PLASMA DERIVED PRODUCTS:
pathogenic safety and innovation technologies

Dr. Nataliya Zubkova
Dr. Alevtina Nikolaeva
Dr. Anthon Perevozchikov
Dr. Oleg Averkin et al.
Blood banks, plasma centers and blood transfusion institutes are the organizations responsible for human plasma collection in RF. About 800,000–1,000,000 liters are stored annually.

Over 50% of fresh frozen plasma (FFP) is available for transfusion as therapeutic products in the hospitals.

Ethanol precipitation technique is a widely used procedure to extract specific plasma proteins. About 300,000 – 400,000 liters of plasma for fractionation is processed annually.

**Microgen**, Russian leader in the manufacture of blood-derived products, processes about 200,000 liters of plasma per year.

There are about 20 plasma fractionation centers in different regions of Russia including blood banks with a manufacturing capacity of 10,000 – 30,000 liters of plasma per year.
DEVELOPMENT OF BIOPHARMACEUTICAL INDUSTRY IN RUSSIA

 ✓ Concept of the Program: Russian pharmaceutical and medical industry development for the period up to 2020 and a further perspective (Pharma and Medical Industry 2020)

REGULATION OF PLASMA COLLECTION and PLASMA FRACTIONATION INDUSTRY


 ✓ Russian Federation State Pharmacopeia.

 ✓ Technical regulations on requirements of security of blood, its products, blood substitutes and equipment, used in transfusion-infusion therapy / approved by RF Government decree No. 29 dated 26.01.2010.

Overall market valuation is 362 millions U.S. $ (including recombinant factors)

The most perspective segment of the market is the intravenous immunoglobulin sector
Plasma derived market is growing permanently

Foreign Manufactures are the main players in the national market:
66% - in packing units.
83% - in Russian Rouble.

Microgen company is the major domestic player in plasma derived market among home manufacturers:
29% - in packing units of total market.
14% - in Russian Rouble.
MICROGEN Scientific Industrial Company was founded in May 2003 to establish a competitive enterprise in the field of medical immunology.

MICROGEN holds the leadership in development and production of immunobiological preparations, diagnostic kits and drugs.

MICROGEN company incorporates the state enterprises producing the immunobiological preparations and laboratories that conducted studies in the field of immunobiology.

The history of some of its branches is over hundred years old.
At present Federal State Scientific-Industrial Company MICROGEN produces more than 300 immunobiological preparations.

60 medicinal preparations are included in the list of vital and essential medicines, over 120 items are immunobiological preparations, vaccines of National immunizations schedule and blood preparations. Plasma derived products (normal and specific immunoglobulins, albumin and others) is about 9% of the total production.
Development and production of innovative biopharmaceutical drugs

- to meet the national healthcare system needs
- to support the national programs of biological and epidemiological safety
MAJOR RISKS ASSOCIATED WITH HUMAN BLOOD PLASMA

Pathogenic agents

Viruses
- HCV, HBV, HIV 1,2, B19V
- CMV, EBV, HTLV1,2, HHV-8
- HAV, HEV
- HGV, TTV, WNV, DENV

Prions
- vCJD, BSE

Protozoa
- Trypanosoma cruzi
- Plasmodium falciparum
- Leishmania
- Babesia

Bacteria
- Spirochetes and Rickettsia
- Gram (-), Gram (+) Bacteria

High level of viremia is possible in infected persons

Blood cell-associated viruses

Short period of viremia.
Low risk of plasma contamination.

Post-transfusion effects are discussed

Blood-borne associated pathogens.
The plasma / whole blood contamination is possible.

Only exogenous contamination

Residual risk of post-transfusion reactions
STARTING MATERIAL:
Plasma for Fractionation

MANUFACTURING PROCESS:
Pathogen Reduction and Inactivation Steps

PLASMA DERIVED PRODUCTS

- Mini-pool testing
- Manufacturing pool testing
- Standardization
- Traditional Steps
- R&D Steps
- Virus Validation Studies
- Quality control and pathogen safety testing
ALGORITHM TO ENSURE VIRUS SAFETY OF PLASMA FOR FRACTIONATION
(plasma collection stage and control of starting materials)

Selection of donors

Screening of donors

Testing for pathogens
Exclusion of positive donations

PCR: HCV RNA, HIV RNA, HBV DNA

Plasma for fractionation/Contracts to supply
Suppliers technical audit of quality system

Donation inspection and registration

ELISA – HBsAg; anti-HCV; anti-HIV1,2/p24; anti-Tr pallidum

Mini pools testing
(24 ± 4 donations)

PCR - HCV RNA, HIV RNA, HBV DNA

Identification and elimination of positive donations

Manufacturing pools testing
(more than 1000 donations)

HBsAg, anti-HCV, anti-HIV1,2/p24, HCV RNA, HIV RNA, HBV DNA, B19 DNA

Quarantine storage: 180 days from the collecting date

Blood banks/plasma centers

MICROGEN company
Development and calibration of reference materials for nucleic acid amplification technique

- Standard Simple of hepatitis C virus RNA calibrated against WHO 3rd Standard of Hepatitis C Virus RNA (HCV RNA) NIBSC code: 06/100
- Standard Simple of hepatitis B virus DNA calibrated against WHO 2nd Standard of Hepatitis B Virus DNA (HBV DNA) NIBSC code: 97/750
- Standard Simple of Immunodeficiency virus RNA calibrated against WHO 2nd International Standard Immunodeficiency Virus (HIV-1 RNA) NIBSC code: 97/650
- Standard Simple of parvovirus B19 DNA calibrated against WHO 1st International Reference Panel for Parvovirus B19 genotypes for NAT based assays NIBSC code: 09/110

Using Standards and Quality Control of molecular genetic methods

- Validation and standardization of nucleic acid amplification technology (NAT) assays
- Development of Positive Controls containing HCV RNA, HIV-1 RNA, HBV DNA for monitoring of NAT testing
- Development of Positive Controls containing DNA D19 for NAT testing to exclude the high positive donations

Figure 1. Microsoft Excel Modulus for calculation of concentration of nucleic acid in the Standard Simples against WHO standard by Parallel-Line Model (European Pharmacopoeia 7.0/Statistical analysis 5.3)
VIRUS REDUCTION STEPS
In the manufacturing process

TRADITIONAL TECHNOLOGIES
✓ Ethanol fractionation by Cohn-Oncley
✓ Pasteurization for albumin
✓ Ion-exchange chromatography- IEC
✓ Solvent-detergent treatment (SD)
✓ Low pH

INNOVATION TECHNOLOGIES
✓ Sodium caprylate treatment
✓ Millisecond Technology (MST) - method of low-temperature short time pasteurization of liquid products. We are realizing the project with Skolkovo innovation center and Millisecond Technologies Co., Ltd.
Ion-exchange chromatography in plasma derived products technology

Chromatographic process for purification of IgG

Hydrophobic resin

Anionic resin

Cationic resin

Sorption of IgG

Deletion of TNBP and aggregates

Extraction of IgA, IgM, ceruloplasmin

Obtaining of purified IgG

List of preparations planned for development:

Highly purified Human normal (polyvalent) immunoglobulin for intravenous administration.

Highly purified hyperimmune globulins to hepatitis B; to tick-borne encephalitis, to tetanus.

Ceruloplasmin.

Complex of IgG, IgA, IgM immunoglobulins for per os and intravenous administration.
## VIRUS VALIDATION STUDY:
Pathogenic and model viruses using in virus validation studies

<table>
<thead>
<tr>
<th>Pathogenic viruses</th>
<th>Model viruses</th>
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<tbody>
<tr>
<td>Hepatitis B virus (HBV)</td>
<td>Duck Hepatitis B virus (DHBV)</td>
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<tr>
<td></td>
<td>Plasma of carriers of hepatitis B virus (model experiments)</td>
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<tr>
<td>Hepatitis C virus (HCV)</td>
<td>Bovine Viral Diarrhea Virus (BVDV)</td>
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<td></td>
<td>Plasma of carriers of hepatitis C virus (model experiments)</td>
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<tr>
<td>human immunodeficiency virus (HIV)</td>
<td>HIV- 1 (cultural)</td>
</tr>
<tr>
<td></td>
<td>(Human immunodeficiency virus (HIV)</td>
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<tr>
<td>Parvovirus B19</td>
<td>Plasma of carriers of parvovirus B19 (model experiments)</td>
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</tbody>
</table>

**Research Centers:**
- FSUC «MICROGEN» MoH (Nyzny Novgorod, Moscow)
- Chumakov Institute of Poliomyelitis and Viral Encephalitides
- Institute of Experimental Veterinary Science of Siberia and the Far East Russian Academy of Agricultural Sciences
- The Republican Research and Practical Center for Epidemiology and Microbiology, Republic of Belarus
### Virus Reduction Steps

<table>
<thead>
<tr>
<th>Virus Reduction Steps</th>
<th>Virus Reduction, $\log_{10}$</th>
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<tbody>
<tr>
<td></td>
<td>DHBV$^{\text{log}_{10}}$ ID 50/ml</td>
</tr>
<tr>
<td>Cohn-Oncley Ethanol Precipitation</td>
<td>-</td>
</tr>
<tr>
<td>Albumin Immunoglobulin G</td>
<td>-</td>
</tr>
<tr>
<td>SD-treatment</td>
<td>≥ 5.0</td>
</tr>
<tr>
<td>Immunoglobulin 0.3% TNBP, 0.2% Sodium Cholate</td>
<td>≥ 5.0</td>
</tr>
<tr>
<td>Caprylate-treatment</td>
<td>≥ 5.0</td>
</tr>
<tr>
<td>5-40 mM, T=18-29°C</td>
<td>6.09±0.05</td>
</tr>
<tr>
<td>pH 4.0-4.5</td>
<td>≥ 5.0</td>
</tr>
<tr>
<td>T=37°C, 24-48 hour</td>
<td>6.09±0.05</td>
</tr>
<tr>
<td>Millisecond Technology (MST)</td>
<td>-</td>
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<tr>
<td>method of low-temperature short time pasteurization</td>
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</tbody>
</table>
The kinetics of inactivation DHBV and BVDV after SD-treatment

Fig. 1. The number of ducks infected DHBV (% of total per group) at end one week after the administration of SD-treated immunoglobulin (1-6 hours)

Fig. 2. The kinetic of inactivation BVDV after application of SD-treatment of immunoglobulin solution
Thank you for your attention