THE PENROSE INQUIRY

Response to the letter from Angus Evans to Susan Murray (CLO), dated 1st April 2011

“The Penrose Inquiry – Heat Treatment to 1985

As you may be aware, the Inquiry visited the old PFC building at the end of 2009 and was provided by Derek Radin with a flowchart and various annotated photographs outlining the equipment and procedures used in the protein fractionation process. The Inquiry was also provided with a floor plan showing the layout of the PFC building. For the flowchart, annotated photographs and floor plan please see the documents attached.

Since then, the Inquiry has obviously developed its understanding of the protein fractionation process at the PFC (both through analysis of the evidence/witness statements and most recently with regard to the manufacturing of albumin products). However, we think it would be helpful – more generally and, in particular, for the Inquiry’s understanding of the above topic – if an appropriate person at the SNBTS could supply a written statement outlining the various steps in the PFC’s manufacturing process.

We appreciate that this process will have changed from time to time; we therefore suggest that the statement deals with the process as it would have existed for the production of Factor VIII as at the end of 1983. We would also like some detail to be added about Factor IX
production at that time. Lastly, it would be helpful to have a
description of the new steps in those processes when heat treatment
was introduced. We would assume that Dr Foster would be the
most appropriate person in first instance, on the understanding that
collaboration with others, for example Dr Cuthbertson, may be
necessary. We are, however, content to leave it to the various PFC
personnel who are assisting with our investigations to determine by
whom such information should be provided, as we are conscious that
particular individuals are bearing very considerable burdens in the
provision of information to us.

The ideal would be if we could be provided with a narrative outlining
the manufacturing process by reference to the steps indicated in the
photographs noted above (and where necessary the flow chart and
the floor plan). For the avoidance of doubt, we are not looking for an
extensive document outlining all of the many complexities of the
manufacturing process, but rather something along the lines of a
relatively simple guide – including graphical information/flowcharts
etc if necessary - which can be understood by lay people and
which the Inquiry can use as a form of reference as its investigations
continue. For your reference, we enclose the document which was
prepared by us following the visit at the end of 2009, to illustrate the
level of description we require. One particular matter we are
interested in is a suggestion which has been made to us that in the
preparation of large plasma pooled products, all the plasma was not
necessarily in together at one time but, rather, that there was a kind
of rolling operation whereby plasma was being added at the top of a
tank at the same time as more plasma was being removed at the
bottom, as it were. Is this correct?”
Statement of Peter Reynolds Foster, outlining the various steps in the PFC's manufacturing process for the production of Factor VIII and Factor IX concentrates at the end of 1983 and new steps when heat treatment was introduced.

1. PRODUCTION AT THE PROTEIN FRACTIONATION CENTRE

i). By the beginning of 1984, the amount of human blood plasma that had been donated in Scotland that was being processed annually at the SNBTS Protein Fractionation Centre was about 60,000 Kg, of which 2/3rds was suitable for the preparation of coagulation factor concentrates of Factor VIII and Factor IX. In addition to producing about 35,000 vials of Factor VIII concentrate and 4,000 vials of Factor IX concentrate, the annual output of plasma products from the Protein Fractionation Centre at this time included approximately 55,000 bottles of albumin products and a total of over 25,000 vials of different immunoglobulin preparations.

ii). A number of non-protein products were also manufactured including, sterile distilled water, sterile physiological saline, hypertonic saline, cryo-protective solution, frozen blood washing solution and acid citrate dextrose anticoagulant solution.

2. PRODUCT MANUFACTURE

i). The manufacture of pharmaceutical proteins from human blood plasma encompassed a number of different elements, including:

- Provision of suitable staff, materials, equipment and facilities;

- Processing of plasma to obtain the desired products;
• Product inspection, labelling, packaging, storage and distribution;

• Application of Quality Control and Quality Assurance procedures.

ii). A film was made in 1995 at PFC which covered all of these activities. Although this film was made after the period of interest to the Inquiry, the scope of much of the work and activities, such as the handling and thawing of plasma, were essentially unchanged. This film (available on dvd) may therefore assist the Inquiry in its understanding of the activities that were undertaken at the SNBTS Protein Fractionation Centre during the period of interest to the Inquiry. A copy of this dvd was given to the Inquiry by the SNBTS in 2009.

iii). This witness statement concerns only the preparative processes to which the plasma was subjected to obtain the desired products (as described in section 2.i) 2\textsuperscript{nd} bullet point); and in particular, the processes used in the preparation of concentrates of Factor VIII and Factor IX.

iv). A set of photographs (Appendix A) and a ground floor plan (Appendix B) of the Protein Fractionation Centre have previously been provided to the Inquiry; therefore, relevant photographs and locations from these documents are identified in the appropriate sections of this statement.

v). A glossary of scientific and technical terms is provided in section 7 (page 29) of this statement.

3. THE OVERALL PROCESS SCHEME

i). The preparative procedures that were established at the Protein Fractionation Centre to produce the desired range of plasma products
are illustrated schematically in a simplified process flow-sheet (see Figure 1).

ii). Processes are shown (Figure 1) for the preparation of six different types of plasma product:

- Factor VIII concentrate,
- Factor IX concentrate,
- Immunoglobulin for intramuscular administration,
  (both as normal immunoglobulin, containing a broad spectrum of antibodies from the general population, and a range of specific immunoglobulin products such as; anti-D for the prevention of haemolytic disease of the newborn and anti-hepatitis B, anti-tetanus, anti-zoster, anti-rubella and anti-vaccinia for the prevention or treatment of these specific infections),
- Human Normal Immunoglobulin for intravenous administration,
- Stable Plasma Protein Solution,
- Human Albumin.

For all of these products, three different process stages can be identified;

3.a. A ‘MAINSTREAM PROCESS’, in which PLASMA was processed in sequential steps to obtain a number of different INTERMEDIATE PRODUCTS.

3.b. These different ‘INTERMEDIATE PRODUCTS’ were the materials from which the desired finished products could be prepared.

At this time, all ‘INTERMEDIATE PRODUCTS’ designated for further processing were held frozen, except for cryoprecipitate which was processed immediately.
3.c. ‘**FURTHER PROCESSING**’ of each ‘**INTERMEDIATE PRODUCT**’ would then be carried out via a series of sequential steps designed to produce a specified ‘**FINAL PRODUCT**’. These steps typically involved further purification and formulation of the protein solution to make it suitable for clinical administration, filtration of the formulated product, for clarification and to remove any bacteria, prior to aseptic dispensing into sterile glass vials, which would be stoppered and sealed. In some instances, where a product was not stable as a solution (such as concentrates of Factor VIII and Factor IX) it was freeze dried in the sterile vial prior to the vials being stoppered and sealed.

4 PREPARATION OF FACTOR VIII CONCENTRATES

4.1. UNHEATED FACTOR VIII CONCENTRATE (NY)

The objective of the preparative method was to provide a specified amount of factor VIII activity per vial (between 200 IU to 250 IU) in a volume (eg. 20 ml) suitable for home therapy, with a high yield and with product characteristics that complied with the British Pharmacopoeia specification for Factor VIII concentrates.

The procedures used for the preparation of unheated Factor VIII concentrate at the Protein Fractionation Centre from late-1979 to late-1984 were based on the method of Newman & Johnson [SGF.001.1913] and are illustrated in Figure 2, column A, in which the process is described in 17 separate steps. A brief explanation of each of these steps is given below, with the step number indicated in each sub-heading.

**Step A01: Warm Plasma to -10°C**

i). Fresh Frozen Plasma from Regional Transfusion Centres was stored at the Protein Fractionation Centre at -40°C in order to
preserve the activity of the most sensitive proteins (eg. factor VIII). Before the plasma could be processed, it was necessary to raise the temperature of the frozen donations from -40°C to within the range -15°C to -10°C.

ii). There were two reasons for this. The first was to assist in the removal of the plastic bag in which the plasma was contained, something that was very difficult to achieve at temperatures colder than -15°C because of a very strong degree of adhesion between plastic and plasma at lower temperatures.

iii). The second reason concerned the avoidance of damage that could occur to proteins if frozen plasma was held at temperatures warmer than -10°C, due to frozen substances within the plasma beginning to become mobile at the molecular level at temperatures warmer than about -10°C.

iv). This process of the controlled warming of frozen plasma was termed ‘plasma conditioning’ and was performed by placing the units of plasma that had been assigned for processing into a controlled temperature environment overnight.

**Step A02: Strip-off Plastic Bags**

Before the frozen plasma could be processed it was necessary to remove each donation of plasma from the plastic bag in which it had been supplied. This was done by cutting each frozen donation in half using a specialised band-saw. Once cut in half, each piece of frozen plasma was squeezed out of the plastic section into a holding container, from which operators would feed pieces of frozen plasma into the next stage of the process.

**Step A03: Crush and Thaw Plasma**
i). The objective at this step was to prepare cryoprecipitate with a high yield of factor VIII activity. In order to achieve this, it was necessary to thaw the plasma as quickly as possible whilst preventing newly formed particles of cryoprecipitate being dissolved due to an increase in temperature. This objective was best achieved using a process of continuous rather than batch thawing.

ii). The first stage involved reducing the pieces of plasma in size, from lumps of about 150g to small particles with the consistency of finely crushed ice, using a hammer mill that was designed to break-up frozen materials. The hammer mill was fed continuously with lumps of frozen plasma and the resultant particles were discharged continuously into a specially designed thawing vessel, in which controlled melting of the plasma-ice took place (the melting point of plasma being -0.5°C).

iii). The hammer mill can be seen (photograph 1, page 3, Appendix A) with its milling shaft uncovered to show the hammer blades arranged in a screw formation. The complete assembly is shown in photograph 4 (page 3, Appendix A), whilst particles of plasma-ice being discharged from the machine into the thawing vessel can be seen in photograph 5 (page 3, Appendix A).

iv). The thawing vessel was a tubular container, surrounded by a hollow jacket through which heated water was pumped to provide a controlled source of heat. The contents of the vessel were mixed using a double helical ribbon impellor which was designed to sweep the vessel wall and to continually mix thawed plasma with particles of plasma-ice to prevent the melted plasma from overheating. The vessel was designed to retain the plasma-ice, whilst allowing the melted plasma, containing particles of cryoprecipitate, to drain-out continuously by gravity into a small holding vessel from which it was pumped continuously into a refrigerated, large-scale, flow-through
centrifuge. The particles of cryoprecipitate would sediment in the centrifuge and be retained, whilst the clarified liquid (the supernatant) would exit the centrifuge.

v). The tubular thawing vessel, together with the helical ribbon impellor, can be seen in photograph 2 (page 3, Appendix A) whilst the assembled vessel, with stirrer motor, cables for temperature monitoring, the small holding vessel and the centrifuge feed pump are all shown in photograph 3 (page 3, Appendix A). [Note: Although the photographs on page 3 were taken more recently, the design of equipment and its operation were essentially the same as in 1983].

vi). This system for the continuous thawing of plasma for the preparation of cryoprecipitate was introduced routinely in August 1979 and replaced a previous batch-tank process. The continuous thawing process was first operating at a thawing rate of 70 litres of plasma per hour, using pilot-scale equipment. This was replaced with a larger-scale unit in January 1981, which operated at thawing rate of 200 litres of plasma per hour.

vii). The temperature of the out-flow from the thawing vessel was monitored continuously and demonstrated that the temperature was maintained close to 0°C at all times, despite the high rate of thawing.

viii). The change from batch to continuous thawing enabled the plasma throughput to be increased relatively easily, as the volume processed could be increased simply by operating over a longer period of time. Regardless of the overall time taken, each particle of plasma-ice was held in the system for only about 15 minutes, thereby minimising the time during which factor VIII activity was most vulnerable to damage.
ix). In comparison with the earlier batch process, continuous thawing of plasma resulted in the yield of factor VIII activity for the overall process being increased by about 50%, together with a commensurate increase in purity and a reduction in the reconstitution time of the final product.

x). Additional information on the design, operation and performance of this process of continuous thawing of plasma is available in Appendices C & D.

**Step A04: Collect Cryoprecipitate**

i). The outflow from the thawing vessel, (located in area 54 on the ground floor plan, Appendix B) which comprised melted plasma containing fine particles of cryoprecipitate, was pumped directly into a refrigerated, multi-chamber centrifuge sited in an adjacent +4 °C cold-processing area (located in area 85 on the ground floor plan, Appendix B).

ii). The centrifuge consisted of a rotating bowl fitted within a steel housing (see photograph, page 6), both of which were refrigerated to maintain the temperature of the contents close to +2°C.

iii). The melted plasma, containing particles of cryoprecipitate, was fed continuously into the rotating bowl and the centrifugal force caused the cryoprecipitate to settle on the vertical walls of two cylindrical chambers within the bowl and be retained in the machine, whilst the clarified cryoprecipitate-depleted supernatant exited into a collection vessel, where it was held for further processing.

iv). When the thawing process had finished, the centrifuge would be run-down and the internal bowl removed (see photograph, page 7, Appendix A). After the bowl had been dismantled, the cryoprecipitate
would be collected as a single mass of about 10Kg of solids for further processing (see photograph, page 10, Appendix A).

v). In this way, the cryoprecipitate that had been retained in the centrifuge was derived from all of the plasma that had been thawed continuously. The mass of cryoprecipitate that had been derived in this way was then processed in its entirety through to a defined batch of finished Factor VIII concentrate. The resultant batch of Factor VIII concentrate was therefore linked directly to the plasma donations from which it had been prepared. At the end of 1983 this was about 4000 donations per batch.

vi). Further processing of the cryoprecipitate was carried out in a separate process area which had been specifically designed for this purpose (area 35 on the ground floor plan, Appendix B; although, it should be noted that at the end of 1983, this process area was located in area 44).

**Step A05: Rinse Cryoprecipitate**
Once all of the cryoprecipitate had been collected, it was rinsed with a 2% solution of ethanol at +2°C to remove residual plasma which might have been remained with the precipitate. This was done to minimise the presence of substances from plasma that could potentially damage factor VIII, such as other coagulation factors or enzymes that degrade proteins.

**Step A06: Cryoprecipitate Extraction**
The rinsed cryoprecipitate was then suspended in a specially formulated buffer solution which was designed to dissolve (extract) most of the cryoprecipitate whilst excluding material that was poorly soluble.

**Step A07: Adjust pH to 7.0**
Factor VIII is very sensitive to conditions that are either acid or alkaline. Therefore, during extraction, the solution was adjusted to neutrality (i.e., pH 7.0) by the slow addition of dilute hydrochloric acid as this pH was considered optimal for the recovery of factor VIII during the extraction process.

**Step A08: Adsorb with Al(OH)₃**

i). Some plasma remains within the cryoprecipitate mass. The presence of coagulation factors II, VII, IX & X in this plasma can cause factor VIII to become unstable, either during processing or in the final product during storage. These unwanted coagulation factors bind to aluminium hydroxide more readily than factor VIII and this difference in behaviour was utilised to remove the unwanted coagulation factors from the factor VIII extract.

ii). Aluminium hydroxide gel (a physically stable emulsion of solid aluminium hydroxide) was added to the extract to remove coagulation factors II, VII, IX & X. The aluminium hydroxide gel also had the property of binding and removing particles of insoluble material, thereby clarifying the extract and making it suitable for subsequent sterile filtration for the removal of any bacteria.

**Step A09: Centrifugation**

The aluminium hydroxide gel was removed from the factor VIII extract by centrifugation. This was done by dispensing the mixture into 1 litre, sterile, polypropylene bottles which were spun in a batch centrifuge at 4000 rpm for 15 minutes at 20°C.

**Step A10: Collect Supernatant**

Following centrifugation, the supernatant solution was decanted from each bottle into a sterile pressure vessel.

**Step A11: filter to 0.45μm**
The solution was then further clarified by filtration through a series of membrane cartridge filters; the last filter in the series having a mean membrane pore diameter of 0.45\( \mu \)m. This was done by attaching the sterile pressure vessel to a sterile filter assembly and applying pressure from a cylinder of sterile inert gas.

**Step A12: Formulate Filtrate with Citrate**
The anti-coagulant tri-sodium citrate was added to ensure that factor VIII would not be de-stablised by any residual activity from trace levels of other coagulation factors and to enhance the solubility of the final product.

**Step A13: Adjust pH to 6.8**
The pH of the solution was adjusted (titrated) to pH 6.8 with dilute hydrochloric acid to obtain the pH that was optimal for the chemical stability of factor VIII activity.

**Step A14: Filter to 0.22\( \mu \)m**
i). The factor VIII solution was filtered through a series of sterile membrane cartridge filters to clarify the solution and to remove bacterial contaminants. The final filter in this series had a mean membrane pore diameter of 0.22\( \mu \)m which was, and still is, the pharmaceutical industry standard for the preparation of aseptic solutions by filtration. This was performed (area 63 on the ground floor plan, Appendix B) using the standard pharmaceutical industry procedures for the preparation, handling and transfer of aseptic solutions.

ii). The sterile 0.22\( \mu \)m filter housing was connected by a sterile line to a sterile receiving vessel which was located in an adjacent aseptic dispensing suite (area 67 on the ground floor plan, Appendix B). The 0.22\( \mu \)m filtered Factor VIII solution passed directly from the sterile
side of the filter into this sterile receiving vessel in the aseptic dispensing area.

**Step A15: Dispense Aseptically**

i). Aliquots of the final solution of factor VIII were dispensed into sterile glass vials using an automated aseptic dispensing system. Typically 40ml of factor VIII solution was dispensed into a 65ml vial for reconstitution in 20ml of distilled water after freeze drying.

ii). After dispensing, each vial was fitted with a raised stopper, especially designed for freeze dried products, and then placed onto special trays for aseptic transfer to the freeze dryer.

iii). Aseptic dispensing at PFC is illustrated in the photographs on page 16 (Appendix A). Although these photographs were taken more recently, the procedures are similar to those employed during the period of interest to the Inquiry.

**Step A16: Freeze Product (cold-shelf)**

i). The product was frozen to -50°C in order to ensure that all constituents were in the solid state prior to freeze drying.

ii). The freeze driers that were used to dry factor VIII concentrate contained a number of shelves, each of which could be refrigerated or heated. The vials were loaded onto the shelves of the freeze drier at ambient temperature (ie. about +20°C) with the stoppers still raised (see left-hand photograph on page 17, Appendix A) and the door of the drier was then closed.

iii). The shelves were then refrigerated to -50°C and the product left overnight to freeze to this temperature.

**Step A17: Freeze Drying (Method 1)**
i). Freeze drying is a process of dehydration which is used to preserve sensitive products. The essential process, known as sublimation, involves the removal of water by heating the material under a vacuum such that the water is transferred from the frozen to the gaseous state without the formation of a liquid phase, in which damage would occur to heat sensitive material such as factor VIII.

ii). The freeze drying process involved two drying stages; first, Primary Drying, during which heat was applied to the freeze drier shelves whilst the product was under a vacuum to remove most of the water, and then Secondary Drying in which the shelf temperature was increased to reduce the residual moisture of the freeze dried product to less than 2% (w/w).

At the end of 1983 the conditions used at the Protein Fractionation Centre to freeze dry coagulation factor concentrates were as follows: 

**Primary Drying**: reduce chamber pressure to 0.2 millibar; raise shelf temperature from -50°C to +10°C; maintain conditions for 1 hour per mm of plug height (typically about 30 hours).

**Secondary Drying**: raise shelf temperature from +10°C to +20°C with chamber pressure below 0.2 millibar and maintain until moisture was no longer being removed, as evidenced by the chamber pressure remaining constant when the freeze drying chamber was isolated from the condenser.

iii). When Secondary Drying was complete, the chamber was raised to atmospheric pressure using nitrogen (an inert gas). The vials were then sealed within the freeze drying chamber using an hydraulic system to lower the shelves such that the special stoppers seated on each vial were closed. The freeze drying chamber would then be opened and the stoppered vials removed (see left-hand photograph, page 17, Appendix A) and then sealed, still under aseptic processing conditions.
iv). This completed the processing of unheated Factor VIII concentrate (NY), with about 1 week having been required to progress from frozen plasma to the freeze dried product. Thereafter, some 3-4 months were required to complete inspection, labelling, packaging, Quality Control testing, Quality Assurance procedures and batch release.

4.2 HEAT TREATED FACTOR VIII CONCENTRATE (NY-HT1)

The objective of the method for preparing heat treated Factor VIII concentrate (NY-HT1) was as described in 4.1 above, except that dry heat treatment at 68°C was applied to the final product for the longest period of time that the product could tolerate without failing to comply with the product specification; in particular, the time for reconstitution (product solubility) that was specified in the British Pharmacopoeia.

All steps in the preparation of Factor VIII concentrate (NY-HT1), heat treated for 2 hours at 68°C, were the same as in 4.1 above except for an additional heat treatment step which is described below.

Step A: 18: Dry Heat, 2 hours at 68°C

i). Sealed vials of freeze dried Factor VIII concentrate were heated for 2 hours at 68°C, as this was the longest that could be tolerated at 68°C before the product failed to meet the specified reconstitution time (solubility), all other Quality Control parameters remaining within specification. These optimal heating conditions were established by heating and testing a number of vials of product taken from a number of different batches and the procedure was implemented routinely from 18th November 1984.
ii). This heat treatment was carried out in the equipment that was normally used for the pasteurisation of albumin and SPPS (see photograph, top-left, page 19, Appendix A).

iii). Although pasteurisation of albumin and SPPS was undertaken at 60°C, this equipment had been designed to function up to 70°C and was therefore able to accommodate heat treatment at 68°C.

iv). During the heat treatment process, the temperature was measured and recorded via a number of ‘indicator’ vials of Factor VIII concentrate fitted with temperature sensors. These ‘indicator’ vials were spread throughout the batch to ensure that the correct temperature was obtained throughout the whole batch for the period of time specified. The ‘indicator’ vials were discarded after heat treatment had been completed.

v). As this procedure was designed to be applied to unheated Factor VIII concentrate (NY), some 12 months stock of unheated Factor VIII concentrate (NY) was heat treated in this manner with the result that Factor VIII concentrate (NY) prepared from donations collected as far back as late-1983 was subjected to heat treatment.

4.3. HEAT TREATED FACTOR VIII CONCENTRATE (NT-HT2)

The objective of the preparative method was as described in 4.1 above, except that the method was modified to extend the length of time that the product was able to tolerate dry heat treatment at 68°C. This was implemented routinely to Factor VIII concentrate prepared from 20th January 1985.

All steps in the preparation of Factor VIII concentrate (NY-HT2), heat treated for 24 hours at 68°C, were the same as in 4.1 above
except for the following steps (see Figure 2, column B).

**Step B12: Formulate Supernatant with Citrate and Sucrose**

The formulation of the factor VIII solution described in step A12, was modified by the inclusion of sucrose to a final concentration of 2%. This modification was made in January 1985, following my experimental discovery that the addition of a small amount of carbohydrate during the formulation of the product would allow 68°C dry-heat treatment to be extended from 2 hours to 24 hours.

**Step B18: Dry Heat, 24 hours at 68°C**

i). Dry heat treatment for 24 hours at 68°C was initially performed as described in step A18 above, except that the period of heat treatment was extended to 24 hours.

ii). From mid-1985 onwards, dry-heat treatment was performed using an oven which had been specifically designed and manufactured for the dry-heat treatment of coagulation factor concentrates. This design was based on a specification drawn up at PFL (Oxford) / BPL (Elstree) in conjunction with a specialist equipment manufacturer see photograph, page 20, Appendix A).

4.4. **HEAT TREATED FACTOR VIII CONCENTRATE (Z8)**

The objective of the preparative method for Z8 was the same as in 4.1 above, except that the product should be able to tolerate dry heat treatment for 72 hours at 80°C. To achieve this degree of heating it was discovered by Dr McIntosh and myself that a new freeze drying method and a new freezing technique were required. In order to accommodate these changes, it was necessary to reduce the volume of factor VIII solution in each vial from the previous 40 ml (see step A15) to 15 ml. To obtain a dose size of 200 iu to 250 iu of factor VIII
activity in this smaller volume it was necessary to increase the concentration of the factor VIII solution, which in turn required increased purification. A new manufacturing process (the Z8 process) was devised by Dr McIntosh and myself for this purpose.

Preparation of Factor VIII concentrate (Z8), dry heat treated for 72 hours at 75°C or 80°C, was begun in August 1986. The method of preparation of Z8 is summarised in Figure 2, column C. Steps which were either modified or new are described below.

**Step C06: Cryoprecipitate Extraction**
The volume and the temperature of extraction were reduced to obtain a greater discrimination between more soluble and less soluble proteins.

**Step C07: Adjust pH to 6.7**
The pH during extraction of cryoprecipitate was reduced to 6.7, instead of 7.0, in order to increase the degree of purification of factor VIII into the extracted solution.

**Step C09: Zinc Precipitation**
Further purification of factor VIII was undertaken by adding zinc acetate to the solution, as this had been discovered by Dr Bier and myself to preferentially precipitate fibrinogen, whilst leaving factor VIII in solution (see Appendix E for further information on this discovery).

**Step C12: Formulate Supernatant with Citrate, Sucrose Calcium & Sodium Chloride**
Calcium chloride and sodium chloride were added to the formulation, in addition to sodium citrate and sucrose, as these chemicals had been discovered by Dr McIntosh (sodium chloride) and myself (calcium and sodium chloride) to enhance the stability of factor VIII (see Appendix F for further information on these discoveries.)
Step C15: Concentration by Ultra-filtration
The more purified factor VIII solution was concentrated using a membrane system known as ultra-filtration which was designed to retain macromolecules, such as proteins, whilst allowing water to be removed through the membrane.

Step 16: Dia-filtration to Remove Zinc and to Formulate the Product
i). Following concentration, the factor VIII solution was re-formulated to establish the final desired concentrations of the various chemicals needed to stabilise factor VIII and to enhance product solubility. These chemicals were sodium citrate, sucrose, calcium chloride and sodium chloride, the concentrations of which were determined by Dr McIntosh to provide a chemical environment that was optimal for the factor VIII molecule and suitable for administration to patients.

ii). This was done by re-circulating the solution in the ultra-filtration equipment of step C15 operated in the dia-filtration mode; whereby the water and small molecules present were removed through the membrane whilst being replaced by the new chemical mixture.

Step C18: Dispense Aseptically
Aseptic dispensing was carried out as described previously, except that the volume of factor VIII solution dispensed was 15 ml per vial for reconstitution in 20 ml of sterile distilled water.

Step C19: Freeze Product (Warm-Shelf)
i). The product was frozen within the freeze drier as described previously, except that a new technique of freezing was used. The objective of the new technique was to obtain a uniform fine-crystal structure after freezing as product composed of fine amorphous crystals, rather than large crystals, had been discovered by Dr McIntosh and Mr Sinclair to withstand heating at 80°.
ii). This type of crystal structure occurs when crystallisation takes place from a solution that is super-saturated. Therefore, a special two-stage freezing procedure was devised by Dr McIntosh and myself to obtain this behaviour.

iii). Firstly, the freeze drier shelves were cooled to -10°C, then vials of factor VIII solution, at 20°C, were placed on the shelves. The factor VIII solution was then allowed to cool slowly to about -5°C, at which temperature nucleation of ice occurred, causing crystallisation to occur virtually instantaneously throughout the vial. The ice matrix formed in this manner in all vials within about 30 minutes.

iv). This procedure resulted in the formation of the desired uniform fine-crystal structure throughout every vial on every occasion. Once the desired crystal structure had formed the shelf temperature was set to -50°C and product was left at less than -45°C for at least 9 hours to ensure that solidification was complete before the freeze drying process was initiated.

**Step C20: Freeze Drying (Method 2)**

i). Freeze drying was carried out as described previously (step A17) except that the conditions for Primary Drying and for Secondary Drying were modified, as follows:

**Primary Drying**: reduce chamber pressure to 0.1 millibar; raise shelf temperature from -50°C to -25°C; maintain conditions for 4 hours after the product temperature had reached -25°C.

**Secondary Drying**: reduce chamber pressure to less than 0.06 millibar, then raise shelf temperature from -25°C to +35°C and maintain for 24 hours.

ii). These new freeze drying conditions were based on a discovery by Dr McIntosh that Factor VIII concentrate freeze dried in this manner
could withstand heat treatment much better than material freeze dried by the method used previously (method 1).

**Step C21: Heat Treatment for 72 hours at 75°C or 80°C**

i). Dry heat treatment was performed in the specialist oven described in section 4.3, step B18 (photograph, page 20, Appendix A).

ii). Although it had been intended that all batches would be heated at 80°C, batch-to-batch variation meant that some batches were not able to tolerate heating for 72 hours at 80°C, but were able to withstand heating at 75°C. As alternative products available in Scotland were heated at lower temperatures, it was considered that batches heated at for 72 hours at 75°C were superior to any other available product.

iii). In order to determine which batches would not survive heat treatment at 80°C, sample vials from every batch were subjected to trial heating for 72 hours at 80°C and those batches whose samples failed to withstand heating for 72 hours at 80°C were subjected to heat treatment for 72 hours at 75°C instead in order to maximise the supply of Z8.

5. **PREPARATION OF FACTOR IX CONCENTRATES**

5.1. **UNHEATED FACTOR IX CONCENTRATE (DEFIX)**

The objective of the preparative method was to provide a specified amount of factor IX activity per vial (about 300 iu) in a volume (10 ml) suitable for home therapy, with a high yield and with product characteristics that complied with the specifications of the British Pharmacopoeia.

The methods used for the preparation of unheated Factor IX concentrates at the Protein Fractionation Centre was based on the
ion exchange purification (see glossary, section 7) method of Middleton, Bennett and Smith (Appendix G) which is illustrated in Figure 3A, where the process is described in 21 separate steps. The resultant product (DEFIX) was produced at the PFC from 1972.

Steps A01 to A04 are as described in section 4.1 above. A brief explanation of the remaining steps is given below.

**Step A05: Formulate Cryo-supernatant**

i). The solution which remained following the removal of cryoprecipitate was formulated to make it suitable for ion exchange chromatography. This required the ionic strength of the solution to be reduced, which was done by diluting the cryo-supernatant with a specified volume of sterile, pyrogen-free distilled water at a temperature of +4°C.

ii). This procedure was undertaken in the cold processing area, room number 85 (Appendix B)

**Step A06: Adjust pH to 6.9**

The diluted solution of cryo-supernatant was adjusted to pH6.9, as this was the pH most suitable for the subsequent ion exchange procedure.

**Step A07: Ion Exchange Adsorption**

A specified quantity of pre-prepared ion exchange gel (DEAE-cellulose, grade 32) was added to the pool of formulated cryo-supernatant at +4°C. The mixture was stirred for 90 minutes during which time factor IX and related proteins became attached to the ion exchange matrix.

**Step A08: Collect Gel by Centrifugation**

i). The ion exchange gel and the proteins that were attached to it
were separated from the proteins remaining in solution by passing the mixture through a refrigerated centrifuge. The particles of ion exchange gel would settle at the wall of the centrifuge and be retained in the machine, whilst the clarified solution would pass through and be collected for subsequent processing by cold-ethanol fractionation to recover immunoglobulin and SPPS/albumin (Figure 1).

ii). Centrifugation was continued for 30 minutes after the last of the mixture had entered the centrifuge to ensure that the ion exchange gel was well packed within the centrifuge rotor. The centrifuge would then be brought to a halt, dismantled and the ion exchange gel removed from the centrifuge rotor.

**Step A09: Suspend Gel in Buffer**
After removal from the centrifuge bowl, the ion exchange gel was suspended in a specified volume of a special buffer ‘wash’ solution containing sodium dihydrogen phosphate and tri-sodium citrate. This was necessary so that the gel could be poured evenly into the chromatography column in a suitable chemical environment.

**Step A10: Add Gel to Chromatography Column**
The suspension of ion exchange gel in wash buffer was added to a chromatography column (a cylindrical vessel) which was fitted with a conductivity sensor at the outflow. Even packing of the gel within the column was critical to ensure that the various solutions to be added would pass through the bed in a uniform manner, without distortion at the interface between different solutions. This equipment was located in a process area which was specially designed for processing coagulation factor concentrates (area 35, Appendix B; formerly area 44).

**Step A11: Treat Column with Wash Buffer**
The original volume of wash buffer was allowed to drain from the
column, such that gel formed a squat column. Residual unbound protein which had been carried over with the gel was removed by flushing the column with a specified volume of wash buffer.

**Step A12: Remove Factor IX with Elution Buffer.**

i). Protein attached to an ion exchange gel can be removed by adding a solution containing an increased concentration of sodium chloride, whereby the chloride ion attaches preferentially to the ion exchange gel, exchanging places with the protein that was bound at a lower concentration of chloride ion. As the protein was originally bound selectively to the gel, then its subsequent removal from the gel formed the purification procedure.

ii). This exchange of ions, known as elution, was achieved by flushing the chromatography column with a special buffer solution which contained sodium chloride as well as sodium dihydrogen phosphate and sodium tri-citrate.

iii). The point at which the coagulation factors began to emerge from the column was detected by the monitoring of a sharp rise in the conductivity of the solution at the out-flow from the column, as the conductivity of the solution is increased by the presence of sodium chloride.

**Step A 13: Collect FIX Eluates E₁ to E₁₀**

Once the solution conductivity began to rise, six different fractions were collected sequentially on a volumetric basis:

- $E₁$ - 100 ml.
- $E₂$ - 300 ml.
- $E₃$ - 300 ml.
- $E₄$ - 100 ml.
- $E₅$ - 100 ml.
- $E₆$ - 100 ml.
Step A14: Freeze & Store Eluates
After taking samples from each fraction for the determination of factor IX, and for other assays, all of the fractions were stored frozen at -40°C.

Step A15: Thaw Selected Eluates
i). Once the factor IX content of each fraction had been determined, and the other assays had been completed, those fractions which met the necessary specification were selected for further processing.

ii). This selection process featured two aspects in particular:
• a suitable concentration of factor IX activity,
• freedom from materials that might cause a thrombogenic reaction in recipients, according to a number of tests that had been specifically designed for this purpose (for further information on these tests see Appendix H).

iii). The selected fractions, in their sealed containers, were allowed to thaw at room temperature.

A16: Pool Selected Eluates
When thawing was complete, the containers were opened, the selected fractions pooled and a sample taken for the determination of factor IX activity.

Step A17: Dilute to Target Potency
When the result of the assay for factor IX activity was available, the solution was diluted, if necessary, to achieve a target factor IX potency of 34 iu / ml.

Step A18: Filter to 0.22μm
The final solution of factor IX was filtered to 0.22μm in the manner
described in section 4.1, step A 14.

**Step A 19: Dispense Aseptically**
The solution was dispensed aseptically into glass vials. This was done in the same manner as factor VIII (section 4.1, step A15), except that 10 ml of solution was dispensed into each vial, for reconstitution in 10 ml of distilled water after freeze drying.

**Step A 20: Freeze Product**
The vials of factor IX solution were frozen as described in section 4.1, step A16.

**Step A 21: Freeze Dry (Method 1)**
The vials of factor IX solution were freeze dried as described in section 4.1, step A 17.

5.2. **HEAT TREATED FACTOR IX CONCENTRATE (HT-DEFIX)**
The method for the preparation of heat treated Factor IX concentrate (HT-DEFIX), dry-heat treated for 72 hours at 80°C was the same as in section 5.1 except for two additional steps (Figure 3B). Preparation of HT-DEFIX by this method was begun on 29 May 1985.

**Step B18: Add Anti-thrombin III**
i). When experimental samples of DEFIX that had been prepared by the method described in section 5.1 were subjected to dry heat treatment, the samples failed to comply with one of the tests for thrombogenic potential that was included in the criteria for product release.

ii). It was discovered by Dr Feldman (PFL, Oxford) and myself that this defect could be corrected by the addition of the human protein anti-thrombin III (see appendix H for further information).
iii). A defined quantity of human anti-thombin III was added to the factor IX solution using pasteurised, clinical grade anti-thrombin III that was obtained from PFL (Oxford) for this purpose.

Step B 23: Dry Heat (72 hours at 80°C)
The freeze dried concentrate was subjected to heat treatment for 72 hours at 80°C using the specially designed oven as described in section 4.3, step B18, which was installed at the Protein Fractionation Centre in mid-1985.

6. APPENDICES

A. Photographs of Plasma Fractionation at the Protein Fractionation Centre; provided to the Inquiry by the SNBTS on 22nd September 2009.

B. Protein Fractionation Centre, Ground Floor Plan 1996; provided to the Inquiry by the SNBTS on 22nd September 2009.


F. Foster PR, Dickson IH, McQuillan TA, Prowse CV, Boulton FE, Greedharry P & Bloom AL. Studies on the stability of VIIIC: during
the manufacture of a factor VIII concentrate for clinical use. Vox.

G. Middleton SM, Bennett IH & Smith JK. A therapeutic concentrate of
coaagulation factors II, IX and X from citrated, factor VIII-depleted

H. Sas G, Owens RE, Smith JK, Middleton S & Cash JD. In vitro
spontaneous thrombin generation in human factor-IX concentrates. Br

I. Littlewood JD, Dawes J, Smith JK, Feldman PA, Haddon ME,
McQuillan TA, Foster PR, Ferguson J & Prowse CV. Studies on the
effect of heat treatment on the thrombogenicity of factor IX

7. Glossary

| Buffer                  | A buffer is a chemical, or mixture of chemicals, which is
designed to regulate the pH of a solution. |
|-------------------------|---------------------------------------------|
| Centrifugation          | A mechanical process for separating solids from liquids
by spinning the mixture at high speed in a rotating
chamber whereby the solid particles settle on the wall of
the chamber. Used extensively in plasma fractionation to
collect protein precipitate fractions. |
| Chromatography          | A process for separating proteins from one another by
passing a mixture of proteins through a bed of solid
particles to which specific chemicals have been attached
which bind selected proteins. After the separation has
been completed, the bound proteins are removed
(eluted) by adding a substance which detaches the
bound proteins from the solid matrix. Applied to the
preparation of factor IX concentrates from the early- |
### Cold-ethanol fractionation

The principal method used to separate plasma proteins into major groups (fractions) according to differences in their solubility. Involves manipulation of the pH, ionic strength, ethanol concentration and temperature to cause a selected fraction of proteins to precipitate out of solution. The major precipitate fractions are: Fraction I (mainly fibrinogen, fibronectin), Fraction II (immunoglobulins), Fractions III & IV (other coagulation proteins and trace components) and Fraction V (albumin). The method was devised by Dr Edwin J Cohn and co-workers and is sometimes known as Cohn fractionation.

### Cryoprecipitate

A gelatinous mass of precipitated protein which forms when frozen plasma is thawed. Mainly composed of fibrinogen (also known as coagulation factor I) and fibronectin (also known as cold-insoluble globulin). Cryoprecipitate contains about 70% of the factor VIII present in the starting plasma (the other 30% remains in the cryo-depleted plasma; the cryosupernatant).

### Cryosupernatant

The solution that remains after cryoprecipitate has been removed from plasma.

### Diafiltration

A membrane filtration process used to change the chemical solution in which a protein is dissolved. Similar to dialysis.

### Elution

The addition of a solution of chemicals to a chromatography column to cause selected substances to be released from the chromatography gel. Collected as a specific fraction or eluate.

### Filtration

Removal of unwanted substances by passing a solution or suspension of protein through a specific type of filter. Widely used in plasma fractionation to remove bacteria.
(by membrane filtration), precipitate particles (by depth filtration), lipoproteins (by depth filtration).

<table>
<thead>
<tr>
<th><strong>Formulation</strong></th>
<th>A chemical environment created for a manufactured protein pharmaceutical (eg factor VIII). The chemical formulation must ensure that the final product is stable for its intended shelf-life as well as being a suitable dose-form for injection into humans to provide a defined amount and concentration of the active constituent.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fraction I</strong></td>
<td>A group of precipitated proteins whose low solubility is exploited by the addition of ethanol to form a precipitate at an early stage of the ethanol fractionation process.</td>
</tr>
<tr>
<td><strong>Fraction II</strong></td>
<td>A precipitate of protein recovered at an intermediate stage of the ethanol fractionation process. Composed mainly of antibodies, fraction II is the basis for the manufacture of a range of immunoglobulin products.</td>
</tr>
<tr>
<td><strong>Fraction V</strong></td>
<td>A precipitate of protein recovered at a late stage of the ethanol fractionation process. Composed mainly of albumin, fraction V is the basis for the manufacture of Human Albumin and other products composed predominately of albumin (eg. Stable Plasma Protein Solution, Plasma Protein Fraction).</td>
</tr>
<tr>
<td><strong>Freeze drying</strong></td>
<td>A process of dehydration in which water is transferred directly from the frozen solid state to the gaseous state (sublimation) by heating at a reduced pressure. Used to stabilise coagulation factor concentrates.</td>
</tr>
<tr>
<td><strong>Ion-exchange</strong></td>
<td>A process used to separate or purify proteins in which selected proteins are bound to a solid matrix and then removed. See chromatography.</td>
</tr>
<tr>
<td><strong>Ionic strength</strong></td>
<td>A measure of the presence of charged molecules in a solution (calculated as $\frac{1}{2} \Sigma C Z^2$ where $C =$ concentration &amp; $Z =$ valency of each substance present)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>A measure of the acidity or alkalinity of a solution. An important parameter in plasma fractionation.</td>
</tr>
<tr>
<td><strong>Precipitate</strong></td>
<td>A state whereby a protein, which is normally dissolved, is in the form of small amorphous particles. Can be produced by adding chemicals (eg. ethanol) which</td>
</tr>
</tbody>
</table>

(protein)
<table>
<thead>
<tr>
<th><strong>PEN.012.1883</strong></th>
<th><strong>dehydrate proteins causing them to become insoluble. Protein precipitation is used widely in plasma fractionation to separate, concentrate and purify proteins.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyrogen</strong></td>
<td>A substance produced by bacteria which causes a rise in the body temperature of humans.</td>
</tr>
<tr>
<td><strong>Sublimation</strong></td>
<td>The process in which a substance in a solid (frozen) state is transferred to the gaseous state directly, without passing through a liquid phase. Used in freeze drying to remove water from heat sensitive biological substances.</td>
</tr>
<tr>
<td><strong>Ultrafiltration</strong></td>
<td>A process for concentrating proteins using synthetic membranes which retain large molecules and allow small molecules to pass through.</td>
</tr>
</tbody>
</table>

**Statement of Truth**

I believe that the facts stated in this witness statement are true.

Signed .......................................................... ..................................................

Dated .......................................................... ..................................................
FIGURE 1
SIMPLIFIED PROCESS FLOW-SHEET FOR THE FRACTIONATION OF PLASMA AT THE PFC AT THE END OF 1983

MAINSTREAM PROCESS | INTERMEDIATE PRODUCT | FURTHER PROCESSING | FINAL PRODUCT
--- | --- | --- | ---
Frozen Plasma | Thawing | Cryoprecipitate | Purification | Formulation | Membrane Filtration | Aseptic Dispensing | Freeze Drying | FACTOR VIII CONCENTRATE

Ion Exchange Adsorption | Ion Exchange Eluates | Eluate-selection | Formulation | Membrane Filtration | Aseptic Dispensing | Freeze Drying | FACTOR IX CONCENTRATE

Cold-Ethanol Fractionation | Fraction I (discard) | | |

Fraction II+III (discard Fraction III) | Fraction II | Depth Filtration | Freeze Drying | Formulation | Membrane Filtration | Aseptic Dispensing | Immunoglobulin for Intramuscular Administration

or

Fraction II | Depth Filtration | Acid/Enzyme treatment | Membrane Filtration | Aseptic Dispensing | Freeze Drying | Immunoglobulin for Intravenous Administration

Fr IV₁ ± IV₄ (discard) | Fraction IV₁ ± IV₄ | Depth Filtration | Vacuum-distillation | Membrane Filtration | Aseptic Dispensing | Pasteurisation | Stable Plasma Protein Solution

or

Fraction V | Depth Filtration | Vacuum-distillation | Membrane Filtration | Aseptic Dispensing | Pasteurisation | Human Albumin

Distillation (for ethanol recovery)
**FIGURE 2 (A, B & C)**

### A (1980-1984)
- A01. Warm plasma to -10°C
- A02. Strip-off plastic bags
- A03. Crush & thaw plasma
- A04. Collect cryoprecipitate
- A05. Rinse cryoprecipitate
- A06. Cryoprecipitate extraction
- A07. Adjust pH to 7.0
- A08. Adsorb with Al(OH)₃
- A09. Centrifugation
- A10. Collect supernatant
- A11. Filter to 0.45μm
- A12. Formulate filtrate with citrate
- A13. Adjust pH to 6.8
- A14. Filter to 0.22μm
- A15. Dispense aseptically
- A16. Freeze product (cold-shelf)
- A17. Freeze dry (method 1)

**Unheated FVIII (NY)**

(A18. Dry heat, 2h at 68°C)

**Heat Treated FVIII (NY-HT1)**
(2 hours at 68°C)

### B (1985-1986)
- B01. Warm plasma to -10°C
- B02. Strip-off plastic bags
- B03. Crush & thaw plasma
- B04. Collect cryoprecipitate
- B05. Rinse cryoprecipitate
- B06. Cryoprecipitate extraction
- B07. Adjust pH to 7.0
- B08. Adsorb with Al(OH)₃
- B09. Centrifugation
- B10. Collect supernatant
- B11. Filter to 0.45μm
- B12. Formulate filtrate with citrate & sucrose
- B13. Adjust pH to 6.8
- B14. Filter to 0.22μm
- B15. Dispense aseptically
- B16. Freeze product (cold-shelf)
- B17. Freeze dry (method 1)

### C (1986-1991)
- C01. Warm plasma to -10°C
- C02. Strip-off plastic bags
- C03. Crush & thaw plasma
- C04. Collect cryoprecipitate
- C05. Rinse cryoprecipitate
- C06. Cryoprecipitate extraction
- C07. Adjust pH to 6.7
- C08. Adsorb with Al(OH)₃
- C09. Zinc precipitation
- C10. Centrifugation
- C11. Collect supernatant
- C12. Formulate supernatant with citrate, sucrose, calcium & NaCl
- C13. Adjust pH to 6.9
- C14. Filter to 0.45μm
- C15. Concentrate by ultrafiltration
- C16. Diafiltration to remove zinc & formulate with tris, citrate, sucrose, calcium & NaCl
- C17. Filter to 0.22μm
- C18. Dispense aseptically
- C19. Freeze product (warm-shelf)
- C20. Freeze dry (method 2)
- C21. Dry heat, 72h at 75 or 80°C

**Heat Treated FVIII (Z8)**
(72 hours at 75 or 80°C)
### FIGURE 3 (A & B)
Outline Processes for the Preparation of Factor IX Concentrate (DEFIX) at PFC

#### A (1972-1984)
- **A01.** Warm plasma to -10°C
- **A02.** Strip-off plastic bags
- **A03.** Crush & thaw plasma
- **A04.** Remove cryoprecipitate
- **A05.** Formulate cryo-supernatant
- **A06.** Adjust pH to 6.9
- **A07.** Ion Exchange adsorption
- **A08.** Collect gel by centrifugation
- **A09.** Suspend gel in buffer
- **A10.** Add gel to chromatography column
- **A11.** Treat column with wash buffer
- **A12.** Remove FIX with elution buffer
- **A13.** Collect FIX eluates E₁ to E₁₀
- **A14.** Freeze & store eluates
- **A15.** Thaw selected eluates
- **A16.** Pool selected eluates
- **A17.** Dilute to target potency
- **A18.** Filter to 0.22μm
- **A19.** Dispense aseptically
- **A20.** Freeze product (cold shelf)
- **A21.** Freeze dry (method 1)

**Unheated FIX (DEFIX)**

#### B (1985-2005)
- **B01.** Warm plasma to -10°C
- **B02.** Strip-off plastic bags
- **B03.** Crush & thaw plasma
- **B04.** Remove cryoprecipitate
- **B05.** Formulate cryo-supernatant
- **B06.** Adjust pH to 6.9
- **B07.** Ion Exchange adsorption
- **B08.** Collect gel by centrifugation
- **B09.** Suspend gel in buffer
- **B10.** Add gel to chromatography column
- **B11.** Treat column with wash buffer
- **B12.** Remove FIX with elution buffer
- **B13.** Collect FIX eluates E₁ to E₁₀
- **B14.** Freeze & store eluates
- **B15.** Thaw selected eluates
- **B16.** Pool selected eluates
- **B17.** Dilute to target potency
- **B18.** Add anti-thrombin III
- **B19.** Filter to 0.22μm
- **B20.** Dispense aseptically
- **B21.** Freeze product (cold shelf)
- **B22.** Freeze dry (method 1)
- **B23.** Dry heat (72 hours at 80°C)

**Heat Treated FIX (HT-DEFIX)**

(72 hours at 80°C)