

Development of a Sensitive and Specific Prototype Prion Assay for Blood Screening

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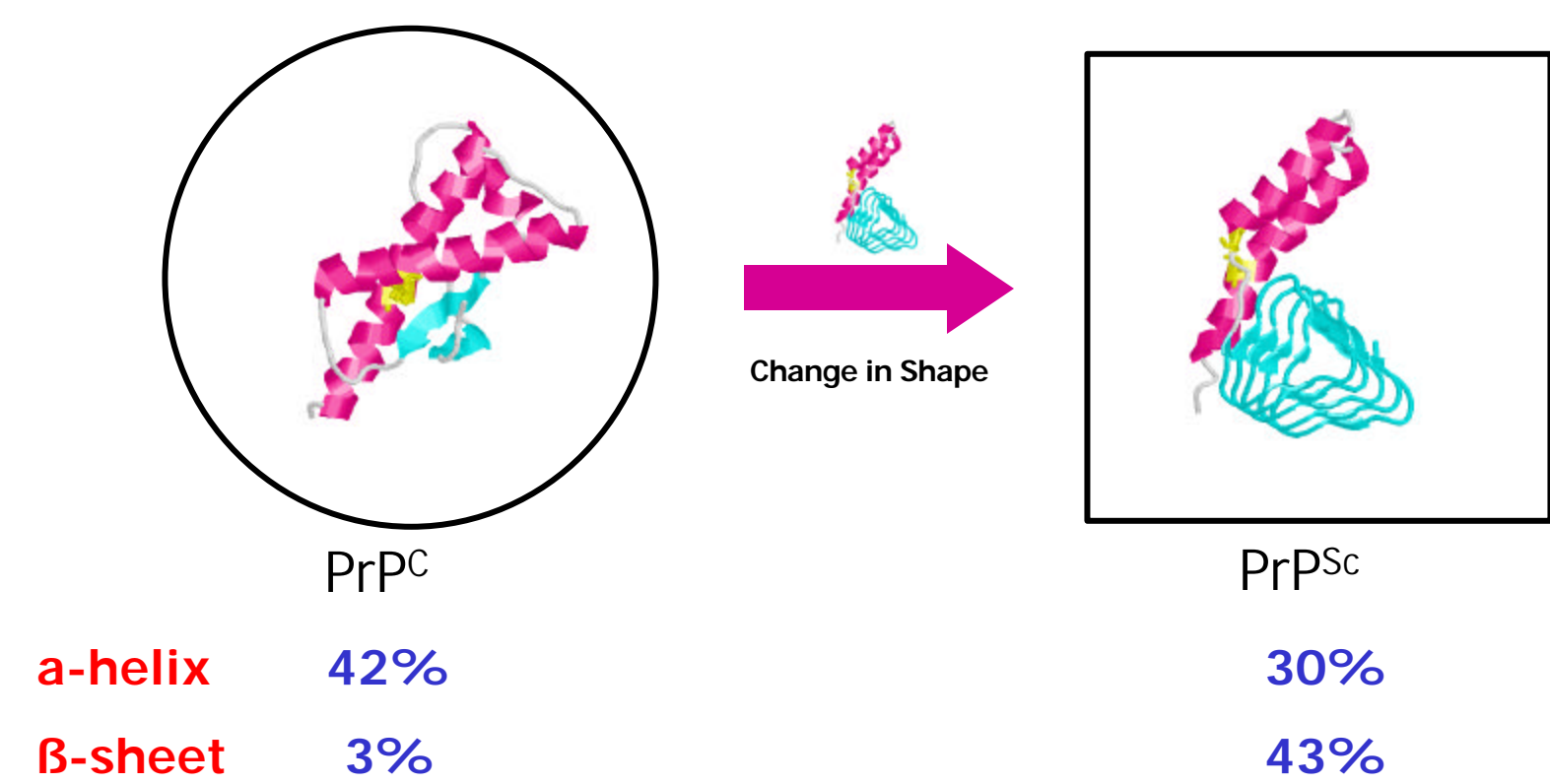
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INTRODUCTION

The isoform (PrP^{Sc}) of a normal cellular prion protein (PrP^C) is an infectious agent that causes Transmissible Spongiform Encephalopathy (TSE, also known as prion disease) disease in animals and human. Scrapie, a prion disease in sheep has been known for more than two centuries with no known transmission to human. Bovine Spongiform Encephalopathy (BSE) was recognized as new prion disease in the 1980s. During this period, the disease passed to human through the consumption of contaminated beef products, leading to the emergence of a new, variant form of Creutzfeldt-Jakob Disease (vCJD). More than one million BSE-infected cows have been slaughtered for human consumption, implying that millions of consumers are at risk of infection. Since there are no clinical signs or symptoms of the disease for many years, there is no accurate way to determine how many people could be harboring the disease. Currently, there is no effective treatment of this fatal, neurodegenerative disease. Increasing evidence indicates that vCJD-associated PrP^{Sc} is transmissible through blood transfusion, which is a large concern for blood safety and public health. Unfortunately, current commercial prion assays are not sensitive enough to detect PrP^{Sc} in the blood due to two major difficulties: extremely low PrP^{Sc} concentration in blood and lack of PrP^{Sc}-specific binding reagents with a high affinity. We report here a prototype prion assay with a high sensitivity and specificity for screening PrP^{Sc} in blood and blood products.

Fig 1. Model For Conversion of PrP^C to PrP^{Sc}



- Unstructured domain in PrP^C is converted into b-sheets resulting in the formation of PrP^{Sc}.
- PrP^{Sc} accumulates in neurons and causes nervous system dysfunction.

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METHODS and RESULTS

Design Concepts for Peptides as PrP^{Sc} Specific Binding Reagents

- Anti prion antibodies inhibits transformation of PrP^C to PrP^{Sc}, which indicates that the regions bound by the antibodies are critical for PrP^C and PrP^{Sc} interaction. -Peretz et al. Nature, 412:739-743, 2001.
- Grafting the critical regions of PrP to the V3 region of a mAb can bind to PrP^{Sc} specifically. -Williamson et al. PNAS, 101:10404-10409, 2004.
- Non-grafted free peptides from PrP also bind PrP^{Sc} specifically -Chiron Data (See Fig 2.)

Fig 2. Peptides Specifically Bind to PrP^{Sc}

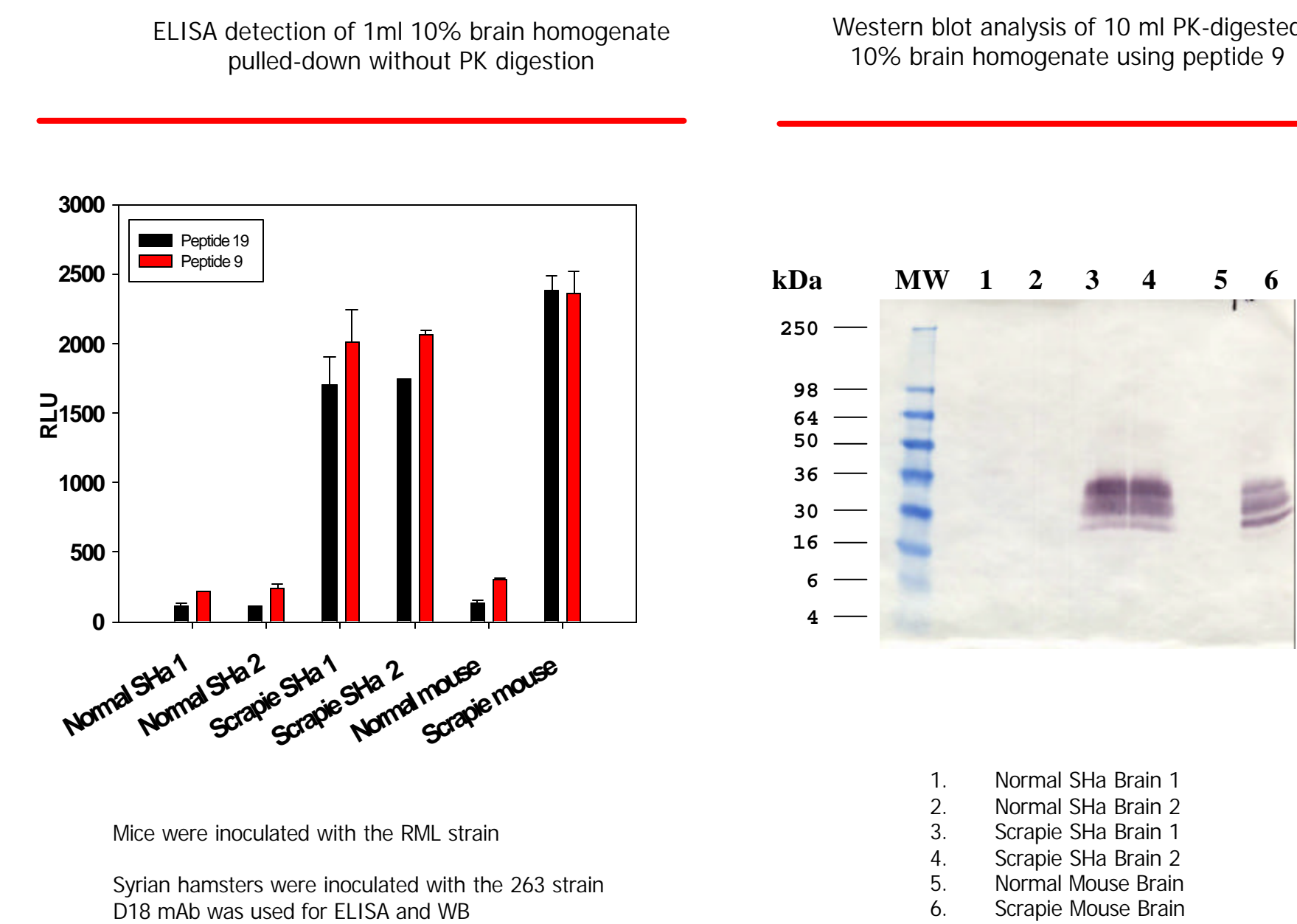
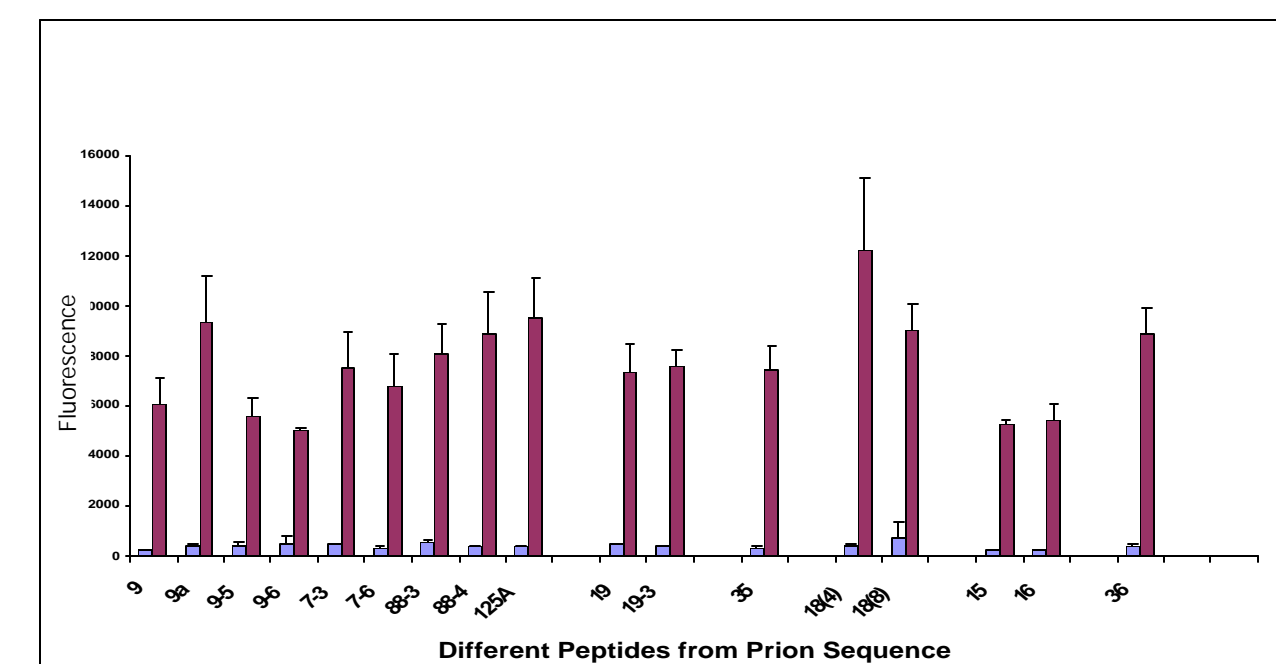


Fig 3. Different Peptides from Prion Sequence

More than 100 different peptides and peptide derivatives were screened for testing PrP^{Sc} from Human vCJD Brain Homogenate. Representative results shown.



We gratefully acknowledge Dr. Jillian Cooper and Dr. Phil Minor of NIBSC for providing the WHO CJD Brain Homogenate reference sample.

Fig 4. Chiron vCJD Assay Format – Pull-down Sandwich ELISA

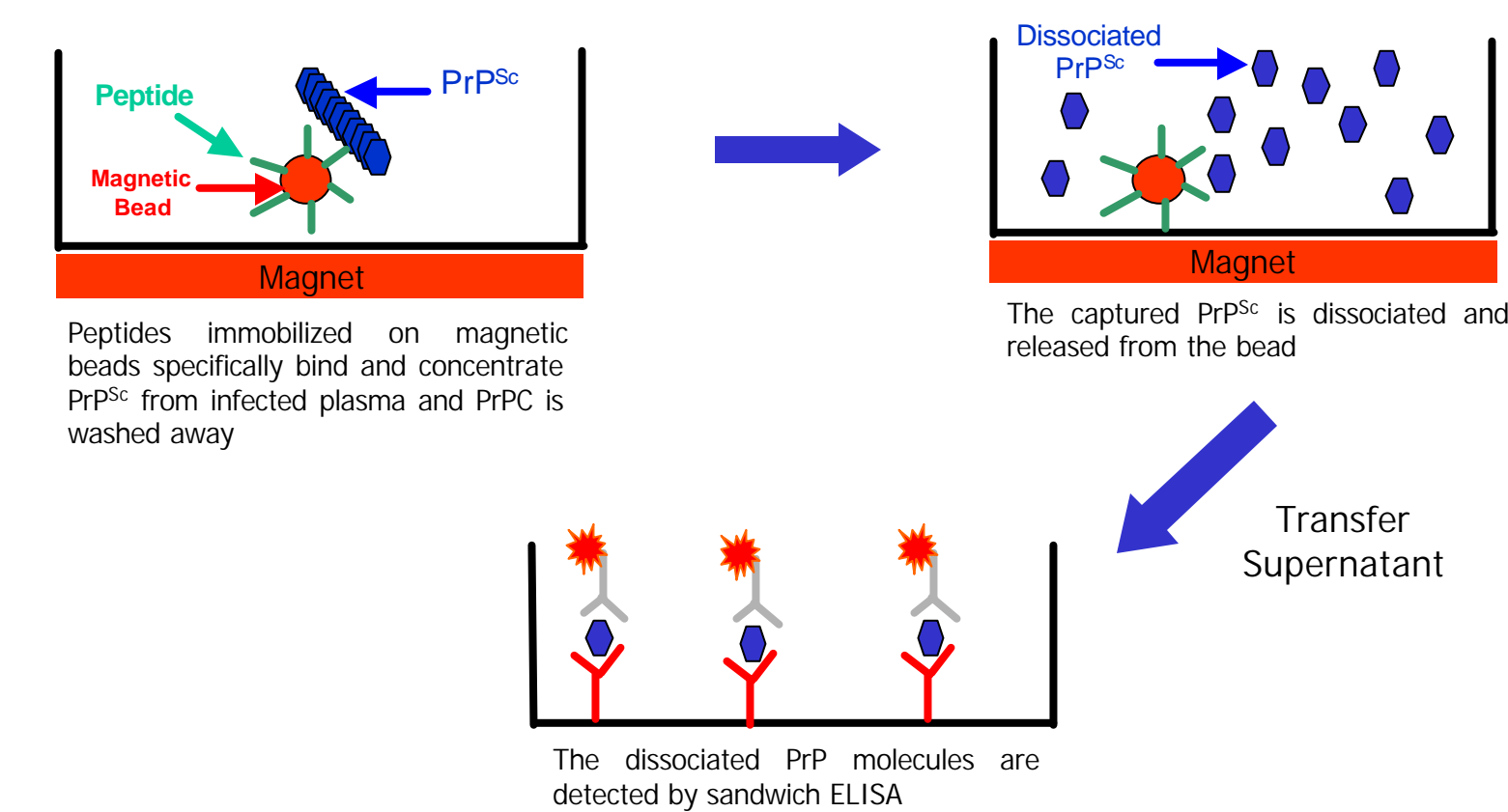
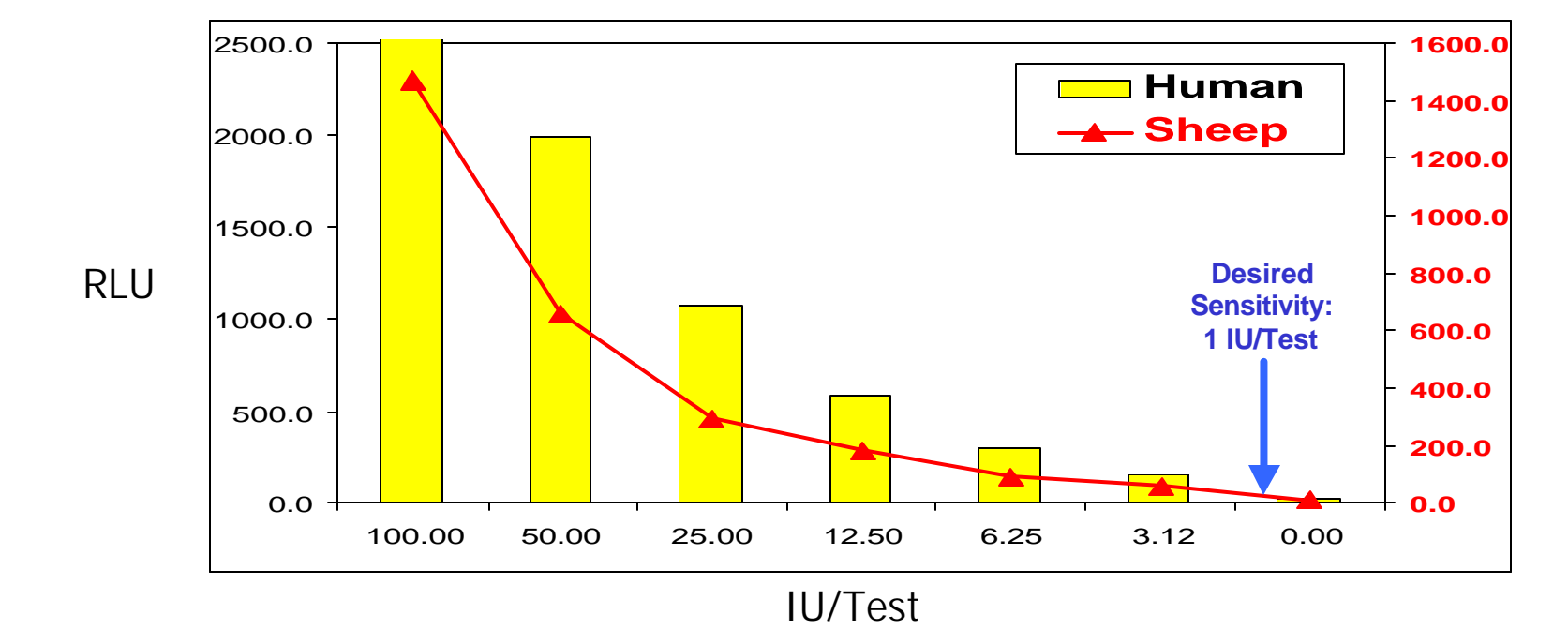


Fig 5. LOD of Current Chiron Prion Assay on Human vCJD and Sheep Scrapie Brain Homogenate

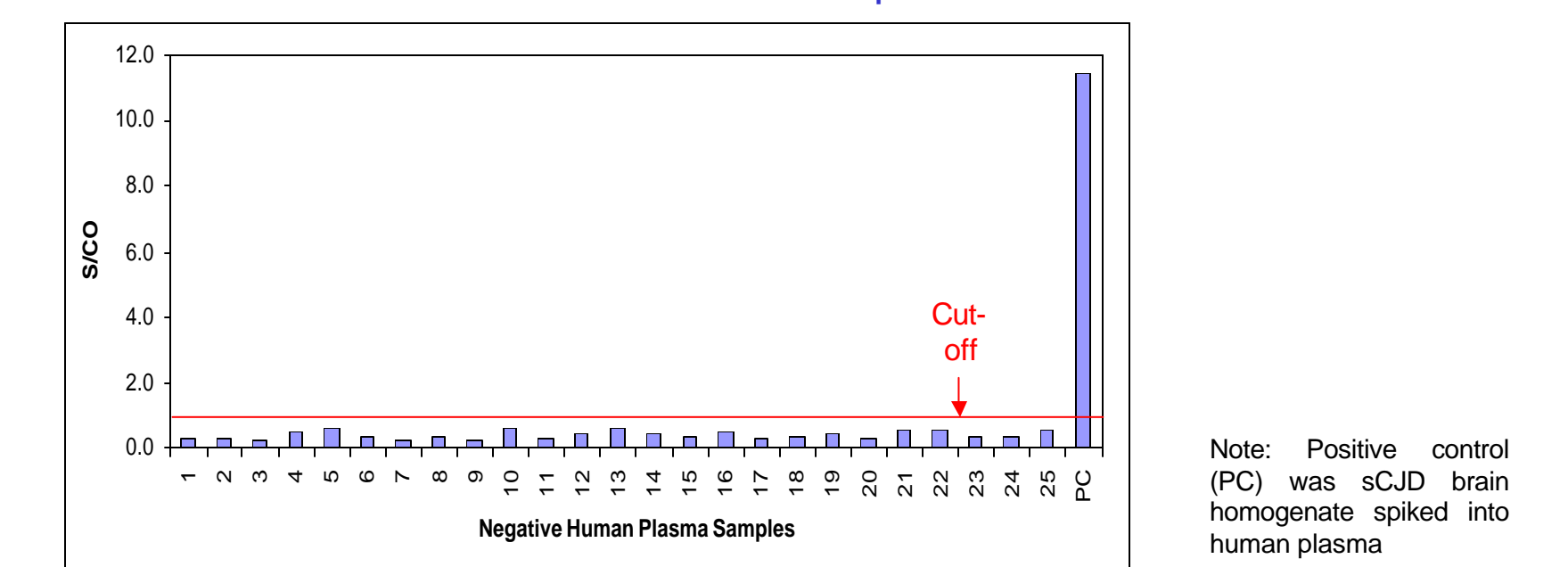


We gratefully acknowledge:
1) Dr. Jillian Cooper and Dr. Phil Minor of NIBSC for providing the vCJD brain homogenate sample.
2) Dr. Katherine O'Rourke of USDA, Pullman, WA, for providing the scrapie sheep brain homogenate.

Fig 6. Detection of PrP^{Sc} in Diseased Sheep Blood

Sheep Scrapie Samples	Rep 1	Rep 2	S/CO
USDA 108-3353	2.2	1.7	
NSPPO 0202	0.8	0.6	
NSPPO 0206	0.7	0.6	
USDA-2603	0.8	0.6	
USDA-2604	0.9	0.9	
USDA-2606	2.7	2.7	
USDA-2608	0.8	0.8	

Fig 7. Specificity of Current Chiron Prion Assay on Normal Human Plasma Samples



SUMMARY

- A vCJD assay for testing blood and blood products must have very high sensitivity and specificity
- Peptide design based on prion sequences work well as PrP^{Sc} specific binding reagents
- The Chiron prion assay demonstrates a sensitivity and specificity approaching the requirement to detect PrP^{Sc} in blood
- The assay detected PrP^{Sc} in plasma samples from diseased scrapie sheep

CONCLUSIONS

- A preliminary prototype vCJD assay has been developed at Chiron for testing donated blood and blood products
- This assay is being further optimized to meet the requirements in specificity, sensitivity and throughput