Critical Quality Attributes for Blood Products

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Disclaimer

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Blood

• A physiological system of interacting components, in which the activity of each component is a function of all the others; a modification in one induces changes in all.
Definition under 21 CFR 640

- Whole Blood - Subpart A, 640.1- 6
- RBCs - Subpart B, 640.10 - 17
- Platelets - Subpart C, 640.20 - 27
- Plasma - Subpart D, 640.30 - 34
- Cryoprecipitated AHF - Subpart, 640.50 - 56
- Source Plasma - Subpart G, 640.60 - 76
Examples of Plasma-Derived Products

- Antihemophilic Factor
- AHF/vWF Complex
- Anti-inhibitor Coagulant Complex
- Coagulation Factor IX
- Factor IX Complex
- Fibrin Sealant
- Thrombin
- Protein C
- α-1 Proteinase Inhibitor
- Anti-thrombin III
- C1-Esterase Inhibitor

- Albumin
- Plasma Protein Fraction
- Immune Globulin: Intravenous and Intramuscular
- Cytomegalovirus Immune Globulin
- Hepatitis B Immune Globulin
- Rabies Immune Globulin
- Rho(D) Immune Globulin
- Tetanus Immune Globulin
- Vaccinia Immune Globulin
- Varicella-Zoster Immune Globulin
Characteristics of Plasma-Derived Protein Therapeutics

- Human protein expressed in active form
- Correct modifications (e.g. glycosylation)
- The “Gold Standard” to which relevant recombinant proteins are compared
- Rapid process development feasible
- Source impurities that do survive the purification process are of human origin
Characteristics of Plasma-Derived Protein Therapeutics

- Poorly defined starting material
  - Source Plasma vs. recovered plasma
  - Different pool size
- Lack of robustness of manufacturing process
  - "Minor" changes with "major" impact
- Generally of low purity
- "Impurities" may be active, may affect activity, immunogenicity
- Often highly complex and heterogeneous proteins
- History of viral transmission with AHF (Human)
Source Material

- Source Plasma
- Unlicensed recovered plasma
- Plasma for fractionation must be properly processed from the time of collection
- Significant effort to prevent the presence of disease-causing agents or contaminants
- PPTA & FDA agreed that final product lots will not represent more than 60,000 donors
Viral Safety

• Screening donors and testing of source material according to FDA requirements
  – Plasma is obtained from U.S. plasma/blood centers only
• In-process test ensures that the level of parvovirus B19V-DNA in the manufacturing pool does not exceed $10^4$ IU/mL
• Validation of viral clearance steps in the manufacturing process
• Assessment of viral safety records: viral transmission during clinical trials or active surveillance of licensed products elsewhere
Requirements for Viral Clearance for Plasma-Derived Products

- Two dedicated orthogonal viral clearance steps with significant viral clearance capacity (> 4 logs)
- Total Log Reduction Factor (LRF) for enveloped viruses > 10 logs
- Total LRF for non-enveloped viruses > 6 logs
The Cohn-Oncley Blood Plasma Fractionation Process

Plasma

freeze, thaw

Cryoprecipitate

ANTIHEMOPHILIC FACTOR

Fraction I

Supernate

pH 7.2
8% ETOH
-2°C

ANTITHROMBIN III

ANION EXCHANGE CHROMATOGRAPHY
HEPARIN AFFINITY CHROMATOGRAPHY

Fraction II+III

Supernate

pH 6.85
20% ETOH
-5°C

Factor IX Complex

Supernate

pH 5.3
18% ETOH
-5°C

Supernate

pH 7.6
20% ETOH
-5°C

Supernate

pH 7.4
25% ETOH
-5°C

Supernate

pH 6.85
20% ETOH
-5°C

Supernate

pH 5.8
40% ETOH
-5°C

Supernate

pH 4.8
40% ETOH
-5°C

Supernate

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

IMMUNE GLOBULINS

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction IV-1

Supernate

pH 5.15
40% ETOH
-5°C

Resuspend

pH 4.8
40% ETOH
-5°C

Resuspend

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction IV-4

Supernate

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction V

Supernate

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction IV-4

Supernate

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction V

Supernate

pH 4.6
10% ETOH
-2°C
Filter
pH 5.15
40% ETOH
-5°C
Resuspend

ALBUMIN

Pasteurization

Fraction V
Plasma Fractionation Process

• Each step forms the starting material for the following step in the process

• Cohn was the control of the process: “No variation in the manufacturing process shall be employed without his approval and each laboratory shall be open to inspection by Dr. Cohn or his representative at all times during working hours.” (Naval Contract Specifications)

• cGMP
Some Factors Known to Affect Product

1. Change in starting material
2. Change in test of starting material
3. “Minor” pH change at one step
4. Change in duration of one step
5. Introduction of viral inactivation
6. Change in formulation
7. Change in physical state
“Minor” changes with “Major” impact

Fraction II + IIIw precipitation

pH 5.4 - stable
pH 5.1 - fragmentation

Fraction III

discard

supernatant III

Fraction II
“Minor” changes with “Major” impact

Stability

Plasminogen → Supernatant
pH 5.1-5.2 (Fraction II)

Plasminogen → Precipitate
pH 5.4 (Fraction III)
Residual Protease in AHF Product

• Removal of Prothrombin Complex by Aluminum Hydroxide Adsorption

• Mistake in Al(OH)₃ preparation
  – More residual protease in the final product
  – More intense product fragment band on SDS-PAGE
  – Fail potency at first stability time-point
“Minor” changes with “major” impact

• Pre-kallikrein activator (PKA) Incident
  – During Fraction II + III precipitation more filter aid was added and the stirring time was lengthened. As a result, there was increased activation of PKA.
  – Elevated PKA in IGIV was associated with increased adverse events: hypotension, chest tightness and wheezing.
Control of Protease Level

• Addition of protease inhibitors in early stages of purification
  – Subsequent removal
    • Validation
    • Specification
• Removal of protease by adsorption or column chromatography
• Validation for process conditions or addition as Specification
Examples of Recombinant Analogs of Blood Products

- **Factor VIII Products**
  - Recombinate (1992)
  - ReFacto (2000)
  - Xyntha (2008)

- **Factor IX Product**
  - BeneFIX (1997)

- **Activated Factor VIIa Product**
  - NovoSeven (1999)

- **Thrombin Product**
  - Recothrom (2007)
Major Post-translational Modifications of Proteins

- Cleavage of signal peptide
- Proteolytic processing of precursor
- Withdrawal of methionine from amino terminus
- Formation of disulfide bridges
- Folding
- Glycosylation (O- and N-)
- Amidation
- Acetylation
- Phosphorylation
- Methylation
- ADP-ribosylation
- \( \gamma \)-Carboxylation
- Hydroxylation
- Sulfation
- Association of subunits
CQA’s for Blood Products

- Potency
- Product-related impurities
  - Not-fully processed
    - Non-cleaved precursors
    - Insufficiently gamma-carboxylated
    - Hypo-sulfated
  - Activated factors
- Process-related impurities
  - In-process reagents – solvent/detergent
  - Affinity ligands
- Aggregation
- Fragmentation
- Host cell proteins
- Endotoxin
- Bioburden
- Sterility
CQA’s for Blood Products

Impurities:

• Proteases
  – Degrade protein of interest
  – Stability
• Cofactors that may stabilize the protein
  – vWF
• Contaminants that may affect efficacy
  – Factor XIII
  – Fibronectin
CQA’s for Lyophilized Blood Products

• Residual moisture

• Appearance of Lyophilized Cake
  – Formulation
  – Lyophilization

• Appearance and Stability of Reconstituted Solution
  – Particulates
  – Aggregates
CQA’s – Specifications?

• Specific activity
• Levels of excipients
Conclusion

• Blood products share many CQA’s with other protein products
• Based on risk assessment
  – Indication
  – Administration
    • Route, Frequency, Size of Dose
• Control of process from source material
• Understand the product as a molecule and its mechanism of action as it relates to and interacts with other components in its milieu
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TSE Safety

• Donor deferral policies since 1999
• TSEAC reviews twice yearly
• Studies reviewed on model TSE agent clearance in plasma derivatives
• Approval of analogous recombinant products made without animal proteins
• Cooperation with WHO in the development of TSE reference materials
• Research on prion detection and decontamination
Immunogenicity due to Impurity

Bovine Thrombin Product for topical use

– Formation of antibodies against bovine thrombin and/or factor V

– Cross-react with human factor V results in factor V deficiency

– Severe bleeding

– Black box warning in the PI