PROLYTIX

Biotherapeutic Experts
from Discovery to Release

# Novel Plasma Fractionation Process to Produce Intravenous Immunoglobulin (IVIG)

Matthew Whelihan Ph.D.

# Session Description and Objectives

- Plasma-derived therapies are critical, life-saving medicines that thousands of people with rare and complex diseases rely on every day around the world.
- Global demand for these therapies, particularly immunoglobulins, has increased dramatically over the last 15 years, and continues to grow.
- Due to subtle differences in the manufacturing processes, IVIG from different manufacturers varies and is tolerated differently by patients
- Define the risks associate with long-term administration of IVIG
- Define the opportunities to improve the current process for plasma fractionation
- Understand how the new process reduces the impact on the environment, reduces the demand for healthy donations, and reduces the risk to patient safety.

# Biography and Contact Information

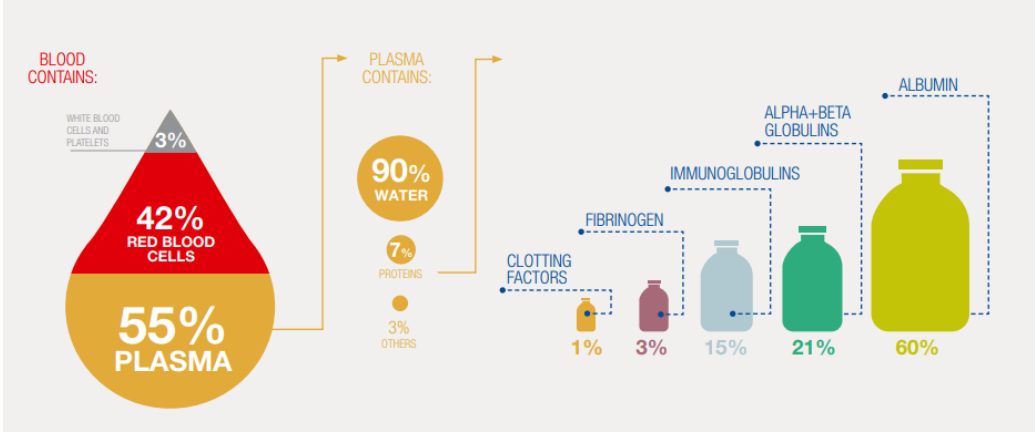
- Matt is a biopharmaceutical industry scientist with 20 years of experience in analytical assay design and purification/process development of plasma-based protein therapeutics.
- Matt earned his Ph.D. in Biochemistry from the University of Vermont where he gained expertise in blood coagulation and protein therapeutics.
- Matt is currently an Associate Principal Scientist at Prolytix where he leads a team of scientists that help solve some of the biopharma industry's biggest challenges all the way from drug discovery to release.

- Contact:

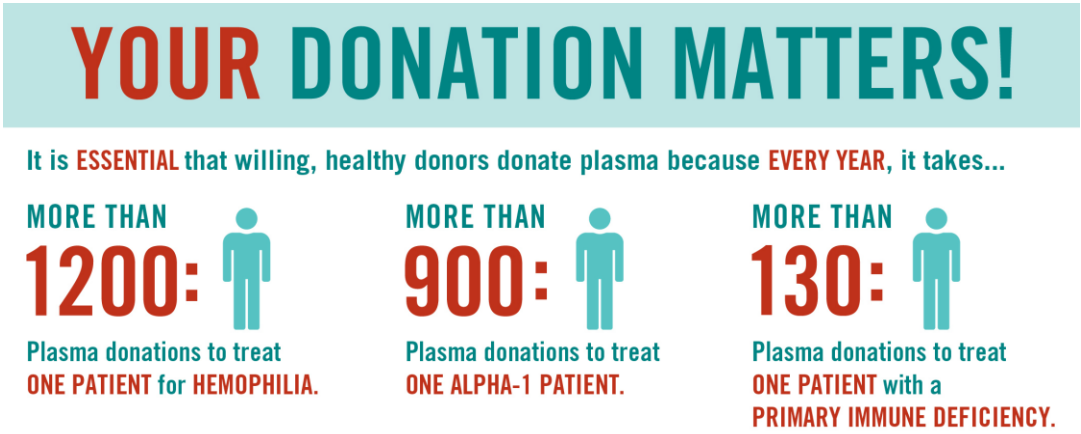
**Matt Whelihan Ph.D.**
Associate Principal Scientist
Prolytix
57 River Rd., Unit 1021
Essex Jct., VT 05452
mwhelihan@goprolytix.com

# Plasma-Derived Biotherapeutics

- Plasma-derived therapeutics start from human blood plasma rather than chemical or synthetic materials
- Human blood plasma is rich in proteins that boost the immune system, and fight infections and inflammation, which makes plasma-derived biotherapeutics useful in treating rare, chronic, and often genetic diseases
- Among the proteins used are immunoglobulins, coagulation factors, alpha-1 antitrypsin, fibrin sealants, and albumin.
- The raw material is human plasma donations
  - Source plasma
  - Recovered plasma




Grifols Integrated Annual Report 2019



# Human plasma is a precious resource

- The demand for plasma-based biotherapeutics continues to outpace the supply of plasma donations
- The COVID-19 pandemic highlighted this shortage as volunteers stayed home instead of traveling to the clinic
- Plasma-derived biotherapeutics are produced through a >70-year-old process called Cohn Fractionation
- Technological advances are needed to keep up with the demand

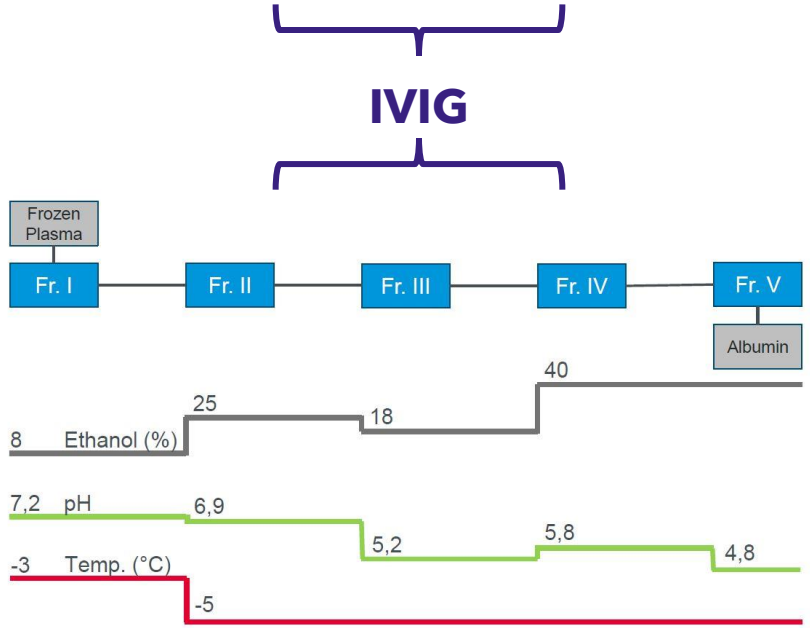
# Cohn Fractionation Process

- The Cohn process, developed by Edwin J. Cohn in the 1940s, is a series of purification steps with the purpose of extracting albumin from blood plasma
  - The Cohn Process involves modifying the pH, ethanol concentration, and temperature to separate proteins through precipitation into five “fractions”
- Gammglobulin (IgG) is a side product derived from fraction II/III
  - The separated proteins undergo an extensive purification process that includes, depth filtration, chromatography, nanofiltration and viral inactivation treatments to produce a sterile, virally inactivated protein product
- 7-10 Day cycling time
- Modern Cohn plants cost hundreds of millions of dollars to construct due to the logistics and safety concerns in dealing with large volumes of ethanol

# Products derived from Cohn fractionation

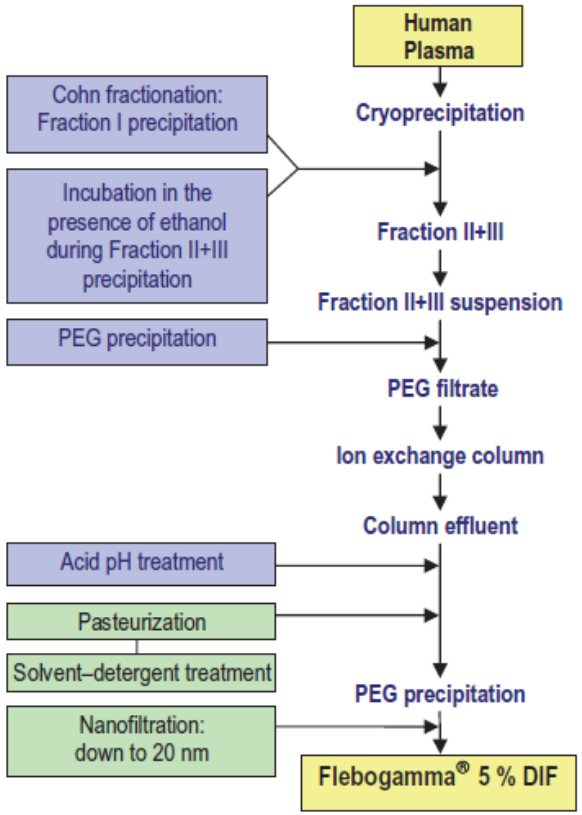
- Albumin (1940s)
  - Blood volume expander for fluid loss
  - Septic shock
  - Renal dialysis
- Hemophilic factors (1960s)
  - Cryoprecipitate
  - Factor VIII and FIX specific products
- Alpha-1 Antitrypsin
- IVIG (1970s)
  - Primary immunodeficiencies
  - Worldwide market cap for IVIG is expected to hit $16 billion by 2025
  - 2021 US market share of $4.7 Billion

| Fraction #: | Fraction I | Fraction II | Fraction III | Fraction IV | Fraction V |
|---|---|---|---|---|---|
| Ethanol %: | 8 | 25 | 18 | 40 | 40 |
| pH: | 7.2 | 6.9 | 5.2 | 5.8 | 4.8 |
| Temperature (°C) | −3 | −5 | −5 | −5 | −5 |
| Protein fraction (%): | 5.1 | 3 | 3 | 3 | 1 |

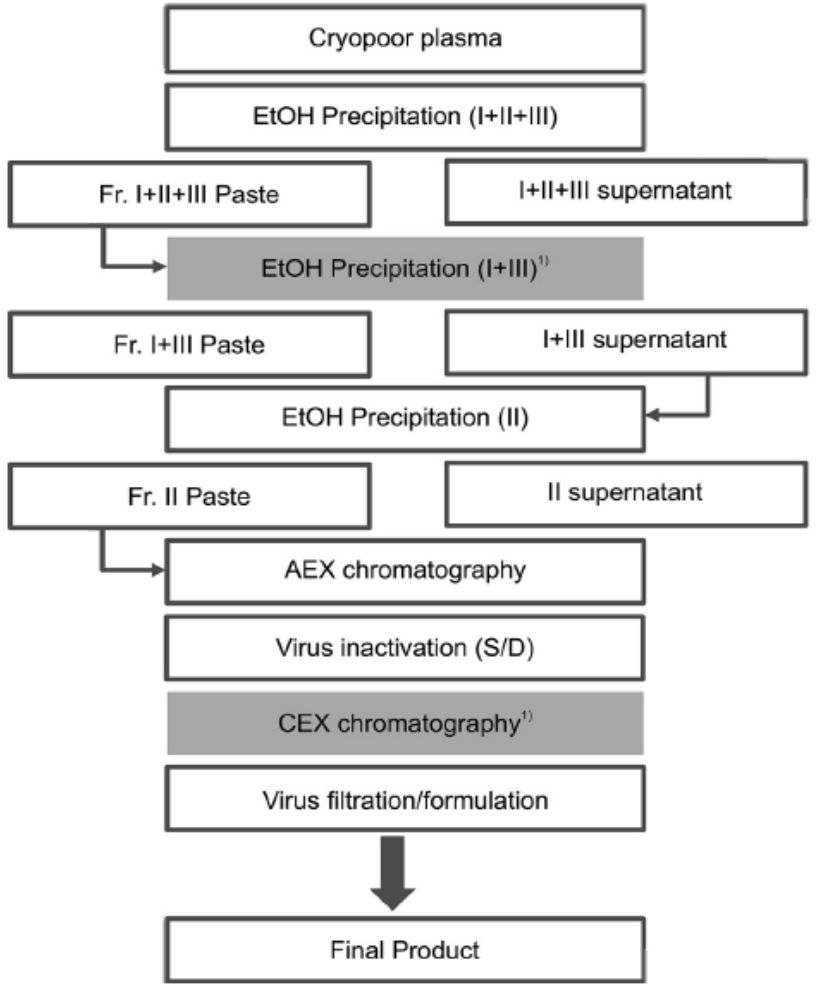


# Modern Purification Improvements

- Other fractionation methods: caprylate and salt
- Chromatography gradually adopted; viral inactivation added
- **While modern IgG yields are a closely guarded secret, they are widely believed to be in the 55-65% range**



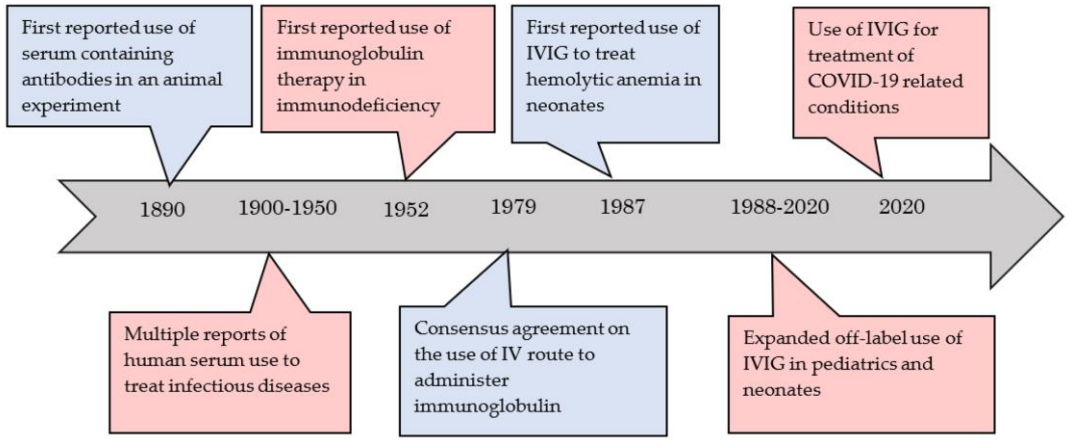

Jorquera, Clin & Exp Immunology, 2009




Park et al., Biologicals, 2017

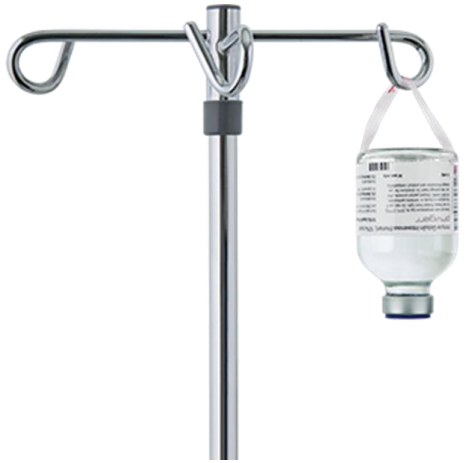
# IVIG Clinical Use

- Polyclonal IgG product: >10,000 donors
- Formulated as 5% (50 mg/mL) or 10% (100 mg/mL) Soln.
  - IVIG
  - IMIG
  - SCIG
- Approved uses: immune deficiencies and several autoimmune conditions
- Off-label: autoimmune disorders are fastest growing market




- Market
  - Tight, susceptible to supply disruptions
  - 2021: $4.6 billion*
  - Forecast: 6.8% CAGR and $10billion in 2028*

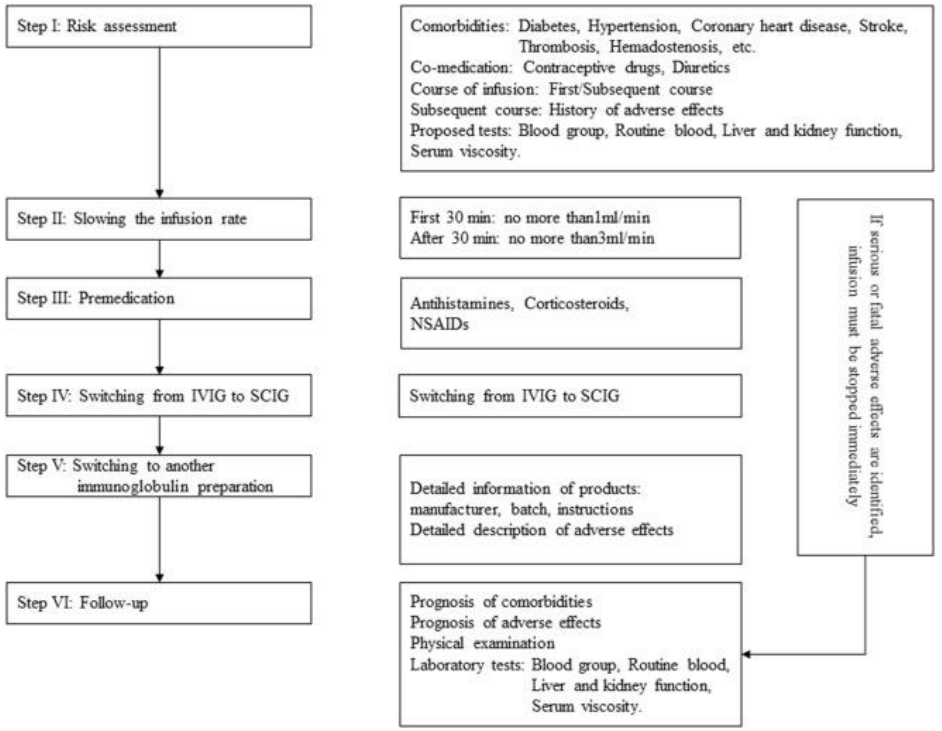
- Typical Adult Doses
  - Humira, rheumatoid arthritis: 40 mg every other week = 80 mg/month
  - IVIG: 25 grams/day for 3 days, every 4 weeks = 75 grams/month



* https://www.researchandmarkets.com/reports/4621728/u-s-intravenous-immunoglobulin-market-size Accessed Apr-13-2022

# Why is IVIG from different producers is tolerated differently by patients?

- Adverse events are reported to be as high as 80% in long-term IVIG administration
  - Preparation-related risk factors
  - Comorbidities
  - Patient-related
- Adverse effects are mostly correlated with high dose infusion
  - Immediate
    - Flu-like symptoms (>80%)
    - Dermatological effects (~6%)
    - Arrhythmia/hypotension (~1%)
  - Delayed (≤1%)
    - Thrombosis
    - Aseptic meningitis
    - Headache
    - Neurological syndromes
    - Electrolyte disturbance
- FDA March 2022 - 29 different lots recalled from 3 major manufacturers due to increased rates of allergic hypersensitivity




Guo et al. Front Immunol 2018

# Example: IgA requirements

- IgA: immunogenicity concern
  - EP: "As determined by a suitable immunochemical method, the content of immunoglobulin A is not greater than the maximum content stated on the label."

Table 4
Pharmaceutical aspects of IGIV: IgA content

| Product | IgA content |
|---|---|
| Flebogamma® 5% | <50 µg/mL |
| Gammagard S/D, Polygam® S/D | <1.2 µg/mL |
| Iveegam EN | <10 µg/mL |
| Gamimune® N, 10% | 270 µg/mL |
| Gamunex® | 46 µg/mL |
| Gammar®–P I.V. | <25 µg/mL |
| Carimune™ NF Nanofiltered | 720 µg/mL |
| Panglobulin® NF Nanofiltered | |
| Octagam® | <100 µg/mL |

600-fold difference

**"Some studies have suggested that IgA-deficient patients may be at a higher risk of adverse effects. Iranian researchers found that the incidence of immunoglobulin-induced adverse effects was higher in patients with primary antibody defects, especially those with low levels of IgA"**

Guo et al. Front Immunol 2018

Siegel, International Immunopharmacology, 2006, 6:523-527.

# Why does IVIG have such a variable safety profile?

- Modern IVIG production processes still use Cohn fractionation as their base fractionation process
  - Prolonged exposure to ethanol and pH extremes tends to denature proteins and create antigenic byproducts
  - Ethanol is a volatile reagent and remains capital intensive for Cohn plants due to safety precautions and recycling of this central reagent
- There is no standardization to the manufacturing process
  - Release metrics are focused on gross purity and immunological issues
  - Prothrombotic markers have only recently been added to the cadre of tests
- A base fractionation process that could fractionate proteins without the use of ethanol would be a major step forward.

# There must be a better way to make IVIG

- In 1940, the Cohn process was originally designed to purify albumin
- Advent of IVIG isolation in the 70's was an afterthought to meet a pharmacologic need and boost profit per unit of plasma
- Today, IVIG is the primary driver for Cohn-based plasma fractionation due to worldwide demand for the product
- A technological advance directed specifically at IVIG production is needed.
  - Eliminate the use of ethanol and pH extremes
  - Streamline the process and increase IgG yields

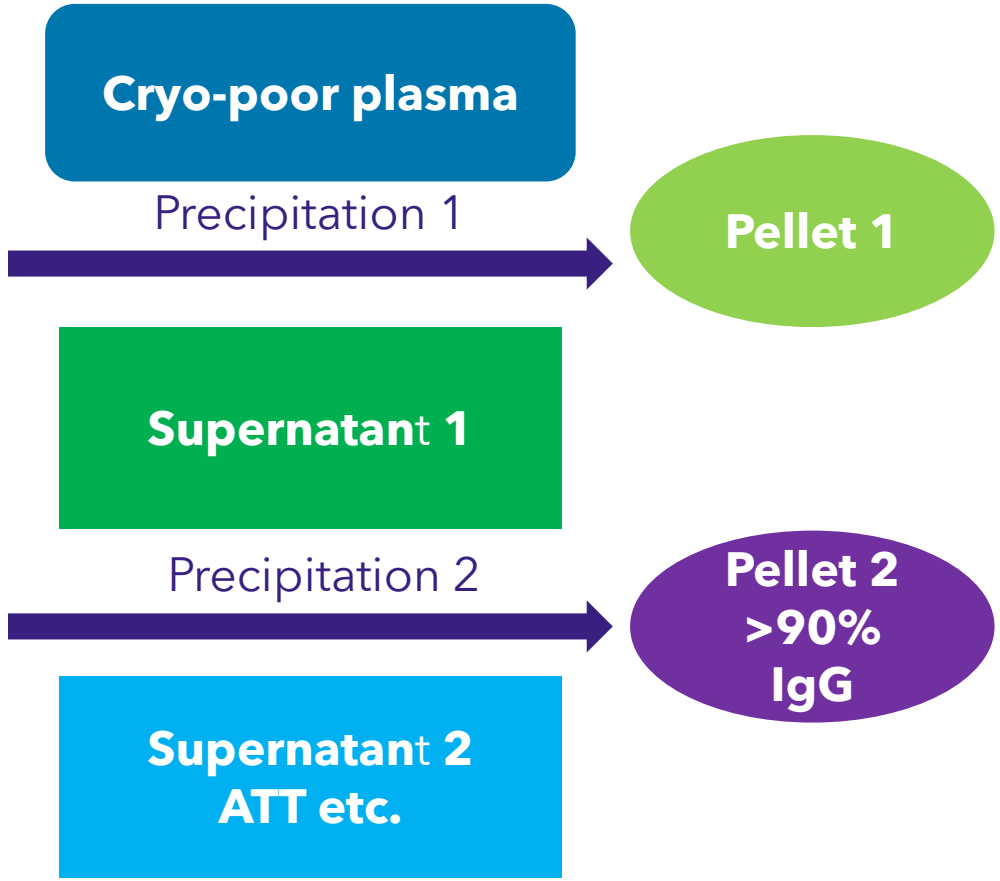
# Salt-based plasma fractionation?

- In 2013, a small S. Carolina startup Plasma Technologies LLC, announced a patented technology to use salt fractionation to achieve "Ultra-High Yields of Alpha-1 Antitrypsin"
- The process utilizes a salt, sodium citrate at neutral pH, to differentially precipitate plasma proteins
  - Increased AAT yields from 7% with Cohn fractionation to ~70%
  - Sodium citrate is gentle on biologics and having been a long used as an FDA-approved preservative
- Is this process capable of delivering increased yields and functionality for other fractionated proteins?

"Plasma Technologies LLC Release: Recently Patented Technology Poised to Disrupt the Plasma Biopharmaceutical Market" 9/10/2013

# Base Fractionation of plasma to produce IVIG

- >90% IgG yield is achieved after two successive precipitations with sodium citrate at neutral pH
- Process takes hours instead of days to complete
- No ethanol or pH extremes
- Resulting precipitate is >60% IgG

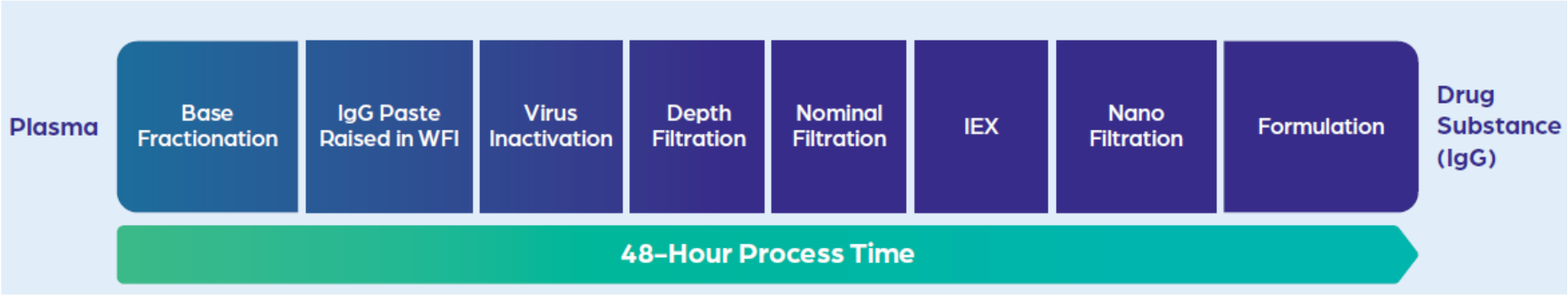


# Plasma Technologies and Prolytix

- March 2020 – Plasma Technologies approached Prolytix with this revolutionary new salt fractionation technology to produce IVIG
- Prolytix's Challenge: Develop an industrially scalable process to produce IVIG from a novel base fractionation process to meet the increased demand during the COVID epidemic
- Prolytix is uniquely suited to this task.
  - Plasma based protein purification experts (specializing in coagulation proteins)
  - Full sweet of instrumentation and know how to produce product batches at the 10L scale
  - In house LCMS capabilities
  - GMP Validated methods for release and stability of IVIG and monoclonal antibody products

# Prolytix offers Integrated process development tools under one roof

- Using an integrative approach, Prolytix was able to create an IVIG drug product at bench scale which passed all FDA and EU release metrics for safety and quality in 18 months
  - LCMS, cell and plasma-based assays were employed to ensure the products safety profile all along process development
  - Process yields in excess of 75% as compared to the starting plasma (>80% is expected at industrial scale)
  - Entire process cycles in just 48 hours
  - Significantly lower cost per cycle



# IGIV Product passes all EP release metrics for safety

- Product lots pass all cell based immunological tests
  - Anti-A/B
  - Anti-D
  - ACA
  - Fc Function
- PKA and FXIa contaminants were selectively removed
- Superior characteristics in LCMS and SEC-HPLC

**Summary of Analytical Testing Results for two sample lots of IVIG produced through the new salt fractionation process**

| Test Method | | Sample Lot # Tested | | | | DP Spec EP Metrics | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Lot 1 | | Lot 2 | | Acceptance Criteria (ACC) | | | |
| Purity by LCMS (Std. Dig.) | | 99.6% | | 99.5% | | No ACC | | | |
| IgG Subclass Distribution | | IgG1 | IgG2 | IgG1 | IgG2 | Starting Plasma | | | |
| | | 62 | 30 | 62 | 30 | IgG1 | IgG2 | IgG3 | IgG4 |
| | | IgG3 | IgG4 | IgG3 | IgG4 | 62 | 28 | 7 | 4 |
| | | 7 | 1 | 6 | 2 | | | | |
| IgA | | ND | | ND | | <4µg/mL | | | |
| IgM | | ND | | ND | | <1 µg/mL | | | |
| TGA, FXIa( mU/mL) | | 0.79 | | 0.27 | | ≤1.0 mU/mL | | | |
| Anti-Complement ($CH_{50}$ U/mg IgG) | | 0.83 | | 0.88 | | ≤1.0 $CH_{50}$ U/mg | | | |
| FC Function | 15 mg IGIV | 129 | | 144 | | ≥60% | | | |
| | 30mg IGIV | 149 | | 134 | | ≥60% | | | |
| PKA (IU/mL) | | 1.48 | | 4.57 | | ≤35 IU/mL | | | |
| NAPTT Clot Time | | >200s | | >200s | | ≥200s | | | |
| SE-HPLC | %Dimer | 2.15 | | 2.43 | | Mono-& di-meric ≥90%<br>Polymeric <2%<br>Fragment <3% | | | |
| | %Monomer | 97.36 | | 96.15 | | | | | |
| | %IGIV | 99.51 | | 99.71 | | | | | |
| | %Polymeric (HMW) | 0.34 | | 0.24 | | | | | |
| | %Fragment (LMW) | 0.15 | | 0.05 | | | | | |

# Industry and patient impact

- The new process uses no ethanol or pH extremes
  - Production/plant costs are lowered significantly
  - Manufacturer safety is no longer a serious concern
- The environmental impact is substantial
  - Significantly less ethanol waste
  - No energy costs associated with ethanol recycling
- Increased yield of IgG reduces the demand for healthy donations
- The resulting IVIG product is of superior yield, purity and quality compared to currently available products.
- This process is currently being evaluated by a major IVIG manufacturer for future implementation

PROLYTIX

Biotherapeutic Experts
from Discovery to Release

# Questions

**Matt Whelihan Ph.D.**
Associate Principal Scientist
Prolytix
57 River Rd.
Unit 1021
Essex Jct., VT 05452
mwhelihan@goprolytix.com