear Dr. Duncan,

Anti-haemophilic Factor - PL 0037/0071

Thank you for your letter of 10th December 1974. We are happy to learn that the C.S.M. has agreed to a Product Licence being granted provided we give our agreement to certain conditions.

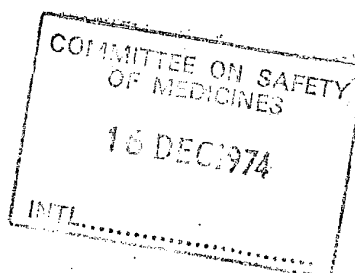
We hereby accept the conditions as detailed in your letter.

Would you please confirm that the word protocols in sub-section 2 (a) of your letter is to be understood as meaning results of assays and tests.

Yours sincerely,

**GRO-C**

J. V. R. Marriott, B.Sc., Ph.D.  
Manager - Regulatory Affairs



Dr. J.V.R. Marriott,  
Manager - Regulatory Affairs,  
Abbott Laboratories Ltd,  
Queenborough,  
KENT. ME11 5EL.

PL/0037/0071

10 December 1974

Dear Mr. Marriott,

Your application for a product licence for Antihaemophilic Factor (Human) Injection has now been considered by the Committee on Safety of Medicines. The Committee has advised that a Product Licence should be granted, provided that we may have your prior agreement, in writing, to the following conditions

1. That potency and dosage are expressed in units with reference to the International Standard.
2. That the following batch release procedure shall apply:-
  - (a) The licensee shall on request furnish to the licensing authority from every batch of the substance, or from such batch or batches as the licensing authority may from time to time specify, a sample of such amount as the authority may consider adequate from any examination required to be made; and the licensee shall, if required by the licensing authority, furnish full protocols of the tests which have been applied;
  - (b) if the licensing authority so direct, the licensee shall not sell or offer for sale any batch in respect of which a sample is or protocols are furnished under the last preceding sub-paragraph until a certificate authorising the sale of the batch has been issued to him by the licensing authority;
  - (c) the licensee shall, on being informed by the licensing authority that any part of any batch of the substance has been found by the licensing authority to be unsatisfactory as regards strength, quality and purity, and on being directed so to do, withdraw the remainder of that batch from sale and, so far as may be practicable, recall all issues already made from that batch;

In this context may I remind you that the material tested in the U.K. under the batch release procedure will be required to satisfy the tests prescribed in the British Pharmacopoeia.

I look forward to hearing from you.

Yours sincerely,

M.E. Duncan



NOT FOR PUBLICATION

COMMERCIAL - IN CONFIDENCE

COMMITTEE ON SAFETY OF MEDICINES

Sub-Committee on Biological Substances

	PL/0037/0071
Report by	Dr. D.P. Thomas
Meeting	November 1974
Therapeutic Class	Blood Product
Date Received	23-8-74

MEDICINES ACT 1968 - APPLICATION FOR A PRODUCT LICENCE

Summary and Report

1. PRODUCT SUMMARY

- 1.1. Name of Product: ANTIHAEMOPHILIC FACTOR (HUMAN)
- 1.2. Description: Lyophilized powder containing dried Human Antihaemophilic Factor. (to be reconstituted with supplied diluent).
- 1.3. Licence to be held by: Abbott Laboratories Ltd.,  
Queenborough,  
Kent.
- 1.4. Period of Validity: 5 years
- 1.5. Manufacturer: Abbott Scientific Products Division,  
Abbott Laboratories,  
5555 Valley Boulevard,  
Los Angeles, California 90032, U.S.A.
- 1.6. Proposed Method of Sale: Through Supply Division of the Department of  
Health and Social Security.
- 1.7. Consideration of this application by other Sub-Committees Not referred

2. CLINICAL USE:

2.1 Recommended clinical use:

Therapy of Haemophilia A (Classical Haemophilia).

2.2 Route of Administration:

Intravenous

2.3 Recommended Dosage:

The dosage must be individualised according to the weight of the patient, the severity of the bleeding, the severity of his blood condition, the source of bleeding, inhibitors present (if any) and other factors as determined by the managing physician or surgeon.

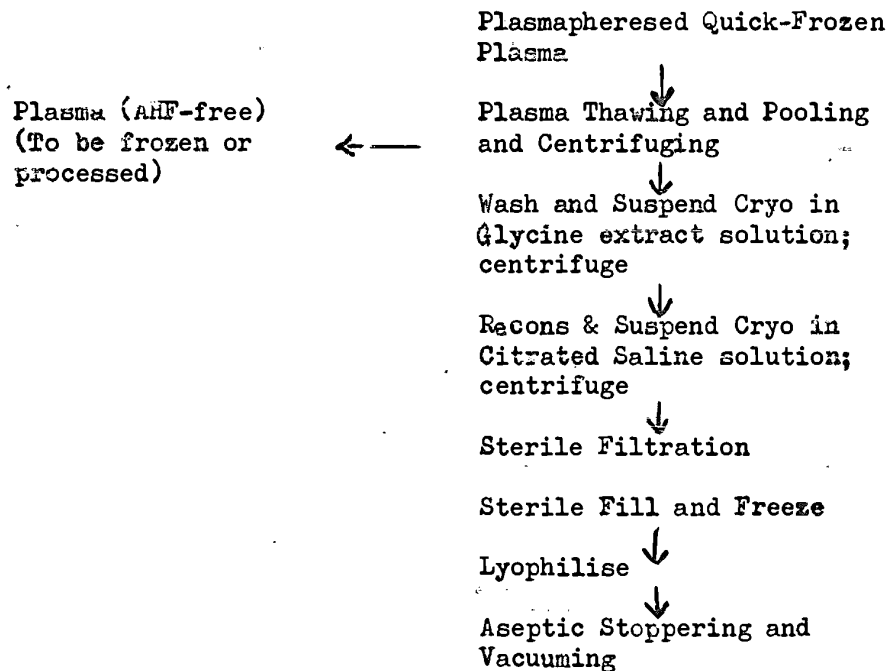
The clinical effect on the patient is the most important factor in the evaluation of adequacy of therapy. It may thus be necessary to administer more Antihaemophilic Factor (Human) AHF that would be estimated in order to obtain the desired result. The dosage requirements of AHF when inhibitors are present are extremely variable, and the dosage can only be determined by the clinical response. Occasionally, low increments of AHF in patients with AHF inhibitors may suffice to produce satisfactory clinical responses.

3. STANDARD PROVISIONS:

No exemption is required from any of the standard provisions.

4. MANUFACTURE AND ASSEMBLY:

4.1 The manufacturing process is summarised in the following flow-sheet:-



4.2 (i) Collection of blood:

The Source Plasma (Human) used in the manufacture of the product

is collected by the United Biologics Donor Centres, owned by Abbott Laboratories. On November 20th, 1973, the United States Food and Drug Regulations for Source Plasma (Human) became effective. As required under these Regulations, applications for licence for Source Plasma (Human) for each location were submitted to the Food and Drug Administration before this date. These locations are in California, Arizona, Texas, Oregon and Washington.

4.3. Manufacture of Dosage Product:

Abbott Scientific Products Division,  
Abbott Laboratories,  
5555 Valley Boulevard,  
Los Angeles, California.

5. QUALITY CONTROL

5.1 Quality control will be exercised as required under the U.S. Food and Drug regulations.

5.2 The licensee will be responsible for deciding if any batch of the product is of acceptable quality for marketing. This function will be exercised by the manufacturer, Abbott Scientific Product Division, at 5555 Valley Boulevard, Los Angeles, California.

6. Containers:

A 30 ml vial for the Antihaemophilic Factor (Human).  
A 30 ml vial for the Sodium Chloride Injection, U.S.P.

Storage should be at temperatures between 2 and 8°C.

The product will be shipped directly to the consignee, by air from the U.S. to the U.K.

7.1 Labelling:

The label and the package enclosures will carry the following warning:

'Single dose container for intravenous administration'

'Discard unused contents'

'This product is prepared from units of human plasma which have been tested and found nonreactive for Hepatitis Associated Antigen. However, it is recognised that presently available methods are not sensitive enough to detect all units of potentially infectious plasma and the risk of transmitting hepatitis is still present'

A date of expiry will be stated corresponding to a shelf-life of 1 year at 2-8°C storage temperature.

8. Method of Sale and Supply:

The product will be made available through the Supply Division of the Department of Health and Social Security.

9. METHOD OF MANUFACTURE:

- 9.1 Specification of starting material: Plasma meets the requirement that each donation shall be individually tested, using the radio-immunoassay method, and found to be non-reactive for hepatitis associated antigen.

9.2 Plasma thawing, pooling and centrifuging:

The Quality Assurance-approved shipments of bottles of frozen plasma are removed from the freezer to the thawing room where they are decapped (but not unstoppered) and examined to sort out the HAA positives, those with missing and illegible bleeding numbers or those that are broken or damaged.

The plasma is thawed in circulating air at 4°C for a time which usually does not exceed 24 hours. The bottles are examined and those showing haemolysis or fat flotation removed. The retained bottles are emptied into the pooling tank with a swirling motion to dislodge precipitated material. The drainings are separately collected and are not used.

The mixture of cryoprecipitate and the liquid is passed through a Sharples super-centrifuge at a temperature of 1-5°C using a special nozzle and at a controlled specific flow rate.

The paste is scraped from the bowl, weighed and immediately put through the next stage.

9.3 Removal of residual plasma:

The weighed paste is ground into 7 times its weight of sterile glycine solution at 0-4°C.

The suspension is then gently agitated for 20 minutes at 0-4°C before being piped to a clean Sharples super-centrifuge through which it is passed under the same conditions as previously described.

The paste is scraped out, weighed and frozen at -65°C.

9.4 Reconstitution and clarification:

To the frozen cryoprecipitate is added citrated saline solution at a temperature not higher than 37°C and the containers are immersed in a water bath at 34-37°C.

The liquid is drained into a jacketed vessel below the grinder before passing the cryoprecipitate through the grinder.

The suspension is stirred for 40-50 minutes in the vessel at 34-37°C. A further 5 minutes stirring at high speed with a non-aerating stirrer is given.

The material is transferred to a source tank and further citrated saline solution added to bring the protein strength to 3.4%, and stirring continued for 20-30 minutes.

Using pressure supplied by sterile nitrogen, the suspension is filtered through a nylon bag into a source tank before passing through an ultracentrifuge (25,000 rpm) cooled to 5°C.

9.5 Sterile Filtration:

The solution is pumped through 0.8 micron prefilters before the 0.3 micron sterile filter and collected in a tank.

9.6 Filling and Freezing:

The sterile solution is dispensed by a Filomatic stainless steel syringe into 50 ml bottles. Total protein, calculated fill, activity units/ml, lead to an adjusted fill in ml/bottles to which the machine is adjusted.

The contents of the bottles are frozen by immersing half-way up in a dry ice-alcohol bath for 3 minutes. The bottles are transferred to a freezer. A Petri dish is placed before each operator, opened when filling starts and covered  $\frac{3}{4}$ -hour later. These will be cultured by Quality Assurance.

9.7 Lyophilisation:

The material is lyophilised in a vacuum of 200 microns at a temperature never allowed to exceed 37°C. Bottles are sealed with processing caps immediately.

9.8 Stoppering and Evacuation:

The processing caps are replaced by the final stoppers with simultaneous evacuation to 30 in Hg. The bottles are sealed with an aluminium seal.

10. FINISHED PRODUCT

10.1 Marketing Formulation:

10.11 AHF Vial Active Constituent: Dried Human Antihaemophilic Fraction.

10.12 Sodium Chloride for Injection Vial: Sodium chloride, USP (9 mg/ml)  
Water for Injection, USP

10.2 Quality Control:

10.21 Specification of Constituents:

10.22 Active Constituent: Plasma for fractionation meets the requirements that each donation shall be individually tested and found to be non-reactive for hepatitis-associated antigen. The Ausria (Abbott) test is used.

The donors meet the criteria of the regulations.

The blood from each donor is tested for syphilis (serological test), for blood group and for rH factor.

11. SELECTION AND SCREENING OF BLOOD DONORS

The controls applied in the collection of plasma for AHF manufacture are detailed in the copies of the forms used to collate the information on the medical history, physical examination and laboratory data of a proposed donor; the Donor medical history cards; the plasma donor list and daily donor rejection list. (see pages 29-37 of the submission).

12. IN-PROCESS CONTROLS:

12.1 Manufacture of AHF: Bottles of thawed plasma are examined for haemolysis and fat and those positive rejected.

During centrifuging, an examination of the out-going fluid for temperature and flow-rate is performed every 20 minutes.

The temperature of the glycine and cryo solution is measured every 5 minutes. During reconstitution of extracted cryo paste, the temperature of the citrated saline and the water bath temperature are checked every 20 minutes. The protein in the reconstituted solution is determined and the concentration adjusted to the desired figure. On the bulk final solution, the following tests are performed: Sterility, Activity, Total protein. The specification for the bulk solution is:

Sterility: Passes FDA Regulations.

Contents of Final Container: 200 units of activity per gram of protein.

12.2 Finished Product Specification:

AHF:- Sterility: }  
Safety: } Pass FDA regulations  
Identity: }  
Pyrogen: }  
Moisture: Does not exceed 2%  
Isoagglutinins: Titre less than 1:32  
Total Protein: Does not exceed 4 g/% upon reconstitution  
Clottable Protein: Does not exceed 4 g % upon reconstitution (but less than total protein)  
Thrombin: Does not clot  
Activity: Minimum 5 AHF units/ml. 15 fold increase of normal human plasma/g protein.  
Identity -  
Labelled Final Specific Product Identification.  
Container:  
Before final release a labelled container from each batch is tested for identity.

13. ANALYTICAL CONTROLS ON FINAL PRODUCT:

AHF:-

13.1 Sterility: The sample consists of 10% of the containers in a batch of less than 200 containers, of 20 containers in a batch of more than 200. The containers are selected at approximately equal intervals during the production run. The powder is restored to approximately 25 ml with sterile diluent and the solution tested for sterility.

13.2 Safety: The sample is one container, randomly selected, per batch.

For each test two mice weighing approximately 20 g and 2 guinea pigs weighing approximately 350 g are injected parenterally, e.g. subcutaneously or intramuscularly. The test dose for each mouse is 0.5 ml and for each guinea pig 5.0 ml. If the injection is subcutaneous, bilateral injections may be made in abdomen area of mice and guinea pigs. A new 22 gauge disposable needle is used for each animal. The weights of mice and guinea pigs before injection and at completion of safety test are recorded.

The animals are observed for seven days and, should neither significant symptoms nor death occur in this period, the product tested passes general safety test.

### 13.3 Identity:

The sample is part of a sample container used for one of the tests.

This test is carried out by the Precipitin reaction by the interfacial technique, i.e. a small amount of antiserum is placed at the bottom of a tube and a layer of diluted antigen placed on top of this. The reaction, if any, occurs at the junction in the form of a "ring" of precipitate. (see p.15 of the submission for further details).

### 13.4 Pyrogens:

The sample is one container, randomly selected, per lot.

All final bulk tanks and a final container from each day's filling are tested for pyrogenic substances.

Three rabbits weighing 1500 g minimum (five rabbits, if a retest) with control temperatures not exceeding  $39.8^{\circ}\text{C}$  are used. The control temperatures before injection are the basis for determining if a substance is pyrogenic. 10 units AHF/kg rabbit body weight are injected. The sample fails if one half or more of all rabbits tested show a temperature rise of  $0.6^{\circ}\text{C}$  or more or if the average temperature rise of

### 13.5 Isoagglutinins: all rabbits used is $0.5^{\circ}\text{C}$ or more.

The sample is one container.

Group A, and Group B prewashed test cells are used to test for saline isoagglutinins. Test cells with a month's dating are purchased from a commercial supplier. If an isoagglutinin titre of AHF is 1:32 or higher the lot must be clinically evaluated prior to release for distribution. (see p.18 of submission for details of the test).

### 13.6 Stability Reports:

The activity in the following subsections was measured by the thromboplastin generation test. The Thromboplastin Generation Assay has an experimental error of  $\pm 20\%$ . This has been allowed for in making the statements following.

At  $32^{\circ}\text{C}$ : Sample assayed over 13 weeks: The rate of loss of activity of this sample was constant over the period, and about a third of the activity was lost.

At  $2-5^{\circ}\text{C}$ : Assayed over 12 weeks; and it was concluded that there was no loss of activity over this period.

At  $-20^{\circ}\text{C}$ : Samples were kept for 5-6 months. Measurements of activity were performed at the beginning and end only. There was no change in activity.

At  $22^{\circ}\text{C}$ : Sample was assayed over 13 weeks. Concluded that after stabilisation after 4 weeks at an activity of 85.5% of the original value, no further decrease occurred over the next 9 weeks.

At  $4^{\circ}\text{C}$  on production material (labelled final containers):

Out of 42 tests only 1 sample showed a loss of activity (over a period at least 3 times the stated shelf-life) of more than 20% of the initial activity when allowance is made for the error on both assays. There is also 1 result showing a gain of more than 20%.

13.7 Proposed Shelf-Life:

A shelf-life of 1 year at a storage temperature of 2-8°C is given.

13.8 Containers:

These are 30 ml glass vials, rubber-stoppered and aluminium sealed.

13.9 Assay of Factor VIII (see p.42 et seq. of submission for details)

A modified thromboplastin generation time test (TGT), a two-stage technique, is the assay used for determining the concentration of Antithaemophilic Factor (Human) activity.

A coagulation timer, as a Fibrometer or equivalent, thermal prep block heating unit and automatic pipette are used for coagulation determinations.

A standard reference plasma is used (no details given of whether or not it is standardized against the International Standard).

14 Medical Comment

The blood used for the preparation of this Factor VIII concentrate is screened for HBsAg by radioimmunoassay. Blood is obtained by plasma-pheresis of commercial donors at 8 centres in the U.S.A. Insufficient information is given on the assay of Factor VIII, particularly in relation to whether or not the International Standard for Factor VIII is used in the assay.

The manufacturer in California has not been inspected by the Licensing Authority.

15. Recommendation

That a Product Licence be granted.



MANUFACTURER

LICENCE HOLDER ALPHA THERAPEUTICS GmbH

PL

ADDRESS

ADDRESS FOR RELEASE CERTIFICATES UNIT 10, LODGE WAY

COMMERCIAL  
NAME

Profilate

FISON WAY INDUSTRIAL ESTATE, THETFORD, NORFOLK IP24 1HE

BIOLOGICAL  
NAME AND UNITAGE Factor VIII

DATE ISSUED 10.9.79.

DATE OF EXPIRY 22.5.80 22.5.85.

Batch Release  
Details

A

Date

4029 0001

BATCH NO(S)	PROTOCOL	SAMPLES			RELEASE		NON-RELEASE	
	Date Received	Date Received	No	Type	Dated	Signed by	Date	Action Taken
AL 3910	24.1.81	24.11.81			21.12.81	GRO-C		BP 0043 A
AL 4370	24.2.82	24.2.82			26.3.82	GRO-C		BP 0095 A
A1-4600	26.4.82	26.4.82			19.5.82	GRO-C		BP 0113 A
A1 4460	8.6.82	8.6.82			23.6.82	GRO-C		BP 0136 A
A1-4810	27.8.82	27.8.82			17.9.82	GRO-C		BP 0013 A
A1-4471	1.9.82	1.9.82			30.9.82	GRO-C		BP 0016 A
A1-4820	22.10.82	22.10.82			4.11.82	GRO-C		BP 0042 A
A1-4850	4.11.82	4.11.82			19.11.82	GRO-C		BP 0056 A
A1-4671	15.11.82	15.11.82			10.12.82	GRO-C		BP 0071 A
A1-4750	9.2.83	7.2.83			21.2.83	GRO-C		BP 0115 A
A1-4510	19.4.83	19.4.83			18.5.83	GRO-C		BP 0168 A
A3-2680	5.5.83	5.5.83			14.6.83	GRO-C		BP 0185 A
A3-2690	5.5.83	5.5.83			9.6.83	GRO-C		BP 0181 A
A3-2820	29.6.83	29.6.83			6.7.83	GRO-C		BP 0215 A
A3-2840	29.6.83	29.6.83			6.7.83	GRO-C		BP 0216 A
A3-3100	23.8.83	23.8.83			12.9.83	GRO-C		BP 0269 A
A3-3080	23.1.84	23.1.84			23.2.84	GRO-C		BP 0365 A
A3-3090	23.1.84	23.1.84			2.2.84	GRO-C		BP 0356 A
A3-3910	5.3.84	5.3.84			27.3.84	GRO-C		BP 0379 A
A3-3940	3.4.84	3.4.84			27.4.84	GRO-C		BP 0011 A

