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SNBTS BRIEFING PAPER

ON

THE DEVELOPMENT OF HEAT TREATMENT

OF COAGULATION FACTORS

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GLOSSARY

AIDS – acquired immunodeficiency syndrome	a disease which damages the ability of humans to fight off other infections. Originally know as Gay Related Immunodeficiency (GRID) and first reported in the USA in 1981. The disease is caused by a blood borne virus, the Human Immunodeficiency Virus (HIV).
albumin	a major protein circulated in the bloodstream.
antibody	a protein produced as part of the body's immune response to a 'foreign invader'. Depending on the infectious agent, antibodies may be effective at eliminating a 'foreign invader' (e.g. measles virus) or less effective (e.g. HIV, Hepatitis B and hepatitis C infection). A reactive antibody to a virus, such as hepatitis C virus, means that the person may at some stage have been infected with the virus. It does not necessarily indicate present infection.
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). Cryoprecipitate can be dissolved in a smaller volume than the volume of plasma that it was prepared from, thereby concentrating the factor VIII about 10-fold over plasma. Cryoprecipitate was not suitable for heat treatment when methods were devised for the heat treatment of factor VIII concentrates.
factor VIII	a blood coagulation factor which is lacking in people with haemophilia A. Factor VIII is a protein which is present in trace quantities in the plasma of normal people e.g. accounting for about 6 parts per million (ppm) of the total protein present in normal human plasma.
factor IX	a blood coagulation factor which is lacking in people with

	haemophilia B.
fibrinogen	a human blood coagulation protein of low solubility which tends to co purify with factor VIII.
fibronectin	a human blood protein of low solubility which tends to co-purify with factor VIII.
fractionation	the process of extracting and separating individual proteins from blood plasma to obtain a number of different medicinal products. Originally used to describe a process devised by EJ Cohn in the early 1940s in which human plasma proteins were separated according to differences in their solubility in the presence of ethanol. The expression is often used to describe the complete pharmaceutical process (or industry) by which blood plasma products are manufactured.
hepatitis A	a form of hepatitis which has a relatively short incubation period and is caused by a virus which is blood borne. Originally known as infectious hepatitis and normally spread by the faecal-oral route (e.g. via contaminated food such as shellfish). Occasionally transmitted via blood products. It usually causes a relatively acute illness with jaundice, but recovery is usually without chronic infection or a long-term carrier state
hepatitis B	a type of viral hepatitis with a longer incubation period than hepatitis A. Caused by a virus which is blood borne and originally known as serum hepatitis because of its transmission by blood serum and other body fluids. Mother-to-infant and sexual transmission are relatively common. Hepatitis B infection may be acute and be followed by recovery, or may lead to chronic infection where the virus is not eliminated from the body. In cases of chronic infection, the individual is at risk of liver damage such as cirrhosis and liver cancer.
hepatitis C	a form of hepatitis with a very long incubation period. Accounts for the vast majority of cases previously known as non-A, non-B hepatitis as well as about 90% of all cases of post-transfusion hepatitis. Most people with hepatitis C

	develop chronic infection, often without any signs. The virus responsible, the hepatitis C virus, was discovered in 1989.
HIV - human immunodeficiency virus	the blood borne virus which causes AIDS. There are different strains of the virus (i.e. HIV-0, HIV-1, HIV-2). HIV-1 was first isolated in 1983 and was proven to be the cause of AIDS in 1984. HIV-2 was discovered in 1986.
immunoglobulin	plasma proteins involved in fighting infections (commonly known as antibodies).
inhibitor	an antibody to factor VIII (or factor IX) which can form in the circulation of haemophilia patients being treated with a coagulation factor concentrate. The development of an inhibitor is a serious complication of haemophilia treatment as the antibody prevents the factor VIII which has been administered from working.
Ion- exchange	a process used to separate or purify proteins in which selected proteins are bound to a solid matrix and then removed.
lyophilisation	a method of stabilising biological substances by a process of dehydration in which water is transferred directly from the frozen state (ice) to the gaseous state (sublimation) by heating at a reduced pressure. Used to stabilise coagulation factor concentrates; also known as freeze drying.
marker virus	a non human virus which is suitable for experimental use in the laboratory and which would be expected to behave in a manner comparable to a human virus of interest (a relevant virus). A number of model viruses have been recommended by the European regulatory authority to simulate the behaviour of HIV, HAV, HBV, HCV and other human viruses in the validation of processes used for the elimination of viruses. Sometimes called a 'model' virus.
Neo- antigens	a new antigenic site on a protein e.g. as a result of damage caused during manufacture and which may result in a patient forming an inhibitor (antibody) to the protein.

NANBH	non-A non-B hepatitis - hepatitis that is not due to either the hepatitis A virus or the hepatitis B virus. Prior to the discovery of the hepatitis C virus, this group would have included infection with the hepatitis C virus as well as hepatitis due to other viruses (e.g. cytomegalovirus, Epstein-Barr virus) and non-viral causes of hepatitis (e.g. obesity, alcohol, certain medications.
Pharmaco-kinetics	a description of the uptake of a medicine by the body and its subsequent elimination from the body.
plasma	the straw coloured liquid portion of blood. Contains proteins (e.g. albumin, antibodies, clotting factors) as well as hormones, fats and dissolved salts and gases.
recombinant	a technique in which a specific segment of DNA is isolated and inserted into a bacterium or other new host (e.g. yeast, animal cell) in a form that allows the DNA segment to be replicated as the new host multiplies. The DNA segment is said to have been 'cloned' because it exists free of the rest of the DNA from which it was derived. Used in the modern treatment of haemophilia in the form of recombinant Factor VIII and Factor IX concentrates in which the human coagulation factor is synthesised by hamster cells.
seroconversion	a response to an infection, usually occurring early in an infection, which denotes the point where an individual goes from having no antibody, to the formation of antibodies against the agent causing infection.
serological marker –	a substance detected in blood serum which indicates the presence of an infection.
Solid- phase poly electrolytes	a substance that can be used to separate proteins from one another. Similar to ion exchange.
thrombotic reactions	formation of a blood clot due to the presence or formation of the protein thrombin.
thrombogenic	a potential to cause thrombosis.

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SUMMARY

The possibility that coagulation factor concentrates might be capable of transmitting viral hepatitis has been known since their inception in the 1940s. Considerable research was undertaken from the outset to try to eliminate this risk, with a variety of physical and chemical methods of virus inactivation being investigated, including heat treatment, all of which were unsuccessful at this time.

Research into heat treatment of coagulation factors was renewed as recognition grew that a type of hepatitis could be transmitted which was neither hepatitis A, nor hepatitis B, after the viruses responsible for hepatitis A and hepatitis B had been discovered. A variety of procedures were devised and evaluated by different manufacturers during the early 1980s. None of these methods was successful in eliminating the transmission of non-A, non-B hepatitis (NANBH), except for a method of pasteurisation (heating of a solution) which had been devised by Behringwerke AG in Germany, but which resulted in such a low yield of factor VIII that the process was not used for the general production of Factor VIII concentrate at this time either by Behringwerke or by any other manufacturer.

It was during this period that the epidemic of AIDS emerged in the USA. Fortuitously, the virus responsible, the human immunodeficiency virus (HIV) proved to be less resistant to heat than the agent(s) responsible for NANBH and heat treatment procedures which had failed to eliminate the transmission of NANBH were found to be capable of destroying HIV in laboratory experiments and were therefore brought into general use in many countries during 1985.

The SNBTS acted on this information immediately, initiating heat treatment of its stock of Factor VIII concentrate in November 1984 and distributing supplies in December 1984, enabling Scotland to be the first country in the world to provide all of its patients with Factor VIII concentrate safe from transmission of HIV.

The SNBTS also introduced heat treated Factor IX concentrate in October 1985, following the completion of essential animal safety studies.

Further research was required to discover a suitable means of inactivating the agent(s) responsible for NANBH. Two procedures for the treatment of Factor VIII

emerged subsequently which, in addition to pasteurisation, were eventually shown to be effective in preventing transmission of NANBH and hepatitis C (HCV); these were:

- heat treatment of the freeze dried product at 80°C for 72 hours, devised at the Plasma Fractionation Laboratory (PFL), Oxford; which was a pilot production facility for the NHS Blood Products Laboratory (BPL) at Elstree and which was unique in being dedicated only to the development and preparation of coagulation factor concentrates.

- chemical (solvent/detergent) treatment, devised at the New York Blood Centre (NYBC) in the USA.

Application of these methods was not straightforward:

- the yield of Behringwerke's pasteurised Factor VIII was only 8% and full manufacture was not established by Behringwerke until 1985, after modifications had been made to increase yield. Although a NANBH safety study of the original product was published in 1987, a second safety study was required to confirm the safety of the modified product, this was published in 1990

  [*Note: a similar product was introduced by Bayer in the USA in 1986 but was replaced in 1991 because of low yield; no safety study was published*].

- dry heating of Factor VIII concentrate at 80°C was an extremely challenging technology, which was difficult to perform successfully in routine, large-scale production and, prior to 1990, was mastered only by the BPL and by the SNBTS

- routine supply of this type of product by the SNBTS was some 18 months later than BPL. This was because the available evidence suggested that pasteurisation was the option most likely to succeed in destroying the agent(s) responsible for NANBH and the SNBTS had therefore continued to focus its research primarily on this technology during 1985. In February 1986 the SNBTS altered its strategy to develop a product comparable to BPL's 8Y in order to provide a higher margin of safety against HIV. The SNBTS developed

its new product as quickly as possible, with material for clinical evaluation being available in December 1986 and routinely available from April 1987

- initial evidence that this procedure might be effective in preventing the transmission of NANBH was provided by BPL in October 1986, by which time the SNBTS had already begun production of a comparable dry heat treated Factor VIII concentrate, named Z8. The effectiveness of the BPL procedure against NANBH transmission was published in 1988 and effectiveness against HCV confirmed in a substantive study published in 1993

- as the SNBTS was able to provide sufficient Factor VIII concentrate for all patients in Scotland, the development of Z8 by the SNBTS made Scotland the first country in the world to be able to provide Factor VIII concentrate for all of its patients that was free from the risk of hepatitis C

- the solvent-detergent (s/d) method of the NYBC was difficult to apply because suitable technology for removing toxic chemicals from the product did not exist and appropriate procedures were not developed until the late-1980s. Evidence that s/d-treatment was effective in preventing transmission of NANBH was published in July 1988.
  [*Note: hepatitis A was subsequently transmitted by a number of coagulation factor concentrates manufactured using s/d-treatment, as this procedure is ineffective against viruses which do not possess a lipid (fat) - envelope, such as the hepatitis A virus*]

- the first commercial Factor VIII concentrate safe from transmission of NANBH (Monoclate-P) was licensed in the UK on 13th December 1989 and the first s/d-treated Factor VIII concentrate was licensed in the UK in 1992

- the earlier generation of commercial heat treated concentrates remained in use until the early 1990s, especially in England where most Factor VIII concentrate used at this time was provided by commercial suppliers. A number of cases of hepatitis transmission were associated with the use of commercial Factor VIII concentrates in England & Wales during the period 1985-1987 (see section 8, Chronology UK).

1. HISTORICAL BACKGROUND (WORLD-WIDE)

1.1 Pasteurisation of Human Albumin

Human proteins in their natural state exist at body temperature and are prone to damage at raised temperatures. Proteins removed from their natural environment may be even more susceptible to heat induced damage, either from heat directly or from an increase in the activity of substances, such as enzymes, which can degrade proteins. Heat causes proteins to denature and become insoluble (e.g. cooked vs. uncooked egg white) and can occur at modest temperatures, well below the boiling point of water, with different proteins differing in their ability to withstand heat. Of the human plasma proteins, albumin is one of the most stable to heat and factor VIII one of the least stable. This property of albumin was crucial to it becoming the first plasma product to be subjected to a heat treatment process.

The development of methods for the preparation of medical products from human blood plasma began in April 1940 when, in preparation for war, Dr Edwin J Cohn was commissioned by the United States National Research Council to investigate the possibility of providing an alternative to blood plasma for the treatment of casualties. Cohn used his extensive knowledge of protein chemistry (Cohn & Edsall, 1943[1]) to devise a method for producing albumin by fractionating plasma into different protein constituents (Cohn *et al.*1946[a]). Successful treatment of casualties at the USA naval base at Pearl Harbour in December 1941 led to the large-scale production of Human Albumin in the USA under the direction of Cohn (Cohn, 1948[a]).

One important requirement was that the solution of Human Albumin should be stable under battlefield conditions; colloquially described as being able to withstand storage in "*a tank in Tobruck*". This led to an investigation of various methods to increase the stability of Human Albumin to heat. The early methods were not ideal, but a chance observation by Dr J Murray Luck led ultimately to the discovery of chemicals which could substantially enhance the heat stability of Human Albumin (Boyer *et al.*1946[a], Edsall, 1984[a]). So profound was the effect, that it was suggested that albumin might tolerate pasteurisation, which could be used to destroy bacterial contaminants instead of using a mercury preservative (Scatchard *et al.*1945[a]). Consequently a modified product that had been heated in solution (pasteurised) for 10 hours at 60°C was introduced in the USA from June 1945 (Cohn, 1948[a]).

Similar research was undertaken by Dr Cohn's group to discover a means of stabilising Human Immunoglobulin, another product of plasma fractionation that was being used to protect USA troops from measles, so that pasteurisation technology could be applied to it too. However, an equivalent method for stabilising immunoglobulins could not be found and the project was abandoned (Surgenor, 2002[a]).

A growing awareness that an infection, known as serum hepatitis, could be transmissible via plasma products led Dr Cohn to recommend that studies should be carried out to determine the heating condition needed to destroy the infective agent responsible. Two studies were funded by the US National Institute of Health for this purpose, both of which used prison volunteers as subjects. In the first study, infectious plasma was added to aliquots of Human Albumin which were then pasteurised for 10 hours at either 60°C or 64°C. None of the recipients of albumin which had been heat treated developed jaundice, by contrast 3 of 5 recipients of unheated material were infected (Gellis, *et al.* 1948[a]).

A substantive study followed, in which albumin and immunoglobulin products were prepared at large-scale from plasma which was known to be highly infectious (Tabor, 1999[a]). No infection was observed in recipients of albumin, produced using fractionation method 6 of Cohn, which had been pasteurised for 10 hours at 60°C, nor in recipients of immunoglobulin produced by Cohn's fractionation method 6/9. By contrast, infection was observed in recipients of unheated albumin and in recipients of immunoglobulin which had been prepared by an alternative method of fractionation (Murray, *et al.*1955[a], Pennell, 1957[a]).

Following the discovery of the hepatitis B virus (HBV), samples retained from these studies were tested for evidence of sub-clinical infection (Barker & Murray, 1972[a]). It was found that most HBV had been eliminated by the fractionation process rather than by the pasteurisation step (Hoofnagle & Barker, 1976[a]). Other investigators found that pasteurisation alone failed to eliminate HBV transmission either in animals (Shikate et al, 1978[a]) or in humans (Soulier et al, 1972[a]).

As a result of these observations, cold-ethanol fractionation became the recommended procedure for the manufacture of albumin and immunoglobulin products with albumin being subjected to pasteurisation for 10 hours at 60°C.

Subsequently, in 1973, hepatitis was transmitted in the USA by a number of batches of Plasma Protein Fraction (PPF), a less purified form of albumin, as a result of the bulk pasteurisation of a large volume of PPF being performed incorrectly (Pattison *et al.* 1976[a]). Consequently, it became a requirement that albumin products should be pasteurised when sealed inside the final container.

1.2. Attempts to Treat Coagulation Factors to Destroy Viruses.

The chemicals that stabilized albumin were not applicable to other proteins, leaving albumin as the only plasma protein to which the original pasteurisation technology could be applied (Edsall, 1984[a]). This opinion was well established and was included in the foremost textbook on biochemical engineering "*Albumin can be pasteurized at 60°C for 10 hours but the other fractions are heat labile*" (Webb, 1964[3]). Considerable research was undertaken in an attempt to discover alternative means of inactivating the agent responsible for transmission of serum hepatitis that could be applied to other plasma products such as coagulation factor concentrates including:

- treatment of plasma with phenol and ether (Sawyer *et al.* 1944[a]).
- irradiation of plasma with ultraviolet light (Oliphant & Hollaender, 1946[a]).
- pasteurisation of plasma for four hours at 60°C (Murray & Deifenbach, 1953[a])
- storage of plasma for six months at 30-32°C (Allen *et al.* 1954[a]).
- evaluation of 550 chemicals for inactivation of viruses in human blood (Hartman *et al.* 1955[a]).
- irradiation of factor VIII with ultraviolet light (McCall *et al.*1957[a]).
- cathode ray irradiation of plasma (Trump & Wright, 1957[a]).
- gamma ray irradiation of plasma (Jordan & Kempe, 1957[a])
- treatment of factor VIII with nitrogen mustard (Pennell, 1957[a]).
- treatment of factor VIII with beta-propiolactone and ultraviolet irradiation (Sgouris, 1969[4])
- treatment of factor VIII with detergent, beta-propiolactone and ultraviolet irradiation and adsorption with silica (Kotitschke & Stephan,1983[5]).

None of these procedures successfully eliminated infectivity from Factor VIII concentrates without causing unacceptable damage to the product.

1.3. Attempts to Physically Remove Viruses from Coagulation Factors

The failure to discover a means of treating coagulation factors to destroy the agents responsible for the transmission of serum hepatitis led investigators to study methods for the physical removal of viruses including:

- adsorption to insoluble silicic acid (Hedlund, 1973[a])
- affinity chromatography (Charm & Wong, 1974[a])
- precipitation of viruses by polyethylene glycol (Johnson *et al.*1976[a])
- adsorption of factor VIII to solid-phase polyelectrolytes (Johnson *et al.* 1978[a])
- hydrophobic interaction chromatography (Einarsson *et al.* 1981[a])

None of these procedures were applied successfully to the routine manufacture of human plasma products and research on them was generally superseded by research into new methods of virus inactivation which emerged in the 1980s.

2. DEVELOPMENT OF HEAT TREATMENT OF COAGULATION FACTORS (WORLD-WIDE)

2.1. Early Heat Treatment Methods

Despite the sensitivity of coagulation factor concentrates to heat, a number of methods were devised by commercial manufacturers of plasma products during the early 1980s, with the objective of inactivating the agent(s) responsible for the transmission of NANBH. These methods involved heating the coagulation factor either dissolved in solution (pasteurisation) or in the form of a freeze dried powder (dry heat).

In general the approach was to apply as much heat as a particular product would tolerate before being damaged unacceptably by, for example, becoming insoluble or exhibiting a reconstitution time which was unacceptably long. Pasteurisation was undertaken for 10 hours at 60°C, whereas methods of dry-heat treatment ranged from 10 hours at 60°C up to 72 hours at 68°C.

To put these heat treatment procedures into context, it may be helpful to note that water becomes too hot for the human body at about 50°C and at 68°C will cause 3rd degree burns in about 3 seconds.

These early heat treatment procedures (summarised in Kasper et al. 1993[a]) included:

Pasteurisation

- heating a solution of Factor VIII for 10 hours at 60°C in the presence of high concentrations of sucrose and the amino acid glycine as stabilisers (Humate-P: Behringwerke, Germany; Koate HS, Cutter Biologicals, Miles Inc. USA).

Dry Heat Treatment

- heat treatment of a freeze dried powder of Factor VIII for 10 hours at 60°C in the presence of steam under pressure (Kryobulin TIM: Immuno Ag, Austria)
- heat treatment of freeze dried powder of Factor VIII for 24 hours at 60°C in the presence of the organic solvent n-heptane (Profilate Heat-Treated: Alpha Therapeutic Corporation, USA).
- heat treatment of a freeze dried Factor VIII concentrate for 30 hours at 60°C (H.T. Factorate: Armour Pharmaceutical Company, USA).
- heat treatment of a freeze dried Factor VIII concentrate for 72 hours at 60°C (Hemofil T: Hyland Division, Baxter, USA).
- heat treatment of a freeze dried Factor VIII concentrate for 72 hours at 68°C (Koate HT: Cutter Biological, Miles Inc. USA).

Although these dry-heated products were approved in the USA from 1983, they were not widely used there because they were not proven to be effective in destroying viruses, there was concern that patients might be harmed and the products were more expensive than established concentrates. Commercial dry-heated concentrates were not licensed for use in the UK until February 1985, for similar reasons.

2.2 Obstacles to the Development of Heat Treatment for Coagulation Factors

Investigators were faced with a number of difficulties, including:

- the sensitivity of factor VIII (and related proteins) to heat, both in plasma and during processing to produce concentrate (Penick & Brinkhous, 1956;[6] Wolf, 1959[7]),
- the difficulty of manufacturing Factor VIII concentrate and the low yields which resulted,

- the high demand for coagulation factor concentrates, because of their value in preventing death and disability in people with haemophilia,
- an emphasis on maximising yield and output so that the use of imported commercial products could be avoided,
- limited availability of human plasma for research & development,
- limited knowledge concerning the structure and properties of factor VIII and related proteins (Hoyer,1981[8]),
- inability to purify and characterise factor VIII before 1984 (Rotblat et al, 1985[a]),
- inaccurate and laborious analytical methods for the measurement of factor VIII activity (Barrowcliffe, 1984[9]),
- lack of knowledge concerning the nature of the agent responsible for AIDS,
- lack of knowledge concerning the heat sensitivity of the agent responsible for AIDS,
- The unavailability of a serological marker to HIV, uncertainty over the nature of the agent(s) responsible for NANBH (Bradley & Maynard, 1983;[a] Tabor, 1985[10]), lack of knowledge concerning the heat sensitivity of the agent(s) responsible for NANBH,
- the unavailability of a serological marker for HCV (before 1990),
- the failure of the chimpanzee model in predicting the effectiveness of heat treatment procedures (see Foster & Bienek, 2008[2], table 20.6),
- concern over potential adverse reactions in patients, including general intolerance to a heated product (Ludlam, 1984[11]), the formation of inhibitors (antibodies) to the relevant coagulation factor (Ludlam, 1983[12], Hann, 1984[13], Bird et al, 1985[14], Mannucci, 2003[15], Evatt, 2006[16]) and thrombotic reactions associated with Factor IX concentrates (Aledort, 1977[17]).

[Note: abnormal outbreaks of inhibitor formation subsequently occurred in recipients of heat treated (pasteurised) Factor VIII concentrates manufactured at Central Laboratory for the Blood Transfusion Service (CLB), The Netherlands (Peerlink et al, 1993[a], Rosendaal et al, 1993[a], Barrowcliffe, 1993[a]) and at Octapharma, Austria (Laub et al, 1997[a], Peerlink et al, 1997[a], Raut et al, 1998[a]).]

2.3. Effectiveness in Preventing Transmission of Human Immunodeficiency Virus

The first heat treatment methods, with the exception of pasteurisation, were not effective in destroying the agent(s) responsible for NANBH and were not therefore widely used, their production representing only a small proportion of the output of Factor VIII concentrate of the respective companies.

Once the virus responsible for AIDS had been discovered (Gallo, et al. 1984[a]) the effect of heat treatment on HIV that had been added to Factor VIII concentrate was studied in laboratory experiments by the USA Centers for Disease Control (CDC), with the studies beginning in Spring 1984 and being completed in collaboration with Bayer (Miles/Cutter) in the late-summer of 1984 (Evatt, 2007[18]).

A summary of the findings was published by CDC on 26th October 1984 (Centers for Disease Control, 1984[a]) and led to the widespread introduction of heat treated concentrates during 1985 (Glied, 1999[a]). Details of this and a similar study were published in peer-reviewed journals in June and August 1985 (Levy, et al 1985[a]; McDougal, et al.1985[a]).

Information on the effect dry-heating Factor VIII concentrate for one hour at 68°C was presented:

- on 2nd November 1984, in an oral presentation by a speaker from CDC at a conference in the Netherlands, at which SNBTS was represented (Foster, 1984[19])

- in September 1985, in a commercial brochure published by Cutter (Cutter Biological, 1985[20]).

Evidence that HIV was relatively heat sensitive and could be inactivated by heating in solution (in 50% serum) was first published in January 1985 (Spire, et al 1985[a]) with a caution from the authors that "*The data cannot, however, be extrapolated to lyophilised products since our experiments were conducted in liquid medium.*"

In February 1985, it was reported that 18 patients who had been treated exclusively with Hemofil® T, dry heat treated at 60°C for 72 hours, were HIV- negative, whereas 5 of 29 patients (17%) who had been treated over the same period with various brands of unheated Factor VIII concentrate from the USA were HIV-positive,

consistent with HIV having been inactivated by the heat treatment method employed (Rouzioux, et al. 1985[21]).

Commercial heat treated Factor VIII concentrates were first licensed for use in the UK during February 1985. However, supplies were insufficient for some months whilst manufacturers made the transition from unheated to heat treated products, with the result that some 40% of UK haemophilia centres were still using unheated concentrates as late as May 1985 (Bloom, et al. 1985[a]).

Although the results of the initial studies of HIV inactivation were encouraging, a subsequent laboratory study in the USA found only a modest degree of inactivation of HIV by dry heat treatment at 60°C, leading the author to advise caution in relying on dry heat treatment for the inactivation of HIV (Prince, 1986[22]).

Factor VIII concentrate prepared by Armour Pharmaceuticals (HT Factorate) that was heated at 60°C for 30 hours was later associated with transmissions of HIV in a number of countries (Wolfs, et al. 1988[a]; Dietrich, et al. 1990[a]; Remis, et al. 1990[a]) including the UK (Williams, et al. 1990[a]), resulting in the UK product licence being withdrawn voluntarily by the company in October 1986.

These events led to the effectiveness of dry heat treatment being questioned and caused most manufacturers to begin to develop solvent/detergent treated products instead. Despite these concerns, it is likely that the early heat treated products were largely successful in destroying HIV, evidenced by the fact that in a major USA study there were no cases of HIV infection in people with haemophilia born after 1984 (Soucie, et al.2001[a]). Nevertheless, a small number of HIV transmissions in the USA were associated with the administration of heat treated concentrates manufactured prior to donor screening (Fricke et al. 1992[23])

Estimates of the date in different countries on which unheated Factor VIII concentrates were generally replaced by heat treated concentrates (Feldman & Bayer, 1999[24]) are shown in the table below.

Table 1. Introduction of Heat Treated Factor VIII Concentrate in Different Countries

Date	Country	Comment
Dec 1984	Scotland	Heat treatment of FVIII begun Nov 1984; general distribution of heat treated FVIII on 10th Dec 1984; all unheated FVIII recalled
Jan 1985	USA	Heat treated FVIII approved 1983/84 but little used; heat treated FVIII advised in Oct 1984; general distribution of heat treated FVIII from Jan 1985; no recall of unheated stocks
Feb 1985	West Germany	Heat treated FVIII mandated Feb 1985; no recall of unheated stocks
Mar 1985	Sweden	Heat treatment of FVIII implemented 22 Feb 1985
Apr 1985	Australia	Dry heat treatment for 12 hours at 60°C begun Nov 1984; use of unheated FVIII continued until April 1985.
Jun 1985	England & Wales	Commercial heat treated FVIII approved Feb 1985; use of heat treated FVIII advised June 1985; 72h/80°C dry heat treated FVIII issued routinely by BPL Sep 1985; no recall of unheated FVIII
Jul 1985	Canada	Distribution of commercial heat treated FVIII from May 1985; distribution of unheated FVIII ceased July 1985; no recall of unheated FVIII.
Jul 1985	Italy	Commercial heat treated FVIII approved from Dec 1984; use of heat treated FVIII mandated July 1985; unheated FVIII recalled May 1988
Oct 1985	Denmark	Heat treated FVIII mandated Oct 1985; no recall of unheated FVIII
Oct 1985	France	Heat treatment of FVIII begun by Centre Nationale Transfusion Sanguine in Sep 1985; heat treatment mandated Oct 1985; no recall of unheated FVIII
Oct 1985	Japan	General distribution of unheated FVIII ceased in July 1985; heat treatment mandated in Oct 1985; voluntary recall of unheated FVIII in Nov 1986 but some sales continued.

2.4 Effectiveness in Preventing Transmission of Non-A, Non-B Hepatitis (NANBH)

The failure of the chimpanzee model to predict hepatitis safety in humans meant that the effectiveness of heat treatment procedures in preventing transmission of NANBH could only be established by monitoring recipients of treated products for absence of infection.

The International Society of Thrombosis & Haemostasis (ISTH) provided a professional forum at which guidance was provided by international experts. At a meeting of the ISTH Factor VIII Sub-Committee in Miami in December 1984, a protocol was designed for this purpose.

There was evidence that patients who had not previously been treated with Factor VIII concentrate were likely to be infected with NANBH on their first treatment with a large-pool, unheated Factor VIII concentrate (Fletcher *et al.* 1983[25], Kernoff *et al.* 1985[26]). Therefore the ISTH protocol recommended that ten batches of a treated product should be administered to twenty patients who had not been treated previously with a blood product, with each batch being assigned to two patients and with each patient being monitored for evidence of NANBH for six months, by liver function tests on blood samples taken every two weeks (Mannucci & Colombo, 1989[a]).

As patients who had previously been treated were ineligible, these studies had to be undertaken predominantly in newly diagnosed patients. Haemophilia is normally diagnosed in infancy. As the prevalence of haemophilia (A + B) is about two births per million population (Rizza *et al*, 2001[a]), then only a small number of suitable patients were available for such studies.

The small numbers of suitable patients and the rigorous sampling regime resulted in studies that were based on the ISTH protocol taking a long time to complete. However, where a heat treatment procedure had little or no effect against NANBH, results were obtained more quickly. For example, early evidence of NANBH infection from dry heat treated Factor VIII concentrate became available during 1983 (Mannucci, 2003[a]).

It was learned that heat treatment procedures devised by three commercial manufacturers had failed to destroy the agent(s) responsible for NANBH, viz:

- heat treatment of a freeze dried Factor VIII concentrate for 72 hours at 60°C; Hemofil T: Hyland Division, Baxter, USA (Colombo *et al*,1985[a]).
- heat treatment of a freeze dried Factor VIII concentrate for 30 hours at 60°C ; H.T. Factorate: Armour Pharmaceutical Company, USA (Preston *et al,* 1985[a]).
- heat treatment of freeze dried powder of Factor VIII for 24 hours at 60°C in the presence of the organic solvent n-heptane; Profilate Heat-Treated: Alpha Therapeutic Corporation, USA (Kernoff *et al,* 1987[a]).

Despite these disappointing results, two heat treatment procedures were subsequently found to be effective in preventing transmission of NANBH in clinical studies, viz:

- heating a solution (pasteurisation) of Factor VIII for 10 hours at 60°C in the presence of high concentrations of sucrose and the amino acid glycine as stabilisers; (Humate- P: Behringwerke, Germany). Results of a NANBH safety study were published by Schimpf *et al,* 1987.[a]
  [*Note: a second NANBH safety study was required because of modifications made by Behringwerke to increase yield (Mannucci et al, 1990[a]).*]

- dry heat treatment of Factor VIII and Factor IX concentrates for 72 hours at 80°C; (8Y and 9A HT: BPL, England). Results of an NANBH safety study were published by Colvin *et al,* 1988.[a]
  [*Note: the initial BPL study did not comply fully with the ISTH protocol and was therefore followed by a second study which was published by Rizza et al, 1993[a]).*]

No transmissions of HCV have been associated with severe dry heat treatment, however, pasteurised Factor VIII concentrate has been implicated in one case of HCV transmission, although intra-familial transmission could not be ruled out (Morfini, et al.1994[a]). Hepatitis B was transmitted by Behringwerke's pasteurised concentrate of Factor VIII (Brackmann & Egli, 1988[a]) and by their Factor IX concentrate (Jantsch-Plunger, et al, 1995[a]) which had been pasteurised in a similar manner.

Because of difficulties associated with heat treatment, most manufacturers of Factor VIII concentrates first achieved safety with respect to hepatitis C by using a

chemical method of virus inactivation which employed treatment with a mixture of solvent and detergent that was devised at the New York Blood Center (NYBC) (Horowitz et al, 1985[a]). This is illustrated in the table below.

Table 2. Development of Factor VIII Concentrates Safe from NANBH/HCV (International)

Year	Manufacturer	Virus Inactivation Method	Safety Study Published	Availability
1979	Behringwerke (Germany)	Pasteurisation 60°C /10 hr	Schwinn et al, 1987[a]	Very limited in Germany
1985	BPL (England)	Dry heat 80°C /72 hr	Colvin et al, 1988[a]	About 30% of FVIII in England prior to 1988, plus a small amount to Scotland in 1986.
1985	NYBC (USA)	Solvent/detergent	Horowitz et al, 1988[a]	Very limited in USA
1986	Cutter (USA)	Pasteurisation, 60°C/10 hr	None	Very limited in USA
1987	SNBTS (Scotland)	Dry Heat, 75-80°C/72hr	Bennett et al, 1993[a]	Generally available Scotland & N. Ireland
1988	Baxter (USA)	Solvent/detergent	Addiago et al, 1992[a]	UK licence 1994
1988	Biotransfusion (France)	Solvent/detergent	Noel et al, 1989[a]	France only, except for small quantity supplied to Scotland in 1991.
1989	Alpha (USA)	Solvent/detergent	Becton et al, 1994[a]	UK licence 1992
1989	Cutter/Bayer (USA)	Solvent-detergent	none	UK licence 1994
1989	Armour (USA)	Pasteurisation 60°C/10hr	none	UK licence Dec 1989
1990	CSL (Australia)	Dry heat 80°C/72hr	none	Australia only

3. PLATFORM TECHNOLOGIES (SNBTS)

Most Factor VIII concentrates produced during the 1970s, including that of the SNBTS, were based on procedures devised by Dr Alan Johnson of New York University Medical Center (Newman et al, 1971[a]).

Subsequently a number of independent discoveries were made by the SNBTS that contributed to the development of heat treatment. These advances are summarised in the chronological order of their discovery.

3.1 Improved Cryoprecipitate by Continuous-Thawing of Plasma

The time taken to re-dissolve freeze dried Factor VIII concentrate is a critical parameter in determining how much heat a particular Factor VIII concentrate can withstand. This re-constitution time is strongly influenced by the solubility of the cryoprecipitate from which the factor VIII has been prepared.

The production of cryoprecipitate is the first step in the process from which Factor VIII concentrate is manufactured. As well as being the point of principal loss of yield, the outcome of this step also influences the composition and quality of the final product.

A process for thawing plasma continuously was devised by the SNBTS (Foster & White, 1978[27]) to provide both faster thawing and better temperature control than was obtained by batch-thawing, with the aim of increasing both yield and process capacity. In addition the solubility of the resultant product was also increased in comparison with Factor VIII concentrate which had been prepared from cryoprecipitate produced by batch-thawing of plasma (Foster et al, 1982[28]).

Evaluation of the performance of pilot-scale equipment which had been designed for this purpose was begun in March 1979. The increase in yield of factor VIII was so dramatic that the established process of batch-thawing was completely replaced by continuous-thawing in July 1979. Larger-scale equipment was designed according to further observations with pilot equipment and was introduced into routine use in January 1981.

Enhancement in the solubility of the resultant SNBTS Factor VIII concentrate (named NY) subsequently enabled this product to withstand dry heating for 2 hours at 68°C without further modification being required.

The fact that this could be done without modification of the product meant that the heat treatment could be applied to stocks of product that had been manufactured previously, including stocks of unheated Factor VIII concentrate that were recalled following the introduction of heat treatment.

As the SNBTS had about 12 months shelf-stock of unheated Factor VIII concentrate, NY, when heat treated Factor VIII concentrate was introduced in December 1984, the ability to heat treat this material meant that factor VIII that had been prepared from earlier blood donations was subjected to heat treatment.

Normally, a lengthy period is required to establish a suitable shelf-stock of a new plasma product. The ability to create a stock of heat treated product quickly, by heating unheated stocks, not only enabled the SNBTS to immediately distribute sufficient heat treated product for all patients but also allowed unheated product that had been issued for use to be returned immediately.

3.2 Separation of Fibrinogen from Factor VIII by Precipitation with Zinc

Fibrinogen co-purifies with factor VIII and could typically constitute up to 60% of the protein present in intermediate-purity Factor VIII concentrates. Fibrinogen is a less soluble protein than factor VIII and is more sensitive to damage by heat. Therefore, it was necessary to remove much of this material before the solution of factor VIII could be subjected either to pasteurisation (MacLeod, 1982[29]) or to severe dry heat treatment. There was no satisfactory means of achieving this (Griffin 1986[30]) as processes available incurred a significant loss of factor VIII.

In 1982, Dr Milan Bier of the University of Arizona visited the Protein Fractionation Centre (PFC) to experiment on new methods for separating proteins from one another. One of these methods involved the use of zinc to selectively cause certain proteins to precipitate out of solution. Dr Bier added zinc to a number of protein mixtures in the PFC research laboratory, including a solution of factor VIII. A heavy precipitate formed which was composed of fibrinogen and fibronectin, another

protein with a low solubility which co-purifies with factor VIII; surprisingly most of the factor VIII remained in solution.

A suitable method for reducing the fibrinogen was needed to support pasteurisation of factor VIII. Scotland's Haemophilia Directors had also advised the SNBTS that a reduction in the fibrinogen content of Factor VIII was desirable clinically.[31] Therefore the use of zinc precipitation for this purpose was studied further. It was subsequently found that the addition of a small quantity of heparin with the zinc resulted in precipitation of some fibronectin as well as fibrinogen (Bier & Foster, 1983[32]), thereby reducing the concentration of another protein of low solubility and further increasing the degree of purification of factor VIII.

Zinc/heparin precipitation of cryoprecipitate extract became an essential step in the preparation of pilot batches of the SNBTS pasteurised Factor VIII concentrate (named ZHT) and later in the preparation of the SNBTS severely dry heated Factor VIII concentrate (Z8).

[*Note: it was whilst studying the use of the SNBTS zinc/heparin precipitation process that scientists at PFL in Oxford discovered an alternative method for the precipitation of fibrinogen and fibronectin, using a much higher concentration of heparin; a procedure which was employed in the preparation of 8Y, a Factor VIII concentrate (Winkelman,1988[a]) which was later found to be able to withstand dry heat treatment for 72 hours at 80°C.*]

3.3 Stabilisation of Factor VIII with Calcium

The instability of factor VIII is well established. It is for this reason that factor VIII is recovered only from plasma which has been frozen shortly after donation (fresh frozen plasma) and is why the final product is freeze dried to prevent its degradation. Despite these measures, processes for the production of Factor VIII concentrate are generally low yielding.

In investigating the mechanisms responsible for loss of yield, SNBTS researchers surprisingly found in late-1980 that the chemical, sodium citrate, which was generally added during processing to stabilise factor VIII by preventing coagulation (Newman et al, 1971[a]), was responsible for making factor VIII even more unstable.

It was considered that this might be due to the preferential binding of calcium to sodium citrate, as this was known to be the mechanism by which citrate acts as an

anti-coagulant, suggesting that removal of calcium might destabilise the factor VIII molecule. This was consistent with an observation that calcium was involved in linking together the sub-units of the factor VIII molecule (Fass et al, 1982[33]).

As the addition of citrate was necessary to prevent coagulation, the possibility of adding calcium to replace ionised calcium that had been removed was explored. It was discovered that the loss of factor VIII activity that had been observed was almost completely prevented, leading to addition of calcium becoming an important ingredient in the mixture of chemicals used to formulate factor VIII solutions (Foster et al, 1983[a]; Foster et al, 1988[a]).

Calcium addition was used by the SNBTS in the preparation of pilot batches of its pasteurised Factor VIII concentrate, ZHT, and in its Factor VIII concentrate, Z8 that was dry heated for 72 hours at 75/80°C (McIntosh et al, 1987[34]).

Information on calcium addition was shared with the BPL who utilised this in the preparation of 8Y (Smith, 1986[35]). The technique was also one of the changes made by Behringwerke in their modified version of pasteurised Factor VIII concentrate, Haemate P (Heimburger et al, 1986[a]).

The addition of calcium to stabilise factor VIII has since become virtually universal in the preparation of Factor VIII concentrates, both from plasma and by recombinant technology.

3.4 Increased Solubility by Addition of Sucrose

As well as studying the effect of calcium addition to factor VIII, other substances which might stabilise factor VIII were also being explored by SNBTS researchers, including the addition of various carbohydrates to the final product formulation (Foster et al, 1988[a]).

In late 1984, all available samples from this research were tested for stability to dry heat and it was discovered that addition of a small amount of sucrose allowed dry heat treatment of the SNBTS Factor VIII (NY) at 68°C to be extended from 2 hours to 24 hours

Preparation of Factor VIII concentrate was largely suspended at the PFC in the period October 1984 to January 1985 in order to facilitate building enhancements. As soon as preparation of the SNBTS Factor VIII concentrate was re-started, the final product formulation was modified to include sucrose.

This knowledge was shared with the PFL/BPL and was included in the formulation of 8Y (Smith, 1986[35]), as well as in the subsequent preparation of Z8 at the PFC (McIntosh, 1987[34]).

3.5 Increased Solubility and Stability by Addition of Sodium Chloride

In experiments performed during 1985, SNBTS researchers discovered that the coagulant activity of factor VIII in solution was lost when the concentration of sodium chloride fell below a critical level (McIntosh & Foster, 1990[36]). A small increase in a low concentration of sodium chloride can also increase protein solubility (Cohn & Edsall, 1943[1]). Therefore, sodium chloride was added to the formulation of the new SNBTS product Z8 to increase both the stability of factor VIII and the solubility of the product (McIntosh, 1987[34]).

[*Note: this procedure is now widely used in the formulation of Factor VIII concentrates both from plasma and recombinant technology.*]

4. SNBTS DEVELOPMENT OF HEAT TREATED FACTOR VIII CONCENTRATE

4.1. Management Processes

Strategies for research and development of the SNBTS coagulation factor concentrates were considered at a number of tiers of management.

National

SNBTS obtained advice on product development from Scotland's Haemophilia Directors and strategies were agreed with haemophilia directors and officials of the Scottish Home & Health Department, either at annual meetings, at meetings of joint working groups or by direct communication with the National Medical Director of SNBTS.

The SNBTS

Statutory responsibility for the provision of blood products derived from blood donors in Scotland lay with the Common Service Agency of the Scottish Health Service, which established a Blood Transfusion Service sub-committee to oversee the work of SNBTS which was a division of the CSA. Although the CSA BTS sub-committee functioned from 1974 to 1989, strategy on matters of a professional nature were largely delegated to the SNBTS. Prior to 1990, the SNBTS was managed by a National Medical Director who utilised meetings of the SNBTS directors to help to establish national policies and to co-ordinate their implementation. Finance for implementation had to be requested from CSA according to the Standing Financial Instructions of the CSA for budgetary developments.

Prior to 1982, the PFC Director acted on advice from the SNBTS National Medical Director either by direct communication or via meetings of the SNBTS Directors.
In January 1982, the SNBTS National Medical Director established a formal mechanism for the management of coagulation factor developments known as the Factor VIII Study Group. The Group comprised senior staff from across SNBTS and was chaired and managed by the SNBTS National Medical Director, who reported progress to the SNBTS Board.

The SNBTS Factor VIII Study Group subsequently encompassed the development of all of the SNBTS plasma products and became known as the SNBTS Product Development Group.

The PFC

Prior to the creation of the SNBTS Factor VIII Study Group, the research and development strategy pursued within PFC was determined by the PFC Director in consultation with the PFC R&D Manager and other senior R&D staff.

These communications continued after the creation of the Factor VIII Study Group to provide advice that was well considered and to progress nationally agreed strategies as efficiently as possible. The arrangements were formalised in 1986 by the creation of the PFC Development Review Group (later named the PFC Development Team) chaired by the PFC R&D Manager which included the PFC Director, the PFC Quality Manager and senior R&D staff.

4.2 Communications between the SNBTS and the BPL/PFL

There were a number of avenues of communication between the SNBTS and the BPL/PFL concerning the development of coagulation factor concentrates during the 1980s. These communications encompassed meetings, correspondence and telephone conversations. Communications most relevant to the development of heat treatment of coagulation factor concentrates are listed in the chronology of events in section 7 below. Communications can be categorised according to the level at which they occurred.

National Directors

The National Medical Director of the SNBTS (Dr JD Cash) and the Directors of the PFC (Mr JG Watt and Dr RJ Perry) communicated with the Director of the BPL (Dr RS Lane) at meetings, by correspondence and by telephone. Whilst there was no formal arrangement between the two services, these regular communications addressed opportunities for scientific collaboration, product developments, mutual support in maintaining product supplies and the consideration of key issues such as product safety and quality.

Heads of R&D

The Heads of R&D at the PFC (Dr PR Foster) and at the SNBTS Headquarters Laboratory (Dr DS Pepper) communicated regularly with the Chief Scientist responsible for coagulation factor developments at the BPL/PFL (Dr JK Smith). These communications included meetings, correspondence, telephone conversations and exchanges of information. Records are available of many of these interactions.

R&D Scientists

The senior PFC R&D scientist working on coagulation factor developments (Dr RV McIntosh) communicated directly with senior R&D scientists at the PFL (Mrs L Winkelman and Mr DE Evans) in meetings, correspondence and telephone conversations. The senior R&D scientist at the Edinburgh Regional Transfusion Centre who was concerned with coagulation factors (Dr CV Prowse) also communicated regularly with staff at the PFL. Similar relationships existed between respective R&D personnel who were concerned with the development of other

products, such as immunoglobulins and albumin, and in related technological developments.

Heads of Quality

The Quality Managers at the PFC (Dr RJ Perry and Dr B Cuthbertson) communicated regularly with the Head of Quality at the BPL/PFL (Dr TJ Snape) in meetings, correspondence and by telephone. Topics encompassed the quality of plasma for fractionation, operational policies, standard operating procedures and quality systems.

Joint Development Projects

There were two specific R&D projects concerning the heat treatment of coagulation factor concentrates which were undertaken jointly between the SNBTS and the BPL/PFL during the 1980s; (a) virus inactivation studies of 8Y and (b) studies on the thrombogenicity of heat treated factor IX concentrates.

(a) Virus Inactivation Studies of 8Y (1986-1988): The SNBTS was one of the first organisations to measure the degree of virus inactivation achieved by heat treatment processes using laboratory experiments with marker viruses, having specialist facilities for this purpose which the BPL/PFL did not possess. Therefore, to obtain laboratory data on the degree to which viruses were inactivated in the heat treatment of its Factor VIII concentrate 8Y, studies were performed for the BPL/PFL by the SNBTS. The project was led by Dr B Cuthbertson of PFC supported by Dr KG Reid with input from Drs PR Foster and RV McIntosh. Input from BPL/PFL was primarily from Dr JK Smith and Mrs L Winkelman. Considerable communication was required between the representatives of the PFC and those of the BPL/PFL to establish procedures which would simulate processes used in the manufacture of 8Y and to agree suitable experimental protocols.

(b) Studies on the Thrombogenicity of Heat Treated factor IX Concentrates (1984-1985). Thrombotic reactions were associated with treatment with Factor IX concentrates and were regarded as a risk of heat treatment which needed to be evaluated before heat treated concentrate was administered to humans (see section 5 below). During the 1970s the SNBTS had pioneered studies of thrombogenic reactions associated with factor IX concentrates using animal models. A canine model was devised as the coagulation system of the dog was comparable to that of humans and the animals were large enough to provide the volumes of blood

samples needed for biochemical analysis. However, the model was expensive and difficult to establish. When it was decided that further animal studies were necessary to determine the safety of heat treated products, the SNBTS invited the BPL/PFL to participate, to provide the BPL/PFL with important information on the safety of its products as well as to share the costs. The project was initially established by the SNBTS National Medical Director (Dr JD Cash) and was led by the senior R&D scientist at the Edinburgh Regional Transfusion Centre (Dr CV Prowse). The animal studies were performed by Drs JD Littlewood and J Ferguson at the Veterinary Schools of the Universities of Cambridge and Glasgow. SNBTS staff at the Headquarters Laboratory (Dr J Dawes) and at the PFC (Dr PR Foster and Mr TA McQuillan) also contributed, with input from the BPL/PFL being provided by Dr JK Smith and Dr PA Feldman. Considerable communication was required between the representatives of the SNBTS and those of BPL/PFL to agree experimental protocols, to organise and schedule the work and to assess the resultant data, so that essential information could be obtained as quickly as possible.

4.3 Background

Factor VIII activity in plasma and concentrates was well known to be temperature sensitive[6,7] but no published information was available on the effect that heating would have on factor VIII during the preparation of Factor VIII concentrate.

When intermediate-purity Factor VIII concentrate (NY) began to be prepared at the PFC in 1975, filtration of the final solution through a 0.22μm membrane filter proved difficult and a loss of factor VIII activity occurred. Dr Alan Johnson was consulted and he advised that filtration should be performed at 30°C rather than 20°C in order to increase the solubility of fibrinogen which he believed was partially blocking the filter, thereby retaining factor VIII and slowing down filtration. The then current preparation of the SNBTS Factor VIII concentrate (NY) was based on the method of Johnson, in which the 0.22μ filtration had been performed at 30°C for this reason (Newman et al, 1971[8]) and this change was therefore made to the SNBTS manufacturing process. However, a comparison at the PFC in 1975 between filtration at 20°C and filtration at 30°C demonstrated an even greater loss of factor VIII activity when filtration was performed at 30°C and the temperature of filtration was returned to 20°C.

These observations confirmed that factor VIII activity could be destroyed by even a modest increase in temperature and it seemed inconceivable that factor VIII could be heat treated at a temperature high enough to eliminate the risk of hepatitis transmission.

The SNBTS did collaborate with Dr Johnson on research into the adsorption of factor VIII to solid-phase poly-electrolytes in order to remove hepatitis virus as well as a means of purification (Johnson et al, 1978[5]). However, the poly-electrolyte reagents were supplied under an agreement with Monsanto which allowed them to be used for research but not for routine production. Monsanto were interested in the possibility of retaining this technology for their exclusive use. Given the uncertainty over the availability of the reagents on which the process was based, the SNBTS decided to shelve the project.

This approach was subsequently overtaken by research into heat treatment and by a fresh collaboration between the SNBTS and Dr Johnson on another method of factor VIII purification which was similar to that involving poly-electrolytes, but based on reagents that were commercially available (Matthews & Johnson,1988[37]).

4.4 Research and Development of Heat Treatment by the SNBTS

Experimental research into heat treatment of coagulation factor concentrates was begun by the SNBTS in September 1981. Eventually a number of different heat treated concentrates were developed. These developments are summarised in table 3, along with the date of key events.

Table 3. Key Dates Concerning the Development of Heat Treated Coagulation Factor Concentrates by the SNBTS

Product	Description	Date	Key SNBTS Event
ZHT	Factor VIII concentrate of increased purity, heat treated in solution in the presence of carbohydrate & amino acids stabilisers. Objective: inactivation of the agents responsible for transmission of NANBH, with albumin pasteurisation as a bench-mark.	02 Sep 1981 08 Feb 1983 14 Jan 1984 20 Mar 1984	First experiment on pasteurisation of FVIII Preparation of first pilot batch of ZHT. Report of adverse reaction in 1 of 3 patients treated. Preparation of fifth pilot batch of ZHT (with revised process

Product	Description	Date	Key SNBTS Event
			conditions).
		24 Sep 1984	Preparation of last pilot batch.
NY HT (1)	Established intermediate-purity Factor VIII concentrate; dry heat treated for 2 hours at 68°C. Objective: to heat the existing product as severely as possible to inactivate HIV.	21 Nov 1983	First experiment on dry heat treatment of FVIII
		18 Nov 1984	Heat treatment of first batches.
		03 Dec 1984	Product issued for clinical evaluation.
		10 Dec 1984	Product distributed for routine use.
		13 Sep 1985	Last issue of product.
		27 Nov 1985	Recall of issued product
NY HT (2)	Established intermediate-purity Factor VIII concentrate with a modified formulation; dry heat treated for 24 hours at 68°C. Objective: to heat the modified product as severely as possible to increase the margin of safety with respect to HIV transmission.	20 Jan 1985	Preparation begun of NY formulated with 2% sucrose.
		14 Mar 1985	Product issued for clinical evaluation (Edin)
		29 Mar 1985	Product issued for clinical evaluation (Gla).
		04 Sep 1985	Product issued for routine use.
		13 May 1987	Last issue of product (Scotland).
		3 June 1987	Last issue of product (N. Ireland).
Z8	Factor VIII concentrate of increased purity, modified formulation and new freeze drying technique. Objective: to increase dry heat treatment to 72 hours at 80°C to further increase the margin of safety with respect to HIV transmission and to possibly inactivate the agents responsible for NANBH transmission.	21 Nov 1985	First experiment to design FVIII for dry heating at 80°C
		27 Feb 1986	SNBTS Management agree proposal to develop this type of product
		23 Jun 1986	First pilot-scale trial batch of Z8 begun
		4 Aug 1986	First production-scale trial batch of Z8 begun.
		22 Dec 1986	Z8 available for clinical

Product	Description	Date	Key SNBTS Event
			evaluation.
		15 Apr 1987	First routine issue of Z8
HT DEFIX	Established intermediate-purity Factor IX concentrate with a modified formulation; dry heat treated for 72 hours at 80°C. Objective: to heat the modified product as severely as possible to provide a high margin of safety with respect to HIV transmission and to possibly inactivate the agents responsible for transmission of NANBH.	Jan 1984	Protocol of dog thromobogenicity study outlined. BPL agree to participate
		July 1984	SNBTS begins to test samples from dog thrombogenicity model
		08 Mar 198	Dog safety study begins on thrombogenicity of HT DEFIX
		1 April 1985	Revised formulate of HT DEFIX proposed
		15 July 1985	HT DEFIX issued for clinical evaluation.
		17 July 1985	First infusion of HT DEFIX
		12 Aug 1985	First routine issue of HT DEFIX

How these developments occurred and the main issues which had to be addressed are described in chronological order.

1981 - 1983

The SNBTS began its research on heat treatment of coagulation factors in 1981 as soon as it became aware of the method of pasteurising factor VIII which had been devised by Behringwerke (Heimburger et al, 1981a[a]). As the yield of the Behringwerke process was reported to be only 8% (Heimburger et al 1981b[a]), the SNBTS research focused on the discovery of modifications to the method to increase yield in order to be able to supply a suitable quantity of product.

Alternative procedures to enhance yield, including the use of sorbitol (Gekko and Morikawa,1981[38]) instead of sucrose as the carbohydrate stabiliser during pasteurisation, as well as the investigation of zinc/heparin precipitation of fibrinogen and stabilisation with calcium as described in sections 4.2 and 4.3 above.

Ultrafiltration technology was also explored as an alternative to precipitation/centrifugation for concentration/formulation of the final product. Pilot preparations of an SNBTS pasteurised Factor VIII concentrate (ZHT) were prepared during 1983 to assess clinical efficacy and tolerability of the product.

Research was also undertaken into methods for the pasteurisation of factor IX concentrate (MacLeod et al, 1984[a]), fibrinogen and immunoglobulin (McLeod et al, 1984[39], Welch et al, 1983[a])

Analytical studies were performed to address concerns that heating might result in harmful neo-antigens being formed (Ludlam, 1983[12]). Although the results of these studies were satisfactory, (Dawes et al 1983[40]) one of three recipients of this material suffered an adverse reaction which was described as unacceptable by the Haemophilia Director concerned (Ludlam, 1984[11]).

Experiments were also performed using the technique of dry heat treatment after this method had been reported (Rubinstein & Dodds, 1982[a]) and it was discovered that Baxter were developing a product of this type with the aim of destroying the agent of NANBH. However, the degree of virus inactivation observed by the SNBTS using marker viruses in laboratory studies was much less than that obtained by pasteurisation, consistent with informal reports that recipients of Baxter's Factor VIII concentrate, which had been dry heated at 60°C for 72 hours, had developed NANBH (Mannucci, 2003[a]).

1984

Further research was undertaken to substantially increase the degree of factor VIII purification to make pasteurisation less difficult to carry out in large-scale production and to remove substances which may have been responsible for the adverse reaction observed. This research was undertaken in collaboration with Dr Alan Johnson at New York University Medical Center who had devised a new procedure for the purification of Factor VIII (Matthews & Johnson, 1988[37]) which required further development and scale-up for large-scale production.

Additional laboratory virus inactivation studies using marker viruses demonstrated that the greatest degree of virus inactivation was obtained in the pasteurisation of albumin (which provided a benchmark for safety) and the lowest with dry heat treatment at 60°C, again consistent with the view that pasteurisation was more likely

to inactivate the agent(s) of NANBH than dry heat treatment. However, the degree of virus inactivation during pasteurising of factor VIII was lower when sorbitol was used as stabiliser rather than sucrose. Alternative pasteurisation conditions, such as heating for a period at 70°C, were therefore explored by the SNBTS to enhance the degree of virus inactivation (MacLeod et al, 1984[a]).

By this time, the virus responsible for AIDS had been identified (Gallo, et al, 1984[a]) and a blood test was under development (Sarngadharan et al, 1984[a]).

Practical work on the scale up of Dr Johnson's new method of factor VIII purification was begun at the PFC following a meeting with Dr J Curling of Pharmacia to identify a commercially available ion exchange resin that would have a much greater capacity for binding factor VIII than the particular resin which Dr Johnson had employed but which was regarded by the SNBTS as unsuitable for large-scale production.

In late October 1984 the SNBTS learned that a number of haemophilia patients who had been treated only with SNBTS Factor VIII concentrate had tested positive for antibody to HIV, indicating that they had been exposed to the virus and demonstrating that HIV had entered Scotland's-donor population. This finding made it imperative that heat treatment be introduced as quickly as possible.

The development of pasteurisation technology and the associated purification technology of Dr Johnson were not sufficiently advanced to be introduced immediately, so further research was begun on the technically less difficult option of dry heat treatment, in case HIV was found to be more susceptible to inactivation by dry heat than the agent(s) of NANBH. Heating experiments on Factor VIII concentrate were performed at both 60° and at 68°C, as these were the temperatures which other manufacturers had employed.

At the beginning of November, USA researchers reported that HIV could be substantially inactivated by dry heating at 68°C for 1 hour (Foster, 1984[19]). It was therefore decided to apply dry heat treatment at 68 °C for 2 hours to the SNBTS Factor VIII concentrate (NY).

A high temperature was desirable, as the inactivation of micro-organisms was believed to be strongly temperature dependent (Aronson, 1982[41]), but caution was

also required because of the possibility that heat-induced damage to protein might result in the formation of harmful neo-antigens (Boulton, 1983[42] ; Ludlam, 1983[12]; Mannucci, 2003[15]; Evatt, 2006[16]). Therefore, 68°C was selected by the SNBTS as it was the highest temperature that had been applied to Factor VIII concentrate by any manufacturer and was known to be well tolerated by recipients. Two hours was specified because this was the longest period of heating that samples of the existing SNBTS Factor VIII concentrate (NY) from a range of different production batches could withstand, as well as being double the period that had been reported to substantially inactivate HIV.

Specialist ovens with the degree of accuracy required for dry heat treatment of coagulation factors did not exist and the SNBTS Factor VIII concentrate (NY) was initially heat treated in a spray cabinet that was used for the pasteurisation of albumin at 60°C, which fortuitously had been specified by PFC staff to function up to 70°C.

Three months supply of Factor VIII concentrate (NY) was heated immediately and the product issued as soon as the necessary clinical evaluation of efficacy and tolerability had been completed. Consequently, the SNBTS was able to supply all of Scotland and Northern Ireland with sufficient dry heat treated Factor VIII concentrate for all patients on 10th December 1984.

Further research undertaken by the SNBTS at this time led to the discovery that the addition of a small quantity of sucrose to the final product formulation of Factor VIII concentrate (NY) enabled heating at 68°C to be increased to 24 hours before the product became insoluble (see para 3.4 above).

The rationale for releasing Factor VIII (NY) heated for 2 hours at 68°C was:

- there was evidence from the USA that this degree of heat treatment could inactivate HIV,
- as about 12 months stock of unheated Factor VIII (NY) was available, heat treatment of this stock meant that heat treated Factor VIII could be supplied from donations that had been collected at an earlier stage of the HIV epidemic, thereby enabling a product to be supplied with a lower theoretical risk than one prepared from donations collected later in the epidemic,
- the product was immediately available for heat treatment, enabling sufficient heat treated Factor VIII concentrate for all patients to be provided very quickly,

- a stock of heat treated product was available to re-fill the supply chain and to have available product in reserve for unplanned use, emergencies and other contingencies,
- waiting for Factor VIII concentrate to be prepared with a revised formulation, to enable the product to tolerate heating for 24 hours, would have delayed the introduction of heat treated Factor VIII concentrate by about 5 months, i.e. the time taken to re-start production, manufacture a fresh batch of Factor VIII concentrate and to then obtain the necessary clinical data concerning efficacy and tolerability.

1985

Preparation of Factor VIII had been largely suspended at the PFC during the period October 1984 to January 1985 to accommodate building enhancements advised by Medicines Inspectors. Therefore all SNBTS Factor VIII concentrate (NY) which had been manufactured earlier, and which did not have sucrose added, was heated for 2 hours at 68°C. Once production re-started (on 20th January 1985) sucrose was added to all SNBTS Factor VIII concentrate (NY), enabling heating to be carried out at 68°C for 24 hours. Experiments were undertaken to determine if stocks of NY could be re-processed to include 2% sucrose, so that 68°C heating could be extended from 2 hours to 24 hours, and to discover if any further changes could be made to the formulation of NY that would allow heating to be increased beyond 24 hours at 68°C, but without success.

The development of a precision oven suitable for controlled, accurate dry heat treatment of bio-pharmaceuticals was being pursued by the BPL. This information was shared with the PFC and an order for the construction of a specialist oven of this design was placed by the PFC in January 1985, with the oven being delivered in July 1985.

Concern remained that factor VIII might be damaged by heat treatment and that neo-antigens might be created which would stimulate the formation of antibodies to factor VIII in patients (Hann, 1984;[13] Bird et al, 1985[14]). Despite these concerns, the SNBTS decided to continue to heat treat all of its Factor VIII concentrate with the time of heating at 68°C being extended from 2 hours to 24 hours as soon as it was possible to prepare Factor VIII (NY) with sucrose added to the product formulation.

Given the continued transmission of NANBH by other dry heated products, it was thought unlikely that the SNBTS Factor VIII dry heated at 68°C would be free from the transmission of NANBH and research towards this objective was continued. Further research was undertaken to increase the degree of dry heating that could be applied to the SNBTS Factor VIII concentrate (NY) but this was not successful.

Clinical studies on pasteurised Factor VIII had been performed by Behringwerke. Although the ISTH Factor VIII sub-committee had criticised the protocol that had been used to establish safety from NANBH, none of the recipients had been infected making this the only Factor VIII concentrate for which clinical data were available on the absence of NANBH transmission (Heimburger et al, 1984[43]).

However, two new procedures were emerging, solvent/detergent treatment devised at New York Blood Center, (Horowitz et al. 1985[a]) and a more severe dry heat treatment of a Factor VIII (8Y) which had been prepared by a new purification procedure devised at the Blood Products Laboratory (BPL) pilot coagulation factor production facility, the Plasma Fractionation Laboratory (PFL) (Winkelman, 1985[44], Winkelman et al. 1985[a], Winkelman, 1988[a], Winkelman et al,1989[a]).

It was unclear if the solvent/detergent method would be effective against the agent(s) responsible for NANBH as there was evidence that one of the agents responsible was a non-enveloped virus (Bradley & Maynard, 1983;[a] Tabor, 1984[10]) against which the solvent/detergent method would be ineffective. How the toxic chemicals could be separated from factor VIII was also uncertain.

The new purification procedure devised by staff at the PFL was the subject of a patent application filed on 7th March 1985 (Winkelman, 1985[44]) a copy of which was provided to PFC. According to this patent application, the main advantage of the purification procedure was that, unlike the zinc precipitation process of SNBTS, it was not influenced by variations in the concentration of citrate, a chemical used to prevent blood donations from clotting, the concentration of which could vary according to the type of anti-coagulant used. Variations in citrate content were much less of an issue in Scotland, where there was little or no variation because the smaller number of Regional Transfusion Centres had standardised on the type of anti-coagulant used.

The PFL patent also noted that the procedure was compatible with heat treatment, either by pasteurisation or by dry heat treatment at 70°C for 24 hours. Compatibility with dry heat treatment at 80°C for 72 hours was subsequently added in March 1986, when the patent application moved from the national to the international phase.

Scientific staff from the PFL/BPL visited the PFC at the end of March 1985 to discuss heat treatment of coagulation factor concentrates. The BPL policy for the routine introduction of heat treatment of Factor VIII was unclear, but it was thought most likely that the existing BPL Factor VIII concentrate (HL) would be dry heat treated at 70°C for 24 hours (designated HT2) to allow more time for the development of the new Factor VIII concentrate 8Y, now being dry heat treated at 80°C for 72 hours (designated HT3).

A number of questions remained concerning the development of 8Y, viz:

- would the product be stable, given that a step (treatment with aluminium hydroxide), previously considered essential for the removal of residual coagulation factors that could degrade factor VIII, was no longer being used?
- would the process performance (i.e. yield, product characteristics and ease of manufacture), be satisfactory and reproducible in routine large-scale manufacture at the BPL, especially with such severe dry heat treatment conditions?
- would the pharmacokinetics, clinical efficacy and tolerability of product manufactured routinely at the BPL be satisfactory?
- would heat treatment at such a high temperature create neo-antigens and cause recipients to form antibodies (inhibitors) to factor VIII?
- what degree of virus inactivation would be obtained (given that no experiments had been performed using marker viruses)?
- would the dry heat treatment employed be effective in preventing transmission of NANBH?

Given the degree of uncertainty associated with alternative options, the SNBTS continued its strategy aimed at producing a high-purity concentrate suitable for pasteurisation, but to which either solvent/detergent-treatment or severe dry heat treatment could be applied instead if they proved to be superior.

SNBTS Factor VIII concentrate heated for 24 hours at 68°C was released for clinical trial on 14th March 1985. Following completion of this clinical trial, it was decided to continue to release SNBTS Factor VIII concentrate heated for 2 hours as this was judged to be in the best interests of patients. The rationale for this decision was as follows:

- the amount of Factor VIII concentrate required to treat patients was uncertain and the level of demand could be uneven, especially when a high level of treatment might be needed to treat a serious bleed or to manage a patient who had developed an inhibitor (antibodies) to factor VIII and who required a much higher dose of Factor VIII to prevent bleeding,
- in order to have necessary supplies available for patients a suitable stock of the new product was needed to fill the supply chain from the PFC to Regional Transfusion Centres to Haemophilia Centres and then to patient's own home supply,
- a system of batch dedication had been established to reduce donor exposure and was regarded as an important part of the overall risk reduction strategy. Maintaining such a system required relatively high overall stocks of product to maintain 15 individual supply chains that had been established within the batch dedication system,
- the risk of batches failing to meet quality specifications is greatest when a new product is first manufactured, making the continued provision of the new product uncertain until a suitable reserve stock has been established,
- it was judged by the SNBTS that, ideally, a 12 month reserve stock of products was required to meet medical emergencies and other contingencies,
- at this time, unheated Factor VIII concentrate was still being used in half of the haemophilia centres in the UK (Bloom, et al. 1985[a]); the Bio-Products Laboratory was not yet producing heat treated Factor VIII concentrate routinely and only a limited supply of heat treated Factor VIII concentrate was available from commercial companies (Bloom, et al. 1985[a]).

In these circumstances it was believed that the ability to treat patients in Scotland might be compromised in the absence of a reserve stock of SNBTS Factor VIII concentrate.

As a result of these considerations, SNBTS Factor VIII concentrate heated for 2 hours at 68°C continued to be released in Scotland until 13 September 1985. SNBTS Factor VIII concentrate heated for 24 hours at 68°C was issued routinely from 4th September 1985 and Factor VIII concentrate heated for 2 hours at 68°C subsequently recalled.

By October 1985, the volume of high-purity factor VIII solution that could be prepared in the PFC research laboratory was sufficient to fill the number of vials needed to perform a trial of freeze drying. Surprisingly, the highly purified factor VIII was destroyed using the established freeze drying conditions that were used by the SNBTS to dry Factor VIII concentrate (NY), leading PFC researchers to design a new cycle from first principles to freeze dry high-purity factor VIII.

The high-purity material tolerated this new freeze drying cycle but, contrary to expectation, a sample of the SNBTS intermediate-purity Factor VIII concentrate (NY), which had been included as an experimental control, not only survived freeze drying but also tolerated 80°C dry heat. By contrast, the samples of high-purity factor VIII failed to withstand 80°C dry heat.

This observation implied that it was the nature of the freeze drying process and not product purity that determined whether or not 80°C/72hr dry heating could be tolerated by Factor VIII. Details of the freeze drying procedure had not been given in the patent application for 8Y (Winkelman, 1985[44]), so further information was sought from the PFL (Foster, 1985[45]). The information supplied (Smith, 1985[46]) confirmed that the new freeze drying cycle devised at PFC was similar in design to that being used to freeze dry 8Y, consistent with this being the key aspect of the 8Y process, rather than the degree of purification.

In addition to this finding, a number of other items of information emerged in late-1985 which, taken together, caused the SNBTS to alter its R&D strategy to focus on the development of an 80°C dry heated Factor VIII concentrate, similar to 8Y of the BPL, rather than the high-purity concentrate. This new information included:

- a pre-publication report from the USA which found HIV to be more resistant to dry heat treatment than earlier experiments had indicated (Prince,1986[22]), leading to uncertainty over the margin of safety being provided by the current SNBTS dry heat treated Factor VIII concentrate,
- a problem of instability that had been identified on the scale-up of SNBTS high-purity Factor VIII, which required to be solved,
- difficulty in dry heat treating high-purity Factor VIII concentrate at 80°C,
- recognition that the sophisticated equipment required for the production of the high-purity Factor VIII concentrate could not be obtained quickly and that its operation would require revised staffing arrangements, the establishment of which was uncertain,
- that full-scale production of 8Y had been achieved successfully at the BPL,
- that the 8Y produced at the BPL had been found to be satisfactory in terms of clinical efficacy and tolerability.

A meeting of PFC Managers was held on 23rd December 1985 specifically to consider these matters (Foster, 1985[54]) and it was agreed that a change in strategy should be recommended to the SNBTS, with the development of an 80°C dry-heat treated Factor VIII concentrate as first priority and the development of a high-purity Factor VIII concentrate as second priority.

1986

This new plan was endorsed at the next meeting of the SNBTS Factor VIII Study Group on 27th February and was agreed with Haemophilia Directors on 5th March, the primary objective being to introduce a new Factor VIII concentrate as soon as possible to provide a greater margin of safety against HIV. It was also important that there be no interruption in the provision of this essential product to patients.

The option of directly transferring the methods and technologies used by BPL was not chosen because a number of uncertainties remained, in particular:

- use by BPL of a chemical (heparin) at a concentration which interfered with the routine method used by SNBTS for measuring factor VIII activity,
- uncertainty over the practicality and time required to replace the SNBTS method of measuring factor VIII activity with the method used by BPL,

- uncertainty over the omission of aluminium hydroxide adsorption in the BPL process and the possibility that minor process variations might result in instability to factor VIII,
- difficulties previously experienced by the SNBTS in the use of precipitation/centrifugation to recover purified factor VIII from dilute solutions,
- the need to purchase, install and become familiar with large-scale size-exclusion chromatography in factor VIII processing.

Therefore, to minimise uncertainty, it was decided to base the method of preparation of the new SNBTS product on technologies with which the SNBTS was already familiar but which were broadly equivalent in their outcomes to the processes being used at the BPL.

The new SNBTS product was named Z8 and the method of preparation (McIntosh & Foster, 1990[36]) included:

- recovery of cryoprecipitate via continuous thawing (see section 3.1),
- partial removal of fibrinogen and fibronectin from cryoprecipitate extract by precipitation with zinc/heparin, using a much lower concentration of heparin than BPL (see section 3.2),
- removal of de-stabilising coagulation factors by adsorption to aluminium hydroxide,
- stabilisation of the intermediate product using calcium (see section 3.3),
- recovery and concentration of factor VIII using ultra-filtration technology, based on experience gained in the preparation of pilot batches of pasteurised factor VIII (ZHT),
- formulation of the final product by dia-filtration with the addition of sucrose and sodium chloride in addition to calcium chloride (see sections 3.4 & 3.5),
- freeze drying of the product using the new type of freeze drying cycle that had been designed by PFC scientists in October 1985 in order to freeze dry research preparations of high-purity factor VIII.

A visual comparison between Z8 and the earlier NY product is shown below. Both vials contain 250 iu of factor VIII activity, but the vial of the NY product, on the left, was freeze dried from 40 mL of solution whereas the vial of Z8, on the right, was freeze dried from 10 mL of solution.

Figure 1. SNBTS Factor VIII Concentrates, NY (left) and Z8 (right)

Suitable process conditions for the manufacture of Z8 were determined in small-volume laboratory experiments that were performed during the first quarter of 1986 and translated to pilot-scale operation in the second quarter of 1986.

By July 1986, progress in the pilot studies was encouraging and there were good stocks of existing 68°C/24hr heat treated Factor VIII concentrate available. It was therefore decided to cease production of the existing product to release production staff and facilities in order to fast-track the development of Z8 at large-scale. Preparation of the first production trial batch of Z8 was begun in August 1986.

Further fine-tuning of conditions for the preparation of Z8 was required at the much larger scale of operation with the new freeze drying process requiring particular attention. Only a small number of vials had been produced in each pilot batch, whereas at full-scale, the number of vials was large enough to fill the freeze dryer shelves. Under these conditions, a significant number of vials failed to withstand 80°C dry heating.

Differences in the crystalline structure of the frozen plug were observed to be associated with this behaviour. Vials with a plug of a uniform fine crystal structure could withstand 80°C dry heating, whilst those with plugs containing larger crystals or a mixture of fine and large crystals did not. This is illustrated in the photograph

in figure 2 below which shows three vials of Z8, all of which were dry heated at 80°C for 72 hours. The first vial (on the left) exhibits a plug of fine crystals, the second (centre) has a mixture of fine and large crystals whilst the third (right) exhibits large crystals. Only the vial on the left was able to tolerate dry heating at 80°C for 72 hours. The failure of the other two vials to withstand severe dry heat treatment is illustrated by the degree of discolouration present.

Figure 2. Vials of SNBTS Factor VIII Concentrate Z8 with different plug crystal structures

Similarly in Fig 1, the crystals in the dried plug of NY (left) are much larger than those of Z8 (right), consistent with the relative heat sensitivity of the different products.

Differences in crystal structure are determined by the rate of freezing and it was postulated that the uniform formation of fine crystals might be a result of super-cooling, a condition at which the vial contents remain liquid below the freezing point of the solution. In this situation a small disturbance is sufficient to cause instantaneous crystal formation (i.e. freezing) with fine crystals being formed.

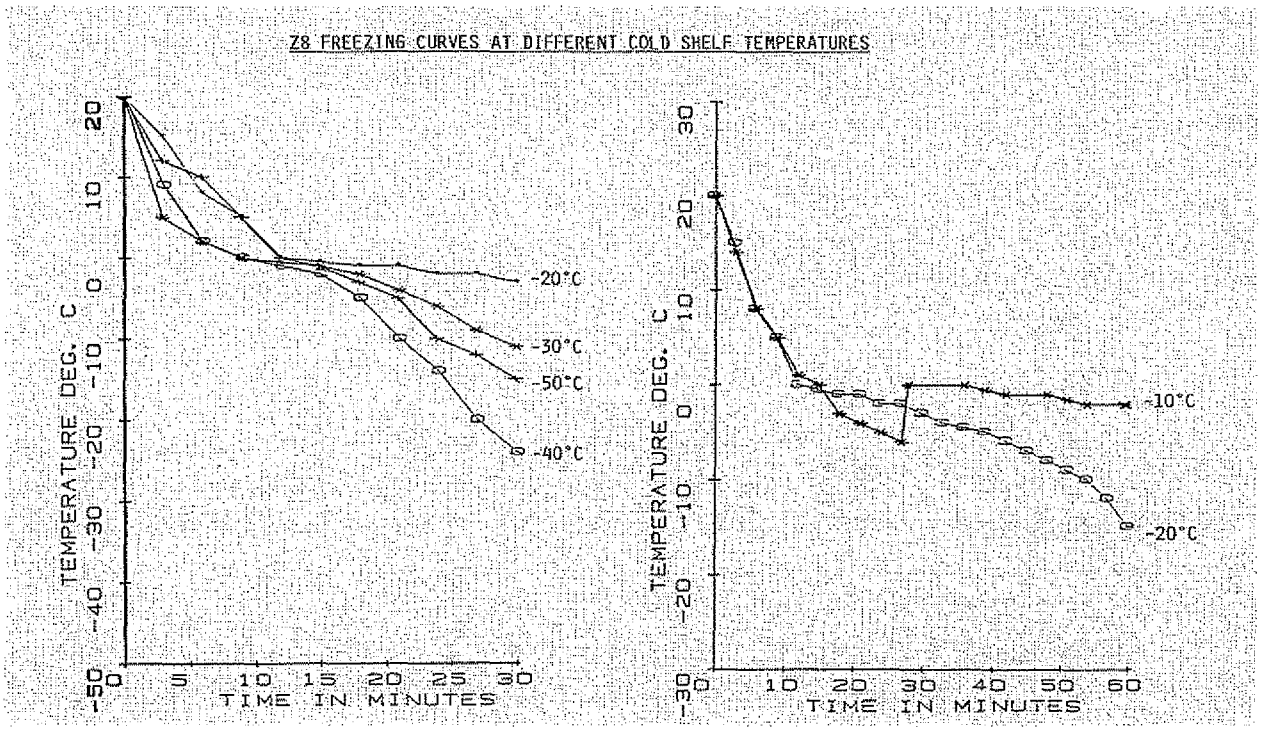
Experiments confirmed this hypothesis and a two-stage freezing procedure was designed whereby the necessary fine crystal structure could be obtained

throughout every vial of every batch of Factor VIII concentrate on every occasion (McIntosh et al, 1987[34]).

Subsequently, careful examination of the product temperature profile obtained during the freeze drying of a sample of BPL's 8Y exhibited a sharp rise in temperature at the point of freezing (Reid, 1988[47]), a characteristic feature of super-cooling (McIntosh et al, 1990[48]) indicating that super-cooling may have occurred adventitiously with 8Y.

This characteristic behaviour is illustrated in Figure 3 below in which the change in temperature of the SNBTS Factor VIII concentrate, Z8, is shown during refrigeration at different temperature settings of the shelf temperature in the freeze drier. At a shelf setting of -10°C, shown in the right hand graph, a sudden rise in temperature can be seen after about 25 minutes cooling. This is the precise point where the supercooled liquid freezes into a solid mass, releasing energy in the form of heat, as freezing is an exothermic process.

Figure 3. Temperatures of Z8 refrigerated on a freeze drier shelf from -50°C to -10°C (from McIntosh et al, 1987[34])



In October 1986, a preliminary report of the safety study of 8Y was presented to the annual meeting of UK Haemophilia Directors, suggesting that 80°C/72hr dry heat treatment might be effective in preventing transmission of NANBH.

A period of about three months was required to prepare and fully test a batch of Factor VIII concentrate, therefore it was not until December 1986 that the first batch of Z8 was available for clinical evaluation.

1987

Before the new product could be released routinely it was necessary to establish that it was clinically acceptable in terms of pharmacokinetic characteristics, efficacy and tolerability. A trial protocol had been agreed with Haemophilia Directors for this purpose. However, agreement to trial the product was withdrawn by the Haemophilia Directors pending receipt of satisfactory assurances from the Scottish Home and Health Department on indemnity for patients. Consequently, the clinical trial data that were needed before Z8 could be issued routinely were not available until April 1987.

In order to minimise the risk of NANBH transmission, the previous SNBTS Factor VIII concentrate had been supplied to patients on a 'batch-dedication' basis, whereby a particular batch was reserved for specific individuals. As the safety of Z8 with respect to NANBH was not certain, the Haemophilia Directors advised that the earlier product should continue to be used until it had been fully consumed, in order that patients could benefit from this particular safety feature. Therefore, the small remaining stock of 68°C/24hr dry heated Factor VIII concentrate continued to be used and was not recalled by the SNBTS.

Dry heating at 80°C presented major challenges to the production of Factor VIII concentrate, as small batch-to-batch variations that had not been of any significance previously now caused unexpected batch failures. This type of behaviour was experienced at both the BPL (Winkleman & Evans, 1987[49]) and at the PFC and led PFC to fine-tune processing parameters to obtain a more robust performance.

At the PFC, most batches which could not withstand 80°C/72hr dry heat were found capable of withstanding heating at 75°C for 72 hours. As this degree of heat treatment was superior to that being applied to the alternative Factor VIII

concentrates available in Scotland at that time, it was decided that it would be preferable to release this material. No cases of HIV, NANBH, or HCV transmission have been associated with 75°C/72hr dry heated Factor VIII concentrate (Z8), suggesting that this procedure was effective in preventing transmission of these viruses.

1988 - 1990

Further experience in routine production demonstrated that Z8 was very sensitive to variations in the composition of cryoprecipitate and on the storage history of plasma. Following the failure of a number of batches, operating parameters were further modified to enable the manufacture of Z8 to tolerate upstream variations of this type. Some commercial Factor VIII concentrate was purchased by Scotland's Haemophilia Directors to cover any potential shortfall from SNBTS.

The yield of factor VIII from BPL's 8Y was also low, averaging 130 iu/kg with the result that despite a new facility at BPL, some 30% of the Factor VIII required for England & Wales was still being obtained from commercial sources (Department of Health & Social Security, 1988[50])

As a result of these experiences the development of a modified version of Z8 was undertaken by the SNBTS (named S8) to provide a process which would be even more robust and which had a much higher yield of factor VIII.

1991-1995

The development of S8 had reached a point where material was being prepared for clinical trial when it was decided instead to again develop a high-purity Factor VIII concentrate, as this was the preference of Scotland's Haemophilia Directors. A high-purity Factor VIII concentrate was then developed by the SNBTS (named Liberate) based on a purification procedure which had been devised at the Regional Transfusion Centre at Lille, France and which used solvent-detergent treatment to inactivate viruses (Burnouf et al, 1991[a]). This collaboration and transfer of technology was carried out under a formal agreement between the Centre Regionale Transfusion Sanguine, Lille, France and the SNBTS.

Scotland's Haemophilia Directors wanted access to a high-purity concentrate of Factor VIII as quickly as possible. Therefore, whilst the SNBTS was developing its new product, it was arranged for high-purity Factor VIII concentrate to be prepared

from Scottish donor plasma at Lille, France. Consequently, high-purity Factor VIII concentrate from local plasma was made available to Scotland's haemophilia doctors in 1991. The equivalent SNBTS product (Liberate) was developed successfully, being approved for clinical trial in 1992 and granted a UK product licence in 1996.

1995 - 2006

Heat treatment was not applied at first to Liberate as solvent/detergent treatment was very effective against both HIV and HCV. This approach was altered when reports emerged of hepatitis A virus (HAV) transmission via solvent/detergent-treated high-purity Factor VIII concentrates similar to Liberate (Foster & Bienek, 2008[2], table 20.8).

It was found by PFC researchers that HAV added to Liberate was readily inactivated by 80°C/72hr dry heat treatment (Hart et al, 1994[a]) and a dry heat treated version of Liberate was therefore developed and was approved for clinical trial in 1996.

This development coincided with the introduction of recombinant Factor VIII concentrate into the UK, the emergence of variant Creutzfeldt-Jakob disease (vCJD), followed by a ban on the fractionation of UK-donor plasma and approval in Scotland for full use of recombinant Factor VIII concentrate. As a result of these events, the SNBTS was unable to complete the necessary clinical trials in Scotland and instead performed these out-with the UK. Despite these complications, the necessary clinical data were eventually obtained, resulting in a product licence for HT Liberate being granted in July 2005, making it one of few plasma derived Factor VIII concentrates in the world to be subjected to two highly effective virus inactivation steps.

5. THE SNBTS DEVELOPMENT OF HEAT TREATED FACTOR IX CONCENTRATE

5.1 Background

The SNBTS has been engaged in research aimed at eliminating the risk of hepatitis transmission by Factor IX concentrate since 1970. The first approach concerned the use of precipitation with polyethylene glycol as a means of separating hepatitis

virus from factor IX and was undertaken in collaboration with Dr Alan Johnson of New York University Medical Center.

The procedure failed to eliminate infectivity in animal studies (Johnson et al, 1976[a]) and additional animal studies raised concern over the possibility that the resultant Factor IX concentrate might cause a thrombotic reaction in patients (Cash et al, 1975[a]). Precipitation conditions were revised and the fractionation of markers of thrombogenicity was examined (Foster et al, 1980[a]) to address these issues.

Factor IX concentrate prepared by the revised method (named Supernine) was supplied by the SNBTS for clinical trial in the early 1980s. However, the Medicines Control Agency did not want to licence two SNBTS Factor IX concentrates (i.e. DEFIX and Supernine). Clinicians did not wish the licence for DEFIX to be withdrawn and there was uncertainty over the ability of precipitation technology used in the preparation of Supernine to effectively remove the risk of hepatitis transmission. Supernine was therefore discontinued in favour of developing a heat treated factor IX concentrate.

5.2 Application of Heat Treatment to Factor IX concentrates

The SNBTS research on the application of pasteurisation was undertaken with factor IX as well as factor VIII (MacLeod et al, 1984[a]).

There was concern that heat could damage the product and cause thrombosis in patients, a serious complication of treatment with Factor IX concentrates, the cause of which was not known (Aledort, 1977[17]).

The SNBTS decided that freedom from thrombogenic reactions should be demonstrated in a suitable animal model (Cash et al, 1975[a]) before heat treated Factor IX concentrate was administered to humans. The animal model was difficult and costly to establish, therefore the BPL were invited to collaborate in the study. The BPL acknowledged the SNBTS view of the importance of the study and agreed to participate.

Whilst the animal model was being established the emphasis changed from NANBH to HIV and from pasteurisation to dry heat following evidence that HIV could be inactivated by this treatment (Centers for Disease Control, 1984[a]).

The established Factor IX concentrates from SNBTS (DEFIX) and from the PFL/BPL (9A) were virtually identical and it was found that on dry heat treatment both products failed to pass an *in vitro* test for thrombogenicity. Researchers at both the SNBTS and at the PFL discovered that this effect could be prevented by a relatively small change to the product formulation. Thereafter, the modified Factor IX concentrates of both the SNBTS and the PFL/BPL were found to be able to withstand dry heat treatment at 80°C for 72 hours. Unlike factor VIII, it was not necessary to establish a new manufacturing process, only to modify the product formulation. Therefore the time required to develop the heated product was determined by the timescales needed to complete the animal safety study, rather than by the time required to establish a new manufacturing process.

The animal safety studies were completed in August 1985 with no increase in thrombogenicity being observed after heat treatment of the modified products (Littlewood et al, 1987[a]). This finding enabled clinical trials to proceed, following which heat treated Factor IX concentrate was issued routinely from the beginning of October 1985. As soon as adequate stocks were in place, the SNBTS recalled all stocks of its unheated Factor IX concentrate (DEFIX) which were destroyed.

A high-purity concentrate of Factor IX (HIPFIX) was subsequently developed based on a procedure developed at the Regional Transfusion Centre, Lille (Burnouf et al, 1989[a]) which utilised solvent/detergent treatment for inactivation of HIV and HCV. However, unlike Lille, the SNBTS chose to retain 80°C/72hr dry heat treatment in addition to solvent/detergent treatment making this product the first Factor IX concentrate in the world to have two defined virus inactivation steps.

Approval for clinical trial was obtained in 1993 and a UK product licence granted in 2001, following completion of clinical trials which had been delayed by a request to modify the product, by the replacement of UK-plasma with imported plasma as a precaution against vCJD and by the necessary clinical trials being undertaken out-with the UK due to the preference of Scotland's haemophilia doctors for recombinant Factor IX.

6. CLINICAL SAFETY OF SNBTS HEAT TREATED CONCENTRATES

6.1 Freedom from Transmission of HIV

All SNBTS heat treated coagulation factor concentrates are considered to have been free from transmission of HIV. Two patients are known to have developed antibodies to HIV after receiving SNBTS heat treated Factor VIII concentrate. However, both patients had previously received unheated concentrates at a point in time consistent with this being the most probable source of infection (Forbes, 1986[51]).

Following the introduction of donor screening for antibody to HIV in October 1985, the earlier donations provided by all of the donors who tested positive were traced to determine how they had been used and archive samples of plasma were tested where available. It was discovered that a number of batches of Factor VIII concentrate which had been heat treated had been prepared from plasma donations where either the archive sample tested positive for antibody to HIV or where infectivity was implied because the associated red cell donation had transmitted HIV. None of these batches transmitted HIV (see table below).

Table 4. HIV positive donations found via look-back to have been used in the preparation of SNBTS coagulation factor concentrates

Date of Donation	Donor	Product	Dry Heat Treatment
23 March 1984	a	FVIII	68°C for 2 hours
10 July 1984	b	FVIII	68°C for 2 hours
9 October 1984	b	FVIII	68°C for 2 hours
25 October 1984	c	FVIII FIX	68°C for 2 hours unheated
26November 1984	d	FVIII FIX	68°C for 24 hours unheated
8 January 1985	b	FVIII	68°C for 24 hours
9 January 1985	e	FVIII	68°C for 24 hours.

The absence of HIV transmission via unheated Factor IX concentrate suggests that the infectivity was eliminated by the manufacturing process or that the recipients were not susceptible to infection.

The fact that an infective donation from March 1984 did not infect people with haemophilia demonstrates the value of dry heat treating the SNBTS stock of Factor VIII concentrate immediately in November 1984, even though the degree of heat treatment was less severe than that used by some manufacturers.

6.2 Freedom from Transmission of Hepatitis C

A protocol to determine the degree to which a coagulation factor concentrate was free from transmission of NANBH was established by the International Society of Thrombosis & Haemostasis in December 1984 (see section 2.3 above). This protocol required 10 batches of a product to be administered to 20 patients, who had not previously been exposed to a coagulation factor concentrate, and who would be monitored for evidence of NANBH infection for 7 months.

Given the difficulties which surrounded this protocol, such studies were not performed on SNBTS products until there was an expectation that the products would be free from transmission of NANBH.

Studies were performed using the SNBTS Factor VIII concentrate, Z8, dry heat treated at 75/80°C for 72 hours, and the SNBTS Factor IX concentrate, HTDEFIX, dry heat treated at 80°C for 72 hours, with approval being obtained from the Ethical Committees of each institution involved. Ten patients were treated with Z8 and three with HTDEFIX with a total of 25 different batches of product being used. No cases of hepatitis were observed and there was no serological evidence for HCV or HIV transmission (Bennett et al, 1993[a]).

7. DETAILED CHRONOLOGY (SCOTLAND)

Names:

SNBTS	PFL/BPL
Dr J D Cash (JDC)	Mr D Evans (DE)
Dr B Cuthbertson (BC)	Dr P Feldman (PF)
Dr P R Foster (PRF)	Dr P Harrison (PH)
Dr A J MacLeod (AJM)	Dr G Neal (GN)
Dr R V McIntosh (RVM)	Dr J K Smith (JKS)
Dr D S Pepper (DSP)	Mrs L Winkleman (LW)
Dr R J Perry (RJP)	
Dr C V Prowse (CVP)	
Dr K G Reid (KGR)	

DATE	EVENT
May 1981	Behringwerke paper on the pasteurisation of factor VIII obtained by PRF and passed to AJM to obtain a translation from V Zolg (Edin Univ), a German research scientist with whom he was collaborating.[52]
2 Sep 1981	AJM performs first PFC experiment on pasteurisation of factor VIII.
28 Jan 1982	First meeting of the SNBTS Factor VIII Study Group, established and chaired by Dr Cash to plan and co-ordinate SNBTS Factor VIII developments
10 Feb 1982	AJM reports results of preliminary studies on pasteurisation of FVIII concentrate, concluding that a more highly purified product is needed
May 1982	Milan Bier (Univ Arizona), visiting scientist to the PFC, discovers precipitation of fibrinogen by zinc, with factor VIII remaining in solution.
5 May 1982	BC & DSP consider obtaining stocks of viruses for laboratory experiments.
2-5 Aug 1982	ISBT Congress, Budapest. Abstracts on dry heat treatment (Rubenstein & Dodd[a]). Baxter announce development of heat treated FVIII, but the method is not disclosed. AIDS in two USA haemophiliacs announced.
10 Sep 1982	BC contacts University of Edinburgh to obtain marker viruses for virus inactivation studies
19 Nov 1982	PRF presentation to British Society for Haematology on use of calcium to stabilise factor VIII.
1 Dec 1982	Abstract from Baxter suggests heating announced at ISBT is not at 60°C/10hr. Information sent to JKS with data on PFC heat treatment

DATE	EVENT
	experiments.
8 Feb 1983	First trial of pilot-scale pasteurisation of the SNBTS factor VIII prepared using zinc precipitation (ZHT-3001).
10 Feb 1983	JKS visits the PFC to discuss coagulation factor R&D
14 Feb 1983	BC performs first PFC study of virus inactivation during pasteurisation of FVIII.
24 Mar 1983	Dr C Ludlam writes to Mr J Watt (PFC Director) to express concern "*about the possibility of neo-antigens developing following heat treatment*".
27 Apr 1983	Letter from PRF to JKS with data on zinc precipitation and details of 2nd pilot production ZHT process. Mentions concern over neo-antigen formation.
16 May 1983	Second pilot preparation of pasteurised Factor VIII (ZHT-3002)
27 June to 1 July1983	Congress of World Federation of Hemophilia, Stockholm. Poster from Armour on testing of heat treated Factor VIII in chimps. Authors reluctantly acknowledge to PRF that method is dry heat. Presentation by B Evatt on epidemiology of AIDS in USA haemophiliacs. PRF approached by A Johnson (NYU) re collaboration on a new method of factor VIII purification.
2-8 July 1983	ISTH Congress, Stockholm. PFC presentations on zinc precipitation, calcium stabilisation & pasteurisation of factor VIII.
29 Aug 1983	Third pilot preparation of pasteurised Factor VIII (ZHT-3003)
8 Sept 1983	JKS visits the PFC to discuss coagulation factor R&D
22-23 Sep 1983	Scientific meeting of the British Society of Thrombosis & Haemostasis. SNBTS presentation on the immunological integrity of pasteurised factor VIII
3 Oct 1983	Fourth pilot preparation of pasteurised Factor VIII (ZHT-3004)
4 Oct 1983	A Johnson (NYU) visits the PFC to discuss joint collaboration on the development of high-purity factor VIII.
2 Nov 1983	JKS visits the PFC to discuss heat treatment of coagulation factors.
15-16 Dec 1983	Inaugural conference of the British Blood Transfusion Society. PFC presentation on the development of a pasteurised Factor VIII concentrate.
21 Dec 1983	First experiments by BC & DSP on dry heating of SNBTS FVIII (NY) at 60°C and 70°C for 72 hours. Product did not survive dry heating at 70°C.

DATE	EVENT
	The degree of inactivation of a marker virus was lower than with pasteurisation.
14 Jan 1984	Letter from C Ludlam to JDC on unacceptable adverse reaction to pilot batch of the SNBTS pasteurised Factor VIII concentrate (ZHT)
19 Jan 1984	JDC & CVP establish outline protocol for dog thrombogenicity study to ensure the safety of heat treated Factor IX concentrate
23 Feb 1984	Split-batch of the SNBTS Factor VIII (NY) prepared with/without added calcium for clinical trial to confirm enhanced stability of factor VIII by addition of calcium.
20-21 Mar 1984	Fifth pilot preparation of pasteurised Factor VIII (ZHT – 4001)
30 Apr-1 May 1984	Sixth pilot preparation of pasteurised Factor VIII (ZHT- 4002)
22 May 1984	JKS writes to PRF seeking further details of PFC research on pasteurisation and notes "*we have stumbled (literally) on an intriguing alternative to zinc.*"
5-6 Jun 1984	Seventh pilot preparation of pasteurised Factor VIII (ZHT-4003).
13-15 June 1984	PRF visits A Johnson at New York University to obtain details of new method of purification of factor VIII and to provide advice on scale-up.
25-26 June 1984	Eighth pilot preparation of pasteurised Factor VIII (ZHT-4004). Observed and photographed by JKS & LW (PFL/BPL) – photos available.
17-18 July 1984	Ninth pilot preparation of pasteurised Factor VIII (ZHT-4005).
July 1984	Studies begin the establish an animal (dog) model to examine the thrombogenic risk of heat treated factor IX concentrates
23 July 1984	PRF presentation on pasteurisation of factor VIII & factor IX at Congress of International Society of Blood Transfusion (Munich)
24 July 1984	PRF & A Johnson meet with Dr J Curling of Pharmacia to specify requirement for a particular ion exchange resin for purification of factor VIII. Dr Curling identifies new Q-Sepharose resin and offers the SNBTS samples for evaluation.
8-9 Aug 1984	Tenth pilot preparation of pasteurised Factor VIII (ZHT- 4006)
22 Aug 1984	B Wormsley (Pharmacia) delivers samples of Q-Sepharose to the PFC.

DATE	EVENT
24-25 Sep 1984	Eleventh pilot preparation of pasteurised Factor VIII (ZHT-4007)
26 Oct 1984	Dr DB McClelland (Director SEBTS) informed by Dr Ludlam that development of antibody to HIV in 3 haemophiliacs might be attributable to SNBTS FVIII
26 Oct 1984	PFC Department Heads discuss AIDS and heat treatment of FVIII
30 Oct 1984	First study of dry heating of SNBTS FVIII at 68°C completed by BC & PRF.
1-2 Nov 1984	Symposium on Plasma Fractionation at Groningen, The Netherlands. Attended by RJP, PRF, CVP & RVM for the SNBTS. Dr Jason (Centers for Disease Control) presents data on heat inactivation of HIV added to Factor VIII. The PFC attendees (RJP, PRF & RVM) agree that the SNBTS should dry heat its existing Factor VIII as soon as possible.
2 Nov 1984	BC recalls batch of the SNBTS FVIII implicated in HIV seroconversions
5 Nov 1984	RJP informs JDC of the data presented at Groningen. JDC accepts the recommendation that the SNBTS should dry heat treat its FVIII as soon as possible.
7 Nov 1984	BC recalls batch of FIX concentrate associated with re-called batch of FVIII
14 Nov 1984	PFC albumin pasteurisation cabinets validated for dry heat treatment of FVIII at 68°C for 2 hours.

DATE	EVENT
15 Nov 1984	Dr DB McClelland writes formally to JDC to inform him that 16 haemophilia patients treated with SNBTS FVIII had developed antibodies to HIV.
18 Nov 1984	Heat treatment of FVIII stocks at 68°C/2h begins.
22 Nov 1984	JDC confirms to RJP that it would be appropriate to dry heat 3 months stock of FVIII at 68°C for 2 hours in advance of clinical trial results.
22 Nov 1984	RJP advises Regional Transfusion directors and JDC that, in order to expedite the issue of heated product, 6 batches of FVIII would be heat treated prior to completion of the clinical evaluation. Urges recall of all product out-with direct RTC control, so that heated product can reach patients at the earliest opportunity.
22 Nov 1984	JKS visits the PFC to discuss heat treatment of coagulation factors.
29 Nov 1984	The SNBTS meets with haemophilia directors to discuss finding of HIV antibodies in haemophiliacs and the introduction of FVIII dry heated at 68°C/2h
3 Dec 1984	FVIII dry heated at 68°C/2h issued for clinical evaluation of efficacy and tolerability
6 Dec 1984	RJP writes to Regional Transfusion Directors to advise them that a stock of 68°C/2h dry heated FVIII will be issued to all centres and that unheated FVIII should be returned.
10 Dec 1984	Issue of 68°C/2hr dry heated Factor VIII (NY HT) to BTS Centres in Aberdeen, Belfast, Dundee, Edinburgh, Glasgow, and Inverness.
8 Jan 1985	RJP informs the Director of the National Institute for Biological Standards that the SNBTS is heat treating Factor VIII for 2 hours at 68°C
16 Jan 1985	The PFC receives the BPL specification for a dry heat treatment cabinet.
20 Jan 1985	PFC production re-started. FVIII formulated with added sucrose enabling dry heat treatment at 68°C to be extended from 2 hours to 24 hours.
4-6 Feb 1985	A Johnson (NYU) visits the PFC & BPL to discuss purification of Factor VIII
14 Feb 1985	JKS sends memo to PRF describing the PFL (BPL) research on high-purity FVIII
19 Feb 1985	JKS visits the PFC to discuss heat treatment of coagulation factors

DATE	EVENT
Feb 1985	BC & KGR perform virus inactivation experiments on 68°C dry heated FVIII and 80°C dry heated FIX.
8 Mar 1985	Start of animal (dog) thrombogenicity safety study of reformulated DEFIX dry heated for 72 hours at 80°C
14 Mar 1985	Issue of 68°C/24hr Dry Heated FVIII (NY HT) to Edinburgh RTC for clinical trial.
15 Mar 1985	Supply of 80°C/72hr dry heated Factor IX (HT DEFIX) for animal safety studies
27 Mar 1985	LW, PF & GN (PFL/BPL) visit the PFC to discuss heat treatment of coagulation factors. LW uncertain over the BPL policy on heating FVIII. She believes it most likely that the BPL will routinely dry heat its existing Factor VIII (HL) at 70°C/24h rather than attempt to fast-track 8Y.
29 Mar 1985	Issue of 68°C/24hr dry heated FVIII to Glasgow Royal Infirmary for clinical trial
1Apr 1985	Scotland's haemophilia directors purchase commercial heat treated FIX concentrate, pending the introduction of heat treated FIX by the SNBTS.
1 April 1985	PRF proposes that antithrombin III be added to DEFIX to prevent thrombogenicity on heat treatment based on discussions with JKS[53].
3 Apr 1985	DEFIX re-formulated with anti-thrombin III for animal safety studies of 80°C/72h dry heated product.
26 Apr 1985	JKS visits the PFC to discuss heat treatment of Factor IX and safety study in animals
29-30 Apr 1985	New batch of 80°C/72hr dry heated Factor IX (HT DEFIX) prepared for animal safety study. Formulated with addition of anti-thrombin III to prevent generation of thrombin.
15 May 1985	Scottish Haemophilia Directors agree that 68°C/24h Factor VIII should not be issued until stocks of 68°C/2h Factor VIII were consumed in order to maintain batch dedication.
15 July 1985	HT DEFIX, re-formulated and dry heat treated for 72 hours at 80°C issued for clinical evaluation.
17 July 1985	First infusion of HT DEFIX
9 Aug 1985	Clinical evaluation of HT DEFIX completed satisfactorily.

DATE	EVENT
12 Aug 1985	HT DEFIX issued to Edinburgh RTC for routine use.
27 Aug 1985	Meeting at the SNBTS HQ lab to discuss results of animal safety study on heat treated factor IX from the PFC and PFL/BPL
28 Aug 1985	RJP advised the SNBTS Directors that unheated FIX would be recalled once stocks of heated FIX have been established
4 Sep 1985	First routine issue of 68°C/24 hr dry heated FVIII (NY HT)
13 Sept 1985	Last issue of 68°C/2hr dry heated FVIII (NY HT).
1 Oct 1985	Routine issue of 80°C/72hr dry heated Factor IX concentrate (HT DEFIX)
21 Oct 1985	RVM discovers that intermediate-purity FVIII withstands 80°C dry heat when dried using new freeze drying cycle devised for high-purity FVIII
28 Oct 1985	Unheated FIX (DEFIX) recalled by BC.
13 Nov 1985	PRF writes to JKS requesting details of the freeze drying cycle used for 8Y
21 Nov 1985	RVM begins experiments to examine the feasibility of applying dry heating to FVIII (ZHT) instead of pasteurisation.
27 Nov 1985	BC recalls SNBTS F VIII (NY) dry heat treated for 2 hours at 68°C.
17 Dec 1985	R Herrington (CSL, Australia) visits the PFC to discuss heat treatment of coagulation factors with PRF & RVM
17 Dec 1985	PRF receives reply from JKS with details of *"recently revamped"* freeze drying cycle for 8Y, which confirms that this is similar to the new freeze drying cycle that had been devised at the PFC.
18 Dec 1985	Memorandum from PRF to RJP summarising progress and options for the development of Factor VIII (Foster, 1985[54])
23 Dec 1985	RJP, BC, PRF & RVM meet to review FVIII development strategy. Agreed that a severe dry heated FVIII should be developed as quickly as possible.
Jan 1985	RVM begins programme of R&D to establish a suitable method for the rapid development of a severe dry heat treated FVIII (named Z8)
27 Jan 1986	DSP reports results of experiments on heating and irradiation of coagulation factors from PFL
11-12 Feb 1986	PRF attends Newcastle conference on AIDS. Dr P Jones suggests that dry heated FVIII has transmitted HIV
20 Feb 1986	Results from DSP on further experiments on irradiation of 8Y and 9A from PFL.

DATE	EVENT
26 Feb 1986	JKS writes to DSP with information on the formulation and product characteristics of 8Y
27 Feb 1986	The SNBTS Factor VIII Study Group endorses the PFC proposal to develop 80°C/72hr dry heated FVIII similar to 8Y as soon as possible
5 Mar 1986	The SNBTS notifies Haemophilia Directors of plan to develop FVIII dry heated at 80°C/72hr
5 Mar 1986	The SNBTS 80°C/72hr dry heated FVIII to be named Z8.
12 Mar 1986	RVM obtains 80% recovery of FVIII after dry heating for 72 hours at 80°C in FVIII samples which had been re-formulated and re-freeze dried using new drying cycle
17 Mar 1986	Meeting between senior staff of the SNBTS & the BPL to discuss virus inactivation studies. Agreed that the PFC would perform virus inactivation studies on 8Y for the BPL.
24 Mar 1986	PRF receives detailed information on freeze drying cycles of 8Y and 9A from the BPL
24 Apr 1986	RVM carries out first experiment with high salt formulation to stabilise FVIII during the preparation of Z8.
25 Apr 1986	BC & KGR perform first virus inactivation experiments on samples of Z8 prepared in the research laboratory and dry heated at 80°C.
30 Apr 1986	BC outlines virus inactivation experiments to be performed with 8Y for the BPL
12-16 May 1986	ISBT Congress, Sydney, Australia. Presentation by PRF on heat treatment of FVIII describes the importance of freeze drying in achieving 80°C dry heat treatment of FVIII concentrate.
8-13 June 1986	Congress of ISTH in San Diego. JKS send his report to PRF.
23 June 1986	First pilot-scale preparation of Z8 (Z8-6-001) begun in the PFC production department
25 July 1986	PFC Management Team decides that production of FVIII (NY-HT) should cease to allow Production Department resources to focus on the development of Z8.
28 July 1986	Second pilot-scale preparation of Z8 (Z8-6-002) begun in the PFC production department

DATE	EVENT
29 July 1986	PRF and JKS meet to discuss freeze drying and heat treatment of FVIII
4 Aug 1986	First trial of full- scale preparation of Z8 in the PFC Production Department
7 Aug 1986	A BPL memo describing changes to freeze drying of 8Y is sent to PRF by JKS
7 Aug 1986	BC & KGR perform first virus inactivation study of dry heating of Z8 in which heating at 80°C and 75°C are compared.
August 1986	RJP obtains 50 vials of 8Y from the BPL at the request of Dr Ludlam and supplies them to Dr Ludlam for the treatment of patients susceptible to NANBH infection.
25 Aug 1986	BC & KGR perform trial virus inactivation study on BPL 8Y dry heated at 80°C.
23 Sep 1986	BC draws up protocol for virus inactivation study of BPL 8Y.
25 Sep 1986	Start of first PFC experiment on virus inactivation in 8Y
25 Sep 1986	Study in PFC production freeze drier of freezing FVIII solution to determine procedures for obtaining super-cooling reproducibly.
9 Oct 1986	PRF meets with JKS to discuss heat treatment of coagulation factors. JKS supplies a copy of his Interim Report on the NANBH safety of 80°C/72hr dry heated 8Y& 9A.
10 Oct 1986	Interim Report on the NANBH safety of 8Y & 9A presented to annual meeting of HCDO (Edinburgh).
5 Nov 1986	BC sends JKS results from the first PFC study of virus inactivation in 8Y
20 Nov 1986	BC receives letter from JKS indicating that the BPL are studying heat treatment of 8Y at 90°C
4 Dec 1986	J Lundblad (Bayer) visits the PFC and indicates to PRF & RVM that Bayer had failed to achieve 80°C dry heat treatment of FVIII and had abandoned their work on this approach
11 Dec 1986	PRF receives report from JKS re variations in composition of 8Y and relationship to plasma thawing conditions.
22 Dec 1986	Issue of 80°C/72hr dry heated FVIII (Z8) to Edinburgh RTC for clinical trial
6 Mar 1987	A Johnson (NYU) visits PFC to discuss research on high-purity FVIII
16 Mar 1987	Report of first PFC study on virus inactivation with 8Y
17 Mar 1987	A Fulton (NYU) visits PFC to discuss research on high-purity FVIII

DATE	EVENT
Mar 1987	PRF receives report on freeze drying of 8Y from JKS which describes problems which arose at BPL early in 1986 and which resulted in batch failures.
2 Apr 1987	H Suomela (Finnish Red Cross) visits PFC to discuss virus inactivation.
15 Apr 1987	First routine issue of Z8 (dry heated at 75°C/72hr).
22 Apr 1987	PRF meets with JKS and LW to discuss dry heat treatment
13 May 1987	Last issue of 68°C/24hr dry heated FVIII (NY HT).
22 May 1987	First routine issue of Z8 (dry heat treated at 80°C/72hr)
9 June 1987	A Johnson (NYU) visits PFC to discuss research on high-purity FVIII
6-10 July 1987	Congress of ISTH, Brussels. RVM presents PFC research on 80°C dry heat treatment of FVIII. RVM explains procedure to Dr A Oats (CSL Australia) who had been unable to achieve 80°C dry heat treatment of the CSL version of 8Y [*Note: CSL went on to introduce their product successfully in 1990.*]
22 July 1987	Letter from JKS to RJP with proposals for further virus inactivation studies
19 Oct 1987	BC, PRF, RVM, RJP & KGR (PFC) meet with DE, PH & LW (BPL) to discuss further programme of virus inactivation studies
3 Nov 1987	LW sends BC draft protocol for further virus inactivation studies of 8Y
2 Dec 1987	PRF meets PF (PFL) to discuss virus inactivation studies
Jan 1988	JKS provides PRF with the first progress report of a study commissioned from Pafra Ltd, Cambridge by BPL of the freeze drying of 8Y.
28 Jan 1988	JKS sends BC further details of virus inactivation studies for 8Y
2 Feb 1988	T Snape, J Williams & GN visit PFC to discuss FVIII preparation and virus inactivation
11-15 Apr 1988	RJP & RVM visit New York University Medical Centre to discuss research into high-purity FVIII and the New York Blood Centre to review solvent/detergent treatment for virus inactivation.
20 July 1988	KGR reports super-cooling in a sample of 8Y (at 8 g protein/L) during a freeze drying study of 8Y at PFC (see fig. 3).
1 Aug 1988	KGR, RVM & PRF review results of PFC virus inactivation studies on 8Y with JKS.
16 Aug 1988	JKS writes to BC requesting that the PFC undertake additional

DATE	EVENT
	experiments on 8Y
15 Nov 1988	PRF & RVM visit BPL to discuss FVIII preparation.
4 Jan 1989	KGR completes the PFC virus inactivation studies on 8Y
16 Mar 1989	F Franks (Pafra, Cambridge) visits PFC to discuss freeze drying of FVIII with PRF & RVM.
7 June 1989	JKS sends memo to PRF & A Johnson describing instability on the further purification of 8Y and examines the use of adsorption with aluminium hydroxide to prevent this.

8. CHRONOLOGY (UK)

DATE	EVENT
July 1983	Committee on Safety of Medicines rejects a proposal to cease importation of FVIII from the USA because of a possible risk of transmission of AIDS
Sep 1983	Committee on Safety of Medicines rejects an application to licence 60°C/72hr dry heat treated FVIII from Hyland/Baxter. Baxter criticised for making an unjustified claim for improved safety from AIDS
Mar 1984	Committee on Safety of Medicines accepts an application to licence pasteurised FVIII from Behringwerke
May 1984	The PFL begin trial issues of 60°C/72h dry heated FVIII[55]
July 1984	Committee on Safety of Medicines rejects an application to licence 60°C/30hr dry heat treated FVIII from Armour.
Dec 1984	The SNBTS issues all FVIII (NY HT) dry heated at 68°C/2hr.
Dec 1984	All unheated SNBTS FVIII (NY) recalled
Feb 1985	Commercial heat treated FVIII concentrates from Alpha (Profilate Heat Treated), Armour (H.T. Factorate), Baxter (Hemofil T), Bayer (Koate HT) & Immuno (Kryobulin TIM), licensed for sale.
Feb 1985	The PFL prepare a trial batch of FVIII (8Y) dry heat treated at 80°C/72 hr for clinical evaluation. The BPL issues some FVIII dry heated for 24 hours at 70°C but finds that not all batches can tolerate heating.[55]
Mar 1985	The PFL submit a patent application for the FVIII purification procedure used in the preparation of 8Y. This is said to be compatible with either pasteurisation or dry heating at 70°C for 24 hours[44].

Apr 1985	The SNBTS cease issue of unheated FIX(DEFIX)
May 1985	The BPL cease issue of unheated FVIII (HL)[55]
May 1985	40% of haemophilia centres surveyed still using unheated Factor VIII concentrate
Aug 1985	The SNBTS issue FVIII (NY HT) dry heated at 68°C/24hr
Sept 1985	The BPL begin to issue all FVIII (8Y) dry heat treated at 80°C/72hr.[55]
Oct 1985	The SNBTS issue all FIX (HT DEFIX) dry heat treated at 80°C/72hr
Oct 1985	The SNBTS recalls unheated FIX (DEFIX)
Oct 1985	The BPL cease issue of unheated FIX (9A)[54]
Oct 1985	The BPL issue all FIX (9A) dry heat treated at 80°C/72hr[54]
Nov 1985	The SNBTS recalls FVIII (NY) dry heated at 68°C/2h
Feb 1986	NIBSC holds first meeting on the virological aspects of the safety of blood products
Mar 1986	Publication of results of clinical study of PFL small pool FVIII dry heated for 72 h at 60°C; no evidence of transmission of NANBH in 3 recipients.[56]
Oct 1986	Armour voluntarily withdraws licence for FVIII (H.T. Factorate) dry heated at 60°C/30hr because of reports of HIV transmission
Apr 1987	The SNBTS begins routine issue of FVIII (Z8) dry heat treated at 75-80°C/72hr
Sep 1987	HCDO meeting receives report of transmission of NANBH (10 cases) and hepatitis B (4 cases) associated with the use of commercial FVIII in England & Wales during 1985-1987 (Craske, 1987[57]).
Sep 1987	Clinical trial protocol for a more 'rigorous' study of the viral safety of 80°C/72h dry heated BPL concentrates, 8Y and 9A, presented to HCDO.
1988	Bayer FVIII (Koate) unheated, no longer licensed for sale.[b]
Mar 1988	NIBSC hold meeting with all suppliers of blood products to the NHS to review product safety (National Institute for Biological Standards and Control, 1988[58])
Oct 1988	Publication of preliminary the BPL safety study of 80°C/72hr dry heating
1989	Cessation of licence for Alpha FVIII (Profilate Heat Treated) heated in solvent at 60°C/24hr.[b]
1989	Cessation of licence for Baxter FVIII (Hemofil T) heated at 60°C/72hr.[b]
Dec 1989	Product licence granted to high-purity FVIII from Armour, pasteurised for 10 hours at 60°C (Monoclate P)[b]
1992	Cessation of licence for Immuno FVIII (Kryobulin TIM) steam heated at 60°C/10hr.

1992	Cessation of licence for Bayer FVIII (Koate HT) dry heated at 68°C/72hr.
1992	First regulatory guidance concerning virus inactivation of plasma products
1992	Product licence granted to Alpha FVIII (Alpha VIII), treated with solvent/detergent for virus inactivation.[b]
1993	Publication of the substantive BPL safety study of 80°C/72hr dry heat treatment.
1993	Publication of SNBTS safety study of 80°C/72hr dry heat treatment.
1993	Product licence granted to Armour high-purity FIX (Mononine) treated with sodium thiocyanate.[b]
1993	Product licence granted to Alpha high-purity FIX (AlphaNine SD) treated solvent/detergent.[b]
1994	Product licence granted to Bayer high-purity FVIII (Koate HP) treated with solvent/detergent.[b]
1994	Product licence granted to Baxter high-purity FVIII (Hemofil M) treated with solvent/detergent.[b]
1994	Product licence granted to BPL high-purity FVIII (Replenate) treated with solvent/detergent
1994	Product licence granted to BPL high-purity FIX (Replenine) treated with solvent/detergent.[b]
1996	Product licence granted to the SNBTS high-purity FVIII (Liberate) treated with solvent/detergent.
2000	Product licence granted to Alpha high-purity FVIII (Alphanate) treated with solvent/detergent.[b]
2001	Product licence granted to the SNBTS high-purity FIX (HIP-FIX) treated with solvent/detergent and dry heat for 72 hours at 80°C

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ADDENDUM

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