INTRODUCTION

Lys-Plasminogen, vapour heated, is a sterile, freeze-dried powder intended for further processing in the production of pharmaceutical specialities. Due to the nature of the product and its intended further processing, the exact format of the Pharmaceutical Expert Report forms is not entirely appropriate. However, the recommended layout is used where possible.

The data in this Expert Report are presented in a sequential manner, starting with the source material, through the extraction and purification stages, to the sterile bulk. Since the form numbers used, differ from those suggested in MAL 2, the list of contents is summarised below, for ease of reference:

Form 8 **PART IIE - CONTROL TESTS ON THE STERILE BULK**
-Specification and Test Methods
Appearance
Identity Tests
Potency Tests
Other Tests
Identification and Quantitative Determination of Other Constituents

Form 9 **PART IIE - STERILE BULK**
-Control Tests on the Sterile Bulk
Analytical Validation

Form 10 **PART IIE - STERILE BULK**
-Batch Analytical Results

Form 11 **PART IIF - STABILITY**
-Information Concerning the Sterile Bulk
Number of batches tested
Storage conditions
Description of the containers
Results of tests
Analytical methods
Proposed shelf life and storage conditions

Pharmaceutical Expert Report
Form 1

PART IIA - COMPOSITION

Description: Page 502 (Enclosure 11)

Lys-Plasminogen is a white to off-white, sterile bulk powder, free from visible evidence of contamination.

Complete Composition: Page 150 (Enclosure 4)

The solution obtained after reconstitution with 11 of Water for Injections ~~In Bulk~~, Ph. Eur, contains: X

Active constituent

Plasminogen	100 µmol

Other constituents

Protein	10.0 g
Sodium chloride	5.84g (0.1 mol)
Sodium dihydrogen phosphate. $2H_2O$	10.3 g
Lysine	2.92g (10 mmol)
Glucose	10.0 g
Aprotinin	50,000KIU

~~pH 7.0 +/- 0.1 (by addition of 0.5N HCl or 0.5N NaOH if required).~~ X

Containers and Closures: Pages 295-329 (Enclosure 7)

The product is filled in portions of 1g into 250ml vials of surface treated soda-lime glass, which complies with the Ph. Eur requirements for hydrolytic glass type II. The vials are sealed with chlorobutyl rubber stoppers which comply with the requirements of DIN 58 367 and the USP requirements for Elastomeric Closures for Injections.

Clinical Trial Formula

Not applicable as the material is to be used in further processing.

Pharmaceutical Expert Report
Form 2

PART IIA - STERILE BULK

Development Pharmaceutics:

Since the product will be used in further processing this section is not entirely relevant. The addition of glucose, and sodium chloride as bulking agents ensures a readily soluble preparation. Sodium hydrogen phosphate is added as a buffer to stabilise the pH of the reconstituted product. ~~Lysine and~~ Aprotinin ~~are~~ added to stabilise the biological activity of the preparation.

lysine,

is

Pharmaceutical Expert Report
Form 3

PART IIB - METHOD OF PREPARATION

Method of Preparation of the Sterile Bulk: Pages 127-157 (Enclosures 3 and 4)

Batch Size: Up to 200kg of Fraction II and III Paste.

Manufacturing Process

Extraction and Purification of Plasminogen

~~Fraction Cohn III is isolated from Fraction II and~~ III paste by precipitation in a buffer solution. Plasminogen is extracted from Fraction III by a lysine-containing phosphate buffer of pH 7.4 and non-plasminogen proteins are removed by cold ethanol precipitation. The plasminogen-containing solution is clarified by filtration, diluted with buffer then purified by affinity chromatography to immobilised lysine (Lysine-Biogel). The plasminogen is then precipitated from solution with ammonium sulphate and ~~dialysed against a sodium chloride/phosphate buffer~~.

X

The activity of ~~the resultant~~ plasmin is determined and neutralised with aprotinin (Step 9). Lysine is added and the solution is freeze dried. The bulk powder is adjusted to a residual moisture of 7-8%(m/m) (Step 10) and is then vapour heated in nitrogen for 10h at 60+/-0.5°C and a pressure of 1220+/-20mbar (atmospheric pressure corrected to 1000mbar plus 220+/-20mbar above atmospheric pressure) (Step 11). The dew point, 48+/-3°C, is continually measured in the vapour heat treatment receptacle to evaluate the resulting partial steam pressure. Temperature and pressure are also recorded throughout the inactivation process and these 3 parameters provide evidence of the consistency and reproducibility of these conditions.

X

Prior to further processing, the bulk powder is stored at 1-5°C, for not more than 8 weeks.

LYS-PLASMINOGEN, VAPOUR HEATED is manufactured from Fraction Cohn II + III. Following the isolation of Fraction Cohn III plasminogen is extracted by a lysine-containing phosphate buffer solution of pH 7.4, and any non-plasminogen proteins are removed by cold ethanol precipitation. The plasminogen-containing solution is subjected to a clarifying filtration. The filtrate is diluted with buffer solution and the plasminogen contained in the diluted filtrate is further purified by affinity chromatography to immobilised lysine (LYSIN-BIOGEL). The eluate is subjected to an ammonium sulphate precipitation and is dialysed against a sodium chloride/phosphate buffer solution.

Pharmaceutical Expert Report
Form 3 continued

PART IIB - METHOD OF PREPARATION

F o r m u l a t i o n o f S t e r i l e B u l k

The required quantities of the additional ingredients are added to the ~~heat-treated~~ material, taking into consideration the amounts already present in the bulk powder. (See Form 1 for the final formulation). The relevant amounts are dissolved in Water for Injections in Bulk, sterile filtered (0.2µm filter, checked by the forward flow test method, both before and after use), then filled in sterile glass containers under laminar flow and fitted loosely with sterile rubber stoppers. The product is frozen at -30 to -50°C and freeze-dried at -35 to +35°C in a vacuum of 0.15 to 0.03mbar. The vials are then sealed under vacuum.

vapour heated ×

Pharmaceutical Expert Report
Form 4

PART IIB - STERILE BULK

Process Validation: Pages 524-609 (Enclosure 13)

H I V I n a c t i v a t i o n

Aqueous solutions of 3 different lots of non-vapour-heated Lys-Plasminogen, were spiked with a quantity of virus stock suspension ~~(10^5/10^6 $TCID_{50}$/ml)~~ of ~~HTLV-III~~/~~H9~~/$HTLV\text{-}III_B$. The mixtures were treated by the same freeze drying and vapour heat methods as used for the manufacturing process. Samples were withdrawn at regular intervals and assayed for virus content using antigen capture and reverse transcriptase activity assays. The manufacturing lyophilisation/vapour heat treatment cycle reduced the virus to below the limit of detection for all 3 batches. Reproducible results were obtained between batches and assay methods.

× ($10^{7.1}$ $TCID_{50}$/ml)
×

M o d e l V i r u s I n a c t i v a t i o n

Aqueous solutions of 3 different lots of non-vapour-heated Lys-Plasminogen were spiked with a quantity of virus stock suspension (~~$10^{4.6}$-5.1 $TCID_{50}$~~ Sindbis virus and ~~$10^{4.7}$-5.7 $TCID_{50}$~~ Lymphocytic Choriomeningitis virus). The mixtures were treated as above and assayed for viral content using an established literature method. At the end of the cycle, no LCM virus titre was detected and the Sindbis virus titre was below the limit of detection. The lyophilisation/vapour heat cycle affords at least a ~~$10^{3.6}$ and $10^{4.7}$~~ titre reduction of Sindbis and LCM respectively.

0.1ml
× $10^{7.1}$-$10^{7.4}$ $TCID_{50}$/
× $10^{6.2}$-$10^{6.8}$ PFU/ml

× $10^{4.1}$ and $10^{4.7}$

T e s t f o r N e o a n t i g e n s

Rabbits were immunised with vapour heated product by S.C. injection at 0, 3 (1st booster) and 5 (2nd booster) weeks. Blood samples were taken 10 days after each booster. The potency of the antisera was tested with a counter immuno-electrophoresis system (SDS-PAGE system followed by Western blot separation and immuno-staining). No neoantigens were detected. The results were confirmed by absorption studies and immuno-staining vs. commercial antisera.

S t e r i l i s a t i o n P r o c e d u r e

Routine methods of sterile filtration, filling and sealing, as regularly used and previously validated, are followed. No additional data are presented here.

Pharmaceutical Expert Report
Form 5

PART IIC - CONTROL OF STARTING MATERIALS

Source Material: Pages 158-171 (Enclosure 5)

The preparation is manufactured from Fraction II and III Paste from US licensed establishments. The paste is obtained from 'Source plasma' and 'Plasma' complying with the requirements of the US Code of Federal Regulations 21 S 640.60 and S 640.30. In addition, the selection criteria for the source material are also in accordance with the requirements of the BP for Albumin. ~~Every individual plasma donation is tested for ALT, HBsAg by RIA and for anti-HIV by ELISA using FDA approved kits. Plasma must be free from HBsAg and HIV-antibody and the ALT value must not exceed 40 U/l.~~ X

The source plasma is then subjected to Cohn Fractionation Method 9, to give Fraction II and III Paste. The following tests are performed on incoming units of Fraction II and III paste, to ensure identity and and composition:

Determination of protein content	~~>24g~~ protein /100g paste by photometric determination ~~of the released photon~~ following combustion and subsequent oxidation of ~~the~~ nitrogen ~~content~~.	X ≥20g X X X X
~~CA-Electrophoresis~~	>37% δ-globulin by cellulose acetate electrophoresis and densitometric scanning ~~vs. a reference plasma~~.	X Electrophoretic composition X X

Every individual plasma donation is tested for HBsAg by RIA and for anti-HIV by ELISA using FDA approved kits. Plasma must be free from HBsAg and HIV-antibody. In addition, every donation is tested for ALT and the ALT value must not exceed twice the upper limit of normal.

Pharmaceutical Expert Report
Form 6

PART IIC - CONTROL OF STARTING MATERIALS

Other Constituents: Pages 172-294 (Enclosure 6)

Other constituents described in a pharmacopoeia

Acetic Acid (glacial)*	Complies with Ph. Eur.	
Aminocaproic Acid*	Complies with B.P.	
~~Ammonium Chloride*~~	~~Complies with Ph. Eur.~~	X
Ammonium Sulphate*	Complies with Ph. Eur.	
Aprotinin	Complies with B.P.	
Ethanol*	Complies with ÖAB.	
Glucose, Anhydrous	Complies with Ph. Eur.	
L-Lysine	Complies with ~~USP~~	X B.P.
Sodium Chloride	Complies with Ph. Eur.	
Sodium dihydrogen-phosphate.$2H_2O$	Complies with Ph. Eur.	
Disodium hydrogen-phosphate.$12H_2O$*	Complies with Ph. Eur.	
Sodium Hydroxide	Complies with B.P.	
Hydrochloric Acid	Complies with ~~B.P.~~	X Ph.Eur.
Water for Injections in Bulk	Complies with Ph. Eur.	

*Used only in the extraction of Plasminogen from Fraction II and III paste.

Other constituents not described in a pharmacopoeia

Baysilone Oil M350* is a polydimethylsiloxane supplied according to a specification previously guaranteed by the manufacturer. On receipt, the identity of the material is confirmed by viscosity and density determinations (Ph. Eur. methods):

Test	Specification
Viscosity	350cst +/-5% at 25°C
Density	0.97 at 25°C

Dialysis tubes* are composed of cellulose with an intermicellar distance of 50Å. The following chemical purity tests are carried out according to the requirements of DAN 88-361, Part 4, Item 5.2:

Pharmaceutical Expert Report
Form 6 continued

PART IIC - CONTROL OF STARTING MATERIALS

Other constituents ≠ described in a pharmacopoeia (Continued)

≠ not

Turbidity and colouration	Clear, slightly opalescent, colourless
Acidity/alkalinity	<10ml 0.1N HCl or NaOH/250ml Test Solution
Residue on evaporation	<12.5mg/250ml Test Solution
Oxidisable substances	<25ml 0.01N $KMnO_4$/250ml Test Solution
Chloride ions	<1.0mg Cl^-/250ml Test Solution
Sulphate ions	<2.5mg SO_4^{2-}/250ml Test Solution
Ammonium ions	<0.5mg NH_4^+/250ml Test Solution
Heavy metals	<0.5mg Pb^{2+}/250ml Test Solution

Lysin-Biogel* is prepared from materials purchased from well-known manufacturers: L-lysine, Biogel P-300, hydrazine hydrate, sodium nitrate and ammonium chloride.The data provided by each supplier, together with an identity test on receipt, ensures that the quality of the ingredients is suitable for their intended use. Prior to use the gel loaded with lysine is tested for its capacity to absorb plasminogen and for germ count:

Test	Specification	
~~Capacity~~ Adsorption capacity	~~≥35% of plasminogen by~~ adsorption of plasminogen in a plasma fraction, followed by determination of the ~~subsequent eluate~~	≥35% of recoverable plasminogen after adsorption of plasminogen present in a plasma fraction, followed by determination of the eluate.
Germs	5 germs/ml by a plate count method.	

X

*Used only in the extraction of Plasminogen from Fraction II and III Paste.

PART IID - CONTROL TESTS ON INTERMEDIATE PRODUCTS

Page 336 (Enclosure 9)

B u l k p o w d e r (Step 9 - see Form 3)

Determination of plasmin content, as a reference value for the addition of aprotinin (by UV absorbance at 405nm of the p-nitroaniline content released from a plasmin specific substrate when heated with plasmin).

B u l k p o w d e r (Step 10 - see Form 3)

Determination of specific activity (limits ⩾50nmol plasminogen / mg nitrogen) by UV absorbance at 400nm of the p-nitrophenol formed from the reaction of plasminogen, activated with streptokinase, and p-nitrophenyl-p'-guanidino benzoate (NPGB).

Determination of residual moisture (limits ⩽5%) by the Karl Fischer method.

B u l k p o w d e r (Step 11 - see Form 3)

Determination of residual moisture (limits 7.5+/-0.5%) by the Karl Fischer method.

Test for loss of activity during vapour heat treatment (limit <20%) by determination of specific activity (as in Step 10 above) and by determination of the protein content by the Kjeldahl method.

Pharmaceutical Expert Report
Form 8

PART IIE - CONTROL TESTS ON THE STERILE BULK

Page 337 (Enclosure 9)

Lys-Plasminogen, sterile bulk powder, is not described in a pharmacopoeia. It complies with the following specification.

Specification and Test Methods

Appearance: Page 502 (Enclosure 11)

White to off-white powder, free from visible evidence of contamination.

Identity Tests: Page 337-8 (Enclosure 9)

A. Positive by the result of the plasminogen assay (UV absorbance at 400nm of the p-nitrophenol formed from the reaction of plasminogen, activated with streptokinase, and NPGB.

B. Positive by determination of Glu-Plasminogen (limits ⩽20% of total plasminogen) by SDS-polyacrylamide gel electrophoresis, followed by staining and densitometric quantitation.

C. Only human protein is detected (gel diffusion literature method).

Potency Tests: Page 337 (Enclosure 9)

Determination of plasminogen (limits 80-120nmol/ml) by UV absorbance at 400nm of the p-nitrophenol formed from the reaction of plasminogen, activated with streptokinase, and NPGB.

Determination of the protein content (limits 8-12mg protein/ml) by the Kjeldahl method.

Determination of the specific activity (limits ⩾50nmol plasminogen/mg nitrogen) based on the results of the above 2 assays.

Other Tests: Pages 337-8 (Enclosure 9)

Test for abnormal toxicity by the method and acceptance criteria of the Ph. Eur.

Test for HBsAg ~~should be~~ non-reactive by RIA. ×

Pharmaceutical Expert Report
Form 8 continued

PART IIE - CONTROL TESTS ON THE STERILE BULK (continued)

Other Tests (continued)

Test for pyrogens by the method and acceptance criteria of the Ph. Eur.

Test for sterility is performed according to the method of the Ph. Eur.

Determination of aminocaproic acid (limits ≤300µg/ml) and L-lysine (limits ≤5mg/ml) by GC with FID.

Determination of plasmin content (limits ≤2% of the plasminogen content) by UV absorbance at 405nm of the p-nitroaniline content released from a plasmin specific substrate when heated with a test solution.

Determination of pH (limits 6.5-8.5).

Determination of residual moisture content (limits ≤3%) by the Karl Fischer method.

Identification and Quantitative Determination of Other Constituents: Page 337 (Enclosure 9)

Determination of sodium chloride (limits 5-7mg/ml) by back titration of a known excess of silver nitrate solution with ammonium thiocyanate.

Determination of ~~disodium hydrogen~~ phosphate (limits ~~8-12mg/ml~~) by photometric determination at 750nm of the colloidal molybdenum blue formed from the reaction of phosphomolybdate (phosphate reaction with sodium molybdate) with p-methylaminophenol sulphate.

content
8-12mg sodium dihydrogenphosphate/ml)

Determination of glucose (limits 8-12mg/ml) by photometric assay at 340nm of the NADH formed by reaction of the glucose with NAD.

Determination of aprotinin (limits ≤50KIU/ml) by measurement of its inhibitory effect on trypsin, and UV absorbance at 405nm of the p-nitroaniline formed by the reaction of the residual trypsin in splitting N-benzoyl-DL-arginine-p-nitroaniline hydrochloride.

equivalent to ≤0.06EPU/ml

Pharmaceutical Expert Report
Form 9

PART IIE - STERILE BULK

Control Tests on the Sterile Bulk

Analytical Validation

The method for the determination of ~~plasminogen~~ has been developed by Beecham Research Laboratories. ~~It is understood that the method has been validated, although data are not presented in the Master File.~~

X the potency of the sterile bulk

The protein content assay is an established laboratory method and the tests for abnormal toxicity, pyrogens and sterility are the Ph. Eur. methods.

The HBsAg RIA method uses a commercial assay manufactured by Immuno Diagnostika. Within limits the amount of HBsAg present in the sample is directly proportional to the amount of ^{125}I labelled HBs-antibody bound to the carrier. The sensitivity of the test system has been examined using reference antigens from the Paul Ehrlich Institute (FRG). The following lower detection limits have been established:
-Subtype ad <1U/ml (PEI) = <1ng/ml
-Subtype ay <1U/ml (PEI) = <1ng/ml

The following coefficients of variation have been determined for the assay methods of the other constituents:

-Sodium chloride	1.7%
~~-Disodium hydrogen phosphate~~	4.5%
-Glucose	1.9%
-Aprotinin	Approx. 10%

Sodium dihydrogen-phosphate

The variation coefficient of this method is 9%.

Pharmaceutical Expert Report
Form 10

PART IIE - STERILE BULK

(11)

Batch Analytical Results: Pages ~~7~~ D 0 → 508 - Ende Encl. 11

Lot number	860787S	86B0188S	86B0288S
Date of manufacture	14 Dec 87	24 Feb 88	14 April 88
Batch size (vials)	244	235	258
Appearance	Complies	Complies	Complies
Solution	Complies	Complies	Complies
Plasminogen (nmol/ml)	105	98	99
Protein (mg/ml)	10.86	10.04	9.96
Specific activity(nmol plasminogen/mg nitrogen)	60.3	61.4	62.4
Glu-plasminogen (% total plasminogen)	<5	<5	<5
Non-human protein	Complies	Complies	Complies
Abnormal toxicity (PhEur)	Complies	Complies	Complies
HBsAg (RIA)	Complies	Complies	Complies
Pyrogens (PhEur)	Complies	Complies	Complies
Sterility (PhEur)	Complies	Complies	Complies
Aminocaproic acid (µg/ml)	<200	<200	<200
L-Lysine (mg/ml)	<4	<4	<4
Plasmin (% plasminogen)	0.005	0.019	0.002
pH	6.95	7.0	7.1
Residual moisture (%)	0.3	0.4	0.5
Sodium chloride (mg/ml)	5.69	5.12	5.83
~~Disodium hydrogen~~ Sodium dihydrogen phosphate (mg/ml)	~~10.07~~ 8.83	~~9.61~~ 8.42	~~10.56~~ 9.26
Glucose (mg/ml)	9.79	10.15	9.96
Aprotinin (KIU/ml)	29.44	25.38	43

COMMENT

Pharmaceutical Expert Report
Form 10 continued

PART IIE - STERILE BULK

Batch Analytical Results: Pages 4 to 5

Lot number	86B0388S	86B0488S
Date of manufacture	24 May 88	25 May 88
Batch size (vials)	255	240
Appearance	Complies	Complies
Solution	Complies	Complies
Plasminogen (nmol/ml)	94	103
Protein (mg/ml)	9.77	9.96
Specific activity(nmol plasminogen/mg nitrogen)	60.4	64.3
Glu-plasminogen (% total plasminogen)	<5	<5
Non-human protein	Complies	Complies
Abnormal toxicity (PhEur)	Complies	Complies
HBsAg (RIA)	Complies	Complies
Pyrogens (PhEur)	Complies	Complies
Sterility (PhEur)	Complies	Complies
Aminocaproic acid (µg/ml)	<200	<200
L-Lysine (mg/ml)	<4	<4
Plasmin (% plasminogen)	0.011	0.009
pH	7.1	7.05
Residual moisture (%)	0.5	0.6
Sodium chloride (mg/ml)	6.39	5.97
~~Disodium hydrogen~~ Sodium dihydrogen phosphate (mg/ml)	~~9.97~~ 8.74	~~10.66~~ 9.34
Glucose (mg/ml)	10.11	10.76
Aprotinin (KIU/ml)	37	38

COMMENT

Pharmaceutical Expert Report
Form 11

PART IIF - STABILITY

Information Concerning Stability of the Sterile Bulk

Pages 507-523 (Enclosure 12)

1543 - 559

Number of batches tested: 3 batches were used for the main stability programme and 3 older batches have been assayed for plasminogen potency only.

Storage conditions: All 3 of the main batches were stored at 2-8°C for up to 12 months and at 20-25°C for up to 12 months. The supplemental batches were stored at 2-8°C for up to 2 years and 9 months.

Description of the containers: All of the batches were stored in the containers proposed for marketing i.e. 250ml vials of surface-treated, soda lime glass, hydrolytic class II, with chlorobutyl rubber stoppers.

Results of tests: No potency losses were observed for any batch, under either of the storage temperatures. No changes in Lys-plasminogen, plasmin or appearance were noted.

Analytical methods: As per Form 8.

Proposed shelf life and storage conditions: Lys-plasminogen is stable for 2 years when stored between 2 and 8°C.

CONCLUSION

Lys-plasminogen, vapour-heated, is a sterile, freeze-dried powder. It is a bulk ~~drug~~ which will be used in the production of pharmaceutical specialities. The powder contains other inactive ingredients, resulting in a simple formulation which is stabilised for biological activity by the addition of ~~lysine and~~ aprotinin. The product is stored in glass vials, suitable for single use, as will be the case for this bulk preparation. The vials are sealed with chlorbutyl rubber stoppers which comply with the relevant requirements of DIN and USP, and have been used in other preparations for many years.

The manufacturing process can be divided into 2 parts:
-extraction / purification of plasminogen from Fraction II and III paste
-preparation of the sterile bulk.

The extraction / purification of plasminogen is based upon its affinity for lysine. This is used in both the extraction and purification stages and provides a selective method for the preparation of plasminogen. Standard precipitation and filtration methods are also used for the separation of plasminogen from other proteins. Any residual plasmin activity is neutralised by the addition of aprotinin and final control of the plasmin content is afforded by the inclusion of a limit test in the Sterile Bulk Specification. ~~Lys-plasminogen is then formed by the addition of lysine.~~

The major critical aspect of the ~~extraction / purification~~ process is the freeze-drying / vapour-heat cycle. Although testing of the source plasma reduces the risk of ~~infection of AIDS and hepatitis~~, it does not completely eliminate ~~the~~ risk, due to the lack of specific or sufficiently sensitive test systems. For example, as a result of the introduction of HBsAg testing, the incidence of hepatitis B transmission has been reduced to 10% of all transfusion-related hepatitis infections. Anti-HIV screening also reduces the potential for AIDS contamination, but cannot provide complete safety as it is an indirect test†. Screening of ALT may reduce the incidence of transfusion-related NANB hepatitis by about one third. NANB hepatitis accounts for 90%of all hepatitis infections through plasma factor concentrates.

† and therefore no absolute proof against HIV-infection.

Conventional methods for virus inactivation, such as dry heat treatment, have not provided safety from virus transmission in IV plasma factor concentrates. In particular, evidence of transmission of HIV and hepatitis in F VIII and F IX has been accumulated. Similarly, the protein stabilisers which are used in solution heat treatment have also been shown to stabilise virus proteins. Methods involving the use of virucidal chemicals were not considered because of the potential of undesirable side effects and the limited inactivation potential. The vapour-heat inactivation method was selected as it allows the use of moist heat, without stabilisers or chemicals and can be developed for specific products.

The vapour heat method has been used by the manufacturer in various plasma factor concentrates. The time, moisture content and pressure have been selected considering the protein properties of the molecule and the composition of the preparation. A F VIII concentrate has been treated using similar parameters, i.e. moist heat at 60°C for 10h. The product was administered to haemophiliacs who had not been treated previously with blood products. ~~24/24~~ patients evaluable concerning NANB and ~~28/28~~ patients evaluable for HIV, remained free of the viruses.

ψ In various clinical studies
× 41/41
× 70/70

In order to ensure that the full conditions of the validated cycle are met, the following parameters are continually monitored during the manufacturing process:

~~Dew point (48+/-3°C)~~
~~Temperature (60+/-0.5°C)~~
~~Pressure (1220+/-20mbar)~~

- Water content of the vapour phase (dew point: 48+/-3°C)
- Temperature of the product (60+/-0.5°C)
- Pressure rise (Δ p 220+/-20mbar)

} ×

The documentation of these parameters ensures consistency and reproducibility of the conditions.

The HIV inactivation has been demonstrated in 3 batches by 2 assay methods. The HT/H9 ~~/HTLV-III$_B$~~ cell line was chosen because of its ability to produce high virus titres in comparison to other clones and cell lines. The antigen capture assay is an immunological ELISA assay which determines the HIV antigen present by the formation of an HIV-anti HIV immunocomplex (limit of detection $10^{0.5}$/ml). The reverse transcriptase method has a resolution of 1 in vitro infectious unit in a given sample. The 2 methods have equal sensitivity but the AC method has certain practical advantages.

×
×
×

The HIV inactivation validation data demonstrate that the vapour - heat cycle reduced the maximum experimental HIV titre to below detectable levels for 3 batches of material, when assayed by 2 methods.

consistently

Controlled laboratory cultivation of the hepatitis B virus has not yet been successful. Sindbis virus and Lymphocytic Choriomeningitis Virus (LCM) have been chosen as model viruses, to study the inactivation of other viruses with similar characteristics to the hepatitis virus:

-they are appropriate for in vitro and quantitative studies
-they are practical for quantitative studies
-they retain residual activity ~~after one hour of a given~~ treatment in order that inactivation kinetics can be studied
-they ~~are not pathogenic to humans~~
-they have similar membrane properties to the hepatitis virus

for a reasonable time during vapour heat

were chosen not to be pathogenic to humans due to possible experimental biassing through previous immunisation

The model virus inactivation data demonstrate that the vapour - heat cycle ~~reduced the titre of Sindbis and LCM virus by at least $10^{3.6}$ and $10^{4.7}$ respectively~~

also reduced the maximum experimental titres of Sindbis and LCM virus to below detectable levels or to zero for 3 batches of material.

The vapour-heat cycle did not result in the formation of neoantigens, although polymerisation did occur. These polymers are also present in the untreated material and in commercially available plasminogen concentrate. No adverse effects of these polymers are anticipated.

The manufacturer of the Sterile Bulk material already has significant experience in the production of sterile products. The sterilisation procedures used in the manufacture of Lys-plasminogen are established methods i.e. heat sterilisation of vials, steam sterilisation of rubber stoppers, sterile filtration of the solution, filling and freeze-drying under laminar flow conditions. All of these procedures have been validated previously, for similar products using the same containers, equipment and premises. ~~It is not considered to be necessary to re-submit this data.~~

The source material is Fraction II and III paste, obtained from US licensed establishments. As required by the BP (Monograph for Albumin), the source plasma is tested according to the requirements of the country of origin. Only FDA approved kits are used for the testing of ~~HIV antigen,~~ HBsAg ~~and ALT~~ and the

HIV-antibodies and

X possibility of viral transmission

type of kit used is recorded on the relevant batch analysis. Hence, the ~~presence of these viruses~~ is controlled using the best methods currently available. The identity and composition of the paste is adequately controlled by protein content and electrophoresis.

The source plasma is also tested for ALT.

The majority of other constituents, including those used only in the extraction process, comply with pharmacopoeial requirements. ~~Aprotinin will comply with the BP 1988 monograph, when implemented.~~ Only constituents used in the extraction process are not described in a pharmacopoeia. Dialysis tubes are controlled for chemical impurities by a DIN standard. Baysilone oil and the constituents of lysin-Biogel are supplied by established manufacturers with guaranteed specifications. This specification guarantee, together with identity testing on receipt, ensures that the quality of the ingredients is suitable for their intended use.

Testing on intermediate products is carried out for neutralisation of plasmin content and to ensure that the material is of adequate activity, prior to further processing.

The control tests on the sterile bulk include 3 identity tests. The assay for plasminogen (as used for i.d. purposes) is specific for plasminogen and plasmin. But since plasmin is also determined separately by another specific assay, the plasminogen content alone can be determined and the identity confirmed. Non-human protein is excluded by a gel diffusion test and the amount of Glu-plasminogen is also controlled in a further i.d. test. Hence, the profile of plasminogen and its related substances can be made.

As stated above, the plasminogen assay also measures the plasmin content. However, since plasmin is also determined separately, the amount of plasminogen alone can be calculated. This method was chosen since it allows the most relevant examination of the product's activity, in view of its intended further processing. The plasminogen limits of 80-120nmol/ml seem rather wide, in view of the batch analytical results. ~~These limits should be reviewed when further~~ batches have been manufactured, as at present, limits of 90-110nmol/ml appear to be relevant. There are no data available on the assay validation, although it is understood that this has been carried out. The validation should be presented and reviewed with the ~~assay limits, when further batches are available.~~

at first sight

However, the relevant quality criterion for further processing is the specific activity which is defined to be at least 50 nmol plasminogen/ mg nitrogen.

~~The determination of the protein content and the~~ lower limit applied to the specific activity test ~~afford further control of the sterile bulk.~~ X

Safety tests i.e. abnormal toxicity, pyrogens and sterility are adequately controlled by the Ph. Eur. methods. Presence of HBsAg is tested for by the use of a commercially available RIA method, which is validated and has an acceptable lower detection limit.

Impurities which may result from the extraction process, aminocaproic acid and L-lysine are adequately controlled by limit tests. pH and moisture content limits are applied as additional controls relevant to the stability of the product.

The plasmin limits of <2% ~~appear to be rather~~ generous in view of the results of batch analysis (max. 0.02%). ~~It is suggested that this limit may be tightened.~~ X ensure that sufficient neutralisation of any residual plasmin activity has been achieved by the addition of aprotinin.

All other formulation constituents are controlled in the Sterile Bulk Specification, with limits applicable to non-active ingredients.

Results of 5 recent batch analysis show excellent reproducibility and full compliance with the specification. ~~As discussed above, it is reasonable that certain limits should be tightened.~~ X

Stability results support a 2 year shelf life, when stored at 2-8°C. ~~Because of some fluctuation in the~~ plasminogen assay results, it is important to compare the assay variability results obtained in the ~~validation with these stability results.~~ X

In summary, it has been demonstrated that the manufacturer can produce Lys-plasminogen of excellent potency by the methods detailed in the application. ~~The assay method validation should be forwarded in order to re-assess limits in the specification and to compare with stability results.~~ X The assay methods have been validated and justify the limits in the Impurities relevant to the extraction process are controlled in the Sterile Bulk Specification, ~~although the plasmin limit is higher than necessary.~~ X quality control specifications. The product is stable for 2 years at 2-8°C.

The key aspect of viral contamination has been carefully assessed as follows:

Individual plasma donations for Fraction II and III Paste	Tested for ~~HIV-antigen,~~ HBsAg and ~~ALT by FDA approved kits~~	X HIV-antibodies and HBsAg by FDA approved kits and for ALT.
Lys-plasminogen powder	Freeze dried and vapour ~~heat treated~~ to inactivate viruses - validated for HIV and ~~HBsAg model viruses~~	X heated X model viruses.
Sterile Bulk Powder	Tested for HBsAg by a commercially available RIA kit (HIV antigen kits are only suitable for plasma).	

It can, therefore, be seen that the manufacturer has combined validated testing of starting materials, with validated methods of specific virus inactivation cycles and validated methods of testing of the Sterile Bulk, in order to add confidence that the material is free from viral contamination.