Plasma Product Biotechnology Meeting 2005

May 9th–12th, 2005
Porto Elounda Deluxe Resort,
Crete,
Greece

Program and Abstract Book

Welcome to Plasma Product Biotechnology Meeting 2005

This meeting has attracted researchers, manufacturers, regulators and others with an active interest in plasma products every other year since 1999. Informative presentations are mixed with lively discussions. Data and ideas change hands. Fresh issues and new developments within the plasma industry are openly discussed by people representing all aspects of the industry.

This time we meet on the Mediterranean island of Crete. Once again, the focus is on creating a relevant, exciting atmosphere to facilitate discussion of the topics that you feel matter most today. The committee's task was to build the event around the topics and presentations you proposed. You shaped and developed the program through your contributions and interactions with the meeting web site – **www.bo-conf.com/ppb05**. Whether you work with plasma product R&D, manufacturing, economics, safety, clinical, regulatory, or any other relevant discipline, you can make an active contribution to PPB05, or simply follow the presentations and discussions.

On behalf of the Organizing Committee, I wish you warm welcome to Plasma Product Biotechnology Meeting 2005 and I look forward to interacting with you here at Porto Elounda in Crete.

Jan Berglöf

Chairman

Organizing Committee

Jan Berglöf, Chairman
GE Healthcare
Sweden

Neil H. Goss
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The Netherlands

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USA

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ZLB Behring GmbH
Germany

Wytold Lebing
Talecris Biotherapeutics
USA

The Fourth Plasma Product Biotechnology Meeting is jointly sponsored by
CSL Bioplasma Ltd, Australia and GE Healthcare, Sweden.

General information

Venue

The meeting takes place at Porto Elounda Deluxe, which is located 70 kms (44 miles) from Heraklion International Airport on the east coast of Crete, 7 km North of Aghios Nikolaos.

Registration

The Registration Desk is open as follows:

Monday	May 9th	16.00–19.00	
Tuesday	May 10th	07.30–13.00	18.00–19.30
Wednesday	May 11th	08.00–13.00	18.00–19.30
Thursday	May 12th	08.00–13.00	14.30–17.30

Please come to the desk if you need any assistance.

Program and Abstract Book

Each registrant is entitled to one copy of the Program and Abstract Book. Additional copies can be picked up on Thursday at the Registration Desk while supplies last.

Oral Sessions

All oral presentations will take place in the main conference room, Isola Di Candia. Consult the program for the presentation times of the different sessions. For those who will give oral presentations, prepare your slides in the Speakers Room outside Isola Di Candia. Hand the carousel or, if using computer projections, your disk to the audiovisual technician at least 30 min prior to session start.

Poster Sessions

Posters should be mounted on Monday afternoon between 16.00 and 19.00 or Tuesday morning before 08.30. All posters should be on display all three days of the Meeting and should be taken down at 14.00 on Thursday afternoon. Poster boards are labelled with a number corresponding to the Abstract Number in the Program Book. Posters will be on display in the Reception Gallery.

Coffee and Lunch Break

Complimentary coffee will be available in the Reception Gallery during coffee breaks in the morning and evening sessions. Consult the program for the times. A light lunch will be available in the Nafsika restaurant.

Social Activities

A Welcome Reception will be held on **Monday, May 9th**, starting after the keynote lecture at 20.00 on the sea side of Elies Restaurant. This is a time to meet old and new colleagues, while enjoying a glass of wine or beer and the BBQ menu with a variety of cold and hot dishes. Greek background music adds to the occasion.

On **Tuesday, May 10th,** a half day tour to the island of Spinalonga is part of the program. Pick up your box lunch and enjoy it on the boat. You will be back at 16.15/16.30 hrs in time for the Focus lecture and the evening session.

On **Thursday, May 12th,** a Crete Night Buffet will be held in the Playiada Square. The event starts at 19.30 with a Sunset Aperitif followed by a buffet of Crete specialties, together with live Greek entertainment.

Dress Code

The meeting and setting promotes communication between participants and informal dress is suggested. For the dinners smart casual attire is recommended.

Message Center

The Registration Desk can receive messages for you on tel: +46 705 336368 or fax: +30 28410 68018, or e-mail: info@elounda-sa.com. A message board will be located by the Registration Desk. Check daily for messages, since those not picked up by Thursday evening at 18.00 will be discarded.

How To Reach Us

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Program

Monday May 09, 2005

19.00–19.15 **Welcome and Introduction**
Chairman: Jan Berglof. GE Healthcare, Uppsala, Sweden.

19.15–20.00 **Keynote Lecture**
"Plasma Protein Products: How Far Have We Come, How Far Do We Go?"
Dr. Graham D. Sher. Chief Executive Officer, Canadian Blood Services, Ottawa, ONT, Canada.

20.00– **Welcome Reception: Elies Restaurant**

Tuesday May 10, 2005

Session 1 – Product Developments and New Technologies

08.30–08.35 **Chairman´s Introduction**
Jan Over. Sanquin Blood Supply Foundation, Amsterdam, The Netherlands.

08.35–09.05 **101 Purification of Plasma-Derived Mannose Binding Lectin (MBL)**
Antje Daehler*[1,4], Teresa Martinelli[1,4], Robert Pike[3,4], and Robyn Minchinton[2,4]
[1]CSL Bioplasma, Australia. [2]Australian Red Cross Blood Service, Australia.
[3]Department of Biochemistry & Molecular Biology, Monash University, Australia.
[4]Cooperate Research Centre for Vaccine Technology, Australia.

09.05–09.35 **102 Fibrin Microbeads (FMB) for Autologous Tissue Regeneration**
Gerard Marx*, Anna Hotovly-Solomon, Ibrahim Kassis, Lila Levdansky, Elena Gaberman, and Raphael Gorodetsky. HAPTO Biotech Ltd., Jerusalem, Israel.

09.35–10.05 **103 Use of X-Ray Photoelectron Spectroscopy for the Direct Assessment of Chromatographic Resin Cleaning**
K. Lyons*[1], G. Seneviratne[1], N. Brack[2], P. J. Pigram[2], and J. Bertolini[1]
[1]CSL Bioplasma, 189–209 Camp Rd, Broadmeadows Vic 3047 Australia.
[2]Centre for Materials and Surface Science and Department of Physics, La Trobe University Vic 3086 Australia.

10.05–10.30 **Coffee break**

10.30–11.00 **104 A New, High Yielding, Affinity Cascade for Sequential Isolation of Plasma Proteins of Therapeutic Value**
Christopher Bryant*[1], Dev Baines[1], Ruben Carbonell[2], Tom Chen[3], John Curling[1], Timothy Hayes[3], Steve Burton[1], and David Hammond[3]
[1]ProMetic BioSciences Ltd., Cambridge, UK. [2]North Carolina State University College of Engineering, Raleigh NC, USA.
[3]American Red Cross, Plasma Derivatives, Gaithersburg, MD, USA.

11.00–11.30 **105 New Adsorbents for Selective Capture of Plasma Proteins from Recovered and Source Plasma**
Dev Baines*[1], Jason Betley[1], Ben Beacom[1], Tom Chen[2], Timothy Hayes[2], Jim Pearson[1], and Pilar Vazquez[1]
[1]ProMetic BioSciences Ltd., Cambridge, UK. [2]American Red Cross, Plasma Derivatives, Gaithersburg, MD, USA.

11.30–12.00 **106 The Application of CaptureSelect Affinity Ligands for Purification of Plasma and Recombinant Plasma Products**
Laurens N. Sierkstra*, Mark ten Haaft, and Pim Hermans. B.A.C. Naarden, The Netherlands.

12.00–16.30 **Lunch box and excursion to the island of Spinalonga**

17.00–17.30 **Focus Lecture 1**
Comparability Protocols, FDA´s Perspective and Experience
Dr. Andrew Chang. Div Hematology, FDA, Rockville, MD, USA.

Session 2 – Recombinant Plasma Proteins

17.30–17.35 **Chairman´s Introduction**
Joe Bertolini. CSL Bioplasma, Broadmeadows, Victoria, Australia.

17.35–18.00 **201 Identification and Production of Recombinant Human Polyclonal Antibody Drugs Reflecting the Natural Human Antibody Diversity**
Søren Bregenholt. Symphogen A/S, Elektrovej 375, DK-2800 Lyngby, Denmark.

18.00–18.25 **202 Transgenic Goats and Cows for the Production of Human Plasma Proteins**
Yann Echelard* and Harry M. Meade
GTC Biotherapeutics, Inc., 5 the Mountain Rd, Framingham, MA 01701, USA.

18.25–18.50 **203 Purification of rHSA on a Pilot Scale**
Bo Ersson*[1], Mikael Berg[1], Joergen Samuelsson[1], Karin Caldwell[1], Makonnen Belew[2], Lise Lundh[2], Lars-Olof Lindquist[2], Li Mei Yan[3], Zhang Wei[3], Wei Jing-Shuang[3], Du Li[3], and Jia Qian[3]
[1]Center for Surface Biotechnology, Uppsala University, Sweden.
[2]GE Helthcare, Uppsala, Sweden.
[3]North China Pharmaceutical Group Corp, P. R. of China.

18.50–19.15 **Coffee break**

19.15–19.40 **204 In-Depth Structural Characterization Can Expedite the Development Process of Plasma Products and their Substitutes**
Nicolas Bihoreau, Emmanuel Nony, François Fenaille, Laurent Siret*, and Sami Chtourou
Laboratoire Français du Fractionnement et des Biotechnologies, Preclinical Development, 3 Avenue des Tropiques, BP305, les Ulis, 91958 Courtaboeuf Cedex, France.

19.40–20.05 **205 Phage Display to Identify Recombinant Proteins as Potent and Selective Proteinase Inhibitors**
Mark de Souza PhD
Sr. Director, Corporate Development, Dyax Corp., 300 Technology Sq., Cambridge, MA 02139, USA.

20.05–20.30 **206 Development of Recombinant Human Thrombin as an Aid to Hemostasis in Subjects Undergoing Surgery**
Jan Ohrstrom MD*, John Forstrom PhD, Linda Zuckerman PhD, and Thomas Reynolds MD PhD
ZymoGenetics, Inc., Seattle, WA, USA.

Wednesday May 11, 2005

Session 3 – Manufacturing

08.30–08.35 **Chairman´s Introduction**

Johan Vandersande. Baxter BioScience, Westlake Village, CA, USA.

08.35–09.05 **301 Computer-Assisted Plasma Logistics**

Gerold Zerlauth. Baxter AG, Plasma Control Europe, Vienna, Austria.

09.05–09.35 **302 Chromatographic Purification of Immunoglobulins at CSL Bioplasma: A Manufacturing Perspective**

John Lontos. CSL Bioplasma, 189–209 Camp Road, Broadmeadows, Victoria, 3047, Australia.

09.35–10.05 **303 Technology Transfer: Practical Answers to Practical Issues**

Andrea Morelli. Kedrion S.p.A., via Provinciale 1, 55027 Bolognana, Gallicano (Lucca), Italy.

10.05–10.30 **Coffee Break**

10.30–11.00 **304 A Comparative Study of Cohn and Chromatographic Fractionation Using a Novel Affinity "Cascade Process"**

John Curling*[1], Dev Baines[1], Christopher Bryant[1], Ruben Carbonell[2], Tom Chen[3], Patrick Gurgel[2], and Timothy Hayes[3]

[1]ProMetic BioSciences Ltd., Cambridge, UK. [2]North Carolina State University College of Engineering, Raleigh NC, USA. [3]American Red Cross, Plasma Derivatives, Gaithersburg, MD, USA.

11.00–11.30 **305 Investigating the Effect of Pumping on Plasma Proteins**

Peter T. Gomme*, Owen C. Tatford, Anna Johnson[1], and Joe Bertolini

CSL Bioplasma 189–209 Camp Road, Broadmeadows, Victoria 3047, Australia.

[1]Current address – CSIRO Food and Health Sciences, 343 Royal Parade, Parkville, Victoria 3052, Australia.

11.30–12.00 **306 Aseptic Filling of Human Albumin Solution into Flexible Container Provides Various Opportunities for Cost Savings**

Theodor Langer. Baxter, Industriestrasse 17–19, 1220 Vienna, Austria.

12.00–13.00 **Lunch: Nafsika restaurant**

13.00–16.00 **Free afternoon**

16.00–17.00 **Poster Session**

17.00–17.30 **Focus Lecture 2**
TSE's and Medicinal Products. Regulatory Aspects in the EU
Dr Sol Ruiz. Spanish Medicines Agency, Madrid, Spain.

Session 4 – Hyperimmune and Intravenous IgG

17.30–17.55 **Chairman´s Introduction**
Wytold Lebing. Talecris Biotherapeutics, Clayton, NC, USA.

17.55–18.20 **401 Production of a Virally Safe Despeciated Equine Botulinum Antitoxin Product**
Hugh Price*, Bill Bees, Lori Soluk, and Andrew Griffiths
Cangene Corporation, 104 Chancellor Matheson Rd, Winnipeg, Manitoba R3T 5Y3, Canada.

18.20–18.45 **402 Immunoregulation of Transplant Rejection Using Intravenous Immunoglobulin**
John Underwood[1], Brian Tait[2], Joe Bertolini*[3], Fiona Hudson[2], Paul Licciardi[1], and Kyle Wilcox[1]
[1]Department of Pathology, University of Melbourne, Australia.
[2]Victorian Transplantation and Immunogenetics Service. Australian Red Cross Blood Service, Australia.
[3]CSL Bioplasma Division, CSL Ltd, Australia.

18.45–19.15 **Coffee break**

19.15–19.40 **403 Purification of SARS Hyperimmune Globulins**
Bing-Lou Wong* and Yi-Wu Xie. Advantek Biologics Limited, Hong Kong, P. R. of China.

19.40–20.05 **404 A High-Yield IVIG Process with Efficient Viral Clearance**
Jaakko Parkkinen*, Anne Rahola, Leni von Bonsdorff, Hannele Tölö, and Esa Törmä
Red Cross Finland Blood Service, Kivihaantie 7, FI-00310 Helsinki, Finland.

20.05–20.30 **405 Emerging Technology in Hyperimmune Therapeutic Manufacturing**
Wolfgang Ruesseler*[1] and Kailing Wang[2]
[1]Corporate VP Life Manufacturing, Life Therapeutics.
[2]Corporate VP Life Bioprocess, Life Therapeutics, 736 Park North Blvd, Suite 100, Clarkston, Georgia 30021, USA.

Thursday May 12, 2005

Session 5 – Pathogen Safety Issues

08.30–08.35 **Chairman´s Introduction**

Bernard Horowitz. Horowitzconsulting, Key Biscayne, FL, USA.

08.35–09.00 **501 Practical Aspects of a Prion Clearance Study**

Dr Olaf Stamm. NewLab BioQuality AG, Max-Planck-Str. 15A , 40699 Erkrath, Germany.

09.00–09.25 **502 Filtration Technologies for Prion Removal from Plasma Products**

Jerold Martin Sr VP
Global Technology Director, BioPharmaceuticals, Pall Life Sciences, 2200 Northern Blvd., East Hills, NY 11548 USA.

09.25–09.50 **503 Application of Filtration Technologies for Prion Removal from Plasma Product Intermediates**

H. Pham*[1], W. Seyfert-Brand[2], M. Alaveras[1], T. Wiegand[2], O. Gnau[2], W. Schäfer[2], A. Gröner[2], and T. Martinelli[1]
[1]Research & Development, CSL Bioplasma, Melbourne, Australia. [2]Virology Department, ZLB Behring, Marburg, Germany.

09.50–10.20 **Coffee break**

10.20–10.45 **504 Validation and Technological Aspects of an Integrated Virus Clearance Platform**

Klaus Tarrach. Sartorius AG, Biotechnology Division, Germany.

10.45–11.10 **505 Development of an *in vitro* TSE Infectivity Assay: Application to Validation of Manufacturing Processes**

B. Flan*[1], G. Le Hir[1], B. You[1], H. Laude[2], and J. T. Aubin[1]
[1]LFB, Laboratoire Français du Fractionnement et des Biotechnologies, 3 Ave des Tropiques – BP 305, LES ULIS, 91958 COURTABOEUF, France.
[2]INRA Jouy en Josas, France.

11.10–11.35 **506 Strategies for the Removal of Blood-Borne TSE Infectivity**

David Hammond*[1], Steve Burton[2], Ruben Carbonell[3], and Robert Rohwer[4]
[1]American Red Cross, USA. [2]ProMetic BioSciences, UK. [3]North Carolina State University, USA. [4]University of Maryland, USA.

11.35–12.00 **507 Detection of PrP^{Sc} with PrP^{Sc} Specific Binding Reagents without Proteinase K Treatment**

M. Michelitsch, D. Peretz, M. Connolly, R. Zuckermann, C. Gao, X. Wang, J. Hall, T. Horn, A. Gyenes, B. Shimizu, D. Dodge, B. Phelps, C. Hu, and Y. Yang*
Blood Testing Business Unit, Chiron Corporation, Emeryville, CA, USA.

12.00–13.00 **Lunch: Nafsika restaurant**

13.00–15.00 **Free afternoon**

Session 6 – Applications of Plasma Products and Clinical Developments

15.00–15.05 **Chairman´s Introduction**

Hubert Heinrichs. ZLB Behring GmbH, Marburg, Germany.

15.05–15.30 **601 Biochemical and Pre-Clinical Characterization of a New 10% Liquid Triple Virally Reduced Human Intravenous Immune Globulin (IGIV, 10% TVR)**

Wolfgang Teschner*[1], Harald Arno Butterweck[1], Wilfried Auer[2], Eva Maria Muchitsch[2], Alfred Weber[3], and Hans-Peter Schwarz[4]

[1]Baxter AG, Industriestr. 131. [2]Baxter AG Industriestr. 20. [3]Baxter AG, Benatzkygasse 2–6.
[4]Baxter AG, Wagramerstr. 17–19, A-1220 Vienna, Austria.

15.30–15.55 **602 Efficacy and Safety of Subcutaneous Immunoglobulin Replacement Therapy at Home in Patients with Primary Immunodeficiency Diseases: Combined Analysis of Two Clinical Studies, One in North America and One in Europe**

Peter Kiessling*[1], Hans D. Ochs[2], Michael Borte[3], Michaela Praus[4], and Hubert Heinrichs[1]

[1]ZLB Behring GmbH, Marburg, Germany. [2]Department of Pediatrics, University of Washington, Seattle, WA, USA.
[3]Department of Pediatrics, Municipal Hospital "St. Georg", Leipzig, Germany.
[4]Biostatistics, Covidence GmbH, Marburg, Germany.

15.55–16.20 **603 Effective Prevention of Ischemia-Reperfusion Injury with Plasma Proteins**

Jaakko Parkkinen. Red Cross Finland Blood Service, Kivihaantie 7, FI-00310 Helsinki, Finland.

16.20–16.45 **Coffee break**

16.45–17.15 **604 WILFACTIN® – Development and Clinical Evaluation of a Triple-Secured and High-Purity von Willebrand Factor Concentrate**

Z. Tellier. Laboratoire Français du Fractionnement et des Biotechnology – Les Ulis – France.

17.15–17.40 **605 Successful Treatment with Sandoglobulin in Patients with Guillain-Barré Syndrome**

Irmgard Andresen MD. Clinical Research, ZLB Behring AG, Berne, Switzerland.

17.40–18.00 **Conclusion of the meeting**

19.30– **Crete Night Buffet: Playiada Square**

701 **Development of a Method to Detect Contaminant Proteins in Immunoglobulin G Preparations IV. A Proteomics Approach**

Peter Gomme*[1], Judith Lysaght[2], Bernie McInerney[2], and Joe Bertolini[1]
[1]CSL Bioplasma 189–209 Camp Road, Broadmeadows, Victoria 3047, Australia.
[2]Australian Proteome Analysis Facility, Level 4 Building F7B, Macquarie University, North Ryde, NSW 2109, Australia.

702 **An Abbreviated Affinity Chromatography Cascade Process for FVIII/von Willebrand Factor Complex and Immunoglobulin G**

Tom Chen*[1], Sonday Allen[1], Dev Baines[2], Jason Betley[2], Davida Blackman[1], Timothy Hayes[1], Guy Harris[2], Bastian Lobezoo[2], and Keith Watson[2]
[1]American Red Cross, Plasma Derivatives, Gaithersburg, MD, USA. [2]ProMetic BioSciences Ltd., Cambridge, UK.

703 **A Combined Proteomics and Well-Characterized Approach to Comparability for the Development of Cascade-Fractionated- and other Plasma-Derived Products**

Timothy Hayes*[1], Tom Chen[1], Dev Baines[2], Tom Busby[1], Kevin Carrick[1], Dale Schmidt[1], and Nadine Ritter[3]
[1]American Red Cross, Plasma Derivatives, Gaithersburg, MD, USA. [2]ProMetic BioSciences Ltd., Cambridge, UK.
[3]Biological Consulting Group, Alexandria VA, USA.

704 **Manufacturing Processes of Plasma-Derived Products Reduce Emerging Pathogens**

Albrecht Gröner. ZLB Behring GmbH, Marburg, Germany.

705 **Screening of Industrial Plasma by HBV NAT: Increase in Viral Safety?**

G. Zerlauth* and M. Gessner. Baxter AG, Plasma Control Europe, Vienna, Austria.

706 **Study on the rHSA Quality Specification**

Jia Qian*, Li Mei Yan, and Xingjun Zhou
New drug R&D center, North China Pharmaceutical Group Corp., shijiazhuang, Hebei, P. R. of China.

707 **Study on Serological Reaction to SARS-CoV of IVIG and Original Plasma from Qualified Donors**

Guo Zhongping, Wang Yu*, and Gou Hongtao
Chengdu Rongsheng Pharmaceutical Co., Ltd, Waidong Baojiang Bridge 610023, Chengdu, Sichuan, P. R. of China.

708 **Characterization of IgG Fragments in Liquid Intravenous Immunoglobulin Products**

Robert V. Diemel[1,2], Hendricus G. J. ter Hart[2], Gerardus J. A. Derksen[2], Anky H. L. Koenderman*[2], and Rob C. Aalberse[1]
[1]Sanquin Research. [2]Sanquin Plasma Products, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands.

709 **Characterization of Plasminogen Removal Gel**

Ingela Blomqvist[1], Inger Lagerlund*[1], Anna Mattsson[1], Roberto Meidler[2], and Israel Nur[2]
[1]GE Healthcare, Uppsala, Sweden. [2]Omrix Biopharmaceuticals Inc., Rehovot, Israel.

710 **Removal of Viruses and Pathogen Agents in the Downstream Processing of Plasma and Biopharmaceutical Products by Nanofiltration with Planova® Filter**

S. Vynck*[1], E. Kederer[1], H. Hisada[2], and T. Sato[2]
[1]Asahi Kasei Planova Europe, Brussels, Belgium. [2]Asahi Kasei Pharma Corporation, Japan.

711 **Advantages of the EBA Method for the First Stage Chromatography Plasma Proteins Re-purification**

G. L. Volkov*[1], S. I. Andrianov, A. Yu. Slominskiy, O. S. Havrylyuk, and T. V. Goroshnikova
Palladin Institute of Biochemistry of the NAS of Ukraine.
[1]Application and Training Laboratory of Amersham Biosciences Representative Office in Ukraine. Leontovicha str., 9, 01601, Kyiv, Ukraine.

712 **Plasma Economics – A Brighter Outlook in the Short Term but Changing Fundamentals Suggest Future Challenges**

Rosemary Cummins. Equity Research, Citigroup, Melbourne, Australia.

713 **Electrophoretic Analysis of Clotting Factor VIII Concentrates**

N. V. Odinokova, M. A. Azhigirova*, and R. N. Khametova
National Research Centre of Haematology, Moscow, Russia.

714 **The Possibility of One-Stage Clotting Method for Determination of Factor VIII Activity in the Purified Antihaemophilic Concentrates**

A. Berkovskiy*, E. Sergeeva, A. Suvorov, M. Ajiguirova, and A. Vorobiov
Hematology Centre, Moscow, Russia.

715 **Is Citrate Deficiency a Common Problem in Transfusion Medicine?**

Edward Shanbrom[1] and William J. Owens*[2]
[1]Santa Ana, CA, USA. [2]University of California, Irvine, Orange CA, USA.

716 **Blood Bank Preparation of Safe Immunoglobulin Concentrate**

Edward Shanbrom[1] and William J. Owens*[2]
[1]Santa Ana, CA, USA. [2]University of California Irvine, Orange, CA, USA.

717 **Innovative Method for Producing Therapeutic Proteins in Plants**

Kimmo Koivu[1] and Timo Virkajärvi*[2]
[1]UniCrop Ltd, Helsinki, Finland. [2]Rintekno Oy, Espoo, Finland.

718 **UVivatec – A Scalable Technology for UV Virus Inactivation in Laboratory and Production**

Dr Sebastian Schmidt*, DI Markus Zamponi, and DI Jörg Kauling
Bayer Technology Services GmbH, D-51368 Leverkusen, Germany.

Abstracts

Purification of Plasma Derived Mannose Binding Lectin (MBL)

101

Antje Daehler*[1,4], Teresa Martinelli[1,4], Robert Pike[3,4], and Robyn Minchinton[2,4]

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[2] Australian Red Cross Blood Service, Australia.

[3] Department of Biochemistry & Molecular Biology, Monash University, Australia.

[4] Cooperate Research Centre for Vaccine Technology, Australia.

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Mannose Binding Lectin (MBL) is a key component of the innate immune system. This plasma protein is considered an important factor in the first line host defense against various pathogens including bacteria, fungi, viruses and parasites.

MBL oligomers form complexes with MBL Associated Serine Proteases (MASP). MBL/MASP complexes bind to specific carbohydrate structures that are found on the surface of these pathogens, but not on mammalian cells. Upon binding its target, the MASP are activated and cleave C2 and C4.

Current research indicates that ~24% of the general population is affected by MBL deficiency due to genetic mutations and carriage of the low promoter polymorphisms and that MBL deficiency has been associated with increased susceptibility to infections. Thus MBL has potential as a therapeutic for a defined group of immunocompromised individuals

MBL/MASP complex can be purified from side fractions of existing plasma fractionation processes by affinity chromatography with carbohydrate resins. As the binding mechanism resembles the binding of MBL/MASP complexes to pathogens, the MASP can become activated during the purification process. These activated MASP could be capable of initiating an immune response independent of the presence of pathogens. To enhance the safety of the MBL product, a method has been developed to separate MASP from MBL.

The fully scalable purification process includes two virus inactivation steps and is capable of delivering a fully functional, MASP depleted MBL.

Fibrin Microbeads (FMB) for Autologous Tissue Regeneration

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Gerard Marx*, Anna Hotovly-Solomon, Ibrahim Kassis, Lila Levdansky, Elena Gaberman, and Raphael Gorodetsky
HAPTO Biotech Ltd., Jerusalem, Israel.

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Fibrin microbeads (FMB) have been fabricated from various source materials including pooled plasma, cryoprecipitate or fraction I paste. The FMB produced by a heated oil emulsion process, were essentially dehydrated fibrin cross-linked with endogenous factor XIII. The FMB production process incorporated a number of viral killing steps including extensive heating at ~70 °C, exposure to solvents such as hexane, acetone and ethanol, as well as γ-irradiation (1.5 Mrad). FMB, which appeared as spheroidal particles with diameters ranging from 50–250 μ, did not exhibit coagulant activity. FMB were sieved to obtain 106–180 μ particles used to culture a variety of cells to high density. For example, FMB were used to separate mesenchymal stem cells from human bone marrow, leading to a ~5-fold increase in the yield of mesenchymal stem cells from bone marrow or peripheral blood versus the yield of cells separated by Ficol and adhered onto plastic plates. The attached pluripotent cells on FMB could be expanded in vitro and driven to differentiate into various cell types, including bone-forming cells and adipocytes. FMB were biodegradable and when implanted sub-dermally into mice, did not generate toxic effects. We also developed a novel dual-channel fibrin pistol which could simultaneously deliver a stream of FMB(±cells) suspended in fibrinogen and a separate stream of thrombin, resulting in a fibrin clot in which FMB (±cells) were entrapped. Currently, our work is focused on demonstrating that FMB, loaded with stem cells derived from bone marrow, can be employed for autologous regeneration of tissue. We are also evaluating potential industrial applications of FMB for growing cells in culture and for using cells-on-FMB for metabolic testing.

References

Gorodetsky *et al.* J. Invest. *Dermatol.* **45** 893–900 (1999).

Gurevich, *et al. Tissue Engineering* **8** 661–672 (2002).

Marx Transfusion. *Med. Rev.* **17** 287–298 (2003).

Use of X-Ray Photoelectron Spectroscopy for the Direct Assessment of Chromatographic Resin Cleaning

103

K. Lyons[1]*, G. Seneviratne[1], N. Brack[2], P. J. Pigram[2], and J. Bertolini[1]

[1] CSL Bioplasma, 189–209 Camp Rd, Broadmeadows Vic 3047 Australia.

[2] Centre for Materials and Surface Science and Department of Physics, La Trobe University Vic 3086 Australia.

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The effectiveness of different solutions to remove waste proteins and other unwanted material that remain bound a chromatographic resin at the completion of a separation is usually assessed by either:

i. Examining the eluate stream of the column for the presence of these molecules.

ii. Extracting the molecules into a liquid phase that can be subsequently analyzed by techniques such as gel electrophoresis or mass spectroscopy.

These methods are indirect and rely on the ability the eluate stream or liquid phase to remove the waste material from the resin for analysis. These methods are not able to directly determine what remains bound to the resin following the cleaning procedure. The current study examines the potential application of a surface analysis technique, X-ray photoelectron spectroscopy (XPS) to overcome this problem. XPS is a sensitive, semi-quantitative technique that is able to directly probe the first 5–10 nm of a surface. Samples are irradiated under vacuum by X-rays, which results in the photoejection of electrons from atoms at the surface of the sample. The energy of the ejected electrons is determined and results in spectra that provide information about the elements present and their relative concentration.

Samples of DEAE Sepharose Fast Flow ion-exchange resin have been exposed to different concentrations of bovine serum albumin (BSA) ranging from $1x10^{-4}$ M to 1 mM under conditions that allow the protein to bind to the resin. These samples were analyzed by XPS to determine the sensitivity of the technique. Results show that by using the nitrogen 1 s photoelectron peak in the XPS spectra to monitor protein bound to the resin it is possible to detect BSA bound to the resin at $1x10^{-3}$ M.

The XPS technique has also been evaluated to determine if it is able to detect differences between DEAE resins that have undergone different regeneration/cleaning processes. Results show that by using the nitrogen 1 s photoelectron peak of the sample spectra the technique is able to detect clear differences between new resin that has never been exposed to protein and resin that has had protein bound and subsequently removed to varying degrees by different solutions such as NaCl and NaOH.

This data suggests that XPS is a useful analytical technique capable of directly detecting material bound to DEAE resin and has application for studying the effectiveness of resin regeneration/cleaning procedures.

A New, High Yielding, Affinity Cascade for Sequential Isolation of Plasma Proteins of Therapeutic Value

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A backbone fractionation system for source and recovered plasma, based on an affinity chromatography cascade (the "Cascade Process") has been developed at 4 litre scale. The process is now in scale up. The protein targets are Factor VIII/von Willebrand Factor Complex, Plasminogen, Fibrinogen, Alpha$_1$-antitrypsin, Immunoglobulin G and Human Serum Albumin. Key results to be presented will include yield and purity data for the cascade process derived from "engineering runs" to protocol and batch record data. The Cascade can be abbreviated for the most needed proteins. Data will also be presented for a FVIII/vWF and IgG process as well as a Factor VIII, IgG and HSA sequence.

New Adsorbents for Selective Capture of Plasma Proteins from Recovered and Source Plasma

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Structural information for seven plasma proteins and selected peptide ligand structure data have been used as the basis for computational chemical, *in silico* design of diversity-optimised virtual libraries using Cerius2 software. Targeted, real, solid-phase libraries were constructed in a 96-well plate format using amine substitution onto a triazine scaffold. Libraries were screened and sub-library generation and screening allowed ligands to be selected and adsorbents developed to the target proteins. Laboratory scale experiments confirm the selectivity, yields and binding capacities for the proteins.

The Application of CaptureSelect Affinity Ligands for Purification of Plasma and Recombinant Plasma Products

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Affinity chromatography is a well-established technology for the purification of molecules from complex source materials. We have developed a technology for the generation of affinity ligands, using small 12 kD fragments derived from single domain antibodies (CaptureSelect) which can be used for purification of products as well as scavenging of impurities. The advantages of our ligands as compared with other affinity technologies is the fact that our technology combines specificity, affinity, stability, short development times, and ease of non-animal derived production. Moreover, BAC has the ability to work with every solid support. The specific ligands are generated from antibody fragment libraries and are recombinantly produced at any scale in *Saccharomyces cerevisiae* (Bakers yeast). The requirements for a ligand for a particular application are incorporated in the screening and subsequently, ligands are selected and tested for affinity, selectivity, and stability. In our presentation we describe the development of affinity ligands for the purification of recombinant proteins, minor components from plasma and IVIG.

Identification and Production of Recombinant Human Polyclonal Antibody Drugs Reflecting the Natural Human Antibody Diversity

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Plasma-derived immunoglobulin products are associated with a number of disadvantages limiting their use such as high production costs, supply constraints, low specific activity, and risk of pathogen transmission. We believe that recombinant polyclonal antibodies could overcome the problems while retaining the efficacy associated with a polyclonal antibody drug. Symphogen's proprietary technology platform, Symplex™, allows the direct cloning and identification of high-affinity cognate antibody VH-VL pairs from human donors. The diversity captured using Symplex™ is much broader than observed with other technologies, thus facilitating the compilation of polyclonal drug leads reflecting the natural human repertoire against a given antigenic structure. Based on a conventional cell banking strategy, the Sympress™ Technology facilitates the manufacturing of such recombinant human polyclonal antibodies in mammalian cells. The Sympress™ Technology has been transferred to an industrial setting for manufacturing of Sym001, a recombinant polyclonal anti-Rhesus D antibody. This work has demonstrated that antigen-specific polyclonal antibody drug leads can be produced in a highly reproducibly and predictable manner. In combination, Symphogen's proprietary technology platforms provide an alternative for identification and manufacture of recombinant polyclonal antibody drug as alternatives to plasma-derived immunoglobulin products.

Transgenic Goats and Cows for the Production of Human Plasma Proteins

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The use of recombinant therapeutic proteins has steadily increased during the last two decades. Clinical applications often require large amounts of highly purified molecules, often for multiple or chronic treatments. The development of very efficient expression systems has been the key to the full exploitation of the recombinant technology. This is particularly true for some of the proteins derived from human plasma, where the combination of a complex structure and large therapeutic dosing have until now precluded the use of traditional bacterial and cell culture bioreactors for recombinant production. Thanks to a careful integration of molecular biology, large animal embryology and protein chemistry, transgenic milk production offers a cost-effective and flexible system for the manufacturing of large amounts of complex proteins. During the last decade, GTC Biotherapeutics has generated transgenic goat herds that yield large amounts of proteins as diverse as: human antithrombin, the C-terminal portion of *Plasmodium falciparum* merozoite surface protein-1 (a candidate Malaria vaccine), monoclonal antibodies, or antibody fusion-proteins. In addition, as an alternative to plasma fractionation, a herd of transgenic cloned cows that yield high levels of human albumin in their milk has been produced. Furthermore, technologies that permit the clinical-grade purification of recombinant therapeutic proteins from the milk of transgenic dairy animals have been developed and implemented. ATryn® (recombinant human antithrombin) the most advanced of the transgenic milk-derived compounds, after several years in clinical development, has completed pivotal trials. This presentation will summarize the current state of the ATryn® program, as well as the latest progress with recombinant human albumin and other recombinant plasma protein programs.

Purification of rHSA on a Pilot Scale

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A complete process for the preparation of recombinant human serum albumin in pilot scale has been developed. Starting material was cell-culture supernatant from a genetically transformed *Pichia pastoris*. The rHSA in the starting material was purified to apparent homogeneity using a combination of three chromatographic steps involving:

1) A capture step using a newly developed salt tolerant cation exchanger, CST I Sepharose™ Fast Flow. The ligand concentration and chromatographic characteristics of this media have been optimised for this particular application, resulting in an adsorbent that binds at least 50 mg rHSA/ml of packed media in the presence of ca 0.25 M salt.
2) An intermediate purification step, based on HIC, using Phenyl Sepharose 6 Fast Flow (high sub) in the column.
3) A polishing step using a newly developed weak anion exchanger called Amino Butyl Sepharose 6 Fast Flow.

A laboratory scale method with 1 g of starting material was first developed to optimize the chromatographic steps. The pilot scale method used 600 g starting material resulting in > 200 g of pure product in every batch. The pilot scale method was designed so that further scaling up should be easy and straightforward. The process also was designed to meet the regulatory demands and for producing a product of standard quality and concentration. Formulation, concentration and other intermediate steps thus were included in the process method development.

The analyses showed that the product fulfilled the demands concerning sterility, content of pyrogens, colour and electrophoretic analyses

In-Depth Structural Characterization Can Expedite the Development Process of Plasma Products and their Substitutes

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The development and registration of biotechnological drug products has required the use of cutting edge analytical technologies to thoroughly characterise their structure. These technologies were mainly used to characterise post-translational modifications, to investigate structure-activity relationships, and to assess batch to batch consistency.

The development of plasma products can also take advantage of the same technologies derived from separation methods and mass spectrometry (MS). They can be used to demonstrate the comparability of products and expedite the development process by avoiding time and resource consuming pre-clinical and clinical development. Different strategies can be undertaken, depending on the study objectives. The primary structure can rapidly be identified or confirmed using peptide mapping with MS/MS sequencing. The same experiment performed in non-reducing conditions can demonstrate protein integrity through identification of disulfide bridges. The glycosylation profile can be assessed qualitatively by MS and quantitatively by capillary electrophoresis. The impurity profile can be assessed using a proteomic-like approach with 2D gel electrophoresis and MS identification of co-purified proteins.

These strategies have been implemented for different proteins, including pd-antithrombin, anti-D monoclonal antibodies and coagulation factor VII. Various methodologies will be presented to illustrate how in-depth structural characterisation can shorten the development and registration process.

Phage Display to Identify Recombinant Proteins as Potent and Selective Proteinase Inhibitors

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Dyax Corp. uses its proprietary phage display technology to discover and develop potent and specific protease inhibitors from its antibody, peptide, and protein libraries. One of the protein libraries is based on a plasma protein scaffold, the first Kunitz domain of LACI-D1, a natural human protease inhibitor. Amino acid residues in the protease binding site of LACI-D1 were variegated to obtain a library of billions of different Kunitz domain proteins. Dyax has selected several potent and specific protease inhibitors from this library. Two of Dyax's Kunitz domains are in clinical trials for three different indications. DX-890, which has a 2 pM Ki for neutrophil elastase is in a Phase II trial for cystic fibrosis. DX-88, which has a 40 pM Ki for plasma kallikrein, is in a Phase II trial for hereditary angioedema. Dyax recently completed a Phase I/II trial with DX-88 to evaluate its ability to prevent blood loss in cardiopulmonary bypass surgery. From its Fab phage display libraries, Dyax has discovered panels of human antibodies that are very potent and selective inhibitors of serine proteases; these antibodies are being evaluated as therapeutics for cancer and inflammatory diseases.

Development of Recombinant Human Thrombin as an Aid to Hemostasis in Subjects Undergoing Surgery

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Thrombin, the terminal protease in the coagulation cascade, is widely used as a topical hemostat to treat surgical bleeding. All currently marketed topical hemostats containing thrombin are derived from pooled human or bovine plasma. Recombinant human thrombin (rhThrombin) has been efficiently produced from precursor recombinant prethrombin-1 derived from cell culture at ZymoGenetics, Inc. The purified rhThrombin product is substantially free of non-thrombin proteins, as assessed by PAGE analysis. LC-MS-MS peptide mapping revealed that rhThrombin comprises the identical amino acid sequence and disulfide-bonding pattern found in native human thrombin. In vivo experiments have demonstrated rhThrombin is effective in achieving hemostasis in a dose-dependent manner in two models of surgically induced bleeding: a rabbit liver excision wound model and a rat hemi-nephrectomy model. In both models, rhThrombin at concentrations 500 U/ml applied using a gelatin sponge significantly reduced time to hemostasis. Recombinant human thrombin was evaluated in 4 concurrent Phase 2 studies in subjects undergoing spinal surgery, liver resection, peripheral arterial bypass, or arteriovenous graft formation for hemodialysis access. All studies were randomized, double-blinded, and were performed in multiple centers. Subjects were treated with either rhThrombin (1000 U/ml) or placebo in combination with an absorbable gelatin sponge applied to a surgical site requiring a topical hemostatic agent. Application of additional open-label rhThrombin was permitted when medically indicated to achieve hemostasis. The primary objective of the study was to evaluate the safety of rhThrombin as determined by incidence and severity of adverse events. Anti-product antibodies were measured by ELISA at baseline and 1 month posttreatment. Time to hemostasis was measured at the surgical site(s) for a maximum of 600 seconds. A total of 130 subjects were enrolled in the four studies. The rate of adverse events observed in subjects treated with rhThrombin was generally comparable to that observed in subjects receiving placebo , and was consistent with the expected rate of adverse events following these types of surgery. There were no serious adverse events that were considered related to administration of rhThrombin. One of 83 subjects (1.2%) exposed to rhThrombin developed low-titer antibodies to rhThrombin. Both hemostasis at 10 minutes and mean time to hemostasis showed a positive trend in favor of subjects treated with rhThrombin. Recombinant human thrombin appeared to be safe and was well tolerated and minimally immunogenic.

Computer-Assisted Plasma Logistics

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Pick-up and transport, storage, screening, and sorting are activities that are necessary before plasma for manufacturing enters the manufacturing process. Given the global nature of plasma sourcing and the high number of individual donations handled in a large manufacturing company, these activities require sophisticated processes.

Consequently, we have developed a computer-assisted plasma logistics system which allows to integrate the mentioned activities into one framework. This system covers each single donation by holding supplier-provided data (e.g. bleed number, bleed date) in a global, centralized database. Such information is fed to the system prior to the actual pick-up of plasma, thereby allowing for a pre-check of the delivery. Delivery-related information is loaded onto a hand-held device, which then is used to verify the material received upon pick-up. In addition, a connection between plasma donation, box number, and palette number can be established, allowing a direct hit of the location of each donation in the warehouse if necessary. The plasma is transported under temperature controlled conditions to the central cold storage warehouse and stored for later use. During the time of storage in the warehouse, the plasma donation database is continuously updated as additional test results and post-donation information become available. Prior to production, the requested plasma is processed through a fully-automated sorting system which removes unsuitable donations. The automated sorting line identifies each donation by barcode scanning, retrieves information concerning the suitability of this donation from the plasma database and drops suitable donations into the manufacturing bin while non-suitable ones are singled out for destruction. The automated sorting line is designed to handle one donation per second. In summary, a system is in place that supports all relevant steps of plasma logistics from donor center to production and, in addition, covers additional functions such as look back, donation tracking, and donation statistics.

Chromatographic Purification of Immunoglobulins at CSL Bioplasma: A Manufacturing Perspective

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The Broadmeadows plant was opened in February 1994. It was designed to purify immunoglobulins using Cohn ethanol fractionation. A few years into operation CSL decided to re-engineer the purification of immunoglobulins via this means and introduce chromatographic purification. The main challenge was to develop and implement a chromatographic process into a plant which was designed for Cohn with minimal disruption and modification to the plant.

In January 2000 CSL commenced with full scale manufacture of chromatographic IVIG (Intragam P). Intragam P is a high purity and high yielding IVIG product. The Immunoglobulin and Albumin process at the Broadmeadows facility are controlled by a high level of automation. CSL will be upgrading its automation system over the next 5 years to achieve an even higher level of automation.

With any chromatography plant there is a requirement for buffers. The Albumin/ IVIG chromatography plants involve 3 automated buffer make tanks and some 16 different buffer storage vessels. On average 300,000 litres of buffers are made per week for the chromatographic process.

The majority of equipment cleaning is performed via automated clean in place systems (CIP). Fixed vessels, portable vessels and fixed pipework are all subjected to a CIP. Recently specialised fully automated CIP units have been installed which clean Ultra filtration units and perform NWP testing. Also the introduction of improved cleaning of ion exchange resin has helped increase the resin life of these gels.

Some vessels downstream of the process undergo an automated steam in place (SIP) procedure. These tanks are in a grade C "Post-VI" area (post viral inactivation) and are usually used for viral inactivation steps such as bulk low pH incubation for albumin, bulk pasteurisation for both albumin and IVIG, and for final formulation purposes.

Successful adoption of chromatography has provided a high purity product with an average yield increase appproximately 25% when compared to Cohn fractionation. It has also resulted in producing a IVIG product with high purity which has very low levels of IgA. Also a high level of automation to drive the process means that the quality and efficiency to manufacture IVIG is reproducibly maintained.

However to gain these advantages processing becomes more complex because of the additional engineering and equipment required to drive a chromatographic purification process. There are fewer processing hold points due to the large volumes involved and the process essentially becomes a continuous one. Buffer manufacture and storage requires a significant quantity of water/ reagents not to mention the plant floor space required to cater for these.

Technology Transfer: Practical Answers to Practical Issues

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Technology transfer is the systematic procedure that is followed in order to pass the documented knowledge and experience gained during development and/or commercialization to an appropriate, responsible and authorised party. Technology transfer, which implies the participation of a sending unit and a receiving unit, can be considered successful when there is the documented evidence that the receiving unit can effectively perform critical elements of the transferred technology meeting pre-defined quality criteria.

In this presentation two examples of tech transfer performed in the biopharmaceutical company Kedrion are shown: a successful transfer of a commercial production between two sites of the same company and an ongoing transfer of two manufacturing processes from R & D Department to a production site. Particularly, the practical solutions that were applied in order to solve the main issues met in these experiences are described.

A Comparative Study of Cohn and Chromatographic Fractionation Using a Novel Affinity "Cascade Process"

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Although major consolidation has occurred in the US fractionation industry with cut backs in plasma throughput there are significant unmet needs in the developing world. New entrants in plasma fractionation need to consider manufacturing technology options as well as local (national needs). This study shows the basic differences between chromatographic plant operation and a standardised Cohn procedure. Data will be presented on capital and operating expenses for a green-field development of a facility to fractionate 500,000 litres of plasma annually.

Investigating the Effect of Pumping on Plasma Proteins

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Manufacture of plasma proteins relies on purification processes that involve multiple pumping steps. As pumps mechanically force product from inlet to outlet there is the potential for protein denaturation and aggregation. In this study it was shown that inefficient lobe pumping promotes albumin aggregation. This is significant for albumin production, as lobe pumps are often used at key stages, such as ultrafiltration. During these steps protein passes through the pump multiple times. Therefore use of an inefficient pump at this point is likely to result in aggregate formation. While downstream purification steps might remove aggregate the accumulation of damaged albumin through the process due to inefficient pumping can have consequences on the long-term product stability. The effect of pumping on protein aggregation however depends on the physico-chemical environment and protein species. This was confirmed in IgG pumping studies with no aggregation detected after extended pumping. These observations highlight the need to investigate individual manufacturing steps for protein damage. Process monitoring, using turbidity, dynamic and static light scattering techniques is therefore recommended to ensure physical conditions which promote protein instability are identified and suitable process changes implemented.

Aseptic Filling of Human Albumin Solution into Flexible Container Provides Various Opportunities for Cost Savings

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Baxter developed the capability of filling human albumin solutions into flexible container. The project started a couple of years ago to increase handling convenience for the customer and reduce costs for manufacturing. Savings will be found in the reduced breakage rate, reduced shipping costs, reduced storage space and in a decrease of the material costs. The challenges were to identify a suitable film material as the product will be stored at room temperature for 2 years. The filling technology was also critical. Albumin cannot be terminal heat sterilized, so an aseptic filling process is required. And the container has to withstand a final container pasteurization as required by CFR and Pharm. Eu. The quality of the seal during bag fabrication and the amount and quality of the extractables allowed to be released into the product were additional areas of concern. Baxter used in house capabilities with the proven Galaxy Seal-Fill-Seal technology and film to solve the issues. End of last year the product was filed to the FDA and in Europe. By using this filling technology Baxter will be able to reduce standard costs up to 25%. Storage space could be reduced up to 50% and weight is reduced by more than 50% per unit.

Production of a Virally Safe Despeciated Equine Botulinum Antitoxin Product

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Cangene Corporation has developed a manufacturing process for a de-speciated equine Botulism Antitoxin product. The process consists of the chromatographic purification of IgG from equine plasma, collected from hyperimmunized horses. The purified IgG is then enzymatically digested with pepsin, which cleaves the antigenic Fc portion from the IgG monomer. The pepsin also digests unwanted plasma protein contaminants, which are then removed, with the pepsin, by diafiltration and anion exchange chromatography. The end product is a highly purified F(ab')2/Fab fraction, which may be safely administered to humans. As the plasma is animal derived, there is the potential for transmission of animal viruses by crossing the species barrier and becoming pathogenic to the user. The manufacture of this product incorporates the following for control of potential viral contamination:

1) Utilization of proper herd management. Herd management, like human donor selection management, is critical to the safety of the finished product.
2) Testing of the plasma to confirm the absence of detectable viruses. Testing is performed to ensure that the plasma pool meets the criteria for viral safety, as outlined in the Code of Federal Regulations (9 CFR), with the addition of testing for West Nile Virus and Eastern Equine Encephalitis.
3) The incorporation of specific virus clearance steps in the manufacturing process. The first step, which is used on the pooled plasma, is the addition of solvent and detergent (SD). The SD step is a well-established virus clearance step, and highly effective for the inactivation of lipid-enveloped viruses. The second step is nanofiltration of the purified product, employing Millipore's NFP (normal flow parvovirus) filter. The nanofiltration step does not distinguish between lipid or non-lipid enveloped viruses, but removes viruses based on the virus size. Both virus clearance steps have been validated as efficient and robust, with log reduction values (LRV) of 4 logs, by scale-down studies using a panel of model viruses. The panel was selected to represent viruses that are potential contaminants of equine plasma, and to represent a wide range of physical and chemical properties in order to ensure clearance of new and emerging viruses.

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Immunoregulation of Transplant Rejection Using Intravenous Immunoglobulin

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Intravenous immunoglobulin (IVIg) is administered for primary immunodeficiency syndromes and also has a recognised immunoregulatory role in treatment of autoimmune diseases including multiple sclerosis, Guillian-Barré syndrome, Pemphigus vulgaris, idiopathic thrombocytopenia purpura and SLE. Anti-idiotype antibodies, anti-cytokine antibodies, antibody-mediated Fc receptor blockade and interference with cellular activation by antibodies to cell membrane signal transduction molecules on lymphocytes represent proposed mechanisms by which IVIg regulates autoimmunity.

Immunoregulatory activity of IVIg has also been demonstrated in solid organ transplant recipients for the reduction or elimination of circulating cytotoxic anti-HLA antibodies. Twenty-five to thirty percent of potential transplant recipients exhibit high PRA (Panel Reactive Antibody) levels. Due to antibody reactivity to a range of different HLA molecules, high PRA levels significantly extend the Transplant Waiting Times which can be life threatening. Multiple doses of efficacious batches of IVIg, administered at 0.5–2 g/kg, has been shown to effectively desensitize high PRA transplant recipients resulting in substantially decreased anti-HLA antibody titres allowing for transplantation.

Using the Luminex multi-analyzer, the presence of idiotype-anti-idiotype antibodies in IVIg have been investigated for the inhibition of cytotoxic anti-HLA antibodies. Following analysis, one of five batches of IVIg examined (20%) produced 33–36% reduction in the HLA specific antibody binding. These preliminary data lend support the anti-idiotype antibody mechanism of action for IVIg inhibition of anti-HLA antibodies in solid organ transplantation and indicate the potential of multiple analyte detectors for the selection of immunoregulatory batches of IVIg

Purification of SARS Hyperimmune Globulins

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Background

Severe acute respiratory syndrome (SARS) is an emerging infectious disease having infected over 8,000 people during the global outbreak in 2003, with a fatality of 9.6%. The effectiveness of current first-line treatments for SARS remains open to question. For more effective treatment and prevention, SARS hyperimmune globulins are purified from human plasma and horse serum.

Study Design and Methods

Forty-seven units of plasma are collected from patients recovered from SARS. Cold ethanol precipitation and ion exchange chromatography are used to process pooled SARS convalescent plasma. To ensure the safety of human hyperimmune globulins, solvent/detergent treatment and nanofiltration are employed to inactivate and remove viruses, respectively. Real-time PCR is used to detect SARS-specific RNA in the human hyperimmune globulins. As human SARS convalescent plasma is very limited, equine serum obtained following challenges with inactivated SARS virus can supplement as an unlimited source of SARS-specific antibodies. The equine serum with anti-SARS antibodies is processed with ammonium sulfate precipitation, pepsin digestion and ion exchange chromatography.

Results

The purified human hyperimmune globulins (5%) have a neutralizing antibody titer specifically against the SARS virus of 1:200. SARS-specific RNA is not detected with quantitative PCR in the human hyperimmune globulins. Other parameters analyzed meet the Chinese Requirements of Biologics for IVIG that are similar to those of FDA and EMEA. The human hyperimmune globulins prepared are subsequently approved for clinical evaluation by the Chinese authority. Equine serum has a much higher SARA neutralizing titer (1:10,280) compared with the human one. The equine serum is found negative on SARS-specific RNA with the quantitative PCR. After removal of the much more antigenic Fc fragments, F(ab')2 fragments are purified and then formulated as a 2% solution.

Conclusion

Purified and concentrated SARS hyperimmune globulins are successfully prepared, which may help to better contain future SARS outbreaks.

A High-Yield IVIG Process with Efficient Viral Clearance

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The caprylic acid process developed by Lebing *et al*. (Vox Sang 2003, 84:193) and the clinical trials carried out with the product have demonstrated the benefits of caprylic acid treatment combined with ion exchange chromatography in the manufacturing of IVIG from human plasma. We have studied possibilities to employ caprylic acid in the manufacturing of IgG which could be filtered with high capacity with a small pore size virus removal filter.

Caprylic acid treatment followed by ion exchange chromatography yielded pure IgG but some polymeric IgG was present which prevented efficacious filtration. By combining caprylic acid treatment with PEG precipitation and a single anion exchange chromatography on ANX Sepharose it was possible to purify polymer-free IgG with high yield. The purified IgG solution could be filtered with a composite virus filter (Viresolve NFP) using a load of up to 10 kg IgG/m^2 with only moderate (50%) decrease in flux under optimized conditions. The yield of IgG in the virus filtration was close to 100% and yield from plasma about 4.5–5.0 g/kg. As another indication of the high purity of the IgG, the solution could be concentrated above 20% without polymerization. When concentrated to a 10% IgG solution, the product proved stable in accelerated stability studies in conventional formulations. A new formulation with trehalose had even improved stability.

When assessed with parvovirus B19 spiking and PCR, the process proved to have high efficacy in removal of non-enveloped viruses. Two process steps (CA+PEG precipitation, virus filtration) removed both about 4 log of parvovirus, total reduction being more than 12 log. The enveloped model virus BVDV was completely inactivated in the caprylic acid treatment.

The results indicate that it is possible to manufacture with high yield from Cohn fraction II+III polymer-free IgG which can be filtered with high capacity through a small pore size virus removal filter.

Emerging Technology in Hyperimmune Therapeutic Manufacturing

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The plasma fractionation market centres on the core therapeutic products Immunoglobulin, Factor VIII and Albumin which account for over 70% of the total plasma protein market. The drivers behind these products center on availability of source plasma and the technical ability to produce a high yielding commercial product that meets all safety and regulatory standards. There are in excess of 1000 proteins found in plasma. Only 10 to 15 of these are currently used as therapeutics, although FDA licensed more than 100 assays for identification of therapeutic candidates. While only three products account for 70% of revenue in the industry, there is considerable scope for growth through the development and commercialization of niche plasma therapeutic products.

Manufacturing capacity and capability is widely regarded as a bottleneck in bringing new therapeutic products to market in a cost effective and timely manner. The intrinsic cost of operating large fractionation facilities demand the number of products sold per liter of plasma to be high in order to provide economic stability for the industry. Few of the products are produced at large scale whereas other proteins are low volume products. Hyperimmunes in addition, while comprising the lowest volume products, often have to be manufactured in few, large batches due to facility/equipment size and one failure in production can put a major percentage of the annual demand at risk. While consequently one would favor to produce Hyperimmunes in separate small scale facilities, this approach can only be profitable if manufacturing cost per dose can be established at or below the cost level of large scale facilities. As the investment required for current state-of-the-art fractionation facilities is prohibitive, the approach of choice is to increase product yield per liter of plasma by deploying new technologies.

Life Therapeutics with a backward integration into specialty plasma donor centers is dedicated to become a niche manufacturer for the Hyperimmune market. The approach will be based on Life Therapeutics' Gradiflow technology, a preparative electrophoresis system that uses the inherent molecular characteristics of size and charge to separate individual proteins from complex biological mixtures. Gradiflow technology combines traditional membrane tangential flow filtration with native electrophoresis. Proof of Principle projects with major fractionators have demonstrated the significant advantages of Gradiflow technology in the area of hyperimmune manufacturing: the ability to deliver greater yields of plasma protein products with high purity; simultaneous fractionation and pathogen removal; and less processing steps resulting in faster processing time.

Practical Aspects of a Prion Clearance Study

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The general principles of a viral clearance study – a spiking experiment using a scaled-down model of the production process – also apply to a prion study. Individual steps within the downstream process are evaluated as to their capacity to inactivate or remove prion proteins. The effectiveness of removal or inactivation is expressed as the logarithmic reduction factor (LRF). However, unlike viral studies, a consensus has not been or is only now being reached on the appropriate source of prions, the preparation of spike material from this source, and the assay method of choice; each will be discussed.

The spike material is normally generated from prion-infected mice or hamsters. These forms of prions are commonly used for research purposes since they are infectious and exhibit shortened incubation times when required in animal studies as compared to bovine and ovine sources.

There is still no cell-culture based assay for the determination of the prion infectivity. Therefore, animal studies using mice or hamsters must be performed or Western blot detection of the proteinase K resistant PrP^{Sc} antigen can also be performed for this evaluation. The bioassay is time consuming taking 12 to 18 months to generate results and requires many animals. To avoid unnecessary animal studies, minimize costs and to obtain the quickest results, the Western blot analysis may be the method of choice for the initial studies. The results are available within a few days and a much more comprehensive study design can be investigated due to the minimized costs. The results from the Western blot study can then help to identify those process steps which are most effective in removing or inactivating prion proteins. Only those steps which show promise in removing or inactivating prions will be tested using animal studies.

Compared to *in vitro* methods, only studies performed in animals are specific for infectious prion particles. A correlation has been shown of the results obtained by Western blot analysis compared to those results obtained in animal studies (Lee *et al*, 2001). This presentation will demonstrate that an optimized Western blot system in combination with the correct spiking material is a fast, cost effective and reliable method for the evaluation of manufacturing processes in regards to their TSE safety. However, when used on their own for regulatory purposes, results obtained from the *in vitro* assays may have to be demonstrated again using animal studies.

Regardless of the method used to detect prions, special attention must be made to the preparation of the prion spike material. Several different preparations of prion material have already been used in the prion clearance studies. These include brain homogenate, brain homogenate treated with detergents and/or filtration, microsomal prion fractions and purified

prion rods. Crude brain homogenate is suitable as the spike material for many inactivation steps. In fact, the use of crude brain homogenate is the best experimental model for plasma originating from an CJD-positive donor. This is due to the fact that in nature prions are mainly membrane-associated and not in an isolated, soluble form. The prions in crude brain homogenate would most resemble this natural state.

Using crude brain homogenate as the spike material is also applicable if the manufacturing process cannot be divided and analysed in separate process steps since it will provide the highest possible titer of prion protein allowing for the highest obtainable LRF. In this case the starting material e.g. plasma will be spiked with the brain homogenate and after each process step a sample will be taken for Western blot analysis. This procedure will require an extremely optimized and sensitive Western blot technique to ensure sufficient dynamic range which is necessary for the demonstration of significant LRFs.

If a nanofilter or a chromatography step needs to be evaluated, prions need to be in a more soluble form. This must be done to avoid clogging of the filter or chromatography column and is accomplished by a detergent treatment followed by pre-filtration. Normally lysolecithin is used for this treatment.

Prion process validation is still a challenge but this challenge can be meet if the investigator is understanding the nature of the prion protein and is using the most sensitive but also valid analytical detection systems.

Filtration Technologies for Prion Removal from Plasma Products

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Since the first cases of vCJD transmission via blood transfusion products were reported in 2004, greater attention is being placed on the potential risks from prions in plasma products. In the U.K. and Europe, where over 150 cases of BSE have been documented, regulatory agencies have mandated removal of prions from blood transfusion products, via white blood cell filtration. The European Union has codified standards for use of animal derivative materials, as has Japan's Ministry of Health, Labor and Welfare. Plasma derivative manufacturers are taking various approaches to qualify elimination of prions from their operations. Filtration and other processes already in use can be demonstrated to remove prions from protein solutions.

Prions are charged, hydrophobic proteins with a strong tendency to aggregate and adsorb to surfaces. While individual prion proteins are too small to easily separate by size from target therapeutic proteins, their surface properties and aggregation states can be exploited for separations purposes. The most widely used filtration technology qualified for prion removal is charge-modified cellulosic depth filters. Mechanism of removal and studies assessing removal of prions from plasma fractions and derivatives will be reviewed.

Virus filters have been shown to be effective for removing more than four logs (>99.99%) of prion proteins. The European Agency for the Evaluation of Medicinal Products has approved the use of a 50 nm rated virus filter to remove prions from intravenous immunoglobulin and US FDA now also allows such claims. Data on prion clearance by size exclusion membrane virus filters will be presented.

Ion-exchange column chromatography has also been demonstrated to remove prions from plasma protein solutions, however for reuse, the columns must be cleaned thoroughly to ensure multi-batch product safety. As an alternative to column chromatography, disposable ion exchange membrane chromatography is being explored to both remove prions and protect columns for reuse.

Application of Filtration Technologies for Prion Removal from Plasma Product Intermediates

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Prion clearance studies performed by the plasma-fractionation industry have demonstrated that process steps used in the manufacture of human plasma-derived therapeutic proteins may well be capable of removing the abnormal prion protein, PrP^{Sc}. In this study, the capability of various filter media including depth, charged-depth and nanofilters to remove PrP^{Sc} in plasma product intermediates was evaluated. Product intermediates generated from a FVIII/VWF process were spiked with two different preparations of abnormal prion protein: a microsomal fraction of hamster brain infected with scrapie agent; and purified PrP^{Sc}. Filtration trials were conducted at laboratory scale with prion removal assessed using the conformation-dependent immunoassay (CDI). Results obtained in this study demonstrate that depth and charged-depth filters are capable of removing up to 2.8 logs of PrP^{Sc}. Nanofiltration was also effective with >3 logs removal of PrP^{Sc} of both microsomal and purified preparations demonstrated. The implementation of specific filters into selected manufacturing processes has the potential to improve clearance of prion protein.

Validation and Technological Aspects of an Integrated Virus Clearance Platform

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The risk of viral contamination is a feature common to all biotechnological products derived from human or animal plasma and from *ex vivo* culture of mammalian cells. Modern manufacturing schemes employ complementary methods, frequently combining active virus removal with virus inactivation step(s). Overall viral titer reductions in excess of 12 $\log_{10}$ are given in some regulatory guidelines. Virus filtration and virus inactivation have traditionally been accepted as complementary and robust methods for viral clearance. Chromatography is also a widely accepted method for viral clearance as it removes viruses regardless of their size and morphology; however, chromatographic removal is contingent upon the chemical composition of the mobile phase and the isoelectric point of each virus species.

Sartorius has developed a novel platform that removes or inactivates viruses based on three orthogonal technologies: virus filtration by nanofiltration, virus inactivation by ultraviolet C (UVC) irradiation, and virus adsorption using membrane adsorbers. These can be used either separately or in combination.

The virus filter (Nanofilter) employs a novel, disposable Polyethersulfone membrane filter. This filter comprises a pleated configuration from 10" to 40" as a standard filter cartridge and 10" to 30" as a disposable MaxiCap®. Membrane qualification by an external virus lab has shown a >4 $\log_{10}$ titer reduction for small non enveloped viruses like porcine parvovirus and >6 $\log_{10}$ titer reduction for retroviruses. Tests were performed using PBS buffered BSA solutions of different concentrations. Membrane integrity of this virus retentive filter is evaluated rapidly following each use with a water-based integrity test. No other media as water is needed for the integrity test, therefore no validation of removal of other wetting agents like IPA is needed. In general, this virus retentive filter is suited for the downstream processing of cell derived- and animal as well as human plasma derived biopharmaceuticals. Scale down & spiking studies can effectively being performed using a 5.3 cm² capsules to minimize volumes and costs.

UVivatec, the continous-flow UVC reactor, designed by Bayer Technology Services, operates at 254 nm and eliminates the need for thin laminar flow films through use of a novel hydraulic spiral flow that generates dean vortices and therefore highly efficient mixing. This allows the dose of UVC irradiation to be delivered evenly and uniformly throughout the solution and, consequently, considerably shorter and homogeneous residence times. Small, non enveloped viruses like porcine parvovirus are especially sensitive to UVC irradiation. Typical flow rates for the inactivation of human IVIG at 35 mg/ml at process scale range from 2–3 hours for a 200 liter batch providing 4 $\log_{10}$ clearance of parvovirus. Proteins solutions including monoclonal antibodies and human plasma derived products are currently being investigated in preclinical trial work. The presentation will provide data from a biochemical assay of an

Immunoglobulin treated with UV light. The presentation will furthermore show data from first experiences with such inactivation technology.

The membrane adsorber technology platform features disposable membrane chromatography matrices consisting of stabilized reinforced cellulose membrane carrying functional groups such as ion exchange (anion exchange, cation exchange). The large pore size of these matrices allows highest flow rates without affecting binding capacity since the mobile phase has complete and immediate access to the solid phase. More than 4 $\log_{10}$ clearance for parvoviruses and 100% virus recovery during the elution process demonstrates its effectiveness and robustness. To support this, experiments were run with a monoclonal antibody solution produced from a CHO-based cell line with ABX-CHO2-P media. Experiments were run in 10 mM TRIS at pH 7. Results showed that elution could be used to recover the spiked virus load, an aspect essential for an adsorption process to claim effectiveness. Furthermore robustness studies have shown multiple use configurations as high as 1.000 cycles for a Sartobind Q membrane adsorbers.

Conclusion. The use or combination of these three virus removal or inactivation technologies, each with a separate principle of operation, offers significant flexibility towards developing new purification processes. Using them in combination should eliminate the potential threat from most if not all viral contaminants that may be present in crude feedstock, whether from human or animal plasma or the result of mammalian cell culture.

Development of an *in vitro* TSE Infectivity Assay: Application to Validation of Manufacturing Processes

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TSE are currently quantified by immunochemical assays or by in vivo infectivity assay (bioassay). Several cell lines have been reported susceptible to prion infection, including both neuronal and non neuronal cell lines. Here we describe the development of a simple and very sensitive in vitro transgenic cell based TSE titration assay whose estimated sensitivity is 4 ID50/ml. This assay was shown to be suitable for evaluating the TSE infectivity removal in plasma product manufacturing processes, using a 15 nm nanofiltration step as a model. Reduction factors correlated quite well with previously reported data obtained by bioassay and Western Blot. Interest of such an assay will be discussed as well as further developments for regulatory recognition.

Strategies for the Removal of Blood-Borne TSE Infectivity

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Animal and recent human cases of probable transmission of variant CJD through blood transmission have highlighted the risk of transmission of variant CJD through the blood supply. Universal leukoreduction of blood donations has been suggested as a precautionary measure against risk of human exposure to transmissible spongiform encephalopathy (TSE) infectivity from blood and blood products. However, recent studies performed with two in-line leukotrap systems, one for whole blood leukofiltration and the other for platelet-red cell leukofiltration, challenged with a full unit of blood from scrapie infected hamsters showed only approximately 40% removal of infectivity.

We present our recent data on our alternative strategy for the removal of infectivity based upon the inclusion of highly selective adsorbents for prion protein. Identification of such materials was initially based upon surveying vast combinations of chemical structures (combinatorial libraries) for structures that specifically bind to prion protein. These structures were synthesized onto inert supports which selectively capture the prion protein thus removing it from blood or blood-derived therapeutics. We present data on the ability of the lead adsorbents to selectively remove human TSEs (including variant CJD and prions from infected animal models) from red blood cells concentrates while leaving red blood cells unaffected. We discuss optimization studies for the selective removal of prion protein and possibilities for incorporation of our lead adsorbent into the processing of blood and blood-derivatives.

Development of this technology may lead to improvements in the safety of blood and blood-derived therapeutics. In conclusion, while this technology is broadly applicable to the selective removal of variant CJD and other blood-borne pathogens it is also applicable to the selective and highly efficient removal and subsequent purification of valuable blood-derived therapeutic proteins.

Detection of PrP^{Sc} with PrP^{Sc} Specific Binding Reagents without Proteinase K Treatment

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Two decades have passed since the BSE epidemic started in the UK, which caused the onset of vCJD in some individuals who consumed contaminated beef. Prion diseases have proven to be tenacious. Over time, there has been an increasing concern that prion diseases may be transmitted through transfusion of blood products. Indeed, recent reports of two cases of vCJD in recipients of blood from donors who were later diagnosed with the disease have underscored the presence of infectious prion in the blood. Since currently there is no reliable test to detect PrP^{Sc} in human blood, these reports have heightened the need for sensitive tests to safeguard donated blood and blood products. There are many challenges in developing such a test. First of all, the level of PrP^{Sc} in blood is believed to be lower than the detection limits of traditional immunoassays. Moreover, it is extremely difficult to induce antibodies specific for PrP^{Sc}. The commonly available antibodies mostly recognize denatured PrP or bind to both PrP^{C} and PrP^{Sc} without much discrimination. Without specific antibodies for PrP^{Sc}, most assays currently rely on the protease resistant nature of PrP^{Sc} and Proteinase K treatment to destroy PrP^{C}. What remains after Proteinase K digestion is taken as PrP^{Sc}. However, some studies indicate that PrP^{Sc} in the blood may be less protease resistant than that in the brain which could lead to a reduction in PrP^{Sc} concentration in blood samples following Proteinase K treatment and an apparent decrease in assay sensitivity. To overcome these challenges, we have developed a series of reagents that specifically bind PrP^{Sc} with high affinity by employing chemical and structural information regarding the prion proteins. Since it is very difficult to secure authentic vCJD patient blood, a panel of human plasma spiked with PrP^{Sc} from homogenates of infected mice or vCJD patient brains has been constructed for use in assay development. Authentic blood samples from infected hamster and sheep have also been evaluated. Our optimum goal is to provide an automated high throughput assay for screening PrP^{Sc} in donated human blood and blood products without Proteinase K treatment.

Biochemical and Pre-Clinical Characterization of a New 10% Liquid Triple Virally Reduced Human Intravenous Immune Globulin (IGIV, 10% TVR)

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The purity, efficacy, safety, pharmacokinetics and toxicity of a new 10% liquid intravenous immune globulin (IGIV,10%TVR) from human plasma was investigated and compared with Gammagard S/D, a licensed lyophilized IGIV.

Purity is demonstrated by a γ-globulin content of ~100%, by absence of amidolytic (plasmin-like) activity and by low content of other Ig-classes. IgG subclass distribution was found comparable to the physiologic range in normal plasma.

The protective activity against systemic bacterial infections of IGIV,10%TVR in mice is at least as good as of Gammagard S/D. This result is supported by the broad antibody spectrum against bacteria and viruses. The functional integrity of the IgG molecule (>90% functional intact IgG) in IGIV,10%TVR is determined by protein A Sepharose and the IgG monomer and dimer content (~100% of the peak area) by High Performance Size Exclusion Chromatography.

The blood pressure lowering effect in spontaneously hypertensive rats and the bronchospastic effect in guinea pigs are used as indicators of the anaphylactoid potential. The influence of the products on vital functions like cardiovascular, respiratory and blood coagulation variables is tested in dogs. In these safety studies and in thrombogenicity studies carried out in rabbits, no adverse effects of IGIV,10%TVR are observed. Pharmacokinetic studies in rats show no statistically significant difference between Gammagard S/D and IGIV,10%TVR.

In acute toxicity studies carried out in mice and rats, IGIV,10%TVR compares favorably to Gammagard S/D.

In conclusion, liquid IGIV,10%TVR combines high purity with excellent efficacy, safety and tolerability in pre-clinical studies.

Efficacy and Safety of Subcutaneous Immunoglobulin Replacement Therapy at Home in Patients with Primary Immunodeficiency Diseases: Combined Analysis of Two Clinical Studies, One in North America and One in Europe

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Introduction

Intravenous infusions of immunoglobulin (IVIG) every 2–6 weeks have been the standard therapy for patients with primary immunodeficiency diseases (PID). Complications of IVIG therapy limit the use of IVIG at home. Weekly self-administered subcutaneous immunoglobulin infusions (SCIG) at home are about to become an alternative therapy regimen. We evaluated a 16% pasteurized, preservative-free liquid human IgG preparation intended for subcutaneous use in two clinical studies with regard to safety and efficacy.

Methods

In two prospective studies, one in the US and Canada (NA study), the other in Europe and Brazil (EU study), 125 PID patients between 3 and 74 years of age self-infused SCIG (Vivaglobin®, ZLB Behring) on a weekly basis at home. The patients began SCIG therapy one week after their last IVIG infusions, and entered a 3-month wash-in/wash-out period followed by the 12-month efficacy period in the NA study and a 6-month efficacy period in the EU study. Clinical endpoints included the annual rate of serious bacterial infections (SBI), annual rate of all types of infections, as well as serum (S) IgG levels observed during the study. Safety variables comprised local and systemic reactions, laboratory investigations and vital signs.

Results

A total of 5,953 infusions were administered to 125 patients in the course of the two studies. The patients received a weekly median dose of 158 mg/kg in the NA study and 89 mg/kg in the EU study during the efficacy phase of the studies. Only three SBIs (pneumonias) were reported during the efficacy phase in the two studies; two in the NA study and one in the EU study, resulting in an identical annualized rate of 0.04 SBI per patient. The annualized rate for any kind of infection was similar in both studies with 4.4 episodes/patient year in the NA study and 4.3 episodes/patient year in the EU study. Sinusitis and upper respirator infections were the most frequently reported types of infection. Mean S-IgG levels increased from 837 mg/dl

to 922 mg/dl at 101% of the previous IGIV dose in the EU study and from 786 mg/dl at start of SCIG to 1040 mg/dl during the efficacy phase at 136% of the previous dose in the NA study. No study drug related serious adverse events were reported in any of the studies. Local injection site reactions, of mostly mild or moderate intensity, dropped rapidly in both studies from initially 85% to about 40% during the course of the NA study and from 65% to about 20% in the EU study. The quality of life improved in SCIG treated patients in two studies.

Conclusion

Two major clinical trials have demonstrated that weekly self-administration of SCIG with a 16% IgG preparation is safe and effective in patients with PID, resulting in normalized stable IgG levels and providing satisfactory protection against severe bacterial infections.

Effective Prevention of Ischemia-Reperfusion Injury with Plasma Proteins

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Ischemia-reperfusion injury (IRI) accompanies various clinical conditions, such as revascularization procedures in cardiac infarction and stroke, angioplastic surgery and organ transplantations. IRI leads to apoptotic and necrotic cell death and inflammation and causes significant morbidity and mortality. By using a well validated mouse model of kidney IRI we have tested various plasma proteins for their efficacy in protection against IRI.

We have found that two plasma proteins, apotransferrin (apo-Tf) and alpha-1 acid glycoprotein (AGP), effectively protected the kidney function (1,2). Redox-active iron was liberated to circulation from the ischemic kidney and it was effectively bound by administered apo-Tf but not by holo-Tf. Similarly, apo-Tf but not holo-Tf prevented leukocyte infiltration. However, both transferrin forms prevented complement activation in the ischemic kidney, indicating iron-dependent and independent protective mechanisms.

AGP also effectively prevented functional impairment of the kidney after IRI. AGP but not apo-Tf effectively prevented apoptosis in the ischemic kidney. Interestingly, a transgenic mouse strain constitutively overexpressing AGP was not protected from IRI whereas administration of exogenous purified AGP was protective. Removal of fucosylated forms from plasma AGP did not abolish the protective effect of AGP.

Recent studies from Fred Hutchinson Cancer Research Center (3) have also indicated highly potent cytoprotective effects for transferrin which are at least partially independent of iron binding. Potent cytoprotective effects have been also described by other research groups for AGP, such as effective prevention of inflammation after resuscitation from hemorrhagic shock (4). Thus, these two glycoproteins which can be readily purified from side fractions of the Cohn process by ion exchange chromatography may offer significant therapeutic potential with low toxicity.

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WILFACTIN ® – Development and Clinical Evaluation of a Triple-Secured and High-Purity von Willebrand Factor Concentrate

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For many years cryoprecipitate has been the mainstay of replacement therapy in von Willebrand disease (VWD); FVIII/VWF concentrates are used since the 80's in severe VWD patients. A more specific approach using a highly purified VWF concentrate with a low Factor VIII content (< 10%) (FACTEUR WILLEBRAND-LFB®) has been used in France since 1989 with a good efficacy/safety profile (1). In order to enhance the biological safety of this product regarding especially non-enveloped viruses and transmissible spongiform encephalopathy agents, a new-generation product named WILFACTIN® was developed with 3 viral safety steps : solvent-detergent treatment, dry heat (80 °C during 72 hours) and nanofiltration 35 nm. In vitro studies demonstrated that nanofiltration and dry heating did not modify VWF multimeric structure neither the in vitro functional activity of VWF present in WILFACTIN®. Two prospective clinical trials were conducted in parallel: in France (French trial) and in Europe (European study) including a total of 62 patients : 26 participated in the pharmacokinetic part, 50 in the efficacy/safety section and 14 in both parts of the trials. The French pharmacokinetic trial showed the bioequivalence between WILFACTIN® and FACTEUR WILLEBRAND-LFB® in 8 type 3 patients in terms of VWF:Rco, VWF:Ag, and FVIII, showing that nanofiltration and dry heat did not impair the pharmacokinetic profile of the product. The European pharmacokinetic trial compared WILFACTIN® to a dual concentrate in 17 patients with different VWD subtypes: the bioequivalence was demonstrated in terms of VWF:Rco, VWF:Ag showing that FVIII content does not modify the pharmacokinetic profile of the product. These studies also showed that the half-life was about 12 h with a recovery of 2 IU/dl/IU/kg. A progressive rise of endogenous FVIII plasma levels was observed with a mean level of 40% 6 hours after infusion with a synthesis rate of 6 IU/dl/h; 113 bleeding episodes were treated in 25 patients including 18 severe episodes in 11 patients and 121 moderate in 21 patients. The outcome was excellent or good in 89% (assessed at 24 hours or at the end of treatment) for severe episodes, and was excellent or good in 88% at 6 hours and 89% at 24 hours for minor episodes. For scheduled surgical procedures an infusion of WILFACTIN® was performed 12 to 24 hours before surgery in order to reach a FVIII plasma level > 60%, 108 procedures were analysed in 44 patients (14 type 3) with 100% excellent or good results. During the clinical development more than 5 million units of WILFACTIN® were administered representing about 2500 infusions; no serious adverse reaction related to the product occurred; 6 mild transient reactions occurred possibly related to the study drug. WILFACTIN® is a new generation, triple secured highly purified VWF with a low FVIII content. Its efficacy and safety are demonstrated for the treatment of VWD when desmopressin is ineffective or contra-indicated.

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Successful Treatment with Sandoglobulin in Patients with Guillain-Barré Syndrome

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Guillaine-Barré-Syndrome (GBS) is the most common acquired autoimmune acute demeyelinating polyneuropathy characterized by acute ascending motor weakness, areflexia, and mild to moderate sensory abnormalities. Treatment for GBS consists of two approaches, which have approximately equal effectiveness, both shortening recovery time by about half and promoting remyelination. Immunoglobulin therapy may help by neutralizing circulating myelin antibodies and down-regulating proinflammatory cytokines, including gamma-interferon and the complement cascade. Plasma-exchange therapy or plasmapheresis helps by decreasing autoantibody production and increasing removal of immune complexes.

We performed a randomised controlled multicenter study in 383 patients with GBS from 1993 until 1995 to demonstrate that high-dose intravenous Sandoglobulin is equivalent or superior to plasma exchange. The results showed that immunoglobulin given at 2 g/kg in 2–5 days is equivalent to PE in reducing the amount of disability at 4 weeks after treatment and that adverse events of immunoglobulin treatment are less frequent than with PE (1). These findings confirm the conclusion of the first trial of PE and immunoglobulins in GBS performed by The Dutch Group (2).

In order to demonstrate the objective efficacy of high-dose intravenous immunoglobulin we collected all the data from the French Cooperative Group (3), which compared standard care with PE. A metaanalysis of the GBS study data and the data of the French study was performed. The objective of this analysis was to assess the comparability on the population included in both studies and to compare efficacy results. Main focus of the efficacy analysis was the time to restart of unassisted walking. The metaanalysis permits an evaluation of high-dose immunoglobulin compared to the best standard care.

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Development of a Method to Detect Contaminant Proteins in Immunoglobulin G Preparations IV. A Proteomics Approach

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CSL Bioplasma manufactures a range of therapeutic proteins extracted from human plasma which include an intravenous immunoglobulin G (IVIG) product (Intragam P). Adverse events, in-patients treated with IVIG solutions might in some cases be related to the presence of contaminating proteins. This poster reports on a comprehensive and sensitive method for identifying contaminant proteins in IVIG solutions. The method is based on a proteomic approach, involving 2D-gel electrophoresis and protein identification by mass spectrometry. One of the difficulties associated with such an approach is the over-abundance of IgG molecules (>99% of total protein), which preclude contaminating proteins being resolved on 2D-gel electrophoresis. To circumvent this, IVIG was prepared by passage through a Protein G column. The flow-through was then concentrated, prior to loading onto an Agilent multi-affinity (MARS) column. The MARS chromatography removed both residual IgG and other prevalent plasma proteins (eg. albumin, IgA, transferrin, haptoglobin). The flow-through from the MARS column was then concentrated prior to 2D-gel electrophoresis. Key spots were then excised, trypsin digested and identified using time of flight mass spectrometry. It is viewed that this approach can be used to to detect and identify trace proteins in protein therapeutics. This would have considerable use in establishing comparability between products and facilitate justifing process changes. By using affinity systems which are sensitive to the structural integrity of the molecule, this technique can also be applied to establishing the effect of processes directly on the molecule of interest. With the use of Protein G which interacts with the Fc portion of the IgG molecule, the effect of pasteurisation (60 °C for 10 hours) on the IgG molecule was investigated. The 2D-gel results indicated that pasteurisation caused some IgG molecules to be denatured in a manner that prevented binding to the Protein G.

An Abbreviated Affinity Chromatography Cascade Process for FVIII/von Willebrand Factor Complex and Immunoglobulin G

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A novel affinity adsorbent has been designed with a fully synthetic (NCE) ligand that selectively binds the Factor VIII/ von Willebrand Factor Complex while allowing essentially quantitative recovery of the immunoglobulin G fraction of recovered and source plasma. IgG is recovered from the Cascade sequence using MAbsorbent A2P®, with or without prior removal of the albumin fraction. The Cascade intermediate can be further processed to remove remaining protein impurities such as IgM and IgA as well as traces of HSA and transferrin. The downstream process is also applicable to other IgG intermediates of the Cohn process.

A Combined Proteomics and Well-Characterised Approach to Comparability for the Development of Cascade-Fractionated- and other Plasma-Derived Products

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As old technology recedes and new technology which provides increased yield and processing efficiency takes its place, change must occur and the need for new safety assurance increases. The genesis of follow-on (generic) plasma products and fractionation in the developing world also drives a need for process safety and knowing the composition of complex biological products for improved communication with regulatory authorities. This assurance will be based on a new paradigm of process and product characterization, comparability studies and process analytical technologies utilizing a risk-based approach. Using process intermediates as illustrations, this presentation will discuss important aspects of raw material, retains, reference standards and plasma protein characterization necessary for old products that must change, intermediates that must be exchanged and new products that must be developed.

Manufacturing Processes of Plasma-Derived Products Reduce Emerging Pathogens

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Emerging zoonotic viruses as West Nile virus (WNV), SARS coronavirus, and avian influenza virus as well as prions, the infectious agent causing neurodegenerative disorders such as scrapie, bovine spongiform encephalopathy, Creutzfeld-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (vCJD), have raised concern about their potential presence in blood/plasma. However, so far only WNV has been clearly demonstrated to be transmitted via blood transfusion. Virus inactivation methods as pasteurisation (heat treatment in aqueous stabilised solution at 60 °C for 10 hours) and removal methods as nanofiltration and precipitation are evaluated for their capacity to reduce these emerging infectious agents. The studies performed by our group demonstrated that WNV was inactivated very effectively by pasteurisation in different plasma derivatives, resulting in an inactivation factor of more than 7 $\log_{10}$ and that BVDV (bovine viral diarrhea virus, as WNV a member of the family Flaviviridae) is a suitable model virus for WNV. Furthermore, coronaviruses and influenza viruses of both avian and human origin were highly susceptible to inactivation by pasteurisation, resulting in complete inactivation of virus infectivity within a short period of time. After establishing a parvovirus B19 (B19V) infectivity assay we could demonstrate effective inactivation of this virus by pasteurisation of intermediates of several plasma products. Based on these results, the suitability of animal parvoviruses employed in virus validation studies as model viruses for B19V has to be critically assessed. In addition, nanofiltration is effectively removing viruses and prions due to size exclusion. Prions, resistant to methods employed during the production of plasma proteins for virus inactivation, are removed from the desired protein concentrate by different adsorption and precipitation steps as well as nanofiltration and are inactivated by cleaning and sanitisation methods routinely used during the production of plasma-derived products. In conclusion, plasma-derived products have multiple strong measures to help assure the safety of these products with regard to emerging pathogens including donor/donation selection and plasma pool testing, efficient pathogen inactivation and/or elimination steps in the manufacturing process, and a thorough batch-to-batch segregation.

Screening of Industrial Plasma by HBV NAT: Increase in Viral Safety?

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HBV infection follows very distinct pattern such that HBsAg screening of single donations comes close to the sensitivity level that is achievable by HBV NAT carried out in minipool format. Pooling of individual donations for NAT to minipools of up to 500 members, however, still is a prerequisite for PCR testing in view of the high number of samples involved and the absence of automation. Due to the relatively small difference in sensitivity between the two assay systems HBsAg screening and HBV NAT in minipool format, the contribution of screening of industrial plasma for HBV by Nucleic acid Amplification Technology (NAT) to the safety margin of plasma for manufacturing has been under debate since the introduction of such screening programs.

However, in screening HBV positive donations are interdicted by HBV NAT that were not detected by HBsAg testing. This can be explained by the existence of HBV escape mutants, which are not detected by HBsAg tests and by the fact that HBV NAT, even in minipool format, is still more sensitive than several of the HBsAg tests commercially available. Consequently, the inclusion of HBV NAT into the NAT screening program for plasma for manufacturing is justified because it increases the viral safety margin. This view will be supported by an analysis of data derived in 10 years of routine HBV NAT screening of plasma for manufacturing.

Study on the rHSA Quality Specification

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rHSA derived from *Pichia pastoris* has been purified and formulated as pHSA substituted. Tentative quality specification was established according to rHSA's characteristic. Biochemical characters of rHSA compared with pHSA were broadly investigated. Purity including residual host protein, DNA and carbohydrate was analyzed by different effective method. Most importantly, pre-clinical animal experiment results have been revealed that rHSA developing by NCPC was functionally identical to pHSA.

Study on Serological Reaction to SARS-CoV of IVIG and Original Plasma from Qualified Donors

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Objective
Exploring the serological reaction to SARS-CoV to see if there is a crossing reaction between common coronal virus infection and SARS-CoV infection, or if there is an asymptomatic SARS-CoV infection in the qualified donors, and whether the serological reaction to SARS-CoV indicates the ability of neutralizing to the SARS-CoV.

Method
Screening the positive plasma from the qualified donors using anti-SARS CoV IgG ELISA approved by SFDA. 5%IVIG was produced from the pooled positive plasma by the cold-ethanol method. The titer of anti-SARS CoV IgG of IVIG was detected by the anti- SARS CoV IgG ELISA, indirect Immunity Fluorescence Assay (IFA) and Viral Neutralizing Plaque Assay (VNPA) of SARS-CoV.

Result
ELISA shows 5.64% of Plasma samples from 7248 qualified donors have a positive reaction. The titer of antibodies against the SARS causing coronavirus is 1:166 for a single positive plasma sample, and 1:30 for a single negative plasma sample by VNPA of SARS-CoV. IVIG made from pooled positive plasma gives positive results to both ELISA and IFA, but a negative one to VNPA of SARS-CoV.

Conclusion
The single plasma sample with high titer of anti-SARS CoV IgG by ELISA shows a high titer of anti-SARS CoV neutralizing antibody both in the indirect IFA and VNPA of SARS-CoV. Further investigations are needed to explain why IVIG made from pooled positive plasma that was anti-SARS CoV IgG positive by ELISA give positive results to both ELISA and IFA, but a negative one to VNPA of SARS-CoV and whether the IgG in IVIG that gave positive serological reactions have a protective immunity to SARS-CoV or not.

Characterization of IgG Fragments in Liquid Intravenous Immunoglobulin Products

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Background

Intravenous immunoglobulin (IVIG) products formulated as a liquid instead of a powder have become commercially available. Preferably, such liquid products should not alter (fragment formation, polymerisation) after storage outside the refrigerator. Therefore, a thorough characterization of IgG at various storage temperatures is required.

Study Design and Methods

Storage experiments with liquid IVIG products from five manufacturers were performed at 4, 25 and 37 °C and IgG fragments were analyzed.

Results

Storage of liquid IVIG products at 4 °C resulted in negligible alterations, while an increase of IgG fragments was observed after prolonged storage at elevated temperatures. SDS-PAGE and Western blot analysis of five liquid IVIG products revealed three IgG fragments (12, 26 and 54 kDa) in all products. Fragments of similar molecular mass were produced upon incubations of IgG with blood-derived proteases. N-terminal amino acid sequencing revealed the cleavage site of these fragments, suggesting human neutrophil elastase to cause the 12 kDa fragment. The presence of elastase in liquid IVIG was confirmed by ELISA. The origin of the 26 kDa and 54 kDa fragments, both with an aspartic acid residue at the cleavage site, could not be determined unambiguously.

Conclusion

IgG fragmentation in liquid IVIG is negligible when stored in the refrigerator. Only after prolonged storage at elevated temperature does proteolytic degradation of IgG become apparent.

Characterization of Plasminogen Removal Gel

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Plasminogen Removal Gel is a new adsorbent for use both in laboratory and industrial scale. The new adsorbent has the ligand trans-4-(Aminomethyl)cyclohexanecarboxylic acid coupled to a Sepharose 4 Fast Flow matrix.

This poster presents some characteristics of Plasminogen Removal Gel, eg. pressure / flow properties in a scale-up column and stability under different conditions.

The use of the new adsorbent will be demonstrated in an application where plasminogen is removed from a protein mixture containing (among others) fibrinogen and plasminogen, with almost complete removal of plasminogen and high recovery of fibrinogen.

Removal of Viruses and Pathogen Agents in the Downstream Processing of Plasma and Biopharmaceutical Products by Nanofiltration with Planova® Filter

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During the last decade nanofiltration has been implemented successfully to production processes of plasma derived products and biopharmaceutical product for improvement of virus safety. Nanofiltration has also the capacity to improve the safety regarding the potential risk of prion disease transmission via medicinal products.

This poster is to present the technology of nanofiltration by Planova® filters under different parameters. Several viral validation studies are introduced, which show the efficient clearance of a wide-range of pathogen particles.

The Planova® filter is reproducible and validatable. Additionally due to membrane material of regenerated cellulose manufactures establish mild purification productions even on high protein application.

A high degree of virus and prion safety can be achieved and nanofiltration has become a widely accepted technology to purify plasma derived and biotechnology products from possible contaminants.

Advantages of the EBA Method for the First Stage Chromatography Plasma Proteins Re-Purification

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The manufacturing of the blood plasma products in Ukraine is founded on exclusive using of the alcohol Kohn's method or its modifications. The most real transition on manufacturing on pharmaceuticals from chromatographically purified and virus-safe plasma proteins is Kohn's fraction re-purification by chromatography methods as with economic, technological, so and with humanitarian standpoint.

However before appearance and broad approbation the EBA chromatography technological difficulties of the re-purification of Kohn's fractions were introduced resistless. For instance, the Factor VIII separation was implied the cryoprecipitate dissolutions, which not always managed because of fibrinogen-fibrin coagulation, which concentration in this fraction was around 80%. The problems of un-solving of the essential part fraction III are expected by technologist in an effort re-purify the IgG. Such conditions require additional and complicated manipulations (filtration of crude particulars, centrifugation, salt precipitation, dissolution, diafiltration for alcohol removing, etc.) and bring essential production expenses, as well as quick and significant proteins activity loosing.

Our studies have shown that introduction to purification technological chain of the EBA method on the first stage allows to avoid the majority of the problems, in accordance with possibility of coagulation or incomplete faction solution. It is suggested that essential increasing of the volume of the solution of the cryoprecipitate fraction greatly reduces formation a fibrin-fibrinogen polymer, which additionally do not prevent the chromatography in extended bed, under which FVIII is captured by STREAMLINE Q XL gel on 93% under practically full saving its activity. In the case of the bad dissolved Kohn's faction III, for instance, the using STREAMLINE SP XL allows effectively to capture the IgG from crude solution, but also pay no regard to presence in solution 1–5% of the alcohol, as well as indissoluble conglomerates of denatured proteins.

A direct comparison of both running and investment cost for the process reveals a cost benefit for the STREAMLINE approach. A cost advantage of about 46 (FVIII) and 52% (IgG) was noted for the STREAMLINE process. This study has shown that a fully optimized STREAMLINE process using STREAMLINE Q XL to capture FVIII from cryoprecipitate solution and STREAMLINE SP XL to capture IgG from large volume alcohol Kohn's fraction III solution was more economical than process involving several types of microfiltration and packed bed ion-exchange chromatography. Further this process will agree history, economic, technological and humanitarian problems of the

Plasma Economics – A Brighter Outlook in the Short Term but Changing Fundamentals Suggest Future Challenges

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The plasma industry appears to be emerging from a cyclic downturn with signs of product tightening. The key factor that contributed to the downturn was an oversupply of product as plasma collections increased and fractionators facilities expanded.

With an estimated 3 m litres of plasma taken out of the industry, the outlook for corporate fractionators has improved. However, looking further forward, there are key questions:

1) can yield improvements balance the higher demand for IVIG products against slower albumin and pd Factor VIII momentum
2) impact of recombinant products on plasma economics
3) new products to improve revenue per litre of plasma
4) potential costs of additional plasma screening tests
5) impact of industry consolidation as number of global players goes from 8 to 5

Electrophoretic Analysis of Clotting Factor VIII Concentrates

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Different preparations of clotting factor VIII concentrates have been investigated. The aim was to detect von Willebrand factor with electrophoretic method. The plasma derived factor VIII concentrates were Agemphil A (National Research Centre of Haematology, Moscow, Russia), Hemophil M (Baxter Healthcare Corporation) and Koate-Dvi (Bayer Corporation, USA).

Electrophoretic analysis was performed with Phast System from Pharmacia-LKB, Uppsala, Sweden). The plates of polyacrylamide gel with gradient 4–15% were used (Pharmacia Biotech). On the first stage of experiment electrophoresis under non-reducing conditions was carried out. On the second stage the electrophoresis was performed under reducing conditions with sodium dodecyl sulphate (Serva), 2-mercaptoethanol (Serva) and previous heating to 1000 °C during 5 minutes. Molecular weight markers were purchased from Pharmacia Biotech (High Molecular Weight Electrophoresis Calibration Kit, 67–669 kDa) and from Sigma (High Molecular Weight Range, 36–205 kDa). The overall concentration of protein in the preparations ranged from 0,25% to 1,25%. The samples stained with Coomassie blue R 250 (0,1%, Merck).

The results of comparative analysis of factor VIII concentrates confirmed the presence of von Willebrand factor in the Agemphil A. The accurate quantitative measurement of the protein constitution of Agemphil A is to be done.

The Possibility of One-Stage Clotting Method for Determination of Factor VIII Activity in the Purified Antihaemophilic Concentrates

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In the Hematology Centre of Science of Russian Academy of Medical Science, Moscow, a method of isolation purified, twice virus inactivated antihaemophilic preparation of the factor VIII from cryoprecipitate of human plasma has been worked out. The factor VIII activity was supervised by a one-stage clotting method and a method with use of a chromogenic substrate (chromogen method).

The aim of work was the choice of such one-stage clotting test-system for determination of the concentrate factor VIII activity which would allow to receive comparable results with a chromogen method. The activity of the factor VIII was measured in keep with advisable NIBSC technique, using for construction of calibrating curve 6th and 7th International Standards Factor VIII Concentrate. Both the standard and tested samples have been preliminary dissolved with haemophilic plasma with normal level of vWF factor, and then with imidasole buffer containing 1% of albumin. Reagents Dapttin (Baxter), Pathromtin (Dade-Behring), Platelin L (Organon), Alexin HS and APTT Reagent (Sigma), STA APTT Kaolin (Stago), Erilyd-kaolin and APTT-ellagic acid (local production) were used as APTT-reagents.

At research it has been received, that the factor VIII activity in the same sample of antihaemophilic concentrate National Standard (the standardized purified preparation sample) does not depend on a method of reception factor VIII deficient plasma (either haemophilic plasma, or artificially depleted plasma). The factor VIII activity of National Standard determined by one-stage clotting method with various APTT-reagents against International Standards, changes from 17.0 up to 26.0 IU/ml. However the factor VIII activity determined in the same sample with the help of a chromogen method and the same International Standards made 13.6 ±0.86 IU/ml. Closer to this magnitude were the values of the activity, re-ceived with the help of Dapttin (Baxter), Pathromtin (Dade-Behring) and Erilyd-kaolin (local production). The modification of test-system Erilyd-caolin for hospholipid concentration from 0.2 to 1.0 mg/ml decreased value of factor VIII activity from 19.6 to 16.0 IU/ml. The change ionized Ca level this system from 25 to 50 mM resulted in decrease factor VIII activity to 13.4 ±1.3 IU/ml. This clotting value was close to the activity of the factor VIII have been received by chromogen method. During factor VIII activity determination in the antihaemophilic concentrates obtained in Hematology Centre by one-stage clotting method with Erilyd-caolin test-system and use the attestated National Standard as calibrator there was no statistically significant difference between the clotting and chromogen method values.

The resulted data testify to a possibility to use one-stage clotting method for definition of activity of the factor VIII in solutions of purified antihaemophilic preparation of the factor VIII.

Is Citrate Deficiency a Common Problem in Transfusion Medicine?

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Those of us involved in plasma fractionation have long suspected that blood should be collected in a higher concentration of citrate. Evidence for this is the activation of clotting factors and observation of microclot formation with the passage of time. Citrate deficiency was suspected, because the final yield of commercial fVIII concentrates was only 25–35% of the fVIII present in plasma although the beginning value was ±100%. This suggested that the procoagulant fVIII was "activated" and therefore unstable. The development of "Supercryoprecipitate" supported these observations. By simply increasing the amount of citrate in the plasma fraction, virtually 100% of the fVIII present in plasma was obtained. To directly demonstrate citrate deficiency, 34 freshly drawn, citrated plasma samples were obtained (minimum volume 4 ml). The samples were divided into four 1.0 ml aliquots. To respective sample aliquots, 0.013 ml, 0.020 ml and 0.027 ml of concentrated potassium citrate solution (75% w/v) was added, giving 1%, 1.5% and 2% added citrate. The aliquots were incubated at 21 °C for a maximum of ten days. Each aliquot was assayed daily for the formation of d-Dimers using the DimerTest latex agglutination assay (American Diagnostica, Stamford, CT). At day 6 of incubation, 41.2% (14) of the non-citrated aliquots were positive for the presence of d-Dimers. By day 7, 100% (34) of the non-citrated aliquots were positive. None of the citrated aliquots had turned positive by day 10 of the incubation period. This evidence suggests that a modest increase in the level of citrate can stabilize plasma proteins and offer superior plasma derived products.

Blood Bank Preparation of Safe Immunoglobulin Concentrate

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A method to obtain maximum yields of cryoprecipitable proteins ("Supercryoprecipitate") has been described collecting plasma in increasing amounts of sodium citrate. Following removal of the cold precipitable proteins (fibrinogen, fVIII, fibronectin, etc), addition of more citrate to the supernatant plasma precipitates immunoglobulin in a linear fashion. At a final concentration of ±15% citrate, virtually all gamma globulin (IgG) is precipitated in its native state which can be pooled and used as is, or further utilized in commercial fractionation. Since aseptic meningitis is a known complication of IVIg administration, the "Cascade Iodination" can be used as an added measure of safety for pathogen inactivation. IgG yield was measured by radial immunodiffusion (86.3% ±12.5%). Protein electrophoresis showed contamination of the product by albumin (9.8% ±3.3%), alpha globulins (1.6% ±0.6%) and beta globulins (3.9% ±1.2%). 1300 ml of gamma globulin was prepared as described above from 5 liters of fresh frozen horse plasma. An equine veterinarian with a special interest in immunotherapy infused product into 2 horses (650 ml in each) over a period of 90 min. with no discernable adverse effects. It would appear from these studies that gamma globulin concentrate could become another blood bank product, perhaps eliminating serious side effects because this "native" protein is not altered by alcohol. Excess citrate, like iodine and iodide, is removed by the "Cascade Iodination" step.

Innovative Method for Producing Therapeutic Proteins in Plants

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In response to the growing demand of the production capacity for virus safe therapeutic proteins, UniCrop provides a low-cost platform for the production of therapeutic proteins/ monoclonal antibodies in a fully contained plant-based system. Since plant-systems are capable of expressing complex proteins this technology offers also the tool to produce non-animal origin ligands for affinity purification of therapeutic proteins.

The transgenic seeds are produced in a greenhouse and the sprouting of the seeds, which is the protein production stage, takes place in an air-lift germination tank. The use of a greenhouse and a germination tank enables good control of the process, consistent product quality, several harvests per year, higher seed yields per harvest. At the same time the environmental risks are minimized.

The use of sprouts enables a flexible process, since the seeds can be stored and used upon demand. The platform enables also a clean process (seeds can be surface sterilized), and a lower volume of fresh biomaterial needs to be processed.

The production host is Camelina sativa, an ancient food crop plant. Camelina seeds have a high storage protein content, the species is no more widely used for food and it is highly self-pollinating. A proprietary transformation method for Camelina sativa has been developed by UniCrop. The model proteins produced so far in laboratory and bench scale include monoclonal antibodies, immunoglobulin fusion protein, human serum albumin as well as enzymes. The protein yields obtained are 1–2 grams of protein of interest per one (1) kilogram of seeds used. Rintekno Oy has performed a concept design to define the process and plant to produce proteins in tens of kilograms scale.

Patents

WO 01/41559 :A process for converting storage reserves of dicot seeds into compositions comprising one or more gene products.

WO 02/38779: A transformation system in Camelina sativa.

WO 02/06498: Molecular control of transgene segregation and its escape by a recoverable block of function (RBF) system.

WO 02/064801: Molecular control of transgene escape by a repressible excision system.

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UVivatec – A Scalable Technology for UV Virus Inactivation in Laboratory and Production

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Contamination of biological fluids by viruses is a growing concern in all pharmaceutical and bio-processes. The virus may either be co-isolated from a natural source or introduced during a biotechnological process. A couple of effective methods for depleting or inactivating viruses are available, but there is still a great need for additional methods as the classic treatment does not address all types of virus effectively. Irradiation with UV-C light is especially known as a very suitable method of inactivating non-enveloped, heat-stable viruses, like parvovirus. But an effective application in technical scale of pharmaceutical productions could not be realized until now.

The UVivatec technology solves the technological problems by a superior kind of flow through apparatus. In a single-use module the fluid moves in a helically wound tube around an UV-C light source. This leads to a homogenous irradiation with a very small residence time distribution and therefore optimal inactivation conditions with low product damage.

It will be shown how this technology is realized in practice. Besides a bench-scale unit for small scale applications and laboratory use (flowrates from 2 to 20 l/h), there is a full scale GMP apparatus for production. In production up to 120 l/h might be processed per module. The scalability of the inactivation of non-enveloped viruses can be shown.

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Notes

imagination at work

CSL

Bioplasma

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