

Plasma-derived products – manufacturing conditions; product release

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Summary:

Proteins acquired through the process of industrial plasma fractionation are invaluable, essential medicinal products that find application in treatment of many diseases. In this article, we presented problems associated with manufacturing of plasma-derived products.

Plasma-derived products made according to the current regulations are the lowest-risk biological therapeutics presently in use.

Key words: plasma, plasma-derived products, plasma fractionation

Plasma – the liquid component of whole blood devoid of its morphotic elements. It is used as a therapeutic product (therapeutic plasma) but it may be also a source material for other invaluable therapeutics.

The works of E. Cohen and his coworkers published in 1946 constituted a crucial landmark that allowed for the development of industrial-scale fractionation of plasma proteins. Techniques they proposed for precipitation and purification of particular proteins have undergone further modifications and improvements in the subsequent years related to changing from laboratory yield to industrial-scale production. The main physico-chemical factors utilized in this process remain: ethanol concentration, pH, ionic strength of used reagents and temperature (1,2).

Plasma used for production of plasma-derived therapeutics may come from the whole blood following the separation of its morphotic components or it may be acquired directly from the donors in a process of plasmapheresis.

This biological complex contains hundreds of proteins that serve important functions in physiological processes. However, some of the roles they play in those processes remain unknown.

Basic proteins used in fractionation are the following: albumins and immunoglobulins are present in the plasma in concentrations 35 g/l and 10 g/l, respectively. They comprise about 80% of all plasma proteins. Other proteins important for industrial processing of plasma are: clotting factor VIII – few ng/l, antithrombin III – 300 mg/l, and α -antitrypsin – 1.5 g/l.

One may state that plasma-derived products manufactured around the world in 35% come from the plasma acquired through centrifugation of the whole blood and in 65% from the plasma obtained via plasmapheresis.

Generally, there are 20 different products, protein preparations essential in treatment of many diseases produced through plasma processing.

Over the past several years plasma fractionation business transformed from small centers manufacturing blood products to cover the needs of local communities into a large, world-wide industry.

Currently 23-28 million liters of plasma are handled in the course of its processing, commonly known as fractionating, in 70 facilities of various sizes around the world.

The modern process of plasma fractionating is conducted with meticulous care and standardized according to international regulations, recommendations and guidelines.

Validated, strictly abided procedures regarding removal and destruction of pathogens, especially viruses, are integral to the process of making plasma-derived products.

All these actions are aimed at releasing high-quality therapeutics with guaranteed virological safety in light of current state of knowledge in this area.

The substrate – plasma – plays an extremely important role in the production process in terms of ensuring their highest quality and safety.

Because of that, a great deal of attention is focused on establishing quality management systems at every site of blood/plasma collection that supplies the fractioning facility.

Activities and procedures applied at the blood/plasma collection facilities exert great influence on the quality and safety of acquired material and, as a result, on the finished therapeutic product. Therefore, it is understandable that plasma “production” is an integral part of the fractionation industry (3, 4, 5).

Every collection center must possess a documented quality management system. Its main goals are presented below:

- to possess implemented operational procedures,
- to have a system for registering and tracking donations,
- to have an implemented management system for printing labels, storage and transportation of donations,
- clearly defined quality specifications for blood and plasma,
- to ensure constant internal quality control,
- appropriate accommodation for blood and plasma collection.

Simultaneously, such collection centers must be subjected to regular formal inspections by appropriate authorities tasked with supervising manufacturing

conditions of medicinal products according to the Good Medical Practice (GMP) requirements.

Plasma fractionation plants are also obliged to audit their partners (plasma suppliers) regularly.

Conduction of audits is aimed at constant, mutual monitoring in order to avoid deviations from the quality parameters of plasma agreed on contracts [6]. Areas of particular interest are the following:

- principles of donor qualification and examination,
- system of labeling and documenting donations,
- storage of acquired material.

Donors must be selected according to the recommendations of the European Council and fulfill health criteria specified by appropriate regulations.

Every donation must be examined using specific, validated diagnostic tests for detection of virological markers such as HBs, HIV 1 and 2, HCV and syphilis.

Individual donations must be negative for anti-HIV and anti-HCV antibodies as well as for HBs antigen. Examination for the presence of non-encapsulated viruses such as HAV and B19 using NAT (nucleic acid-based test) assays is also obligatory. (7)

A system should be created that would allow for constant collection of data regarding:

- fulfillment of donor selection criteria,
- information on potential positive results of virological tests in a donor,
- verification of performed tests,
- information on infections caused by the use of plasma-derived products,
- occurrence of the symptoms of Creutzfeldt-Jakob disease,
- appearance of post-transfusion infections caused by the treatment with blood or its products.

Plasma collection bags constitute an important safety factor. Therefore, their manufacturer, serial number and the type of used anticoagulant as well as sterilization methods applied during their production should always be noted.

Temperature is a crucial element influencing protein content in collected plasma, especially of clotting factor VII.

Obtained fresh frozen plasma (FFP) must be frozen at a temperature of -20°C, -25°C or -30°C for 6-8 hours after it was collected depending on local requirements.

There is one exception to this rule: plasma intended for production of albumin or immunoglobulins

may be frozen at a temperature of -20°C within 72 hours from its collection.

Frozen plasma may be stored at a temperature of -20°C or lower for several months (maximally over a dozen). Plasma storage and transportation temperature should be kept constant.

Information on all of these actions as well as storage conditions and local epidemiological data are contained in the Plasma Master File (PMF), which is also an annex to the registration dossier of every plasma-derived therapeutic [14].

It contains a set of data and information required by law regarding collection and further processing of plasma. Despite it being a part of the registration dossier, it is an independent document. The scope and type of data contained in this document is determined by appropriate regulations.

The content of Plasma Master File must be updated by the responsible entity every year, regardless of the validity period for the authorization for marketing (registration).

According to the requirements, plasma is pooled before the beginning of plasma processing/fractionation. Every donation included in the pool must be registered. Typical plasma pool contains 2000 – 4000 liters.

All documents together with plasma samples must be stored for at least one year after the end of the validity period for the therapeutics that were produced from it.

Simultaneously, each plasma pool must undergo re-testing by the fractionator in order to ascertain that it is free of viruses mentioned previously.

Slowly unfreezing the plasma pool constituting a particular batch begins a technological process of isolating consecutive fractions (intermediate points) that may be stored, mixed and even sent to other plants for the manufacture of the ultimate plasma-derived drugs. Care must be taken to validate all actions ensuring the virological safety of the end product. Also, stability testing of particular fractions stored at given conditions should be performed.

After isolating specific fractions in the initial stage of production, obtained proteins undergo further processing in order to attain the greatest possible chemical purity while sustaining their biological activity.

The following methods are used at this stage of production:

- 1) physical methods: e.g. centrifugation; acquiring cryoprecipitate – the source material used for production of Clotting Factor VIII, purified and formed into an end medicinal product through a series of steps including precipitation, absorption and use of chromatographic techniques.
- 2) physico-chemical methods: concern mainly fractionating with the use of ethyl alcohol. This technique is used in isolation and purification of albumins and immunoglobulins. Further purification of proteins is done in the next steps of this process with the use of chromatographic techniques, i.e. molecular filtration chromatography, ion exchange, affinity, immunoaffinity, ultrafiltration and microfiltration [12, 13].

One may say that main goals of above mentioned chromatographic techniques are:

- 1) obtaining products of the highest purity,
- 2) acquisition of proteins that occur in small (trace) concentrations,
- 3) limiting the losses of valuable proteins – increase in efficiency,
- 4) removing reagents from the end product that have been previously used for virus inactivation.

Currently produced plasma-derived therapeutics must be virologically safe. Therefore, various techniques and procedures for virus inactivation are used according to the pharmacopeial requirements [8, 9, 10, 11].

The following are some of the recognized and required methods of viral inactivation:

- heating of water solutions – mainly used in production of albumins,
- heating of lyophilized end products – mainly used in the process of production of clotting factors,
- inactivation of viruses with detergents – this technique is used for destroying encapsulated viruses in production of the intermediate product,
- using nanofiltration for removal of viruses,
- conditioning of the intermediate product in low pH (about 4).

All these techniques require validation in production conditions of every fractionating plant and may be also treated as successive steps of the technological process.

In the production process, a lot of attention is focused on interoperative monitoring. The fractionator is required to provide precise descriptions of the used equipment and instruments as well as sampling techniques and storage of collected and examined material.

Actions such as: plasma pooling, testing of the source material and virological markers must be fully validated.

Monitoring of parameters important for the technological process such as: pH, temperature, ethanol concentration and sterility control including testing for bacterial endotoxins must be thoroughly documented.

Quality control of the end products must fulfill pharmacopeial requirements. If the manufacturer is using his own testing and monitoring methods, he is required to conduct the validation process in such way that would prove their full compatibility with the methods described by the European Pharmacopoeia.

Individual stages of production must be validated and documented. An assessment of technological "efficacy" and productivity of the applied process as well as biological activity of the product must be conducted.

It is especially important to give proof of effective removal of undesirable components such as: chemical reagents used in the process and natural biological factors dangerous for the well-being of the patient, e.g. compounds determining blood types or active forms of clotting factors.

Use of chromatographic techniques, especially in case of affinity chromatography, requires proof that undesirable substances are not released from the columns.

Washing and disinfection effectiveness is also an incredibly important factor for validation of the production process.

Effectiveness of the steps of the technological process as well as methods of inactivation and removal of viruses must be assessed based on strictly specified model virus strains, both encapsulated and non-encapsulated. However, the fundamental

condition is that the following viruses must be used in conducted tests: HIV 1 and 2, HCV, B 19.

For the greater certainty with regard to the quality and safety of plasma-derived products introduced into the market, according to separate regulations, each batch of the finished product is required to undergo a formal preliminary release. The manufacturer covers the costs.

According to those requirements each batch of the product together with a batch report and testing samples must be delivered to an official laboratory designated by a competent body.

According to the requirements of the Polish Act on Pharmaceutical Law, all blood-derived products, i.e. clotting factors, clotting inhibitors, fibrin glue, human albumin, human immunoglobulin, or inactivated plasma must undergo serial preliminary control which, within the European Union, is abbreviated to OCABR (Official Control Authority Batch Release).

Only after obtaining a certificate confirming that the quality of the product conforms with its registered specification may the manufacturer introduce the plasma-derived therapeutic into the market.

Summarizing the briefly discussed problems associated with plasma fractionation, one may state that plasma-derived products, despite their high price, are indispensable and irreplaceable therapeutics used in treatment of many diseases.

At the same time, constant development of methods and techniques for the safety of produced preparations as well as continuation of clinical studies that would allow for further extension of indications for their therapeutic use pose an endless challenge for the plasma fractionation industry

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