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GRO-C: C R Bishop

cc This = Alpha Data to Med Dept

Inactivation of AIDS-associated viruses in antihemophilic factor products:

The effectiveness of heat treatment

A professional service from

Cutter Biological

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Introduction

Ever since K. M. Brinkhous discovered plasma factor in 1947, the blessing of blood coagulation product therapy has been tainted by the threat of viral contamination. Recently, that threat became more evident and more serious than ever before with the outbreak of Acquired Immunodeficiency Syndrome (AIDS) and its rising incidence in the hemophiliac population.

As it became clear that AIDS could be transmitted through blood and blood products, hemophiliacs began to feel as though they were taking their lives in their own hands with each infusion. Science was at a loss for an explanation, leaving hemophiliacs frightened, confused, and sometimes reluctant to take their factor.

Then in 1984, three independent research groups identified a virus (or viruses) that seemed to be the causative agent in AIDS. Since many other infectious viral agents were known to be heat-sensitive, scientists at Cutter immediately suspected that the AIDS viruses might be inactivated in coagulation products through usage of the heat process. The challenge, then, was to determine whether the process did, in fact, inactivate the AIDS viruses.

Research was conducted to determine the ability of AIDS-related viruses to withstand heat treatment. The Cutter heat treatment process was found to inactivate the highly infectious agents implicated in the transmission of AIDS, without compromising product efficacy. With the heat treatment, along with the intensive donor

screening procedures Cutter performs, a safer, highly effective and dependable product can now be produced. The usage of heat-treated products has been recommended by both the Centers for Disease Control and the National Hemophilia Foundation.

"In view of the accumulating evidence for the heat sensitivity of HTLV-III [Human T-cell Lymphotropic Virus], and the apparent lack of untoward effects attributable to the heat treatment, we recommend that physicians should strongly consider prescribing heat-treated coagulation factor concentrates for the treatment of patients with severe hemophilia, with the understanding that protection against AIDS is yet to be proven."[1]

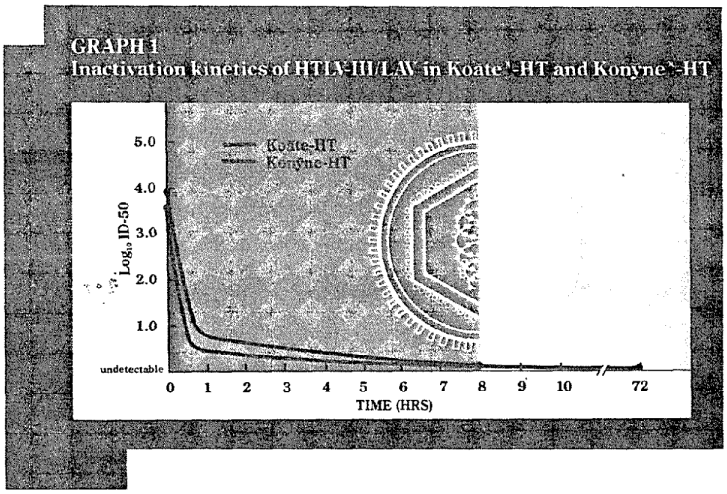
Medical and Scientific Advisory Council

The National Hemophilia Foundation

The following are summaries of three studies conducted to determine the effectiveness of heat treatment in inactivating viruses that have been identified as being associated with AIDS. These viruses, HTLV-III (Human T-cell Lymphotropic Virus), LAV (Lymphadenopathy-associated Virus) and ARV (AIDS-related Virus) were each identified separately by three independent research groups, and are believed to be either the same virus or very similar to one another.

Viral inactivation of HTLV-III/LAV

In an independent study by the Centers for Disease Control in Atlanta, Georgia, Drs. J. Steven McDougal, Bruce L. Evatt and their colleagues provided Cutter Laboratories with seeded cultures of HTLV-III/LAV. The virus was added to Factor VIII and Factor IX concentrates, then lyophilized and heated according to manufacturing specifications. The virus titer was determined at time intervals from one to 72 hours of the heat treatment. HTLV-III/LAV was rendered undetectable when tested after eight hours of heating, and at given time intervals up to 72 hours. (See Graph 1)

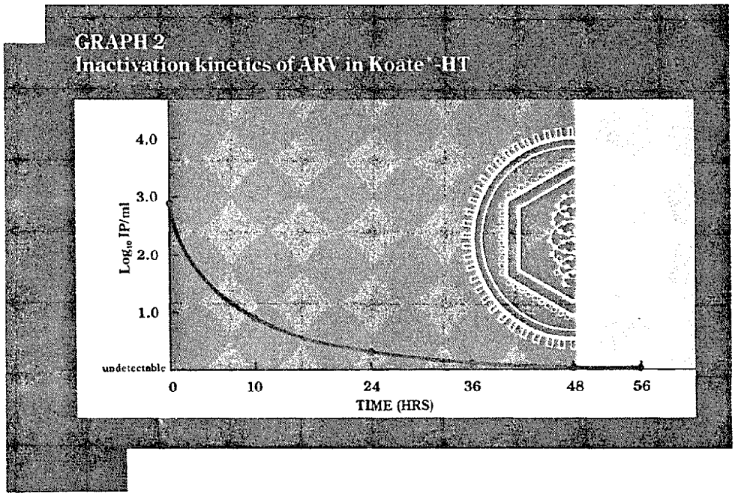


Dr. J. Steven McDougal, et al Centers for Disease Control, Atlanta

"...both in vitro and vivo data indicate that the use of heat-treated products will reduce (and hopefully terminate) AIDS transmission by antihemophilic factor."[2]

Viral inactivation of ARV

Another *in vitro* study was conducted by Dr. Jay A. Levy of the University of California, San Francisco, who first isolated the ARV virus. Dr. Levy and his group added titers of ARV to human plasma. A Factor VIII filtrate was separated from the "spiked" plasma and found to have infectious ARV after lyophilization. The factor was then heat treated at 68°C. ARV was undetectable after 48 hours, and at time intervals up to 56 hours of the treatment.* (See Graph 2)



"...the results confirm the ability of infectious retroviruses to withstand the procedures used to purify FVIII from plasma ...[and] suggest that heating lyophilised FVIII for 72h at 68° C...will eliminate infectious ARV..."[3]

Dr. Jay A. Levy, et al
University of California,
San Francisco

*Cutter process extends heat treatment to 72 hours to add a greater margin of safety.

Clinical results: Heat-treated Factor VIII

A 12-month clinical trial was conducted in Europe by Dr. C. Rouzioux of the Hôpital Claude Bernard, Paris, Dr. S. Chamant, Institut Pasteur, Paris, and Dr. L. Montagnier, University of Milan, Italy. A group of 18 previously untreated patients with hemophilia A were given heat-treated Factor VIII concentrate. A matched control group of 29 previously untreated hemophilia A patients were given equivalent doses of non-treated commercial concentrate.

None of the 18 test group patients was positive for HTLV-III/LAV antibodies upon testing after six and 12 months of treatment. However, five of the 29 controls were anti-HTLV-III/LAV positive. These five patients had no risk factor for AIDS other than treatment with the untreated Factor VIII concentrate. (See chart below.)

CHART
Absence of Antibodies to LAV in Patients Receiving Heat-Treated Factor VIII Concentrate

	Test group (n = 18)	Control group (n = 29)
Mean age in years (range)	9 (0.25–58)	13 (2–50)
Factor VIII concentrate	Heated (US)	Non-heated (US)
Period of treatment	Dec 1982–June 1984	1982–1984
Mean total dose (IU) (range)	9711 (240–66 720)	7700 (1000–83 540)
Antibodies to LAV	0/18	5/29 (17%)

"It is possible that precautionary measures...such as accurate donor screening...have also helped to prevent contamination of plasma pools. The absence of seroconversion to anti-LAV [anti-HTLV-III] in the test group may therefore be due to a combination of precautionary measures and heat treatment."[4]

Dr. C. Rouzioux, et al
Virology Laboratory
Hôpital Claude Bernard
Paris, France

Antihemophilic Factor (Human) Heat-Treated Koāte®-HT

INDICATIONS AND USAGE

Koāte-HT is indicated for the treatment of classical hemophilia (hemophilia A) in which there is a demonstrated deficiency of activity of the plasma clotting factor, Factor VIII. Koāte-HT provides a means of temporarily replacing the missing clotting factor in order to correct or prevent bleeding episodes, or in order to perform emergency and elective surgery on hemophiliacs.

Koāte-HT is not indicated in the treatment of von Willebrand's disease.

CONTRAINDICATIONS

None known.

WARNINGS

Antihemophilic Factor (Human), heat-treated, Koāte®-HT concentrate is a purified dried fraction taken from large pools of fresh human plasma obtained from many paid donors. Although each unit of plasma has been found nonreactive for hepatitis B surface antigen (HBsAg) using a U.S. Federally approved test with third-generation sensitivity, the presence of hepatitis viruses in such pools must be assumed.

Kasper and Kipnis have concluded that those who have had little exposure to blood products have a higher risk of developing hepatitis after introduction of clotting factor concentrates. For such patients, especially those with mild hemophilia, they recommend single donor products. For patients with moderate or severe hemophilia who have received numerous infusions of blood or blood products, they feel that the risk of hepatitis is small. They believe that the clotting factor concentrates have so greatly improved the management of severe hemophilia that these products should not be denied to appropriate patients.

Isolated cases of Acquired Immune Deficiency Syndrome (AIDS) have been reported in hemophilics who have received blood and/or coagulation factor concentrates including Factor VIII concentrates. It is not known if the disease is due to a transmitted specific agent, secondary to multiple antigenic exposures, or to some other mechanisms. The physician and patient should consider that Factor VIII concentrates may be associated with the transmission of AIDS and weigh the benefits of therapy accordingly.

PRECAUTIONS

General

1. Koāte-HT is intended for treatment of bleeding disorders arising from a deficiency in Factor VIII. This deficiency should be proven prior to administering Koāte-HT, since no benefit may be expected from its use in treating hemorrhages due to other causes.
2. Administer promptly (within 3 hours) after reconstitution. Do not refrigerate after reconstitution.

NOTE: Although Koāte-HT is fully stable, without potency loss, for at least 24 hours at room temperature after reconstitution, the recommendation to administer promptly after reconstitution is intended to avoid the ill effect of any possible bacterial contamination occurring during reconstitution.

3. Administer only by the intravenous route.
4. A filter needle should be used prior to administering.
5. Koāte-HT contains levels of blood group isoagglutinins which are not clinically significant when controlling relatively minor bleeding episodes. When large or frequently repeated doses are required in patients of blood groups A, B or AB, there is a possibility of intravascular hemolysis. If hemolytic anemia develops, administration of type O packed red blood cells should be considered.
6. Administration equipment and any reconstituted Koāte-HT not used should be appropriately discarded.

Pregnancy Category C

Animal reproduction studies have not been conducted with Koāte-HT. It is also not known whether Koāte-HT can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Koāte-HT should be given to a pregnant woman only if clearly needed.

ADVERSE REACTIONS

Allergic reactions may result from the administration of AHF preparations.

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Canadian License No. 24

Factor IX Complex Konyne®-HT

INDICATIONS AND USAGE

The main indication for using Factor IX Complex, Konyne®-HT is in the treatment of conditions caused by Factor IX deficiency. In the treatment of specific coagulation disorders involving Factors II or X, and even cases of mild hemophilia B, the use of fresh, frozen plasma should first be considered. The use of Konyne-HT should be reserved for these patients if treatment with fresh frozen plasma is not feasible or has proven ineffective.

Konyne-HT is not indicated for use in the treatment of Factor VII deficiencies.

Konyne is appropriate for use in:

1. Hemophilia B (Christmas disease); demonstrated Factor IX deficiency in children or adults with real or impending bleeding episodes. Spontaneous bleeding can occur even in the absence of any trauma.
2. Reversal of coumarin anticoagulant induced hemorrhage; in situations where prompt reversal is required (e.g., preceding emergency surgery, trauma, etc.), administration of fresh, frozen plasma should be initially considered as treatment; however, Factor IX Complex—Konyne®-HT may be considered as a secondary approach if the risk of transmitting hepatitis is considered justifiable in the face of a life-threatening situation.

In addition to coumarin anticoagulant induced deficiencies, low levels of Factors II, VII, IX and X may be found in vitamin K deficiency, in patients with gut sterilization due to oral antibiotics, in patients with liver disease, and in those with nephrotic syndrome. However, Factor IX Complex is not indicated in these situations and treatment should be aimed at correcting the primary condition.

CONTRAINDICATIONS
None Known.

WARNINGS
1. Hepatitis
Factor IX Complex a plasma fraction obtained from many paid donors. The presence of hepatitis viruses should be assumed and the hazard of administering Konyne-HT should be weighed against the medical consequence of withholding it, particularly in persons with few previous transfusions of blood or blood products. Single donor fresh plasma may be appropriate in such circumstances.
2. AIDS
Isolated cases of Acquired Immune Deficiency Syndrome (AIDS) have been reported in hemophiliacs who have received blood and/or coagulation factor concentrates including Factor VIII concentrates. It is not known if the disease is due to a transmitted specific agent, secondary to multiple antigenic exposures, or to some other mechanisms. The physician and patient should consider that Factor IX complex may be associated with the transmission of AIDS and weigh the benefits of therapy accordingly.
3. Thrombosis
Cases of patients developing post-operative thrombosis after treatment with Factor IX Complex have been described. Although thrombosis is a well known risk of the post-operative period, it is found to be greater in these patients. No other data are presently available. Until further surveys and more conclusive studies are available, Konyne-HT is only advised for patients undergoing elective surgery where the expected beneficial effects of its use outweigh the increased risk of the possibility of thrombosis. This applies especially to those who may be predisposed to thrombosis.
Do not use in cases of known liver disease where there is any suspicion of intravascular coagulation or fibrinolysis.

PRECAUTIONS
General
1. Reconstitute only with Sterile Water for Injection, USP.
2. Administer promptly (within three hours) after reconstitution. Do not refrigerate after reconstitution.
3. Administer only by the intravenous route.
4. The administration equipment and any reconstituted Factor IX Complex, Konyne®-HT not immediately used should be discarded.
5. ε-amino-caproic acid should not be administered with Factor IX Complex as this may increase the risk of thrombosis.
6. Patients who receive Konyne-HT either post-operatively or with known liver disease should be kept under close observation for signs and symptoms of intravascular coagulation or thrombosis. Any suspicious findings of this nature indicate the dosage should be markedly decreased if the patient's condition is such that the treatment cannot be discontinued entirely. In the event of thrombo-hemorrhagic disorders occurring, reduction in dosage should be considered, and treatment with heparin may be warranted. Although this preparation does not contain heparin, it has been suggested that reconstitution with heparin in a concentration of 2 to 5 IU per mL may reduce the risk of development of thrombosis. However, thrombosis can occur even in the presence of heparin.
7. Patients receiving Konyne-HT for prolonged periods should be continually monitored at least for levels of Factors II, IX and X. The same comments as in 6. above are indicated.

Pregnancy Category C
Animal reproduction studies have not been conducted with Konyne-HT. It is also not known whether Konyne-HT can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Konyne-HT should be given to a pregnant woman only if clearly needed.

ADVERSE REACTIONS
In some patients the rapid administration of Konyne-HT can cause transient fever, chills, headache, flushing or tingling.

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Canadian License No. 24

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1. *Hemophilia Information Exchange: AIDS Update*. New York, National Hemophilia Foundation, Medical Bulletin No. 21, Chapter Advisory No. 26, April 12, 1985.
2. McDougal JA, et al: Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotropic virus III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. *J Clin Invest* 76: 0001-0003, 1985.
3. Levy JA, et al: Inactivation by wet and dry heat of AIDS-associated retrovirus during Factor VIII purification from plasma. (Letter) *Lancet* 1: 1456-7, 1985.
4. Rouzioux C, et al: Absence of antibodies to AIDS virus in haemophiliacs treated with heat-treated Factor VIII concentrate. (Letter) *Lancet* 1:271-2, 1985.
5. Data on file, Cutter Biological.

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Cutter heat treatment and product integrity

Maintaining product integrity is a major concern when developing viral inactivation techniques. The Cutter process has been found not only to preserve but to enhance the clarity and purity of Koāte®-HT Antihemophilic Factor (Human) and Konyne®-HT Factor IX Complex.

Cutter heat-treated coagulation products offer these important advantages:

- ☐ *HTLV-III, LAV, ARV inactivated*—optimal patient protection from AIDS-related viruses
- ☐ *Long mean half-life*—10.2 hours for Koāte-HT, Konyne-HT has a mean half-life of 24 hours
- ☐ *Low fibrinogen*—minimal risk of platelet dysfunction or side effects
- ☐ *Heparin free*—avoids misleading coagulation profiles
- ☐ *Rapid reconstitution*—less than 2 minutes
- ☐ *Hepatitis B surface antigen (HBsAg) tested*—every unit of plasma and each lot of final product tested for HBsAg using FDA-approved method

Other infectious agents inactivated by Cutter heat treatment[5]

- ☐ feline leukemia virus
- ☐ Simian AIDS (SAIDS)
- ☐ mouse C retrovirus
- ☐ cytomegalovirus (CMV)
- ☐ herpes simplex 1
- ☐ vesicular stomatitis
- ☐ Sindbis virus

Koāte®-HT
Antihemophilic Factor (Human)

Konyne®-HT
Factor IX Complex

Cutter Biological
Berkeley, CA 94710 USA
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Elkhart, IN 46514 USA

To the Medical and Pharmaceutical Professions only
DRIED FACTOR VIII FRACTION, HIGH PURITY, HEAT-TREATED

1. Dried Factor VIII Fraction, high purity, heat-treated (type 8Y) is a concentrate prepared from large pool fresh frozen plasma obtained from blood donors of the National Blood Transfusion Service. Each unit of plasma has been found negative for HBsAg using a third generation test capable of detecting at least 2 BSU/ml.

2. The product has been prepared using a new highly selective procedure to remove most of the fibrinogen and fibronectin, leaving factor VIII in solution. After recovering the factor VIII from the supernatant by precipitation with glycine and sodium chloride, the factor VIII is redissolved at high potency.

   Precipitants are removed by gel filtration and are replaced by a solution of lower ionic strength containing stabilisers. The solution is sterilised by filtration, lyophilised and heated in the dry state in the vial at 80°C for 72 hours to inactivate viral agents (including hepatitis and AIDS viruses) but it cannot be assumed to be free from viral infection.

3. Sucrose, at a level not greater than 200 mg/vial is added to the product prior to lyophilisation. This may be of significance in the interpretation of the results of some biochemical tests.

4. Each vial of product contains the Factor VIII activity stated on the label, expressed in International Units (iu). The product should be reconstituted before use, using the volume of Water for Injections stated on the label.

5. The conditions of heating have little or no effect on the factor VIII content or solubility of the concentrate.
   Additionally, after heating

   a) Prekallikrein activator activity and pH are unchanged and no other parameter measured in conventional pharmacopoeial tests (including animal tests for pyrogen and acute toxicity) is altered.

   b) The product has a specific activity in excess of 2 iu factor VIII/mg protein, some ten times greater than intermediate purity concentrates HL or 8CRV.

   c) There is little or no effect on the electrophoretic properties of the concentrate.

   d) Preliminary studies indicate that the in vivo recovery and clearance of Factor VIII:C activity are unaffected.

6. VIRUS INACTIVATION

   Published experiments now suggest that heating lyophilised Factor VIII concentrates at 68°C for 72 hours will inactivate infectious human AIDS - associated retrovirus (1); but this remains to be confirmed by prospective studies.

   The heating conditions used for this product (80°C for 72 hours) are more severe than those used in published attempts to inactivate NANBH (2)(3) and would be expected to inactivate the "model" retrovirus used by Levy et al (4).

Freedom from transmission of virus can be confirmed only by follow-up studies after clinical infusion.

7. STORAGE

The label carries the instruction: "Store in the dark below +6°C". Short periods of storage at normal ambient temperature are not detrimental.

8. POTENCY

The label carries a statement of the activity, determined by assay (5), of blood coagulation factor VIII expressed in International Units (iu), as established by the World Health Organisation.

9. DOSE

The number of units needed and duration of treatment depend on the lesion being treated. If the rise in the concentration of factor VIII in the plasma following administration of concentrate is expressed in international units per 100 ml plasma and the total dose given in international units of factor VIII per kg body weight is calculated, "the response" is defined as follows:

$$\text{Response} = \frac{\text{Rise in plasma factor VIII (in iu per 100 ml)}}{\text{Dose in iu/kg body weight}}$$

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

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

Lesion	Concentration of factor VIII desired in patient's blood immediately after transfusion (iu per 100 ml)	Initial dose of factor VIII (iu/kg body weight)
Minor spontaneous haemarthrosis, and muscle haematoma	15 to 20	7 to 13
Severe haemarthrosis and muscle haematoma, haematoma in potentially dangerous situations; haematuria	20 to 40	9 to 25
Major surgery	See below	

A dose of 1 iu/kg will give, on average, a rise of about 2 iu/100 ml plasma. If the desired concentration or clinical response is not

achieved, another dose should be given the same day. If an abnormally low response persists, carry out a test for specific antibody to factor VIII. The doses mentioned are only rough guides since there is considerable variation in response from patient to patient. It is usual to give the contents of the number of whole vials nearest to the calculated dose. Doses may be repeated at intervals of 8, 12 or 24 hours as needed to maintain the desired concentration of factor VIII.

10. MAJOR SURGERY

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

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

11. RECONSTITUTION

The container of concentrate and the Water for Injections should be brought to between 20°C and 30°C, prior to removal of the "flip-off" closures. The volume of Water for Injections indicated on the product label is drawn up into a suitable syringe, and the syringe transferred to the vial containing the product. On piercing the seal, water will be drawn into the vial which is under vacuum. (In the unlikely event of there being no evidence of a vacuum, the vial must not be used and the defect should be reported to BPL Quality Control at the address given below). The container is agitated gently until solution is complete before releasing the vacuum. A clear or slightly opalescent solution is usually obtained in about fifteen minutes or less. If a gel or clot forms discard the solution and inform BPL Quality Control. Should more than one vial be required to make up the dose, the contents of the required number are pooled. The solution should be used immediately, and in any event within 3 hours.

12. ADMINISTRATION

The solution should be drawn from the vial into a plastic disposable syringe through the filter needle supplied with the product. For administration, a Number 21 "butterfly" needle is convenient. Although the material is unlikely to cause side effects, the dose, especially the first dose, should be given slowly (approximately 3 ml per minute). The solution must not be stored and infusion should be completed within three hours of reconstitution. It should not be given by "continuous infusion", over many hours, and it must not be added to or mixed with any other fluid given, including whole blood.

13. WARNING

(1) The material contains blood group antibodies derived from the starting plasma in amounts which are insignificant in the normal treatment of haemarthroses and muscle haemorrhage. If very high dosage is necessary in patients with blood groups A, B or AB, the patient should be monitored for signs of intravascular haemolysis.

(2) Patients congenitally deficient in factor VIII may develop antibodies to factor VIII following treatment. This risk does not appear to be increased by the use of concentrate (6) but patients should be monitored from time to time, especially if there is any doubt about the clinical effectiveness of a dose of factor VIII.

14. CAUTION

Circumstances outside the control of BPL can reduce the efficacy of the product. It is important that instructions for storage and handling and reconstitution are followed.

Where possible, pre and post-infusion factor VIII assays should be carried out, at least for the first course of treatment. It is recommended that where the patient's treatment history permits, follow-up studies be made to confirm freedom from viral infection.

15. REFERENCES

(1) Levy et al. Inactivation by wet and dry heat of AIDS-associated retrovirus during Factor VIII purification from plasma. Lancet 1456-1457, June 22, (1985).

(2) Dolana et al. Continued observations on the effect of a heating procedure on the inactivation of NANBH and HB viruses in clotting factor concentrates.
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(3) Mozen et al. Heat inactivation of viruses in AHF concentrates.
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(4) Levy et al. Recovery and inactivation of infectious retroviruses added to factor VIII concentrates.
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(5) British Pharmacopoeia (1980) II 847-848.

(6) Biggs, R. Jaundice and antibodies directed against factor VIII and IX in patients treated for haemophilia or Christmas disease in the United Kingdom. British Journal of Haematology, 26, 313-329 (1974).

These notes are intended for guidance in the use of Dried Factor VIII, Type 8Y, manufactured and distributed by:
BLOOD PRODUCTS LABORATORY, ELSTREE, HERTS WD6 3BX

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