CSM/Biol/94/5/KA

RESTRICTED - COMMERCIAL

TYPE OF APPLICATION: 1. Application for a UK Product Licence (Abridged)	NUMBER: PL 08801/0031-0033
PROPOSED CERTIFICATE/LICENCE HOLDER: BPL, Bio Products Laboratory Dagger Lane Elstree Herts, WD6 3BX	PRODUCT NAME: **Replenate 250, 500 and 1000iu/vial**
MANUFACTURER OF DOSAGE FORM: BPL, Bio Products Laboratory Dagger Lane Elstree Herts WD6 3BX	THERAPEUTIC CLASSIFICATION: **Blood Product**
LEGAL STATUS: POM / SALE/SUPPLY: POM	RECEIVED: 23 March 1994
	MEETING: July 1994
Summary Box High Purity Factor VIII, Solvent/detergent-treated to inactivate lipid-enveloped viruses. Purified by immunoaffinity chromatography with a murine monoclonal antibody. For use in the treatment of haemophilia A for the prevention and control of haemorrhage.	COMMITTEE ON SAFETY OF MEDICINES
	SUB-COMMITTEE ON BIOLOGICALS
	CONSIDERATION BY OTHER SUB-COMMITTEES:

INDEX:	Page
Pharmaceutical Assessment Report	2
Company's Expert Report & Summary Data	12
Summary of Product Characteristics	79
Application Documents	87
Draft Recommendations	95

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PHARMACEUTICAL ASSESSMENT REPORT

1. Introduction

Replenate is a high purity Factor VIII manufactured by BPL. The manufacturing process and formulation were developed by Baxter. The Baxter product, Hemofil M, has already been seen by this Committee and is about to be licensed.

BPL already market a high purity Factor VIII product, 8SM, under transitional arrangements following the end of Crown Immunity. 8SM is a slightly different product equivalent to a Kabi product Octanativ. (Kabi have not applied for a licence for Octanativ in the UK). Kabi manufacture 8SM for BPL using BPL's cryoprecipitate. Both Octanativ and 8SM are based on the Baxter process and product.

The chemistry and pharmacy expert report gives a clear and thorough account of the product's development, manufacture, quality control, validation and formulation. This Secretariat's report will concentrate on areas warranting further expansion and comment.

2. Removal/Inactivation of Viruses

The process incorporates a solvent/detergent treatment which is effective in inactivating lipid-enveloped viruses eg HIV 1 and 2, hepatitis C and hepatitis B. It is not specifically designed to deal with non-enveloped viruses eg hepatitis A and parvovirus B19.

The CPMP Background document on blood products issued in March requires processes to be validated for removal/inactivation of non-enveloped viruses including hepatitis A. Where validation studies reveal that the process does not adequately remove/inactivate non-enveloped viruses, a modified process is to be developed.

The immunoaffinity process does have some ability to remove non-enveloped viruses but, as stated by the expert, it does not have the same reliability as a validated chemical or heat treatment. There is no viral spiking study using hepatitis A or possibly a parvovirus. The Company have indicated that work is being carried out to validate the removal of hepatitis A and it is hoped that this will be completed by the end of 1994 (additional letter from Company dated 17 May 1994).

Validation studies have been carried out with EMC, polio and are on-going with hepatitis A. Since parvovirus can be transmitted by blood products does the Committee consider that a parvovirus should be investigated?.

The Company should indicate whether they are considering any modification of the process to further improve safety against non-enveloped viruses. Possible options, timescales and need for additional clinical work should be addressed.

The Committee will wish to consider whether the partition occurring in immunoaffinity chromatography is considered a reliable method of removing non-enveloped viruses or whether they would wish to encourage the Company to develop a further process.

It would be inconsistent to withhold a licence from this product when the very similar product 8SM is already on the market. BPL should be required to carry out the additional work to address the issue of hepatitis A and any other validation work on non-enveloped viruses the Committee may consider necessary on an on-going basis.

An appropriate warning statement should be included in the product literature. The Company should take account of the Summary of Product Characteristics (SPC) warning statement proposed in the CPMP Background document although they would not be required to adopt it exactly.

3. Summary Of Product Characteristics And Product Literature

3.1. Maximum Storage Period at Ambient Temperature

The original literature referred to 'short periods' of storage at ambient temperature being permissible. The Company have now modified their literature to indicate storage for up to 4 weeks at ambient temperature (25°C) (letter of May 17 1994). This is supported by stability data in Volume 4 which indicates a predicted stability of 6 months at 25°C.

3.2. Method a) for Reconstitution

The Company have modified their literature to indicate that 10ml of diluent should be added to reconstitute the vial (letter of May 17 1994). This is a clearer description than the previous add 'required' volume.

3.3 Suitable injection/infusion sets for administration of Replenate

In the original application, the SPC stated that only 'approved' injection/infusion sets should be used but did not specify which were approved. The data sheet did not specify this at all.

The Company have now modified their product literature to specify the plastic syringes and venisystem which have been shown to be compatible with Replenate (letter of 17 May 1994). Data are provided to support this statement in the original application. The Company should confirm that the syringes and venisystem they are recommending are approved by the Medical Devices Directorate (MDD). The stability data refers to an administration set being supplied but this is not indicated in the product literature. The Company should clarify this.

3.4. Storage of the reconstituted product

The expert report states that the reconstituted product should not be refrigerated. The Company were asked to comment on the lack of inclusion of this in the product literature. They have responded that since the reconstituted product is recommended to be used immediately or within one hour there is no need to refrigerate the product. On the other hand, if the product were to be refrigerated, there would be no deleterious effect. (Letter of 17 May 1994).

4. **Kits for Testing for Viral Markers**

Test kits for use in the UK are all approved for use at donor centres by the National Blood Authority. The Company should provide brief information on how the NBA approves test kits eg how PHLS evaluations are used and any pilot studies.

5. **Post-Collection Advice**

The Company have provided information on their policy if it is found retrospectively that donation(s) should have been excluded from processing (letter 17 May 1994, copy of policy attached).

The Company have been asked to clarify why, under 1.1.4 iii), only the test for HBsAg would currently be considered useful. Why have results of HIV antibody tests on immunoglobulins and intermediates in their production been excluded?

They have also been asked to indicate what factors will influence the consideration given to the need for a recall and justify the approach taken (1.1.5 of their post-collection advice). The Company have been asked to justify the approach taken in 1.2.

6. Donation Centres

BPL have responded to the UK questionnaire on donation centres which was based on the draft headings for notice to applicants on control of starting materials for the production of blood derivatives. Their response is appended. They state under point 9 that BPL has no defined mechanism for interaction with CPMP in the event of failure of a donation centre being discovered. Since BPL only supply the UK market this is not relevant at this time. Information received by the UK could be passed on through the European alert system, where relevant.

BPL have provided the list of transfusion centres. With the ending of Crown Immunity, these transfusion centres have had to apply for Manufacturer's Specials Licences. Not all sites have obtained their licences at this time but the Inspectorate will be asked to confirm that outstanding issues would not preclude the continued use of donations collected through these centres. Testing of individual donations for virus markers (HBsAg, antibodies to HIV 1 and 2 and HCV and serological test for syphilis) is performed at the transfusion centres. An outline has been provided of the quality assurance system in place at transfusion centres.

BPL PROCEDURE ON POST-COLLECTION ADVICE

Appendix III: Abstract from a BPL procedure on post-collection advice

1.1 **Action to be taken following notification from a BTC that a plasma donation, previously sent to BPL for fractionation, tests positive for a mandatory virus marker, using the test method in use when the donation was first tested[1]:**

1.1.1 unfractionated plasma will be recovered from stock without delay, identity confirmed by both bar-code and eye-readable identifiers, and will be placed in quarantine, pending return shipment to the BTC for reconciliation and destruction;

1.1.2 intermediate and finished products will be identified and held, and the batch manufacturing record(s) annotated with cross-reference to the incident file;

1.1.3 released product on inventory will be held; no action will be taken at this point in respect of product already issued;

1.1.4 the incident file, and relevant batch manufacturing records, will be reviewed jointly by the Technical Director (BPL) and the Medical Director (BPL), with particular attention to:

i. the results of screening and confirmatory tests reported by the BTC;
ii. the results of virus marker tests on the plasma pool (BPL & NIBSC);
iii. the results of virus marker tests on intermediate and finished products (only the test for HBsAg would currently be considered useful);
iv. the conditions of the relevant product licences, and current regulatory guidelines and requirements.

1.1.5 If the review confirms the presence of virus marker in the original donation, in a sample of the pool, in an intermediate, or in the finished product, or indicates non-compliance with regulatory requirements, those intermediates/products will be destroyed, and consideration will be given to recall of affected products at issue.

1.2 **Action to be taken following notification from a BTC that a plasma donation is compromised by circumstances in which the donor is identified as being in a high risk category, has developed symptomatic virus infection post-donation, or is implicated in an episode of post-transfusion virus infection:**

1.2.1 an incident file will be opened, and the BTC will be asked to review the record of tests performed on the donation at time of release.

1.2.2 If review of the record of original testing is satisfactory, no further action will be taken and the incident file will be closed.

1.2.3 If review of the record of original testing highlights irregularities in testing, the BTC will be asked to perform a repeat test on a retained sample.

1.2.4 If this repeat test is negative, no further action will be taken and the incident file will be closed.

1.2.5 If the repeat test is positive, the procedure under 1.1 will be followed.

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1. The status of a plasma donation must be judged in the context of the technical standards applying at the time the original donation was tested. If retest of a retained sample is considered appropriate, the retest shall be performed using a test with the same specificity/sensitivity characteristics as that use for the test on a sample at time of donation; sample preparation for the test shall be the same as was applied at the time of donation. The use of later generation tests, with enhanced specificity/sensitivity, or techniques for concentration of the plasma sample, are inappropriate.

BPL RESPONSE TO UK QUESTIONNAIRE ON DONATION CENTRES

1. Names and addresses of donor centres:

We have assumed that, in this context, you are referring to transfusion centres. It would not be possible to list "donation centres" used by the transfusion centres in the UK; these are not fixed sites. None of the transfusion centres operates sub-contractor sourcing.

A list of transfusion centres is appended (Appendix I). These are the centres used previously; they are also those required for BPL's Product Licences.

2. Testing of individual donations for virus markers is performed at the transfusion centres, and at no other sites. The list appended in respect of question 1 applies (Appendix I).

3. An outline of the quality assurance system in place at transfusion centres is appended (Appendix II).

4. The low level of initial reactivity in new donors indicates that transfusion centres in the United Kingdom are not collecting from a high risk population for blood-borne infections. This is confirmed by the National Blood Authority's (NBA) National Quality Assurance Manager, and supported by evaluation of epidemiology at transfusion centres, data from which is collated nationally.

There is continuing evaluation of the epidemiology at collection centres, including reporting of seroconversion rates in regular donors, and percentage positive in new donors, for markers of infection which are required to be tested. This evaluation is the responsibility of the NBA's National QA Manager. Reports are available for inspection.

5. The NBA operates a peer review audit system (with BPL as a fully participating member).

Audits, which are investigative in nature, are performed by two trained auditors, drawn from centres other than the centre under inspection. Audits are formally reported. BPL has free access to audit reports.

Audit of donation and testing centres is performed on an annual basis.

6. BPL is an operational unit of the NBA, and as such has no commercial contract with transfusion centres which are part of the same oragnisation. In the context of the annex to the guide to GMP, a quality contract exists in the form of a specification for plasma for fractionation.

The current plasma specification, agreed between BPL, the Central Blood Laboratories Authority (CBLA) and the National Directorate of the Blood Transfusion Service, forms part of Annex IIC/1 to all BPL's Marketing Authorisations. A revised specification is currently in preparation, which will take into account the formation of the NBA, and the disappearance of CBLA and the National Directorate.

This specification deals with:

i. quality systems
ii. quality specification for plasma for fractionation (which is defined in the context of:
    a. Plasma for Fractionation, *Ph Eur*
    b. the annex to the EC Guide to GMP
    c. UK "Guidelines for the BLOOD TRANSFUSION SERVICE"
iii. collection of plasma for fractionation
iv. testing of plasma for fractionation
v. labelling
vi. storage and transportation
vii. documentation
viii. failure to meet specification (including a mechanism for the reporting of post-collection information)

7. Transfusion centres are subject to independent inspection by the MCA.

8. Donation centres providing source material for a batch are clearly identifiable. The label on individual donations carries eye-readable centre identification and a machine readable donation number. Individual donations entering each plasma pool are uniquely traceable using these two identifiers.

9. In the event of a serious failure being discovered, the MCA (as the licensing authority) would be immediately informed, and would be consulted as to the reasonableness of proposed actions.

BPL has no defined mechanism for interaction with CPMP in this regard.

7. **Batch Release**

BPL have indicated that they will submit batches of Replenate to NIBSC for batch release. BPL have been informed that both the product and the albuminused in it will be subject to batch release including provision of samples of plasma pools.

8. **Immunoaffinity Chromatography**

Both Baxter and BPL reuse the immunoaffinity column. Baxter, for Hemofil-M, set a limit for re-use based on cumulative use. BPL intend to assess 5-lot running means for:

Factor VIII loss in flow-through (replace column if >1iu/ml); Trends in mouse IgG concentration in Q-Sepharose flow-through (no specific limit), and in final product (NMT 0.01 ng/iu). Trends in fibrinogen concentration in final product (NMT 5 mg/iu).

The Company will be asked to consider setting more in-process limits for markers of column deterioration e.g. mouse IgG and fibrinogen. This will provide a more sensitive indicator of column change than tests on the finished product. Although the Company have only a limited amount of experience at the present time, they should be able to draw on data from Kabi and Baxter to establish appropriate in-process limits. A shelf-life limit based on the age of the reagent should also be considered.

The immunoaffinity reagent is purchased form Baxter. Baxter have had two main sources of immunoaffinity reagent, one where the monoclonal antibody was grown by Celltech and one where it was grown by Hayward. Equivalence between the use of these reagents was addressed in the Hemofil-M application. The expert states that it is understood that only the Celltech source has been used in the development and manufacture of Replenate. BPL should clarify whether they will be changing to the Hayward source. They should also ensure that the documentation provided to them by Baxter contains information on the source of the monoclonal antibody in view of the complexity of this reagent and the complicated history associated with this monoclonal antibody. Any future changes to the method of manufacture or quality control of this reagent would require a variation to the Replenate licence (as well as that for Hemofil).

9. **Raw Material Specifications**

BPL have indicated that they intend to change to TNBP of an improved specification. They will be asked to confirm this.

10. **Finished Product Specification**

Fibrinogen residues provide a useful marker of the purity of the product and should be included in the finished product specification. (The Hemofil M specification includes a fibrinogen limit).

The specific activity is incorrectly stated in their expert report to be not more than certain limits. In Part IIE 1.3 these are correctly expressed as not less than. The expert states that these limits are in line with those for Hemofil M. In fact, Hemofil M has higher lower limits for specific activity and includes an upper limit. BPL will be asked to consider setting a range for specific activity and tightening or justifying their lower limit. (NB Since specific activity is affected by the albumin stabiliser, it is not a critical parameter as it would be for a pure protein).

Zenalb, BPL's albumin, added as a stabiliser has a green colouration which it would be expected would be seen with this product. This does not appear to be the case and the Company have been asked to comment.

PHARMACEUTICAL ASSESSORS CONCLUSIONS

1. **Viral Inactivation**

1.1 An assurance should be given that the results of the Hepatitis A validation study on the immunoaffinity step will be provided by the end of 1994. In the event of circumstances proving this impossible, the Company would contact the MCA at the earliest opportunity to explain the situation and agree a new timetable.

1.2 As assurance should be given that reports will be given to the MCA on a 6 monthly basis (June and December) on progress towards the addition of a further viral inactivation step to the process to further improve safety against non-enveloped viruses.

2. **Immunoaffinity Chromatrography**

2.1 Consideration should be given to setting appropriate in-process limits for markers of column performance to monitor re-use of the immunoaffinity column.

2.2 A shelf-life limit based on the age of the reagent should be considered.

2.3 The detailed sourcing of the immunoaffinity reagent now and in the future should be specified ie cell banks, site of culture and site of coupling. It should be confirmed that the Company routinely receive this information with batches of reagent.

3. **Raw Material Specification**

3.1 It should be confirmed that TNBP of the improved specification will be used in future.

4. **Finished Product Specification**

4.1 An appropriate limit for fibrinogen residues, justified by results of batch analyses, should be included in the finished product specification.

4.2 Consideration should be given to setting a range for specific activity and tightening or justifying the lower limits.

4.3 Comment is required on whether the Zenalb imparts a green colouration to this product.

POINTS FOR CONSIDERATION BY THE BIOLOGICALS SUB-COMMITTEE

1. The immunoaffinity process removes non-enveloped viruses from the Factor VIII but, as stated by the Part II expert, it does not have the same reliability as a validated chemical or heat treatment.

Does the Committee wish to encourage the Company to develop a further process to inactivate/remove non-enveloped viruses?

2. Given that parvovirus infection has been associated with some blood products, do the Committee consider that the immunoaffinity chromatography step should be challenged with a parvovirus or do they consider that the work with EMC, polio and on-going with hepatitis A will suffice?

3. Do the Committee consider that the approach proposed at 1.1 and 1.2 for Hemofil is an acceptable way forward for dealing with the issue of non-enveloped viruses? If this is the case, licensing of other blood product could be handled in a similar way.

PRODUCT LICENCE APPLICATION (PLA08801/0030-32) FOR BPL "REPLENATE"

PART 1. EXPERT REPORT ON THE CLINICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION

CONTENTS

		Page
PRODUCT PROFILE		1
INTRODUCTION		3
1.	COMPOSITION	3
1.1	Ingredients	3
1.2	Container and Closure	4
1.3	Clinical trial formulation	4
1.4	Development pharmaceutics	4
1.5	Bioavailability	6
2.	METHOD OF PREPARATION	6
2.1	Manufacturing Formula	6
2.2	Manufacturing Process	7
2.3	Method of Preparation	10
3.	CONTROL OF STARTING MATERIALS	15
3.1	Plasma	15
3.2	Other Ingredients	15
3.3	Plastic Containers	17
3.4	Packaging Materials	17
4.	CONTROL TESTS ON "INTERMEDIATE PRODUCTS"	18
5.	CONTROL TESTS ON THE FINISHED PRODUCT	18
6.	STABILITY	21
6.1	Replenate in Zenalb	21
6.2	Studies on Hemofil-M	22
6.3	Studies on Octonativ/8SM	23
6.4	Stability of Reconstituted Replenate	24
6.5	Stability of Frozen Cryoprecipitate and Q-Sepharose Eluate	24
7.	OTHER INFORMATION: VIRUS INACTIVATION	24
8.	CONCLUSIONS	29
9.	REFERENCES	30
10.	INFORMATION ON THE EXPERT	31

000035

Product Profile

Replenate, A High Purity Factor VIII

This product is manufactured at BPL using a process developed by Baxter Hyland to replace their previous, heat-treated, intermediate purity product. Major steps in this process are:

1. Production of "cold cryoprecipitate" solution from human plasma, using conventional techniques.
2. The addition of an organic solvent/detergent mixture to the "cold cryoprecipitate" solution (viral inactivation).
3. Anti-FVIII:C immunoaffinity chromatography (purification of Factor VIII and removal of contaminants and viral particles).
4. Ion exchange chromatography (removal of contaminants and reagents).
5. Dilution and adjustment of the Factor VIII potency and addition of product constituents.
6. Sterile filtration, aseptic, filling, lyophilisation and packaging.

a) Type of Application

This is a National UK Application consisting of a "Standard Abridged" application for Replenate 250 iu vial and "Piggy back" (simple abridged) applications for Replenate 500iu and 1000iu vials.

The application is for a known, monographed, biologically active substance, Human Factor VIII, and is essentially similar to Hemofil-M (Baxter Hyland) and 8SM (BPL).

b) Chemical and Pharmacokinetic Properties

The active ingredient in Replenate is Factor VIII (Human), a glycoprotein required for the clotting of blood via the intrinsic pathway of blood coagulation. Replenate contains high purity, plasma-derived, Factor VIII stabilised by the addition of human albumin. The structure of Factor VIII protein in Replenate has been characterised as being comparable to Factor VIII protein in other plasma-derived products.

Replenate is a lyophilised preparation which readily dissolves in 10ml Water For Injections. Administration should finish within one hour of reconstitution. Reconstituted product should not be refrigerated.

Sensitisation studies in guinea pigs indicate an acceptable safety margin between sensitising doses of mouse IgG (hybridoma-derived anti-factor VIII antibody) and the actual product levels.

Residuals of TNBP and Octoxynol-9 (as Triton (R) X-100) used for the solvent/detergent viral inactivation procedures, and hybridoma-derived DNA are present at such low levels in the final product that there is no toxicological risk to be expected.

000036

13

c) Indications and Classification

Replenate is indicated in the replacement therapy of patients with congenital or acquired haemophilia A. It may be of significant value in patients with factor VIII inhibitors. It must not be used in the treatment of von Willebrands Disease.

It has been shown in a canine model of haemophilia A that the preparation is efficacious in normalising a cuticle bleeding-time test.

This product is classed as an anti-haemorrhagic. The mode of action is considered identical to that of endogenous factor VIII.

d) Significant Precautions/Warnings Undesirable Effects

With products derived from human blood, despite screening donors for HBsAg and antibodies to HIV-1, HIV-2 and HCV, the risk of infection from blood-borne viruses cannot be entirely excluded.

e) Marketing/Post-Marketing

Factor VIII products produced by the Method-M process are licenced (Hemofil-M and 8SM) in:-

Germany	(Hemofil-M)	USA	(Hemofil-M)
UK	(8SM)	Japan	(Hemofil-M)
Netherlands	(Hemofil-M)	Sweden	(Hemofil-M)
Italy	(Hemofil-M)	Canada	(Hemofil-M)
Spain	(Hemofil-M)		

Hemofil-M, an equivalent product to Replenate, has been used extensively in the USA since February 1988 with no cases of hepatitis transmission attributable to the Method-M product.

000037

INTRODUCTION

This Application is for Replenate, a factor VIII concentrate identical in many respects to the currently licenced product, 8SM (PL 08801/0015). The modifications are (a) those imposed by a change in the manufacturing site, from Kabi-Pharmacia in Sweden to BPL in England and (b) minor changes consequent on choices of formulation; these represent a reversion to the design of Baxter/Hyland "Hemofil-M" on which 8SM was based. Annex II is therefore presented as a description of the new process and product, Replenate, emphasising its similarity to 8SM or Hemofil-M and locating the precise differences. Where there is complete identity, e.g. in the validation of the immunoadsorbent, the relevant section of the 8SM licence Application is quoted with acknowledgement and becomes part of this application. Although this Replenate Application is free-standing, BPL have less experience with this process and product than has been accumulated for 8SM and Hemofil-M since 1988. Where appropriate, limited data on Replenate are supported by data on precursor products. In each case, this support is justified by detailed consideration of the most critical process or product similarities. IIA.Introd.

This Expert Report will follow a similar pattern. Annex II has been fully assessed as a free-standing description of Replenate, but the Report emphasises the significance of modifications from the parent processes.

1. COMPOSITION

Format 1

1.1 Ingredients

Replenate is a freeze-dried preparation of human plasma proteins, containing a nominal 250, 500 or 1000 iu human blood coagulation factor VIII, for reconstitution in 10 ml Sterilised Water for Injections (also supplied). The product is intended only for the intravenous treatment of factor VIII deficiency and no therapeutically useful amounts of other coagulation factors are present.

The reconstituted product contains approximately 10 mg total protein per ml, mostly human albumin added as a stabiliser. Albumin replaces the carrier protein, von Willebrand factor (vWF) which is greatly depleted during the process. Specific activity of the factor VIII, if it were processed without addition of albumin, would be >2000 iu/mg protein. With the inclusion of albumin, specific activity of Replenate is approximately 2.5 -10 iu/mg. Residual vWF in Replenate is approximately equimolar with factor VIII, but is therapeutically insignificant; Replenate is not indicated for replacement therapy in von Willebrand's disease. Additional buffering capacity to pH 6.8-7.4 is provided by L-histidine, present in redissolved Replenate (all dose sizes) at 7.8 mg/ml. Polyethylene glycol (PEG) IIA.1 IIB.3.2.3

at 1.0mg/ml stabilises factor VIII by inhibiting its adsorption and inactivation at surfaces; PEG also facilitates resolution of the freeze-dried plug. Calcium chloride at 0.44 mg/ml stabilises factor VIII activity, probably by providing Ca^{2+} as a metal-ion "bridge" between the heavy and light chains of the active protein. Sodium chloride at 8.3 mg/ml adjusts the ionic strength of the solution, making the infusion slightly hypertonic.

All formulating agents have been in common use in other coagulation factor concentrates, at comparable concentrations, for many years.

1.2 Container and closure **Format 1**

The Application is made in respect of three pack sizes, 250 iu, 500 iu and 1000 iu, each to be redissolved in 10ml. The container is a glass vial, type I Ph.Eur., 30 ml. The closure is a synthetic bromobutyl rubber freeze-drying stopper, Ph.Eur. type I, over-sealed with an aluminium cap with an integral polypropylene flip-off centre. Vials are vacuum-sealed after freeze-drying, integrity of the container vacuum being specified in the data sheet as essential for safe use. IIA.2

The container/closure combination provides a tamper-evident seal, which maintains the container vacuum and resists adventitious contamination of the product. The flip-off polypropylene centre facilitates entry with the needle for addition of Water for Injections and removal of reconstituted solution via the filter needle.

1.3 Clinical trial formulation **Format 1**

The formulation used in clinical trials (pharmacokinetics) was identical with that described for the product, as routinely manufactured for marketing, in 1.1 above. Only a single batch of the 500 iu dose size was used in the study. IIA.3 Where this application is supported by clinical trials on alternative versions of the concentrate, such as Hemofil-M or Octonativ/8SM, the appropriate product or batch specification is tabulated. IIH.1 Minor differences of product content and formulation are discussed below. IIA.3.Tab 1

1.4 Development pharmaceutics **Format 2.1-2.3**

The purification of factor VIII from human plasma in the Replenate process follows very closely the 8SM process (PL 08801/0015) with minor, validated modifications whose significance is discussed later. The Octonativ/8SM process is itself based firmly on Hemofil-M (PLA 0016/0236-8) with minor modifications validated in PL 08801/0015. **Format 2.1** IIA.4

In the design of Replenate, BPL elected to revert to the pharmaceutical "finishing" strategy of Hemofil-M, particularly a formulation which provides a common fill volume for all dose sizes of 250, 500 and 1000 iu, each redissolving in 10 ml to give the same concentrations of excipients and differing virtually only in factor VIII potency. In order to achieve this, the following changes were made to the process described in the 8SM licence (PL 08801/0015):-

(1) In accordance with the Hemofil-M process, factor VIII was eluted from the Q-Sepharose column at pH 5.4-5.6, rather than at pH 6.75-6.95. This acidic eluate, was collected into a neutralising buffer, and may be stored frozen before sterilisation and filling.

(2) Factor VIII potency was adjusted with diluent, prior to sterilisation and filling. 8SM is redissolved in 2.5 x the fill volume. Replenate is redissolved in the same volume as the filled dose.

One effect of these modifications is seen in comparisons of product specification. Although 8SM and Replenate contain very similar, low concentrations of protein and reagent contaminants, the specifications can be strictly compared only at the same factor VIII potency. For this reason, concentrations of contaminants in w/iu supplement earlier expressions as w/ml. IIA.3. Tab.1

In addition to support for these changes adduced by reference to the established Hemofil-M product, their possible effects have been found to be neutral or benign by several validation studies. These include:

Comparisons of contaminants removed by Q-Sepharose chromatography - virtual identity between 8SM and Replenate. IIB.3.1.12-14

Stability of factor VIII in Q-Sepharose eluate - stability on frozen storage, and on repeated freezing and thawing. IIB.3.1.15

Freeze-drying protocol - approximately 100% yield of factor VIII. IIB.3.1.16

Comparison of product characteristics - close identity between 8SM and Replenate (excepting deliberate formulation choices). IIB.3.2.

Molecular integrity of factor VIII IIB.3.2.2

The choice of materials for the vial and stopper is appropriate for the method of preparation and use. Water adsorbed to the stopper during sterilisation is removed by extensive drying. The compatibility of the freeze-dried product with the glass vial is inferred from the stability in Annex IIF. In studies of the redissolved product in contact with the vial only, or with the vial and stopper, the only change in the product over 24 hours at 4°C and 20°C was a slow decay of factor VIII activity at the expected rate. Format 3 IIA.5.1

1.5 Bioavailability Format 3

In the context of plasma protein concentrates, bioavailability is usually interpreted as immediate recovery of the injected substance in the circulation and its rate of disappearance. The following studies are included in Part IV of this Application and evaluated in the Clinical Expert Report:-

A single-product pharmacokinetic study on Replenate in 17 patients with severe haemophilia A.

A cross-over study in 18 patients given alternately 8SM and 8Y (BPL's intermediate-purity concentrate).

The bioavailability of Replenate reflects the metabolic properties of native factor VIII in normal plasma and is not influenced by the design of the product or pharmaceutical processes.

The bioavailability of factor VIII in Replenate is identical with that in 8SM and 8Y.

Conclusions from this Section

The active ingredient of Replenate is human factor VIII. Human albumin at 10 mg/ml replaces protective proteins, particularly vWF, removed during purification. The formulation and composition of Replenate are essentially identical with those of Hemofil-M. The formulating ingredients are appropriate for the preservation of factor VIII activity and for intravenous injection.

2. METHOD OF PREPARATION

Formats 4.1-4.8

2.1 Manufacturing Formula Format 4.1

Batches are usually started from pools of approximately 7000 kg plasma. Frozen cryoprecipitate or Q-Sepharose eluates may be pooled for further processing. No limit is applied to the number of donations in the plasma pool from which a finishing batch is derived, but full accountability of every donation in the effective starting pool is maintained. Since the manufacturing process starts with the pooling of single donations for the removal of cryoprecipitate, there is no opportunity to sample the starting pool itself; samples are taken at the first accessible point, cryoprecipitate supernatant (CPS), to represent the starting pool in tests for virus markers, HBsAg and antibodies to HCV, HIV-1 and HIV-2. Typically, the weight of frozen cryoprecipitate taken for one batch is 80-160kg, yielding several thousand vials of product containing a nominal 250, 500 or 1000 iu factor VIII; stabilisers and excipients, including added human albumin; and very low concentrations of residual plasma proteins and process reagents. Re-solution is in 10 ml Water for Injections, for all dose sizes.

2.2 Manufacturing process **Formats 4.1-4.8**

A simplified diagram of the process stages and parallel control tests is provided.

Plasma conditioning at -10 to -15°C is necessary for removal of frozen plasma from the plastic pack and subsequent crushing to reduce particle size during thawing. A controlled-temperature room is used for this purpose.

Cryoprecipitation is achieved by thawing at -0.5 to +2°C. The cryoprecipitate is harvested in cooled, continuous-flow centrifuges. Bulk cryoprecipitate is frozen in liquid nitrogen and stored below -40°C for batching. (Formerly, this cryoprecipitate was transported to Kabi for further processing and finishing as 8SM, equivalent to Kabi Octonativ). Consistency of manufacture justifies storage of cryoprecipitate for at least 4 months at -40°C. **Format 4.2** IIE4.1

Re-solution and "cold precipitation" of cryo-precipitate **Format 4.2**

Frozen cryoprecipitate is re-dissolved in 2-4 volumes of 50 µM calcium chloride at 28 ± 2°C. The permitted range of re-solution volumes is slightly wider than in the 8SM process but falls within the range adopted for Hemofil-M to compensate for variations in quality of cryoprecipitate. Re-solution in calcium chloride solution is preferred to re-solution in water, followed by addition of concentrated calcium chloride solution, as in 8SM; this is intended to eliminate local high concentrations of Ca^{++} during addition. The concentrations of poorly soluble proteins, fibrinogen and fibronectin, are greatly reduced by adjusting the pH to 6.5-7.1, cooling to 10 ± 1°C and removing a "cold precipitate" by continuous centrifugation. The target pH, 6.7 ± 0.1, is the same as is used for 8SM, but the tolerance reverts to that validated for Hemofil-M. Conditions have been optimised to remove the maximum amount of insoluble protein without losing unacceptable amounts of factor VIII in the precipitate.

Adjustments prior to virus inactivation **Formats 4.2, 4.8**

The aim of this stage is to define conditions of temperature, pH, ionic strength, protein concentration, TNBP and Triton X-100 concentrations within the ranges validated earlier for Hemofil-M and 8SM, and now specifically for Replenate. The "cold supernatant" is warmed to 18-26°C and sodium chloride and calcium chloride are added. The solution is filtered through a tested 0.45 µ membrane to remove any aggregates within which virus particles might be sequestered. Protein concentration is estimated by absorption at 280 nm and must be in the range 5-35 A_{280}. A prepared mixture of TNBP and Triton X-100 is added, to final concentrations of 0.3% v/v and 1% v/v respectively. pH is adjusted to within the range 6.5 to 7.5 and conductivity is checked as falling within the range 55-75 mS/cm.

The mixture is stirred for 20 min before transfer under pressure to a sterile vessel within the Virus-Safe Area. A sample of the solution is removed for retrospective analysis of TNBP and Triton X-100 content.

Virus inactivation under defined and validated conditions

Format 4.3, 4.8

The mixture is incubated at 18-25°C for a minimum of 30 minutes, but this may be increased to 5 hours if it is not convenient to continue processing immediately. The following conditions are considered critical for repeatable virus inactivation, and fall within limits validated in small-scale experiments:-

IIH.2.1.1.1

Condition	Range	Verification
pH	6.5-7.5	In-process adjustment and check
Conductivity, mS/cm	55-75	In-process adjustment and check
Protein concentration, A280	5-35	In-process check
TNBP, % v/v	≤0.25	Retrospective analysis
Triton X-100, % v/v	≤0.8	Retrospective analysis
Temperature, °C	18-25	Continuous recording
Incubation period, min.	30-300	Timer, BMRs

These conditions have been validated as inactivating 6 logs of Sindbis within less than 15 seconds. These conditions differ from those used in 8SM only in tolerances, which have been fully validated. They are identical to those used for Hemofil-M except for definitions of incubation time. Virus inactivation is reviewed more comprehensively in 7. (below).

Chromatography on MAb-Sepharose

Formats 4.3, 4.5 4.6, 4.7

The virus-inactivated "cold supernatant", filtered again to 0.45 µ, is applied at a controlled loading to a column of anti-F.VIII antibody covalently bound to a solid matrix, Sepharose. Factor VIII, already dissociated from vWF in a buffer of high ionic strength and high calcium concentration, attaches very strongly to the immobilised antibody. Virtually all other proteins, solvent/detergent reagents and some virus particles (if present) are not bound and are washed through the column by 48 column volumes (cv) of a wash buffer solution.

Factor VIII is then eluted in a buffer containing 40% ethylene glycol as a chaotrope to break the protein-antibody bonds, and

including albumin and PEG to stabilise the almost-pure factor VIII.

This chromatographic stage takes about 36 hours at ambient temperature. Microbiological safety is assured by checking the endotoxin content of column washings before use and by rigorous cleaning after use. The applied factor VIII-rich solution has been filtered to 0.45µ and contains solvent/detergent reagents which are bacteriostatic as well as virucidal. Chromatographic buffers are sterilised by filtration.

Separation specificity is assured by checking the most important characteristics of buffer solutions before use; by limiting loading and flow-rates; by measuring unbound factor VIII; and by specifying minimum wash volumes.

The methods described for recycling the column appear to be efficient and conserve the immobilised antibody.

Chromatography on Q-Sepharose FF

Formats 4.3, 4.5 4.6, 4.7

The eluate from MAb chromatography is applied at a controlled loading to an equilibrated column of the anion-exchanger, Q-Sepharose FF. Factor VIII is bound strongly to the ion-exchanger under the selected conditions. Components of the MAb eluate which are not desired in the final product - including ethylene glycol and traces of leached mouse anti-F.VIII - do not bind and are washed through the column with 30 cv of a glycine buffer containing other stabilisers. This is followed by 10 cv of a similar buffer, omitting glycine which is not desired in the final product.

Factor VIII is finally eluted at high ionic strength in a buffer of pH 5.5 containing the formulating stabilisers histidine, albumin, PEG and calcium chloride. In order to neutralise and pre-dilute this eluate, it is collected into a buffer containing the same components, but at pH 8.2. This pre-diluted eluate may be stored frozen for batching-up prior to pharmaceutical finishing.

Microbiological safety of this process stage is assured by checking the endotoxin content of column washings before use and by rigorous cleaning after use. Batch-to-batch transfer of viruses on the Q-Sepharose column is unlikely, since re-cycling includes a wash with 0.5M sodium hydroxide. Chromatographic buffers are sterilised by filtration. Separation specificity is assured by checking the most important characteristics of buffer solutions before use; by limited loading; by limiting flow-rates; by measuring unbound factor VIII; and by monitoring trends in the concentrations of contaminants in the final product.

Pharmaceutical finishing

Formats 4.3, 4.5 4.6, 4.7

Based on an assay of factor VIII potency in the Q-Sepharose eluate, the solution is diluted to target potency (25, 50 or 100 iu/ml) with a buffer containing the formulating stabilisers. The formulated solution is sterilised by filtration through an integrity-tested 0.22µ filter. 10 ml doses are dispensed by weight into sterile vials and closed using sterilised

freeze-drying stoppers, in the first position. The vials are freeze-dried in a pre-sterilised freeze-dryer, using freezing, sublimation and secondary drying programmes which consistently yield a product with unaltered factor VIII activity and low moisture. These programmes acknowledge inevitable differences in the characteristics of the freeze-dryers used at BPL and at Kabi. However, the most critical parameters - sublimation and secondary drying temperatures - are identical. The vials are sealed by pressing the stoppers to the second position while still under vacuum in the freeze-dryer; oversealed on removal; inspected; machine-labelled with batch number, expiry date and potency; packed and stored at 2-8°C.

Homogeneity of the batch of vials rests on automatic check-weighing of each dose, filled from a homogeneous, sterile bulk solution. It is assumed that freeze-drying introduces no vial-to-vial heterogeneity and adherence to GPMP eliminates other sources of variation.

Specification and control of processing parameters ensure that the Replenate process differs only in small, practical details from the licensed 8SM process. Since formulation and finishing choices, from the Q-Sepharose stage onwards, revert to the Hemofil-M strategy, the finished Replenate is virtually identical to that parent product.

2.3 Method of preparation. Process Validations **Formats 5.1-5.4**

Many of the experiments cited in this Application were designed to demonstrate equivalence of the Replenate process to the corresponding licenced parent processes Octonativ/8SM and Hemofil-M. Where equivalence has been established, the extensive documentation and experience built up on the parent processes and products may be expected to apply equally to Replenate. **IIH Appendix 1**

The types of plasma used by BPL for Replenate and the method of harvesting cryoprecipitate are identical to those used for 8SM. BPL prefer to re-dissolve cryoprecipitate directly in 50 µM calcium chloride rather than add the reagent to cryoprecipitate already dissolved in water; it has been shown that this change has no influence on factor VIII yield. The only other modification by BPL at this stage is allowance of a wider range of cryo-resolution volumes than is used for 8SM. This can have only a neutral or beneficial effect on the yield of factor VIII and, although it varies the protein concentration of "cold supernatant", virus inactivation has been shown to be effective at a wide range of protein concentrations. **IIB.3.1.2**

<u>Validations relating to solvent/detergent treatment</u> **Formats 5.1, 5.2**

Comprehensive experiments have demonstrated rapidly effective mixing of the cold supernatant with solvent and detergent in BPL equipment, i.e. using selected tanks filled to different levels, and nominated stirrers at optimal settings. There **IIB.3.1.3.3**

are therefore no gradients or pockets of solute concentrations or temperature during virus inactivation of the mixture. Control of temperature during formal incubation has also been confirmed. IIB.3.1.4

In Annex IIH, it is shown that all the minor differences in the critical virus inactivation parameters (protein concentration, solvent/detergent concentrations, ionic strength, pH, temperature and time) between Replenate, 8SM and Hemofil-M are without significant influence on the rate of inactivation of Sindbis. The range of equivalent effectiveness of each parameter was sufficient to encompass minor differences in the target values and tolerances for all these related processes. IIH.2.1.1.1

It was further shown that the 0.45µ filter used prior to MAb chromatography did not significantly reduce the concentrations of solvent/detergent in the virus-inactivated cold supernatant. This means that, after the formally validated incubation period, the medium remains lethal to viruses for many hours until solvent/detergent reagents are washed through the column. IIB.3.1.6

Validations relating to efficiency of immunoaffinity chromatography **Format 5.2**

The same source of MAb-Sepharose is used in Replenate as in Octonativ/8SM and in Hemofil-M. Validations relating to the manufacture and safety of this reagent are not reassessed in this Report. The factor VIII solution applied to the column in the three related processes and the specification of wash and eluant solutions differ only in small details which would not be expected to alter the efficiency of separation. IIB.3.1.9 Appendix IIC/8,/9,/10 IIE.4.2

New data on the Replenate process confirm equal efficiency in the removal of 99.9% solvent/detergent reagents by 40 cv of wash buffer, with an inference that 48cv (preferred by BPL) can only enhance clearance. The partitioning of factor VIII between column fractions during washing and elution confirms that, across the range of loadings adopted for Replenate, less than 5% of the applied factor VIII is lost in the flow-through and wash fractions, even when the wash volume has been increased to 48 cv. This increase in wash volume from 8SM improves the margin of confidence in the clearance of fibrinogen, to at least the factors calculated for Octonativ/ 8SM and Hemofil-M. Consistency data on the final product which admittedly reflect clearance on both immunoaffinity and ion-exchange columns, also support the conclusion that clearance of fibronectin and vWF is equally efficient in the Replenate process and in the parent processes. Taken together, these validation experiments justify all minor variations of Replenate from 8SM at this stage. IIB.3.1.7 IIB.3.1.8

The original Hemofil-M strategy for re-use of this column has been reviewed by BPL. Rather than relying on chronological age or cumulative use (set for Hemofil-M at 10.5×10^6 iu total F.VIII loaded per litre of gel). IIB.3.1.10

BPL intend to assess 5-lot running means for:

F.VIII loss in flow-through (replace column if >1 iu/ml).
Trends in mouse IgG concentration in Q-Sepharose flow-through (no specific limit), and in final product (≯0.01 ng/iu).
Trends in fibrinogen concentration in final product (≯5 mg/iu).

This modified policy is not yet strictly validated as correlating with process efficiency and product quality, since it has not yet been challenged. However, these criteria seem to be more practically related to column function than simple replacement after an arbitrary cumulative use or age. They should predict some kinds of column deterioration not already covered by microbiological monitoring.

Validations relating to efficiency of Q-Sepharose chromatography — Format 5.3

Replenate uses the same ion-exchanger as Octonativ/8SM and identical loading, wash buffers and flow-rates; it has also been shown that there is no significant change in the nature of the immunoaffinity eluate applied to the Q-Sepharose. The clearance of solutes into flow-through and wash fractions should therefore be identical in both processes. However, in reverting to the Hemofil-M formulation, Replenate requires an eluting buffer of pH 5.5, lower than that used for 8SM, and the eluate is neutralised by collection into a pre-diluting buffer originally used for Hemofil-M. This modification could conceivably alter the concentrations of proteins, etc. co-eluted with factor VIII from Q-Sepharose.

New data on the Replenate process confirm that, as in Hemofil-M and Octonativ/8SM, mouse IgG is cleared to lower than the detectable concentration (equivalent to 0.01 ng/iu) in the Q-Sepharose eluate. Similarly, ethylene glycol in the immunoaffinity eluate is cleared to less than the permitted limit concentration in the Replenate process (0.5 µg/iu). Triton X-100 and TNBP, which are not completely removed by immunoaffinity chromatography, are also cleared to undetectable concentrations (approximately <20 ng/iu and <4 ng/iu respectively) after Q-Sepharose. — IIB.3.1.12, IIB.3.1.13

For the reasons outlined above, the partitioning of factor VIII during the Replenate Q-Sepharose stage is to be compared with Hemofil-M rather than with Octonativ/8SM. It was confirmed in six batches of Replenate that less than 2% of the applied F.VIII was lost in the flow-through and wash and that 60-100% was recovered in the first 4.3 column volumes of eluate; this demonstrates adequate consistency, within the limitations of factor VIII potency assays.

In the specification of Octonativ/8SM, Q-Sepharose was replaced after 10 uses. BPL apply additional functional criteria for replacement of the ion-exchanger in the — IIB.2.5.6

Replenate process, but this policy has not yet been challenged.

Additional studies have validated two optional holding points during the Replenate process. The virus-inactivated cold supernatant may be held up to 40 hours at 20-25°C, without heavy losses of factor VIII activity, before application to the immunoaffinity column. The Q-Sepharose eluate may be stored frozen for 13 weeks, or even undergo an additional freeze-thawing cycle, before finishing. These options offer some flexibility in routine operations and appear to be justified. IIB.3.1.5 IIB.3.1.12

The automated freeze-drying programme adopted by BPL includes no unusual features. The demonstrated high recovery of factor VIII and low residual water in the product are an adequate indication of performance, and the ultimate test is long-term stability (see below). IIB.3.1.16 IIF

Product Characteristics **Format 5.3**

Since minor modifications from the Hemofil-M and Octonativ/8SM processes do not appear to have impaired the selectivity and efficiency of the chromatographic stages, Replenate should not differ from either of these parent products in its main product characteristics, except insofar as these are affected by deliberate choices of the Hemofil-M formulation. In addition to the parameters measured as part of the batch specification, other characteristics of the related products have been compared with Replenate in auxiliary experiments. Residual imidazole and glycine were determined in several batches of product, using appropriately sensitive methods. Imidazole was undetectable (<0.03 mM) in Replenate and in Hemofil-M. Glycine was found in similar concentrations in both products (0.1 - 1.7 mM). The low residual concentrations of human IgG, IgA, fibrinogen, fibronectin and vWF in Replenate were found to fall within the ranges reported for Hemofil-M and Octonativ/8SM. IIB.3.2 IIB.3.2.3 IIB3.2.4

BPL have also confirmed that the native molecular (sub-unit) structure of F.VIII in Replenate is well preserved after multi-stage processing, as might be predicted from the relatively gentle methods used. The method of assessing F.VIII fragmentation was originally described for Hemofil-M (PLA 0016/0236-8). SDS-PAGE analysis followed by immuno-blotting confirms the consistent absence of degraded fragments (43 and 52 kDa, or any others capable of recognition by the anti-F.VIII antibody). A modest degree of aggregation attributable to freeze-drying is seen in both Replenate and Hemofil-M. The bands representing the functionally intact heavy (90-200 kDa) and light (80 kDa) chains of normal factor VIII appear in the same predicted zones when Hemofil-M, Replenate and Octonativ/8SM are compared. Although the quality of SDS-PAGE analysis is somewhat limited by the presence of albumin in the products, redissolved product is the only relevant sample for these studies. Immuno-blotting gives only semi-quantitative information, but is the most IIB.3.2.2

specific method applicable to the study of factor VIII molecular size.

Validation of the post-virus-inactivation environment **Format 5.4**

After virus inactivation, the manufacturing process is carried out in a Virus-Safe Area (VSA) intended to exclude contamination of the "downstream" operations with fluids and equipment which might still be contaminated with blood-borne viruses. The interfaces of the VSA with the upstream processing area and the finishing area have been well planned, but rely on vigilance and audit for continuous validation. Precautions include: controlled access; segregated air handling and other services; and use of dedicated, sterilised or sanitised equipment. IIB.3.3.2 IIB.3.3.1

The critical dispensing environment is monitored to conventional standards by DOP- and velocity-testing of LAF units. Vials and stoppers are sterilised by appropriate dry-heating and autoclaving respectively, and the formulated factor VIII solution is sterilised by passage through a 0.22µ filter, integrity-tested before and after use. The microbiological security of the finishing area is challenged regularly by broth fills. The freeze-dryers, including condensers, are sterilised between charges. The integrity of vial sealing can be checked by detection of the vacuum at the point of use. IIB.3.3.2.4

Regular off-line testing and within-batch monitoring of steriliser performance are conventional and raise no unusual issues. IIB.3.3.2.2

Conclusions from this section

The method of preparation departs only in minor details from that used for Octonativ-8SM and Hemofil-M. During processing, factor VIII is not exposed to extremes of pH temperature or high concentrations of aggressive reagents. The Replenate process is more comprehensively controlled, particularly during crucial virus-inactivation using solvent/detergent. Process reagents and redundant plasma proteins are separated from factor VIII by immunoaffinity chromatography, followed by ion-exchange. The chromatographic stages are described in detail and, since they contribute to virus safety, are closely controlled. Comparisons of Replenate with Hemofil-M, in respect of factor VIII properties and contaminant levels, establish effective identity with the parent process. The most critical processing stages have been validated, and are at least as efficient and effective as the corresponding stages in the parent process. Consistency of manufacture has been demonstrated over 5 consecutive batches.

3. CONTROL OF STARTING MATERIALS

Formats 6, 12.1 -12.3, 14, 16

3.1 Plasma

Format 6

The plasma used as starting material for Replenate has been provided solely from unremunerated blood donors to the Regional Transfusion Centres of the UK. The collection and transfer of donations to BPL is subject to NBTS/NIBSC guidelines (1993) and BPL's specification, agreed with each RTC. Each donor completes a health questionnaire designed to exclude high-risk donors. Blood or plasma is taken into licenced bags containing only approved anti-coagulant solutions. Each donation is tested singly at the RTC for virus markers HBsAg, anti-HCV, anti-HIV-1 and -2. The validated test kits and confirmatory tests used at the RTCs and BPL are considered to be the most specific and sensitive for screening UK donors for the most important blood-borne viruses. Minimum sensitivity for HBsAg is 0.1 iu/ml(1). However, it is acknowledged that potentially infective donations may escape the screening tests because of limited sensitivity and there is no screening for viruses currently considered less dangerous in large-pool concentrates.

Appendix IIC/4
IIC.1.1-1.4
Appendix IIC/1
IIC.1.2
IIC.1.4

Changes from 8SM (PL 08801/0015) in respect of approved tests kits are tabulated. Plasma pools are sampled only indirectly. The earliest representative sample of the pool is cryo-supernatant, which is tested for the same virus markers, using the most sensitive tests available. These additional tests are performed to detect an infective donation included in the pool as a consequence of human error, rather than test insensitivity. Whereas tests for HBsAg are validated for sensitivity using an International Standard, "sensitivity" of an anti-HIV or anti-HCV test can be defined only in terms of its response to a panel of reference plasmas. Proficiency standards are circulated for use in the UK by PHLS, but these are not yet calibrated or authorised by WHO.

IIC.Introd.

IIC.1.5

No quarantine period between plasma receipt at BPL and pooling for fractionation is now specified. The period between donation and release of product is commonly at least six months, allowing time for reassessment of any suspect donor or donation. Bar-code identification of each pack permits identification of each donation contributing to a finished batch of Replenate. Review and issue of stored plasma for manufacture are otherwise controlled as for 8SM.

3.2 Other ingredients

Formats 14, 16

Five substances (albumin, histidine, PEG 4000, calcium chloride and sodium chloride) added to Replenate as formulating ingredients are purchased to a pharmacopoeial grade and fully tested in-house to the respective specification, sometimes using acceptable alternatives to the pharmacopoeial tests.

IIC.2.1

Albumin (Zenalb) meets the Ph.Eur. test limits for Human Serum Albumin. Polyethylene glycol is purchased as PEG 4000 and meets BP specification for Macrogol 4000, with rational adjustments for molecular weight. The BP's specification relates to topical use of PEG and the tests are undemanding for an ingredient in an i.v. injection. However, a very similar grade of PEG was used by Baxter in toxicology studies reported in Annex III.

Water for Injections is tested to Ph.Eur.

Six substances (glycine, Triton X-100, hydrochloric acid, glacial acetic acid, sodium hydroxide and ethanol) added during processing but which are not part of the final formulation are purchased to a pharmacopoeial grade and fully tested in-house to the respective specification, sometimes using appropriate alternatives to the pharmacopoeial tests. IIC.2.2

Six substances used during processing, but which are not part of the final formulation, are not described in a pharmacopoeia:

Tris(hydroxymethyl)aminomethane is purchased as Reagent grade, identified by melting point and IR spectrum and assessed as 99.5% pure by acidimetric titration. It is tested, to appropriate limits and by appropriate methods, for water-insoluble residues, chloride, sulphate, arsenic, lead, iron and copper. IIC.2.2.1

Ethylene glycol is purchased as AR grade and identified by IR spectrum. It is tested for residual water and sulphated ash. IIC.2.2.2

Imidazole is purchased as Reagent grade, identified by melting point and IR spectrum and assessed as 99% pure by titration with perchloric acid. It is tested for sulphated ash. IIC.2.2.3

TNBP is purchased to manufacturer's specification, identified by IR spectrum and assessed as better than 98% pure by GLC. It is tested for specific gravity and acidity. Although the control of this reagent is not elaborate, it appears to match the specification used by Baxter in toxicity studies reported at Annex III. IIC.2.2.4

The applicant has recently found a supply of purer TNBP, but this has not been used in any of the batches described in this Application. The proposed specification appears to be more appropriate.

MAb-Sepharose CL-2B is purchased from Baxter. Manufacture, specification and testing are described in Appendix IIC and were comprehensively evaluated in the Hemofil-M PLA 0016/0236-8. Appendix IIC/8-/10
Is understood (IIC 3.1) that only one source of the immunoaffinity reagent (Genetics Institute source, grown by Celltech, coupled to Sepharose by Baxter) has been used in the development and manufacture of Replenate. However, Annex IIE 4.2 makes the case for equivalence of performance, in all essential respects,

between immunoadsorbents from this source and a second approved source (grown by Hayward), sometimes used in the development and manufacture of Hemofil-M. BPL test only for particle size by microscopy and by a small-scale functional test which mimics its application to factor VIII binding in the manufacturing process.

Q-Sepharose FF is purchased from Pharmacia, whose specification and testing are described in an Appendix. BPL test only for particle size by microscopy and by a small-scale functional test which mimics the application to factor VIII binding in the manufacturing process. The approach to validation of these chromatographic materials is exactly as for 8SM. Appendix IIC/3

Except where indicated, bacterial TVC is not carried out routinely on all process reagents.

3.3 Plastic containers

Containers used to store intermediates are purchased to Ph.Eur. specification, are accompanied by the manufacturer's compliance statement, and are not further tested at BPL.

3.4 Packaging materials **Format 16**

Containers are glass type I vials purchased to Ph.Eur. specification, tested by BPL only by visual inspection for defects and for critical dimensions. Stoppers are siliconised, bromobutyl rubber bungs, type I, purchased to Ph.Eur. specification and tested by BPL for identity by IR spectrum and aqueous extractables. Compatibility of Replenate with this vial and stopper has been established. The transfer needle, filter needle, and administration set are purchased to a specification approved under DoH Sterile Devices Scheme, and have been validated for compatibility with reconstituted Replenate. IIC5 IIA5.1 IIC5.7 IIA5.21-2

The vial of Sterilised Water for Injections in the product pack is a licenced product, purchased and tested by BPL to Ph.Eur. IIC5.6

Conclusions from this section

Sourcing and testing of plasma are essentially the same as for 8SM and follow well-established national protocols. The quality of the Active Ingredient, factor VIII, is assured by the method of manufacture and its controls, discussion in (2) above. Sourcing of other ingredients and process reagents is to pharmacopoeial grade, where available, or to the best obtainable grade. Testing is by pharmacopoeial methods or acceptable equivalents. Non-pharmacopoeial process reagents, which are substantially removed before formulation, are tested to BPL standards similar to those used for pharmacopoeial reagents. Packaging which comes in contact with Replenate is appropriately specified and is compatible with the product.

4. **CONTROL TESTS ON "INTERMEDIATE PRODUCTS"** — Format 17.1

It is impossible to obtain representative samples of the plasma pool or bulk cryoprecipitate. The earliest sample representing the plasma pool is cryo-supernatant, which is re-tested for markers of the important blood-borne viruses. — IIC.1.5
Bio-burden (TVC) is assessed on the cryo-suspension; "warning" and "action" limits are 10 and 150 CFU/ml respectively, but this is primarily a retrospective test, for information on manufacturing trends. — IID.1

Pooled Q-Sepharose eluate, which may be regarded as the "active ingredient" presented for pharmaceutical finishing, is sampled for factor VIII potency assay, providing a basis for subsequent dilution to the required dose potency. — IID.2.7
TVC is assessed retrospectively on the formulated solution just prior to sterilising filtration; counts >50 CFU/ml would fail the batch unless a rational explanation of a false-positive result were forthcoming. — IID.1

5. **CONTROL TESTS ON THE FINISHED PRODUCT** — Formats 17.1-17.3 and 18.1-18.3

After re-solution of the freeze-dried product in 10 ml Water for Injections, the potency of the active ingredient, factor VIII, is determined by a two-stage clotting assay. The validation of this "skilled" assay and the interpretation of — IID.3.2
results are clearly set out, and the statistical validity of the "vial contents" (over-printed on the packaging) is soundly based. The molecular state of the factor VIII is inferred from development studies and consistency of — IIB
manufacturing, and is not assessed on each batch. The Specific Activity of the active ingredient (iu/mg protein) is dominated by the re-addition of stabilising albumin to the processing solutions and formulation. No attempt is made to assess the "without-albumin" specific activity of each batch.

The examination of each batch of final product is carried out — IIE.1.1
essentially to Ph.Eur. specification. This Report focuses on additions to the pharmacopoeial assessment and on differences from 8SM (PL 08801/0015). — IIE.1.2

The potency of the finished product is fixed by its potency at dispensing and all dose sizes (250-1000iu) are filled and redissolved in 10 ml, whereas various dose sizes of 8SM were dispensed in different volumes at a single potency and vials were redissolved in 2.5 x the dispensed volume. Consequently, some comparisons of e.g. limit concentrations of contaminants are better assessed on a "w/iu" basis rather than "w/ml".

All excipients (also called formulating reagents, ingredients or stabilisers) are subject to upper and lower limits with which each batch must comply. Residual process reagents are subject to upper limits, mostly quite close to the LOD or reflecting the inherent performance of the manufacturing

method. The process contaminants glycine and imidazole, and the residual plasma proteins fibrinogen, fibronectin, IgG, IgA and vWF, are not determined on each batch; low concentrations, consistent with those in the parent concentrates, were established in development studies. Required tests on the final product for human identity, HBsAg, anti-HIV and haemagglutinins appear to be redundant in Replenate but are properly applied. **Compared with 8SM**, Replenate has the following distinguishing features: IIB.3.2.3-4 IIE.1.2 IIE.1.3

Re-solution time has been specified to a tighter limit, 1 min rather than 3 min.

Total protein and **albumin** (g/L) limits are now the same for all dose sizes but **Specific Activity** (iu/mg) is now dose-related, for the same reasons. These limits are the same as those for Hemofil-M.

Sodium, chloride and **calcium** ions are now specified as falling within defined upper and lower limits, since they are formulating ingredients, and they are the same as those for Hemofil-M.

Potassium has been omitted as redundant.

Moisture (RWC) has been specified to a tighter limit of ≯2% rather than ≯2.5%. The non-pharmacopoeial method of measuring moisture (Karl Fischer) has been validated as equivalent to Loss on Drying for protein concentrates.

Anti-A and anti-B haemagglutinins have been specified to a tighter limit, 1/32 rather than 1/64.

Histidine and **PEG** limits reflect the Hemofil-M formulation and specification.

Mouse IgG limit remains at ≯0.01 ng/iu, but the LOD of the method requires that 250 iu doses of Replenate be specially redissolved in 5 ml rather than 10 ml for this determination.

Triton X100 has been specified to a tighter limit, ≯25 rather than ≯37.5 ng/iu.

TNBP limit has been confirmed at ≯12.5ng/iu.

Ethylene glycol limit has been confirmed at ≯0.5mg/iu.

The specified concentrations of solutes in the final product are appropriate to the frequent intravenous replacement of factor VIII in haemophilia. Residual concentrations of process reagents are fixed at very conservative limits in relation to the perceived toxicity of these substances. The toxicity of mouse IgG, TNBP and Tween X-100 are quantitively examined in Annex III; the limits applied to their concentrations in Replenate provide generous safety factors of 100 or more.

Where a reagent has been deliberately added as an ingredient in the final formulation, it is now accorded both upper and lower limits. In each case, these acknowledge the reproducibility of the test method and the precision of reagent addition to the process. Although the concentrations of these stabilisers are probably much less critical to the quality of Replenate than the limits seem to imply, compliance within narrow limits helps to show that the process is in control and in line with the parent processes. It has been shown that, over 5 batches, these limits are consistently achieved. Format 18.3

The upper limit for PEG is justified by its inertness and efficacy as a stabiliser and aid to re-solution. This reagent has been present at comparable concentrations in some factor VIII concentrates for about 20 years, albeit as a residual precipitant, notably in the successful Hyland Method 4 concentrate. Its use is amply supported by the toxicity studies on Hemofil-M reported in Annex III. All other ingredients are normal constituents of human plasma. Delivered in the low dose volume permitted by the high potency of factor VIII in Replenate, any increase in normal plasma concentrations of albumin, histidine, calcium, sodium or chloride would be imperceptible.

Many of the Test Methods used to evaluate finished batches of Replenate are those of the Ph.Eur., or identical with those applied to 8SM. Having been judged appropriate for a virtually identical product, they are not reassessed here. IIE.2

BPL's validation of these methods is inferred, in some cases, from validations established for other plasma protein concentrates. This is appropriate, since the added human albumin, Zenalb, is by far the preponderant protein present in Replenate. Others have been re-validated for application to Replenate, because it was suspected e.g. that other solutes present in the sample might influence the specificity or sensitivity of the standard test method. The scope, precision and therefore limitations of the Test Methods are summarised. The reproducibility of each Test Method is compatible with the range of concentrations of that solute permitted in the product specification. Where a Test Method measures the upper limit permitted for a residual solute, the validated LOD is lower than the specified upper limit. The factor VIII potency estimate on each batch, i.e. the vial contents printed on packaging, carry confidence limits derived directly from the assay set in which the batch was compared with a calibrated standard. IIE.3 Format 18.2 IIE3.5, IID2.7

The two-stage factor VIII assay, used extensively in the Development Pharmaceutics section of this application, has a within-set precision of approximately 5% and between-set reproducibility of 13%, consistent with published data from other expert laboratories(2). Consequently, where experiments cannot be indefinitely repeated, apparent differences between observed factor VIII potencies have to be of the order of 20% IID.2.7

(or greater where two potencies are expressed as a ratio) to acquire "statistical significance". The interpretations of factor VIII-related data in this Application are statistically correct. More importantly, observations which approach the confidence limits of the assay have been reasonably evaluated, and no claim or inference strains a common-sense assessment of "significance".

Conclusions from this section

The specification of Replenate is essentially identical with, or better than, those of 8SM and Hemofil-M. Ingredients are assigned both upper and lower limits. Permitted residual levels of process reagents and protein contaminants offer wide margins of safety in relation to their known toxicity. None of these residues is likely to affect the stability of Replenate or limit its safety at the recommended dose in either adult or young patients. Test Methods are Ph.Eur or accepted equivalents, are described in a repeatable form, and have been extensively validated for use in Replenate. Consistency of manufacture has been demonstrated over 5 consecutive batches. The batch of Replenate users in Clinical Trials meets the standard specification.

6. **STABILITY**

Stability Studies **Format 20.3**

Format 20.2 copies Table 1 of the Introduction to Annex IIF, as a summary of the studies considered relevant to this Application. Real-time stability of Replenate at 4°C (IIF4) is the most important element in this Annex but the interim results from this study may be extrapolated with some support from studies on the parent concentrates.

The Applicant's proposal is for a dating period of 24 months at 2°C-8°C, for Replenate formulated in Zenalb after processing which includes 48 c.v. wash of the MAb-Sepharose column, all as defined in Annex IIB-IIE.

6.1 Replenate in Zenalb (IIF.4) **Format 20.1-2.**

Three batches processed with 40 c.v. wash cover three other conceivable variables - dose strength (250, 500, 1000 iu), batch size (80 and 130 kg cryoprecipitate) and the inclusion (or not) of frozen storage of Q-Sepharose eluate. I agree that minor deviations from specification in two of these batches are immaterial to the study.

Three batches processed with 48 c.v. wash cover the same potential variables; all batches met specification.

The study will eventually assemble evidence on 24-36 month stability of Replenate at the recommended storage temperature of 4°C. Since the study is incomplete (1.5-6 months)

at the time of Application, it includes an attempt to predict long-term stability at 2-8°C by examining samples held at higher "stress" temperatures, 25°C, 37°C and 56°C. Eight parameters are being monitored as indicators of stability. The most important of these indices is factor VIII potency (only 2-stage assays assessed here), which is always measured directly against a control sample stored at -40°C. This design greatly enhances the sensitivity of the study in finding statistically significant changes.

The criterion that all specified and implied parameters should remain within original specification limits over the dating period seems likely to be met by all the minor variants of Replenate represented in this study. Ideally, all properties of Replenate which could conceivably determine stability (e.g. moisture content) might be subjected to similar scrutiny, using batches near or beyond the extreme ends of the specified limits. However, the scope of the present study is realistic and capable of answering the most important questions.

No sample stored at a temperature up to and including 37°C has lost potency at a rate exceeding 1% per year. None of the factor VIII potencies measured after 1.5 - 6 month storage at 56°C has differed significantly from the control, but an apparently non-linear physical response - yellowing of the powder - suggests that data at stress temperatures should be interpreted with caution. Application of the Arrhenius equation does not acknowledge the common experience that changes in proteins may be discontinuous rather than showing a linear increase at elevated temperatures; such constructs and the derived stability estimates applied to "real" temperatures should be regarded as pessimistic. In the present study, the minimal changes observed in factor VIII potency scarcely justify statistical treatment of data from higher temperatures of storage. However, DEGTEST analysis of the single batch showing any change suggests the loss of only 0.01% factor VIII potency per year at 8°C and the combined data for the 40 c.v. subgroup predicts no significant change in factor VIII potency. Intuitively, it seems highly probable that there will be no abrupt change in the (zero) slope of factor VIII decay over time, and that a dating of 24 months at 2-8°C is secure. No inference is to be drawn about storage at 20°C until the study has proceeded for 12-24 months.

6.2 Studies on Hemofil-M

Further studies IIF1.1-1.3 are quoted from the Hemofil-M PLA0016-0236-8. These offer particularly strong support for Replenate, in that this Application has established a very close identity between the two processes and products, in terms of formulation, composition, specification, starting materials and in-process control limits. These studies examine

batches of Hemofil-M including minor variants in the source of MAb-Sepharose, the type of ion-exchanger used for the second chromatographic stage, the stopper and the drying of stoppers before use. The stability-indicating variables were similar to those selected in IIF.4, the most critical being factor VIII potency. The most important conclusion was that, even in batches inadvertently exposed to moisture levels exceeding 4%, there was no significant loss of factor VIII potency or other deterioration in quality at temperatures up to and including 30°C over 24 months (IIF.1.1). Application of the Arrhenius equation to data obtained at "stress" temperatures up to 53.5°C pointed to a shelf-life of at least 18 months at 5°C plus 6 months at 30°C, with loss of less than 10% factor VIII potency (IIF1.2). In study IIF1.3, no factor VIII potency fell below 90% of the original assigned potency after storage at temperatures up to 35.5°C for up to 18 months.

Direct application of these results from Hemofil-M to Replenate is inhibited only by limited data on their respective freezing and freeze-drying programmes, which are difficult to reproduce in every detail. There is a strong inference that none of the minor variations between these Hemofil-M batches affected stability in the freeze-dried state.

6.3 Studies on Octonativ/8SM

Study IIF2.3 is a completed experiment on six lots of Octonativ, quoted from the 8SM PL08801-0015. Study IIF 3.1 is an on-going trial at BPL on six lots of 8SM.

Only modest claims are made for the relevance of these studies to Replenate, since the formulation of the two products differs during the critical stages of freezing and freeze-drying (there are also differences between different dose strengths of 8SM). These differences are likely to have a greater influence on product stability than differences in site of manufacture, albumin formulation, plasma source, etc. However, I agree with the Applicant's interpretation (IIF3.1) the real-time data at 12 months on 8SM, stored at 2°C and 8°C, support a two year shelf-life for 8SM. The source of albumin stabiliser appears to be immaterial. IIF2.1 and 2.3 confirm the stability (less than 10% loss of factor VIII potency) of three batches of Octonativ 500 iu for 36 months at 2-8°C, including up to 2 months at 25°C. Data on Octonativ 1000 iu are incomplete at 12 months but show no unusual pattern of factor VIII loss.

It is reasonable to conclude that the inherent stability of factor VIII prepared by the Hemofil-M "family" of processes is more important than details of formulation.

Close examination of effects on two of the 8SM batches at "stress" temperatures (IIF3.1) underlines earlier reservations about interpretation of accelerated degradation data. The applicant's claim does not rely on data derived from storage at high temperatures.

6.4 Stability of Reconstituted Replenate

In the course of tests on packaging compatibility, (IIA5.1) reconstituted Replenate remained within 80-125% of the initially assigned factor VIII potency over 24h at 4°C or at 20°C. The mean rates of potency loss were 0.37% per hour at 4°C and 0.36% per hour at 20°C. This supports the applicants claim for stability of Replenate in solution.

6.5 Stability of Frozen Cryo-precipitate and Q-Sepharose Eluate

See sections 2.2 and 3.2 of this report.

Conclusions from this section

Real-time studies of Replenate at 4°C, although incomplete, offer the most direct support for the claimed shelf-life of 24 months at 2-8°C. Studies on other, related concentrates strongly imply that interim data on the stability of Replenate may be extrapolated confidently to 24 mo.

None of these studies suggested an increase during storage (freeze-dried or in solution) in the concentration of any component likely to affect the toxicity of Replenate.

In IIF4, 2-stage factor VIII assays were supplemented with 1-stage and chromogenic assays. None of the comparisons between these assays suggests that factor VIII in Replenate is significantly activated. The stability of reconstituted Replenate over many hours at 4°C or 20°C does not suggest the presence of pre-formed VIIIa in the dried concentrate or any unusual susceptibility to activation after reconstitution.

7. OTHER INFORMATION: VIRUS INACTIVATION

Formats 21.1-

In this Application, the viral safety of Replenate is correctly identified as resting on several pillars: selection of blood donors and testing of each donation; inactivation of the most important blood-borne viruses by treatment with solvent/detergent; physical removal (clearance) of virus particles during processing, particularly the chromatographic processes; and avoiding recontamination of virus-inactivated solutions with infective material from earlier stages.

Testing of blood and plasma donations

Each donation is tested, at the time of collection, for HBsAg, anti-HCV and anti-HIV-1 and -2, markers of the most important blood-borne viruses HBV, HCV and HIV. In the UK, it has been concluded that additional testing for hepatitis viruses, e.g. by anti-HBc and serum aminotransferase determinations, would not significantly improve the sensitivity and selectivity of donor exclusion (3). It is also considered that screening for other conceivable contaminating viruses, such as HTLV-1, would not be cost-effective (4). The sensitive tests applied

to every donation are the main line of defence against transmission of the most important viruses by blood cellular components which cannot be sterilised efficiently by chemical or physical methods. The risks to the recipient of untreated, single-donor components are infection by viruses not excluded by medical screening of the donor; not detected because no testing was attempted for certain viruses; or missed (false-negatives) because tests were not sufficiently sensitive to detect a low, but infective titre of virus. Clerical and human errors have also occurred. The risks of a plasma pool containing an infective concentration of virus, and the consequences of recipients of large-pool products, obviously multiply with the number of donations in the pool. Additional measures to protect recipients of large-pool products are required.

Virus inactivation by treatment with solvent/detergent

This family of methods, using the solvent TNBP and various optional detergents including Triton X-100, was invented by New York Blood Center and has been thoroughly proven in laboratory and clinical trials over a decade. Appendix I to Annex IIH provides a useful summary. The earlier clinical studies on "non-A, non-B hepatitis" were of variable design and quality. However, they carry more conviction than more recent studies on concentrates made from plasma which was screened at donation level for anti-HCV and anti-HIV as well as HBsAg. There is only a supposition that these products would have been infected, even without solvent/detergent treatment. Laboratory spiking experiments are therefore essential to quantify the probable efficiency of any virus-inactivation system, but complementary clinical trials are still advisable. In most cases, the "laboratory" limit of detection of viruses is much higher than the probable minimum concentration required to infect a susceptible individual. Inactivation of HIV has been demonstrated both by clinical trial and by "spiking" experiments in the laboratory. Effectiveness against HBV and HCV can be established only by clinical trial, although Sindbis is sometimes used as a surrogate virus, resembling HCV in its susceptibility to solvent/detergent and heat treatment (5).

Claims for the viral safety of Replenate rest heavily on the laboratory and clinical evidence assembled over 5 years for Hemofil-M (PLA 0016/0236-8) and the derived concentrates Octonativ/8SM. None of these concentrates has been reported as having transmitted HBV, HCV, or HIV either in formal prospective clinical trials or as detected in regular testing of patients who receive these products. Early laboratory studies on the Hemofil-M process, quoted in this Application, established that high titres of lipid-enveloped viruses, including Sindbis and HIV-1 and -2, were completely inactivated within the time required to mix in the solvent/detergent reagents, and certainly within three minutes. Additional laboratory studies on the similar Octonativ process, quoted in this Application, confirmed that 6.2 logs of HIV-1 were inactivated within one

IIH.2.3.2

minute of solvent/detergent addition. It is widely agreed that no more than 10^5 infective doses per ml could plausibly occur in a large plasma pool as a result of test failures. Inactivation of >5 logs of HIV in the first few minutes of solvent/detergent treatment in itself meets the requirement for "inclusion of an effective virus inactivation stage". IIH.2.3

The effectiveness of the TNBP Triton X-100 system in the inactivation of HIV-1 and Sindbis strongly suggest that it can also inactivate the maximum expected titre of HBV and HCV which might contaminate cryoprecipitate. Earlier solvent/detergent systems (eg. TNBP-Tween) which inactivate Sindbis efficiently have been shown by chimpanzee and human clinical trials to render factor VIII concentrate non-infective with these viruses, which cannot be studied directly in model "spiking" experiments(6).

Replenate acquires the assurance offered by Hemofil-M and Octonativ/8SM data through two groups of new studies, both using Sindbis as a convenient surrogate for the hepatitis viruses and human immunodeficiency viruses, comparably sensitive to solvent/detergent inactivation.

(1) Standard conditions were chosen for virus inactivation on the manufacturing scale. Variables which could possibly influence the efficiency of virus inactivation were identified as concentration of Triton X-100 and TNBP, pH, ionic strength and protein concentration of the medium, and temperature and time of incubation. Target processing limits for these variables were chosen as lying within or very near the range of conditions established for Hemofil-M and Octonativ/8SM. Each of these variables in turn was varied across a wide range and the effect of the change on virus inactivation efficiency was measured. In this way, it was established that inactivation of Sindbis was extremely effective over a broad range of conditions on either side of the set parameters, and including all conditions used in the manufacture of Replenate, Hemofil-M and Octonativ/8SM. IIH.2.1.1.1.

Supporting experiments confirmed that the conditions of reagent addition, mixing, etc. used in manufacture were accurately reflected in the small-scale experiments which necessarily had to be undertaken in the Virology Research Lab. IIH.2.1.1.5

Exhaustive validation of the manufacturing process has confirmed that the process limits set for all these critical variables in the solvent/detergent stage are consistently achievable in routine manufacturing. IIB.3.1.3-4

(2) In small-scale spiking experiments, challenge titres of 4-6 logs of Sindbis were completely inactivated within 15 seconds of adding the solvent/detergent mixture, linking the susceptibility of the Replenate cold-supernatant to that of the corresponding intermediate in the Hemofil-M and Octonativ/8SM processes. IIH.2.1.1.5

The incubation period of the formal, validated virus inactivation stage therefore appears to be at least 10^2 x greater than the minimum requirement to inactivate 5 logs of HIV. In addition, these time-course experiments have confirmed that virus-lethal conditions are maintained for many hours thereafter, during loading of the solvent/detergent -treated incubate onto the MAb column.

In the writer's opinion, these experiments on Replenate justify the conclusion that Replenate, like Hemofil-M and Octonativ/8SM, will not transmit lipid-enveloped viruses HBV, HCV and HIV. They further increase confidence in the margins of safety provided by this solvent/detergent process.

It is difficult to assess the rate of inactivation of lipid-enveloped viruses in the TNBP/Triton X-100 system without resorting to reagent dilution in order to slow the reaction. Many studies have shown that rates of virus inactivation, whether by chemical or physical means, are seldom strictly first-order, and the limit of detection of residual virus may be greater than human infective dose. It was demonstrated that in the Replenate process, virus inactivation in the defined system is complete within the first 15 seconds of the 30 minute incubation, disposing of any serious concern about "tailing" of the rate of inactivation. However, this Application prudently avoids claiming more than "complete inactivation of X logs of virus within the defined virus inactivation stage".

While it is extremely effective against lipid-enveloped viruses, protein-enveloped viruses such as parvovirus B19 or hepatitis A virus (HAV) are virtually unaffected by solvent/detergent treatment. In the earlier Hemofil-M studies, quoted in this Application, the surrogate virus EMV, which is not lipid-enveloped, was used to demonstrate this. Transmission of such viruses, which are known to occur infrequently in donated plasma, must be addressed either by exclusion of infective donors or by physical removal of viruses during processing. Screening for these viruses at donor level has not been introduced in the U.K.

Physical removal (clearance) of viruses during processing

Although it is not established directly in this Application, other virus clearance studies in plasma fractionation suggests that a modest partitioning of blood-borne viruses will have occurred, even before the solvent/detergent stage, e.g. during cryo-precipitation and cold precipitation (7,8).

Immunoaffinity chromatography in the Replenate process might be predicted to achieve partitioning of factor VIII from contaminating viruses - the factor VIII is bound very tightly, while contaminants are washed off at a high ionic strength which suppresses some non-specific interactions with the solid phase. Earlier studies on Hemofil-M and Octonativ/

8SM processes, quoted in this Application, confirmed that HIV, Sindbis, VSV and EMC were cleared by factors of 2.4-8.4 logs on immunoaffinity chromatography at laboratory scale. The only non-lipid-enveloped virus in this group, EMC, was cleared more effectively than the lipid-enveloped viruses, possibly because it is less susceptible to non-specific binding at high ionic strength, e.g. by hydrophobic interactions. Additional experiments on Replenate tend to confirm this observation; non-enveloped polio virus was depleted on MAb chromatography by 4.9 logs, compared with 3.7-4.2 logs for Sindbis. IIH.2.2.2. 2.3.2. IIH.2.1.2

Earlier studies on Hemofil-M indicated that the ethylene glycol eluant contributed about 1 log to inactivation of HIV at this stage.

The Hemofil-M studies were extended to partitioning of viruses on the Q-Sepharose column. It was concluded that the clearance was quite limited and virus-specific, i.e. that clearance of the important blood-borne viruses could not be predicted from measured clearances of surrogate viruses.

Although it is fortunate that non-lipid-enveloped viruses (which escape inactivation by solvent/detergent) may be susceptible to clearance in chromatographic stages, the mechanisms of clearance are less clearly understood and therefore less controllable than those of chemical inactivation. In-process removal is virus-specific and may well be affected by scale(9). There are published instances of virus transmission by concentrates which claim efficient clearance factors in the manufacturing process (10,11,12). It is not claimed that Replenate is safe from transmitting non-lipid-enveloped viruses. There is no well-proven alternative or supplementary process in common use for the selective inactivation of these viruses.

Avoidance of recontamination with viruses after inactivation with solvent/detergent

Before 30 minutes' incubation with solvent/detergent under the defined, validated conditions, the factor VIII solution is transferred from the downstream processing area to a "Virus-Safe Area" (VSA) where services and perimeter access have been specially designed and are carefully controlled. Factor VIII leaves the VSA as a sterile bulk solution, to be dispensed and freeze-dried aseptically in a Finishing Area also used for handling products which have not yet been virus-inactivated. This Application briefly describes the features of plant design and operation, services, equipment sterilisation and movement of personnel which establish control over the VSA's upstream and downstream interfaces. These controls have been thoughtfully planned and, with constant vigilance, are capable of maintaining security against cross-contamination of the Replenate process after the solvent/detergent stage.

Conclusions from this section

The Application acknowledges guidance on the validation of the virus removal and inactivation procedures given in Vol III Addendum II of the series "The Rules Governing Medicinal Products in the European Community". The virus safety of Replenate rests primarily on the solvent/detergent stage, which inactivates the lipid-enveloped viruses HBV, HCV and HIV with very high efficiency.

Inactivation of lipid-enveloped viruses by solvent/detergent treatment is identical with that used for Hemofil-M, which has a good record of virus safety and clinical use. Equivalent inactivation patterns have been demonstrated for appropriate model viruses. Removal of some non-lipid-enveloped viruses during the immuno-affinity chromatography stage is a welcome indication of enhanced safety but does not eliminate the risk of transmitting such viruses in the concentrate.

8. CONCLUSIONS OF THIS REPORT

This Application describes Replenate, a factor VIII concentrate processed from plasma in essentially the same way as Octonativ/8SM and Hemofil-M. The specification of the product is identical, in all important respects, with the specification of the parent concentrates. Equivalent safety and effectiveness may therefore be predicted with confidence.

Replenate meets Ph.Eur. specification for human factor VIII. The procurement and viral testing of blood and plasma, process design, formulation and validation are in accordance with the Note for Guidance "Medical Products Derived from Human Blood and Plasma" issued by the CPMP ad hoc Working Party on Biotechnology/Pharmacy and the Annex to the EC Guide to GMP "Manufacture of Products Derived from Human Blood or Human Plasma".

9. REFERENCES

1. Barbara J.A.J. Questions of Quality: How Much HBsAg Is There in This Sample and Is Our Assay Sensitive Enough to Detect It? Vox Sang (1993); 65: 249-250.

2. Barrowcliffe T.W. Standardisation and Assay. Sem. Thromb. Hemostasis (1993); 19: 73-79.

3. Reesink H.W., Nydegger U.E. Blood Donor Screening or 'Over-Screening': How Far to Go in Avoiding Transmission of Infectious Agents? Vox Sang (1992); 63: 59-69.

4. Editorial. Human T Cell Leukaemia/Lymphoma Virus and Blood Donation. Routine Screening of Blood Donors is not currently indicated in Britain. Brit. Med. J. (1993); 307: 1224.

5. Horowitz B., Prince A.M. Laboratory and Preclinical Evaluation of the Virus Safety of Coagulation Factor Concentrates. Develop. biol. Standard., (1987); 67: 291-302.

6. Prince A.M., Horowitz B., Brotman B. Sterilisation of Hepatitis and HTLV-III Viruses by Exposure to Tri(n-Butyl)Phosphate and Sodium Cholate. Lancet. (1986); 706-710.

7. Morgenthaler J-J. Effect of Ethanol on Viruses. In Morgenthaler J-J (ed): "Virus Inactivation in Plasma Products." Basel: Karger, (1980); pp 109-121.

8. Levy J.A., Mitra G.A., Wong M.F., Mozen M.M. Inactivation by Wet and Dry Heat of AIDS Associated Retroviruses During FVIII Purification from Plasma. Lancet (1985); I:1456-1457.

9. Schreiber A.B., Hrinda M.E., Newman J., Criss Tarr G., D'Alisa R, Curry W.M. Removal of Viral Contaminants by Monoclonal Antibody Purification of Plasma Proteins. In Morgenthaler J-J (ed): "Viral Inactivation in Plasma Products." Basel: Karger, (1989); pp 146-153.

10. Berntorp E., Nilsson I.M., Ljung R., Widell A. Hepatitis C Virus Transmission by Monoclonal Antibody Purified Factor VIII Concentrate. Lancet (1990); 335: 1531-32.

11. Mariani G., Di Paolantonio, T., Baklaya, R., Morfini, M., Mannucci P.M. and the Ad Hoc Study Group of the Fondazione Dell'Emofilia. Prospective Study of the Evaluation of Hepatitis C Virus Infectivity in a High-Purity, Solvent/Detergent-Treated Factor VIII Concentrate: Parallel Evaluation of Other Markers for Lipid-Enveloped and Non-lipid-Enveloped Viruses. Transfusion (1993); 33: 814-818.

12. Mannucci, P.M. Outbreak of Hepatitis A Among Italian Patients with Haemophilia. Lancet (1992); 339: 810.

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Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIA: COMPOSITION

Product Description Volume 2 Page(s)	(For National Authority Use Only) COMMENTS
A sterile, freeze-dried, white or pale-yellow powder which, on addition of 10 ml Water for Injections yields a clear solution containing a nominal 25, 50 or 100 iu/ml of coagulation factor VIII activity.	

Complete Composition: Volume 2 Page(s)

Active ingredients	Unit and/or Percentage formula
Human Factor VIII	25, 50 or 100 iu/ml nominal
Other Ingredients (all in 10ml)	
Albumin	100 mg
L-histidine	78 mg
Polyethylene glycol	10 mg
Calcium chloride	4.4 mg
Sodium chloride	83 mg

Trace concentrations are present of other human plasma proteins, mouse IgG and reagents used in processing. For limits and typical concentrations, see Formats 17.2 and 12.3.

Container (brief description) Volume 2 Page(s)

Vial Injection bottle, glass Type 1 Ph.Eur., 30 ml for all fill sizes.
Stopper Halobutyl stopper, sealed with flip-off seal.

Clinical trial formula Volume 2 Page(s)

Active ingredients	Unit and/or Percentage formula
Factor VIII	500 iu nominal

Formulation was as for standard batches, above.

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIA: DOSAGE FORM - DEVELOPMENT PHARMACEUTICS Product Development Studies Summary: Volume 2 Page(s)	(For National Authority Use Only) COMMENTS
Replenate was developed from an established method, Baxter Hemofil-M, and includes modifications made by Kabi in the product 8SM (Octanativ). Process modifications from Hemofil-M include minor details of cryoprecipitate re-solution; validated re-definition of conditions for virus inactivation by solvent/detergent; and the use of Q-Sepharose in the ion-exchange step. In order to fill all dose sizes in 10 ml, Replenate departs from the 8SM formulation, reverting to that of Hemofil-M. No formal development studies are reported.	
Explanation of choice of the composition.	
Factor VIII: active therapeutic ingredient. Albumin, human: stabiliser; increases protein concentration L-histidine: stabiliser; buffers pH. Polyethylene glycol: stabiliser; facilitates re-solution. Calcium chloride: stabiliser; integral part of functional factor VIII molecule. Sodium chloride: adjusts ionic strength (tonicity).	
Explanation of optimisation of concentrations of the additives in the composition	
These concentrations of additives were established in the original Hemofil-M method as sufficient to provide stability (in the freeze-dried state and in solution) and safe on intravenous injection at the maximum expected dose of factor VIII. Albumin increases the protein concentration of the solution and preserves factor VIII activity during purification and after reconstitution, partly by inhibiting adsorption of	
Summary of studies on compatibility data with other products (if necessary): Volume 2 Pages	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIA: DOSAGE FORM - DEVELOPMENT PHARMACEUTICS Product Development Studies Summary: Volume 2 Page(s)	(For National Authority Use Only) COMMENTS
Explanation of choice of the composition.	
Explanation of optimisation of concentrations of the additives in the composition	
factor VIII to surfaces. Histidine is added at a concentration which contributes to pH buffering and stabilisation of factor VIII activity. PEG is added at a concentration optimal for re-solution of the dried powder, and to prevent surface inactivation of factor VIII during processing. Calcium chloride is added at approximately physiological concentration, stabilising factor VIII activity which requires a "calcium bridge" between the light and heavy chains.	
Summary of studies on compatibility data with other products (if necessary): Volume 2 Pages	
Addition of factor VIII to other injectable products is not recommended and no compatibility studies are reported.	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIA: DOSAGE FORM - DEVELOPMENT PHARMACEUTICS

Summary of studies on compatibility with the container/ closure: Volume 2 Pages	**(For National Authority Use Only) COMMENTS**
The choice of Type 1 glass minimises the likelihood of interactions between the container and the freeze-dried product. Stoppers are dried after sterilisation to avoid desorption of water and its migration into the product. The reconstituted product was stored at 4°C for up to 24h, in contact with the vial alone or, by inversion, in contact with both the vial and the closure. There was only a very slow loss of factor VIII potency at either temperature, in contact with the vial or closure. All other specified parameters remained constant. (IIA.5.1)	
Summary of in vivo bioavailability/bioequivalence studies: Volume 2 Pages	
In vivo recovery of factor VIII in injected Replenate and its rate of disappearance from the circulation are assessed in Annex IV. This study used many of the same patients who contributed to a cross-over study using 8SM and an intermediate-purity concentrate (8Y), quoted here from PLA 08801/0015.	
In vitro dissolution data on products used in the in vivo bioavailability studies. Volume 2 Pages	
The freeze-dried powder dissolves in water in less than 1 minute.	

FORMAT 3

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIB: METHOD OF PREPARATION

Manufacturing Formula : Volume 2 Page(s)	(For National Authority Use Only) COMMENTS
Batch size: typically 20,000 - 50,000 donations, (7-15 tonnes) of FFP, or 80-160 kg cryo-precipitate. Finishing batch size: unspecified, dependent on dose size. Formula: Factor VIII nominal 250, 500 or 1000 iu reconstituted in 10ml water. Albumin: 100 mg per vial. L-histidine: 78 mg per vial. Polyethylene glycol: 10 mg per vial. Calcium chloride: 4.4 mg per vial. Sodium chloride: 83 mg per vial.	
Manufacturing process (including in process control and assembly: Volume 2 Pages	
The Replenate process is described schematically in the following pages 4.2, 4.3 and 4.4. The purpose of each operation is briefly indicated on the left and control operations on the right. Tests done on in-process samples are italicised.	

Flow Chart: Stages in Manufacture, Replenate and in-process controls

Fresh-Frozen, Platelet-Poor Plasma

RECOVER CRUDE FACTOR VIII-RICH FRACTION FROM PLASMA

Cryoprecipitation
Thaw at less than 10°C
Sample suspension for *TVC*
Centrifuge, wash precipitate in Tris/ethanol
Sample cryo-supernatant for *HBsAg, anti-HIV, anti-HCV*

Cryoprecipitate

STORE FOR BATCHING-UP

Weigh, Double-wrap, freeze and store
-40°C ± 5°C

Cryoprecipitate

REDISSOLVE CRUDE FRACTION

Cryoprecipitate Extraction
Dissolve in 3 vols *50μM Calcium chloride*
Temperature 28°C ± 2°C
Adjust to *pH 6.5-7.1*

Cryoprecipitate Extract

"COLD PRECIPITATION" REMOVES POORLY - SOLUBLE PROTEINS

Removal of Fibrinogen, Fibronectin
Cool to 10°C ± 1°C
Centrifuge, 9-14°C outflow

Cold Supernatant

SOLVENT-DETERGENT REAGENTS ADDED, IONIC MEDIUM CONTROLLED

Preparation for Virus Inactivation
Warm to 18°C-26°C
Add Sodium Chloride to 0.8M
Add Calcium Chloride to 50mM
Filter to 0.45μ; test filter integrity
Check A_{280} = *5 - 35*

(continues)

Addition of solvent/detergent
Add a mixture of Tri-n-butyl-phosphate (3 parts) Triton X-100 (10 parts) to a final concentration of *0.3% TNBP/1% Triton X-100*
Mix 20 minutes
Check pH *(pH 6.5-7.5)*
Check *conductivity (55-75mS)*

TRANSFER TO VIRAL SECURE AREA

Filtrate in TNBP / Triton X-100

VALIDATED CONDITIONS FOR VIRUS INACTIVATION

Incubate ≤30 minutes, 18°C - 25°C for virus inactivation

Solvent/Detergent-Treated Filtrate

PURIFICATION OF FACTOR VIII BY IMMUNO-AFFINITY, STABILISATION BY BUFFER COMPOSITION

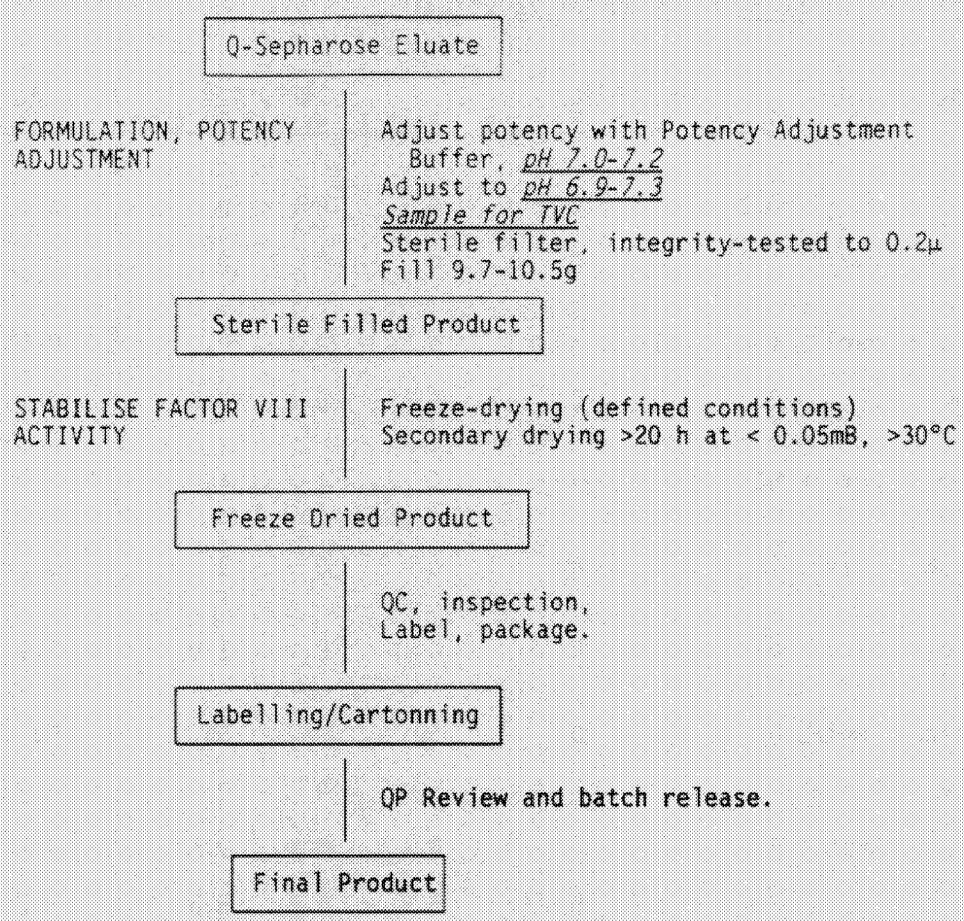
Factor VIII Separation on MAb-Sepharose
Wash column before use; *LAL*
Equilibrate *pH 6.5-7.5*
Load via 0.45 µ filter
Loading time ~24 hours, ≥3cv/h
Sample flow-through *(FVIII <1iu/ml)*
Wash with ≤48 column-volumes, ≥5 cv/h of MAb Wash Buffer, *pH 6.3-6.5*
Elute in MAb Elution Buffer, *(pH 6.1-6.9)*, ≥2 cv/h

MAb Eluate

ION-EXCHANGE REMOVAL OF MOUSE IgG AND UNWANTED REAGENTS

Q-Sepharose recovery of FVIII
Regenerate column before use; *LAL*
Equilibrate column prior to loading, *pH 6.3-6.5*
Load onto Q-Sepharose FF column, ≥6 cv/h
Sample flow-through *(FVIII <1iu/ml)*
Wash Q-Sepharose FF column, *pH 6.3-6.5*
Total Wash of ≤40 column-volumes
Elute Q-Sepharose column pH *5.4-5.6*, ≥2 cv/h
Collect eluate into Pool Dilution Buffer, *pH 8.1-8.3*

(continues)



Nominal content of critical buffer solutions

Reagent	Unit	MAb column			Q-Sepharose			Pool	Potency
		Equil.	Wash	Elution	Wash 1	Wash 2	Elution	Diln.	Adj.
TNBP	%v/v	0.3	0	0	0	0	0	0	0
Triton X-100	%v/v	1.0	0	0	0	0	0	0	0
Imidazole	mmol/L	50	50	50	0	0	0	0	0
Sodium chloride	M	0.8	0	0	0.15	0.15	0.6	0	0.15
Calcium chloride	mmol/L	50	40	40	1	1	4	5	4
Ethylene glycol	%v/v	0	5	40	0	0	0	0	0
Albumin (human)	%w/v	0	0	0.1	0.1	0.1	1.0	1.0	1.0
Histidine	mmol/L	0	0	0	50	50	50	50	50
Glycine	M				1	0	0	0	0
PEG	%w/v	0	0	0	0.1	0.1	0.1	0.1	0.1
pH		7.4	6.4	6.4	6.4	6.4	5.5	8.2	7.1
Conduct-ivity	mS/cm	65	9	3.5	14	15	50	1.5	15

Tabulation of in-house process controls and their significance

(1) Controls on solutions used in process

Solution	Use	Test
Calcium chloride, 50μm	Dissolves cryo-ppt.	Ca++ (retrospective) conductivity
Calcium chloride, 5M	Stock solution added to cold s/n	Ca++ (prospective) conductivity
MAb equilibration	Ionic equilibration before loading	pH, conductivity TNBP and Triton X-100 (retrospective)
MAb wash	Post-load wash	pH, conductivity
MAb elution	Elute F.VIII in stabilisers	pH, conductivity
Q equilibration	Ionic equilibration before loading	pH, conductivity
Q Wash 1	Post-load wash	pH, conductivity
Q wash 2	Remove glycine before elution	pH, conductivity
Q elution	Elute F.VIII in stabilisers	pH, conductivity
Pool dilution	Adjust pH of eluate	pH, conductivity
Potency adjust	Dilute to fill potency	pH, conductivity

(2) Control tests on samples taken from intermediate stages of the process

(Many of the controlled operations in the manufacturing scheme (Formats 4.2-4.4) are in-process adjustments and the run does not proceed until adjustment is successful. These controls are not tabulated here.)

Stage	Tests	R/P	Significance of non-compliance
Cryosuspension	TVC	R	Investigation
Cold s/n, adjusted	A_{280}	P	(3) Critical for virus inactivation
SD addition	TNBP, Triton X-100	R	(3) virus inactivation
SD incubation	Conductivity, pH, temperature	P	(1) virus inactivation
Pre-incubation filter	Integrity, 0.45µ	P	(1)
MAb pre-wash	LAL	P	(1)
MAb flow-through	F.VIII (trend)	R	Replace column
Q-Sepharose pre-wash	LAL	P	(1)
Q-Sepharose flow-through	F.VIII (trend) Mouse IgG (trend)	R R	Replace column
Q-Sepharose eluate	F.VIII	P	Potency adjustment
Pre-Sterile filtration	TVC pH	R P	(4) (1)
Sterile filtration	Integrity, 0.22µ	P	(1)

Key:

R - Results assessed retrospectively.
P - Results assessed prospectively before process continues.
(1) - Repeat operation, or make other authorised, until condition is satisfied.
(2) - Check that parameter is within limits for optimal efficiency
no immediate action on non-compliance unless parameters are out of limits at a later stage.
(3) - Batch fails if control limits are not met.
(4) - Consider with other evidence at Batch Review.

(3) **Summary of critical in-process controls**

A batch fails if the following conditions are not met (and documented) after examination of the bulk solution or a sample.

Stage	Condition	Limits
Incubation with solvent-detergent in controlled medium (validated conditions)	A_{280}	5-35
	pH	6.5-7.5
	Conductivity	55-75 mS
	TNBP	≤0.25% (v/v)
	Triton X-100	≤0.8% (v/v)
	Temperature	18°C - 25°C
	Incubation period	≤30 min.

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIB: METHOD OF PREPARATION - PROCESS VALIDATION Summary of experimental studies: Volume: 2 Page(s)	(For National Authority Use Only) COMMENTS
Where validation studies have been reported before, in relation to Hemofil-M or 8SM PLAs, reference is made to the explanatory section as copied into this Application. Headings (1)-(13) summarise validation and optimisation studies to characterise the process during development. Validation of the critical virus inactivation stage using solvent/detergent treatment is specifically discussed in Annex IIH, summarised in Formats 21.1-21.4. Heading (14) demonstrates consistency of product characteristics with those of Hemofil-M and 8SM. Headings (15)-(19) summarise validations which provide continuing assurance of controlled processing environment and services. **(1) Dissolution of cryoprecipitate in 50 µN calcium chloride solution (IIB. 3.1.2.)** A minor change in procedure plausibly introduces Ca++ more safely than bolus addition and is shown here to have no adverse effect on F.VIII yield. **(2) Mixing of solvent/detergent reagents to homogeneity (IIB 3.1.3.3.)** Extending previous data on 8SM and Hemofil-M, mixing of large and small volumes in BPL's processing equipment is demonstrated to be complete in less than five minutes. **(3) Temperature control during virus inactivation in solvent/detergent (IIB.3.1.4)** Temperature control at all points in BPL's processing equipment, at minimum and maximum tank contents, is shown to be within the validated range of 18 - 25°C over 30 minutes. (Other aspects of virus inactivation validation are described in Annex IIH).	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIB: METHOD OF PREPARATION - PROCESS VALIDATION Summary of experimental studies: Volume: 2 Page(s)	For National Authority Use Only) COMMENTS
(4) Stability of F.VIII in the presence of solvent/ detergent (IIB.3.1.5). Extending previous data on 8SM and Hemofil-M, it is shown that Replenate suffers a similar, modest loss of F.VIII activity during incubation with solvent/detergent, about 0.4% per hour. These data justify the option of holding the mixture for up to 40h at 20-25°C, should this prove necessary. **(5) Adsorption of solvent/detergent reagents to pre-MAb filter (IIB.3.1.6)** This study shows that, even after the formal, validated virus inactivation incubation, the concentrations of reagents remained lethal to viruses and are not significantly diminished by pre-filtration prior to immuno-affinity chromatography. **(6) Clearance of solvent/detergent reagents on immuno-affinity (MAb) columns (IIB.3.1.7)** This validation study shows that, after 40 column volumes wash buffer, the concentration of these reagents in the eluted F.VIII is reduced by a factor >10^3. (Further clearance occurs on Q-Sepharose chromatography). **(7) Plasma protein reduction by immuno-affinity chromatography (IIB.3.1.8)** Extending data on 8SM and Hemofil, it is shown that the Replenate process achieves a similar purification of F.VIII and that the patterns of F.VIII partition are very similar in all three closely related processes. **(8) Leakage of DNA from the MAb-Sepharose (IIB.3.1.9)** Previously presented for 8SM, PL08801/0015. The same reagent is used in the production of Replenate. **(9) Re-use of MAb column (IIB.3.1.10)** Re-use of MAb column (IIB.3.10). Product characteristics,	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIB: METHOD OF PREPARATION - PROCESS VALIDATION Summary of experimental studies: Volume: 2 Page(s)	For National Authority Use Only) COMMENTS
microbiological monitoring and loss of F.VIII in flow-through are reviewed against criteria shown to predict good column performance in the Hemofil-M prcess, which uses the same MAb-Sepharose. **(10) Removal of mouse IgG (MAb) by ion-exchange chromatography (IIB.3.1.12)** Extending previous data on Hemofil-M (reduction to <0.1ng/iu), new data on five Replenate batches demonstrate reduction to less than the detection limit in both the final Q-Sepharose wash and eluted factor VIII; this is equivalent to <0.01 ng/iu in the final product, as in 8SM. **(11) Removal of other contaminants by ion-exchange chromatography (IIB.3.1.13 and 3.1.14).** Extending previous data on Hemofil-M and 8SM, new data on 3-6 batches of Replenate demonstrated satisfactory partition of F.VIII and reduction of ethylene glycol, TNBP and Triton X-100 to below detection limits in the product. **(12) Stability of FVIII in Q-Sepharose eluate (IIB3.1.15).** New data on frozen storage of eluate in polyethylene containers. There is no significant change in FVIII activity after storage at -40°C or -70°C for up to 13 weeks in any format. An additional freeze-thawing cycle causes no loss of F.VIII. **(13) Definition of freeze-drying protocol (IIB.3.1.16).** The defined cycle for Replenate, including sublimation below -32°C and secondary drying at +30°C, gave a consistently high yield of F.VIII and rwc <0.3% in 6 batches. Annex IIF offers further support for the success of the defined drying cycle. **(14) Product characteristics (IIB.3.2.).** Extending previous data on Hemofil-M which compared this high-purity concentrate with conventional concentrates, immuno-blotting analysis of the subunit structure of	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIB: METHOD OF PREPARATION - PROCESS VALIDATION Summary of experimental studies: Volume: 2 Page(s)	For National Authority Use Only) COMMENTS
factor VIII in Replenate shows batch-to-batch consistency and identity with that of Hemofil-M. Concentrations of residual plasma proteins in Hemofil-M, 8SM and Octanativ are compared with those in Replenate: vWF, IgG, IgA, fibrinogen and fibronectin in Replenate are in similar ranges to those found in the related products. Residual concentrations of glycine and imidazole, not routinely measured in the control of Replenate, are shown to be consistently very low. **(15) Integrity of the Virus-Safe Area (IIB.3.3.1)** This is a description of the design, construction and operation of the VSA to preclude cross-contamination of virus-inactivated intermediates with other materials and equipment not yet subjected to virus inactivation. **(16) Replenate production environment (IIB.3.3.2)** This describes environmental monitoring in Sterile Operations; DOP- and velocity-testing of air filters; criteria for broth fills; and testing of liquid-sterilising filters before and after use. **(17) Sterilisation of equipment (IIB.3.3.2)** There is frequent, conventional validation of the performance of autoclaves and dry-heat sterilisers. Sterilisation of freeze-dryers prior to each run is described. **(18) Sanitisation (IIB.3.3.2.5)** Defined clean-in place (CIP) cycles are checked for efficiency by microbiological and chemical testing. **(19) Services (IIB.3.3.2.6)** Generation of WFI-bulk and its microbiological, particulate and chemical analysis to Ph.Eur. specification. Generation and delivery of deionised water and its microbiological testing. 0.2µ filtered compressed air. Generation, delivery and filtration of clean steam and its testing for dryness and non-condensable gases.	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		
PART IIC: CONTROL OF STARTING MATERIAL - ACTIVE INGREDIENTS		For National Authority Use Only) COMMENTS
Specification and routine tests: Volume 3 Page(s)		
Fresh-frozen plasma (BP 1993). Human plasma for fractionation Ph.Eur. 1993.		
Summary of Specifications and Routine Tests - Volume 3.		
Characteristics: Page(s)		
Donors are selected according to current UK NBTS/NIBSC Guidelines for the Blood Transfusion Services in the United Kingdom, Appendix IIC/4. Each unit of plasma meets BPL's current specification, described in IIC 1.1-1.4 and Appendix IIC/1.		
Identification tests: Page(s)		
Not relevant		
Purity Tests: Page(s)		
Physical: Weight Chemical: Not relevant Biological/Immunological Total viable count. (cryo-suspension). Virus markers; HBsAg, anti-HCV, anti-HIV-1 and -HIV-2 (cryo-supernatant).		
Other Tests : Page(s)		
Not relevant.		
Assay/Other evaluation of potency: Page(s)		
No homogeneous plasma pool sample available for testing.		

Name of Company Bio Products Laboratory	Summary Table Referring to Part of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIC: CONTROL OF STARTING MATERIALS, ACTIVE INGREDIENTS - SCIENTIFIC DATA (IMPURITIES) (Volume 3)

Potential impurities arising from the starting material: Pages	Test procedures and their limits of detection or limits of quantitation:
Fibronectin Fibrinogen vWF:Ag IgG, IgA	Very low concentrations of these residual plasma proteins were measured by ELISA tests in Replenate, in the course of a product validation study (IIB.3.2.3). The limits of detection were not reported. These measurements do not form part of
Potential impurities arising from the route of synthesis Pages	**Test procedures and their limits of detection or limits of quantitation:**
Potential impurities arising during the production and purification	**Test procedures and their limits of detection or limits of quantitation:**

COMMENTS - (For National Authority Use Only)

FORMAT 12.1

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61

Name of Company Bio Products Laboratory	Summary Table Referring to Part of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIC: CONTROL OF STARTING MATERIALS. ACTIVE INGREDIENTS - SCIENTIFIC DATA (IMPURITIES) (Volume 3)

Potential impurities arising from the starting material: Pages	Test procedures and their limits of detection or limits of quantitation:
	the product specification. Data on Hemofil-M and 8SM are compared with Replenate in IIB 3.2.3 and IIA4.2
Anti-A, anti-B	Indirect test using anti-human globulin. LOD not reported.
Potential impurities arising from the route of synthesis Pages	**Test procedures and their limits of detection or limits of quantitation:**
Mouse IgG	(ELISA). LOD 0.4 ng/ml.
TNBP	(GLC). LOD 0.1144 ppm.
Triton X-100	(HPLC). LOD 0.45 ppm.
Ethylene glycol	(GLC). LOD 10ng/ml.
Glycine	(colorimetric method). LOD not reported. Not part of product specification.
Imidazole	(HPLC). LOD 0.03mM. Not part of product specification.
Potential impurities arising during the production and purification	**Test procedures and their limits of detection or limits of quantitation:**
Factor VIII "fragments"	Immuno-blotting [IIB.3.2.2.]. No LOD reported. Not part of product specification.

Impurities and structural deviants actually found (with indication of amounts): Page(s) -

See Table, Format 12.3 overleaf.

COMMENTS - (For National Authority Use Only)

Impurities and structural deviants actually found (with indication of amounts):
Page(s)

Substance	Unit	Hemofil-M No. Range Batches	Octonativ M No. Range Batches	BSM No. Range Batches	Replenate No. Range Batches
Fibrinogen	µg/ml	n = 49 0.033-0.244	n = 4 0.13-0.29	-	n = 7 0.022-0.128
Fibronectin	ng/ml	n = 18 <17.2 - 43.7	n = 4 43.9 - 117.12	-	n = 4 14 - 48
vWF:Ag	iu/ml	n = 3 0.65- 2.38	n = 4 1.4-1.8	n = 56 1.24-3.13	n = 7 0.44-2.24
Human IgG	µg/ml	n = 5 <99	n = 4 <20	-	n = 3 <7
Human IgA	µg/iu	n = 5 <39	n = 4 <20	-	n = 3 <10
Human IgM	µg/iu	n = 5 <54	n = 4 <20	-	-
Anti-A, anti-B		-	-	n = 5 neg at 1:64	n = 6 neg at 1:32
Mouse IgG	ng/iu	n = 49 0.14 - 0.03	n = 4 }0.01	n = 56 }0.01	n = 6 }0.01
TNBP	µg/ml	n = 15 <1	n = 5 <1	n = 23 <0.2	n = 6 <0.2
Triton X-100	µg/ml	n = 18 0-0.45	n = 10 1.3 - <0.2	n = 56 0.4 - <1.0	n = 6 <0.5 - <1.0
Ethylene glycol	µg/ml	n = 18 0 - 13.25	n = 10 <5 - 46	n = 56 <10 - <40	n = 6 <10
Glycine	mmol/ml	n = 18 0.25 - 1.25	-	-	n = 15 0.1 - 1.9
Imidazole	mM	-	n = 3 <0.03	n = 3 <0.03	n = 3 <0.03

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIC: CONTROL OF STARTING MATERIALS. OTHER INGREDIENTS - SPECIFICATIONS & ROUTINE TESTS (VOLUME 3) Pages

(1) Other ingredients described in a Pharmacopoeia

Albumin	Ph.Eur. test limits (Licensed product Zenalb).
L-histidine	USP. Tested in-house to USP.
PEG 4000	BP. Tested in-house to BP (Macrogol 4000)
Calcium chloride	Ph.Eur. Tested in-house to Ph.Eur.
Sodium chloride	Ph.Eur. Tested in-house to Ph.Eur.

(1a) Processing reagents (not formulation ingredients) described in a Pharmacopoeia

Glycine	Ph.Eur. Tested in-house to Ph.Eur.
Triton X-100	USP. Tested in-house to USP.
Hydrochloric acid	Ph.Eur. Tested in-house to Ph.Eur.
Glacial acetic acid	Ph.Eur. Tested in-house to Ph.Eur.
Sodium hydroxide	Ph.Eur. Tested in-house to Ph.Eur.
Ethanol	BP. Tested in-house to BP.

(2) Processing reagents (not formulation ingredients), not described in a Pharmacopoeia

Tris (hydroxymethyl)aminomethane	Reagent grade
Ethylene glycol	Reagent grade
Imidazole	Reagent grade
TNBP	Reagent grade
MAb-Sepharose - see Appendix IIC/8-/10	Baxter
Q-Sepharose - see Annex IIC 3.2	Kabi-Pharmacia

Specifications and in-house testing of non-pharmacopoeial reagents are consistent with the manufacture of a product for intravenous injection and are described in Annex IIC 2.2 to IIC3.2.

(For National Authority Use Only) COMMENTS

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIC: CONTROL OF STARTING MATERIALS. PACKAGING MATERIAL (IMMEDIATE PACKAGING)

Specification and routine tests: Volume 3 Page(s)	(For National Authority Use Only) COMMENTS
Container Glass vial, type 1 Ph.Eur., injection bottle, 30 ml. Tested in-house only by visual and dimensional inspection for defects, to BS 6001. Closure Bromobutyl rubber freeze-drying stopper, Ph.Eur. type 1. Tested in-house to Ph.Eur. VI 2.3.1 for aqueous extractables and infra-red spectrum. Overseal Flip-off aluminium and propylene seal. Transfer needle IV_2 20 gauge needle (Sherwood) registered under DoH Sterile Devices Scheme as RO043SP. Purchased to manufacturer's compliance statement. Administration set Supplied separately to the product. Previously described in PL 08801/0015 and described in Annex IIC.5.5. Sterile Water for Injections - Ph.Eur. Purchased from Phoenix Pharmaceuticals, product licence number PL 1502/0003. Tested in-house for nominal volume and to monograph Sterilised Water for Injections - Ph.Eur.	
Summary of development studies on packaging materials: Volume Page(s)	
Batch analysis (Analytical results) Volume Page(s)	

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IID: CONTROL TESTS ON INTERMEDIATE PRODUCTS:

Volume 3 Page(s)

(For National Authority Use Only) COMMENTS

The only storage of "intermediates" is as cryoprecipitate or (optionally, and as validated in IIB.3.1.15) as Q-Sepharose eluate. It is difficult to procure a representative sample of cryoprecipitate and no control tests are specified. The pooled Q-Sepharose eluate, pre-diluted in a stabilising solution, is assayed for FVIII potency, providing the basis for calculation of final potency adjustments before sterile filtration.

PART IIE: CONTROL TESTS ON THE FINISHED PRODUCT:

Volume 3 Page(s)

Each batch of Replenate is released against the specification in Format 17.2.

In addition, the following typical concentrations of residual substances were found during characterisation studies [IIB3.2.3 and 3.2.4], but do not form part of the specification for release:

Substance	Quantity found
Glycine, mM	0.1 - 1.9
Imidazole, mM	<0.03
Fibrinogen, ng/iu FVIII	0.8 - 1.6
Fibronectin, ng/iu FVIII	0.6 - 0.8
vWF:Ag/Factor VIII, iu/iu	0.022 - 0.033

Product specification & control methods

The analytical specification follows in Format 17.2. Control tests are described in Annex IIE.2. The specification and test methods satisfy the requirements of the monograph Human Coagulation Factor VIII, Ph.Eur. except that moisture content is determined by Karl Fischer titration rather than loss of weight on drying.

Identification & determination of excipients

The following ingredients may be considered to be excipients and their concentrations in the product form part of the release specification of each batch of Replenate: Albumin, polyethylene glycol, L-histidine, calcium chloride, sodium chloride.

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Bio Products Laboratory		
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

Control Tests on The Finished Product

Test	Compliance Reference	Limit
Sterility	Ph.Eur.	Pass
Pyrogenicity, °C/n rabbits	Ph.Eur.	Pass
Abnormal Toxicity	Ph.Eur.	Pass
HBsAg	Ph.Eur.	Pass (none detected)
Anti-HIV (1 and 2)	BPL (>Ph.Eur.)	Pass (none detected)
Description	Ph.Eur.	Complies
Solubility 20°C, min	Ph.Eur.	≤1
Appearance of Solution	Ph.Eur.	Complies
Clarity of solution, NTU	BPL	≤17
Stability 20°C, h	Ph.Eur.	≥3
pH at 20°C	Ph.Eur.	6.8-7.4
Factor VIII potency	Ph.Eur.	80%-125% of label
Factor VIII potency, iu/ml	BPL (>Ph.Eur.)	≥20
		≥40
		≥80
Factor VIII potency, iu/vial	Ph.Eur.	200-312.5
		400-625
		800-1250
Total protein, g/L	BPL	7.5-12.5
Albumin, g/L	BPL	7.2-12.5
Specific Activity, iu/mg protein	BPL (>Ph.Eur.)	≥1.6
		≥3.2
		≥6.4
Sodium ion, mmol/L	BPL	125-160
Chloride ion, mmol/L	BPL	≥180
Calcium, mmol/L	BPL	3.4-4.6
Histidine, mmol/L	BPL	40-60
Triton X-100, ng/iu FVIII	BPL	≤25
TNBP, ng/iu FVIII	BPL	≤12.5
Ethylene glycol, µg/iu FVIII	BPL	≤0.5
Polyethylene glycol, mg/ml	BPL	0.7-1.1
Moisture content, % w/w	Ph.Eur.	≤2.0
Mouse IgG, ng/iu FVIII	BPL	≤0.01
Human Identity	Ph.Eur.	Human (positive)
Haemagglutinins	Ph.Eur.	Negative at 1:32

The only differences between the three product presentations is in their potency.

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIE: CONTROL TESTS ON THE FINISHED PRODUCT - SCIENTIFIC DATA

Summary of analytical development & validation studies: Volume 3 Page(s)	(For National Authority Use Only) COMMENTS
The test methods are described in Annex IIE.2 and their validation in IIE.3.	
BATCH ANALYSES (Page(s))	

Batch Tested:	FHD	FHE	FHE	FHE	FHE
Batch (lot) number:	4235	4244B	4247A	4250	4252
Date(s) of manufacture (assayed)	20/8/93	13/9/93	24/9/93	4/10/93	8/10/93
Place(s) of manufacture:	BPL	BPL	BPL	BPL	BPL
Batch size: (vials)	3628	1257	1933	3284	1768
Use of batch:	<——— Clinical ———>				

Results of Batch Analyses: Page(s)

Critical results are summarised here:

Tests:

Biological Safety Tests:	Pass	Pass	Pass	Pass	Pass
Factor VIII Potency, iu/ml	24.8	48.9	48.6	55.5	114.3
Specific Activity, iu/mg	2.6	5.1	4.9	4.7	10.9

Reference Standard

Name of Company Bio Products Laboratory	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product: Replenate		
Name of Active Ingredient: Human Factor VIII		

PART IIE: VALIDATION OF TEST METHODS

Summary of analytical development & validation studies: Volume 3 Page(s)

(For National Authority Use Only) COMMENTS

The most important data for critical evaluation of experimental studies and product specification are summarised here.

Test Method	LOD(1)	Precision(2) CV%	Reproducibility(3) CV%
Clarity	0.02 NTU	0.75	0.83
HBsAg	0.1 iu/ml	-	-
Factor VIII	10^{-4} iu/ml	5	13
Albumin	-	2.63	1.53
Sodium	-	0.183	0.896
Chloride	-	0.82	0.95
Calcium	-	2.07	6.24
Histidine	-	1.09	3.33
PEG	0.05mg/ml	4.9	14
Triton X-100	0.45ng/ml	8.8	17.6
TNBP	0.11ng/ml	7.4	21.5
Ethylene glycol	3.3µg/ml	9.3	7.5
Mouse IgG	0.4 ng/ml	5	5
Moisture	0.228%	1.96	3.6
LAL	0.01EU/ml	-	-
pH	-	0.05	0.05

(1) Limit of Detection
(2) Within assay Relative Standard Deviation (CV)
(3) Between-assay Relative Standard Deviation (CV)

Results of Batch Analyses: Page(s)

Reference Standard

Consistency of Manufacture

	FHD4235	FHE4244B	FHE4247A	FHE4250	FHF4252
Sterility, Pass	Pass	Pass	Pass	Pass	Pass
Pyrogenicity, °C/n-Pass	0.28/3, Pass	0.24/3, Pass	0.7/3, Pass	0.65/3, Pass	0.65/3, Pass
Abnormal toxicity, Pass	Pass	Pass	Pass	Pass	Pass
HBsAg, Pass	Pass	Pass	Pass	Pass	Pass
Anti-HIV, Pass	Pass	Pass	Pass	Pass	Pass
Description, Complies	Complies	Complies	Complies	Complies	Complies
Solubility 20°C, min	<0.5	<0.5	<0.5	<0.5	<0.5
Appearance of Solution Complies	Complies	Complies	Complies	Complies	Complies
Clarity, NTU	2.9	6.2	5.2	3.9	7.3
Stability 20°C, h	>3	>3	>3	>3	>3
pH at 20°C	7.0	7.1	6.9	7.0	7.0
Factor VIII, % of label	100	98	97	111	114.5
Factor VIII, iu/ml	24.8	48.9	48.6	55.5	114.3
Factor VIII, iu/vial	250	490	485	555	1145
Total Protein, g/L	9.7	9.5	10.0	11.9	10.5
Albumin gl/L	9.6	9.5	9.8	11.8	10.4
Specific Activity, iu/mg	2.6	5.1	4.9	4.7	10.9
Sodium ion, mmol/L	142	135	145	144	136
Chloride ion, mmol/L	157	146	155	154	146
Calcium, mmol/L	3.83	3.80	4.08	4.03	4.15
Histidine, mmol/L	50.8	49.4	55.2	55.4	55.4
Triton X-100, ng/iu FVIII	<20.2	<10.2	<10.3	<9.0	<4.4
TNBP, ng/iu FVIII	<8.06	<4.09	<4.12	<3.60	<1.75
Ethylene glycol, µg/iu FVIII	<0.4	<0.2	<0.21	<0.18	<0.09
PEG, mg/ml	0.93	0.80	0.95	0.90	0.90
Moisture, % w/w	<0.03	<0.03	<0.03	<0.03	<0.03
Mouse IgG, ng/iu FVIII	<0.01	<0.01	<0.01	<0.01	<0.01
Human Identity, Positive	Positive	Positive	Positive	Positive	Positive
Haemagglutinins, Pass	Pass	Pass	Pass	Pass	Pass

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIF: STABILITY TESTS ON THE FINISHED PRODUCT

Replenate Batches Tested

BATCH NUMBER	NOMINAL POTENCY	FILL DATE	AGE AT START OF TRIAL, m	PROCESS VARIABLES	REPORT STATUS, m
Group 1 batches;					
FHD4143	250iu	23/4/93	1 m	40-CV wash 80kg cryo	6 m
FHD4199	500iu	26/3/93	2 m	40-CV wash 130kg cryo	6 m
FHF4222	1000iu	11/6/93	.5 m	40-CV wash frozen Q-eluate	4.5 m
Group 2 batches:					
FHF4244A	1000iu	10/9/93	1 m	48-CV wash 130kg cryo	1.5 m
FHF4235	500iu	20/8/93	2 m	48-CV wash 80kg cryo	1.5 m
FHD4244B	500iu	13/9.93	1 m	48-CV wash frozen Q-eluate	1.5 m

Stability Study Methods

Real time Studies (Temperature °C, % RH, Light)

4°C protected from light, external RH uncontrolled, vials under vacuum. Controls stored -40°C. Planned duration 36 months.

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

STABILITY TESTS ON THE FINISHED PRODUCT

Studies under other conditions

Stress Temperatures 25°C, 37°C and 56°C.

Characteristics studied

Physical: Description, solubility, appearance of solution, stability of solution, clarity, pH.
Microbiological: None.
Chemical: factor VIII potency (14 other parameters at longer intervals, see IIF4.1).
Packaging: None.

Evaluation Methods and Validation

All characteristics listed above were evaluated by Test Methods applied to final products, described with validations in Annexes IID and IIE.

Results of Tests

No significant change of factor VIII potency or other measured characteristics after 1.5 - 6 months at 4°C, 25°C or 37°C. Only one batch, FHE4199, shows a significant change of potency at 56°C, corresponding to a loss of 0.01% factor VIII potency per year at 8°C (Arrhenius equation).

Interpretation of Results

There is no evidence of significant change in Replenate stored at 4-37°C for up to 6 months. Data on related products (IIF-3) suggest that there will be no abrupt deterioration for up to 24-36 months at these temperatures, sufficient to constitute non-compliance with the original specification limits.

Shelf-life

24 months at 2-8°C, protected from light. 1h at RT after reconstitution.

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIF: STABILITY TESTS ON THE FINISHED PRODUCT

SUMMARY CHARACTERISTICS OF PRODUCTS FOR WHICH STABILITY STUDIES ARE PRESENTED

CHARACTERISTIC	HEMOFIL-M	OCTONATIV-M	TYPE 8SM	Replenate^{tm}
Manufacturer	Baxter	Kabi	BPL (Kabi)	BPL
Site	Glendale	Stockholm	Stockholm	Elstree
Plasma source	US	Sweden	UK	UK
Rec Vol, ml (250iu)	10ml	n/a	5ml	10ml
Rec Vol, ml (500iu)	10ml	5ml	5ml	10ml
Rec Vol, ml (1000iu)	10ml	10ml	10ml	10ml
MAb Column wash	40CV	40CV	40CV	40CV
				48CV
Albumin source	Baxter	Kabi	BPL HAS	
			BPL Zenalb	BPL Zenalb
Shelf-life claim	24m	36m	36m	24m
Annex Reference	IIF.1	IIF.2	IIF.3	IIF.4

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIH: OTHER INFORMATION Volume

1. Batch Analysis Data (IIH.1) 2 Page(s)

A detailed description is given of the properties of all batches of Hemofil-M, Octonativ, 8SM and Replenate used in clinical trials and stability trials quoted in this application, whether as primary or supporting evidence.

Minor differences between these products, affecting the specification or the performance of individual batches against specification, are described. Where the specifications (e.g. of limit concentrations of residual reagents) of related products are expressed in different units, conversion factors are presented to facilitate comparisons.

2. Virus inactivation and removal (IIH.2)

2.1 Virus inactivation by controlled incubation with TNBP and Triton X-100

The highest achievable titres of selected viruses were "spiked" into cryoprecipitate supernatant, incubated under the virus inactivation conditions specified for Hemofil-M, and residual concentrations infective viruses determined on appropriate dilutions of the incubate, at selected intervals. Controls ensured that quantitation of infectivity was not inhibited by residual reagents.

Comment - (For National Authority Use Only)

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIH: OTHER INFORMATION Volume

i) Batch Analysis Data (IIH1) 2 **Page(s)**

Virus inactivation and removal (IIH.2)

2.1.1 New studies on Replenate (IIH2.1)

Virus Studied: Sindbis

Results

(1) 4-6 logs of Sindbis were completely inactivated within 15 seconds of incubation, under the conditions specified for Replenate.

(2) The rate of inactivation of Sindbis was not significantly affected by varying critical parameters, within the following ranges around the values specified for Replenate. (IIH2.1.1.1).

Condition	*Process Limits*	*Rates unchanged within Range*
TNBP, % v/v	±0.25	0.15-0.60
Triton X-100, % v/v	±0.80	0.5-2.0
Temperature, °C	18-26	15-30
Incubation Time, min.	±30	±2
Protein, A280	5-35	3-42
pH	6.5-7.5	5.8-7.8
Conductivity, mS/cm	55-75	32-111

(3) Appropriate scaling down of production-scale virus inactivation to these experiments in the Virology Research Lab. was validated by showing that solvent/detergent addition to cold supernatant was equally effective in inactivating Sindbis, whether the mixing was done in the production plant at full scale or in the Virus Research Lab. on a small sample of the same supernatant.

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIH: OTHER INFORMATION Volume

Virus inactivation and removal (IIH.2)

2.1.2 Earlier studies on Octonativ/8SM process (IIH2.2), quoted in PL 08801/0015

Viruses studied
Vesicular stomatitis virus (VSV)
Human immunodeficiency virus-1 (HIV-1)

Results
(1) 3.5 logs of VSV were completely inactivated within <1 minute, under the conditions specified for the 8SM process.
(2) 6.2 logs of HIV-1 were completely inactivated within <1 minute, under the conditions specified for the 8SM process.

2.1.3 Earlier studies on Hemofil-M process (IIH2.3), quoted in PL 08801/0015

Viruses studied
Vesicular stomatitis virus (VSV)
Sindbis
Human immunodeficiency virus-1 (HIV-1)
Human immunodeficiency virus-2 (HIV-2)
Pseudorabies virus (PRV)
Encephalomyocarditis virus (EMC)

Results
(1) In experiments carried out using appropriate concentrations of Triton X-100 and TNBP, Sindbis and VSV in a Hemofil-M intermediate were inactivated by >4 logs, as predicted by New York Blood Centre, the originators of the method (IIH2.3.2A).

(2) 5.2 logs of Sindbis were completely inactivated in 3 min of incubation under the conditions specified for Hemofil-M (IIH2.3.2.A).

(3) 3.3-11 logs of five lipid-enveloped viruses (VSV, Sindbis, HIV-1, HIV-2 and PRV) were completely inactivated within 1 min of incubation, under the conditions specified for Hemofil-M. The protein-enveloped virus EMC was unaffected (IIH3.32B, G, K).

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIH: OTHER INFORMATION Volume

Virus inactivation and removal (IIH.2)

2.2 Virus clearance during immuno-affinity (MAb) and Q-Sepharose chromatography

In laboratory-scale experiments, the highest achievable titres of several viruses were "spiked" into samples corresponding to those applied to chromatography columns in the production process. (In order to provide a high challenging titre of viruses, TNBP and Triton X-100 were omitted from the cold supernatant in most of these experiments; otherwise, the protein and ionic conditions were comparable). Virus titres were measured on the flow-through/wash fractions and the eluted factor VIII fraction.

2.2.1 New studies on Replenate process (IIH2.1.2)

Viruses studied: Sindbis, Polio (not lipid-enveloped)

Results
Immuno-affinity chromatography achieved reductions of 3.7 - 4.2 logs Sindbis and 4.9 logs polio. The small-scale chromatographic model was shown to clear fibrinogen by a similar factor to that achieved in routine production.

2.2.2 Earlier studies on Octonativ/8SM process (IIH2.2.2)

Virus studied: VSV

Results
$10^{9.5}$ TCID50 of VSV were applied to the MAb column. Recovery of VSV in the F.VIII eluate indicated clearances of 4.3 and 6.3 logs in two experiments.

Name of Company	Summary Table Referring to Part 2 of the Dossier	(For National Authority Use Only)
Name of Finished Medicinal Product:		
Name of Active Ingredient: Human Factor VIII		

PART IIH: OTHER INFORMATION Volume

Virus inactivation and removal (IIH.2)

2.2.3 Earlier studies on Hemofil-M process (IIH2.3.2)

Viruses studied

EMC, Sindbis, VSV, HIV-1

Results

(1) On scaled-down MAb chromatography, the clearance of HIV was estimated by p24 antigen as 3 logs, and by infectivity assay as 4 logs. Ethylene glycol in the eluting buffer made a minor contribution to the inactivation of HIV.
(2) On scaled-down MAb chromatography, clearances of non-lipid-enveloped EMC were 8.2 logs - 8.4 logs in two experiments.
(3) On scaled-down MAb chromatography, clearance factors for lipid-enveloped viruses Sindbis and VSV were 4.3 logs and 2.4 logs respectively.
(4) On scaled-down Q-Sepharose chromatography, clearance of Sindbis was 2 logs and clearance of EMC was 2.4 logs.

Summary of Product Characteristics

1. **Name of Medicinal Product**

REPLENATE™.

2. **Qualitative and Quantitative Composition**

2.1 Qualitative Composition

REPLENATE™ is a high purity human Factor VIII stabilised by buffers and albumin.

2.2 Quantitative Composition

a. There are three product strengths:

The 250i.u. nominal has a potency range of 200-312.5i.u./vial.
The 500i.u. nominal has a potency range of 400-625i.u./vial.
The 1000i.u. nominal has a potency range of 800-1250i.u./vial.

b. REPLENATE™ has a specific activity of

≠ 1.6 i.u./mg of protein (250 i.u. vial)
≠ 3.2 i.u./mg of protein (500 i.u. vial)
≠ 6.4 i.u./mg of protein (1000 i.u. vial)

3. **Pharmaceutical Form**

This product is manufactured from venous plasma obtained from voluntary, unpaid donors in the United Kingdom.

REPLENATE™ is presented as freeze-dried powder for reconstitution with 10ml of supplied Sterilised Water for Injections, Ph.Eur. The reconstituted product is administered intravenously.

4. **Clinical Particulars**

4.1 Therapeutic Indications

REPLENATE™ is used in the treatment of haemophilia A for the prevention and control of haemorrhage. REPLENATE™ is not indicated for the treatment of von Willebrand's disease.

4.2 Posology and Method of Administration

4.2.1 Posology

There is considerable variation in response between patients. It is recommended that the following guidelines be adopted.

The number of units needed and the duration of treatment depend on the condition being treated. If the rise in the concentration of

Factor VIII in the plasma following administration of concentrate is expressed in International Units per 100ml plasma and the total dose given in International Units of Factor VIII per kg body weight is calculated, "the response" is defined as follows:

$$\text{Response} = \frac{\text{rise in plasma Factor VIII (in i.u. per 100ml)}}{\text{dose in i.u. kg body weight}}$$

The "theoretical value" of 2.4 for this ratio is rarely reached. It is variable even in the same patient; a range of 1.6-2.2 is usual but values outside this range may be found. A low value may indicate that the patient's plasma contains an antibody to Factor VIII and appropriate tests should be done.

The following table indicates the approximate levels of Factor VIII required for haemostasis in various circumstances.

Condition	Concentration of Factor VIII desirable in plasma immediately after injection (i.u. per 100ml)	Initial dose of Factor VIII (i.e./kg body weight)
Minor spontaneous haemarthrosis and muscle haematoma	30	15
Severe haemarthrosis and muscle haematoma, haematoma in potentially serious situations; haematuria	40 - 50	20 - 25
Major surgery	See below	

A dose of 1 i.u./kg will give, on average, a rise of about 2 i.u./100ml plasma. If the desired concentration or clinical response is not achieved another dose should be given the same day. If an abnormally low response persists, test for specific antibody to Factor VIII. The doses mentioned are only rough guides since there is considerable variation in response from patient to patient. It is usual to give the contents of the number of whole vials nearest to the calculated dose. Doses may be repeated at intervals of approximately 8, 12 or 24 hours as needed to maintain the desired concentration of Factor VIII.

Dosage in Children

In the case of children a dose of 1 i.u./kg will possibly give a reduced rise, on average, of about 1.5 i.u./100ml plasma.

Dosage in Major Surgery:

Major surgery should be undertaken in a centre which has facilities for assaying Factor VIII for ascertaining the patient's response to treatment. The patient's plasma should be tested for Factor VIII antibodies. If antibody is not present, a pre-operative dose of 35 to 50 i.u. per kg is given to raise the plasma Factor VIII concentration to 80 i.u. or more per 100ml plasma. During the first few days after operation the plasma Factor VIII concentration is monitored and the dose repeated 6-hourly or 8-hourly as needed, so that the concentration does not fall below 30-50 i.u. per 100ml plasma. After the first few days the frequency of the dose may be reduced. The course of treatment is usually continued for ten days or longer.

As indicated previously, if the Factor VIII concentration does not reach the expected level, or falls off with a reduced half-disappearance time (less than 12 hours), the presence of an antibody to Factor VIII should be suspected and the appropriate laboratory tests done. The treatment of patients with antibodies to Factor VIII is outside the scope of these notes.

DO NOT EXCEED RECOMMENDED DOSE.

Administration

Reconstitute before administration, as instructed in 6.6. The reconstituted solution should be administered immediately or at least within one hour of reconstitution. After cleaning the stopper with a spirit swab the solution should be drawn from the vial into a plastic disposable syringe (approved syringes are Becton Dickinson, Sabre and Terumo) through the sterile filter needle supplied which will remove particulate matter. For administration a Number 23 "butterfly" needle (Abbott venisystem) is approved for use with this product. Inject the product intravenously up to a rate of 10ml per minute.

The pulse rate should be monitored before and during infusion and if a significant increase occurs the rate of infusion should be slowed or temporarily halted until the pulse is normal.

Patients who are to receive the contents of more than one vial may pool these contents into an appropriate size syringe by drawing up the contents of each vial through a separate sterile filter needle. Sterile filter needles are intended to filter the contents of a single bottle of REPLENATE™.

4.3 Contra-indications:

Known hypersensitivity to mouse protein is a contra-indication to the use of REPLENATE™.

4.4 Special Warnings and Special Precautions for Use

If allergic or anaphylactic reactions occur the injection/infusion

should be stopped immediately and the reaction treated according to guidelines on shock therapy.

REPLENATE™ contains blood group antibodies derived from the starting plasma in amounts which are insignificant in the normal treatment of haemarthrosis and muscle haemorrhage. If large doses are necessary in patients with blood groups A, B or AB, the patient should be monitored for signs of intravascular haemolysis.

Patients congenitally deficient in Factor VIII may develop antibodies to Factor VIII after treatment. This risk does not appear to be significantly increased by the use of REPLENATE™, but patients should be monitored as a routine procedure. Failure to achieve the anticipated clinical response may indicate the development of Factor VIII antibodies. Patients undergoing major surgery should be tested for Factor VIII antibodies prior to surgery. The presence of antibodies requires specialist advice and clinical management.

Where possible, Factor VIII assays should be performed before and after infusion, particularly in the first course of treatment.

4.5 Interaction with Other Medicaments and Other Forms of Interaction

No interactions of REPLENATE™ with other medicinal products are known so far.

4.6 Pregnancy and Lactation

The safety of REPLENATE™ for use in human pregnancy has not been established in controlled clinical trials. Experimental animal studies are insufficient to assess the safety with respect to reproduction, development of the embryo or fetus, the course of gestation, and peri and post-natal development. Therefore REPLENATE™ may only be used if clearly needed during pregnancy and lactation (category B2).

4.7 Effects on Ability to Drive and Use Machines

There are no indications that REPLENATE™ may impair the ability to drive or to operate machines.

4.8 Undesirable Effects

Apart from specific hypersensitivity reactions to mouse protein already described in the "Contra-indications" section, only occasional side effects have been reported, which include flushing, nausea, increased pulse rate and headache; these are controlled by slowing or stopping infusion. Patients should be aware of the early signs of hypersensitivity reactions including hives, urticaria, tightness of the chest, wheezing, hypotension and anaphylaxis, and are advised to discontinue use of the product. In some patients antibodies to Factor VIII may develop, in these cases the efficacy of the product will diminish. Any side effect must be reported to a doctor.

REPLENATE™, high purity Factor VIII, is manufactured from human venous plasma. All plasma donations are tested by validated procedures and found non-reactive for hepatitis B surface antigen and antibodies to HIV-1, HIV-2 and HCV. The process includes an organic solvent/detergent viral inactivation step which is effective against enveloped viruses such as HIV, hepatitis B and Hepatitis C. The process also includes a chromatographic step which is effective in removal of both enveloped viruses and non-enveloped viruses, like hepatitis A. However, the risk of infection from blood-borne viruses cannot be entirely excluded.

In patients receiving replacement therapy with coagulation factors by concentrates derived from human plasma it is recommended that, where relevant, the patient's immune status to blood-borne viruses is determined by the treating physician with a view to taking appropriate action.

4.9 Overdose

No specific side effects have been reported with overdosage of REPLENATE™ since the short half-life of Factor VIII, about 12 hours, means the Factor VIII:C activity in the patient's plasma will rapidly decline.

5. Pharmacological Properties

5.1 Pharmacodynamic Properties

REPLENATE™ is a high purity Factor VIII with no significant associated von Willebrand's Factor.

Factor VIII is responsible for the coagulation activity. As a cofactor for factor IX it accelerates the conversion of Factor X to activated Factor X. Activated Factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot can be formed. The Factor VIII activity is greatly reduced in patients with haemophilia A and therefore substitution therapy is necessary.

5.2 Pharmacokinetic Properties

After injection of the product approximately two thirds to three quarters of the Factor VIII remain in the circulation. The level of Factor VIII activity reached in the plasma should be between 80%-120% of the predicted plasma Factor VIII activity.

Plasma Factor VIII activity decreases by a two-phase exponential decay. In the initial phase, distribution between the intravascular and other compartments (body fluids) occurs with the half-life of elimination from the plasma of 3 to 6 hours. In the subsequent slower phase (which probably reflects the consumption of Factor VIII) the half-life varies between 8-20 hours, with an average of 12 hours. This appears to correspond to the true biological half-life.

5.3 Preclinical Safety Data

Human plasma coagulation Factor VIII (from REPLENATE™) is a normal constituent of the human plasma and acts like the endogenous Factor VIII. Single dose toxicity testing is of no relevance since higher doses result in overloading.

Repeated dose toxicity testing in animals is impracticable due to interference with developing antibodies to heterologous protein.

Even doses of several times the recommended human dosage peer kilogramme body weight show no toxic effects on laboratory animals.

Since clinical experience provides no hint for tumourigenic and mutagenic effects of human plasma coagulation Factor VIII, experimental studies, particularly in heterologous species, are not considered imperative.

6. Pharmaceutical Particulars

6.1 List of Excipients

Each vial contains approximately, 77.5mg L-histidine, 10mg polyethylene glycol, 4.4mg calcium chloride, 83mg sodium chloride and 100mg human albumin added as a stabilising agent. The product contains less than 0.01ng of mouse protein per i.u. of Factor VIII.

6.2 Incompatibilities

REPLENATE™ should not be mixed with other medicinal products as their effects have not been established. Only approved injection/infusion sets should be used because efficacy may be reduced due to adsorption of Factor VIII to the internal surfaces of some unapproved infusion equipment.

6.3 Shelf Life

Unopened stored at 2°C-8°C	2 years
Unopened stored at Ambient (25°C)	up to 4 weeks
Opened	1 hour

6.4 Special Precautions for Storage

Sterilised Water for Injections, Ph.Eur. should be stored between 2°C and 25°C and must not be used beyond the expiry date printed on the label or if signs of particulate matter are visible.

REPLENATE™ should be stored between 2°C and 8°C in its carton in the dark, however, it may be stored for up to 4 weeks at ambient temperatures (25°C). REPLENATE™ must not be used beyond the expiry date printed on the label. Where REPLENATE™ is for home use, a domestic refrigerator is suitable for storage.

6.5 Nature and Contents of Container

REPLENATE™ is supplied in single dose vials of either 250 i.u., 500 i.u. or 1000 i.u. nominal for reconstitution in 10ml of Sterilised Water for Injections, Ph.Eur. supplied with the product. Supplied in packs of 10 x REPLENATE™ vials and 10 x Sterilised Water for Injections, Ph.Eur. vials. Single vials and home-treatment packs are also available.

The vial is made of neutral glass, Type I, Ph.Eur. stoppered with a synthetic rubber stopper and oversealed with a tamper proof ring.

6.6 Instructions for Use/Handling

Reconstituted REPLENATE™ should be used within one hour. Sterilised Water for Injections, Ph.Eur., is only to be used for reconstituting REPLENATE™ and must not be injected on its own.

The container of Factor VIII concentrate and the Sterilised Water for Injections, Ph.Eur. should be brought to between 20°C and 30°C, prior to removal of the "flip-off" closures. Remove the caps from the concentrate and Sterilised Water for Injections, Ph.Eur. and clean stoppers with a spirit swab. Either of the following methods of reconstitution can then be used:

a) Using a sterile disposable needle and syringe draw up a 10ml volume of Sterilised Water for Injections, Ph.Eur. and transfer to the vial of REPLENATE™. On piercing the seal of the REPLENATE™ vial, the water will be drawn into the vial which is under vacuum.

NB THE FILTER NEEDLE PROVIDED MUST NOT BE USED TO DRAW UP THE WATER FOR INJECTIONS.

or

b) Remove the cover guard from one end of a double ended transfer needle and insert through the stopper into the vial of Sterilised Water for Injections, Ph.Eur. Remove the other end of the needle guard, invert the water vial over the product vial and insert the free end of the needle through the stopper into the vial of REPLENATE™. On piercing the seal of the REPLENATE™ vial the water will be drawn into the vial, which is under vacuum. A small amount of water will remain in the water vial.

If the water to be used for reconstitution is not drawn into the vial containing the REPLENATE™, this indicates loss of vacuum. If the vial does not contain a vacuum or if the reconstituted REPLENATE™ forms a gel or a clot, the vial must not be used.

The container should be agitated to wet the product and the vacuum then released by either:

a) Removing the syringe from the needle before removing the needle from the product vial.

or

b) Disconnecting the two vials by first removing the transfer needle from the water vial and then removing the transfer needle from the product vial.

Continue to agitate gently until dissolution is complete. A clear or slightly opalescent solution should be obtained within 15 minutes. If a gel, or clot forms, discard the vial.

Any used materials, unused solution or any unused Sterilised Water for Injections, Ph.Eur. should be discarded by approved means.

6.7 Name and Address of the Holder of the Market Authorisation

BPL, Bio Products Laboratory
Dagger Lane
ELSTREE
Herts, UK
WD6 3BX

Tel: 081 905 1818.

7. **Market Authorisation Number**

REPLENATE™ POM

Product Licence Number: PL 8801/0031-0033.

Sterilised Water for Injections, Ph.Eur. PL 1502/0003, supplied by Phoenix Pharmaceuticals Ltd.

8. **Date of Approval/Revision of SPC**

May 1994.

I:SOPC.REPLENAT

Application for a marketing authorization of a medicinal product

(continued)

Part I A; Administrative data

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1. Proposed name of the medicinal product in the concerned member state:

   If different names in different member states are proposed in a Community Procedure, these should be listed:

Country:	Name:
UK	REPLENATE, a high purity Factor VIII

1.1. **Name of the active ingredient(s)** (INN, Ph. Eur., National Pharmacopoeia, trivial name and chemical description)

Freeze-Dried Human Coagulation Factor VIII

1.2. **Pharmacotherapeutic classification** (use ATC classification system, WHO Collaborating Centre for Drug Statistics Methodology)

B02B

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2. **Pharmaceutical form and strength** (Please use CPMP Standard Classification according to III/3593/91):

Powder for reconstitution for injection, nominal 250 iu / vial

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2.1 **Route of administration** (Please use CPMP Standard Classification according to III/3593/91):

Intravenous use

2.2 **Container and closure** (Please use CPMP Standard Classification according to III/3593/91):

2.2.1 **Package sizes** nominal 250 iu in a 30 ml glass vial, Type 1 glass Ph.Eur.

2.2.2 **Shelf life** 24 months

2.2.3 **Shelf life (after first opening of container)**
one hour

2.2.4 **Shelf life (after reconstitution)**
one hour

2.2.5 **Storage conditions** 2°-8°C in the dark

Application for a marketing authorization of a medicinal product

(continued)

2.3 Way of dispensing/classification proposed by the applicant

o without prescription*

- o promotion intended to the general public
- o promotion intended to physicians only

o for prescription only

- ✓ medicinal products on prescription, which may be renewed
- o medicinal products on prescription, which may not be renewed
- o medicinal products on special prescription
- o medicinal products on restricted prescription, reserved for certain locations with specific equipment

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3. **Applicant (= proposed marketing authorization holder/proposed person responsible for placing the product on the market):**

Name: BPL, Bio Products Laboratory.
Address: Dagger Lane, Elstree, Herts. WD6 3BX. UK.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.1 **Person from the applicant responsible for technical data of the dossier (where required):**

Name: Dr. T. J. Snape
Address: Bio Products Laboratory, Dagger Lane, Elstree, Herts. WD6 3BX.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.2 **The following person is authorized for communication on behalf of the applicant during the procedure:**

Person of contact: Dr. A.J.West-Watson, Manager Regulatory Affairs,
Address: Bio Products Laboratory, Dagger Lane, Elstree,Herts.WD6 3BX.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

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* For applications submitted to Germany, Ireland, the Netherlands and the United Kingdom, applicants must specify whether they wish to obtain a general sale or pharmacy only licence.

date 1/2/94

Application for a marketing authorization of a medicinal product

(continued)

3.3 Address for communication and commercialization after licensing if different from 3./3.2

Name:
Address: as for 3.2
Country:
Telephone:
Telefax:

3.4 Manufacturer of the finished product

Name: BPL,Bio Products Laboratory,
Address: Dagger Lane, Elstree, Herts. WD6 3BX.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.4.1 Other sites of manufacture (name and site(s) of the manufacturer(s)).

For each manufacturer and manufacturing site, please give:

Name: NONE
Address:
Country:
Telephone:
Telefax:

3.4.2 Storage

Name: BPL Bio Products Laboratory,
Address:Dagger Lane, Elstree, Herts. WD6 3BX
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.5 Other manufacturer(s):
(including a description of the steps they perform)

NONE

Name:
Address:
Country:
Telephone:
Telefax:
Steps performed:

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date. 1/2/94

Application for a marketing authorization of a medicinal product

(continued)

3.5.1 Other sites of manufacture of other manufacturers (name and site(s) of the manufacturer(s)).

For each manufacturer and manufacturing site, please give:

Name: NONE
Address:
Country:
Telephone:
Telefax:

3.6 The manufacturer/importer responsible for batch release in the EEC.

Name: BPL,Bio Products Laboratory,
Address: Dagger Lane , Elstree, Herts. WD6 3BX.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.6.1 Qualified person responsible for batch release in the EEC.

Name: DR.T.J.Snape
Address: Bio Products Laboratory,Dagger Lane,Elstree,Herts. WD6 3BX.
Telephone: 081 905 1818
Telefax: 081 207 4824

3.6.2 Site where batch release takes place.

Name: Bio Products Laboratory
Address: Dagger Lane ,Elstree,Herts. WD6 3BX.
Country: UK
Telephone: 081 905 1818
Telefax: 081 207 4824

3.7 Manufacturer(s) of the active ingredient(s):

Not Applicable as the Active Ingredient is Plasma

Name:
Address:
Country:
Telephone:
Telefax:
Drug Master File ref. (Pharm. Eur. Certificate No.:]
Date of submission:

date. 12/94

Application for a marketing authorization of a medicinal product

(continued)

3.8 Contract companies used in development:

For each contract company, please give:

Name: NONE
Address:
Country:
Telephone:
Telefax:
Duty performed according to contract:

4. Marketing applications for this medicinal product in the EEC (i.e. from the same company or daughter/sister/mother company of the same corporation/holding company or licencee containing the same active ingredient for a comparable indication):

Authorized:	country: date of authorization: authorization number: product name:	NONE
Pending:	country: date of submission: application number:	NONE
Rejected:	country: date of rejection: application number:	NONE
Withdrawn (by applicant before authorization)	country: date of withdrawal: application number: reason of withdrawal:	NONE
Withdrawn (by applicant after authorization)	country: date of withdrawal: authorization number: reason of withdrawal:	NONE
Suspended/revoked/ withdrawn (by competent authority)	country: date of suspension: authorization number: reason of withdrawal:	NONE

date 1/2/94

Application for a marketing authorization of a medicinal product

(continued)

No.	Name of the	Quantity	Unit	Norm/Reference to standards
	- active ingredient(s)			
1	Human Factor VIII	250 (nominal)	iu	Ph.Eur.
	- excipient(s)			
1	Human Albumin (Zenalb-20)	100	mg	HSE
2	L-Histidine	77.5	mg	USP
3	Polyethylene glycol	10	mg	
4	Calcium chloride	4.4	mg	Ph. Eur.
5	Sodium chloride	83	mg	Ph. Eur.
6	Hydrochloric acid	0-5	mol	Ph. Eur.
7	Sodium hydroxide	0-0.5	mol	Ph. Eur.
8	Glacial acetic acid	0-1	mol	Ph. Eur.
9	Imidazole	ND		HSE
10	Ethylene glycol	less than 156.25	mcg	HSE
11	Glycine	less than 250	mcmol	Ph. Eur.
12	Triton X-100 (Octoxynol-9)	less than 7.81	mcg	USP
X 13	Tri-n-buty-phoshate	less than 3.91	mcg	HSE

Details of any overages: - these should not be included in the Formulation Columns but stated in this section.	
-	active ingredient(s)
-	excipient(s)

date. 1/2/94

92

Application for a marketing authorization of a medicinal product

(continued)

No.	Name of the	Quantity	Unit	Norm/Reference to standards
	- active ingredient(s)			
1	Human Factor VIII	500 (nominal)	iu	Ph.Eur.
	- excipient(s)			
1	Human Albumin (Zenalb-20)	100	mg	HSE
2	L-Histidine	77.5	mg	USP
3	Polyethylene glycol	10	mg	
4	Calcium chloride	4.4	mg	Ph. Eur.
5	Sodium chloride	83	mg	Ph. Eur.
6	Hydrochloric acid	0-5	mol	Ph. Eur.
7	Sodium hydroxide	0-0.5	mol	Ph. Eur.
8	Glacial acetic acid	0-1	mol	Ph. Eur.
9	Imidazole	ND		HSE
10	Ethylene glycol	less than 312.5	mcg	HSE
11	Glycine	less than 500	mcmol	Ph. Eur.
12	Triton X-100 (Octoxynol-9)	less than 15.62	mcg	USP
13	Tri-n-buty-phoshate	less than 7.81	mcg	HSE

Details of any overages: - these should not be included in the Formulation Columns but stated in this section.

-	active ingredient(s)
-	excipient(s)

Dated 16/5/94 in substitution for page 9 dated 1/2/94

Signed.. GRO-C

Application for a marketing authorization of a medicinal product

(continued)

No.	Name of the	Quantity	Unit	Norm/Reference to standards
	- active ingredient(s)			
1	Human Factor VIII	1000 (nominal)	iu	Ph.Eur.
	- excipient(s)			
1	Human Albumin (Zenalb-20)	100	mg	HSE
2	L-Histidine	77.5	mg	USP
3	Polyethylene glycol	10	mg	
4	Calcium chloride	4.4	mg	Ph. Eur.
5	Sodium chloride	83	mg	Ph. Eur.
6	Hydrochloric acid	0-5	mol	Ph. Eur.
7	Sodium hydroxide	0-0.5	mol	Ph. Eur.
8	Glacial acetic acid	0-1	mol	Ph. Eur.
9	Imidazole	ND		HSE
10	Ethylene glycol	less than 625	mcg	HSE
11	Glycine	less than 1000	mcmol	Ph. Eur.
12	Triton X-100 (Octoxynol-9)	less than 31.25	mcg	USP
13	Tri-n-buty-phoshate	less than 15.62	mcg	HSE

Details of any overages: - these should not be included in the formulation Columns but stated in this section.

	- active ingredient(s)
	- excipient(s)

Dated 16/5/94 in substitution for page 9 dated 1/2/94

Signed... GRO-C

94