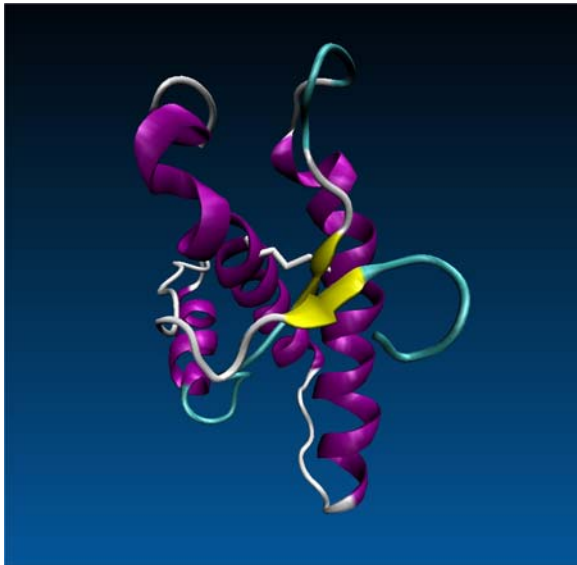




# Reduction of prion from red cells, plasma and plasma products using specific affinity ligands



Steven J. Burton  
Chief Executive Officer  
ProMetic BioSciences Ltd



## Pathogen Removal and Diagnostics Technologies Inc. (PRDT)

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- Joint venture between:

- ProMetic Biosciences
- American Red Cross



- Scientific founders:

- David Hammond (ARC), Ruben Carbonell (NCSU), Robert Rohwer (VA Medical Center, Univ. Maryland).

- Partnership with MacoPharma SA: prion-reduction device for leuco-reduced red blood cell concentrate (P-Capt™).

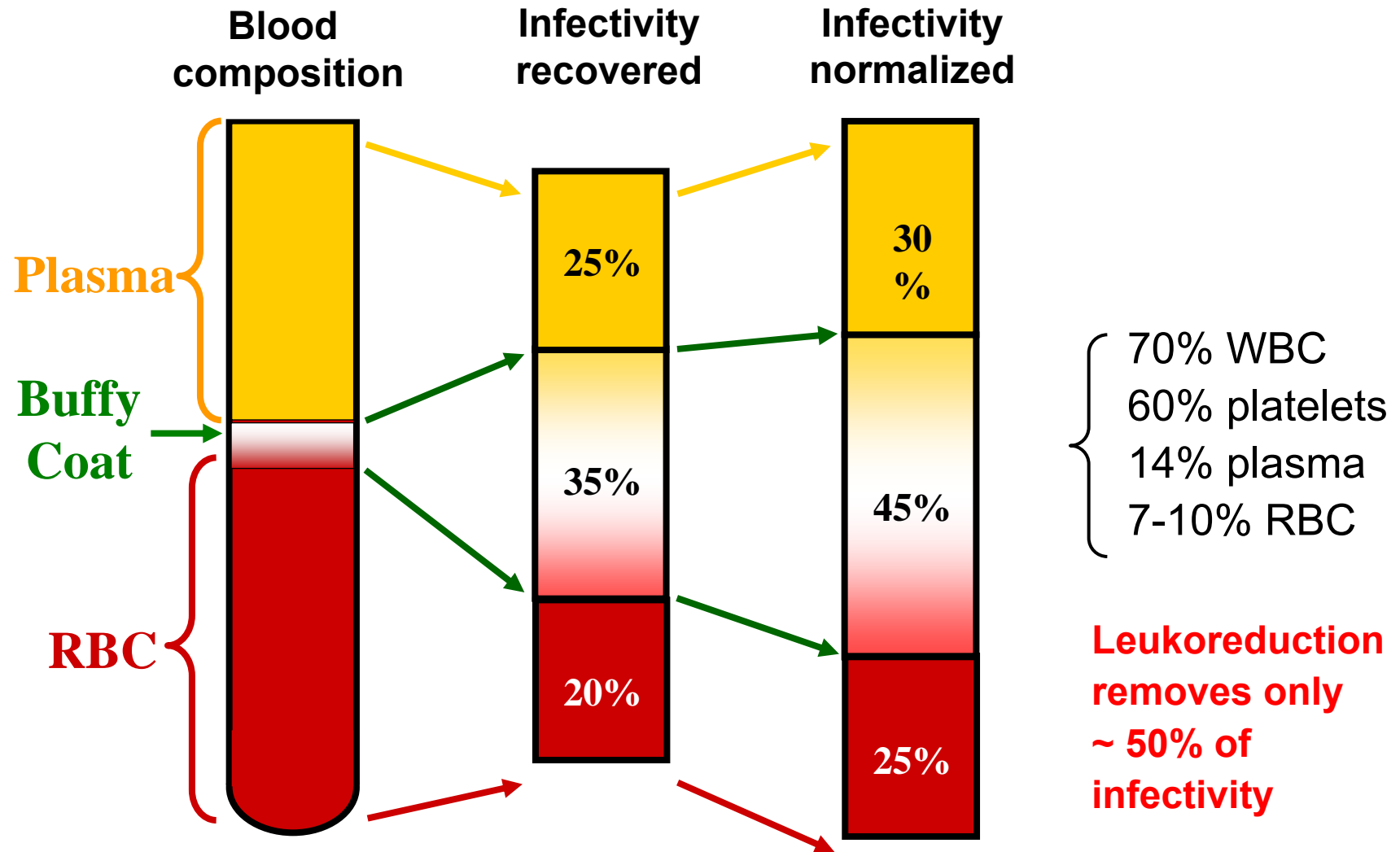


- Collaboration with Octapharma AG: prion-reduced S/D Plasma (Octaplas® LG).





# Distribution of TSE Infectivity in Blood Components





# Approaches to increasing the prion safety of blood and plasma products

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FDA (May, 2007) and EMEA (February, 2007) have both issued statements that there is “an unquantifiable but low risk [of TSE transmission] associated with plasma derived products”.

CPMP: “Guidelines for the Investigation of Manufacturing Processes for Plasma-Derived Medicinal Products with Regard to vCJD Risk” (CPMP/BWP/CPMP/5136/03).

 Prion detection



Current prion assays lack sensitivity;  
Issues surrounding false-positives.

 Prion inactivation



PrP<sup>res</sup> is significantly more stable than  
cellular components and plasma proteins

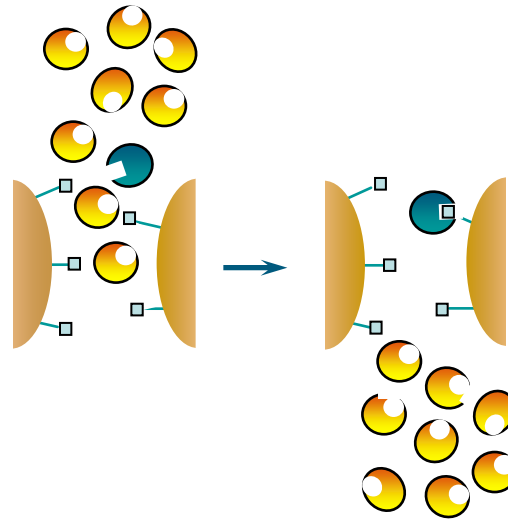
 Prion removal



Viable approach with appropriate  
affinity ligands

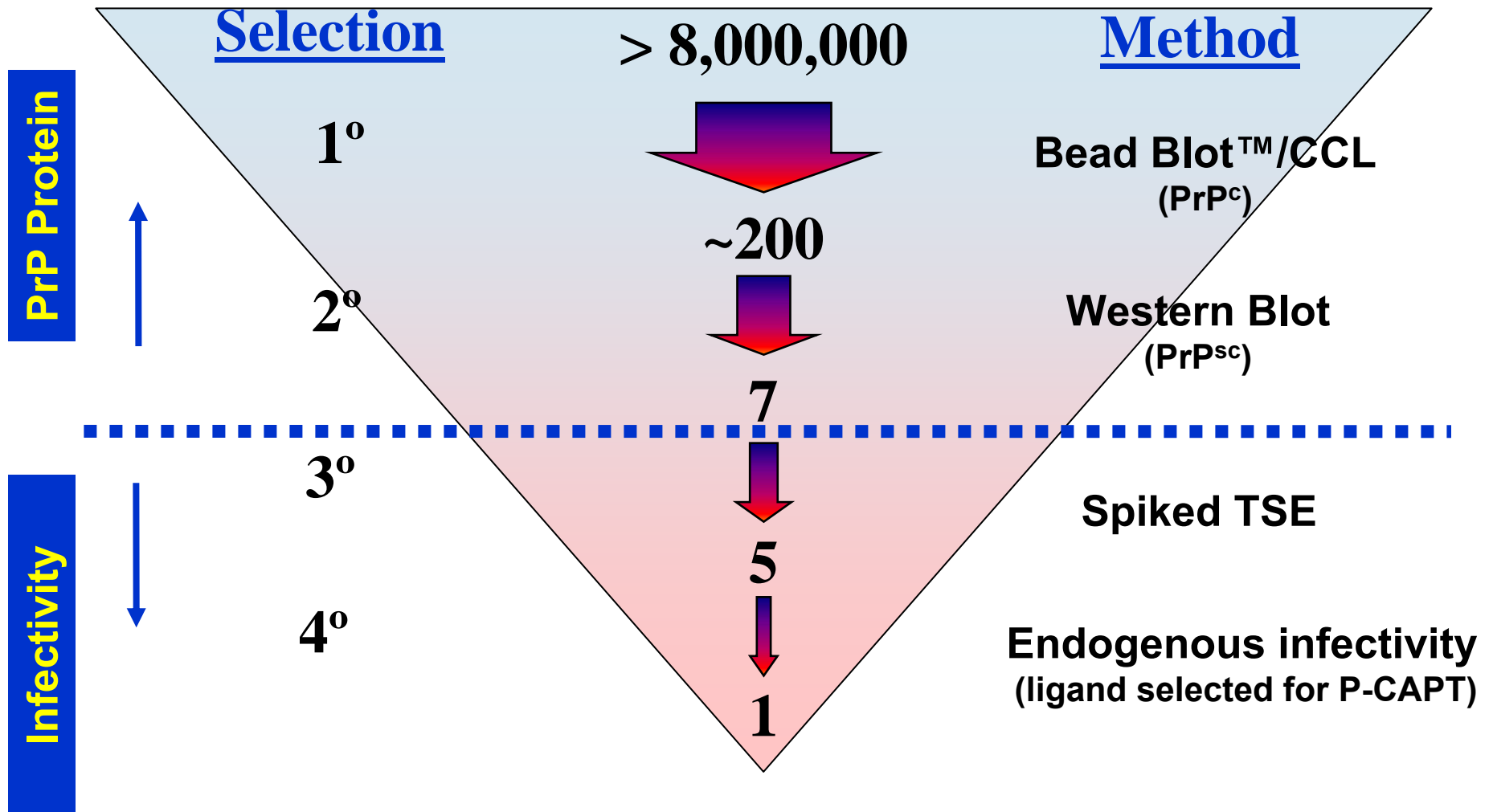


# Prion removal with affinity ligands



- Ligand specific to prion proteins (ideally different types of prion protein)
- Minimal interaction/binding of biological product (low impact on product yield)
- Inexpensive ligands/adsorbents (single-use disposable)
- Synthetic ligands (Mimetic ligands<sup>TM</sup>, peptide ligands)
- Viable option for specific removal of problem pathogens/impurities

# Selection strategy for prion binding ligands

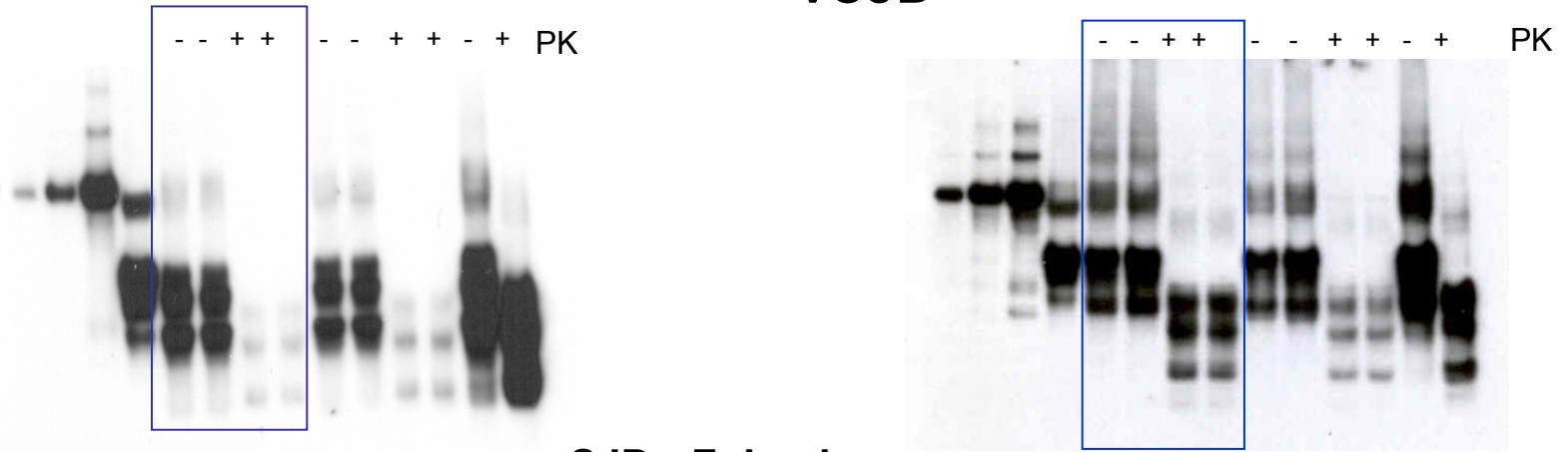




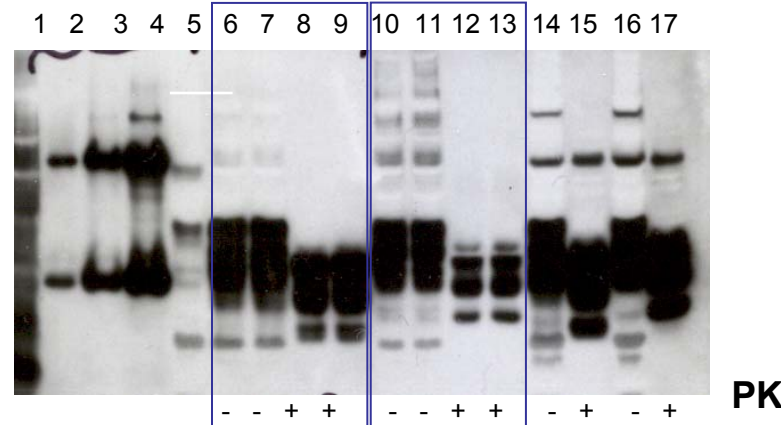
# Capture of TSEs by selected affinity ligands

spCJD

vCJD

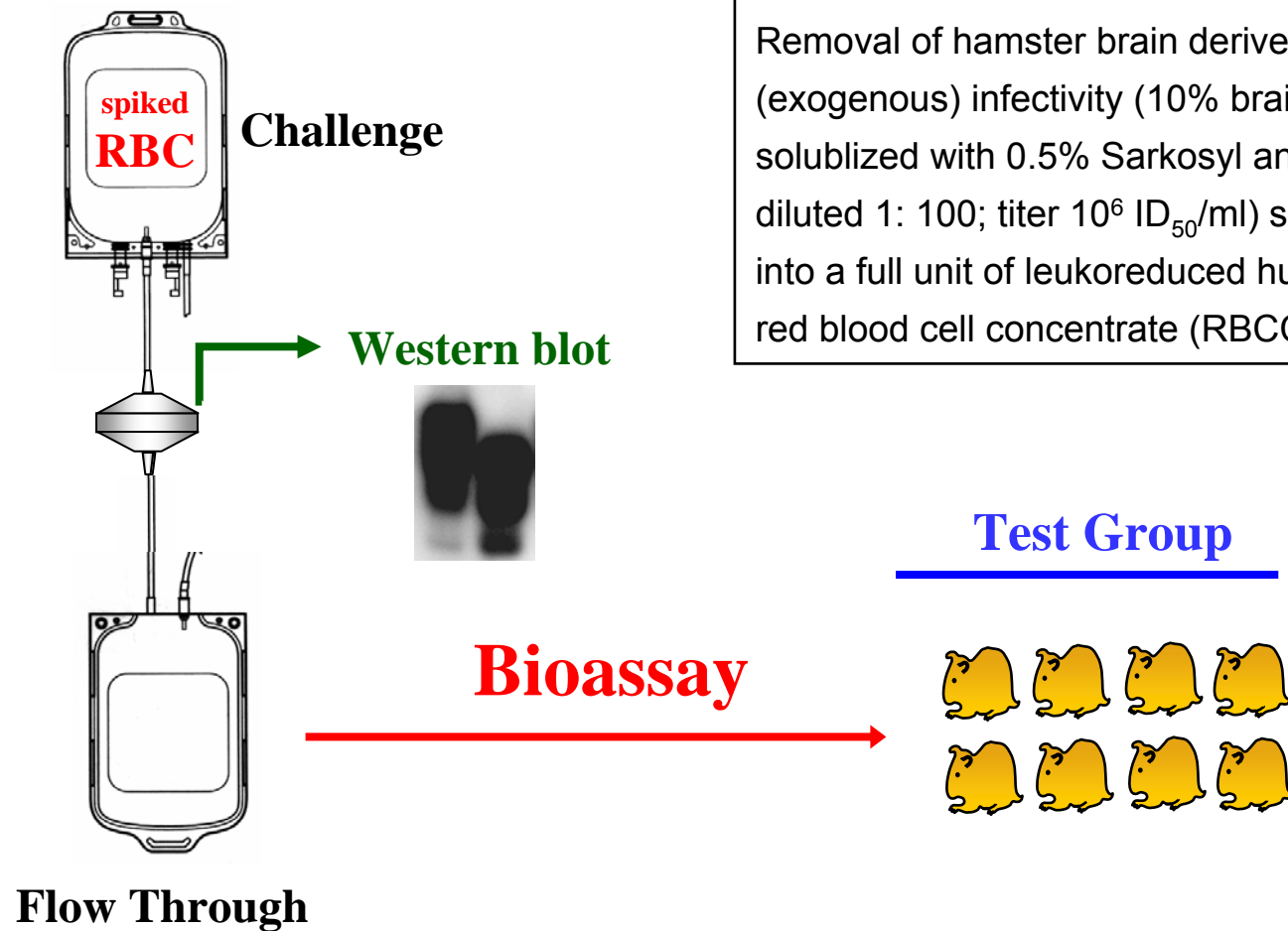


vCJD Fukuoka



**Only ligands that bound different forms of TSE with minimal non-specific binding were selected for further study**

# RBCC spiked with scrapie-infected brain homogenate



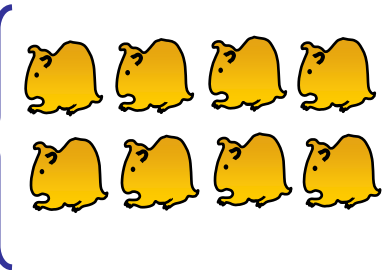
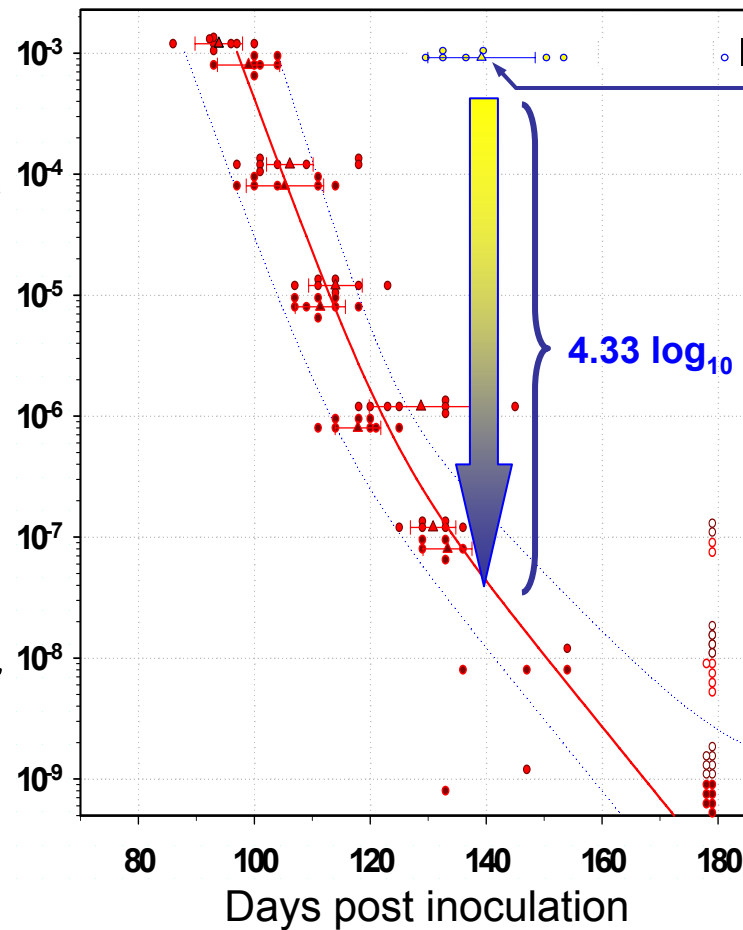
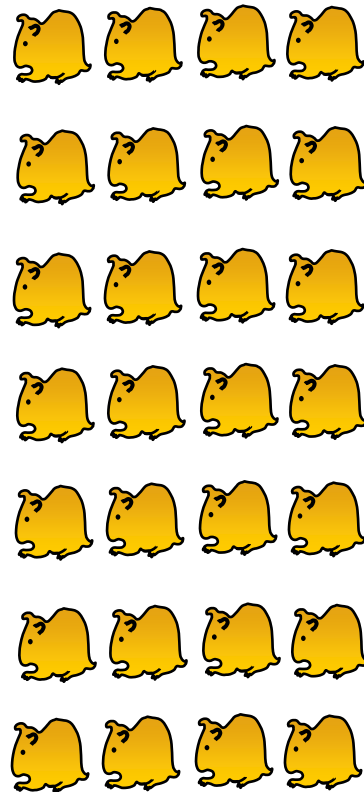
Removal of hamster brain derived (exogenous) infectivity (10% brain solublized with 0.5% Sarkosyl and diluted 1: 100; titer  $10^6$  ID<sub>50</sub>/ml) spiked into a full unit of leukoreduced human red blood cell concentrate (RBCC).

# Incubation time bioassay for control and treated RBCC

## Dose Response Standard Curve

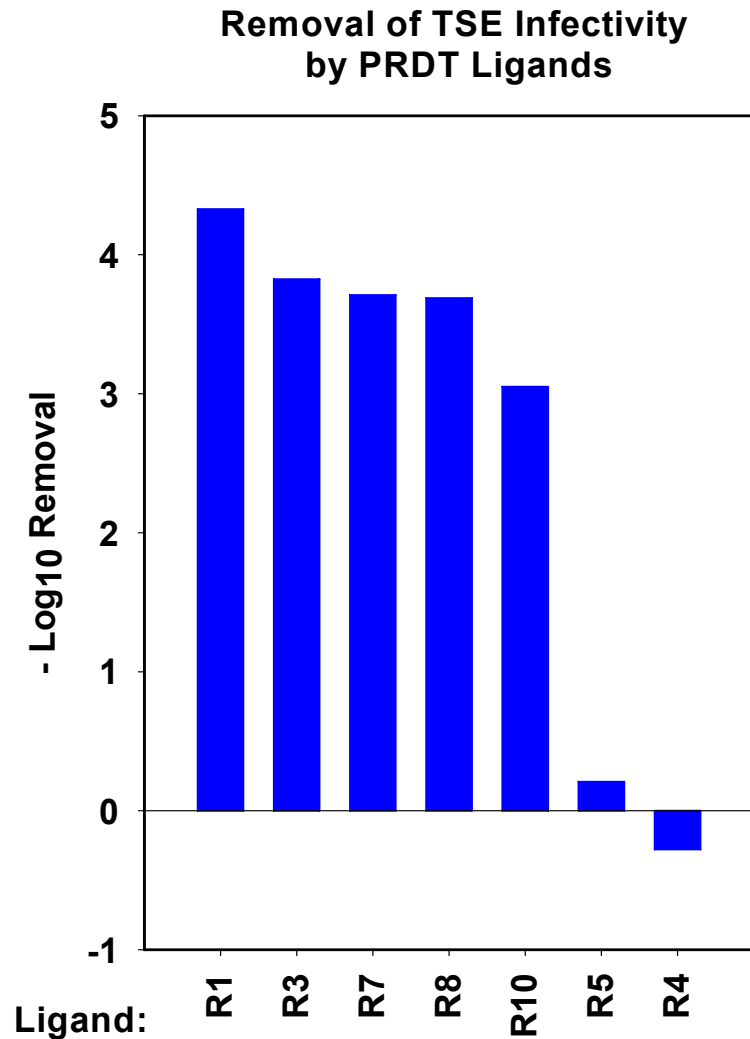
## Test Group

Dilution relative to whole brain





# Reduction of brain derived infectivity spike in human RBCC by PRDT ligands

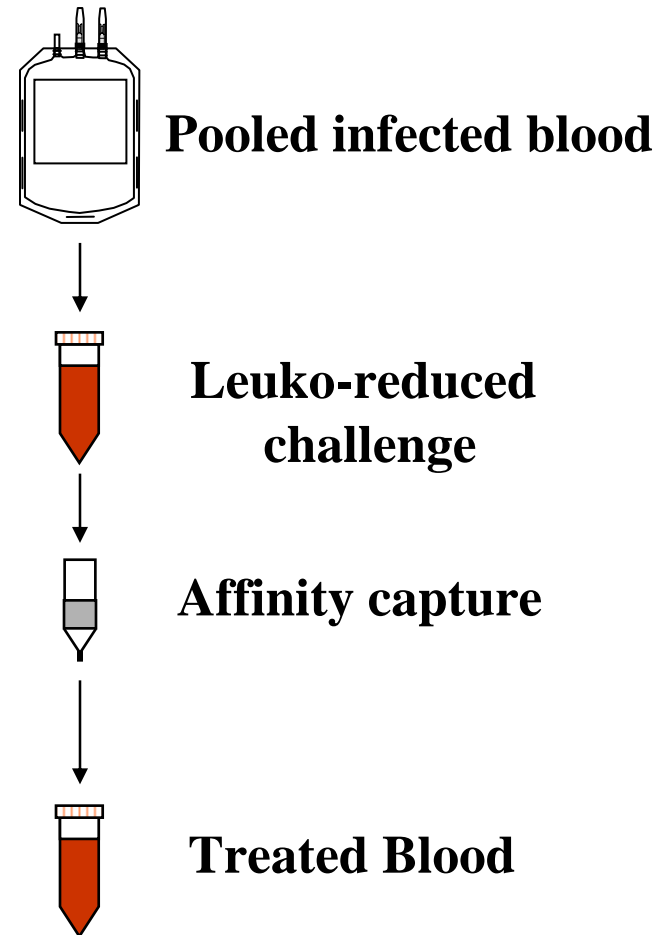


- Challenge titer = 1 million ID/ml
- Blood titer is 10 ID/ml
- R1, R3, R7, R8 and R10 are selected PRDT resins
- R5 and R4 are negative controls

[Gregori *et al.*, Transfusion, **46**, 1152-1161, 2006]

# Reduction of endogenous blood-borne infectivity

- Lead ligand (P-CAPT) tested for PrP<sup>sc</sup> binding.
- Scale-down experiment with leukoreduced endogenous scrapie-infected hamster (263K) whole blood.
- Prion infectivity determined by limiting dilution titration of the challenge and post-affinity capture sample.
- 0.05 ml injected intra-cranially per animal. Recipient animals observed for 550 days post inoculation.





# Reduction of endogenous blood-borne infectivity

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	Whole Blood	Leukoreduced whole blood challenge	Treated blood
No. infections	21/47	15/99	0/100
Poisson Titre (ID/mL)	11.8 ± 2.2	3.3 ± 0.8	<0.2 ± 0.2

**Endogenous infectivity reduction  $\geq 1.2 \log_{10}$**

[Gregori *et al.*, Lancet 2006, **368**, 2226-30]

# P-Capt™ prion reduction filter

- Sterile, single use prion-reduction device.
- Incorporates selected PRDT prion-binding affinity resin trapped between supporting membrane layers.
- Stand-alone filter
- Designed for use with human leuko-reduced rbcc.
- Device is CE Mark approved in Europe.





# Risk of vCJD transmission by plasma and plasma-derived products

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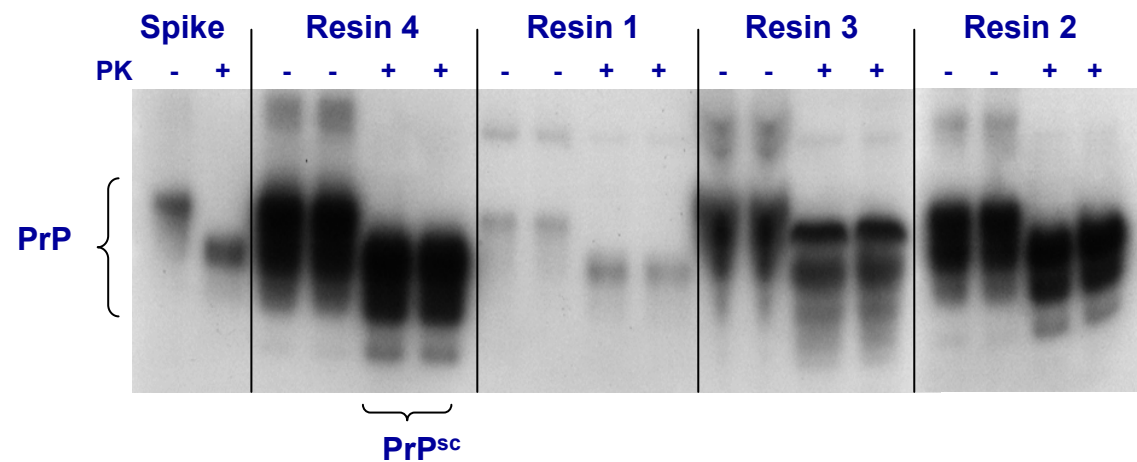
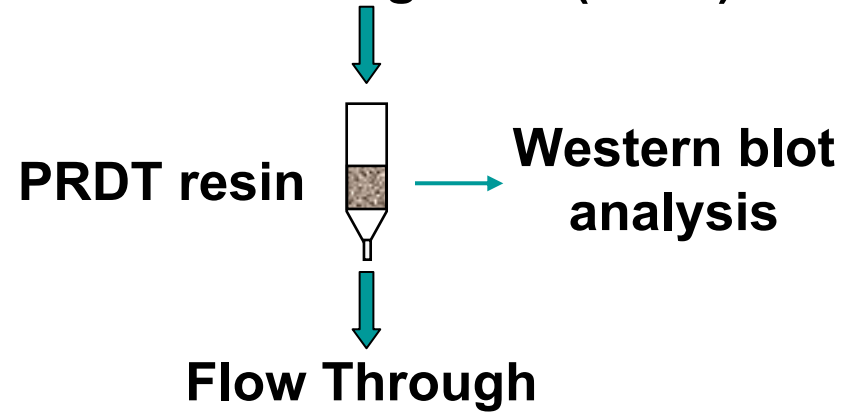
- No reported plasma-derived transmission cases to date
- Low level of contamination (approx. 3 ID<sub>50</sub>/ml based on 263K hamster studies)
- Large plasma pools (many 1000's of units)
- Dilution of infectivity does not eliminate the risk
- Precautionary measures for a potential risk





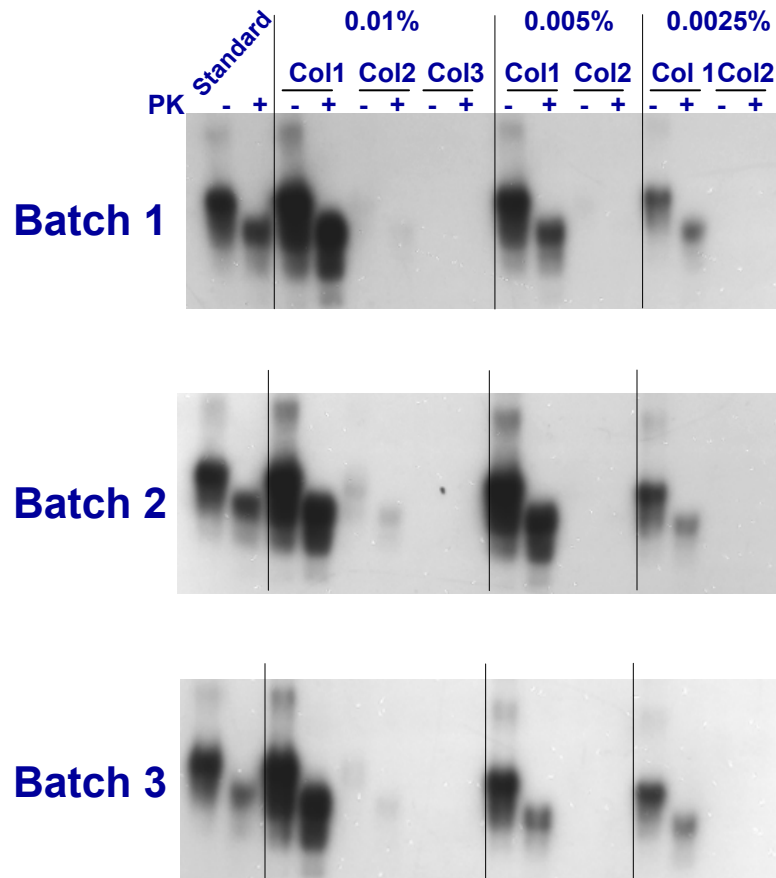
# Prion reduction from S/D treated plasma

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





# Prion reduction from S/D treated plasma

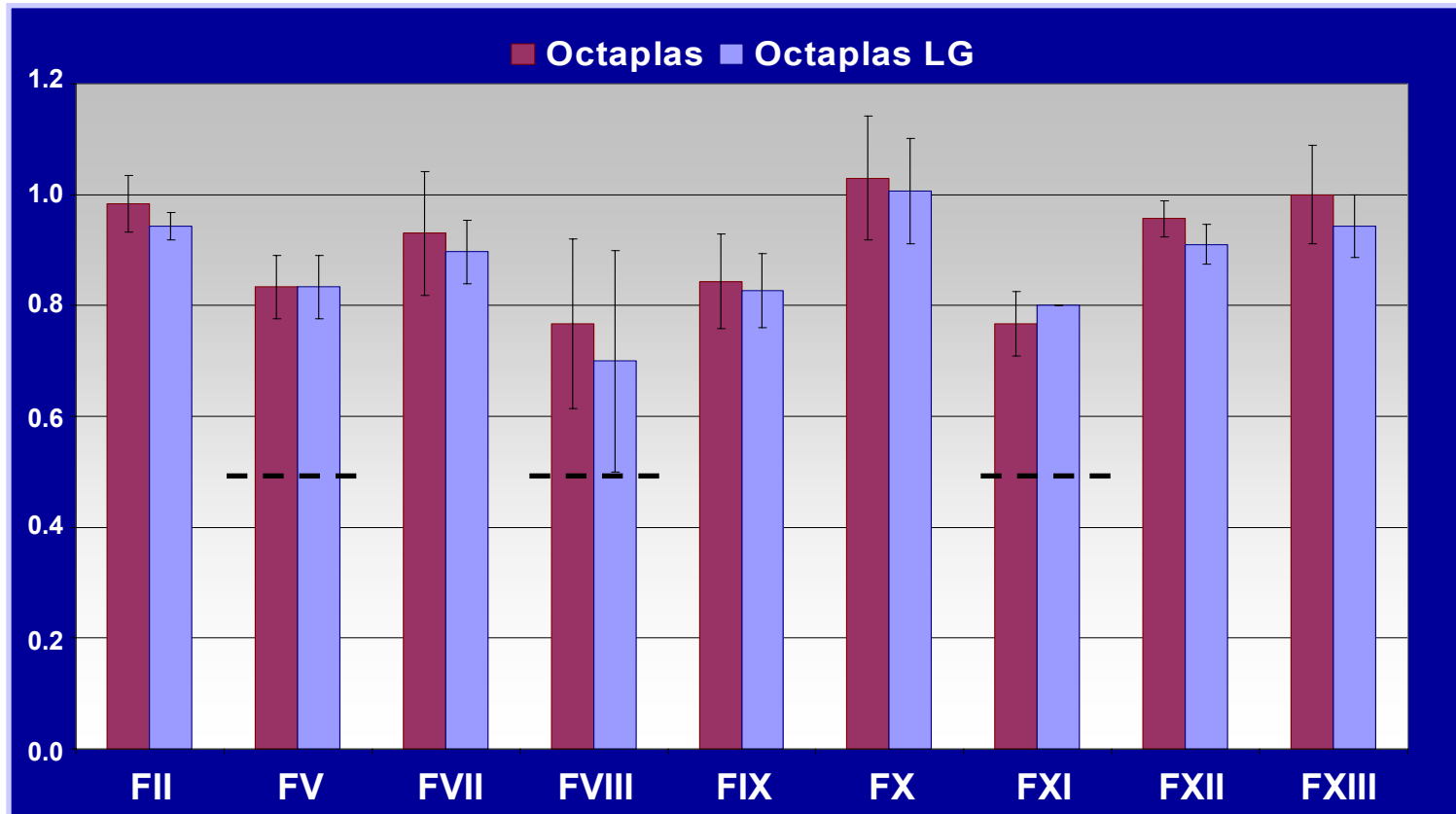


- PrP<sup>sc</sup> binding studies with 0.5ml columns of resin and 10ml S/D treated plasma.
- PrP<sup>sc</sup> present in 0.005% scrapie homogenate spike is bound by a single column.
- Spike titer =  $5 \times 10^4$  ID<sub>50</sub>/ml challenge
- Input infectious doses =  $5 \times 10^5$  ID<sub>50</sub>
- PrP signal recovered on 0.5 ml resin ~ 100% input
- **Indicated resin capacity ~ 6 log<sub>10</sub> ID<sub>50</sub>/ml**



# Effect of prion reduction step on coagulation factor activity

Coagulation factors [IU/ml]

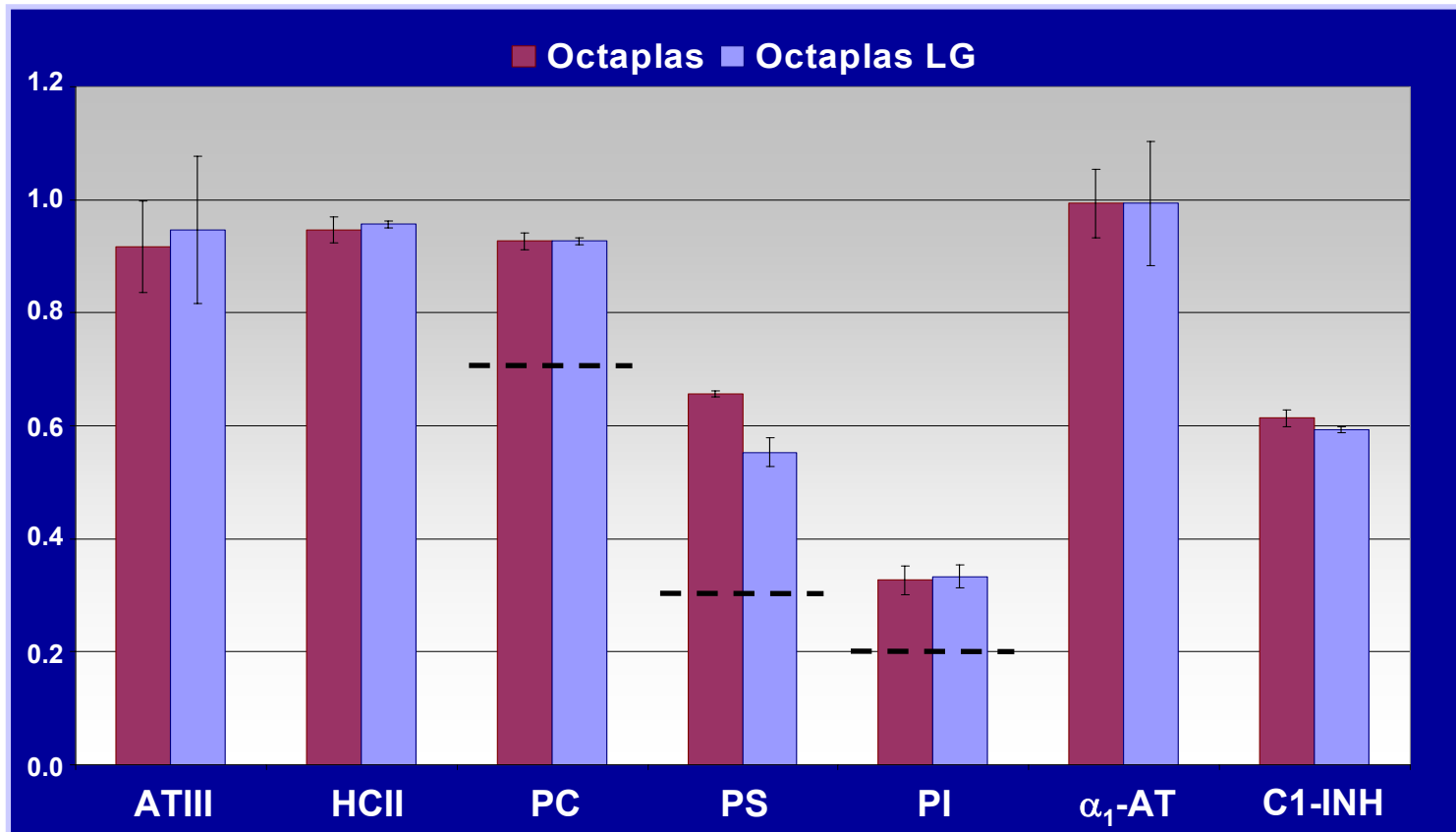


Broken lines, Ph.Eur. specification levels for coagulation factors V, VIII and XI (FV, FVIII and FXI)  
[Ph.Eur. 5.0, 01/2005:1646]

Data courtesy of A. Heger, Octapharma AG

# Effect of prion reduction step on protease inhibitors and cofactors

Protease inhibitors and cofactors [IU/ml]



Broken lines, specification levels for protein C (PC), protein S (PS) and plasmin inhibitor (PI) in the process of being included into the Ph.Eur. [Ph.Eur. 6.2, 07/2008:1646]

Data courtesy of A. Heger, Octapharma AG



# Estimated safety margin

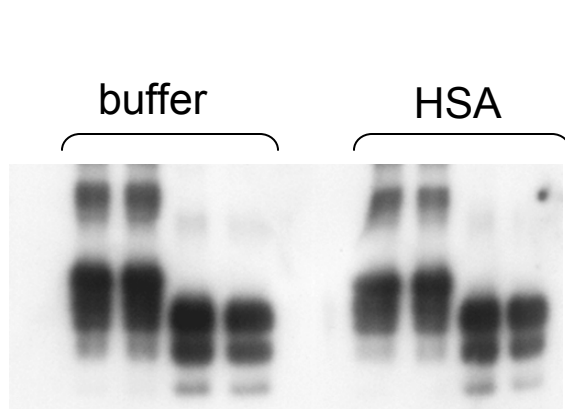
Possible prion infectivity (ID/mL plasma)	20
Plasma bag size (mL)	600
Estimated Prion Load for a single donor (ID)	12,000
Infectivity binding capacity (ID <sub>50</sub> /mL PRDT resin)	≥6.0 log <sub>10</sub>
Infectivity binding capacity (ID/3.8L column)*	≥9.4 log <sub>10</sub>
Safety margin assuming 1 infectious plasma bag	≥5.3 log <sub>10</sub>
Safety margin assuming 10 infectious plasma bags**	≥4.3 log <sub>10</sub>

\* ID = ID<sub>50</sub> x 0.69; \*\*Equivalent to a hypothetical 1.6% prevalence among blood donors

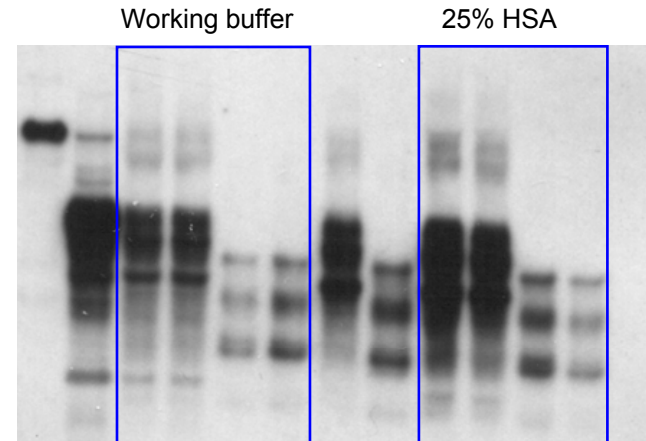
**“After implementation of this novel technology, Octaplas® has the same clinical safety and efficacy profile, except for the further enhanced safety margin in terms of prion disease transmission such as vCJD”**



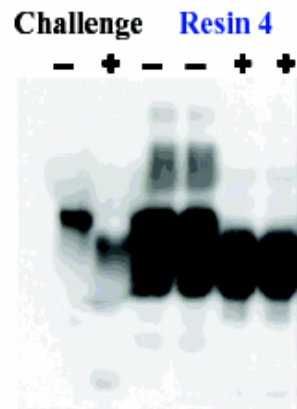
# Prion capture from protein solutions



Capture of HaPrP<sup>sc</sup> from 25% w/v HSA



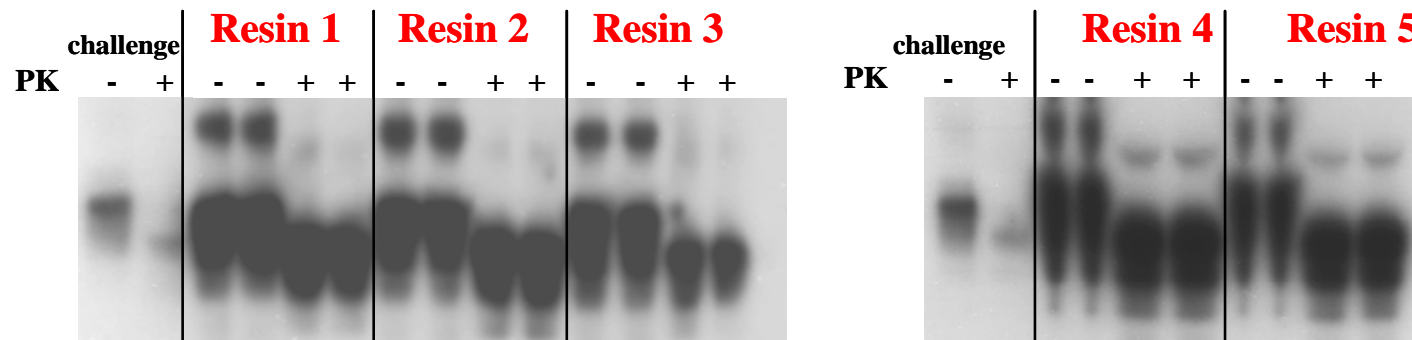
Binding of spCJD in presence of 25% w/v HSA



Capture of HaPrP<sup>sc</sup> from 3% w/v IVIG



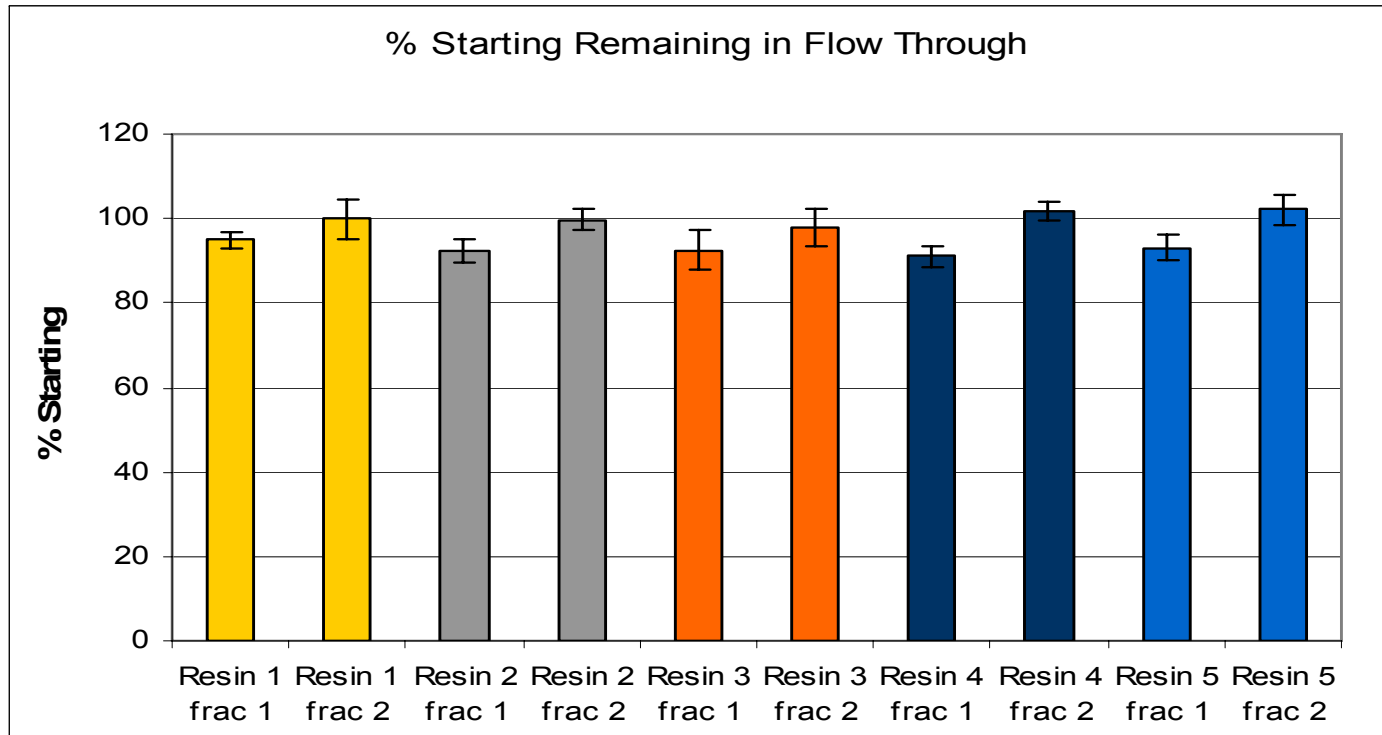
# Prion capture from 25% w/v HSA solution: PRDT resin performance



Western blot of PrP<sup>res</sup> binding to different PRDT affinity resins in the presence of 25% w/v HSA.



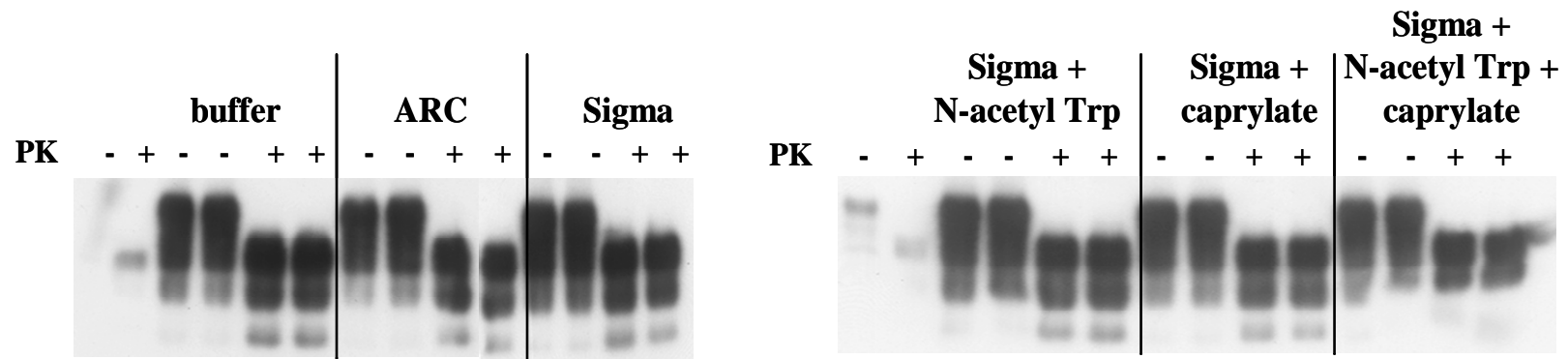
# Prion capture from 25% w/v HSA solution: protein recovery



10 mL of 25% w/v albumin were applied to 0.5 mL resin packed in a column. Two 5 ml flow through fractions were collected and albumin quantified in the challenge and the flow through fractions by nephelometry.



# Prion capture from 25% w/v HSA solution: effect of excipients



Western blot of PrP<sup>res</sup> bound to PRDT Resin 3 challenged with different 25% w/v HSA formulations.



# Summary of prion-binding ligand performance

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- ✓ PRDT has developed five prion-binding ligands which are very effective for use in prion capture applications.
- ✓ Selective for mammalian PrP<sup>c</sup> and PrP<sup>res</sup> (mouse, hamster, sheep, monkey & human brain spikes).
- ✓ High affinity for prion protein ( $K_a > 10^9 \text{ M}^{-1}$ ).
- ✓ Infectivity binding capacity:  $\sim 10^6 \text{ ID}_{50}$  per mL (brain derived infectivity).
- ✓  $\geq 1.2 \log_{10}$  removal of endogenous infectivity (removal to LOD) from leuco-reduced whole blood.
- ✓ Effective for whole blood, red blood cell concentrate, plasma and plasma proteins.
- ✓ No impact on red blood cells or activation of coagulation factors, platelets or complement; minimal binding of other plasma proteins.
- ✓ Can be used to reduce the risk of transmission of prion infectivity from products isolated from human or animal sources.



# Acknowledgements

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