

Recombinant protein : Factor VIII

A background image showing a laboratory setting. On the left, a glass pipette is shown with a small drop of liquid hanging from its tip. On the right, there is a blurred image of a petri dish or a similar laboratory container. The overall scene is brightly lit, suggesting a clean and professional laboratory environment.

Recombinant proteins

1. **Human Growth Hormone** : somatotropin or somatropin, is a peptide hormone that stimulates growth, cell reproduction and regeneration.
2. **Insulin** : Insulin is a hormone made by the pancreas that allows your body to use sugar (glucose) from carbohydrates in the food.
3. **Follide Stimulating Hormone** : a hormone secreted by the anterior pituitary gland which promotes the formation of ova or sperm.
4. **Erythropoietin** : a hormone secreted by the kidneys that increases the rate of production of red blood cells in response to falling levels of oxygen in the tissues.
5. **Tissue Plasminogen Activator** : Tissue plasminogen activator is a protein involved in the breakdown of blood clots.
6. **Factor VIII** : Essential blood clotting protein

FVIII

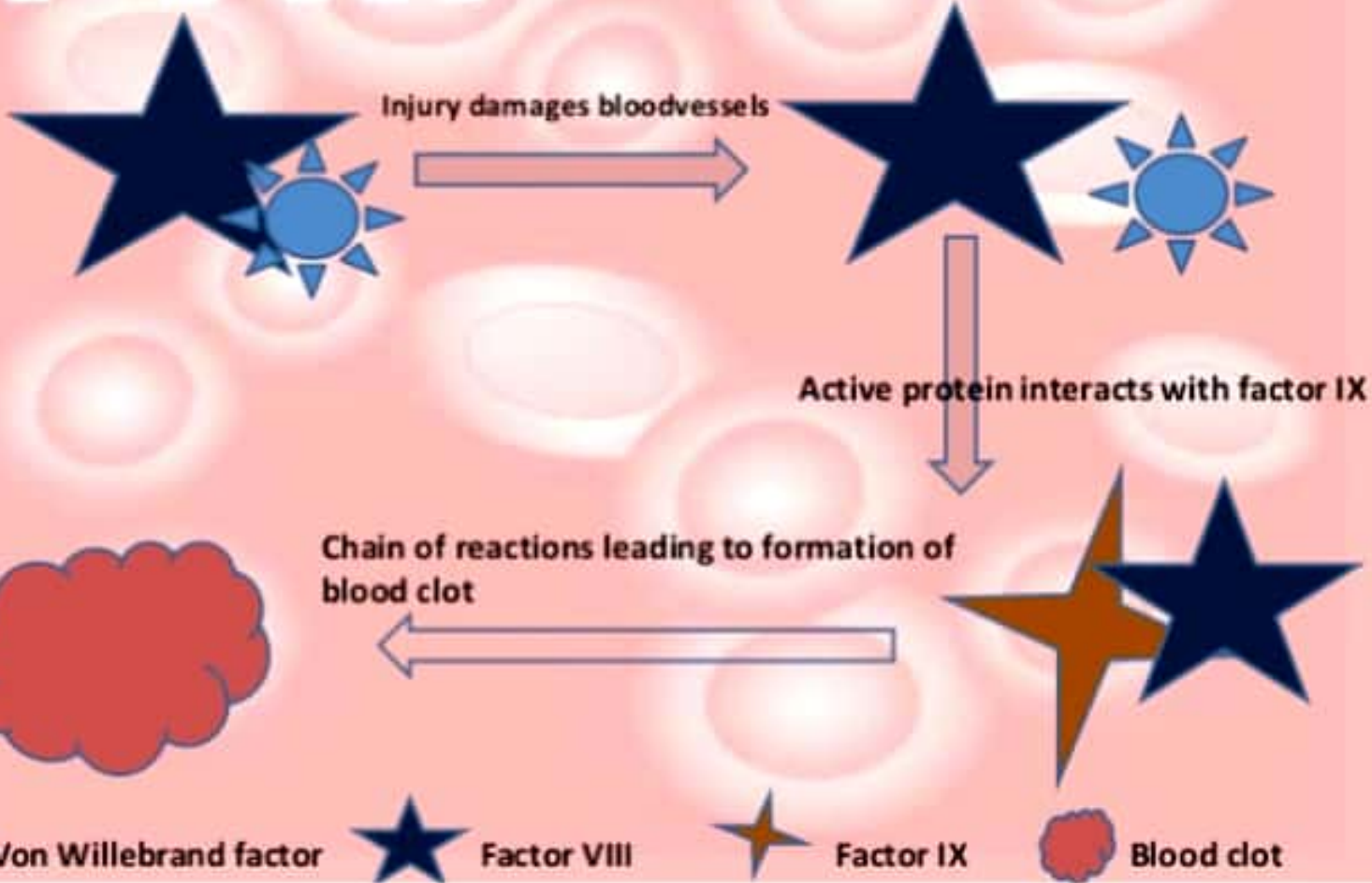
It is an essential blood-clotting protein, also known as anti-hemophilic factor (AHF). In humans, factor VIII is encoded by the F8 gene

This protein circulates in the bloodstream in an inactive form, bound to another molecule called von Willebrand factor, until an injury that damages blood vessels occurs.

In response to injury, coagulation factor VIII is activated and separates from von Willebrand factor.

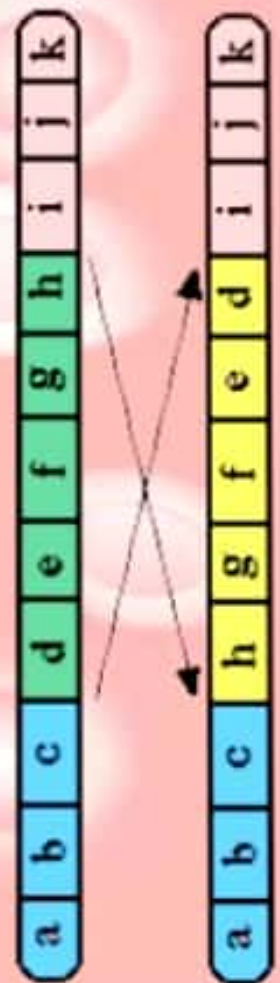
The active protein (sometimes written as coagulation factor VIIIa) interacts with another coagulation factor called factor IX. This interaction sets off a chain of additional chemical reactions that form a blood clot.

FVIII



FVIII

- The *F8* gene provides instructions for making a protein called coagulation factor VIII.
- Mutations in the *F8* gene lead to the production of an abnormal version of coagulation factor VIII or reduce the amount of this protein.
- The altered or missing protein cannot participate effectively in the blood clotting process. As a result, blood clots cannot form properly in response to injury.
- Some mutations, such as the large inversion described above, almost completely eliminate the activity of coagulation factor VIII and result in severe hemophilia.
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- Other mutations reduce but do not eliminate the protein's activity, resulting in mild or moderate hemophilia.



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Introduction

- Since the early 1990s, recombinant human clotting factor VIII (rhFVIII) produced in hamster cells has been available for haemophilia A treatment.
- All these proteins have been produced in either Chinese hamster ovary (CHO) or baby hamster kidney cells (BHK).
- (While these products have been able to alleviate concerns about supply shortages and show good pathogen safety profiles, they display a non-human pattern of post-translational modifications (PTMs).
- However, the post-translational modifications of these proteins are not identical to those of native human FVIII, which may lead to immunogenic reactions and the development of inhibitors against rhFVIII.
- For the first time, rhFVIII produced in a human host cell line is available.

•FVIII is subjected to multiple PTMs, especially glycosylations, and is considered the largest and most complex marketed protein produced by recombinant DNA technology to date.

•Incorrect reproduction of these PTMs in a nonhuman expression system may trigger immune reactions and lead to the formation of inhibitors against FVIII, which may render FVIII replacement therapy ineffective

•Inhibitor development occurs throughout life in haemophilia patients and causes considerable distress to the patient and equally considerable costs to healthcare systems.

•It is one of the main concerns regarding FVIII therapy,



The human embryonic kidney cell line HEK 293 has been used. Successful attempts to produce active rhFVIII in HEK 293-based cell lines in laboratory scale have recently been reported.

in 2003, HEK 293 cells were finally adapted to grow in suspension culture and in the absence of serum by Invitrogen, and this cell line was called HEK 293 F.

Octapharma chose HEK 293 F as a host cell line and subsequently developed all the necessary methods for industrial recombinant protein production in these cells, based on extensive previous experience with the parental HEK 293 cells as well as with CHO and BHK hamster cell lines.

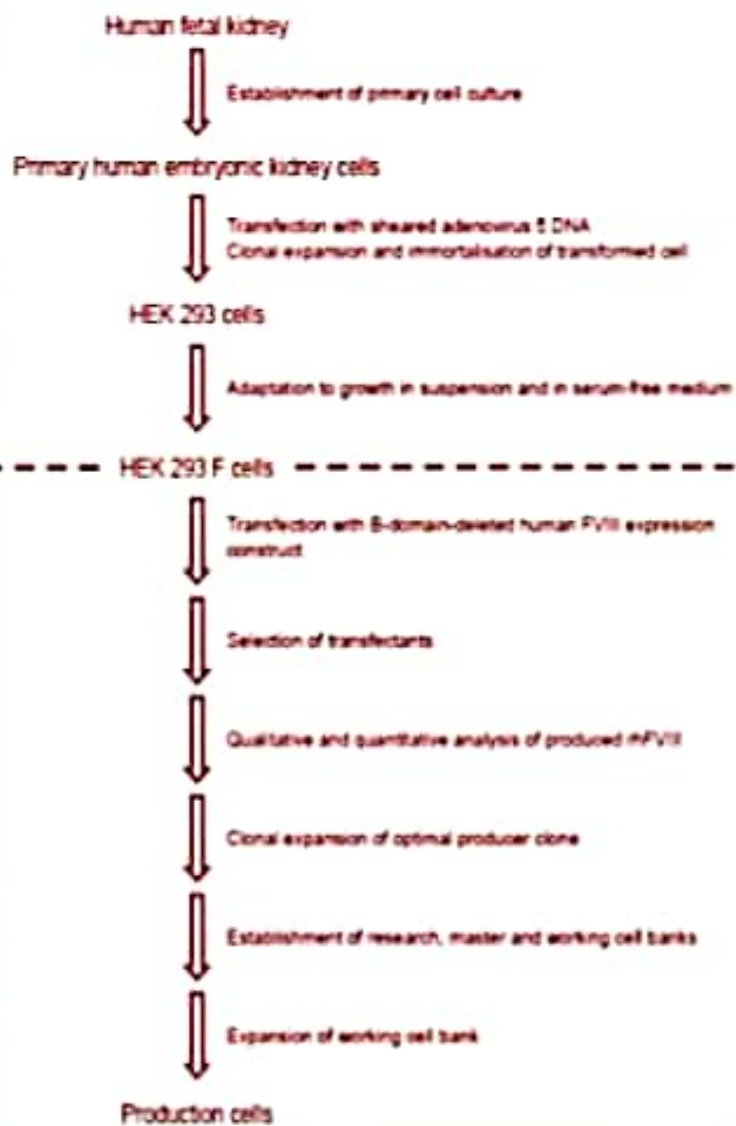


HEK293 cells

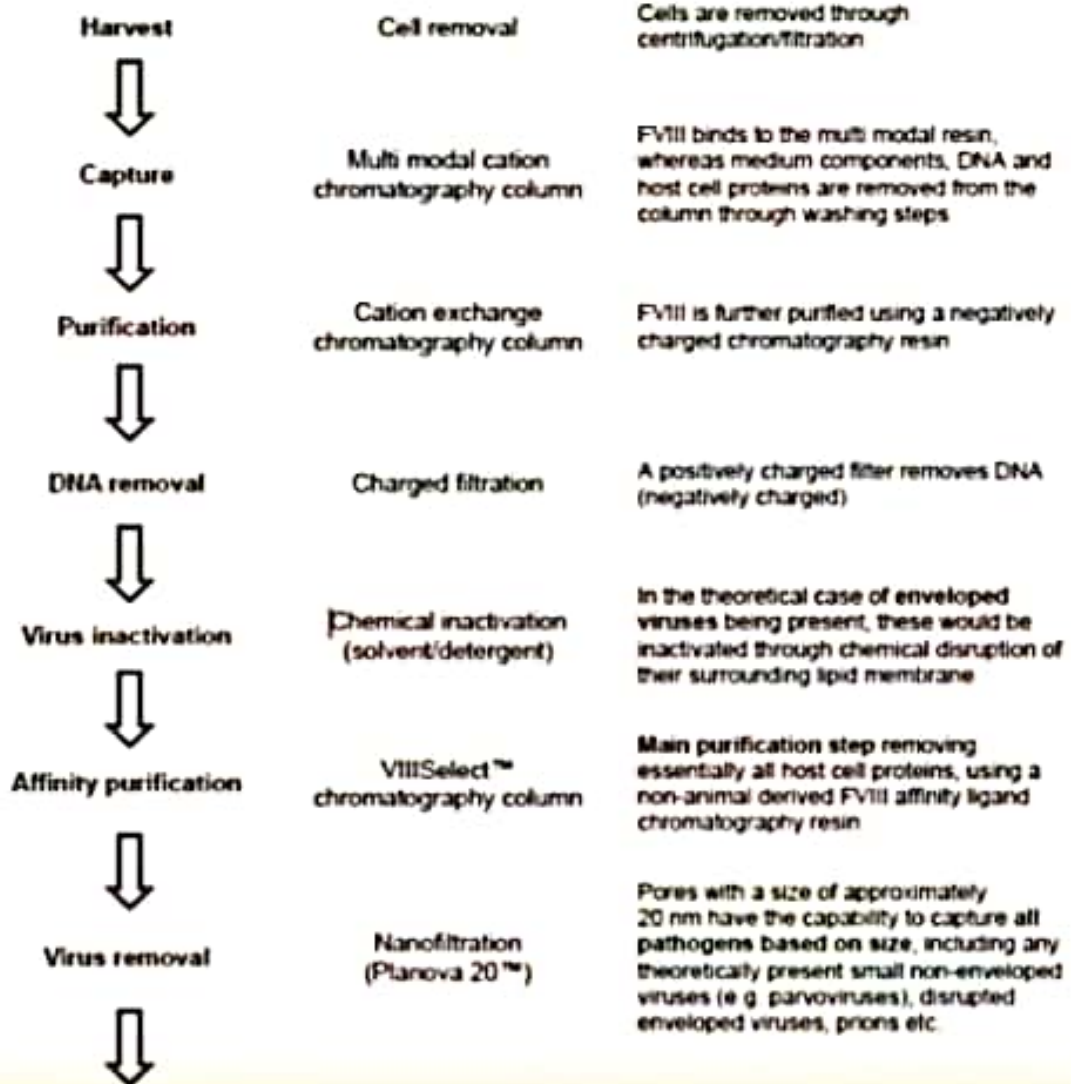
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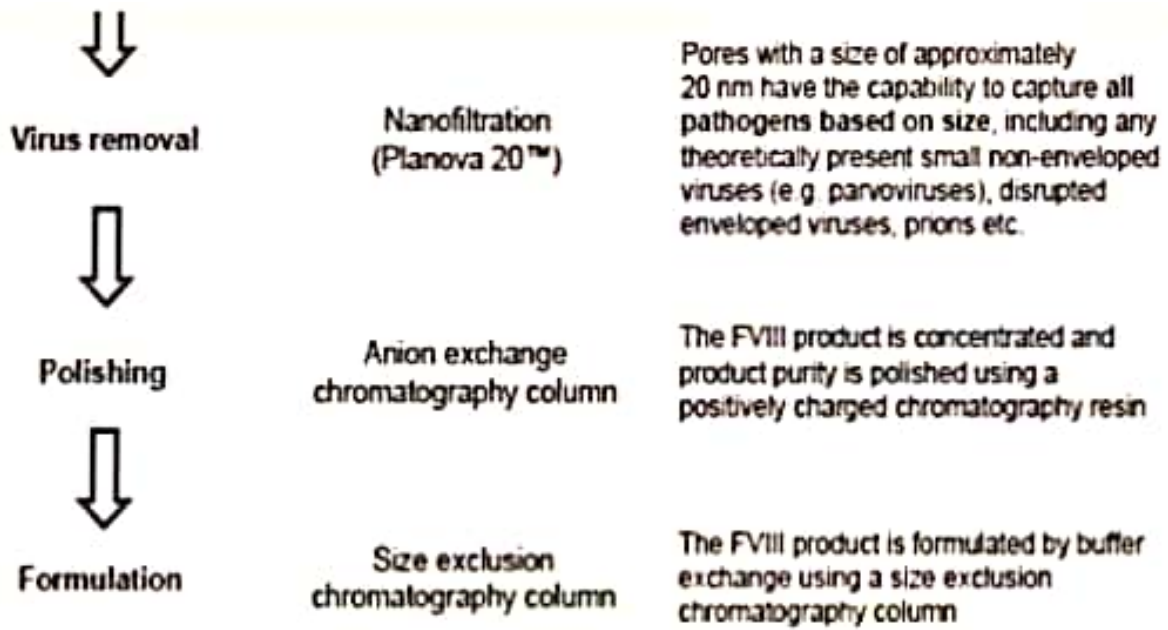
Methodology



Purification Process



Purification Process



Clones with superior growth properties, stability, productivity levels and quality of the secreted FVIII were expanded and cryopreserved as a research cell bank (RCB).

This bank was used to generate a master cell bank (MCB), from which the working cell bank (WCB) was produced.

Table 1. Currently available factor VIII concentrate products.

Product	Manufacturer	Prepared	Purified	Viral inactivation methods
Alphanate	Alpha Therapeutic	Pooled human venous plasma	Heparine agarose chromatography	Solvent, detergent and heat treatment
Hemofil-M	Baxter	Pooled human venous plasma	Immunoaffinity chromatography using murine monoclonal antibody	Solvent and detergent.
Monarc-M	American Red Cross	Pooled human venous plasma	Immunoaffinity chromatography using murine monoclonal antibody	Solvent and detergent.
Monoclote-P	Aventis	Pooled human venous plasma	Affinity chromatography using murine monoclonal antibody	Pasteurization.
Humate-P [®]	Aventis	Pooled human venous plasma	-	Pasteurization
Korate-DVI		Pooled human venous plasma	-	Solvent and detergent
Hyate c	Speywood	Porcine plasma	Polyelectrolyte fractionation	-
Helixate	Aventis, Behring	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody immunoaffinity chromatography	-
Kogenate FS	Bayer	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody, immunoaffinity chromatography	-
Helixate FS	Aventis, Behring	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody immunoaffinity chromatography	Solvent and detergent
Biociate	Aventis, Behring	Recombinant, Chinese hamster ovary cells	Column chromatography and monoclonal antibody immunoaffinity chromatography	-
Recombinate	Hyland	Recombinant, Chinese hamster ovary cells	Column chromatography and monoclonal antibody immunoaffinity chromatography	-