0566F/1/PV

CSM 1990/190

COMMERICAL IN CONFIDENCE	NUMBER: PL 04447/0005
APPLICATION FOR A Product Licence	PRODUCT NAME: Profilate - SD
LICENCE HOLDER: Alpha Therapeutic UK Ltd Howlett Way Fison Way Industrial Estate Thetford, Norfolk	THERAPEUTIC CLASSIFICATION: *BLOOD PRODUCT*
	RECEIVED: 1 November 1989
MANUFACTURER OF DOSAGE FORM: Alpha Therapeutic Corporation 5555 Valley Bvd, Los Angeles, California 90032, USA	MEETING: January 1990
	COMMITTEE ON Safety of Medicines
LEGAL STATUS: POM	SUB-COMMITTEE ON Biologicals
SALE/SUPPLY: POM	CONSIDERATION BY OTHER COMMITTEES:
	ASSESSED BY: Dr F Rotblat Mrs G Silvester

VARIATION

This is the first application for a solvent/detergent treatment for viral inactivation *for a Factor VIII product.*

(1)

1. BACKGROUND

Profilate is Dried Factor VIII Fraction BP. This variation application is for a change to the virus inactivation process. Currently, virus inactivation is achieved by suspending freeze-dried powder in n-heptane and heating at 60°C for 20 hours (Profilate Heat-Treated). The proposed method is a solvent-detergent method using TNBP (tri-n-butyl phosphate) and Tween 80. This will replace the n-heptane and heat treatment. The company state that they wish to make the change because published studies indicate that blood products treated with solvent and detergent are more pure and safer from viral transmission than Profilate Heat-Treated.

This is the first application to market a solvent/detergent treated Factor VIII in the UK. Alpha currently hold a Clinical Trial Exemption (CTX0444?/0019A) to allow them to undertake clinical trials in 'virgin patients'. Alpha purchased the technology for the new process from the New York Blood Centre where it had been developed.

2. PHARMACEUTICAL COMMENT AND RECOMMENDATION

2.1 Introduction

The Company have provided one volume of data containing quality, safety and efficacy data in support of the proposed change. A summary of the new data provided on quality is included in this report. The evaluation of the Company's data is presented under the following sections:

Viral Inactivation ie.	manufacturing procedure including time/temp/concentrations and in-process controls. Viral inactivation studies to demonstrate inactivation of HIV and model viruses.
Effect on the product ie.	product characterisation particularly activity and neo-antigen formation. Batch analyses and compliance with BP/in-house specifications. Stability of the product.
New materials used in the process ie.	quality control of the solvent and detergent. Levels of and tests for residues of these. Purification steps to remove them.
Other changes relating to the main variation ie.	name change, label/leaflet amendments, dextrose dropped from formulation and PEG level in f.p.s. reduced.

2.2 Viral Inactivation

The solvent/detergent treatment is carried out on the filtered supernatant from the first polyetheylene glycol (PEG) precipitation (3.5% PEG). Tri-n-butyl phosphate (TNBP) and polysorbate 80 are added to the filtered anti-haemophilic factor (AHF) solution to a final concentration of 0.30 ± 0.2% TNBP v/w and 1.00 ± 0.05% $^{w}/_{w}$ polysorbate 80. The pH of the mixture is adjusted to 6.3 ± 0.8 with dilute acetic acid. The mixture is maintained at 27 ± 3°C for not less than six hours. The solvent/heat-treatment virus inactivation step is no longer carried out.

In-vitro virus inactivation studies have been carried out with Vesicular Stomatitis Virus (VSV), Sindbis virus and HIV-1.

In the VSV/Sindbis study, virus stock solution was added to the supernatant from the 3.5% PEG precipitation in a dilution of 1:10. TNBP and polysorbate 80 were added to give concentrations of 0.3 and 1%, respectively. After incubation for 30 mins and 3 hours at 22-24°C, aliquots were taken and precipitated with 12% PEG. Controls were treated in the same way with the omission of the TNBP/polysorbate 80 step. Precipitate was suspended in media and plaque assays carried out on FL cells and Vero cells for VSV and Sindbis, respectively. The sensitivity of the assays was 2 log pfu/ml. 6.82 logs of VSV were present in the control samples. 3.4 logs remained after 30 minutes solvent/detergent treatment and no virus was detected after 3 hours. 6.41 logs of Sindbis were present in the control samples. No virus was detected after 30 mins or 3 hours solvent/detergent treatment.

Two inactivation studies have been carried out with HIV-1. The first investigated virus inactivation in different matrices (cryoprecipitate suspension, 3.5% PEG supernatant (AHF), 3.5% PEG in saline and 0.9% NaCl) using 0.3% TNBP with 1% polysorbate 80 or 1% Triton X - 100. The matrices were spiked to contain > 9 $\log_{10}$ $TCID_{50}$/ml HIV-1. No HIV-1 was recovered from any of the mixtures after 3 hours at 30°C. Release of infectious HIV-1 was determined by detection of infected cells (H9) exhibiting characteristic cytopathic effect (CPE) and for release of HIV-1 in culture supernatants by reverse transcriptase assay or expression of viral antigens by the antigen capture assay. Positive and negative controls were used and it was demonstrated that a 10^{-2} dilution of the solvent and detergent were non-toxic and non-virucidal in the detection system.

In the second study a 3.5% PEG AHF supernatant as produced in routine production of Profilate - SD was spiked to contain > 11.5 $\log_{10}$ $TCID_{50}$/ml HIV-1 and incubated with 1% polysorbate 80 and 0.3% TNBP at 24°C. The virus was diluted 1:10 in the supernatant. Samples were taken for detection of virus after 30 minutes, 1 hour and 3 hours. Very low levels of viral antigens were detected in test samples T30 (titer of $10^{1.16}$), T60 and T180 (titers of $10^{0.50}$) as determined by the antigen capture assay. Only the 60 min test sample was confirmed by the reverse transcriptase assay to contain low levels of infectious virus (titer of $10^{0.16}$).

Continued /

These three studies demonstrate that the solvent/detergent process is an effective viral inactivation procedure for lipid-enveloped viruses. The major weakness of the solvent/detergent approach to viral inactivation is the inability of the process to inactivate non-lipid-membrane-coated viruses. (This was discussed by Prince et al. in the article reproduced on pp 299-310 of the Alpha submission.)

The recent work on hepatitis C virus suggests that it may be similar to the togaviridae or flaviviridae, both of which have lipoprotein membranes and indeed part of the evidence for this conclusion in the sensitivity of the agent to organic solvents (Choo et al., Science, 244, 359-364 (1989)). This is encouraging for the likely effectiveness of solvent/detergent treatment against one agent of nonAnonB hepatitis (NANBH) but does not preclude the possible existence of other agents which may be resistant. However, this has to be weighed against the current imperfect situation with heat treatments for viral inactivation which vary in their ability to reduce hepatitis transmission. This was reviewed by Mannucci and Colombo (The Lancet, October 1, 1988, pp782-785). The Alpha product (60°C, 20 hours, heat and heptane) was associated with cases of NANBH (3 out of 11 patients studied). The same review included the promising findings that no case of NANBH developed in 12 patients infused with solvent-detergent treated Factor VIII from the New York Blood Centre (TNBP/sodium cholate, 4 hours at 22°C).

The possibility of human parvovirus transmission was discussed by Prince et al. (pp299-310 of the Alpha submission). They considered the occasional transmission of these agents to be a minor disadvantage when offset against the good viral inactivation activity against lipid-enveloped viruses.

The Committee will wish to consider this lack of activity against non-lipid-membrane-coated viruses. There does not seem to be any benefit in asking the Company to carry out any spiking studies with such agents as this would only be expected to confirm a lack of activity.

One point poorly covered by Alpha is the effect of variability in the conditions of the solvent/detergent treatment stage on the effectiveness of viral inactivation. This has been investigated by the New York Blood Centre. They found that increasing the concentration of TNBP from 0.1-0.3%v/v in the presence of 1% Tween 80 improved the reproducibility of viral inactivation. Increasing the concentration of Tween 80 accelerated the rate of inactivation. Increasing the temperature of incubation also increased the rate of inactivation and reduced the initial lag time seen in the kinetics of the inactivation process. (Horowitz B et al, Transfusion, 25, No 6, pp 516-522 (1985). This reference is incompletely reproduced in the submission by Alpha (pp 295-298).) In a further study, the kinetics and degree of inactivation of marker viruses added to antihaemophilic factor were adversely affected by increased protein concentration especially at concentrations of 20mg/ml and above. The authors speculate that this decreased inactivation may be a function of lipids in this concentrate and not protein per se as this phenomenon was not observed with some other blood products that were investigated. They found that the decrease could be overcome by increasing the concentration of TNBP. (Edwards C. A. et al., Vox Sang. 52 pp 53-59 (1987). Reproduced on pp 311-317 of the Alpha submission.)

Alpha do not specify the protein concentration used in their process nor do they indicate the levels in their own viral inactivation studies. The protein concentration should be defined and justified by information on the levels present in their inactivation studies. The allowed variability in the concentration of TNBP seems unjustifiably wide (0.1-0.5%~/w) considering the findings of Horowitz. The Company's viral inactivation studies all state that a concentration of 0.3% TNBP was used. The variability allowed in the concentration of polysorbate 80 (0.95-1.05%~/w) is more reasonable. The mixture is held at a temperature between 24 and 30°C for not less than six hours. Since the Company's viral inactivation studies with VSV and Sindbis used temperatures of 22-24°C, the lower temperature appears justified. The higher temperature limit and possible incubation beyond six hours will be considered later under the effect on the product.

The solvent/detergent treatment is carried out after a polyethylene glycol (PEG) precipitation procedure. The Company have not commented on whether the concentration of PEG in the medium affects viral inactivation. The pH of the mixture is controlled to 5.5-7.1 by the addition of dilute acetic acid. This is probably a reasonable variation but again is not discussed by the Company.

A filtration step is carried out prior to the solvent/detergent treatment to remove any solid particles. This is good practice and brief details should be provided of this step to complete the information we hold on the process. The Company have validated the mixing process used prior to incubation of the solvent/detergent mixture.

2.3 Effect on the Product

2.3.1. Characterisation

The prime evidence of improved product purity arising from the new manufacturing procedure is that derived from the specific activity results. The batch analyses provided on page 51 show specific activities (U/mg protein) of 7.25, 12.11 and 13.75. This compares with an average specific activity from the last twelve lots of the current solvent/heat process of 4.92.

The Company have provided a Weinstein blot showing separation according to molecular weight for the Factor VIII polypeptide material of Profilate-SD as evidence of the integrity of the molecule. (An original photograph was included with the covering letter to the variation.)

Also provided is a photograph of the results of SDS-PAGE using a gradient gel under non-reducing conditions using Coomassie Blue stain (p.39). Two lots of Profilate-SD were run at various dilutions. (Original photograph with covering letter to variation.) The photograph illustrates that the major impurity is fibrinogen. Cellulose Acetate Membrane Electrophoresis (CAME) results of Profilate Heat-Treated and Profilate-SD are provided on pp 39-40.

This information is encouraging but alone is certainly not conclusive evidence that the product has not been affected by the new agent. The evidence from the half-life and recovery study will be discussed under the Medical Comment section of this report. The Company have not reported any studies to investigate possible neoantigen formation with the new process. One of the literature reports provided includes results of the examination of the proteins present in an AHF concentrate by crossed immunoelectrophoresis following treatment with TNBP and 0.2% sodium cholate for 6 hours at ambient temperature. Using agarose as the separation medium and anti-normal human serum as antibody, at least 19 protein peaks were detected. The great majority of these were unchanged as a consequence of treatment, while 2 peaks differed. One peak was identified as alpha-1-lipoprotein using monospecific antiserum. The identity of the second was unknown. (Horowitz B. et al., Transfusion, 25, No 6, 516-522 (1985).)

The Committee will wish to consider whether they would require the Company to undertake any studies of potential neoantigen formation for their own process and if so whether they would require these to be done before the variation to allow the solvent/detergent process is granted or on an on-going basis.

When considering product integrity, the variability in process conditions the Company are allowing themselves has to be taken into account. There is no information on the exact conditions used for the batches for which characterisation data are provided but bearing in mind the fairly crude nature of these checks there is little value in pursuing this. Nevertheless, the Company should be asked to define how far beyond 6 hours they would allow the solvent/detergent stage to continue and if this is significant comment on possible effects on the product. Similarly, the duration of incubation at 30°C should be discussed.

2.3.2 The Finished Product Specification and Batch Analyses

The current and proposed finished product specifications are included in this report. The limits for Polysorbate 80 and TNBP will be discussed in the following section "New Materials Used in the Process". The limit for PEG has been reduced as a result of the additional washing stage introduced. None of the changes to the specification would compromise compliance with the BP monograph. Batch analyses have been provided for three batches of product on p.51 of the Alpha submission. This does not include all the tests in the finished product specification although generally the main tests of interest are included. Fibrinogen levels are omitted but the Company do state on p.35 that fibrinogen is well within the BP limit. The results given comply with the specification except for the moisture content of one batch. This batch would not be released for clinical use.

2.3.3 Stability of the Product

The batches for which batch analyses have been provided were used in stability studies. Six months data at 5°C and 30°C are given. The first results for a further two batches should have been available in November 1989. Fiducial limits are not provided for the Factor VIII:C assay results. The available data are summarised in this report.

The Company have made no direct comparison of the stabilty of the solvent/heat treated product and the solvent/detergent treated product. A comparison with the data currently available to the Secretariat (submitted October 1987) on the solvent/heat treated product suggests that the new product may be less stable. The Company should properly address this and, if necessary, modify the current storage recommendations. A commitment will also be required that they will immediately report to the Licensing Authority any on-going stability results that are outside of specification. This is particularly important in view of the low assay result at 30°C for one batch (73% of initial) which the Company have put down to an erroneously high initial assay result.

2.4 New Materials used in the process

2.4.1 Quality control of the solvent and detergent

Tween 80 is tested by an in-house specification which is provided. The Company give an assurance that it would, if tested, comply with the BP monograph. This is satisfactory.

There is no BP specification for a pharmaceutical grade of tri-(n-butyl)-phosphate. An in-house specification is provided. There is no discussion of the choice fo tests and limits but the specification is probably reasonable.

2.4.2 Purification steps to remove solvent and detergent

The New York Blood Centre changed from the use of Tween 80 as the detergent to sodium cholate because of problems with the removal of Tween 80 from the product. They reported that Tween 80 tended to form micelles which interfere with its removal by dialysis or ultrafiltration and that some of the Tween 80 appears to bind firmly to plasma proteins. (Prince, A. M., et al., Vox Sang, 46, 36-43 (1984) reproduced on pp. 319-326 of the Alpha submission.) It is, therefore, interesting to see how Alpha coped with this problem.

Alpha have introduced an additional wash with glycine/citrate/heparin solution to remove TNBP and Tween 80. The manufacturing method (p.11) states that this may be repeated a further time but does not specify when this option will be taken. The Company should clarify this.

2.4.3 Levels of and tests for residues of solvent and detergent.

The finished product specification limits Polysorbate 80 to NMT 2.5 ug/FVIII:C Unit and TNBP to NMT 0.4 ug/FVIII:C Unit. The results provided on page 51 show levels of Polysorbate 80 (ug/FVIII:C Unit) of 1.66 in one batch and <1 in the other two batches. TNBP levels (ug\FVIII:C Unit) were 0.146, 0.21 and 0.2. The specification limits can be readily met and it would appear that the Company may be able to reduce the limits with further experience. The Company address the toxicology of TNBP and Polysorbate 80 in their submission. This will be dealt with in the medical/scientific section of this report.

2.5 Other Changes Relating to the Main Variation

The change of name, label amendments, the reduction in PEG levels in the finished product specification and the dropping of dextrose from the formulation are all satisfactory.

2.6 Pharmaceutical Recommendation

The variation is considered basically satisfactory provided that the process variables are tightened up and suitable shelf-lives based on stability are set. The draft recommendations are on the final page of the report.

MEDICAL ASSESSMENT

This is an application to vary the product licence for Profilate HT a Factor VIII product prepared from pooled donor plasma and used for the treatment of Haemophilia.

The main purpose of the variation is a change in the viral inactivation procedure.

1. Introduction

Profilate HT is virus inactivated by heat treatment of lyophilised material suspended in a heptane slurry. It has been marketed in the UK since 1985.

The new product to be known as Profilate SD is inactivated by mixing with Tri-(n-butyl)-phosphate (TNBP) and Polysorbate 80. This solvent/detergent process disrupts the viral lipid envelope.

The solvent/detergent inactivation method was developed by Horowitz at the New York Blood bank, from where the company have purchased the technology. It is widely used in the USA by the Red Cross Fractionation Service.

This process is also used by the French Transfusion Service for all their Factor VIII, France being self sufficient.

Solvent detergent treatment is also widely used in Germany and Scandinavia.

Alpha Profilate SD is marketed in Germany and the USA.

Most viruses transmitted by blood products have lipid envelopes, including Hepatitis B and C and HIV. Parvovirius which has occasionally been associated with Factor VIII products may not be inactivated by this process.

The company have carried out a comparative pharmacokinetic study, of Profilate HS and SD showing bioequivalence for recovery and T½ (p 4[o]).

These are the only clinical data supplied.

Surveillance of patients treated with other Solvent/Detergent inactivated products indicates that HIV and Hepatitis are inactivated. No transmission of these diseases has been recorded. The company have considered this - see below.

Absolute confidence concerning lack of viral transmission by a coagulation factor product derived from human blood is probably unobtainable. It has been calculated that ignoring the possibility of batch variability and applying the "rule of three" for zero numerators [7] a total of 60 patients without evidence of non-A, non-B hepatitis would need to be studied to show with 95% confidence that the product carries less than a 5% risk of transmitting non-A, non-B hepatitis. Because they would have to be previously untreated with blood products, it is impracticable to obtain this number of haemophiliac patients for study. Horowitz et al [8] recently reported the results of a clinical study of solvent/detergent treated Factor VIII concentrate in 20 haemophiliac patients not previously exposed to blood products and 17 of these were followed for at least 6 months. No evidence of transmission of HIV-1 or non-A, non-B hepatitis was seen. While the data is certainly reassuring Alpha would wish to obtain similar data on Profilate-SD. To this end the company has recently applied for a CTX application to enable a "virgin patient" study to be carried out.

It will of course take a long time perhaps at least 2 years before the data from this study is complete. Alpha Therapeutic is firmly convinced, however, that the considerable weight of evidence which already exists concerning the safety of solvent/detergent treated coagulation factor products justifies the approval for marketing of Profilate-SD in advance of the virgin patient study data being available. This conviction is also held by the Licensing Authorities in the major industrialised nations where solvent-detergent treated Factor VIII products are licensed e.g. USA, W.Germany, France, Holland, Spain, Norway and Sweden and where the growing body of evidence clearly points to the treatment process being safe and effective.

Solvent/Detergent inactivation has been widely taken up, as the process of choice for 2nd generation Factor VIII products. It gives a reasonable balance between safety and recovery of intact Factor VIII, losses being apparently less than with pasteurisation.

2. **Toxicology**

No data have been presented, but a summary of the toxicity of TNBP and Polysorbate is included (p 37).

3. **Data Sheet (p 48)**

As usual for Factor VIII data sheets, it is overlong and has been mixed up with the package insert, (ie lots of information on how to get the needle out of the packet). However it is adequate.

4. **Medical Recommendation**

The variation should be granted.

Manufacturing Summary
Antihaemophilic Factor (Human)
Heparin PEG - Inactivation Method C

Antihaemophilic Factor (AHF) is manufactured from pooled human plasma [Source Plasma, Source Plasma Salvaged, Recovered Fresh Frozen, etc.] by a Polyethylene Glycol (PEG) procedure. Each unit of plasma included in the pool for the manufacture of this product has been individually tested and found nonreactive for hepatitis B surface antigen, HIV antibody and screened for levels of alanine amino tansferase. For use in manufacture, the frozen plasma is thawed at controlled temperatures. The cryoprecipitate is removed by centrifugation.

Cryoprecipitate is either further processed or frozen for future processing. Cryoprecipitate is suspended in heparinized distilled water (80 ± 20 heparin units per ml) and the pH of the solution is adjusted to 7.0 ± 0.2 with dilute hydrochloric acid and mixed at 28 ± 5°C until well suspended. The volume of heparinized distilled water used is 3 ± 2 litres per kilogram of cryoprecipitate.

PEG is then added to the AHF Solution to a final concentration of 3 ± 2% and is mixed at 25 ± 5°C. The pH of the AHF suspension is then adjusted to 6.3 ± 0.8 with dilute acetic acid. The suspension is mixed at 25 ± 5°C for not less than 15 minutes. The precipitate formed is removed by centrifugation.

The recovered supernatant from centrifugation is filtered to remove any solid particles. Tri-n-butyl Phosphate (TNBP) and polysorbate 80 are added to the Filtered AHF Solution to a final concentration of 0.30 ± 0.2% TNBP v/w and 1.00 ± 0.05% w/w polysorbate 80. The pH of the mixture is adjusted to 6.3 ± 0.8 with dilute acetic acid. The mixture is maintained at 27 ± 3°C for not less than six hours.

Additional PEG is added to the SD AHF Solution to a final concentration of 12 ± 4%. The suspension is mixed for not less than 15 minutes at 25 ± 5°C. The AHF precipitate formed is then recovered by centrifugation.

The PEG-Precipitated AHF is suspended at 5 ± 6°C in 1.6 ± 0.4M glycine solution containing 0.23 ± 0.05M citrate and 13 ± 3 U/ml of heparin. The volume of glycine solution is 20 ± 15 litres per kg of the precipitated AHF. When the suspension has been mixed to homogeneity, it is centrifuged to recover the AHF precipitate. An additional wash of the AHF precipitate is carried out using the glycine/citrate/heparin solution as above and this may be repeated. The washed AHF precipitate is recovered by centrifugation.

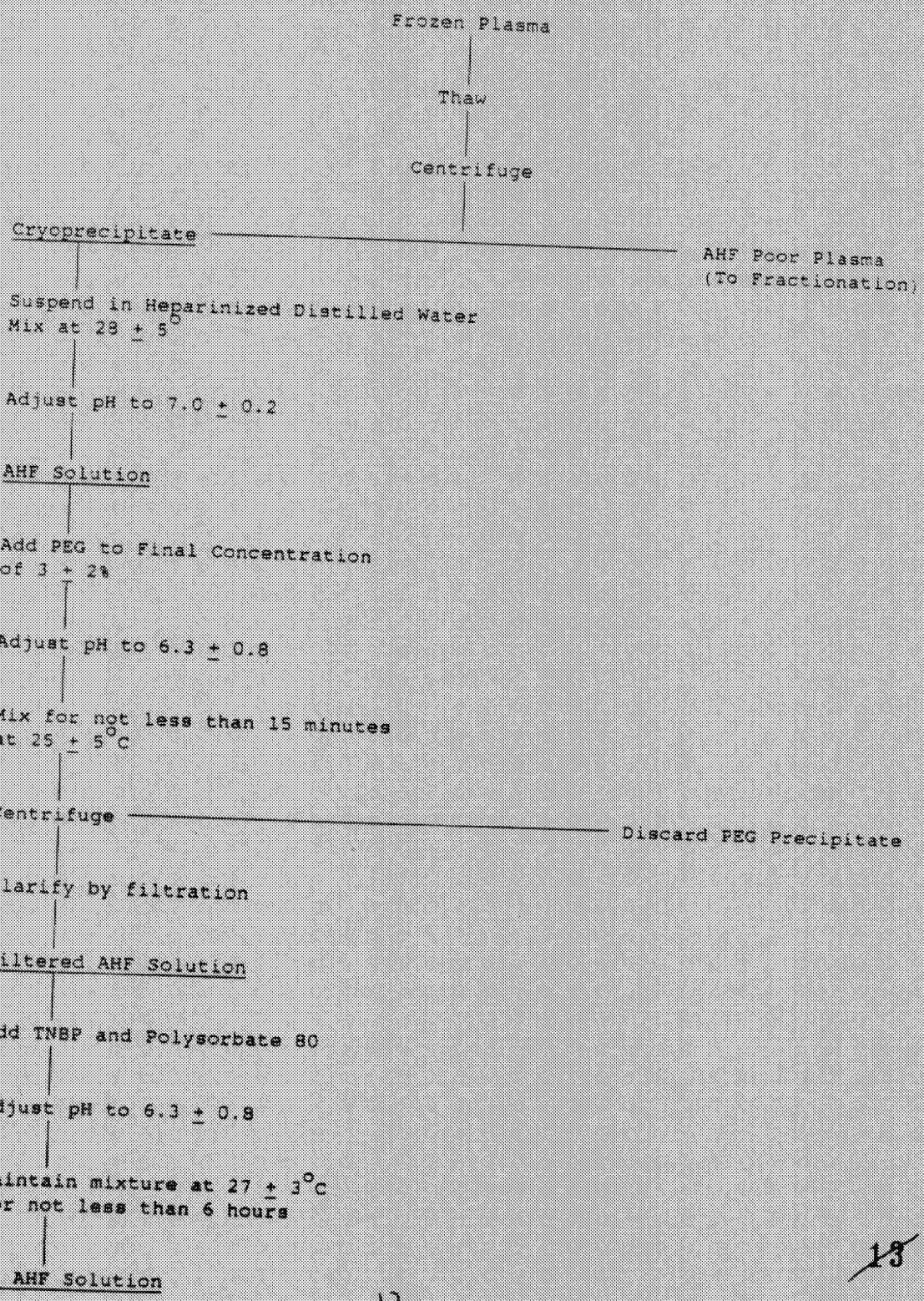
The Glycine-Precipitated AHF is dissolved in sodium citrate buffer solution at 20 ± 5°C. The pH of the solution is adjusted to 7.2 ± 0.2 with dilute sodium hydroxide or dilute hydrochloric acid solution. The volume of glycine/citrate/heparin solution used is 20 ± 15 litres per kg of PEG precipitated AHF. Depending on the final container AHF potency requirements, the final AHF solution may be diluted further with water for injection or glycine/sodium citrate/heparin solution prior to sterile filtration.

The Final AHF Solution is then filtered through previously sterilized bacteria retentive membrane or cartidge filters. The resulting filtered AHF solution is maintained as a sterile bulk solution in preparation for filling into final containers.

The Sterile AHF Bulk Solution is transferred into the asceptic filling area. Samples for sterility are taken by Quality Control. The sterile bulk is filled into clean sterilized vials, frozen and dried under vacuum.

Final Container AHF is then tested by Quality Control. When test results are within all applicable specifications, Quality Control releases the lot.

Antihaemophilic Factor (Human)
Heparin PEG - Inactivation Method C
Process Flow Diagram



Process Flow Diagram (continued)

SD AHF Solution

Add PEG to Final Concentration at 12 ± 4%

Mix for not less than 15 minutes at 25 ± 5°C

Centrifuge ——— Discard Effluent

PEG-Precipitated AHF

Suspend in 1.6 ± 0.4 M glycine, citrate, heparin solution

Centrifuge ——— Discard Effluent

Glycine-Precipitated AHF

Resuspend in 1.6 ± 0.4 M glycine, citrate, heparin solution

Centrifuge ——— Discard Effluent

Glycine-Precipitated AHF

Dissolve in sodium citrate buffer solution at 20 ± 5°C

Adjust pH to 7.2 ± 0.2

Final AHF Solution

Sterile Filter

Sterile AHF Bulk Solution ——— Quality Control Sterility

Fill

Freeze Dry

Final Container AHF - Quality Control

Notes on Manufacturing Summary

1. It should be noted that this product is produced from a highly variable starting material. Furthermore the manufacturing summary covers the production of final product ranging in potency from 250 I.U. Factor VIII per vial to 1000 I.U. Factor VIII per vial. Hence it is necessary to have fairly wide limits for quantities of reagents used during the production process.

2. The solution used to suspend the PEG-precipitated AHF contains :

Sodium Citrate	0.23 ± 0.05M
Glycine	1.6 ± 0.4M
Heparin	13 ± 3 Units per ml

The pH of this solution is 6.7.

13

DEVELOPMENT PHARMACEUTICS

1. Virus Inactivation Method

Towards the end of the 1970's most of the commercial plasma fractionators in the USA began development of a variety of heat treatment processes for use during the manufacture of coagulation factor products. It was hoped that these treatments would prevent the transmission by Factor VIII and Factor IX concentrates of certain viral infections and in particular viral hepatitis which had been reported in the literature. The appearance of AIDS in the early 1980's prompted the rapid introduction of heat-treatment for these blood products. Alpha Therapeutic Corporation had already been developing a combination virus inactivation process involving the use of an organic solvent, n-heptane, and heat treatment at 60°C for 20 hours. This process was therefore introduced for their proprietary Factor VIII and Factor IX products (Profilate and Profilnine).

Of some concern at the time was the protein denaturing which resulted from heat-treatment and the effect this had on neoantigen formation and final product purity. However as experience with heat-treated products increased the much more serious concern arose of outbreaks of virus transmission following use of Factor VIII products. Some Factor VIII concentrates heat-treated in the lyophilised state (dry heat-treated) became implicated, first in the continued transmission of hepatitis and subsequently in the transmission of HIV-I. On the other hand heat-treatment of Factor VIII in a liquid phase (wet heat-treatment) appeared to offer more certain protection against HIV-I and hepatitis. A clinical study was conducted in "virgin" haemophiliac patients using the wet heat-treated product, Profilate Heat-Treated. The absence of transmission of HIV-I and hepatitis B was confirmed but a small percentage (13%) of patients had liver enzyme markers indicative of non-A, non-B hepatitis. At the time this study demonstrated the superiority of Profilate Heat-Treated over certain other available Factor VIII products. Although manufacturers developed the heat-treatment process further and some additional clinical studies were performed it now emerges that all UK licensed commercial Factor VIII concentrates whether wet or dry heat-treated have been implicated by clinical study or anecdotal report in the tranmission of viral infection.

The need for an alternative virus inactivation process for coagulation products is clearly evident. Since more severe heat-treatment jeopardises both yield (and thus worldwide availability) as well as final product purity a different virus inactivation method was sought.

A combination of detergent and ethyl ether is known to inactivate (1) lipid-enveloped viruses. On the basis of this fact, Prince et al. tested the ability of a mixture of 20% ether and 1% polysorbate 80 to inactivate both hepatitis B virus and Hutchinson strain non-A, non-B hepatitis virus. Normal chimpanzee serum containing either 10^6 chimpanzee infectious doses (CID) of hepatitis B virus or 10^4 CID of non-A, non-B hepatitis virus treated with 20% ether, plus 1% polysorbate 80 was innoculated into two chimpanzees each. The four chimpanzees remained free of serologic and biochemical evidence of hepatitis during a 6-month follow-up period. The animals were shown to be susceptible to infection by challenge with the original untreated innocula.

Prince and co-workers then investigated other detergent: organic solvent combinations mainly because ether is explosive but also they did not then have a method for removing the large micelles of polysorbate 80 from the AHF concentrates. In 1986, Prince et al [2] demonstrated that a combination of 0.3% tri-(n-butyl)-phosphate (TNBP) and 0.2% sodium cholate inactivated at least 10^4 CID of hepatitis B virus, 10^4 CID of Hutchinson strain non-A, non-B hepatitis virus and $10^{4.2}$ tissue culture infective doses of HTLV-III (HIV). These results were obtained by treating a Factor VIII preparation containing the viruses with the TNBP-sodium cholate mixture for six hours at 30°C for the hepatitis viruses and for 20 minutes at 24°C for the HIV. It should be noted that the amount of virus inactivation given above is a minimal estimate whose magnitude is limited by the amount of virus which could be added to the innocula. In the examples given all the virus added was inactivated.

Horowitz et al [3] presented data which demonstrated that a combination of 0.1% TNBP and 1% polysorbate 80 at room temperature inactivated three model viruses (VSV, Sindbis and Sendai) more rapidly than the combination of ether and Tween which was shown to inactivate the hepatitis viruses [1]. In addition, in the presence of 0.3% TNBP, 1% polysorbate 80 was shown to be more efficient than 0.2% sodium cholate and equal to 1% sodium cholate in inactivating these model viruses. A more recent report [4] demonstrates that 0.3% TNBP and 1% polysorbate 80 inactivates VSV and Sindbis virus in Fibronectin and Factor IX complex with equal or greater efficiency than 0.3% TNBP and 0.2% sodium cholate does in AHF and Albumin.

Polysorbate 80 has the advantage of doing little or no damage to the Factor VIII since the recovery of Factor VIII was 114% while with sodium cholate the recovery was only 83% with 0.2% sodium cholate and just 2% recovery with 1% sodium cholate.

Based on the growing body of evidence to support the efficacy and safety of a combination of solvent and detergent for viral inactivation, Alpha Therapeutic Corporation purchased the technology from the New York Blood Centre where it had been developed. Studies were begun to introduce the procedure into the manufacturing process for Profilate. Once this had been achieved and the process optimised a series of <u>in vitro</u> virus inactivation studies were begun in order to validate the method. Included in this variation application are reports of three separate in vitro studies.

The first study was performed using model viruses to demonstrate the general lethality of the process to lipid envelope viruses. The results of this study show that 6.82 $\log_{10}$ of Vesicular Stomatitis Virus (VSV) are inactivated within 3 hours and 6.41 $\log_{10}$ of Sindbis virus are inactivated within 30 minutes of treatment with 0.3% TNBP and 1% Polysorbate 80 at 30°C.

The second study looked at the lethality to HIV-I of two solvent/detergent combinations and four process admixtures ('matrices'). 1% Tween 80 or 1% Triton X-100 were used in combination with TNBP and inactivation of HIV-I, spiked at a level of greater than 9 $\log_{10}$ was determined using observation of infected cells for cytopathic effect (CPE), by reverse transciptase assay and by expression of viral antigens by antigen capture assay. The process

admixtures investigated were cryoprecipitate suspension, 3.5% PEG supernatant containing AHF, 3.5% PEG in saline or 0.9% sodium chloride (saline). No infectious virus was recovered from any of the spiked process admixtures samples incubated with Tween 80/TNBP or Triton X-100/TNBP mixtures for 3 hours at 30°C. The overall reduction of HIV-I infectivity was > 9 $\log_{10}$ $TCID_{50}$/ml.

A further study is presented in which a 3.5% PEG AHF mixture as produced in routine production of Profilate-SD was spiked to contain >11.5 $\log_{10}$ $TCID_{50}$/ml HIV-I. Infectivity was reduced by >10 $\log_{10}$ $TCID_{50}$/ml after incubation with 1% Tween plus 0.3% TNBP for 30 minutes or longer at 24°C.

These studies are included in the section of data entitled "Process Validation".

Further reassurance concerning the ability of TNBP/Detergent mixtures to produce a safe Factor VIII concentrate is provided by a report of clinical follow-up of 17 haemophiliac patients for at least 6 months following administration of TNBP and sodium cholate Factor VIII concentrate.[5] No transmission of either HIV or NANB hepatitis was observed.

Together these studies provide excellent assurance about the ability of the process to inactivate viruses in vitro. Alpha is fully confident therefore that Profilate-SD will prove to be a safe, reliable product for clinical use.

2. Removal of Dextrose

As alluded to earlier, dextrose was included in the original formulation of Profilate Heat-Treated to assist with the dissolution of the low purity lyophilised plug of Factor VIII. The original specification for specific activity of Profilate Heat-Treated was not less than 0.5 Factor VIII units per mg protein. Process improvements have increased this to not less than 3.0 Factor VIII units per mg protein. It is anticipated that the introduction of solvent detergent treatment will result in much improved final product purity. The specific activity of, for example, the three stability lots were in the range 7.0 - 14.0 Factor VIII units per mg of protein and it is possible that even these levels will be exceeded routinely. This improvement in purity will undoubtedly result in improved dissolution characteristics of Profilate-SD and it makes the use of dextrose unnecessary. Dextrose has been implicated on the rare occasions when customers have complained about a pinkish tinge in Profilate Heat-Treated. It is a logical step therefore to remove dextrose from the formulation of Profilate-SD.

3. Evidence of Improved Purity

The prime evidence of improved product purity arising from the new manufacturing method is that derived from the specific activity results. The currently licensed product Profilate Heat-Treated has a specific activity in the range 4.0 - 6.0 based on the APTT assay value. The average of the last twelve lots is 4.92. Although production experience using the new solvent detergent method is still limited, the values for specific activity for Profilate-SD which have

been achieved are significantly improved with the current average value being about 10.0. It is highly likely that even this value will routinely be improved upon as experience develops.

As evidence of the integrity of the molecule included overleaf is a Weinstein blot showing the separation according to molecular weight for the Factor VIII polypeptide material of Profilate-SD. This serves to confirm the evidence of functional integrity as shown by the half-life and recovery study reported in the clinical section of this application.

Also included overleaf is a photograph of the result of SDS-PAGE carried out using a gradient gel under non-reducing conditions using Coomassie Blue stain. Two lots of Profilate-SD are included on the same gel (Lots AR9002A and AR9003A) at various dilutions. This technique is a fairly crude indicator of product purity but it does illustrate that the major impurity is fibrinogen which nevertheless is well within the B.P. limit. Cellulose Acetate Membrane Electrophoresis (CAME) results of Profilate Heat-Treated and Profilate-SD are also included for comparison.

References

Note: These references are available at the rear of this volume.

1. Prince,A.M., Horowitz,B., Brotmas,B. et al., "Inactivation of Hepatitis B and Hutchinson Strain Non-A, Non-B Hepatitis Viruses by Exposure to Tween 80 and Ether" Vox Sang 1984: 46 ; 36-43.

2. Prince,A.M., Horowitz,B. and Brotmas,B., "Sterilization of Hepatitis and HTLV-III Viruses by Exposure to Tri-(n-butyl) Phosphate and Sodium Cholate" Lancet 1986: i ; 706-710.

3. Horowitz,B., Wiebe,M.E., Lippin,A. and Stryker,M.H., "Inactivation of Viruses in Labile Blood Derivatives; 1. Disruption of Lipid-Enveloped Viruses by Tri-(n-butyl) Phosphate Detergent Combination". Transfusion 1985: 25 ; 516-527.

4. Edwards, C.A., Piet,M.P.J., Chin,S. and Horowitz,B., "Tri-(n-butyl) Phosphate/Detergent Treatment of Licensed Therapeutic and Experimental Blood Derivatives". Vox Sang. 1987: 52 ; 53-59.

5. Horowitz,M.S., Horowitz,B., Rooks,C., Hilgartner,M.W. "Virus Safety of Solvent/Detergent-Treated Antihaemophilic Factor Concentrate". Lancet 1988: ii; 186-9.

PROCESS VALIDATION

1. Virus Inactivation

The solvent detergent processing step used during the manufacture of Profilate-SD has been validated using a series of in vitro studies. Virus was deliberately added to solutions containing partially processed AHF and the reduction in virus titre was recorded during treatment with 0.3% TNBP and 1% Polysorbate 80 for a total of 6 hours at 27 ± 3°C. HIV-I (Strain III-B), Sindbis and Vesicular Stomatitis viruses were used in the studies in order to demonstrate the efficacy of the process against a range of viruses.

Reports on three studies are provided in support of this variation application. The first study was performed using model viruses to demonstrate the general lethality of the process to lipid envelope viruses. The results of this study show that 6.82 $\log_{10}$ of Vesicular Stomatitis Virus (VSV) are inactivated within 3 hours and 6.41 $\log_{10}$ of Sindbis virus are inactivated within 30 minutes of treatment with 0.3% TNBP and 1% Polysorbate 80 at 30°C.

The second study looked at the lethality to HIV-I of two solvent/detergent combinations and four process admixtures ('matrices'). 1% Tween 80 or 1% Triton X-100 were used in combination with TNBP. Inactivation of HIV-I, spiked at a level of greater than 9 $\log_{10}$ was determined using observation of infected cells for cytopathic effect (CPE), by reverse transciptase (RT) assay and by expression of viral antigens by antigen capture assay. This latter method involves the use of an ELISA kit manufactured by Cellular Products Inc. of Buffalo, New York. The test is immunologically specific for HIV-I and provides confirmation of the CPE and RT results. The process admixtures investigated were cryoprecipitate suspension, 3.5% PEG supernatant containing AHF, 3.5% PEG in saline and 0.9% sodium chloride (saline). No infectious virus was recovered from any of the spiked process admixtures samples incubated with Tween 80/TNBP or Triton X-100/TNBP mixtures for 3 hours at 30°C. The overall reduction of HIV-I infectivity was >9 $\log_{10}$ $TCID_{50}$/ml.

A further study is presented in which a 3.5% PEG AHF mixture as produced in routine production of Profilate-SD was spiked to contain >11.5 $\log_{10}$ $TCID_{50}$/ml HIV-I. Infectivity was reduced by >10 log $TCID_{50}$/ml after incubation with 1% Tween plus 0.3% TNBP for 30 minutes or longer at 24°C. Thus the rate of inactivation is extremely rapid.

Full reports on each of these studies are included in the Appendix as follows:

- Inactivation of Sindbis and VSV in Profilate-SD.
- Inactivation of HIV-I spiked cryoprecipitate suspension or 3.5% PEG supernatant (AHF) or 3.5% PEG in saline or 0.9% NaCl (Saline) using Tween 80 and TNBP or Triton X-100 and TNBP.
- Inactivation of HIV-I spiked 3.5% AHF PEG using 1% Tween 80 plus 0.3% TNBP at 24°C.

- Package Insert for CPI Retro-Tek HIV p24 antigen ELISA as used in virus inactivation studies.

The data from these studies by themselves fully supports the use of Polysorbate 80/TNBP for virus inactivation in Profilate-SD. The use of high titre (>9 $\log_{10}$ $TCID_{50}$/ml) HIV-I innocula in these experiments provides reassurance concerning the lethality of the process and confirms the data obtained by Alpha's own experimentation using relatively low titre HIV innocula (data presented to support CTX 4447/0017).

Alpha Therapeutic is fully confident and secure that, based on these experiments conducted in two different laboratories, at two temperatures in the presence and absence of protein, PEG and buffer, the viral inactivation method used for Profilate-SD is safe and effective.

2. Komax Mixer Validation

The treatment of Factor VIII with Polysorbate 80 and TNBP takes place in a dedicated reaction tank. Solutions of 3% TNBP/10% Tween 80 and Factor VIII in 3.5% PEG are held in separate tanks before being passed together through a Komax mixer where they are blended to a homogenous mixture and pumped into the reaction tank. Homogenicity is maintained in the reaction tank which has its own stirrer system.

CONSTITUENT SPECIFICATIONS

veen 80 (pages 24-31)

Appearance	Viscous liquid
Colour	Amber to yellowish
Identity (IR)	Pass
Specific gravity at 25°C	1.06-1.09
Water content	NMT 3.0%
Residue on Ignition	NMT 0.25%
Arsenic	NMT 1ppm
Heavy metals	NMT 0.001%
Acid value	Pass
Hydroxyl value	65-80
Saponification value	45-55
Viscosity (centistokes at 25°C) (Ostwald-Type viscometer)	300-500
Reducing substances	Pass
Pyrogens	Pass

Tri-n-butyl phosphate (pp. 17-23)

Appearance	Clear mobile liquid
Colour	NMT 20 APHA
Identification (IR)	Pass
Specific gravity at 25°C	0.972-0.976
Refractive index at 25°C	1.422-1.424
Residue on ignition	NMT 0.25%
Heavy metals	NMT 0.001%
Water content	NMT 1.0%
Endotoxin level	NMT 0.25 EU/ml

Introduction

Included overleaf is the current finished product specification for Profilate Heat-Treated (with heparin) approved as a variation to PL 4447/0005 in September 1988. This is followed by the proposed finished product specification for Profilate-SD.

The differences between these specifications and the reasons for the changes are highlighted below.

Dextrose

Dextrose was originally included in the product formulation to improve the solubility characteristics of Profilate Heat-Treated. However the recent process improvements, firstly the use of heparin (variation approved September 1988) and now the use of solvent-detergent in place of n-heptane/heat treatment has resulted in a purer product. Dextrose is no longer included in the formulation because Profilate-SD has excellent solubility characteristics.

Heptane

The viral inactivation process as proposed in this variation is solvent (Tri-n-butyl phosphate) and detergent (Polysorbate 80). Treatment with n-heptane will not be used in future and hence a limit test is no longer necessary.

Polysorbate 80

A limit test for Polysorbate 80 is clearly appropriate for product produced by the new process.

Tri-(n-butyl) Phosphate (TNBP)

A limit test for TNBP is clearly appropriate for product produced by the new process.

Protein Identity

This test has now formally been extended to exclude protein of porcine origin.

Polyethylene Glycol (PEG)

Following solvent/detergent treatment and PEG precipitation an extra glycine/citrate/heparin wash stage has been introduced relative to the current manufacturing process. This additional step is necessary to ensure extremely low levels of Polysorbate 80 and TNBP in the final product. However a benefit appears in the improved removal of PEG from the product. This has enabled a reduction in the limit for PEG in tne final product from 45 to 20 ug/FVIII:C unit.

PROFILATE HEAT-TREATED

HEPARIN PRODUCTION METHOD

PRODUCT SPECIFICATION

TEST REQUIRED	SPECIFICATION	PROCEDURES
Individual Donor Plasma		
ALT	NMT 2X upper limit of normal range	FDA approved test
Anti-HIV	Nonreactive	FDA approved test
HBsAg	Nonreactive	FDA approved test
Final Bulk		
Sterility	No growth of any organism	LSP S-500-044
Final Container		
Appearance	White or slightly yellow friable Powder of relatively uniform texture and consitency	LSP C-659-474
Citrate	NMT 55 mmoles/L	LSP C-540-122
Coagulation	No clot formation within 3 hours of reconstitution at 20-25°C	LSP C-659-474
Dextrose	NMT 4 mg/FVIII:C Unit	LSP C-549-139
Factor VIII:C	For Specific Activity calculation; 80-125% of label; 64-156% fiducial limits of assay error	LSP C-522-104; LSP C-597-262
Fibrinogen	NMT 80% of total protein	LSP C-513-095
Glycine	NMT 750 ug/FVIII:C Unit	LSP C-579-205
HBsAg	Nonreactive	LSP C-525-107
Hemolysins	Negative	LSP C-529-111
Heparin	NMT 2.0 Units/mL	LSP C- *
Heptane	NMT 0.5 mg/Vial	LSP C-611-313
Isoagglutinins	Saline:NMT 1:256 Antiglobulin:NMT 1:32	LSP C-529-111

TEST REQUIRED	SPECIFICATION	PROCEDURE
Moisture	NMT 2.0% w/w	LSP C-523-105
pH	6.8 - 7.4	LSP C-512-094
Polyethylene Glycol	NMT 45 ug/FVIII:C Unit	LSP C-578-204
Product Identity	Reduces clotting time of hemophilic plasma AHF Heparin PEG pattern	LSP C-522-104 LSP C-597-262 LSP C-562-180
Protein	For Specific Activity calculation	LSP C-515-097
Protein Activity	Anti-Human reactive Anti-Bovine nonreactive	LSP C-532-114
Pyrogen	3 Rabbit Test: No rabbit temp rise $\geq$ 0.6°C and total temp rise NMT 1.4°C; 8 Rabbit Test: NMT 3 rabbits with a temp rise $\geq$ 0.6°C and total temp rise NMT 3.7°C	LSP P-505-236
Safety	No indication of toxicity	LSP T-505-270
Sodium	NMT 10 mEq/Vial	LSP C-504-086
Solubility	NMT 5 minutes	LSP C-659-474
Specific Activity	NLT 3.0 FVIII:C Units/mg Protein	LSP C-604-290
Sterility	No growth of any organism	LSP S-500-044
Visual Inspection	Defective vials to be removed from lot	MSP 416-007

* See text in introduction to this section.

PROFILATE-SD

SOLVENT DETERGENT PRODUCTION METHOD

PRODUCT SPECIFICATION

TEST REQUIRED	SPECIFICATION	PROCEDURES
Individual Donor Plasma		
ALT	NMT 2X upper limit of normal range	FDA approved test
Anti-HIV	Nonreactive	FDA approved test
HBsAg	Nonreactive	FDA approved test
Final Bulk		
Sterility	No growth of any organism	LSP S-500-044
Final Container		
Appearance	White or slightly yellow friable Powder or relatively uniform texture and consistency	LSP C-659-474
Citrate	NMT 55 mmoles/L	LSP C-540-122
Coagulation	No clot formation within 3 hours of reconstitution at 20-25 °C	LSP C-659-474
Factor VIII:C	For Specific Activity calculation; 80-125% of label; 64-156% fiducial limits of assay error.	LSP C-522-104; LSP C-597-262
Fibrinogen	NMT 80% of total protein	LSP C-513-095
Glycine	NMT 750 ug/FVIII:C Unit	LSP C-579-205
HBsAg	Nonreactive	LSP C-525-107
Hemolysins	Negative	LSP C-529-111
Heparin	NMT 2.0 Units/mL	LSP C-664-489
Isoagglutinins	Saline:NMT 1:256 Antiglobulin:NMT 1:32	LSP C-529-111
Moisture	NMT 2.0% w/w	LSP C-523-105
pH	6.8 - 7.4	LSP C-512-094
Polyethylene Glycol	NMT 20 ug/FVIII:C unit	LSP C-578-204

TEST REQUIRED	SPECIFICATION	PROCEDURE
Polysorbate 80	NMT 2.5 ug/FVIII:C Unit	LSP C-672-076
Product Identity	Reduces clotting time of hemophilic plasma AHF Heparin PEG pattern	LSP C-522-104 LSP C-597-262 LSP C-562-180
Protein	For Specific Activity calculation	LSP C-515-097
Protein Identity	Anti-Human reactive Anti-Bovine nonreactive Anti-Porcine nonreactive	LSP C-532-114 LSP C-656-466
Pyrogen	3 Rabbit Test: No rabbit temp rise $\geq 0.6^{\circ}C$ and total temp rise NMT $1.4^{\circ}C$; 8 Rabbit Test: NMT 3 rabbits with a temp rise $\geq 0.6^{\circ}C$ and total temp rise NMT $3.7^{\circ}C$	LSP P-505-236
Safety	No indication of toxicity	LSP T-505-270
Sodium	NMT 10 mEq/Vial	LSP C-504-086
Solubility	NMT 5 minutes	LSP C-659-474
Specific Activity	NLT 3.0 FVIII:C Units/mg Protein	LSP C-604-290
Sterility	No growth of any organism	LSP S-500-044
Tri (n-butyl) Phosphate (TNBP)	NMT 0.4 ug/FVIII:C Unit	LSP C-671-066
Visual Inspection	Defective vials to be removed from lot	MSP 405-006

BATCH ANALYSIS

PROFILATE-SD

Antihaemophilic Factor (Human)

Test Description	Units	AR8001A	AR8002A	AR9001A
Factor VIII:C	Units/ml	46.4	43.6	44.0
Glycine	ug/FVIII:C Unit	290	250	260
HBsAg (nonreactive)	-	Pass	Pass	Pass
Heparin	Units/ml	0.21	0.12	0.12
Isoagglutinins (Saline) Anti-A and Anti-B	-	1:32	1:32	1:32
Moisture	% w/w	1.55	0.66	2.84*
pH	-	7.13	7.23	7.38
Polyethylene Glycol	ug/FVIII:C Unit	<0.86	<0.96	<1.0
Product Identity	-	Pass	Pass	Pass
Protein	g/Vial	0.62	0.09	0.08
Pyrogen	-	Pass	Pass	Pass
Safety	-	Pass	Pass	Pass
Sodium	mEq/Vial	0.87	0.78	0.92
Solubility	minutes	1	1	1
Specific Activity	Units/mg protein	7.25	12.11	13.75
Sterility	ug/FVIII:C Unit	Pass	Pass	Pass
TNBP	ug/FVIII:C Unit	0.146	0.21	0.20
Polysorbate 80	ug/FVIII:C Unit	1.66	<1.0	<1.0

*Note: This lot is outside specification for moisture content and would not be released for clinical use by Q.C. However it was considered suitable for investigation of product stability.

JK/002/001

29

STABILITY STUDIES

Batches studied and packaging

Three batches manufactured during an early production run (batch analyses p.51). Date of manufacture 20.12.88 (2 batches) and 24.1.89 (one batch). Two further batches were put on stability in July. Containers are 100ml Type 1 glass with West 4416/50 grey butyl rubber stoppers. The diluent is 25ml of Water for Injections.

Storage conditions

Six months at 5°C and 30°C available. Testing schedule is at 3 month intervals in the first year and every six months during the second year. Testing will be continued up to 3 years. Samples from 5°C storage are tested at 1 and 2 years to determine stability of Factor VIII:C activity after reconstitution. Similarly, samples from 30°C are tested at 6 months.

Evaluation methods

Factor VIII:C activity, pH, solubility, vacuum, appearance and moisture. (Pyrogenicity and safety are tested at the end of the shelf life at each temperature only.)

Results of tests

Results are given on the following pages.
Factor VIII:C activity:- Samples retained 84, 88 and 91% of their original activity when stored at 5°C for approx. 6 months. Samples retained 80, 73 and 86% of their original activity when stored at 30°C for approx. 6 months. The Company comment that they believe that the low 73% value is due to an erroneously high estimate of original potency.

Samples tested within specification for pH, solubility, vacuum and appearance at both 5 and 30°C. One batch failed the initial moisture test.

After reconstitution:- Lots stored at 30°C for 6 months showed a drop in AHF activity with storage time of the reconstituted solution of approximately 10% (81, 92.5 and 92.5%) after storage of the reconstituted solution for 4 hours at room temperature.

Proposed shelf life and storage conditions

Two years when stored between 2 and 8°C protected from light. May be stored at room temperature not to exceed 30°C for up to 6 months. Do not freeze. Use within 3 hours after reconstitution. Discard any unused contents.

TABLE I

Product: Profilate^{R}-OSD
Lot No.: AR8001A
Mfg. Date: 12-20-88
Stability Start Date: 01-23-89

Test

Test Date	Storage Time (months)	Storage Temp. °C	Factor VIII:C (U/vial)	Protein (g/vial)	Specific Activity (U/mg protein)	pH	Solubility (min.)	Vacuum	Appearance	Moisture (% w/w)	Pyrogenicity	Safety	Reconstitution Stability Factor VIII:C (U/vial) 0 h	2 h	4 h
1/89	0	5°	1160	0.16	7.25	7.13	1.0	*	*	1.55	Pass	Pass	*	*	*
4/89	2	5°	1130	0.16	7.06	7.13	1.0	Pass	White	*	*	*	*	*	*
		30°	1030	0.16	6.44	7.12	1.0	Pass	White	*	*	*	*	*	*
6/89	4	5°	1140	0.16	7.13	7.12	1.0	Pass	White	*	*	*	*	*	*
		30°	900	0.16	5.63	7.12	1.0	Pass	White	*	*	*	*	*	*
8/89	6	5°	970	*	*	7.13	1.0	Pass	White	*	*	*	*	*	*
		30°	930	*	6.64	7.14	1.0	Pass	White	0.73	**	**	930	860	750

*Test not performed
**Test results not yet available

0831X:9

TABLE II

Product: Profilate^{R}-OSD
Lot No.: AR8002A
Mfg. Date: 12-20-88
Stability Start Date: 01-23-89

Test

Test Date	Storage Time (months)	Storage Temp. °C	Factor VIII:C (U/vial)	Protein (g/vial)	Specific Activity (U/mg protein)	pH	Solubility (min.)	Vacuum	Appearance	Moisture (% w/w)	Pyrogenicity	Safety	Reconstitution Stability Factor VIII:C (U/vial) 0 h	2 h	4 h
1/89	0	5°	1090	0.09	12.11	7.23	1.0	*	*	0.66	Pass	Pass	*	*	*
4/89	3	5°	860	0.09	9.56	7.22	1.0	Pass	White	*	*	*	*	*	*
		30°	800	0.09	8.89	7.21	1.0	Pass	White	*	*	*	*	*	*
4/89	Retest	5°	940	0.09	10.44	*	*	*	*	*	*	*	*	*	*
		30°	820	0.09	9.11	*	*	*	*	*	*	*	*	*	*
6/89	4	5°	830	*	*	7.20	1.0	Pass	White	*	*	*	*	*	*
		30°	840	*		7.20	1.0	Pass	White	*	*	*	*	*	*
8/89	6	5°	960	*	*	7.21	1.0	Pass	White	*	*	*	*	*	*
		30°	800	*	8.0	7.22	1.0	Pass	White	**	**	**	800	**	740

*Test not performed
**Test results not yet available

0831X:10

[redacted]

TABLE III

Product: Profilate^{R}-OSD
Lot No.: AR9001A
Mfg. Date: 01-24-89
Stability Start Date: 02-01-89
Bottle size: 100 mL

Test

Test Date	Storage Time (months)	Storage Temp. °C	Factor VIII:C (U/vial)	Protein (g/vial)	Specific Activity (U/mg protein)	pH	Solubility (min.)	Vacuum	Appearance	Moisture (% w/w)	Pyrogenicity	Safety	Reconstitution Stability Factor VIII:C (U/vial) 0 h	2 h	4 h
2/89	0	5°	1100	0.08	13.75	7.38	1.0	*	Pass	2.84#	Pass	Pass	*	*	*
4/89	2	5°	1030	0.08	12.88	7.25	1.0	Pass	White	*	*	*	*	*	*
		30°	970	0.08	12.13	7.26	1.0	Pass	White	*	*	*	*	*	*
6/89	4	5°	1020	*	*	7.26	1.0	Pass	White	*	*	*	*	*	*
		30°	990	*	*	7.24	1.0	Pass	White	*	*	*	*	*	*
8/89	6	5°	1000	*	*	7.22	1.0	Pass	White	*	*	*	*	*	*
		30°	950	*	9.5	7.25	1.0	Pass	White	0.57	**	**	950	910	880

*Test not performed
**Test results not yet available
#Highest of four tested values-NDMR filed

0831X:11

TABLE IV

Product: Profilate^{R}-OSD
Lot No.: AR9002A
Mfg. Date: 07-27-89
Stability Start Date: 08-04-89
Bottle size: 100 mL

Test

Test Date	Storage Time (months)	Storage Temp. °C	Factor VIII:C (U/vial)	Protein (g/vial)	Specific Activity (U/mg protein)	pH	Solubility (min.)	Vacuum	Appearance	Moisture (% w/w)	Pyrogenicity	Safety	Reconstitution Stability Factor VIII:C (U/vial) 0 h	2 h	4 h
8/89	0	5°	1270	0.16	7.94	7.17	1.0	*	*	0.14	Pass	Pass	*	*	*
11/89	3	5° 30°													

*Test not performed

0831X:14

TABLE V

Product: Profilate^{R}-OSD
Lot No.: AR9003A
Mfg. Date: 07-27-89
Stability Start Date: 08-04-89
Bottle size: 100 mL

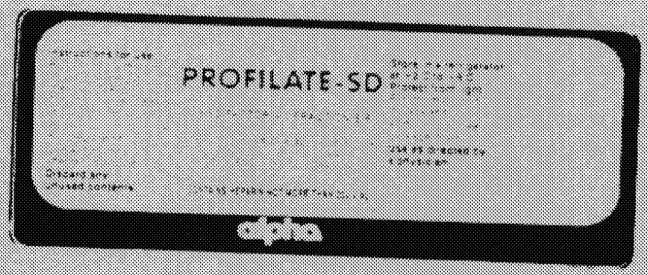
Test

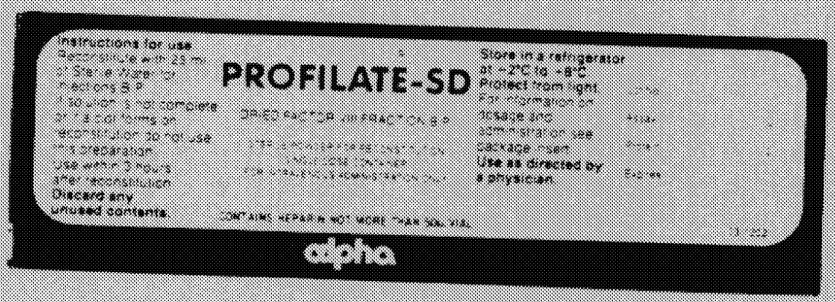
Test Date	Storage Time (months)	Storage Temp. °C	Factor VIII:C (U/vial)	Protein (g/vial)	Specific Activity (U/mg protein)	pH	Solubility (min.)	Vacuum	Appearance	Moisture (% w/w)	Pyrogenicity	Safety	Reconstitution Stability Factor VIII:C (U/vial) 0 h	2 h	4 h
8/89	0	5°	1250	0.17	7.35	7.16	1.0	*	*	0.23	Pass	Pass	*	*	*
11/89	3	5° 30°													

*Test not performed

0831X:15

PROPOSED LABELLING
(MOCK-UP ONLY)





NOTE: Actual labels will contain PL number, an indication that the product is a prescription only product (i.e. POM) and the maximum level of fibrinogen in the product.

JK/002/001

PRE-CLINICAL

1. Toxicology of Tri(n-butyl) Phosphate

Vandekar and coworkers[1] found that a single dose of 80 mg/kg administered into rat tail veins elicited incoordination and mild anaesthesia within 1 hour and pronounced weakness after 4 hours. A dose of 100 mg/kg was lethal, causing respiratory failure. Intraperitoneally, the dose with the lowest effect in mice was 63 mg/kg.[2]

In a study performed by Oishi et al[3] actively growing rats were fed a pellet diet containing 0.5% TNBP for 9 weeks. As compared with controls, rats fed a diet including TNBP exhibited lower body weight and increased liver weight, expressed as weight per se and as weight per 100g of body weight. No statistically significant difference in the absolute weight of the kidneys or testes was observed, though both were increased when expressed per unit of body weight. Spleen weight was lower in absolute terms but not when normalized to body weight. No statistically significant differences were observed in blood cell indicators (leukocyte or erythrocyte count, haemoglobulin, haematocrit, or mean corpuscular volume) or in 8 of 9 plasma indicators (total protein, cholesterol, triglyceride, bile acids, sodium, potassium, prothrombin time, or kaolin-PTT). Blood urea nitrogen was elevated in the rats fed TNBP. No significant difference was observed in serum enzyme levels (GOT, GPT, AIP, CLE). The authors concluded that the only consistent response to phosphoric acid esters was a weak effect on the liver. These results generally confirm an earlier study by the same authors[4], except in the earlier study coagulation time was prolonged in rats fed TNBP.

Hanna and Dyer investigated[5] the mutagenicity of TNBP and 139 other organophosphorus compounds. The tests with bacteria were similar to those described by Ames et al utilising specially selected strains of S. typhimurium and E.coli. TNBP was applied onto the bacterial layer and the number of prototropic revertants determined after 48 and 72 hours. Using this test, TNBP was not found to be mutagenic in either strain of bacteria. In drosophila, male flies containing a revertant to the normally recessive lethal Cy/B1L genotype were mated with normal female flies which were allowed to oviposit on standard food medium containing TNBP. Emerging males were mated to Cy/B1L virgins and suspected lethals were confirmed by obtaining an F_1 generation. The drosophila population exposed to TNBP was not significantly different from the control in the accumulation of mutations. However, the rate of sterility among males at the first mating was 12.8% as compared to 1.0% for the control. The authors concluded that they could not determine whether the apparent higher incidence of sterility resulted from an effect on spermatogenesis or on reduced sexual activity.

A similar material, trimethylphosphate, has been demonstrated by Jackson et al[6] to have a sterilising action when administered intraperitoneally or orally, and five consecutive daily doses of 100 mg/kg to the male rat seem to be the minimal amount required for clearcut action. In subsequent studies, these authors commented that other tri-n-alkyl phosphates do not affect fertility in the male rat.[7]

Monodealkylation of tri-alkyl phosphates has been reported to be their principal metabolic reaction[8]. Metabolism of tri(n-butyl) phosphate

has been shown to give rise to di(n-butyl) phosphate, and S-butyl-cysteine. Administration of ^{32}P-triethylphosphate to rats resulted in essentially complete recovery of radioactivity in the urine within 96 hours. The excretion and metabolism of ^{14}C-TNBP administered intraperitoneally and orally was recently studied.[9] 90% of label was excreted in 5 days, mostly in the urine.

2. Toxicology of Polysorbate 80

Hopper et al[10] reported the LD_{50} in mice for polysorbate 80 by intravenous injection to be 5.8 grams per kilogram body weight (24 hours observation).

Krantz et al[11] determined that the LD_{50} value in rats by intraperitoneal injection was between 8 and 9 cc of polysorbate 80 per kilogram body weight.

The LD_{50} value of polysorbate 80 by the intravenous route of administration in fasted (16 hours) male and female rats was determined in the Atlas Toxicology Laboratory. They obtained the following results when polysorbate 80 was administered as a 25% w/v solution in saline:

Animal Sex	Average Body Weight (grams)	LD_{50} (g/kg)*	95% Confidence Limits	Slope
Male	145	2.18	1.78 - 2.67	1.45
Female	148	1.72	1.57 - 1.88	1.11
M & F	147	1.79	1.57 - 2.04	1.38

*Calculated at 7 days in terms of polysorbate 80 according to the method of Litchfield and Wilcoxon.

Krantz et al[11] also found an allergic depressor response in the dog, but not in the cat, rabbit or Rhesus monkey (Macaca mulatta) when polysorbate 80 is injected intravenously. This is a characteristic reaction to various polyoxyethylene compounds in members of the canine family of animals but not in other species, including man, cat, monkey, rabbit, rat, chicken and guinea pig.[12,13]. Neither was the polyol hydrolysate from polysorbate 80 nor those from other polyoxyethylene types of partial esters studied found to elicit the allergic response in the dog when injected intravenously.[15] For example, no significant effect resulted from the intravenous administration to the dog of 5cc. of a 5% solution of the polyol hydrolysate from polysorbate 80.

Glas et al[14] studied the effect, on experimental fat embolism in rabbits, of intravenous doses of 10 cc of 5% polysorbate 80 solution per animal before, with, and after 1.0 cc doses of intravenous fat. No significant effects were obtained.

Tobler and Kimball[15] concluded from an extensive review of the literature that Polysorbate 80 is not mutagenic in bacterial systems and is not clastogenic in the in vitro and in vivo mammalian systems tested.

Kellner et al[16] administered polysorbate 80 (20% in buffered saline solution) to rabbits by intravenous injection, with no recorded effects using doses below 1 cc of this solution per kilogram body weight. Single doses of 2.5 cc of the solution per kilogram were also well tolerated, except for an apparent intravacular haemolysis, which disappeared with repeated injections and a transitory increase in the blood cholesterol and phospholipid which returned to normal within 24 to 48 hours unless the injections were repeated. Doses as high as 4 cc of the solution per kilogram resulted in the death of the animals.

Payne and Duff[17] also studied the repeated intravenous injection of very large amounts of polysorbate 80 in rabbits: 10 cc and 15 cc of a 20% polysorbate 80 in saline solution were injected twice a day for 40 to 65 days to each of 10 rabbits weighing 2.4 to 3.5 kilos with 6 deaths after 40 to 61 days of treatment. Histologic examination of the survivors showed greatly enlarged spleens with tremendous foam cell accumulation in the reticuloendothelial system and marked lipid infiltration of the renal tubular epithelium.

3. Conclusion

It is the considered opinion of Alpha Therapeutic, based on the above results that the extremely low residues of Polysorbate 80 and TNBP found in Profilate-SD are unlikely to be the cause of any adverse events arising from the chronic use of the product. This conclusion is supported by the excellent tolerability of solvent/detergent treated Factor VIII as shown by Horowitz et al[18] and as shown by Alpha's own post-marketing surveillance of its solvent detergent Factor VIII product marketed in the USA, W.Germany and Sweden.

PHARMACOKINETIC DATA

A single study compares Profilate HT (current UK product) with solvent detergent inactivated product Profilate SD.

12 haemophiliacs at two centres participated. (Orthopaedic Hospital Los Angeles and University of Nebraska Medical Centre). All were severe haemophiliacs on long term Factor VIII treatment.

Computation of AHF Dose

The dose of Factor VIII concentrate administered to each Subject was that amount which was necessary to bring the level of Factor VIII to 1 Unit/mL plasma. The plasma volume of each Subject was estimated using the equation:

Plasma Volume (mL) = B mL/Kg x body weight (Kg)

where B is 40 for adults and 55 for Subjects under 18 years of age.

Each patient received both preparations, none were bleeding at the time of administration. Administered doses were between 2400-4400 units.

$T\frac{1}{2}$ was similar for the two products at a mean of 11.45 hours for SD and 11.71 for HT. This is also in line with $T\frac{1}{2}$ for other Factor VIII products.

Recovery was lower in Nebraska than LA, which may be due to some Nebraska patients having low level inhibitors. There were no significant differences between products.

In Vivo Recovery of Factor VIII

For each infusion of the product concentrate, in vivo recovery was calculated from the equation below and expressed as a percent.

$$\text{Percent Recovery of AHF} = \frac{\left|\text{AHF level in plasma 10 min after end of infusion}\right| - \left|\text{AHF level in plasma before infusion}\right| \times (\text{Plasma volume})}{(\text{Number of Units of AHF administered})^*} \times 100$$

*The dose was computed from the claimed potency on the product label.

ATC 88-22

Table X

IN VIVO RECOVERY OF INFUSED FACTOR VIII CONCENTRATE

Subject	Product Infused	Plasma Volume (mL)	Factor VIII Infused (Units)	Plasma Factor VIII Level (U/mL)§ Baseline	Plasma Factor VIII Level (U/mL)§ 10 Min	Percent Recovery¶
Orthopaedic Hospital						
101 CD	Profilate® OSD	4340	4338	0.01	1.23	122
	Profilate® HP	4380	4378	<0.01	1.35	135
102 JM	Profilate® OSD	2960	2962	0.02	1.30	128
	Profilate® HP	2940	3086	0.02	1.30	122
103 RF	Profilate® OSD	2820	2819	0.01	1.31	130
	Profilate® HP	2840	2842	0.02	1.29	127
104 SL	Profilate® OSD	2940	2850	0.03	1.29	130
	Profilate® HP	2860	2778	0.03	1.19	119
105 DB	Profilate® OSD	3200	3200	0.03	1.22	119
	Profilate® HP	3180	3180	0.02	1.20	118
106 RH	Profilate® OSD	2600	2601	0.01	1.57	156
	Profilate® HP	2600	2598	0.02	1.41	139
Means:	Profilate® OSD	3143.3 ± 618.1	3128.4 ± 624.2	0.018 ± 0.010	1.320 ± 0.128	130.8 ± 13.1 ¤
	Profilate® HP	3133.3 ± 638.6	3143.7 ± 640.6	0.018 ± 0.010	1.290 ± 0.085	126.7 ± 8.7 ¤
University of Nebraska Medical Center						
201 TD	Profilate® OSD	3160	3160	<0.01	1.10	110
	Profilate® HP	3160	3124	0.03	1.07	105
202 KP	Profilate® OSD	3680	3680	<0.01	1.25	118
	Profilate® HP	3720	3719	<0.01	1.00	100
203 BH	Profilate® OSD	2760	2760	0.02	0.88	86
	Profilate® HP	2760	2756	<0.01	0.76	77
204 RR	Profilate® OSD	2840	2840	<0.01	1.25	125
	Profilate® HP	2840	2825	<0.01	1.00	100
205 JR	Profilate® OSD	2400	2400	0.02	1.00	98
	Profilate® HP	2400	2397	0.04	0.76	72
206 TD	Profilate® OSD	2760	2758	0.05	1.20	115
	Profilate® HP	2760	2756	<0.01	1.00	99
Means:	Profilate® OSD	2933.3 ± 438.7	2931.7 ± 439.5	0.015 ± 0.020	1.113 ± 0.150	106.7 ± 14.3 ¤
	Profilate® HP	2940.0 ± 452.4	2929.7 ± 450.9	0.012 ± 0.018	0.932 ± 0.136	92.2 ± 13.9 ¤
MEAN OF ALL SUBJECTS:						
	Profilate® OSD	3038.3 ± 522.6	3030.1 ± 524.8	0.017 ± 0.015	1.217 ± 0.171	119.8 ± 17.5 ¤
	Profilate® HP	3036.7 ± 537.2	3036.7 ± 539.9	0.015 ± 0.014	1.111 ± 0.216	109.4 ± 21.1 ¤

§ Factor VIII concentration determined using the Thromboscreen Standard.

¶ Percent Recovery calculated using the number of Units of Factor VIII infused as computed from the labeled potency.

¤ Mean Percent Recovery calculated from the individual Percent Recovery values.

(ref:8822-9.tbl/A)

0673t/19

TABLE XI

STATISTICAL SUMMARY OF IN VIVO RECOVERY OF INFUSED FACTOR VIII CONCENTRATE

	n	Mean (%)	Std'	SEM'	Paired t Degrees of Freedom	Paired t t statistic	Paired t p'	Wilcoxon Signed Rank p'
Orthopaedic Hospital								
Profilate® OSD	6	130.8	13.1	5.4	5	1.00	0.363	0.313
Profilate® HP	6	126.7	8.7	3.5				
University of Nebraska Medical Center								
Profilate® OSD	6	108.7	14.3	5.8	5	4.81	0.005	0.031
Profilate® HP	6	92.2	13.9	5.7				
All Subjects								
Profilate® OSD	12	119.8	17.5	5.0	11	3.25	0.008	0.012
Profilate® HP	12	109.4	21.1	6.1				

1: Standard Deviation
2: Standard Error of Mean
3: Two-tailed p value

Table XII

CONCENTRATION OF FACTOR VIII IN PLASMA

(U/mL)

Subject	Product Infused	Time from End of Infusion (Hours) 0	0.17	0.50	1.0	1.5	3	6	12	24	Half-Life (Hours)
Orthopaedic Hospital											
101 CD	Profilate® OSD	0.01	1.23	1.06	0.96	0.86	0.70	0.55	0.25	0.20	9.92
	Profilate® HP	<0.01	1.35	1.20	1.04	0.86	0.82	0.57	0.29	0.19	9.37
102 JM	Profilate® OSD	0.02	1.30	1.17	1.02	0.89	0.73	0.44	0.35	0.23	11.24
	Profilate® HP	0.02	1.30	1.14	0.96	0.91	0.83	0.76	0.25	0.16	8.29
103 BF	Profilate® OSD	0.01	1.31	1.16	0.98	0.84	0.76	0.60	0.47	0.24	12.11
	Profilate® HP	0.02	1.29	1.21	1.04	0.87	0.75	0.64	0.55	0.34	16.09
104 SL	Profilate® OSD	0.03	1.29	1.16	0.96	0.86	0.66	0.57	0.29	0.24	11.50
	Profilate® HP	0.03	1.19	1.06	0.85	0.77	0.68	0.55	0.42	0.21	11.92
105 DB	Profilate® OSD	0.03	1.22	1.11	0.87	0.75	0.68	0.55	0.28	0.18	10.10
	Profilate® HP	0.02	1.20	0.99	0.91	0.80	0.74	0.56	0.26	0.15	8.68
106 RH	Profilate® OSD	0.01	1.57	1.31	1.04	0.84	0.68	0.57	0.35	0.15	8.78
	Profilate® HP	0.02	1.41	1.24	1.04	0.86	0.78	0.63	0.24	0.19	9.14
Mean:	Profilate® OSD	0.018	1.320	1.162	0.972	0.840	0.702	0.547	0.332	0.207	10.60 §
	Profilate® HP	0.022	1.290	1.140	0.973	0.845	0.767	0.618	0.335	0.207	10.33 §

§ Mean half-life values were computed by linear regression analysis of the common log of the mean values of factor VIII concentration for the 1-24 hour time points.

(ref:8822-10.tbl/A)

AIC 88-22

Table XII (continued)
CONCENTRATION OF FACTOR VIII IN PLASMA
(U/mL)

Subject	Product Infused	Time from End of Infusion (Hours) 0	0.17	0.50	1.0	1.5	3	6	12	24	Half-Life (Hours)
University of Nebraska Medical Center											
201 TD	Profilate® OSD	<0.01	1.10	1.08	1.00	0.98	0.78	0.67	0.51	0.27	12.53
	Profilate® HP	0.03	1.07	1.00	0.78	0.78	0.73	0.61	0.46	0.27	14.76
202 KP	Profilate® OSD	<0.01	1.25	1.10	1.05	1.00	0.86	0.51	0.41	0.21	10.05
	Profilate® HP	<0.01	1.00	0.87	0.79	0.76	0.68	0.51	0.35	0.20	11.52
203 BH	Profilate® OSD	0.02	0.88	0.78	0.76	0.76	0.70	0.54	0.47	0.29	16.58
	Profilate® HP	<0.01	0.76	0.72	0.59	0.57	0.54	0.45	0.29	0.21	14.82
204 RR	Profilate® OSD	<0.01	1.25	1.10	1.00	0.90	0.79	0.52	0.37	0.17	9.23
	Profilate® HP	<0.01	1.00	1.04	0.90	0.87	0.72	0.47	0.30	0.16	9.15
205 JR	Profilate® OSD	0.02	1.00	0.81	0.80	0.73	0.63	0.48	0.33	0.18	10.93
	Profilate® HP	0.04	0.76	0.81	0.73	0.70	0.46	0.42	0.33	0.20	13.28
206 TD	Profilate® OSD	0.05	1.20	1.25	1.05	1.05	1.05	0.82	0.63	0.36	14.44
	Profilate® HP	<0.01	1.00	1.35	0.87	0.82	0.72	0.62	0.46	0.26	13.55
Means:	Profilate® OSD	0.030	1.113	1.020	0.943	0.903	0.802	0.590	0.453	0.247	12.04 §
	Profilate® HP	0.035	0.932	0.965	0.777	0.750	0.642	0.513	0.365	0.217	12.55 §
MEAN OF ALL SUBJECTS:											
Profilate® OSD		0.022	1.217	1.091	0.957	0.872	0.752	0.568	0.392	0.227	11.32 §
Profilate® HP		0.026	1.111	1.052	0.875	0.797	0.704	0.566	0.350	0.212	11.30 §

§ Mean half-life values were computed by linear regression analysis of the common log of the mean values of factor VIII concentration for the 1-24 hour time points.

(ref:8822-10.tbl/A)

0673t/23

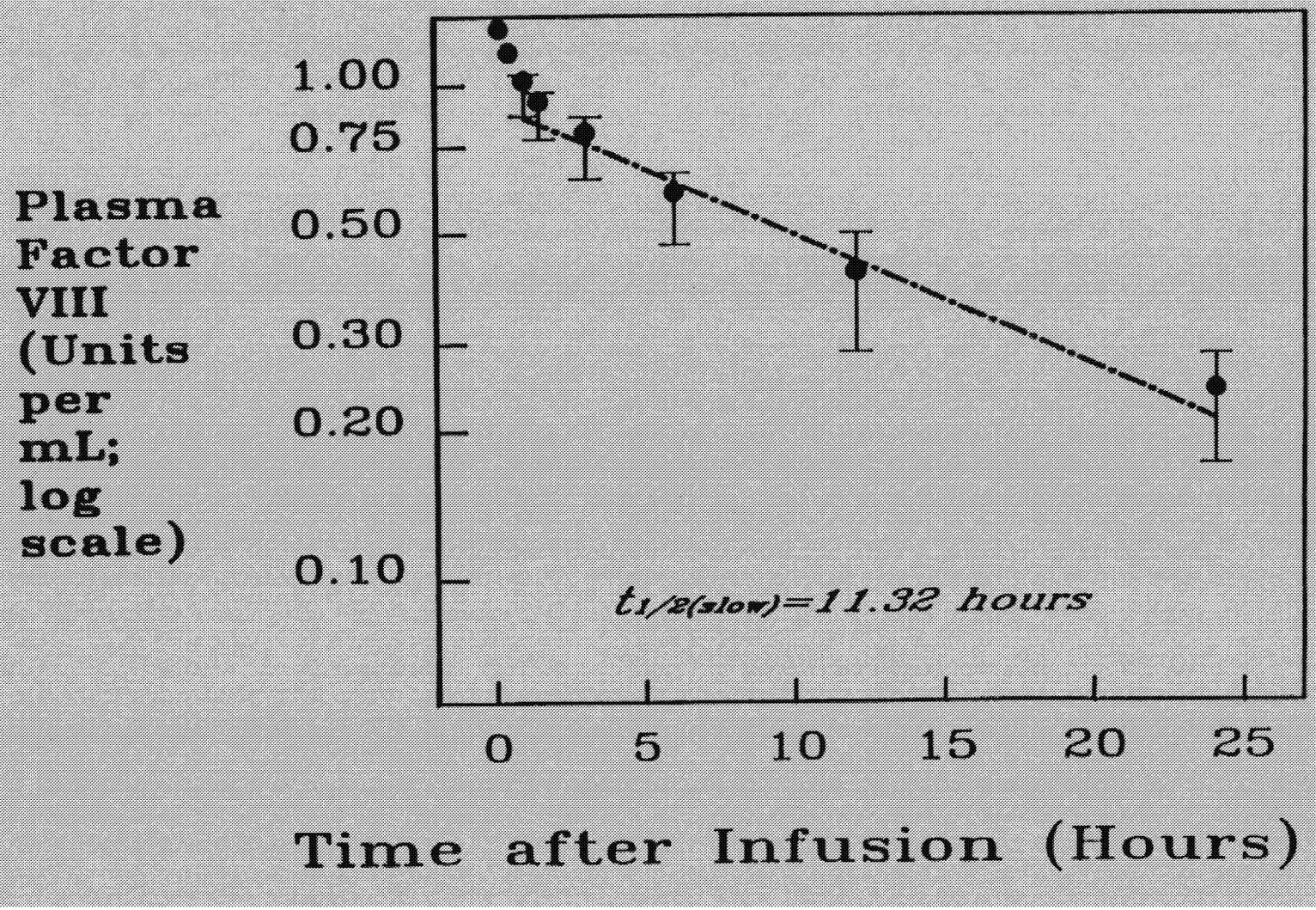
TABLE XIII

STATISTICAL SUMMARY OF FACTOR VIII HALF-LIVES

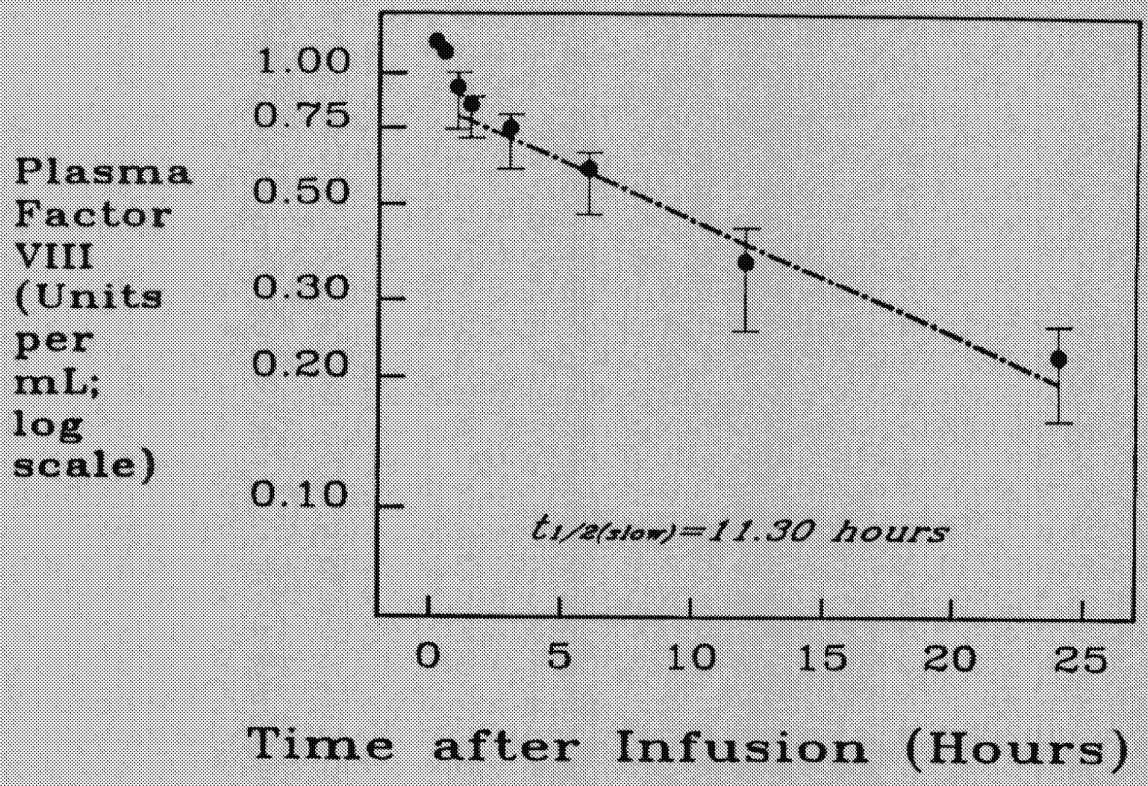
	n	Mean (Hrs)	Std'	SEM'	Paired t: Degrees of Freedom	Paired t: t statistic	Paired t: p'	Wilcoxon Signed Rank: p'
Orthopaedic Hospital								
Profilate® OSD	6	10.61	1.23	0.50	5	0.03	0.979	0.844
Profilate® HP	6	10.58	2.99	1.22				
University of Nebraska Medical Center								
Profilate® OSD	6	12.29	2.80	1.15	5	-0.79	0.464	0.563
Profilate® HP	6	12.85	2.18	0.89				
All Subjects								
Profilate® OSD	12	11.45	2.24	0.65	11	-0.46	0.652	0.754
Profilate® HP	12	11.71	2.76	0.80				

1: Standard Deviation
2: Standard Error of Mean
3: Two-tailed p value

Decay of Plasma Factor VIII Concentration: Mean Values for All Subjects with Profilate® OSD



Decay of Plasma Factor VIII Concentration: Mean Values for All Subjects with Profilate® HP



Safety

There were no adverse reactions seen and no changes in vital signs in the patients during or after infusion of either product.

PRESENT

DATA SHEET ALPHA

PROFILATE HEAT-TREATED

Presentation

Profilate Heat-Treated is a preparation of Dried Factor VIII Fraction B.P. It is a stable, highly purified, freeze-dried concentrate of Factor VIII (Antihaemophilic Factor, Antihaemophilic Globulin) which has been wet heat-treated using an organic solvent.

Profilate Heat-Treated is a sterile preparation intended for intravenous administration. Each vial is a single dose container having the Factor VIII activity expressed on the label in International Units (I.U.). One International Unit approximates to the activity of one ml of fresh pooled human plasma. The specific activity of the product is at least 3.0 I.U. of Factor VIII per milligram of protein. Profilate Heat-Treated contains not more than 4 milligrams of dextrose per I.U. of Factor VIII. The reconstituted product contains not more than 2 units of heparin per ml and not more than 20 milligrams of fibrinogen per ml.

Uses

For the prevention and control of bleeding in patients with moderate or severe Factor VIII deficiency (classical haemophilia A) or acquired Factor VIII deficiency.

Dosage and Administration

Dosage:

The dose of Profilate Heat-Treated to be administered must be adjusted according to the needs of the patient. The patient's plasma level of Factor VIII should be determined and monitored during treatment. The formula below provides a guide to dosage

PROPOSED

DATA SHEET ALPHA

PROFILATE-SD

Presentation

Profilate-SD is a preparation of Dried Factor VIII Fraction B.P. It is a stable, highly purified, freeze-dried concentrate of Factor VIII (Antihaemophilic Factor, Antihaemophilic Globulin) which has been treated during manufacture with an organic solvent Tri-(n-butyl) phosphate (TNBP) and a detergent, Polysorbate 80 in order to inactivate certain viruses known to be transmissible by human blood.

Profilate-SD is a sterile preparation intended for intravenous administration. Each vial is a single dose container having the Factor VIII activity expressed on the label in International Units (I.U.). One International Unit approximates to the activity of one ml of fresh pooled human plasma. The specific activity of the product is at least 3.0 I.U. of Factor VIII per milligram of protein. Profilate-SD, when reconstituted contains not more than 2 units of heparin per ml and not more than 20 milligrams of fibrinogen per ml.

Uses

For the prevention and control of bleeding in patients with moderate or severe Factor VIII deficiency due to classical haemophilia A or acquired Factor VIII deficiency.

Dosage and Administration

Dosage:

The dose of Profilate-SD to be administered must be adjusted according to the needs of the patient. The patient's plasma level of Factor VIII should be determined and monitored during treatment. The formula below provides a guide to dosage

calculations:-

Bodyweight in kg × 0.5 × Desired increase in Factor VIII (as a percent) = Number of Factor VIII Units Required

Example

50 × 0.5 × 30 = 750 I.U. Profilate Heat-Treated

Mild to moderate haemorrhages may usually be treated with a single administration sufficient to raise the plasma Factor VIII level to 20 - 30 percent. In the event of more serious haemorrhage the patient's plasma Factor VIII level should be raised to 30 - 50 percent, infusions are generally required at twice daily intervals over several days.

Surgery in patients with Factor VIII deficiency requires that the Factor VIII level be raised to 50 - 80 percent with the level maintained at or above 30 percent for approximately two weeks post-operatively. For dental extractions, the Factor VIII level should be raised to 50 percent immediately prior to the procedure; further Factor VIII may be given if bleeding recurs.

In patients with severe Factor VIII deficiency who experience frequent haemorrhages, Profilate Heat-Treated is administered prophylactically on a daily or every other day schedule so as to raise the Factor VIII level to approximately 15 percent.

Reconstitution:
Use only the diluent provided. Use aseptic technique.
1. Warm diluent and concentrate bottles to at least room temperature (but not above 37°C).
2. Remove plastic flip-off caps from the diluent and concentrate vials.
3. Swab the exposed rubber surfaces with alcohol. Do not leave excess cleaning agent in indentation on the stoppers.
4. Remove covering from one end of a double ended needle to expose the short needle. Insert this exposed short needle through

calculations:-

Bodyweight in kg × 0.5 × Desired increase in Factor VIII (as a percent) = Number of Factor VIII Units Required

Example

50kg × 0.5 I.U./kg × 30% = 750 I.U. Profilate-SD

Mild to moderate haemorrhages may usually be treated with a single administration sufficient to raise the plasma Factor VIII level to 20 - 30 percent. In the event of more serious haemorrhage the patient's plasma Factor VIII level should be raised to 30 - 50 percent, infusions are generally required at twice daily intervals over several days.

Surgery in patients with Factor VIII deficiency requires that the Factor VIII level be raised to 50 - 80 percent with the level maintained at or above 30 percent for approximately two weeks post-operatively. For dental extractions, the Factor VIII level should be raised to 50 percent immediately prior to the procedure; further Factor VIII may be given if bleeding recurs.

In patients with severe Factor VIII deficiency who experience frequent haemorrhages, Profilate-SD should be administered prophylactically on a daily or every other day schedule so as to raise the Factor VIII level to approximately 15 percent.

Reconstitution:
Use only the diluent provided. Use aseptic technique.
1. Warm diluent and concentrate bottles to at least room temperature (but not above 37°C).
2. Remove plastic flip-off caps from the diluent and concentrate vials.
3. Swab the exposed rubber surfaces with alcohol. Do not leave excess cleaning agent in indentation on the stoppers.
4. Remove covering from one end of a double ended needle to expose the short needle. Insert this exposed short needle through

the depression in the centre of the stopper in the vial of diluent.

5. Remove plastic cap from the upper end of the double ended needle now seated in the stopper of the diluent vial. Hold concentrate vial in one hand, invert the vial of diluent in the other hand and push the exposed end of the needle through the depression in the centre of the stopper, making certain that the diluent is always above the vial of concentrate. There should be enough vacuum in the vial to draw in all the diluent.

6. Disconnect the two vials by removing needle from the concentrate vial stopper. Swirl the concentrate vial until all concentrate is dissolved. Reconstitution normally requires less than five minutes. When the reconstitution procedure is strictly followed a few small particles may occasionally remain. The microaggregate filter will retain particles and the labelled potency will not be reduced.

Administration:
Profilate Heat-Treated is intended for intravenous administration only and should be used within 3 hours of reconstitution.

By Syringe:- Use aseptic technique.

1. Peel cover from microaggregate filter package and securely install the syringe into the exposed luer inlet of the filter using a slight clockwise twisting motion.
2. Remove filter from packaging. Remove protective sleeve from the spike end of the filter using clockwise twisting motion.
3. Pull back plunger to aspirate sufficient air into the syringe to allow reconstituted product to be withdrawn as described in the next step.
4. Insert the spike end of the filter into the reconstituted concentrate vial. Inject air and aspirate the reconstituted product from the vial into the syringe.
5. Remove and discard the filter from the syringe. Attach syringe to an infusion set, expel air from syringe and infusion set. Perform venipuncture and administer slowly.
6. If the patient is to receive more than one vial of concentrate, the infusion set will allow this to be performed with a single venipuncture.

the depression in the centre of the stopper in the vial of diluent.

5. Remove plastic cap from the upper end of the double ended needle now seated in the stopper of the diluent vial. Hold concentrate vial in one hand, invert the vial of diluent in the other hand and push the exposed end of the needle through the depression in the centre of the stopper, making certain that the diluent is always above the vial of concentrate. There should be enough vacuum in the vial to draw in all the diluent.

6. Disconnect the two vials by removing needle from the concentrate vial stopper. Swirl the concentrate vial until all concentrate is dissolved. Reconstitution normally requires less than five minutes. When the reconstitution procedure is strictly followed a few small particles may occasionally remain. The microaggregate filter will retain particles and the labelled potency will not be reduced.

Administration:
Profilate Heat-Treated is intended for intravenous administration only and should be used within 3 hours of reconstitution.

By Syringe:- Use aseptic technique.

1. Peel cover from microaggregate filter package and securely install the syringe into the exposed luer inlet of the filter using a slight clockwise twisting motion.
2. Remove filter from packaging. Remove protective sleeve from the spike end of the filter using clockwise twisting motion.
3. Pull back plunger to aspirate sufficient air into the syringe to allow reconstituted product to be withdrawn as described in the next step.
4. Insert the spike end of the filter into the reconstituted concentrate vial. Inject air and aspirate the reconstituted product from the vial into the syringe.
5. Remove and discard the filter from the syringe. Attach syringe to an infusion set, expel air from syringe and infusion set. Perform venipuncture and administer slowly.
6. If the patient is to receive more than one vial of concentrate, the infusion set will allow this to be performed with a

7.Discard all administration equipment after use.

By Administration Set:- Use aseptic technique.
1.Close clamp on the administration set.
2.With vial upright, insert piercing pin straight through stopper centre. Do not twist or angle.
3.Immediately invert vial to automatically establish proper fluid level in drip chamber (half full).
4.Attach infusion set, open clamp and allow solution to expel air from tubing and needle, then close clamp.
5.Perform venipuncture and adjust flow, not to exceed 10ml/minute.
6.Discard all administration equipment after use.

Contra-indications, warnings etc.
Contra-indications:
There are no known contraindications to the use of Profilate Heat-Treated.

Warnings:
This product is prepared from pooled units of human plasma which have been individually tested and found nonreactive for hepatitis B surface antigen and antibody to Human Immunodeficiency Virus (HIV). The plasma used in the preparation of this product has been screened for alanine aminotransferase (ALT) levels in an effort to reduce the risk of transmission of non A, non B hepatitis. Each unit used in the manufacture of this product has been found to have an ALT level less than 2 times the upper limit of normal for the test. Other screening procedures are used to eliminate high risk plasma donors and a heat-treatment step in the manufacturing process is designed to reduce the risk of transmitting viral infection. The effectiveness of the heat-treatment step has been demonstrated by in vitro inactivation studies using live viruses added to Profilate Heat-Treated.

However, testing methods presently available are not sensitive enough to detect all units of potentially infectious plasma and treatment methods have not been shown to be totally effective in eliminating viral

single venipuncture.
7.Discard all administration equipment after use.

By Administration Set:- Use aseptic technique.
1.Close clamp on the administration set.
2.With vial upright, insert piercing pin straight through stopper centre. Do not twist or angle.
3.Immediately invert vial to automatically establish proper fluid level in drip chamber (half full).
4.Attach infusion set, open clamp and allow solution to expel air from tubing and needle, then close clamp.
5.Perform venipuncture and adjust flow, not to exceed 10ml/minute.
6.Discard all administration equipment after use.

Contra-indications, warnings etc.
Contra-indications:
There are no known contraindications to the use of Profilate Heat-Treated.

Warnings:
This product is prepared from pooled units of human plasma derived from fully screened donors. Each unit has been individually tested and found nonreactive for hepatitis B surface antigen and antibody to Human Immunodeficiency Virus (HIV). The plasma used in the preparation of this product has been tested for alanine aminotransferase (ALT) levels in an effort to reduce the risk of transmission of non A, non B hepatitis. Each unit used in the manufacture of this product has been found to have an ALT level less than 2 times the upper limit of normal for the test. Incubation in an organic solvent/detergent mixture during the manufacturing process is designed to reduce the risk of transmitting viral infection. The effectiveness of the heat-treatment step has been demonstrated by in vitro inactivation studies using live viruses added to Profilate-SD.

However, testing methods presently available are not sensitive enough to detect all units of potentially infectious plasma and no treatment method has yet been shown to be totally effective in eliminating viral

infectivity from this product. Despite all precautions taken by the manufacturer it cannot be assumed that this product is totally free of HIV or hepatitis virus. As with all drugs the risks associated with the use must be weighed against the benefits of therapy.

Precautions:
Profilate Heat-Treated should not be administered at a rate exceeding 10ml/minute. Rapid administration may result in vasomotor reactions.

Some patients develop inhibitors to Factor VIII. The risk does not appear to be increased by the use of concentrate. In patients with inhibitors, the response to Profilate Heat-Treated may be much less than would otherwise be expected and larger doses are often required. The management of patients with inhibitors requires careful monitoring, especially if surgical procedures are indicated.

Nursing personnel and others who administer this material should exercise appropriate caution in handling because of the risk of exposure to viral infection such as hepatitis.

Discard any unused contents. Discard administration equipment after single use. Do not resterilize components. Do not use any vials which on reconstitution appear to totally lack vacuum.

Adverse Reactions:
Adverse reactions may include urticaria, fever, chills, nausea, vomiting, headache, somnolence or lethargy. Some patients develop reactions of a mild nature following the administration of Factor VIII concentrates. Adverse reactions may be on an allergic basis. If a reaction is noted and the patient requires additional Profilate Heat-Treated, product from a different lot should be administered. Massive doses have rarely resulted in acute haemolytic anaemia, increased bleeding tendency or hyperfibrinogenaemia.

Profilate Heat-Treated contains blood group specific isoagglutinins and when large and/or

infectivity from coagulation factor products. Despite all precautions taken by the manufacturer it cannot be assumed that this product is totally free of HIV or hepatitis virus. As with all drugs the risks associated with use must be weighed against the benefits of therapy.

Precautions:
Profilate-SD should not be administered at a rate exceeding 10ml/minute. Rapid administration may result in vasomotor reactions.

Some haemophiliacs develop inhibitors to Factor VIII. In these patients the response to Profilate-SD may be much less than would otherwise be expected and larger doses are often required. The management of patients with inhibitors requires careful monitoring, especially if surgical procedures are indicated.

Nursing personnel and others who administer this material should exercise appropriate caution in handling because of the risk of exposure to viral infection such as hepatitis.

Discard any unused contents. Discard administration equipment after single use. Do not resterilize components. Do not use any vials which on reconstitution appear to totally lack vacuum.

Adverse Reactions:
Occasionally, mild reactions occur following the administration of Factor VIII concentrates. These may include allergic reactions, urticaria, fever, chills, nausea, vomiting, headache, somnolence or lethargy. If a reaction is noted and the patient requires additional Profilate-SD, product from a different lot should be administered.

Massive doses of Factor VIII concentrate have rarely resulted in acute haemolytic anaemia, increased bleeding tendency or hyperfibrinogenaemia. Profilate-SD contains blood group specific isoagglutinins and when large and/or frequent doses are required in patients of blood group A, B, or AB, the

frequent doses are required in patients of blood group A, B, or AB, the patient should be monitored for signs of intravascular haemolysis and falling haematocrit. Should this condition occur, thus leading to progressive haemolytic anaemia, the administration of serologically compatible type O red blood cells or the administration of Dried Factor VIII Fraction B.P. produced from group specific plasma should be considered.

Pharmaceutical Precautions
Profilate Heat-Treated should be protected from light and stored at temperatures between 2° - 8°C. Do not freeze. May be stored at room temperature not to exceed 30°C for up to six months.

Legal Category
P.O.M.

Package Quantities
Profilate Heat-Treated is supplied in cartons containing ten vials of freeze dried concentrate. The potency is stated on each vial label. Accessory packs containing either 10ml or 25ml vials of diluent are provided with each carton of concentrate.

Further Information
The process used in the manufacture of Profilate Heat-Treated involves heating a liquid suspension of the product at 60°C for 20 hours and is designed to reduce the risk of transmission of hepatitis, Human Immunodeficiency Virus (HIV), and infection by other viruses. This heat treatment process has been shown to inactivate a minimum of 4.3 logs of HIV when the virus was intentionally added to the product.

Chimpanzee studies demonstrate that the heat-treatment step is effective in inactivating at least 500 chimpanzee infectious doses (CID) of hepatitis B virus. The studies also showed that the process inactivated an undetermined quantity of at least one type of non-A, non-B hepatitis present in the Dried Factor VIII Fraction.

The incidence of post-infusion non-A, non-B hepatitis in patients receiving a first exposure to unheated Factor VIII concentrates

patient should be monitored for signs of intravascular haemolysis and falling haematocrit. Should this condition occur, the administration of serologically compatible type O red blood cells or the administration of Dried Factor VIII Fraction B.P. produced from group specific plasma should be considered.

Pharmaceutical Precautions
Profilate-SD should be protected from light and stored at temperatures between 2° and 8°C. Do not freeze. Profilate-SD may be stored at room temperature not to exceed 30°C for up to six months.

Legal Category
P.O.M.

Package Quantities
Profilate-SD is supplied in cartons containing ten vials of freeze dried concentrate. The potency is stated on each vial label. Accessory packs containing either 10ml or 25ml vials of diluent are provided with each carton of concentrate.

Further Information
The organic solvent detergent treatment process has been shown to provide a high level of virus kill without compromising protein structure and function. The susceptibility of human pathogenic viruses such as human immunodeficiency virus (HIV), hepatitis B virus, non-A, non-B hepatitis virus and marker viruses such as sindbis virus and vesicular stomatitis virus to inactivation by organic solvent detergent treatment has been discussed in the literature. In vitro inactivation studies sponsored by Alpha Therapeutic Corporation to evaluate the organic solvent detergent treatment step used in the manufacture of Profilate-SD show inactivation of greater than 10 logs of HIV as well as 6.82 logs of vesicular stomatitis virus (VSV) and 6.41 logs of sindbis virus.

approaches 100%. However the wet heat treatment process used in the production of Profilate Heat-Treated has been shown to be effective in reducing the risk of transmission of non-A, non-B hepatitis.

Product Licence Number PL 4447/0005

Date of Preparation
October 1988

Alpha Therapeutic UK Ltd
Howlett Way, Fison Way Industrial Estate,
Thetford, Norfolk IP24 1HZ. 19-0102

Product Licence Number
PL 4447/0005

Date of Preparation
October 1989

Alpha Therapeutic UK Ltd
Howlett Way, Fison Way Industrial Estate,
Thetford, Norfolk IP24 1HZ. 19-1201

Date: January 1990 CSM/BIOLS/90/ Meeting Appendix

Number:
PL 04447/0005

Company:
Alpha Therapeutic
UK Ltd

Product:
Profilate-SD

Therapeutic Class
Blood product

Active Constituent:
Factor VIII

Key Words:
Solvent/detergent
treatment

SUB-COMMITTEE ON BIOLOGICALS
DRAFT RECOMMENDATIONS

On the evidence before them, the Sub-Committee recommended the grant of a variation to the Product Licence for this preparation on condition that:

1. The variability allowed in the conditions of the viral inactivation stage is reduced or justified. Factors to be considered should include: solvent, detergent and protein concentration; temperature and time of incubation; polyethylene glycol concentration and pH of the medium.

2. Stability
2.1 A comparison of the stability of product from the new and old processes is provided and the shelf life of the new product is reduced of justified.

2.2 An assurance is provided that any results of on-going stability studies that are outside of specification are immediately reported to the Licensing Authority.

Remarks to the Company

1. Clarification is required of when the glycine/citrate/heparin wash in the manufacturing procedure is repeated.

2. Brief details are required of the filtration step prior to the solvent/detergent treatment.

Remark to the Licensing Authority

1. It is recommended that a product orientated inspection of the manufacturing site is undertaken.

Remark to the Biologicals Sub-Committee

1. The solvent/detergent process is unable to inactivate non-lipid-membrane-coated viruses. The Committee may with to consider this disadvantage compared with the benefits of the process.