



**Plasma Product  
Biotechnology  
Meeting 2009  
Menorca, Spain**

**Prion Infectivity  
Removal from Human Plasma  
and Plasma Products Using  
Affinity Ligands**

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# TSEs and Blood Transfusion

- **Unequivocal demonstration with**
  - **Different experimental strains of the TSE agent**
  - **Diverse experimental animal models**
  - **Natural TSE strains**
    - **Sheep**
    - **Human – 4 transfusion transmissions of vCJD**
    - **Deer**
- **Transmission by pre-clinical blood**
- **High transmission rate**

# TSE Blood Infectivity

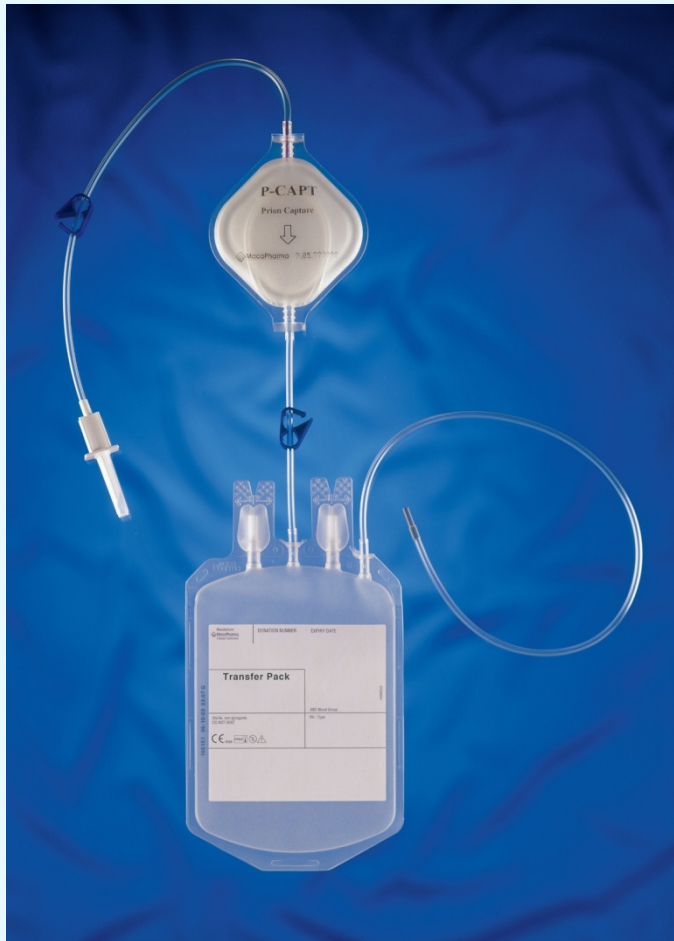
## The Hamster Model

- **Hamster scrapie (263K) blood infectivity is ~ 10 ID/mL**
  - **Limiting dilution titration method**
- **~ 40-50% in buffy-coat**
- **~ 5-8 ID/mL in plasma**

# Transfusion Transmission Risk Management

- **Leucoreduction**
  - 42% endogenous blood infectivity is removed by leucoreduction (Gregori *et al.* Lancet, 2004)
  - 5,600 ID/unit → 3,200 ID/unit
  - Leucoreduction is necessary, but not sufficient to significantly reduce the risk
- **Removal of infectivity by affinity ligands**
  - P-CAPT<sup>®</sup> filter

# P-Capt<sup>®</sup> Prion Removal Device



- PRDT resin reduced brain spiked infectivity by  $> 3 \log_{10} ID_{50}$  (Gregori *et al.*, Transfusion, 2006)
- PRDT resin removed all endogenous infectivity (to LOD of bioassay) (Gregori *et al.*, Lancet, 2006)
- Resin incorporated into P-CAPT<sup>®</sup>
- CE marked in September 2006
- Manufactured and commercialized by MacoPharma
- Designed for use with human leucoreduced RBC

# Infectivity in Plasma

- Pooling of plasma potentially increases the risk for spread
- Dilution of infectivity reduces infectivity per volume unit, but does not eliminate the risk
- Potential risk of amplification
- Low level of contamination
- One presumed case of transmission through a plasma product
  - Hemophiliac, pre-clinical
- Measures to reduce risk are needed

# Infectivity in Plasma PRDT solution

- P-CAPT<sup>®</sup> filter was not developed for plasma
- Plasma and plasma products do not have some of the constraints posed by RBC
- Resins in column format are suitable for use with plasma

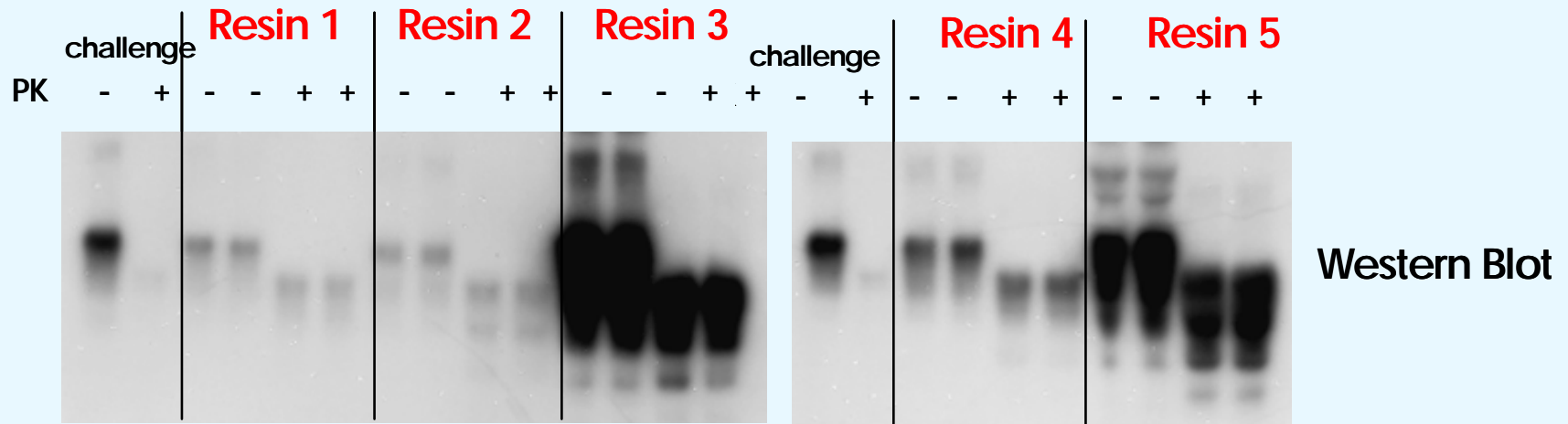
# Infectivity in Plasma PRDT solution

- Select PrP<sup>sc</sup> ligands that demonstrated
  - $> 3 \log_{10} ID_{50}$  of infectivity reduction
  - Wide range of species/strain specificity
    - Hamster (Scrapie), Mouse (Fukuoka)
    - Squirrel Monkey
    - Human (sCJD and vCJD)
- Establish a ligand panel
  - Resins adsorb PrP<sup>sc</sup> from plasma or plasma products
  - Minimal/no impact on therapeutic application of the product

# Infectivity in Plasma PRDT solution

- Single chromatographic removal step
- Single use resin
- Flexible implementation at various stages of the manufacturing process

# Prion Removal from FFP



- Not all ligands clear prions from FFP
- 2 resins have good performance

# Plasma Protein Binding to P-CAPT<sup>®</sup> resin

Assay	Factor II (Coag) IU/ml		Factor VIII (Coag) IU/ml)		Factor VIII (chrom) (IU/ml)		Factor VII (Coag) (IU/ml)	
Normal Plasma		~1		~1		~1		~1
Col1 Frac 2	0.321		0.628		0.74		0.938	
Col2 Frac 2	0.416		0.666		0.76		0.897	
Col3 Frac 2	0.281	0.339	0.568	0.621	0.75	0.750	0.878	0.904
Col 1 Frac 3	0.457		0.665		0.8		1.001	
Col 2 Frac 3	0.529		0.676		0.8		1.04	
Col 3 frac 3	0.42	0.469	0.636	0.659	0.81	0.803	1.011	1.017

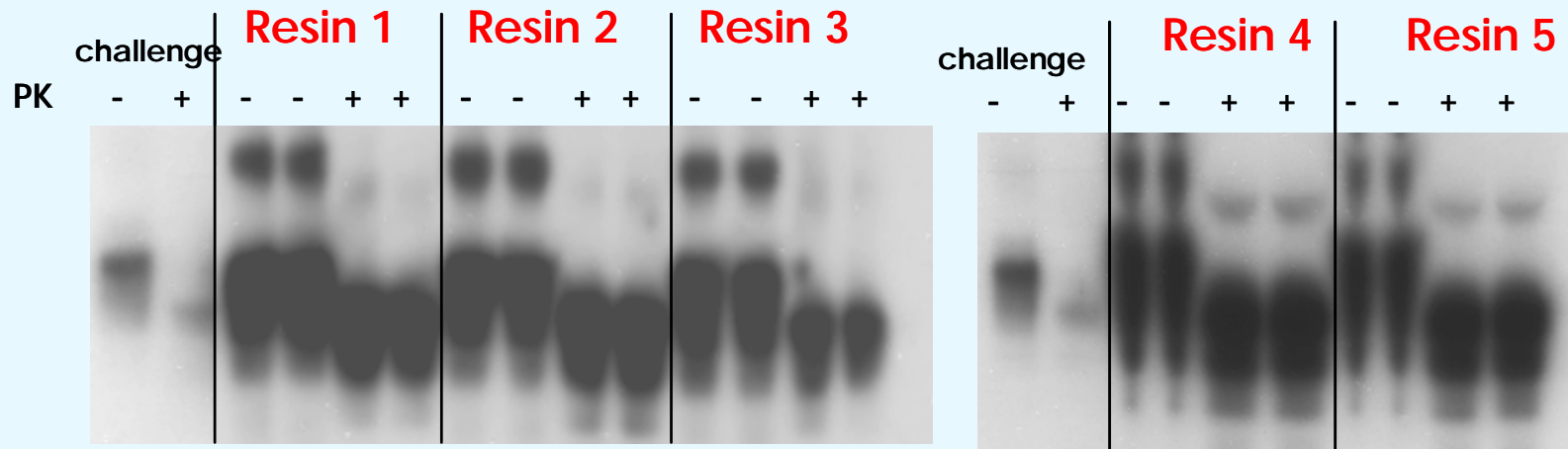
Assay	Factor IX (Coag) (IU/ml)		Factor XI (Chrom) (mg/ml)		API (ug/ml)	
Normal Plasma		~1		~1		1500-2000
Col1 Frac 2	0.132		0.71		1468.4	
Col2 Frac 2	0.258		0.747		1511.6	
Col3 Frac 2	0.111	0.167	0.731	0.729	1573	1517.7
Col 1 Frac 3	0.276		0.754		1482.6	
Col 2 Frac 3	0.257		0.747		1514.6	
Col 3 frac 3	0.219	0.251	0.743	0.748	1550.6	1515.9

- The resin used in the P-CAPT<sup>®</sup> filter binds high amounts of Factor II and Factor IX

# Plasma Protein Binding to PRDT resins

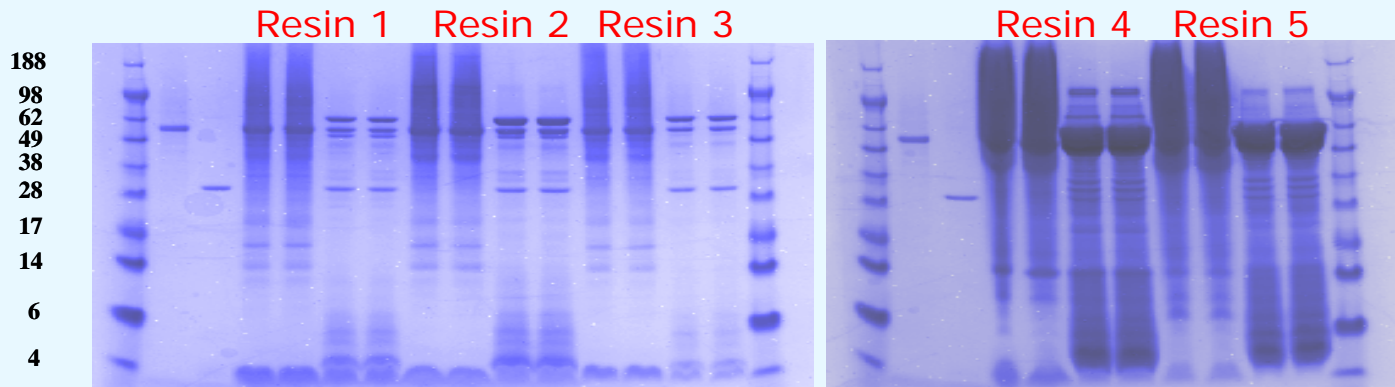
Sample	F II (Coag) (U/ml)	F VII (Coag) (U/ml)	F VIII (U/ml)	F IX (U/ml)	F XI (mg/ml)
Untreated	1.03	1.079	0.754	0.827	0.865
Resin A	1.195	1.341	0.844	0.949	0.917
Resin B	0.865	0.965	0.705	0.746	0.825
Resin C	0.984	1.049	0.771	0.861	0.833
Resin D	0.996	1.051	0.749	0.791	0.788
Resin E	0.977	1.019	0.71	0.824	0.771
Resin F	0.955	1.054	0.707	0.761	0.832
Resin F	1.101	1.141	0.749	0.855	0.972
Resin G	0.194	0.77	0.685	<0.1	0.866
PCapt	0.49	0.977	0.631	0.293	0.878

# Binding of Prion in Presence of 25% Albumin



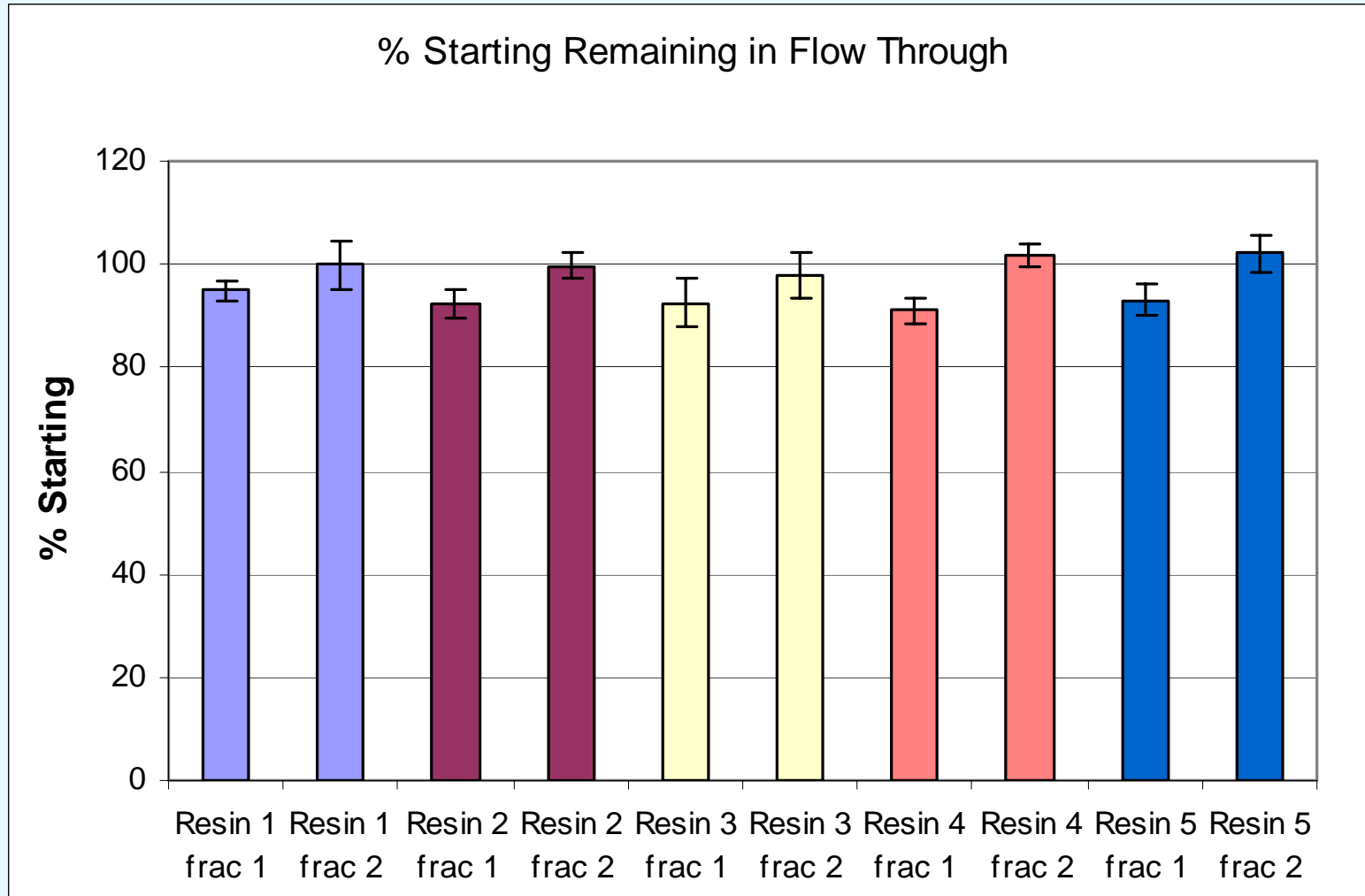
- PRDT resins are able to bind and concentrate prions in the presence of 25% albumin

# Bound Protein Profile

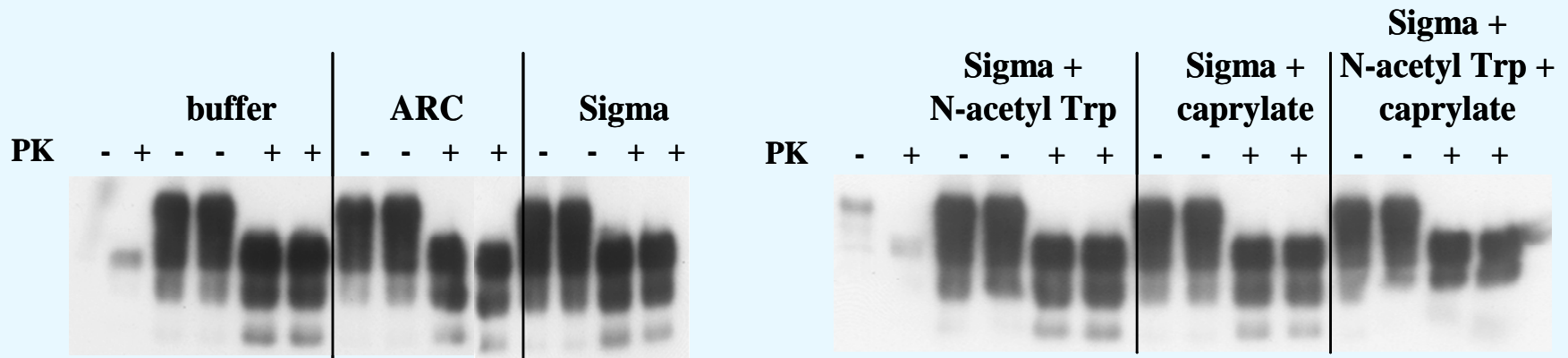


- 25% Albumin
- Hamster Brain Homogenate spike
- Resins 4 and 5 display a high total protein binding profile

# Albumin Loss During Prion Removal

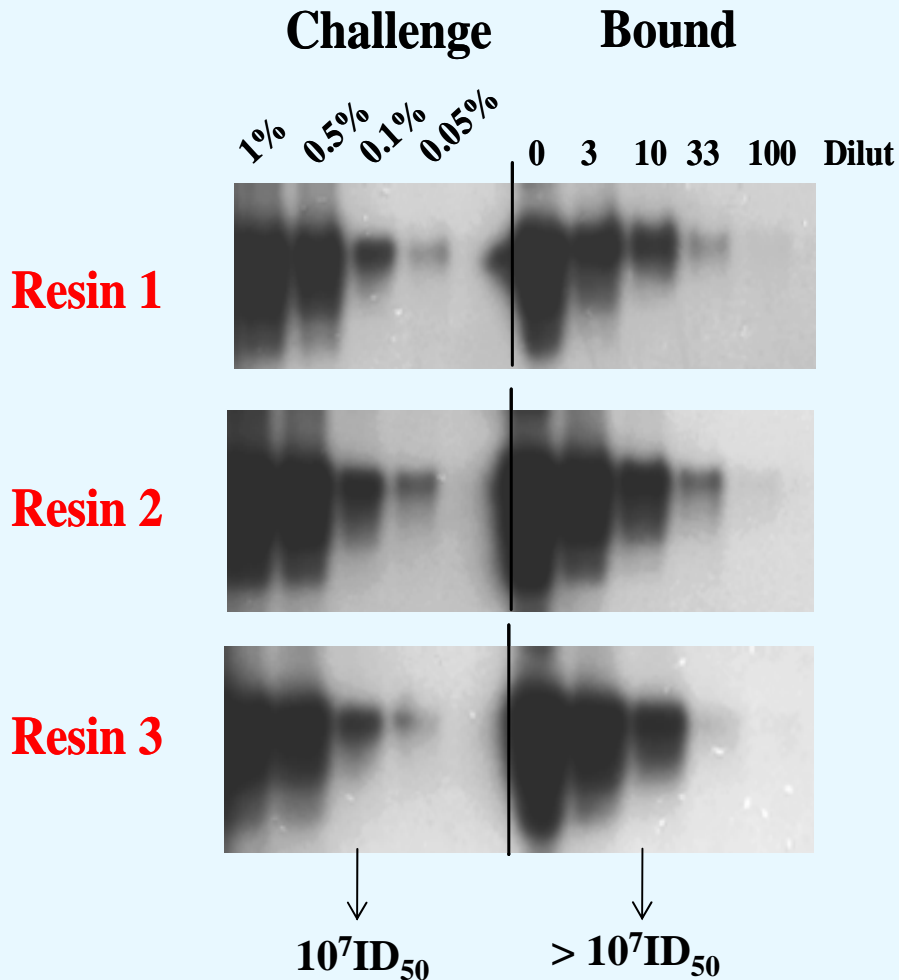


# Influence of Additives on Prion Binding in 25% Albumin



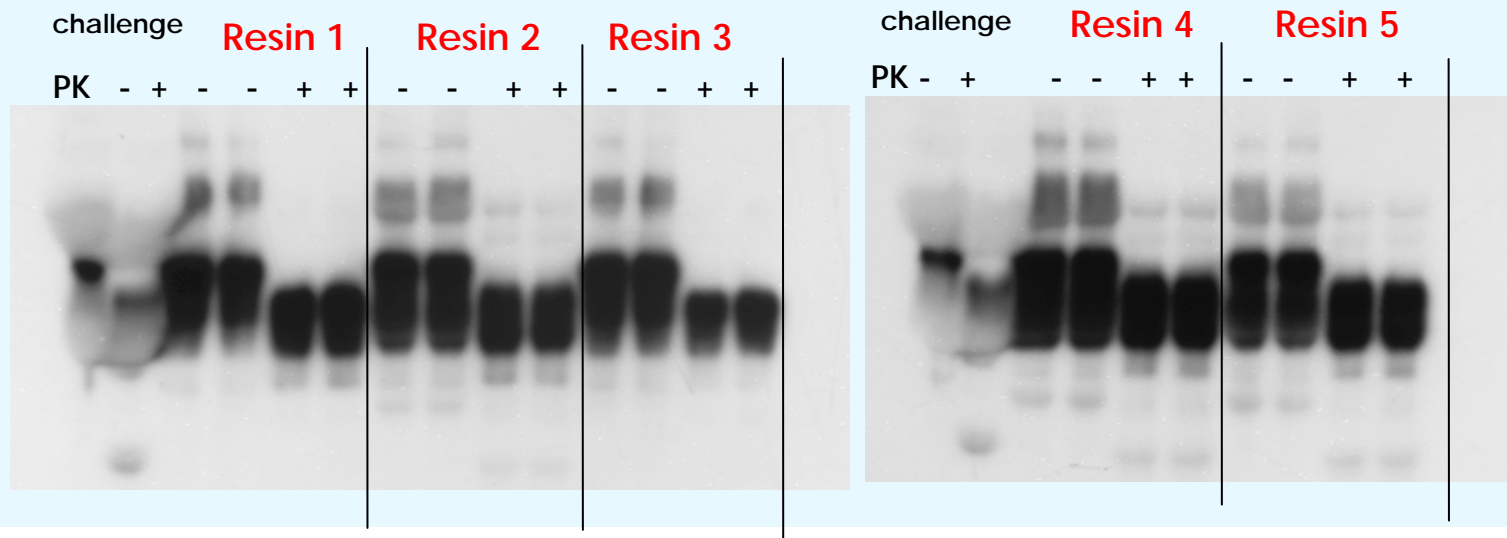
- No noticeable influence of common additives present in 25% albumin solutions

# Semi-Quantitation of Prion Clearance in Presence of 25% Albumin



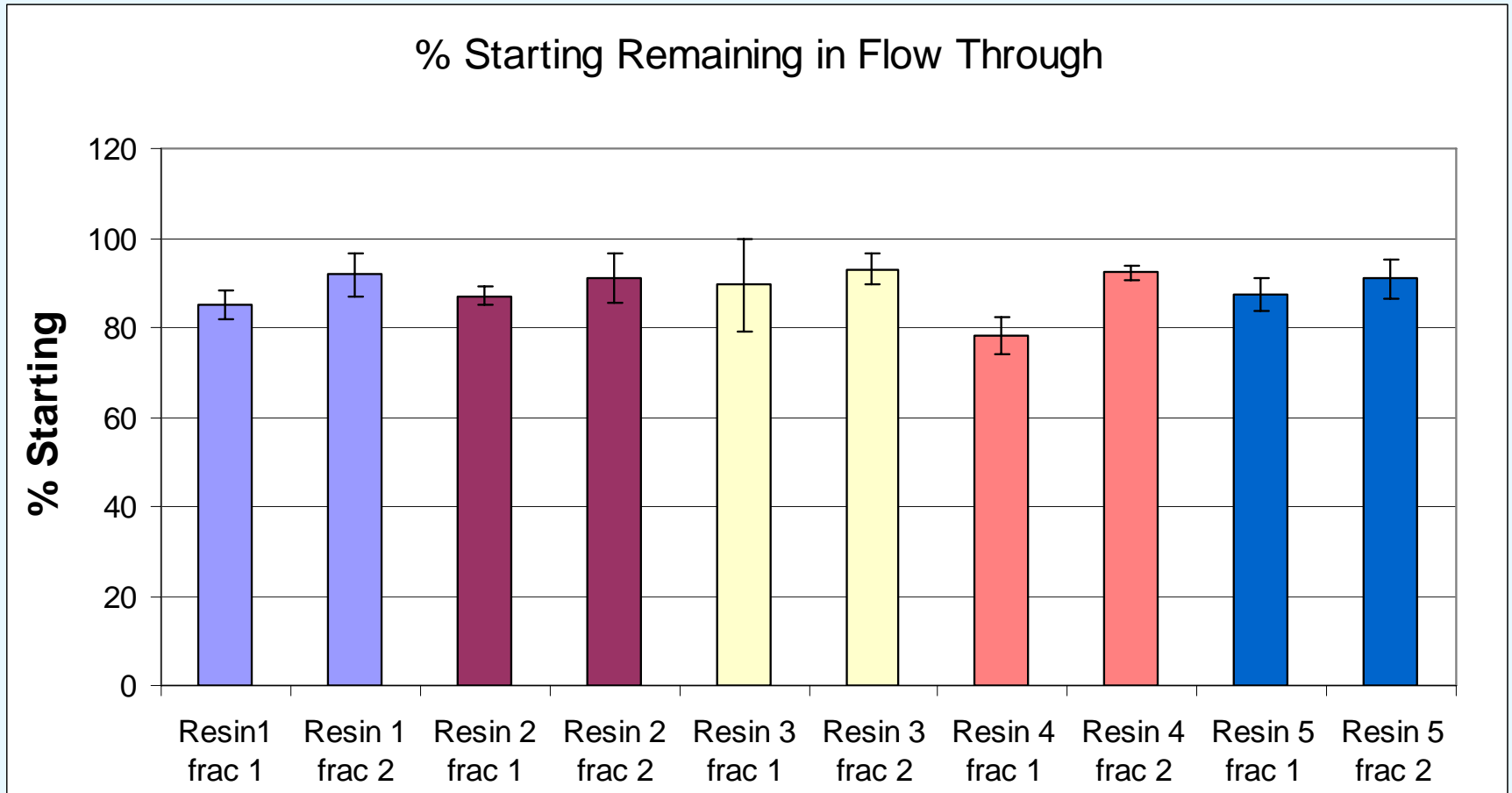
- Resins 1, 2 and 3 bound  $\sim 10^7$  ID in a 0.5-mL column
- Prion was removed in the presence of a  $10^7$  excess of competing protein

# Binding of Prion in Presence of 3% IgG



- PRDT resins are able to bind and concentrate prions in the presence of 3% IgG

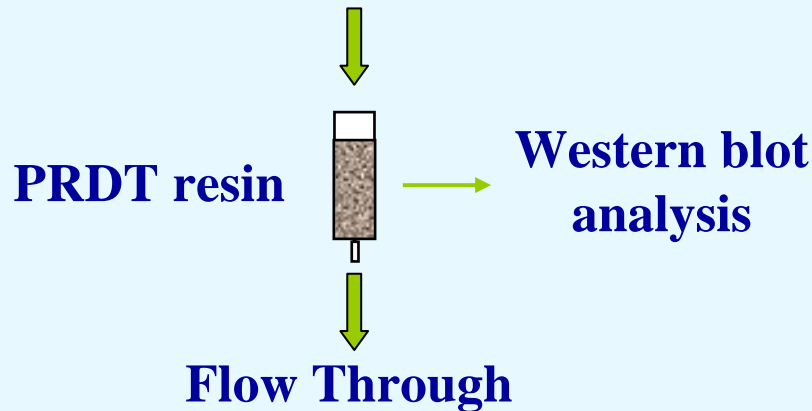
# IgG Loss During Prion Removal



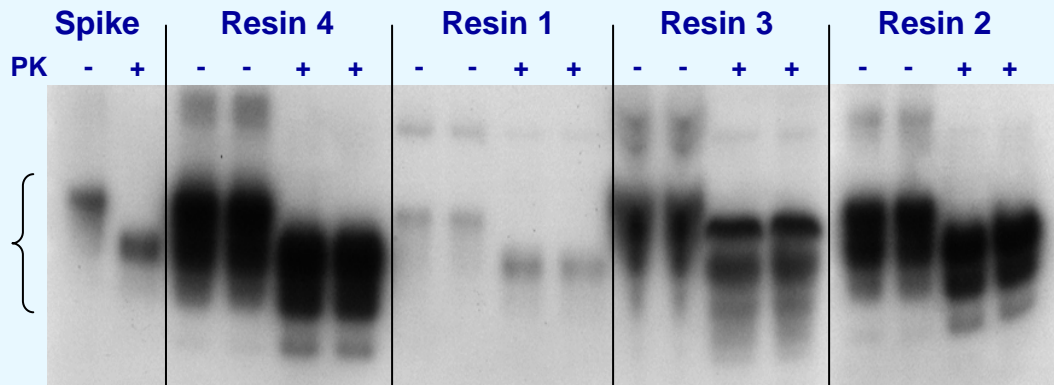
# Binding of Prion in Presence of FVIII

- Several preparations at different stages of process were tested
- Solutions were spiked with infectious hamster brain homogenate
- Depending on the preparation, 2 to 5 PRDT resins performed well
- Binding was estimated to be around  $10^6$  ID<sub>50</sub>/0.5 mL resin

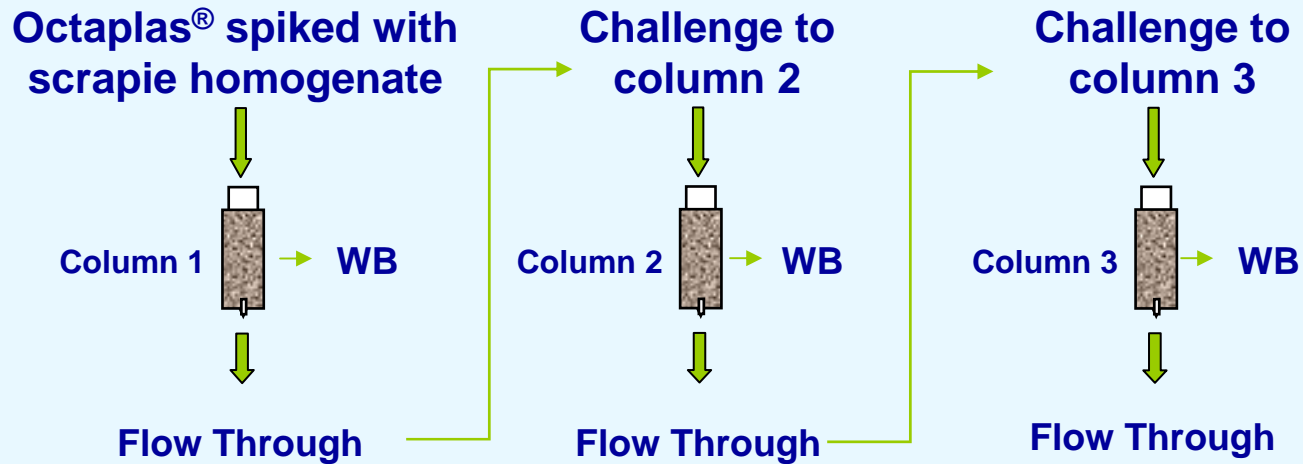
# Screening of PRDT Ligand Panel with Post-SD Octaplas<sup>®</sup>



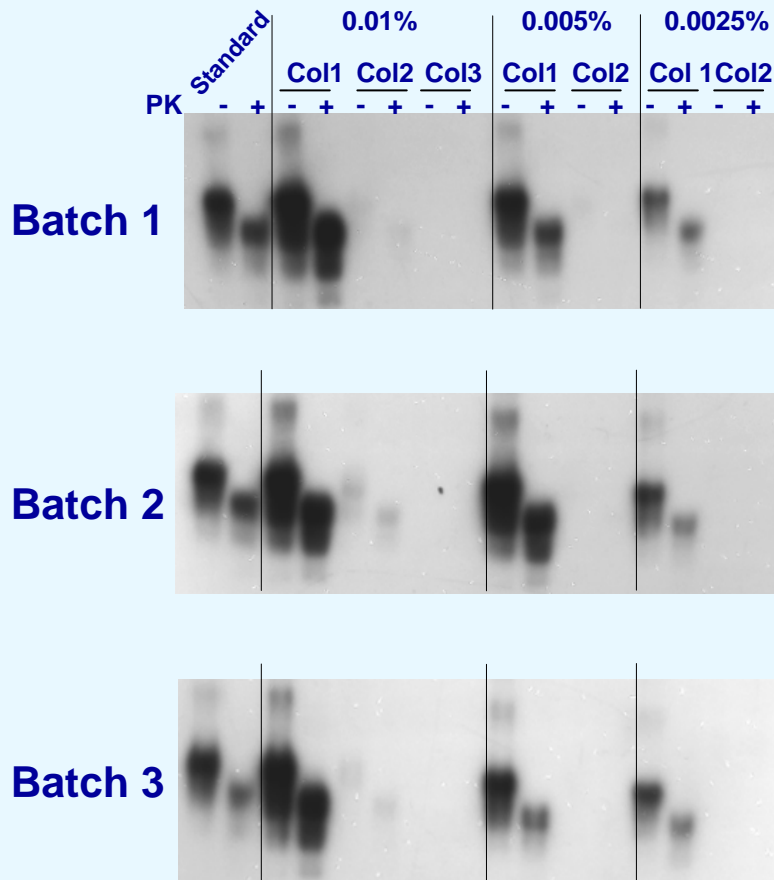
- Octaplas<sup>®</sup> spiked with hamster scrapie brain homogenate (263K)



# Resin Capacity with Octaplas<sup>®</sup> Methodology



# Resin Capacity and Batch Reproducibility



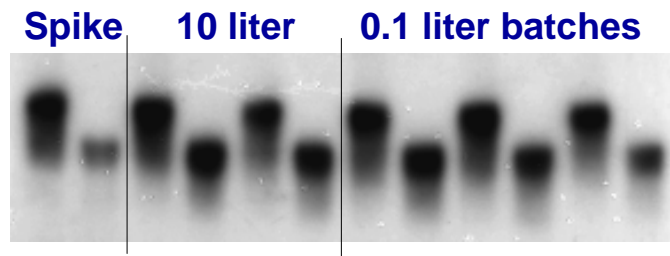
- **0.005% scrapie homogenate optimal conditions**
- **Spike titer  $5 \times 10^4$  ID<sub>50</sub>/mL challenge**
- **Input infectious doses =  $5 \times 10^5$  ID<sub>50</sub>**
- **PrP signal recovered on 0.5 ml**
- **Resin ~ 100% input**
  
- **Resin capacity ~  $6 \log_{10}$  ID<sub>50</sub>/ml**

# Current Status

- Octapharma is currently implementing the addition of a prion removal step into its industrial process for manufacturing SD-treated plasma

# Resin Manufacturing Scale-up

- Resin manufactured at ProMetic Biosciences, Cambridge, UK
- 0.1L scale → 10 Liter scale



- MTD/EMD acute/escalating dose toxicity studies on resin leachates
- Bacterial mutagenicity (AMES)
- Hematology

# Resin Stability Studies

- Two storage conditions (dry and in buffer)
- Two temperatures (4 °C and 25 °C)
- Controlled humidity
- 0, 4, 12, 26, 52 weeks (August 2008)
- Tests
  - PrP<sup>Sc</sup> binding, PrP<sup>Sc</sup> binding capacity, FTIR spectra, leachates, bioburden, endotoxin, microscopy
- Resin can be stored dry up to 26 weeks

# ProMetic Manufacturing Facility

- **Products manufactured in ISO9001:2000 accredited site under GMP**
- **Products are for use in pharmaceutical and biopharmaceutical industry**
- **Products manufactured to required industry scales**
- **Regulatory support packages are available**

# Summary

- Plasma products are a transmission risk of vCJD
- PRDT resin panel is able to bind infectious prion efficiently in the presence of
  - Albumin, IgG, FVIII
- Binding is in the order of  $10^6 - 10^7$  ID<sub>50</sub>/0.5 mL resin
- Selected resin removes infectious prions from SD-treated plasma
- Prion removal step is under implementation by Octapharma

Thank you

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