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DEVELOPMENT OF A DEVICE FOR REDUCTION OF PRION INFECTIVITY FROM RED BLOOD CELL CONCENTRATE

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WP TTID Annual Meeting
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Pathogen Removal and Diagnostic Technologies - PRDT

- Joint venture of ProMetic and the American Red Cross
- R. Carbonell and R. Rohwer are co-founders
- MacoPharma is a partner

PRDT™

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American Red Cross Biomedical Services

MacoPharma

NC STATE UNIVERSITY

University of Maryland 1807 School of Medicine

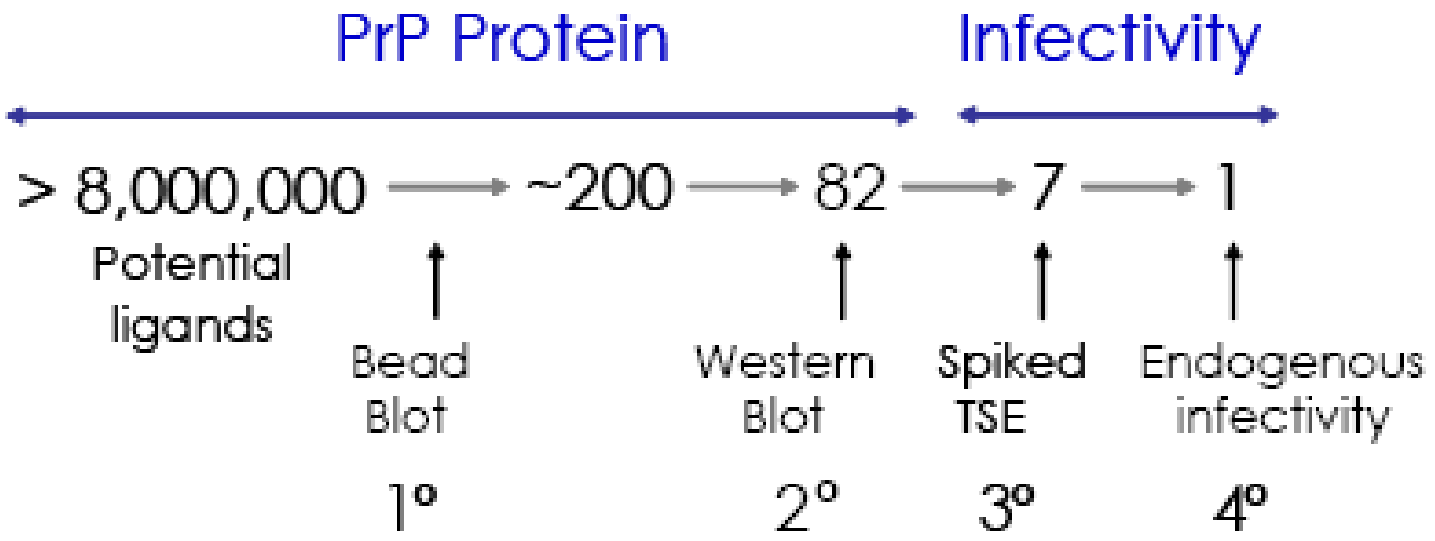
Transfusion Transmission of vCJD

- vCJD is transmissible through blood transfusion
- Four cases have been reported, thus far
- Short incubation times suggest high titer, efficient route
- Study has estimated about 380 possible blood donors as infected with vCJD in the UK
  - Hilton et al, J Pathol 202 (2004)
- Leukofiltration removes only 50-70% of infectivity

PRDT Solution

- Develop an affinity technology-based device that can reduce endogenous infectivity from red blood cell concentrates (RBCs) while maintaining the integrity of the product
  - Development of ligand selection methodology
  - Screening involving different spikes and ligand sources
  - Infectivity bioassays
  - Hemocompatibility
  - Development of filter device

Ligand selection



Lathrop et al (2007) Anal Biochem 361:65-76

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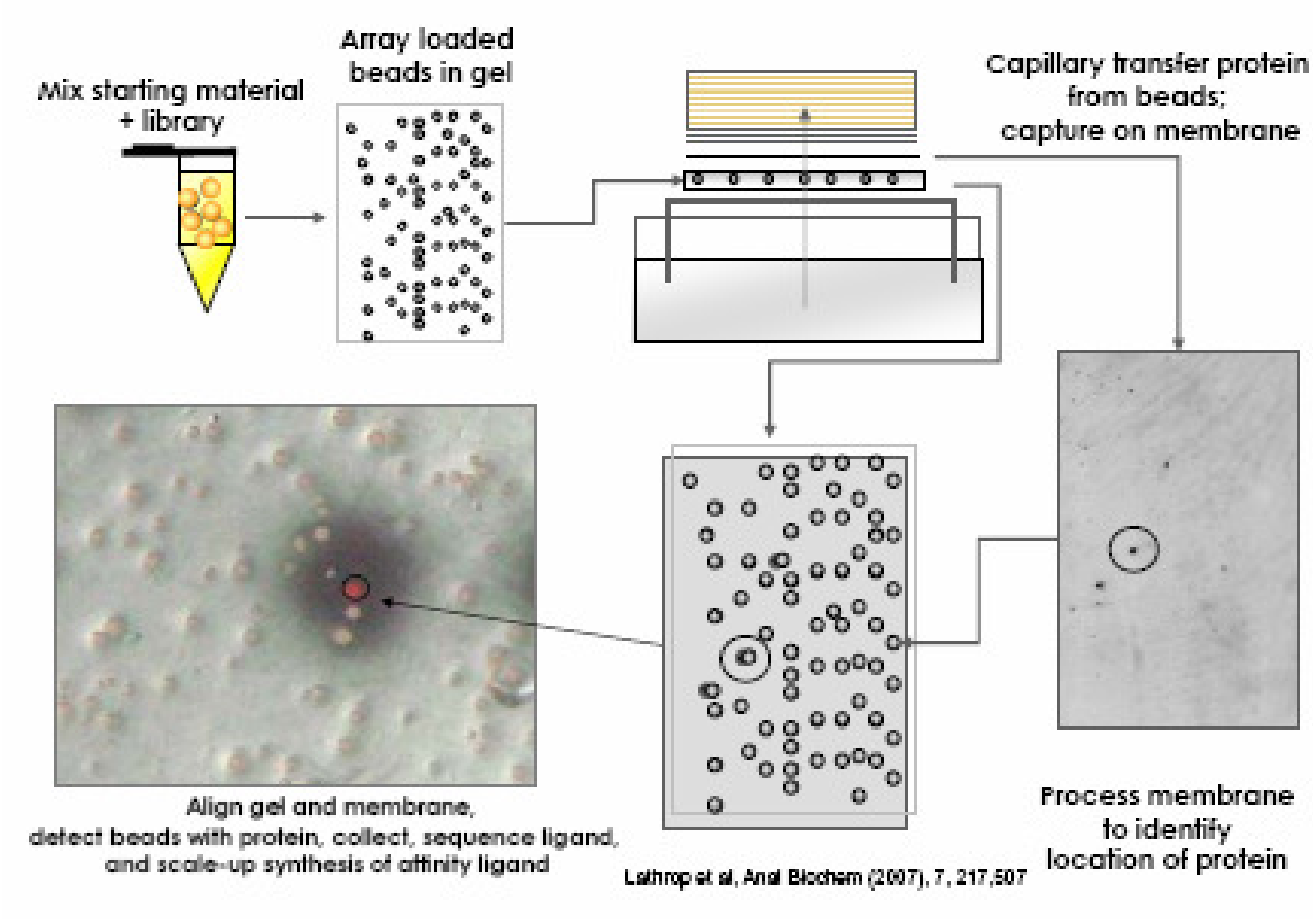
Ligand Selection

- Peptide ligands
  - 1-6 amino acid residues investigated
  - Solid-phase libraries
  - Millions of possible sequences
- Polymers
  - Commercially available
- Mimetic ligands
  - Triazine-based ligands
  - Library design based on peptide library results

Ligand Selection

- Primary Screening - Bead Blot



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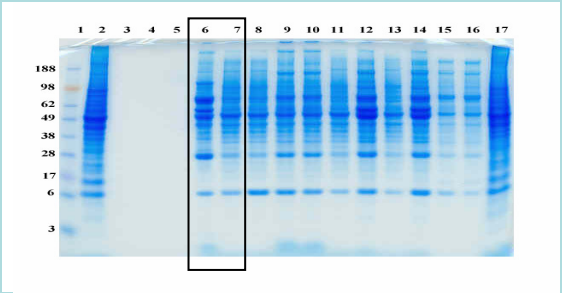
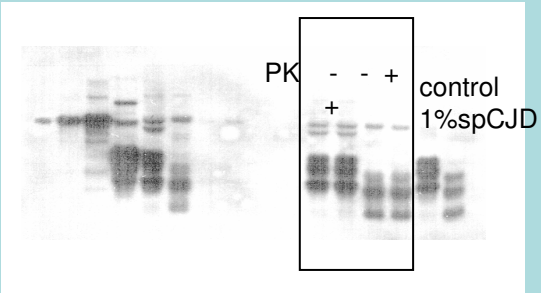
Ligand Selection

- Secondary Screening – Western Blots and SDS-PAGE Gels
  - Different spikes
  - Small chromatographic columns

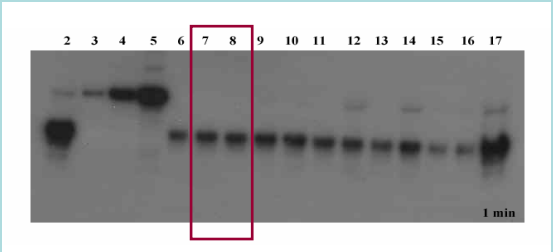
Hamster Brain PrPc Total protein staining

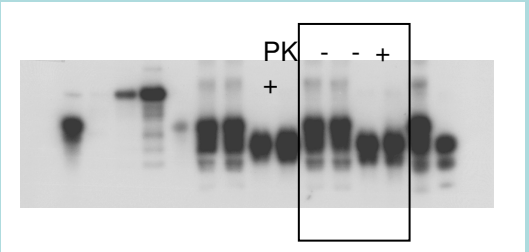




Human Brain PrPsc

Hamster Brain PrPc





Hamster Brain PrPsc

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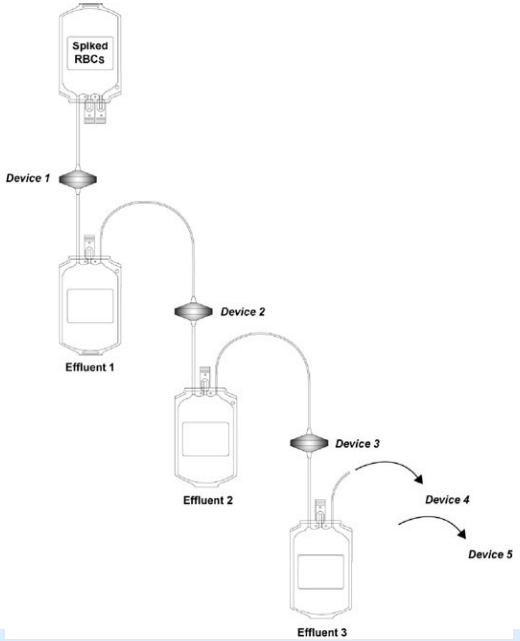
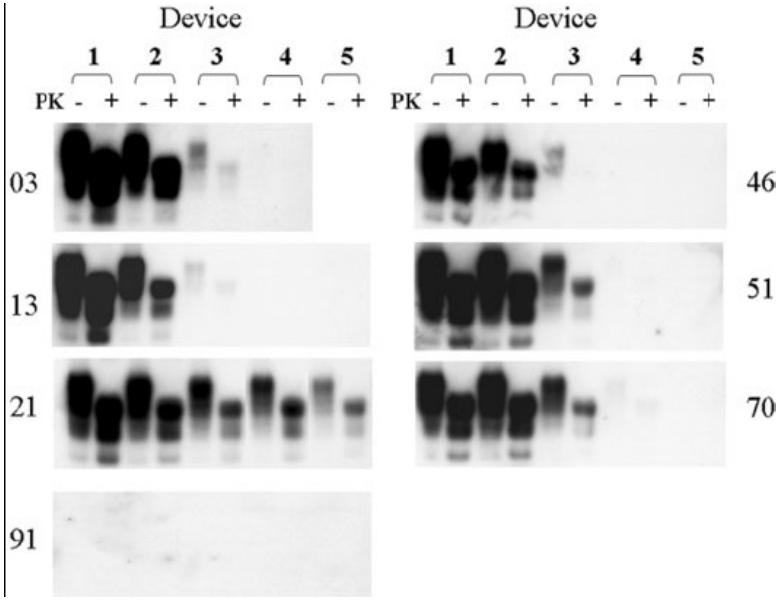
Ligand Selection

- Tertiary Screening – Infectivity Study
  - Removal of hamster brain derived infectivity spiked into human leukoreduced red blood cell concentrate
    - Gregori et al. (2006) Transfusion 46:1152-1161





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Ligand Selection

- Quaternary Screening – Infectivity Study
  - Removal of endogenous infectivity from scrapie-infected hamster leukoreduced whole blood
    - Gregori et al. (2006) Lancet 368:2226-2230

	Whole blood	LR WB Challenge	Flow through
Infected/Total animals	21/47	15/99	0/100
Poisson Titer ID/ml	11.8 ± 2.2	3.3 ± 0.8	< 0.2 ± 0.2
Reduction			> 1.2 $\log_{10}$
%Leukoreduction		72%	

Device removed all detectable infectivity from challenge

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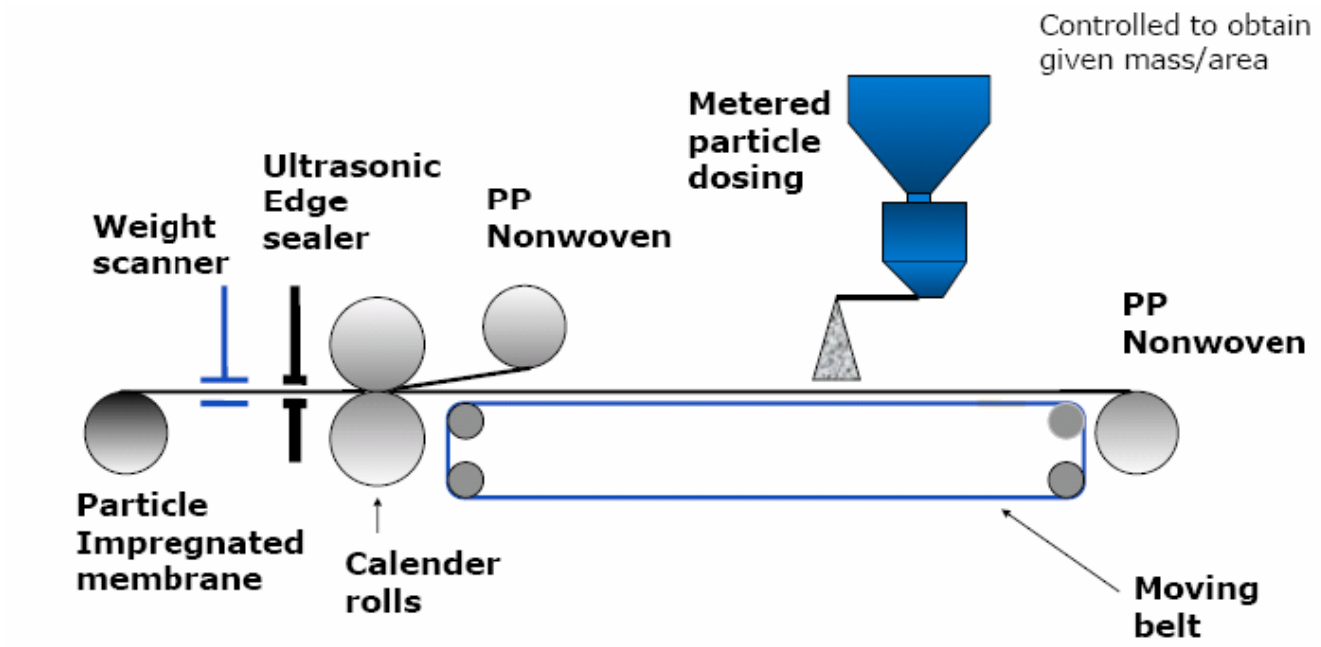
Device Development

- Particle-impregnated membrane (PIM) produced as below
- Multiple layers of PIM are stacked, fused together and encased, forming the final device



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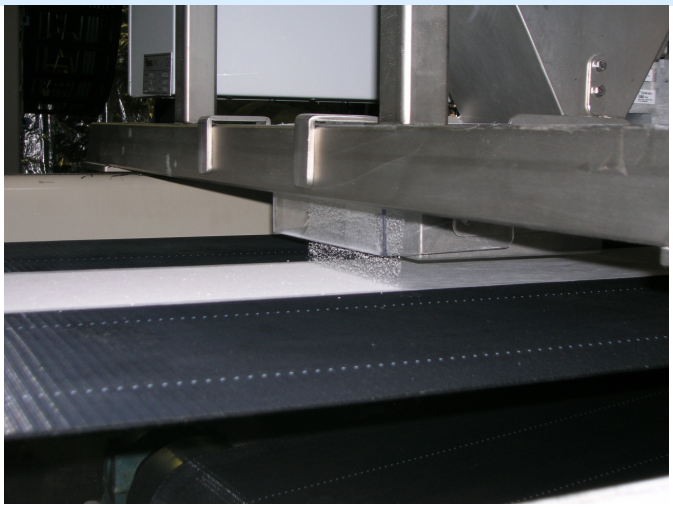
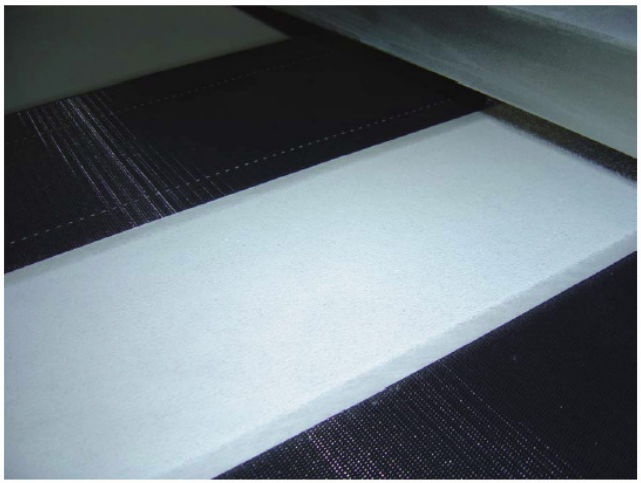
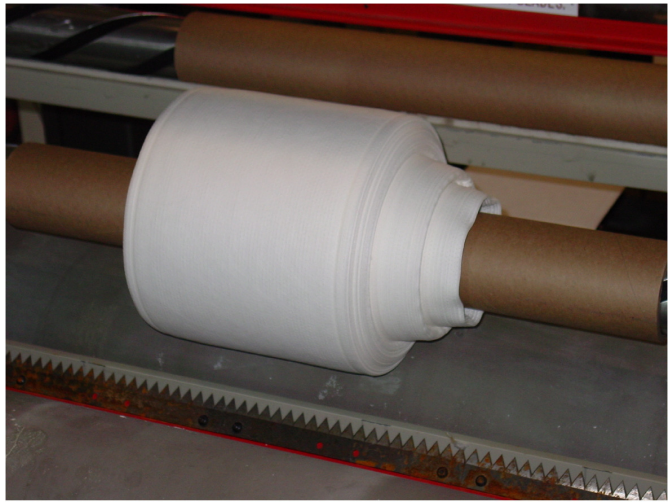
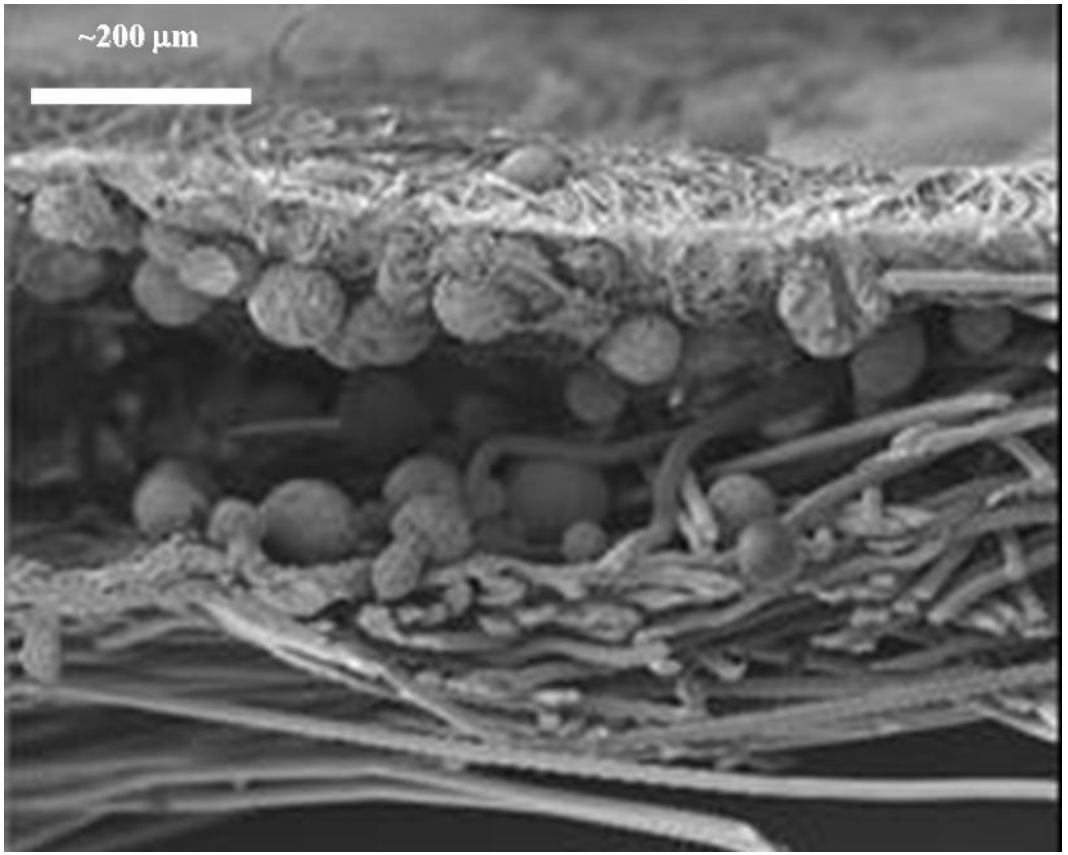
Device Development







PIM Characterization



- SEM of particle-impregnated membrane

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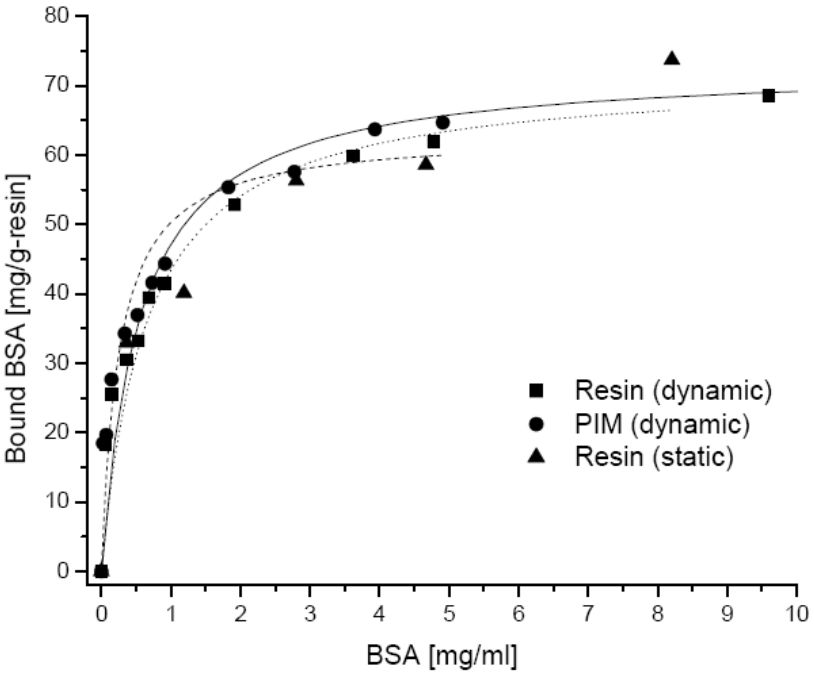
PIM Characterization

- Binding Isotherms
  - PIM has the same binding behavior as a packed bed column



Material	Q_{max} [mg/g]	K_d [M]
PIM (dynamic)	68.1	
Resin (dynamic)	72.9	$\sim 10^{-6}$
Resin (static)	71.8	

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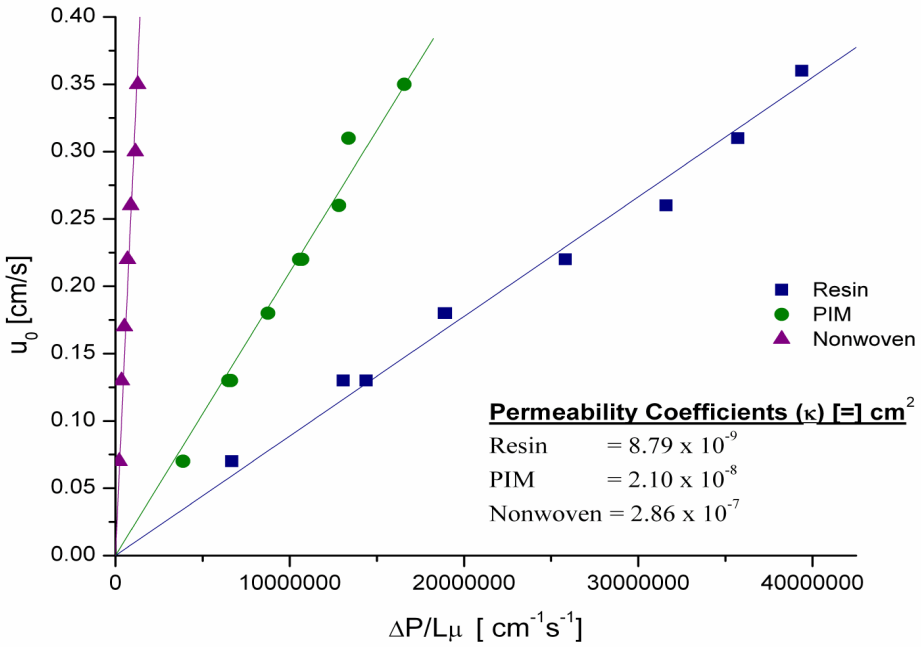
PIM Characterization



- Higher permeability than packed beds
  - Allows for the passage of particulate material, such as red blood cells

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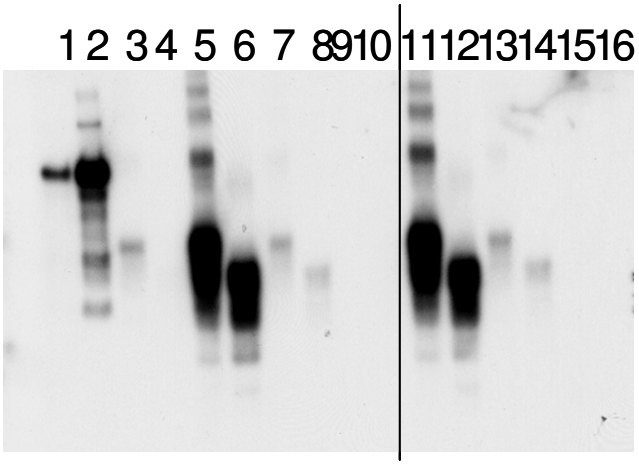
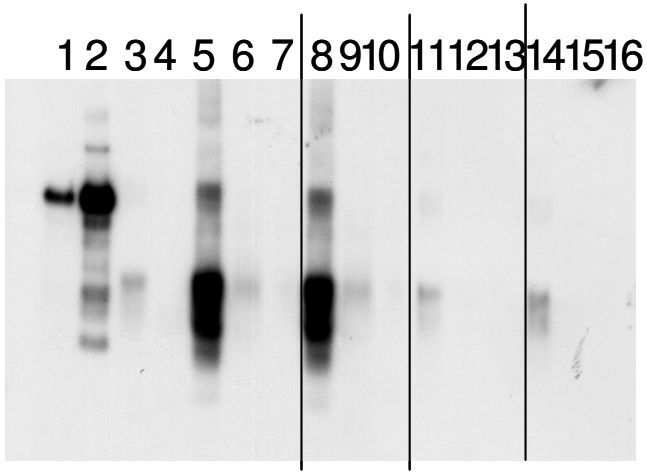
Binding of PrP to Device

- Binding of spiked PrP in RBC by resin columns and P-CAPT device
  - Packed columns and device bind PrPsc similarly



Resin in columns



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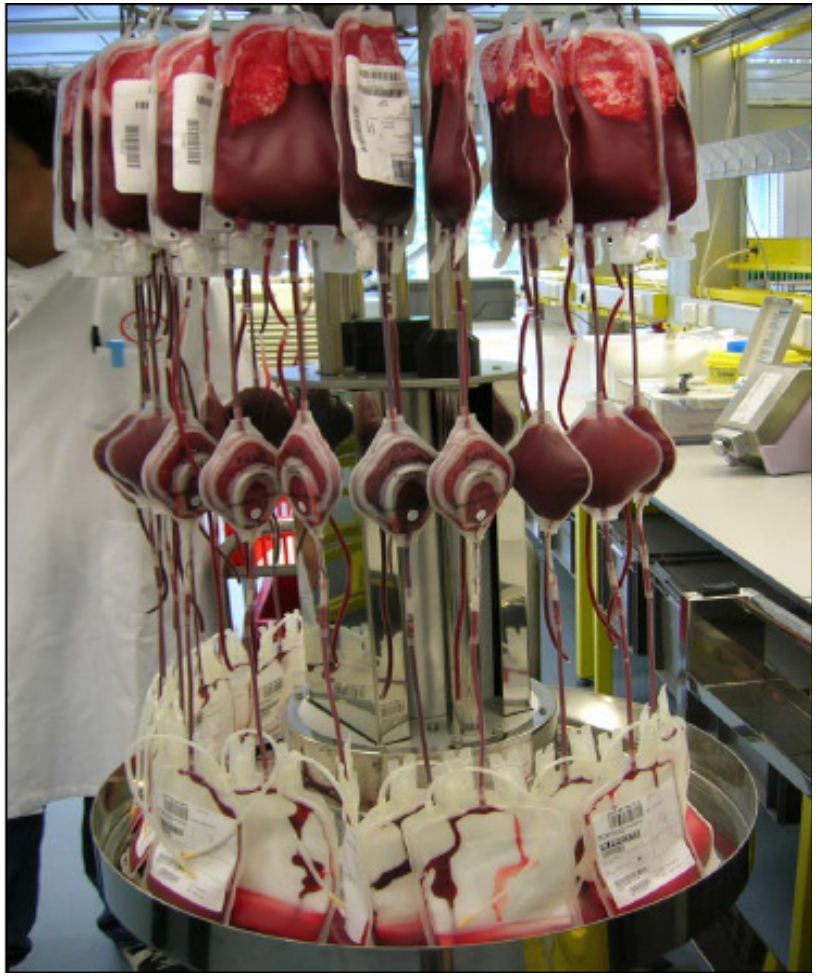
Hemocompatibility

- Hemocompatibility of resin with whole blood showed no negative effects
  - No hemolysis
  - No platelet activation
  - No complement activation
  - No factor VII activation
- RBC yields are within the acceptable limits



P-Capt Device

- Approved for commercialization in Europe (CE mark)
- Efficacy of Removal
  - >3 $\log_{10}$ reduction of exogenous brain spike infectivity in RBC containing 2,000,000 times the level of infectivity expected in RBC
  - Removal of all detectable endogenous infectivity from whole blood
- No impact on red blood cells or activation of coagulation factors, platelets or complement
- Neoantigenicity and Red Cell Recovery and Survival studies have been completed
- No adverse effects detected in Human Safety trials

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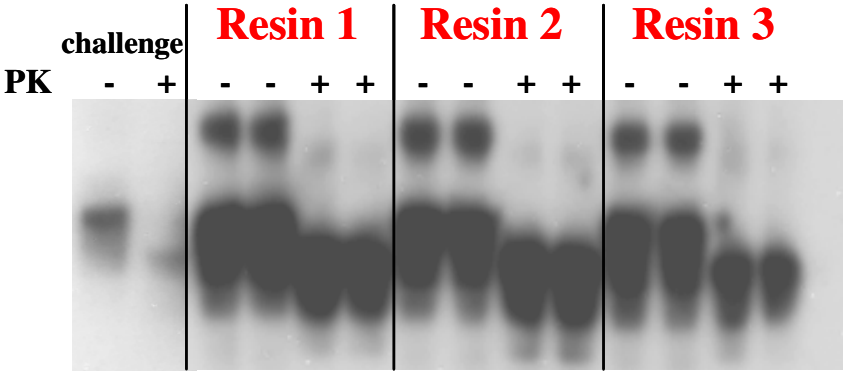
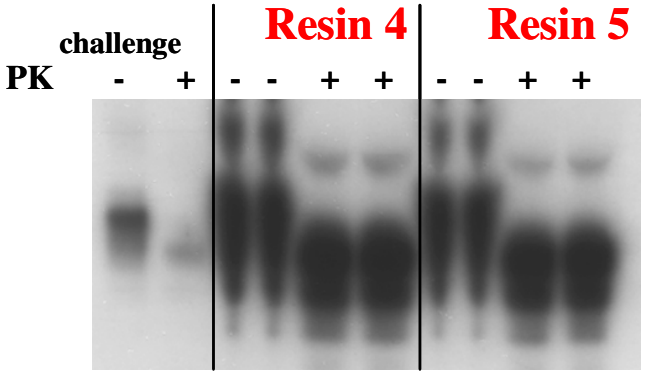
Prion Removal from Plasma Products

- One presumed plasma-derived transmission case to date
  - Patient was a hemophiliac
- Low level of contamination (approx. 3 ID_{50}/ml based on 263K hamster studies)
- Large plasma pools (many 1000's of units)
- Dilution of infectivity does not eliminate the risk
- Precautionary measures for a potential risk

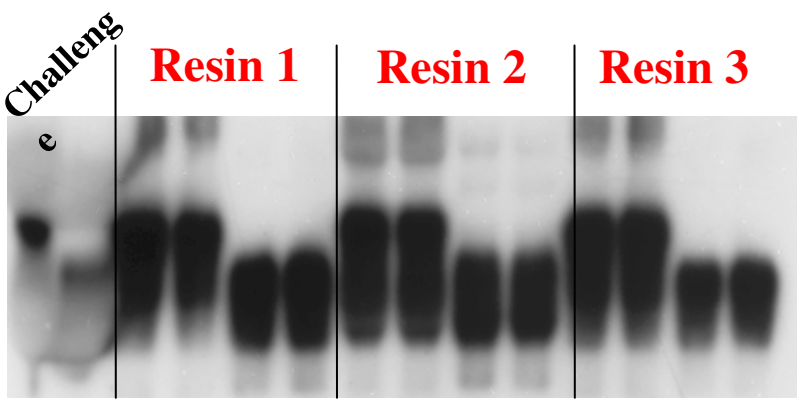
Prion Removal from Plasma Products

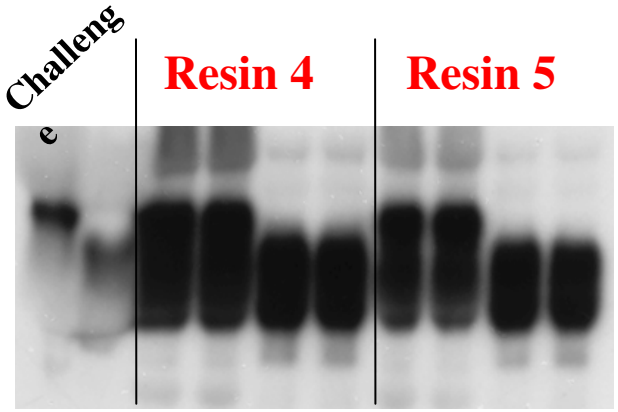
PRDT resins challenged with 25% HSA





PRDT Resin 3 challenged with 3% IgG





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In Conclusion

- P-Capt, a device for removal of prion infectivity from Red Blood Cells
  - Demonstrated infectivity removal at endogenous levels
  - Device is safe and effective
  - Currently available for adoption by Blood Services

Acknowledgements

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  - Ruben Carbonell, Omon Herigstad
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