

Chapter 7: Fractionated Blood Products and Associated Pathogen Safety

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BACKGROUND

Originally all plasma fractionation products were derived from pooled human plasma. Increasingly, many plasma proteins are manufactured by biotechnology as recombinant proteins, without need for donated plasma; depending on the plasma protein product, either a recombinant or fractionated product, or both are available in Canada.

This chapter presents in general terms the various methods and principles by which plasma protein products are manufactured for use in patients. It is complemented by chapters 3, 4, 5 and 6 of this [Guide](#).

RECOMBINANT PLASMA PROTEIN PRODUCTS

Recombinant plasma protein products are made by culturing mammalian cells which are transfected with nucleic acid vectors carrying the gene of interest. The transfected cells make the plasma protein and secrete it into the culture medium. The culture medium is harvested, and specified proteins are extracted, purified and formulated for therapeutic use.¹

The evolution of manufacturing processes, based on degree of elimination of plasma and albumin from production and product formulation steps, has achieved incremental reduction in risk of viral contamination. "First generation" recombinant products incorporate a small amount of residual human plasma protein, usually albumin, used during the cell culture process and final formulation steps. "Second generation" recombinant products eliminate use of plasma proteins from the final formulation steps, while "third generation" recombinant products (e.g. Advate (rFVIII), or Niasase RT® (rFVIIa), or Nuwiq® (B-domain deleted rFVIII)), are manufactured without use of human or animal proteins in either cell culture or final product formulation.^{2,4}

Longer acting recombinant FVIII (e.g. Eloctate®), and FIX (e.g. Alprolix®) products achieve longer product biologic half-life by various means, such as conjugating clotting factors to polyethylene glycol (PEG) or fusing clotting factors to albumin or the immunoglobulin G (IgG) constant region (Fc).⁵⁻⁷

Multiple manufacturing and quality assurance processes ensure the safety, potency and efficacy of recombinant products. Virus inactivation/reduction procedures, such as solvent detergent treatment, nanofiltration, or heat/pasteurization, are incorporated into the manufacturing process of most recombinant concentrates.⁸

PLASMA-DERIVED PROTEIN PRODUCTS

Government-licensed private biotechnology companies make plasma-derived protein products by pooling plasma collected from large numbers of donors (typically >10,000) and then separating or fractionating the different constituents. Since the introduction of the Cohn fractionation process in the 1940s, involving varying protein concentration, ethanol concentration, ionic strength, temperature, and pH concentration to precipitate various plasma fractions in a stepwise manner,⁹ fractionation processes have evolved to include newer technologies such as chromatography, monoclonal affinity columns, and nanofiltration to further improve purity, diversity, and yield of extracted products.¹⁰⁻¹²

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TRANSFUSION-TRANSMISSIBLE DISEASE PREVENTION STEPS

Manufacturers incorporate multiple steps before, during and after the fractionation process that cumulatively achieve a very safe end-product.¹⁰⁻¹⁶ Plasma is sourced from carefully selected donors who are screened by sensitive serologic and nucleic acid tests capable of detecting a wide range of blood-borne infectious agents, including HIV, hepatitis B and hepatitis C viruses.¹⁶ Additional validated manufacturing steps, with strict adherence to good manufacturing processes,^{10, 13, 16} effectively render these products extremely safe from risk of infection. There has not been a single case in Canada of transmission of HIV, hepatitis B, or hepatitis C caused by plasma protein products since the introduction of modern manufacturing practices during the 1980s and 1990s.¹⁷ The estimated risk of known blood-borne infectious agents in plasma-derived products ranges from less than one in a million to less than one in 10 million or even lower.¹⁸

Donor screening and donation testing

Donor health screening includes a health assessment at the time of each donation; persons with specified risk factors for blood borne infections, including potential exposure to prion diseases (e.g. Creutzfeldt-Jacob Disease (CJD) or variant-CJD), are excluded. Each donated unit is laboratory-tested for specific pathogens, including nucleic acid and serologic testing for HIV, hepatitis B and hepatitis C viruses; donated units that test positive are discarded.^{11, 12}

Donor eligibility criteria also aim to protect donors' health. For example, additional safeguards are in place to protect the health of apheresis plasma donors, with a defined weight-dependent, maximum donation volume, cumulative annual donation volume and annual number of donations; these donors require a serum protein measurement to be performed with each donation, along with protein electrophoresis every 4 months, and an annual physical examination.

Most fractionated plasma products distributed in Canada are made from source plasma collected from foreign donors—the majority from paid plasma donors from the United States. Despite some differences in donor screening procedures or tests as performed in Canada, all plasma protein products distributed in Canada meet Health Canada, and/or Council of Europe (CE) and/or United States Food and Drug Administration (FDA), licensing requirements. Strict safety protocols at all stages of plasma collection, testing and production ensure that manufactured plasma protein products from paid and unpaid donors are equally safe.^{13, 16, 17} In addition, some plasma protein products that are currently unlicensed by Health Canada and not distributed in Canada may be accessible to clinicians on a case-by-case basis through Health Canada's Special Access Program.

See [Chapter 6](#) of this Guide for details of donor selection and transmissible disease testing.

Pathogen reduction and inactivation processes for fractionated products

A variety of methods available during the manufacturing process further decrease the risk of pathogen transmission (Table 1). Most manufacturers use a combination of two or more complementary processes.¹⁰⁻¹⁵ Their degree of effectiveness is validated by determining pathogen recovery from microbially-contaminated test samples after pathogen inactivation treatment. Various manufacturing steps have also been shown to effectively remove prion protein from plasma derivatives.¹⁹

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Table 1: Pathogen reduction and inactivation methods for fractionated products

Fractionation process	Decreases bacterial, viral and probably prion contamination, as the changes in pH, temperature and ethanol concentration keep microbial contamination low and physically disassociate viruses from proteins. ²⁰
Chromatography and nanofiltration	Achieve further incremental pathogen reduction and enhance the purity of the product. ^{10, 14}
Heat treatment	Can be dry, steam or wet (pasteurization) depending on the product. The specific temperature, pressure and length of time are predetermined for each product so that specific pathogens are inactivated without undue loss of product biological activity. ^{13, 15}
Caprylate or solvent-detergent treatment	Effective against lipid enveloped viruses such as HIV, hepatitis B and hepatitis C viruses. ^{13, 21} Water-immiscible solvents are used in combination with detergents to disrupt the lipid membrane of viruses; these reagents are then removed at a later stage in the manufacturing process.

Additional transmissible disease testing

In addition to donor screening that is done before the plasma is sent for fractionation, further tests may be performed by the plasma fractionators ("in-process" testing). These include tests for non-lipid enveloped viruses, such as parvovirus B19 and hepatitis A virus (HAV), which are more resistant to inactivation during fractionation. Nucleic acid testing of plasma pools for parvovirus B19 and HAV has been implemented by most manufacturers; this testing enables identification of plasma units that have a very high titer of parvovirus B19 or that test positive for HAV, and these units are removed from the manufacturing process.

All plasma-derived products are also tested at the end of the process to ensure that they are sterile and pyrogen-free.

CONCLUSIONS

Although the potential risk of disease transmission cannot be completely eliminated, recombinant and plasma-derived fractionation products are manufactured according to Good Manufacturing Practices using validated processes and appropriate quality control testing to ensure that they meet very high standards for purity, potency, efficacy and safety.

For information on plasma protein products available in Canada and best clinical practice on when and how to use these products, please see [Chapter 3](#) for **albumin**, [Chapter 4](#) for **immune globulin products**, and [Chapter 5](#) for **coagulation factor concentrates**.

CONTINUING PROFESSIONAL DEVELOPMENT CREDITS

Fellows and health-care professionals who participate in the Canadian Royal College's Maintenance of Certification (MOC) Program can claim the reading of the Clinical Guide to Transfusion as a continuing professional development (CPD) activity under Section 2: Self-learning credit. The reading of one chapter is equivalent to two credits.

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