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 of donated plasma; depending on the particular plasma protein product, a recombinant or a 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 Chapter 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 particular 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

“First generation” recombinant products used plasma proteins in the production, purification and final product formulation, and a small amount of residual human plasma protein, usually albumin, to provide product stability. Manufacturing processes have evolved: “third generation” recombinant products [e.g. Advate® (rFVIII), or NiaStase® (rFVIIa)], are manufactured without exposure to human or animal proteins other than the production cell line (e.g. hamster), and contain no human protein.²³ More recently, human cell line-derived recombinant products [e.g. Nuwiq® (B-domain deleted rFVIII)], with no animal or human protein additives, are being marketed.⁴

Longer acting recombinant FVIII (e.g. Eloctate™), and FIX (e.g. Alprolix™) products are also available. Longer product biologic half-life is achieved by methodologies such as conjugation of clotting factors to polyethylene glycol (PEG) or fusing of clotting factors to albumin or the constant region (Fc) of immunoglobulin G (IgG).⁵⁷

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 outside of Canada 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. The Cohn fractionation process, developed in the 1940’s, varies protein concentration, ethanol concentration, ionic strength, temperature, and pH concentration to precipitate various plasma fractions in a stepwise manner.⁹ The supernatant from one precipitation step becomes the starting material for the next. Each fraction is then processed separately to remove impurities, stabilize the product, inactivate and/or remove pathogens and ensure sterility. The basic Cohn fractionation process is very efficient and with modifications, is still in use today. Manufacturing improvements such as liquid chromatography, monoclonal affinity columns, and nanofiltration further increase the yield and purity of final products.¹⁰

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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. Plasma is sourced from carefully selected donors who are screened by sensitive tests capable of detecting a wide range of blood-borne infectious agents, including HIV, hepatitis B and hepatitis C viruses. Additional manufacturing steps 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 modern manufacturing practices were introduced decades ago. The estimated risk of infection from various known blood-borne infectious agents ranges from less than one in a million to less than 1 in 10 million or even lower.

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.

Donor eligibility criteria are designed to also protect donors’ health. For example, additional safeguards are in place to protect health of apheresis plasma donors, who may donate up to 31 litres of plasma per year; these donors require a serum protein measurement to be performed with each donation, along with quarterly protein electrophoresis, and an annual physical examination.

Not all fractionated products available in Canada are derived from plasma sourced from Canadian donors. Commercial products made with plasma from non-Canadian donors may not have the exact same screening procedures or tests as performed in Canada. They do however, meet local and/or Council of Europe (CE) and/or United States Food and Drug Administration (FDA) licensing requirements for donor screening and testing. Products available in Canada meet licensing requirements of FDA or CE in addition to Health Canada.

See Chapter 6 of this Guide for details of donor selection and transmissible disease testing.

Pathogen reduction and inactivation processes for fractionated products

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

Pathogen reduction and inactivation methods include:

The fractionation process itself: 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.

Chromatography and nanofiltration: achieve further incremental pathogen reduction and enhance the purity of the product.
**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.

**Solvent-detergent treatment:** effective against lipid enveloped viruses such as HIV, hepatitis B and hepatitis C viruses. 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 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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We’re here to answer your questions about the Clinical Guide to Transfusion. We’d also appreciate your ideas on how to improve the Guide. Please contact us through the Clinical Guide feedback form.

**REFERENCES**

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