

PRINCIPLES OF CLOTTING FACTOR THERAPY IN HEMOPHILIA REVISED 2005

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A congenital deficiency or dysfunction of one of the following plasma clotting factors results in a hemorrhagic disorder:

PLASMA FACTOR	INHERITANCE OF DEFICIENCY	INCIDENCE OF DEFICIENCY
Factor I (fibrinogen)	Recessive	Rare
Factor II (prothrombin)	Recessive	Rare
Factor V	Recessive	Rare
Factor VII	Recessive	Rare
Factor VIII (AHF, AHG)	Sex-linked	Common (Hemophilia A)
Factor IX (Christmas factor)	Sex-linked	Common (Hemophilia B)
Factor X	Recessive	Rare
Factor XI	Recessive	Uncommon (Hemophilia C)
Factor XIII	Recessive	Rare
Von Willebrand Factor (VWF)	Dominant in most families, with variable expression	Common (Von Willebrand Disease, VWD)

Fibrinogen is the only clotting factor commonly measured by weight. The others are commonly measured in functional (biological) assays. The total amount of a factor ("antigen"), both functional and dysfunctional, may be measured in an immunologic assay.

Clotting factors are measured in "units". A unit is the amount in one milliliter (ml) of AVERAGE fresh normal plasma, separated from blood anti-coagulated with sodium citrate solution in a 9:1 volume ratio. Average normal plasma is said to contain 100% of each factor, which is the same as one unit / ml.

One unit/ml = 100 % = 100 units/deciliter (dL)

Thus, an average normal person who has a plasma volume of 3000 ml has 3000 units of any clotting factor circulating in his plasma at one time, a unit for each ml of plasma.

An international standard for factors VIII and VWF and another standard for factor IX are made of lyophilized citrated plasma pooled from many normal persons. Standards are calibrated by comparing their factor levels to those of fresh plasma from 15 normal adults in each of 25 or more laboratories around the world. Thus, the international standard reflects the average factor levels of about 300 normal people.

FACTOR VIII AND IX LEVELS IN NORMAL PERSONS AND IN HEMOPHILIA A AND B

Average normal person	100 %, by definition	1 unit/ ml plasma
Range in normal persons	40 – 200 %	0.4 to 2 units/ ml plasma
"Severe" hemophilia	<1 %	<0.01 units/ ml plasma
"Moderate" hemophilia	1-5 %	0.01 – 0.05 units/ ml plasma
"Mild" hemophilia	>5 but < 40 %	0.051-0.39 units/ ml plasma
Average carrier of hemophilia	50 %	0.5 units/ ml plasma
Range in carriers of hemophilia	1 – 200 %	0.01 – 2 units/ ml plasma

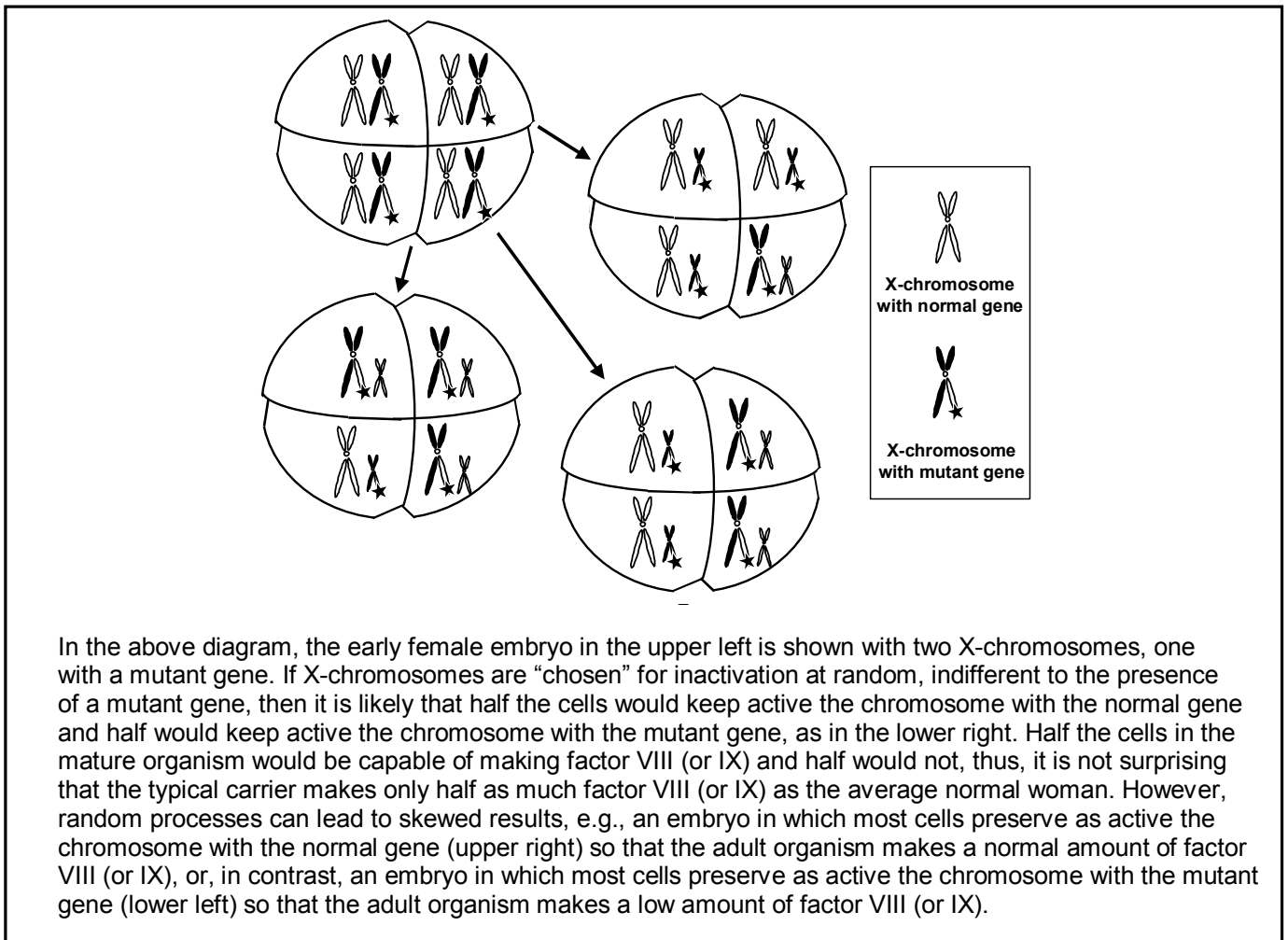
The severity of hemophilia A or B also can be distinguished by clinical manifestations. Very small amounts of clotting factor make a big clinical difference:

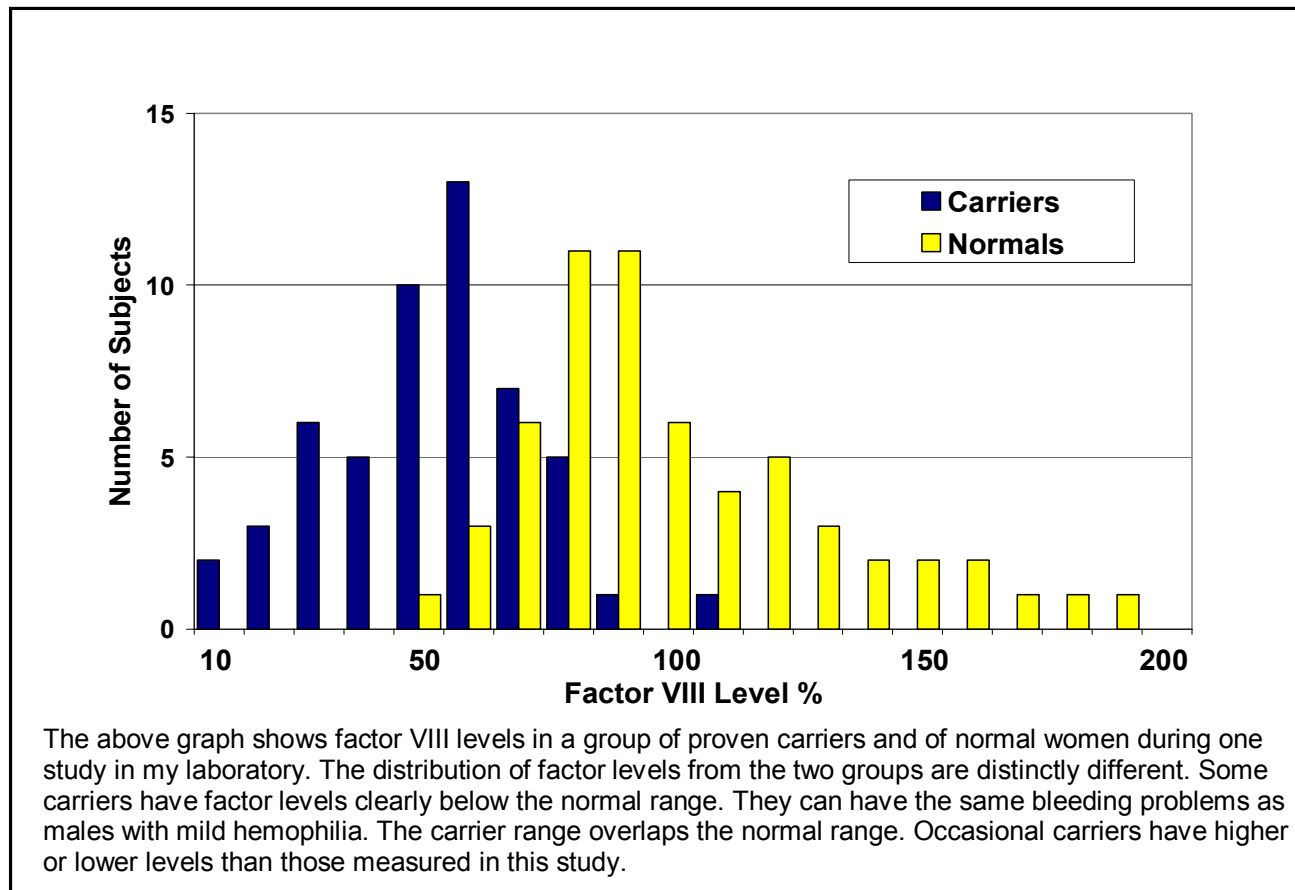
Severe hemophilia	Joint & muscle hemorrhages without obvious trauma; multiple joints typically involved
Moderate hemophilia	Joint & muscle hemorrhages with minor trauma; a damaged joint may bleed repeatedly
Mild hemophilia	Hemorrhages with surgery or serious trauma; a damaged joint may bleed repeatedly

Patients with severe deficiencies (<1 %) or dysfunction of other clotting factors typically have much less frequent joint bleeding than do patients with severe hemophilia A or B.

Heterozygotes for the bleeding disorders transmitted as autosomal recessives usually have levels of the relevant factor that are about half of the mean normal level, which is sufficient for normal clotting in nearly all instances. Thus, these heterozygotes usually are asymptomatic.

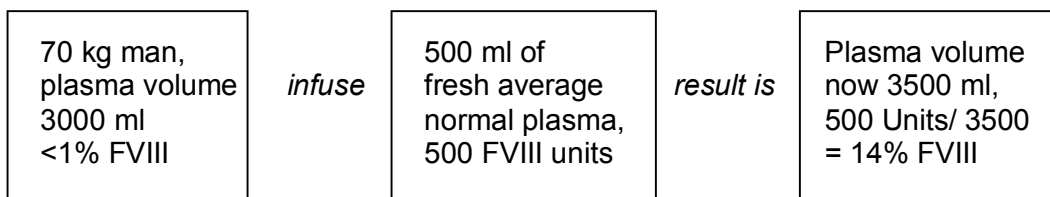
Hemizygotes (female carriers) of sex-linked hemophilia A and B also, on average, have about half the mean normal level of the relevant factor. In contrast to the situation with autosomal disorders, carriers have a wide range of factor levels because of inactivation of one of the X-chromosomes in each of the cells of female embryos. At an early stage, an X-chromosome in each of the cells is “inactivated”, that is, “chosen” to be non-influential in that cell and in all its daughter cells. The inactive X-chromosome is seen on the periphery of the nucleus as the dense-stained Barr body.



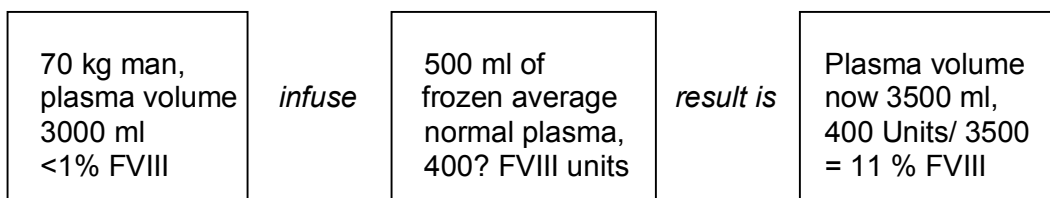


CALCULATION OF DOSAGE

If whole plasma is given (fresh, fresh-frozen or freeze-dried), as was common before 1970 for all patients and is still used for rare clotting factor deficiencies, it expands the patient's plasma volume. Only a young, lean person with elastic vasculature and a normal heart and lungs can tolerate much volume expansion. In older or compromised patients, plasma can be given by exchange-plasmapheresis to avoid volume overload. Restrictions on volume expansion limit the plasma factor levels attained, as follows:



However, fresh plasma is rarely available. We use fresh-frozen, or freeze-dried (lyophilized) plasma. Some of the factor VIII deteriorates in storage and handling.

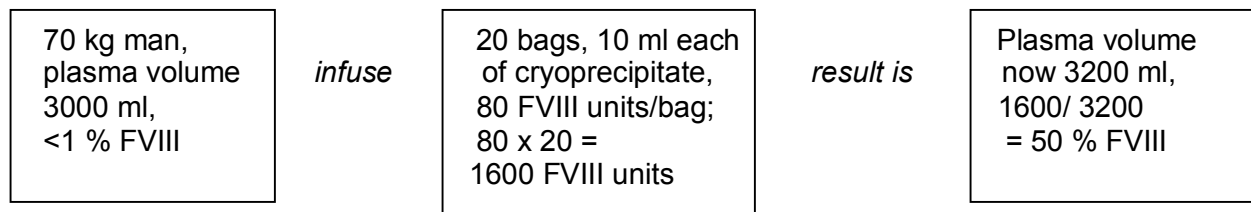


Thus, if one infuses whole plasma without exchange plasmapheresis, it is not possible to raise the level of a severely-deficient factor into the normal range. This sub-optimal therapy may stop a hemorrhage,

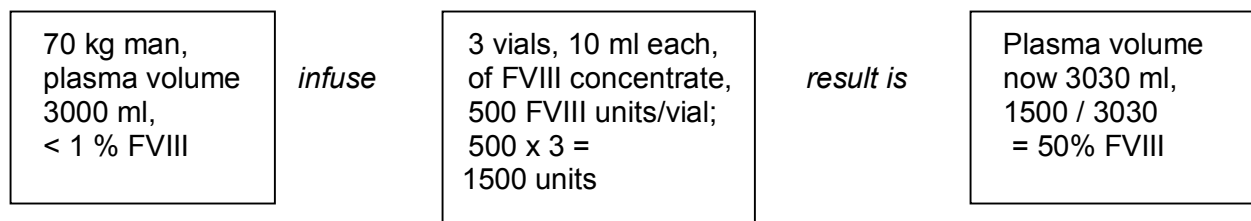
but it may not. Repeated doses of plasma may be effective. After having been infused with plasma on many occasions, patients may have allergic reactions (asthma, hives) with subsequent infusions and need anti-histamines.

Cryoprecipitate was developed in the mid-1960's. Dr. Judith Pool observed that if frozen plasma was thawed slowly (at 4° C.) and not quite completely, the remaining unattractive stringy frozen matter contained about half the factor VIII, VWF and fibrinogen from the starting plasma. Those factors are the last to thaw. Under routine production conditions in a blood bank, a 200-ml bag of plasma, recovered from a whole blood donation, yields about 80 units of factor VIII in only 10 ml or so of residual plasma. Any blood bank with a freezer (to freeze the plasma), a refrigerator (to thaw it slowly, usually overnight) and a refrigerated blood-bank centrifuge can make cryoprecipitate. The centrifuge forces the stringy precipitate into the bottom of the plastic bag. The thawed plasma is siphoned off to make other plasma components such as gamma globulin and albumin. The precipitate is re-frozen for storage.

In some communities, cryoprecipitate remained the standard therapy for hemophilia A into the 1990's. Its advantages are that it can be made locally, the cost of its production is low if calculated as an addendum to whole blood collection, a sufficient amount can be given to achieve a normal factor VIII level without volume overload, it is effective in Von Willebrand Disease and in fibrinogen deficiency, allergic reactions are fewer than with plasma, and any undetected local epidemic viral infection is spread by its use only within the local community. Other communities are unaffected as long as cryoprecipitate is not transferred from one place to another. (Communities which were not epidemic for AIDS at its outset, and depended on local cryoprecipitate for management of hemophilia A, such as Seattle, had a much lower rate of HIV transmission to persons with hemophilia than did communities dependent on concentrates made from plasma pooled from various geographic areas.) Disadvantages are that cryoprecipitate must be transported and stored frozen and that viral-inactivation is problematic. Cryoprecipitate available nowadays in most of the world is NOT viral-inactivated. Avoidance of transmission of viral infections depends solely on donor screening.



Freeze-dried concentrates of clotting factors were made using several methods, including precipitation with various agents (cryoprecipitation is one such method) and chromatography. Early products, developed in the 1960's and available by about 1970, were not much more purified than cryoprecipitate and were not viral-inactivated. They contained Von Willebrand Factor and fibrinogen. Factor VIII concentrates available today may contain 250 to 1500 units per vial (the assayed quantity is stated on the label), to be reconstituted at the time of use by adding small amounts of sterile water, e.g. 10 ml.



With cryoprecipitate and concentrate, the added volume was so small, it could be ignored in dosage calculations. To reach a desired plasma factor VIII level, e.g. 50%, in severe hemophilia, multiply that desired level, 0.5 units/ml, by the plasma volume, 3000 ml, to get the needed dose, 1500 units. To reach a desired plasma factor VIII level, e.g. 50%, in mild hemophilia, subtract the patient's own factor level, e.g.

10%, from the desired level: $50 - 10 = 40$ or 0.4 units/ml, and multiply by the plasma volume, e.g. 3000 ml, or, $3000 \times 0.4 = 1200$ units needed. OR:

Infuse one unit of factor VIII/kg for each 2 percentage-points increase in plasma factor VIII wanted.

For hemophilia B, concentrate was introduced in 1970 containing prothrombin, factor VII, factor IX and factor X (factors that travel through fractionation together), called “prothrombin complex concentrate” or “PCC”. It is assayed in terms of its factor IX content. During fractionation, some of the clotting factors are activated, which may be the cause of the major serious side-effect. The concentrate is associated with an increased frequency of thrombosis in vulnerable patients, such as those with immature or failing livers, or with massive tissue trauma, or undergoing surgical operations. In 1990, the first concentrate containing factor IX alone was introduced. Such concentrates are not associated with excessive thrombosis.

Factor IX, alone among all the clotting factors, has a mysterious property. When it is infused, as plasma or as concentrate, about half disappears instantly. This phenomenon has not yet been explained. A given volume of fresh-frozen plasma raises the factor IX level in hemophilia B only half as much as the same volume of fresh-frozen plasma raises the factor VIII level in hemophilia A. To achieve a desired plasma factor IX level with concentrate, the dose of factor IX concentrate must be twice as high as that used with factor VIII concentrate in hemophilia A.

70 kg man,
plasma volume
3000 ml,
<1 % factor IX

infuse

500 ml of
normal plasma,
 $500 / 2 = 250$, i.e.
250 recoverable
units of factor IX

result is

Plasma volume
now 3500 ml,
 $250 / 3500$
= 7 % factor IX

70 kg man,
plasma volume
3000 ml,
< 1% factor IX

infuse

3 vials, 10 ml each,
factor IX concentrate,
1000 units/vial,
 $\times 3 = 3000$ units,
 $3000/2 = 1500$
recoverable units

result is

Plasma volume
now 3030 ml,
 $1500/ 3030$
= 50 % factor IX

To reach a desired plasma factor IX level, e.g. 50% or 0.5 units/ml, multiply that by the plasma volume, e.g. 3000, and then double it: $0.5 \times 3000 = 1500 \times 2 = 3000$ factor IX units needed, OR:

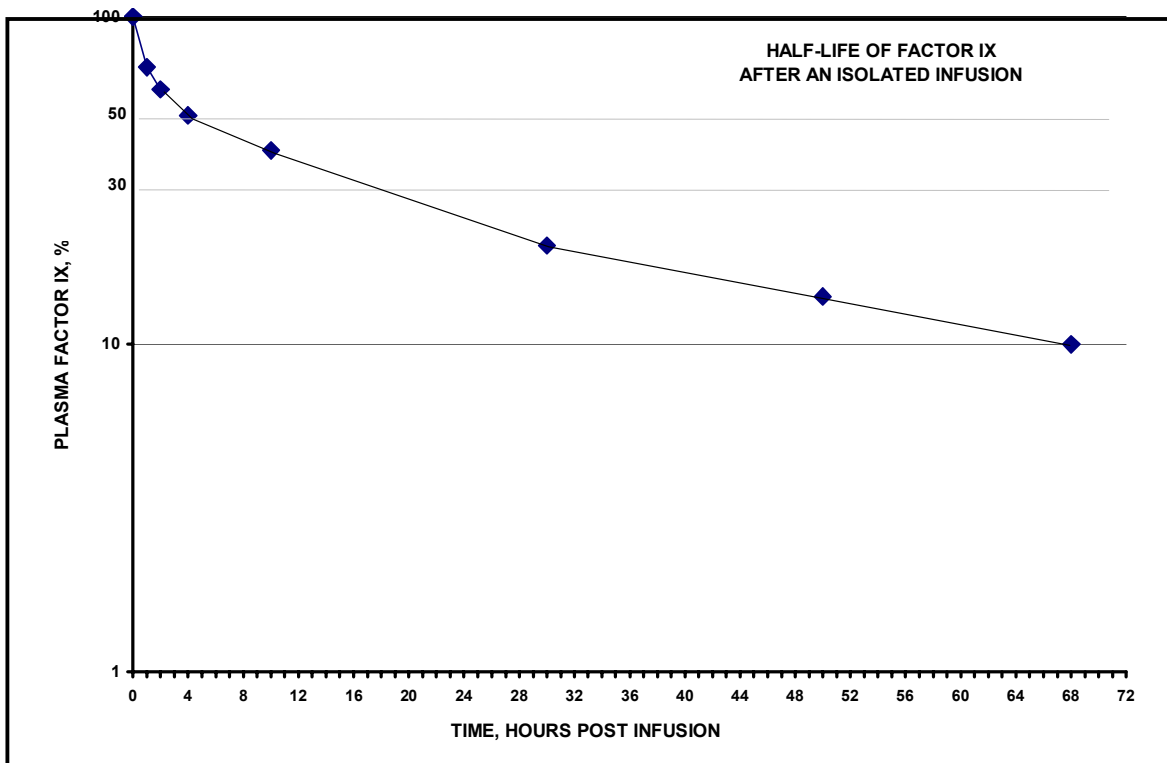
Infuse one unit of factor IX/kg for each percentage-point increase in plasma factor IX wanted.

In Vivo Recovery and Half-Life

Factor VIII and factor IX concentrates are assayed by their manufacturers against an international standard made of factor VIII or of factor IX **concentrate**, respectively. "One unit" of a clotting factor in a concentrate standard is about the same as in a plasma standard. If a given amount of factor VIII concentrate, labeled, e.g., 1500 units, is infused into a man with <1% factor VIII / ml and a 3000 ml plasma volume, we expect to "recover" 1500 units in his plasma, "*in vivo*", a few minutes after the infusion, as soon as the concentrate has mixed with the plasma in circulation. . That is, we expect that his post-infusion factor VIII peak level, e.g. 50% or 0.5 units/ml, multiplied by his plasma volume, 3000 ml, to be approximately the same, 1500, as the number of units infused, or, 100% of the infused units.

If factor IX concentrate, labeled as containing 1500 units, is infused into a man with <1% factor IX/ml and a 3000 ml plasma volume, we expect to "recover" 750 units (or a bit less) in his plasma. That is, we expect that his post-infusion factor IX peak level, e.g. 25% or 0.25 units/ml, multiplied by his plasma volume, 3000 ml, will be approximately half (750) of the infused units (1500).

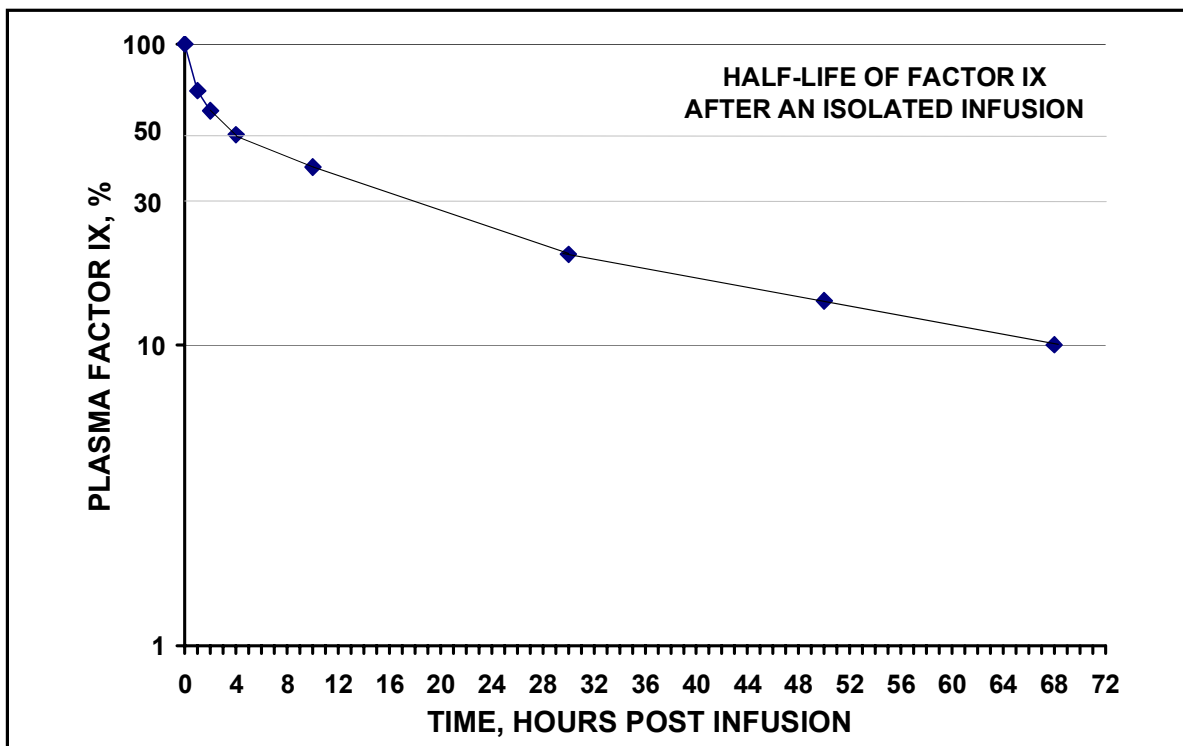
Actual *in vivo* recoveries are acceptable in the range of 80-120% of the expected recovery. Inaccuracies result from incorrect estimations of plasma volume, incomplete injection of concentrate, assay variability and so on. If a patient has *in-vivo* recoveries notably below the acceptable range on a consistent basis, he should be investigated for the presence of an inhibitor. If a given concentrate is associated with *in vivo* recoveries notably below the acceptable range on a consistent basis in most or all patients, and the patient-plasma assay system is calibrated against the international plasma standard, one should seek help from the manufacturer. Some newer recombinant concentrates are associated with lower-than-expected *in-vivo* recovery (see below). The assay system or the dosage may need adjustment.



The above graph illustrates plasma factor VIII levels after a single isolated infusion of factor VIII at 50 units/kg in a patient with severe hemophilia A.

The infused clotting factor is distributed throughout the circulation within a few minutes and then equilibrates with the extravascular space. During the equilibration phase, the plasma level of the factor drops rapidly; typically, half the infused factor VIII or IX disappears from the plasma in the first four hours.

When equilibration stabilizes, the plasma level of the factor drops according to its rate of catabolism. The second, “metabolic” or “catabolic” phase of the half-life usually is the phase defined in package inserts. The catabolic-phase half-life of factor VIII is 12 hours, or a bit less; the catabolic-phase half-life of factor IX is 24 hours or a bit more.



The above graph illustrates plasma factor IX levels after a single isolated infusion of Factor IX concentrate, 100 units/kg, in a patient with severe hemophilia B.

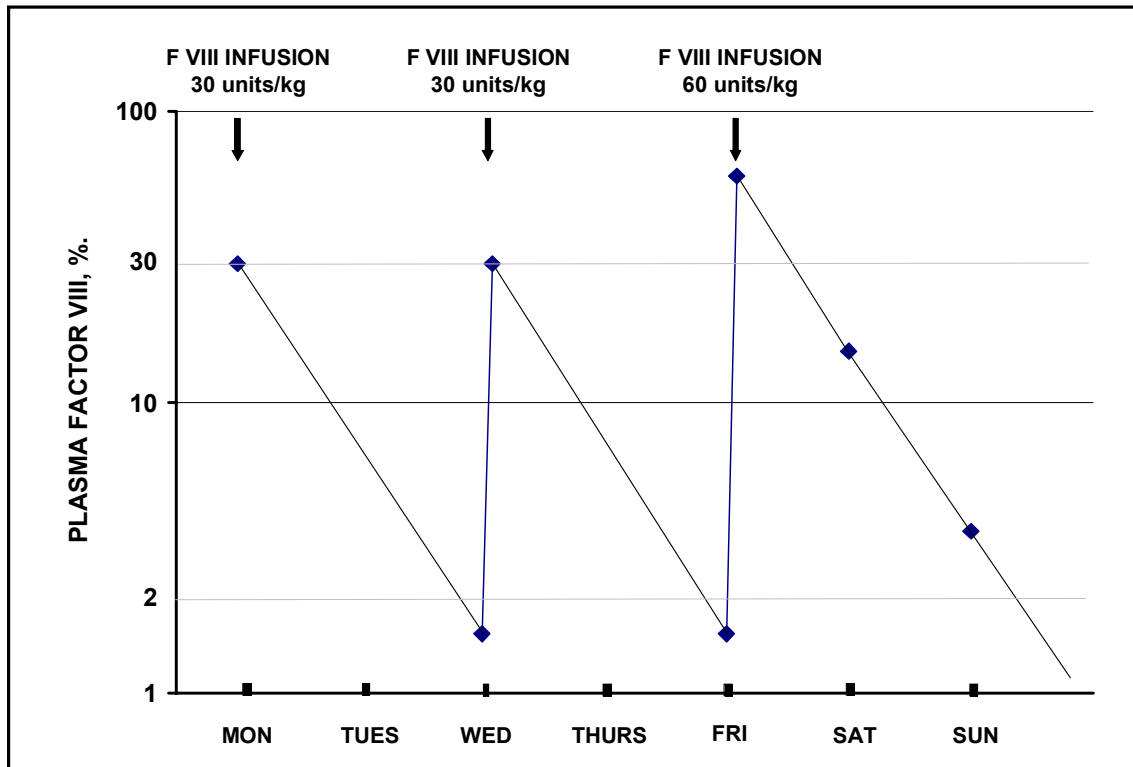
Dosage for Acute Hemorrhages.

Blood clots within a few minutes. For an acute hemorrhage, a bolus infusion of concentrate is given reasonably rapidly to achieve a peak level of the factor in the plasma. “Adequate” dosage is a topic of endless debate. A peak of factor level of 15-20% may suffice for early hemorrhages in sites of low danger, without inflammation, such as superficial soft tissue. For hemorrhages in the most dangerous areas such as the CNS or retro-pharyngeal area, levels of at least 50% and often 100% are used. For average hemorrhages into joints, levels of 30-50 % may be sought. Repeated bolus doses are always given for hemorrhages in dangerous locations and often for serious hemorrhages in chronically-inflamed joints (“target” joints), in the ileopsoas muscle, from the gastro-intestinal tract, or in other areas known for recurrent or persistent bleeding. In general, bleeding caught early in a site which has not sustained previous damage and is not inflamed is likely to be controlled with lower levels of clotting factor whereas bleeding of many hours’ duration in a damaged or inflamed area is likely to require higher doses. Affluent countries use the higher doses in the range suggested in order to achieve hemostasis with the first dose in nearly every instance. Less-affluent countries which use lower doses tolerate a slightly lower probability of hemostasis with the first dose.

Most patients with severe hemophilia, in countries with economies good enough to afford some concentrate use, have been trained to give themselves concentrates intravenously at home. Mild to moderate hemorrhages may be treated entirely at home, according to protocols designed by the supervising doctor. Home treatment usually means much earlier treatment of bleeding than if the patient had to travel to a clinic or hospital. Patients with serious hemorrhages sometimes are given one dose of concentrate at home while arranging transportation to a hospital, to stop the bleeding as early as possible.

Dosage for Prophylaxis

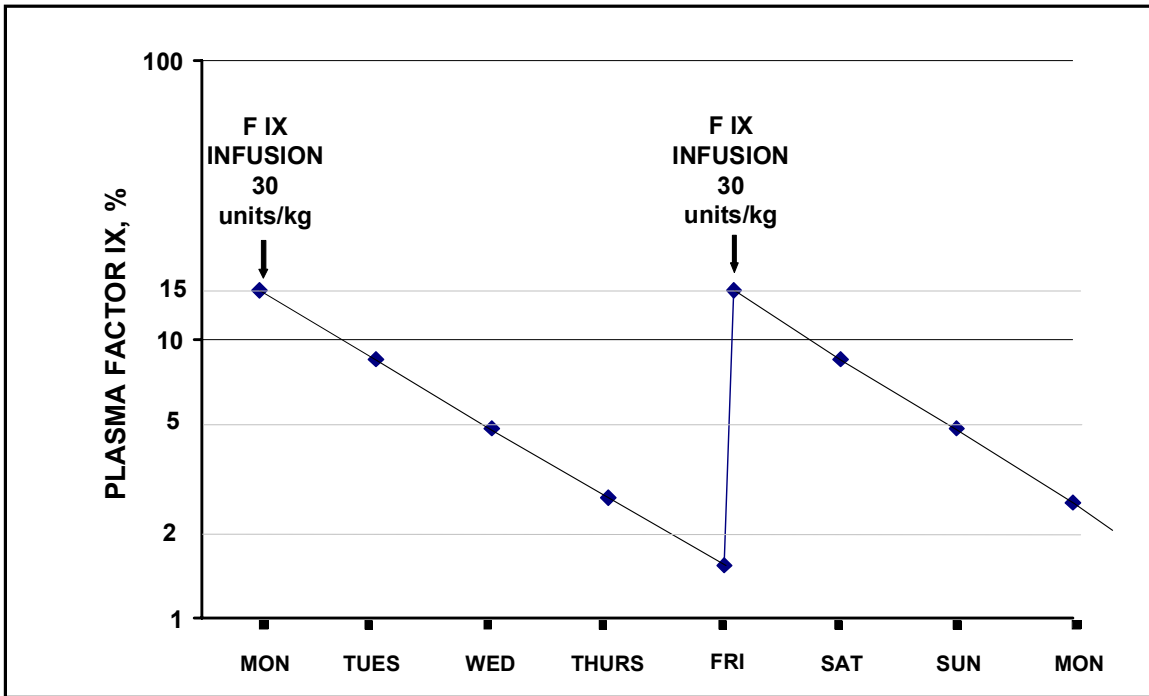
When one gives regular infusions of concentrate to maintain the patient's baseline factor level above a certain minimum, for example, above one percent, equilibration is stable and the rate of catabolism determines the frequency of needed infusions. For example, in a patient with severe hemophilia A and a catabolic half-life of infused factor VIII of 12 hours in the steady state, one might raise the plasma factor VIII level to about 30% every 48 hours to maintain a minimal level above one percent (see graph below). Thrice-weekly doses are typically used; if inadequate, a larger dose is given for the weekend or every-other-day dosing is used. Some adults with excellent veins use smaller daily doses. (Small doses given frequently are more cost-effective than large doses given at greater intervals, because the disappearance of the factor is exponential. Thus, the ideal prophylaxis for cost-effectiveness would be a continuous infusion into a venous access device using a small pump.) Catabolic rates vary somewhat from patient to patient, trough levels should be checked from time to time.



Hypothetical prophylaxis doses and factor levels, steady state, severe hemophilia A

Low-level prophylaxis sometimes is started in early childhood (e.g. age 1-2 years) in severe hemophilia, “primary” prophylaxis, in the hope of preventing joint damage completely. In an older patient, “secondary” prophylaxis may be given for a short or a long term to control recurrent bleeding and to allow rehabilitation. Slightly higher minimal factor levels may be needed to assure hemostasis in patients with weak muscles and damaged joints.

Prophylaxis for hemophilia B is relatively easy because the catabolic half-life is long. Patients may be infused twice a week to maintain factor IX levels above 1% (see graph below). Many find that once-weekly dosage suffices to prevent most bleeding.



Hypothetical prophylaxis doses and factor levels, steady state, severe hemophilia B

Dosage for Physiotherapy

A dose of concentrate, to raise the plasma factor level to 25-50% immediately before the therapy session, may be desirable at the outset. As the patient gains strength and flexibility, he may no longer need pre-therapy concentrate. Where resources are limited, or, for patients with inhibitors, physiotherapy can be given gently and gradually without concentrate.

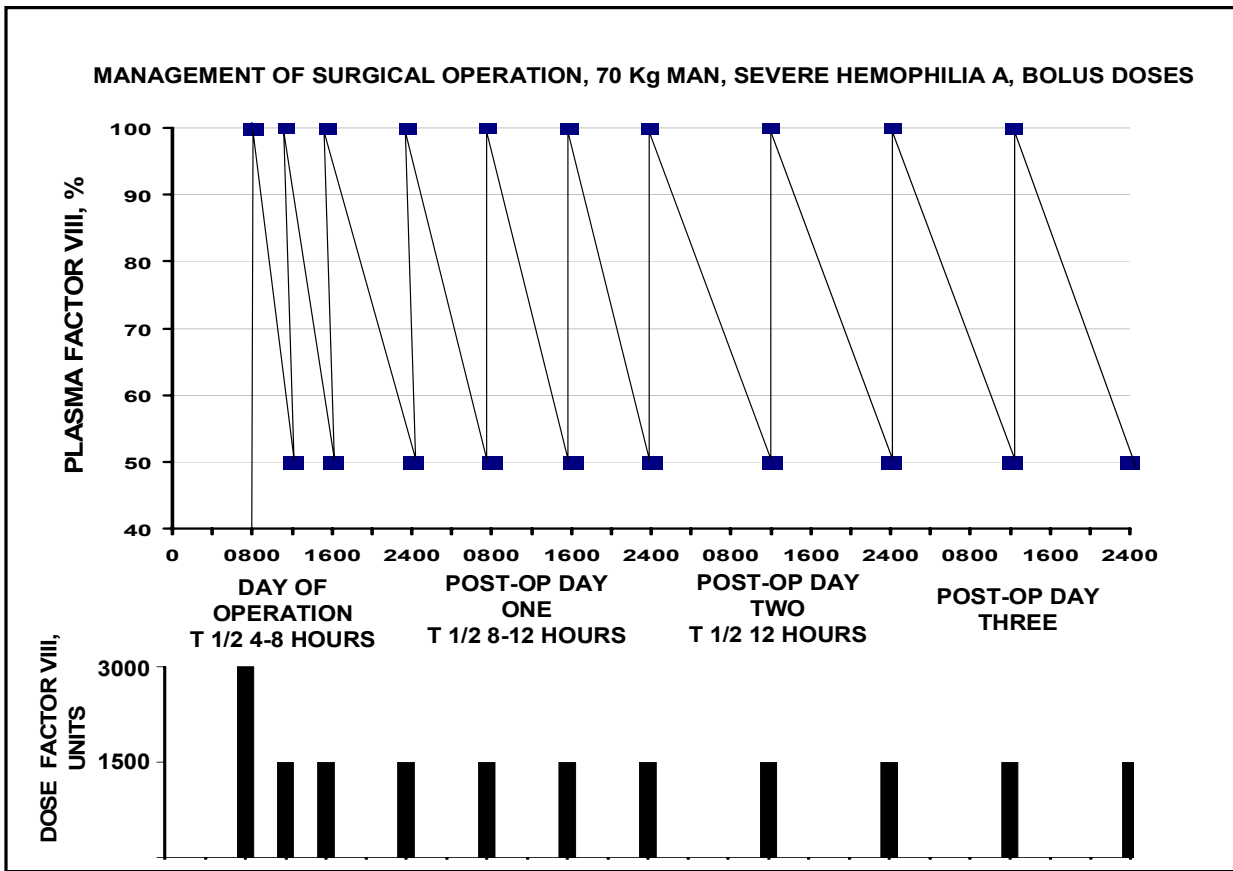
Dosage for Dental Procedures

The most dangerous aspect of dental restorations (fillings, etc.) are injections of local anesthetics into the highly-vascular angle of the jaw for "regional block" anesthesia. Occasionally, a rapidly-expanding hemorrhage results, which may dissect down the neck and press on the trachea. A pre-injection dose of concentrate, to a 30 to 50% plasma level, is advised. Some hospital-based dental chairs are equipped for light general anesthesia so that multiple restorations can be accomplished without regional block.

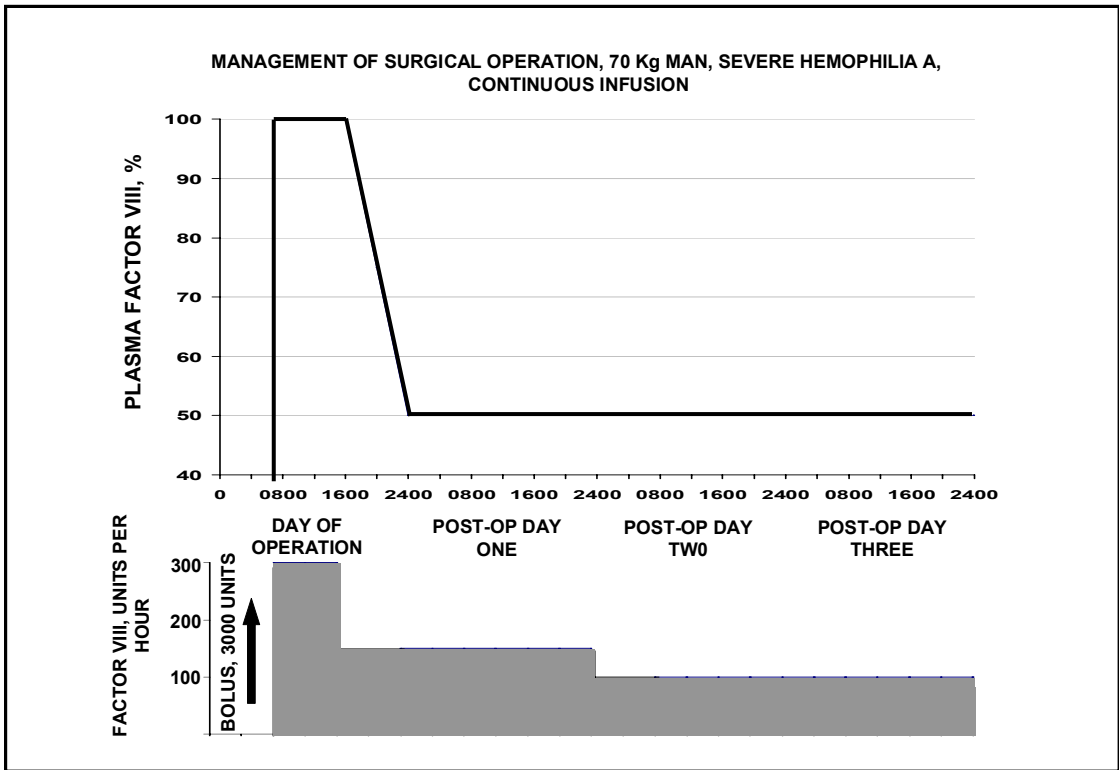
For tooth extractions, the plasma factor level is raised to 30 to 50% immediately beforehand. Anti-fibrinolytic drugs, epsilon-amino-caproic acid ("Amicar") or tranexamic acid ("Cyclokapron") are especially helpful in preventing clots in the mouth from breaking down. They are given before an extraction and for 7-10 days afterwards.

Dosage for Surgical Operations

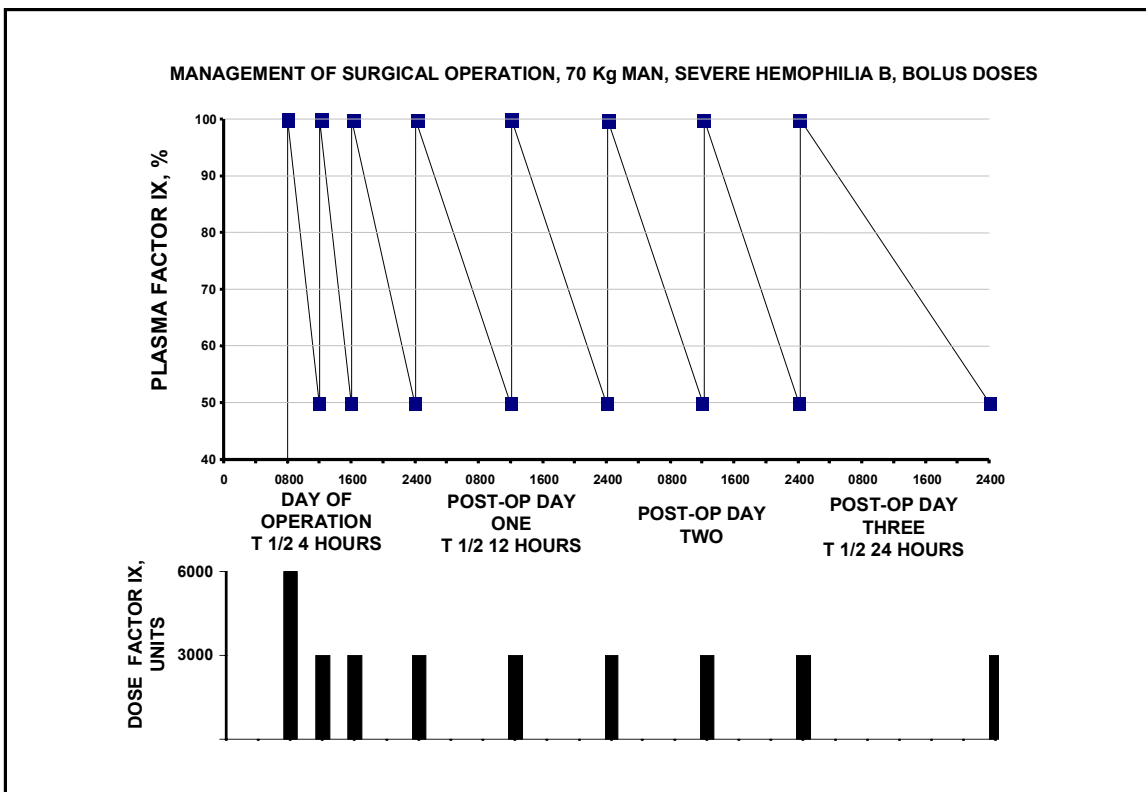
Concentrate is given immediately prior to major surgical operations and also afterwards, for at least ten days. It is continued, at lower doses, if physiotherapy is being given until that therapy is completed. Post-operative re-bleeding is most likely to occur about 5 - 7 days after an operation and is uncommon after 10 days. Nowadays, in affluent countries, the level of the deficient factor is maintained during the surgical operation at about 100% and during the post-operative period at a minimum of about 50%. I successfully used lower levels (50% for the operation, 30% minimum afterwards) in Los Angeles 30-35 years ago, when concentrate first became available. Such doses continue to be used successfully in less-affluent countries. Appropriate dosage is debated. Higher doses give physicians a feeling of security, especially if clotting factor assays are difficult to obtain. Lower doses can be used with close observation and frequent assays.



Hypothetical doses and factor levels for a surgical operation in severe hemophilia A. Note that dose intervals widen as sites of equilibration are saturated. In an actual patient, trough factor levels should be assayed. Factor VIII catabolic rates vary from patient to patient.



Hypothetical doses and factor levels for a surgical operation in severe hemophilia A using a pre-operative bolus dose followed immediately by continuous infusion of concentrate. Note that lower doses are needed as equilibration sites become saturated.



Hypothetical doses and factor levels for a surgical operation in severe hemophilia B using bolus doses. Note that dose intervals widen as sites of equilibration are saturated. In an actual patient, trough factor levels should be assayed. Factor IX catabolic rates vary from patient to patient.

Concentrate can be given by intermittent bolus doses or by continuous infusion after the first bolus dose. With bolus doses, trough factor levels are assayed from time to time to adjust dosage. With continuous infusion, blood for factor level assays can be drawn at the same time as blood is drawn for other laboratory tests. A larger amount of concentrate is needed to maintain minimal plasma factor levels early in the post-operative course than later, after the clotting factor saturates all its sites of distribution. Necessary dosage is adjusted as indicated by factor assays. Patients with heavy surgical blood loss, which has not been replaced entirely, may become anemic. Infused concentrate is diluted in a greater plasma volume, thus, the dose of concentrate may need adjustment upwards.

EVOLUTION OF CONCENTRATES

Testing Donations

Transmission of hepatitis through plasma products was recognized when plasma treatment became common in the 1950's but it could not be studied until causative agents were identified and blood tests for markers of infection in donors were developed. In the 1960's and 1970's, for patients **rarely** requiring factor VIII replacement, cryoprecipitate appeared safer than concentrate because its use exposed the patient to the possibility that one of only a few donors had a viral infection. In contrast, concentrate was made from the pooled plasma of large numbers of donors and thus could spread an infection from one highly-viremic donor to many recipients. Patients with severe hemophilia A who were treated **frequently** with cryoprecipitate (as in Seattle) were exposed to a large number of different donors and had the same near-universal serologic evidence of past hepatitis B as did patients treated with concentrate (as in Los Angeles) when compared in the mid-1970's.

Before 1972, the only serologic test available for donors was that for syphilis. In 1972, the first serologic test for hepatitis B surface antigen was introduced for blood donations. Other specific serologic

tests introduced were HIV antibody in 1985, HTLV-1 in 1989, and hepatitis C antibody in 1990. The latter was introduced for whole blood before its use was permitted to test plasma obtained by plasmapheresis, because a government official believed that the antibody might be protective (like hepatitis B surface antibody) rather than, predominantly, a marker for infection (like HIV antibody). The window period is the time interval between infection and the appearance of a positive serologic test.

USA Blood Donor Serologic Screening Tests

Infection/test	Date begun	Window period	Comment
Syphilis	1940's		Spirochetes do not survive freezing or long refrigeration. Test is a "life-style marker".
Hepatitis B surface antigen	1972	56 days	Indicates acute or chronic infection
HIV antibody	March 1985	22 days	Current or previous infection
ALT (alanine amino transferase)	1987		Non-specific marker for hepatitis
HTLV 1	1989	51 days	Current or previous infection
Hepatitis C antibody in whole blood donations	May 1990	82 days	Current or previous infection
Hepatitis C antibody in plasmapheresis donations	March 1992	82 days	Current or previous infection
HIV 1 & 2 antibody	1992	22 days	Current or previous infection
HIV p24 Antigen	1996	16 days	Current or previous infection
HTLV 1 & 2	1998	51 days	Current or previous infection

Some of these tests now are being supplemented by direct tests for viral RNA, called "NAT" for nucleic acid testing, by procedures similar to PCR amplification. AFTER serologic testing, small pools of plasma intended for concentrate manufacture in the USA is tested for HBV, HCV, HIV and often also for HAV and B-19 parvovirus. Direct viral tests do NOT substitute for serologic ones. The utility of the ALT test and of the HIV p24 antigen test, now that more specific tests are available, are being questioned seriously. As tests for HIV by NAT are licensed, manufacturers gain FDA permission to drop the p24 antigen test.

Viral Inactivation

By the late 1970's, work had begun to try to kill hepatitis viruses in plasma products with **heat**. Clotting factors deteriorate in heat, so stabilizers were added to (partially) protect the clotting factors. The first such product was made in the late 1970's in Germany by heating concentrate at 60° C for 10 hours, while still in solution, a process called "pasteurization" (which turned out to be effective against HIV, HBV and HCV). The pasteurization process sometimes is indicated in a product name by the suffix "-P" or by "HS" meaning "heated in solution". The first heated product made in the USA, licensed in early 1983, was baked after it was lyophilized in the sealed final vial, a process commonly called "dry heat". The initial method employed (60° C for 72 hours) turned out to be effective against HIV (although that virus had not been identified at the time) but not against the hepatitis viruses for which it was developed. The dry-heat process sometimes is reflected in product names with the term "HT" for heat-treated. With further improvements in stabilizing clotting factors in the 1990's, dry-heating at much higher temperatures (80° C for 72 hours to 100° C for 30 minutes) became feasible and proved adequate to kill hepatitis viruses. Another heat process, used by an Austrian company, in which lyophilized concentrate blown into hot steam vapor under increased barometric pressure, called vapor-heated or "VH", also was effective against hepatitis viruses as well as HIV.

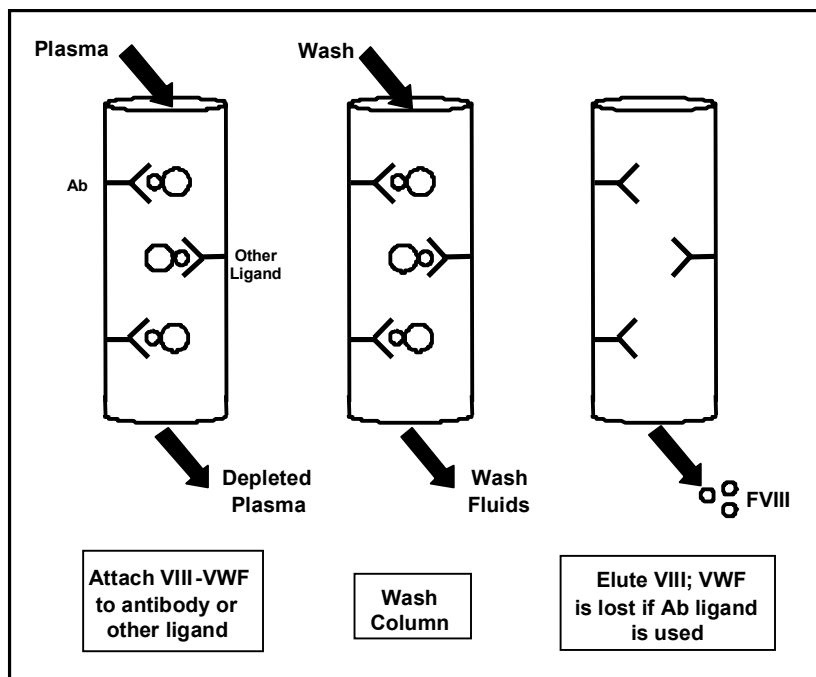
Another highly-effective method of killing viruses was introduced in the mid-1980s. Plasma or partially-fractionated liquid products could be treated with non-toxic **solvent-detergent** combinations, such as tri-n-butyl-phosphate and polysorbate 80, which dissolved the lipid envelopes of HIV, HBV and HCV very

effectively, with minimal damage to clotting factors. Non-enveloped viruses including HAV were not killed. The suffix “SD” is sometimes added to product names.

Some concentrates now are treated with two methods of viral-inactivation. When factor VIII is so treated, solvent-detergent treatment usually is combined with dry-heating. For factor IX-containing concentrates, the second method usually is **nanofiltration** to allow the very small factor IX molecule to be separated from larger viruses.

Methods of Plasma Fractionation

Initially, the major methods of extracting clotting factors from plasma were precipitation (as with cold, or glycine, or polyethylene glycol) for factor VIII, and absorption (as on tri-calcium phosphate or DEAE Sephadex) for prothrombin complex. Gel chromatography and ion exchange chromatography also were used. Affinity chromatography was introduced in the late 1980’s and permitted much greater separation of the desired clotting factor from other plasma proteins. As an attractant-ligand, a monoclonal antibody to factor VIII/VWF (or factor IX) can be used. The prefix “mono” or suffix “M” were used to designate products produced by immuno-affinity chromatography with monoclonal antibodies. Later, another fractionator used a heparin-ligand attractant, rather than an antibody, to separate FVIII-VWF in affinity chromatography.



Schematic representation of affinity column chromatography using an antibody or another ligand to capture a clotting factor, in this example, FVIII-VWF, from plasma or an intermediate fraction. After capture, the column is washed to remove as much of the unwanted plasma components as possible. The wanted factor is then eluted. When antibody ligands are used, VWF is lost; the resulting FVIII concentrate contains negligible amounts of VWF. Major brands include “Monoclote P” and “Hemofil M”.) When heparin agarose ligand is used, VWF is retained (“Alphanate”).

In the late 1980’s, a few studies suggested that some substance in less-highly-purified FVIII concentrates was suppressive to the immune system, but other studies contradicted that assertion. It now appears likely that transforming-growth-factor-beta may have been present in disproportionately high levels in some less-highly-purified concentrates, no longer marketed today. The original evidence, presented at a time when the death-rate from AIDS of persons with hemophilia was very high, led to widespread adoption of highly-purified concentrates, especially for patients with HIV infection.

The term “specific activity” (SA) describes the degree of purification of clotting factors from plasma. The specific activity of a clotting factor is the amount of that factor (in units) per mg of total protein. Cryoprecipitate has a specific activity of about one. Early factor VIII concentrates had specific activities of two or three. As factor VIII became more highly separated from other plasma proteins, it had to be stabilized, usually by adding human plasma albumin. The added albumin increased the total protein. Traditional calculations of specific activity no longer reflected the level of separation from miscellaneous proteins. Nowadays, we may subtract added albumin from the total protein before calculating specific activity when comparing purification levels of different concentrates.

Factor VIII concentrates that retain VWF, a heavy molecule, may appear to have low levels of purification when specific activity is calculated. Some scientists suggest that the weight of VWF, as well as that of added albumin, should be subtracted from total protein before calculating specific activity. For example, VWF is not retained with immuno-affinity (“monoclonal”) chromatography but is retained with heparin-ligand chromatography. If the weight of the VWF molecule were subtracted from total protein, then concentrate made with heparin-ligand-affinity-chromatography would have a specific activity similar to that of immuno-affinity purified concentrate.

There is NO official terminology to denote low, intermediate or high purification levels, indeed, the International Society on Thrombosis and Hemostasis, which regulates nomenclature, has deliberately avoided any such designations because of disagreement about the desirability of subtracting remaining VWF or added albumin from total protein before calculating specific activity. Among many patients and doctors, a strong demand remains for concentrate of “high-purity”, despite the difficulty of that definition and disputes about its importance.

Recombinant Concentrates

To make a recombinant concentrate, hamster cells, from a stable cell line that replicates well, are transfected with normal human genes for the desired clotting factor. More than one gene can be transfected into each cell. Other genes that stimulate production or help stabilize the clotting factor may be co-transfected. The gene can be modified to be expressed more easily or have other desirable characteristics. In one instance, the large factor VIII gene was shortened, by taking out the un-necessary B-domain, before being transfected into the cell. A shortened factor VIII molecule is made (ReFacto®).

Stable transfected cell lines are stored deeply-frozen. For production, the cells are allowed to propagate in culture medium in stainless-steel vats under carefully-controlled conditions. The clotting factor is released from the cells into the medium, which, from time to time, is harvested and purified using immuno-affinity chromatography and other methods. (A given recombinant concentrate may be marketed under two trade names, that of its actual manufacturer, and that of the patent-holder for the immuno-affinity process, which is ZLB-Behring. For example, Kogenate® is manufactured and sold by Bayer; the same product, made by Bayer and labeled Helixate®, is sold by ZLB-Behring.) “First-generation” recombinant factor VIII was made with several animal and human proteins in the culture medium. Albumin derived from human plasma was used as a stabilizer. Animal- or, especially, human-derived protein use in stabilizing recombinant concentrates has been diminished or eliminated in more recent products. Stabilization in the final vial is provided by sugars for some products.

The major advantage of recombinant concentrate over plasma-derived concentrate is the decreased use or absence of human-derived material, thus, theoretically, increased safety from human blood-borne diseases, known and not yet known. Another advantage is the potential to modify the transfected gene in order to make clotting-factor “designer-molecules”, which may convey advantages.

Disadvantages of some recombinant concentrates have been: (1) lesser stability of factor VIII in sugar instead of albumin and (2) difficulty in reconciling assays of concentrates performed by the manufacturer (*in vitro*) with factor levels observed after infusion of the product in the patient (*in vivo* recovery). The latter problem is seen with B-domain-deleted recombinant factor VIII as an artifact of the use of the “chromogenic” assay by the manufacturer and the “one-stage” assay by most clinical laboratories. The problem is seen with

recombinant factor IX because there are inherent differences between factor IX as produced naturally in the human body and recombinant factor IX produced in hamster cells: as follows: (1) nuclear DNA determines the sequence of amino acids assembled in the cytoplasm (“translation”) to make a protein, (2) the protein is modified in the cytoplasm (“post-translational modification”), e.g. glycolylation (the addition of sugar moieties) by mechanisms dependent on the host, or hamster, cell mechanisms. Cytoplasmic mechanisms are not the same in hamsters as in humans. Post-translational modification in hamster cytoplasm results in less glycosylation, and other modification, than in human cytoplasm. The recombinant molecule behaves differently in the manufacturer’s *in vitro* assays than it does in the human body as measured by *in vivo* recovery.

Safety of Concentrates Nowadays

HIV has not been transmitted by a US-manufactured concentrate since early 1987. (No HIV transmission occurred at any time in the USA with any concentrate that was made from HIV-antibody tested plasma and that also was viral-inactivated.) In the USA, the CDC has tracked HIV serology in persons with hemophilia since the test became available and, in recent years, also has tracked hepatitis serology and thoroughly investigated suspected sero-conversions. Transmission of HBV and HCV from concentrate has disappeared. Hepatitis A, a rare contaminant of blood, can be transmitted by concentrates treated with solvent-detergent only or with the less-strenuous heat-treatments, but has not been documented in concentrate recipients in the USA in recent years. B-19 parvovirus is capable of surviving all current viral-inactivation methods.

CHOICE AMONG CONCENTRATES

Products for hemophilia A include recombinant factor VIII concentrates (Kogenate FS®, Helixate FS®, Recombinate rAHF®, Advate rAHF PFM® and ReFacto®) and viral-inactivated plasma-derived concentrates (in the USA, Alphanate®, Hemofil M AHF®, Humate P®, Koate DVI®, Monarc M® and Monoclate P® as of 2005) and, in some developing countries, cryoprecipitate. In affluent countries, most physicians and consumer groups advocate the use of recombinant products, especially for children and newly-diagnosed patients. Many adults remain on plasma-derived concentrates, either from personal choice, or because of shortages of recombinant products. Patients with mild hemophilia A also may use desmopressin (DDAVP, Stimate®) which releases factor VIII and VWF from storage sites. Details are provided in a monograph on VWD.

Products for patients with inhibitors to factor VIII include human factor VIII, PCC (in the USA, Bebulin VH®, Profilnine SD®, Proplex-T®), activated PCC (FEIBA VH®) and recombinant activated factor VII (NovoSeven®, known in Canada as Niasase®). Strategies for choosing among these products are described in a monograph on inhibitor management. Plasma-derived porcine factor VIII is no longer on the market but a recombinant porcine molecule is in clinical trials.

Products for hemophilia B include prothrombin complex concentrates and factor IX concentrate, which is available as a recombinant concentrate (BeneFIX®) and as viral-inactivated highly-purified plasma-derived concentrates (in the USA, Alphanine SD® and Mononine®). Plasma is used in some developing countries. Factor IX concentrate is advocated over PCC for patients at risk of thrombosis, and, in affluent countries, is rapidly replacing PCC for routine management of hemophilia B. The recombinant product is widely advocated for children and newly-diagnosed patients.

Products for patients with inhibitors to factor IX include factor IX, PCC, activated PCC and recombinant activated factor VII. The latter (NovoSeven®) is the only appropriate product for those patients whose inhibitor reacts with infused factor IX in such a way as to cause severe allergic reactions.

Products for Von Willebrand Disease (VWD) include factor VIII concentrates which also contain Von Willebrand Factor; all such concentrates now available are plasma-derived. In the USA, only Humate-P®

is licensed for use in VWD. Other products containing VWF (Alphanate®, Koate DVI® have been used off-label for VWD. A concentrate of VWF with little FVIII has become available in France. Cryoprecipitate is used in developing countries.

Patients with factor V deficiency are treated with plasma. Pooled, solvent-detergent treated plasma is available in Europe but is no longer available in the USA. No factor V concentrate exists.

Patients with factor VII or X deficiency may be treated with PCC in the USA. Some patients with factor VII deficiency are treated with recombinant activated factor VII concentrate “off-label”. Concentrates of factor VII are made in France and in Austria for domestic and export use, but are not licensed in the USA.

Patients with factor XI deficiency may or may not have an increased tendency to hemorrhage. Those with bleeding problems may be treated with plasma, with or without exchange plasmapheresis. Given the long half-life of factor XI, 48-72 hours, patients also may be prepared for surgery by receiving twice-daily infusions of the tolerated volume of whole plasma until the factor XI level climbs into the low-normal range, that is, for two or three days. Concentrates of factor XI are made in England and in France but are not available in the USA. For surgery, an initial bolus dose is given to bring the plasma level to 80% and half that dose is given every two to three days afterwards.

Patients with severe factor XIII deficiency have been managed successfully with prophylactic plasma infusions given monthly, for the half-life of factor XIII is six days. A factor XIII concentrate is made in France but is not licensed in the USA.

COST OF CARE

The cost of hemophilia care astounds the uninitiated. In 1995, an audit was conducted in California showing an average yearly cost of medical care of \$140,000 for persons with hemophilia. Nearly all of that cost was for concentrate. The subgroup of patients with inhibitors cost four times as much as the subgroup without inhibitors. Since 1995, the figure has risen with the introduction of more costly new or improved products and with the acceptance of prophylaxis as standard care for children with severe hemophilia. Given those figures, it is not surprising that the cost is a major issue in choice of concentrates where financial resources are limited.

The following were the July 2005 maximum rates, in U.S. dollars, that the State of California would reimburse pharmacies for concentrate dispensed for patients on State-supported medical programs. When slightly different reimbursement rates were quoted for vials of different sizes, a mean or median is given.

BRAND (Factor VIII)	US dollars / unit	BRAND (Factor IX, etc)	US dollars / unit
<i>Plasma-Derived:</i>		<i>Plasma-Derived prothrombin complex:</i>	
Alphanate	0.66	Bebulin VH	0.73
Hemofil M	0.64	Profilnine SD (1000+ unit vial)	0.41
Humate-P	0.83	Proplex-T	0.48
Koate DVI	0.71	<i>Plasma-Derived factor IX (only):</i>	
Monarc-M	0.48	Alphanine SD	0.79
Monoclote P	0.59	Mononine	0.87
<i>Recombinant:</i>		<i>Recombinant factor IX:</i>	
Advate	1.12	BeneFIX	0.96
Helixate FS	0.97	<i>Activated prothrombin complex:</i>	
Kogenate FS	1.10	FEIBA VH	1.31
Recombinate	1.03	<i>Activated recombinant factor VII:</i>	
ReFacto	1.01	NovoSeven	1.00 per microgram