

# **Prion2008**

*October 8-10, 2008 – Madrid, Spain*

## **Specificity and capacity of the P-Capt<sup>®</sup> filter for prion protein and prion infectivity**

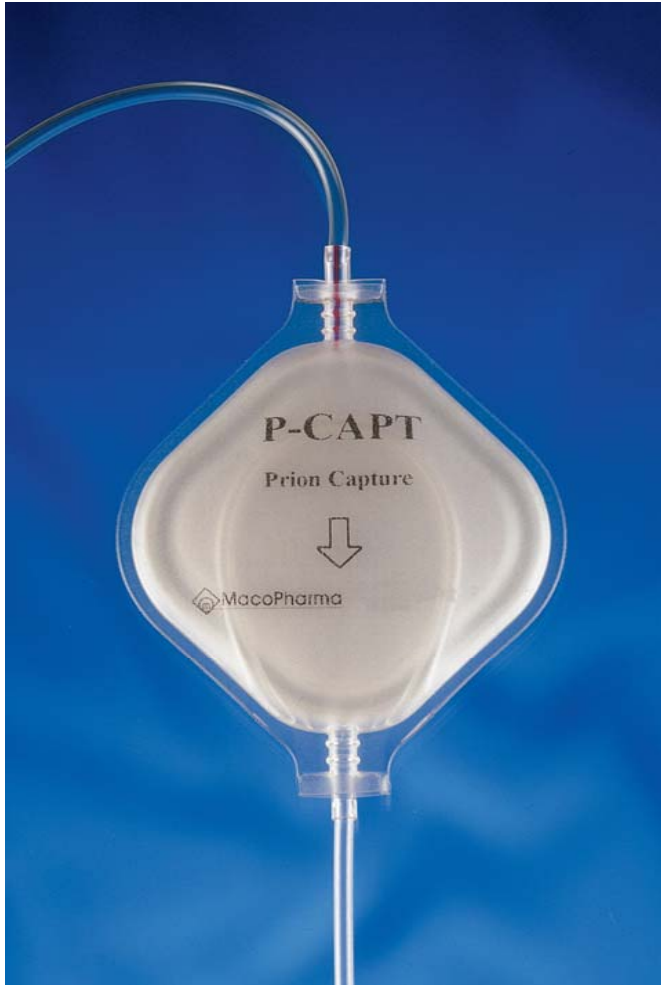
**Gregori L<sup>1</sup>, Lathrop JT<sup>2</sup>, Gurgel PV<sup>3</sup>, Lescoutra N<sup>4</sup>,  
Rohwer RG<sup>1</sup>**

**<sup>1</sup>VAMC and UM, Baltimore, USA, <sup>2</sup>Prolias, LLC, Rockville, USA, <sup>3</sup>Prometic Life Sciences, Montreal, CA, <sup>4</sup>CEA, Fontenay-aux Roses Cedex, FR and MacoPharma Inc. Tourcoing, FR**

# Background

- **Affinity ligands to reduce TSE infectivity from blood**
- **Application of proprietary technologies to screen millions of ligands for PrP<sup>TSE</sup> binders**
- **One ligand was tested with 263K infectivity:**
  - **> 3 log<sub>10</sub> reduction of brain-derived infectivity (Gregori *et al.* Transfusion, 2006)**
  - **> 1.2 log<sub>10</sub> (to the limit of the bioassay) reduction of endogenous blood infectivity (Gregori *et al.* Lancet, 2006)**

# P-Capt<sup>®</sup> Filter

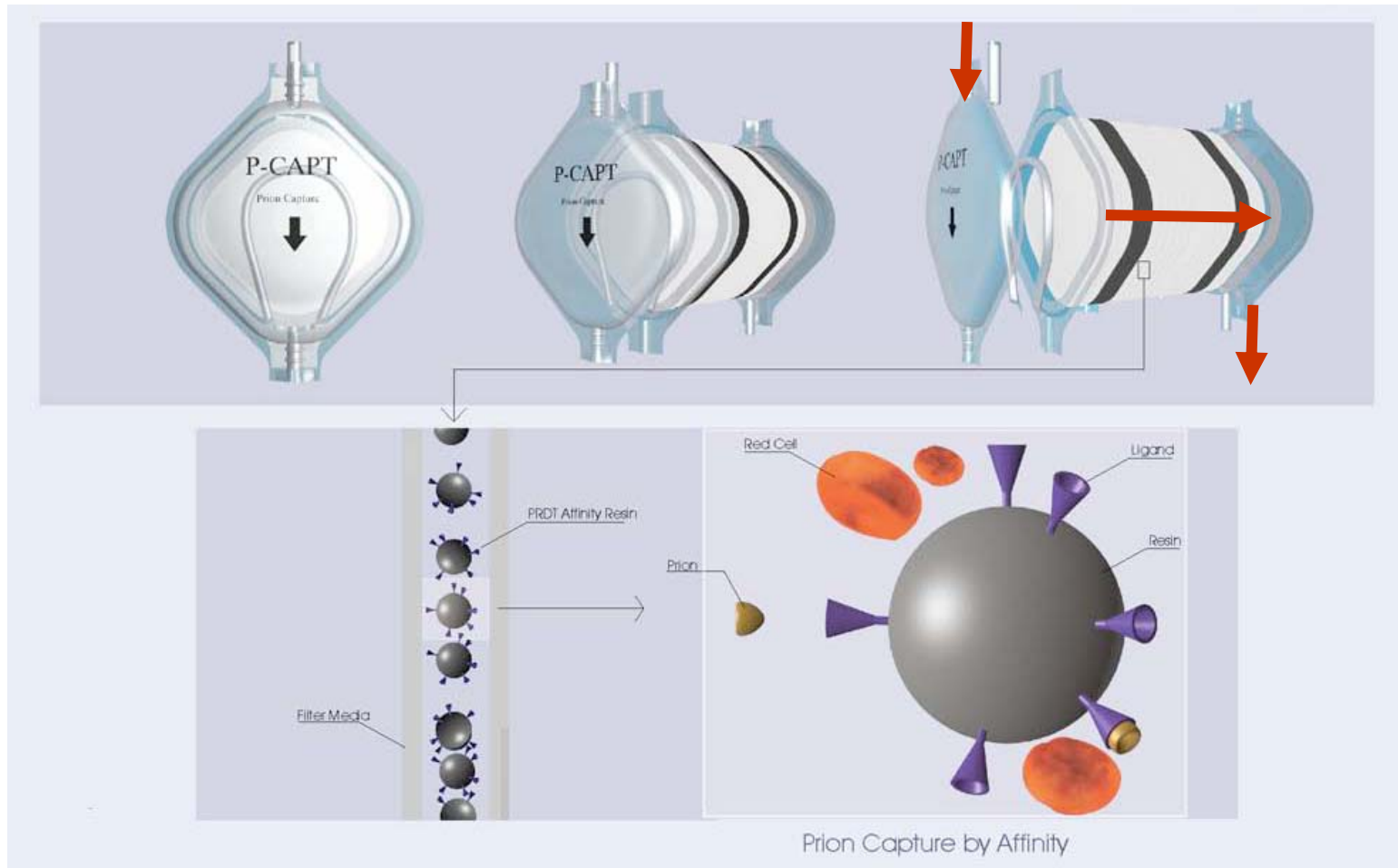


- **Resin incorporated into the P-Capt<sup>®</sup> prion reduction filter**
- **MacoPharma manufacturer and marketing company**
- **CE mark as a Class IIB device (September 2006)**
- **Stand-alone device**
- **Approved for use with LR human RBC**

# P-Capt<sup>®</sup> Filters in Use (MacoPharma)



# P-Capt<sup>®</sup> Filter



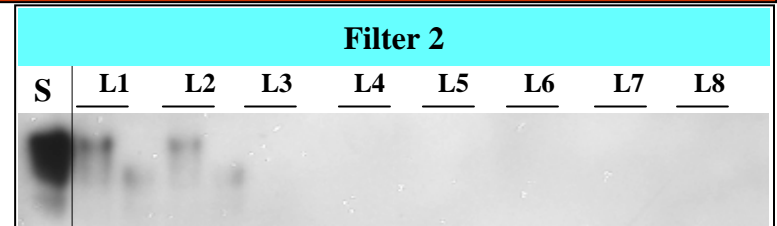
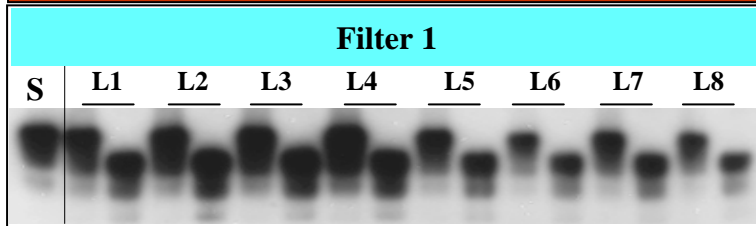
# **P-Capt<sup>®</sup> Filter capacity with hamster scrapie brain**

- **Human leukoreduced RBC unit spiked with various concentrations of 263K hamster scrapie brain**
- **Application to two P-Capt<sup>®</sup> filters in series**
- **Recovery of resin from each filter layers**
- **Elution of bound proteins, PK digestion**
- **Western blot of resin-bound proteins**

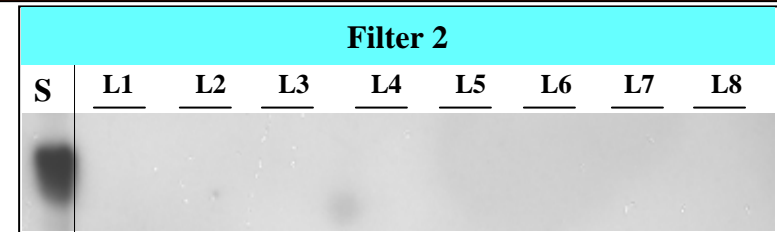
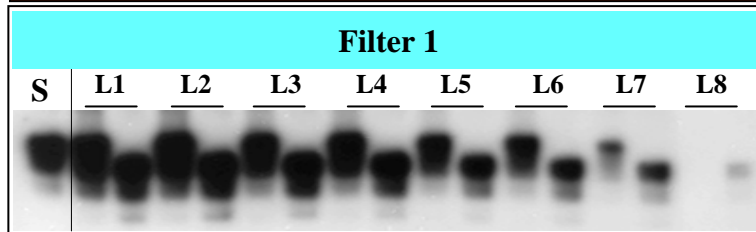
# PrP<sup>sc</sup> bound to filter layers

Stock hamster scrapie brain homogenate titer =  $10^8$  ID<sub>50</sub>/ml

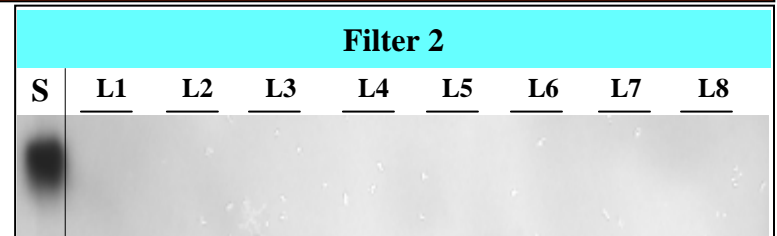
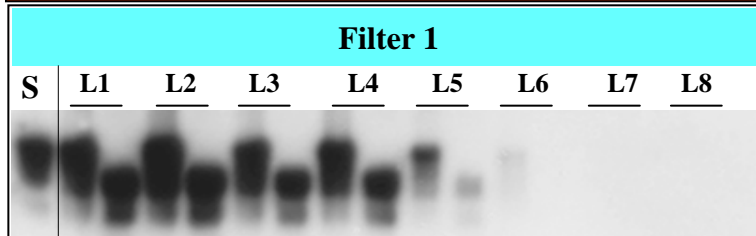
0.02% Spike = 60,000,000 ID<sub>50</sub> or 7.8 log<sub>10</sub> ID<sub>50</sub>/Filter 1



0.01% Spike = 30,000,000 ID<sub>50</sub> or 7.5 log<sub>10</sub> ID<sub>50</sub>/Filter 1



0.005% Spike = 15,000,000 ID<sub>50</sub> or 7.2 log<sub>10</sub> ID<sub>50</sub>/Filter 1

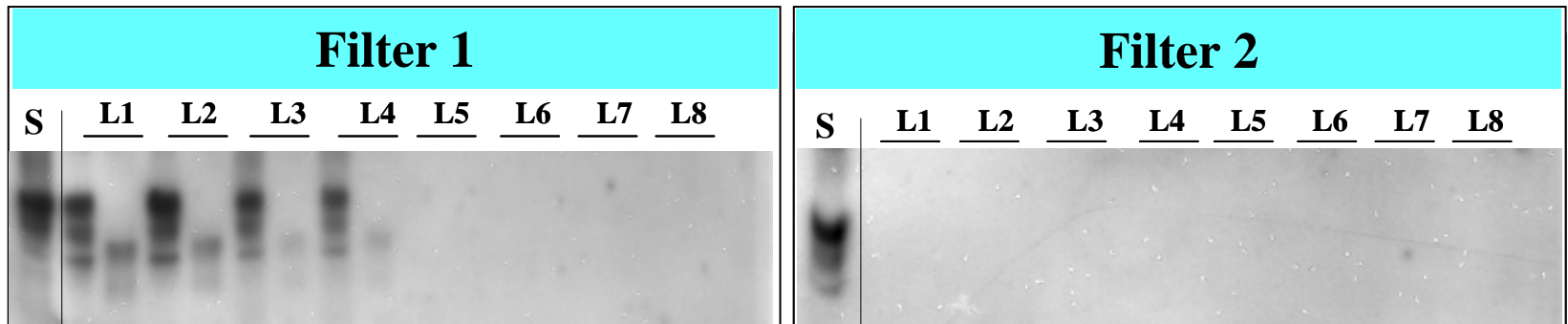


# TSE specificity of the P-Capt<sup>®</sup> Filter

- **Screening of the resin with brain PrP<sup>TSE</sup> from**
  - **Hamster with 263K scrapie (UM)**
  - **Mouse with mouse-adapted GSS (ARC)**
  - **Human with sCJD (ARC)**
  
- **Human leukoreduced RBC unit spiked with brain PrP<sup>TSE</sup> from various species**

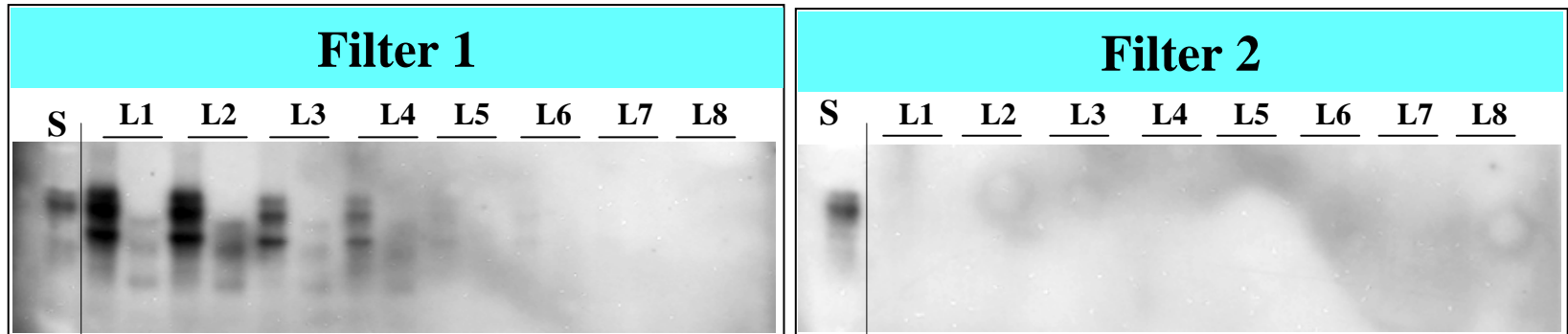
# Mouse-adapted BSE Strain

**0.01% 301V MOUSE brain homogenate in  
human LR RBC**

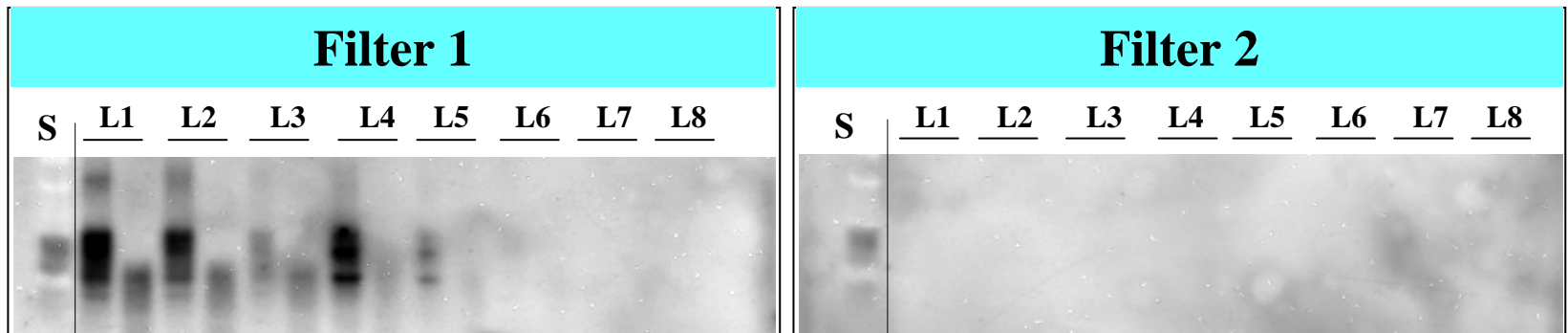


# Human vCJD and sCJD Strains

**0.01% vCJD HUMAN brain homogenate in human LR RBC**

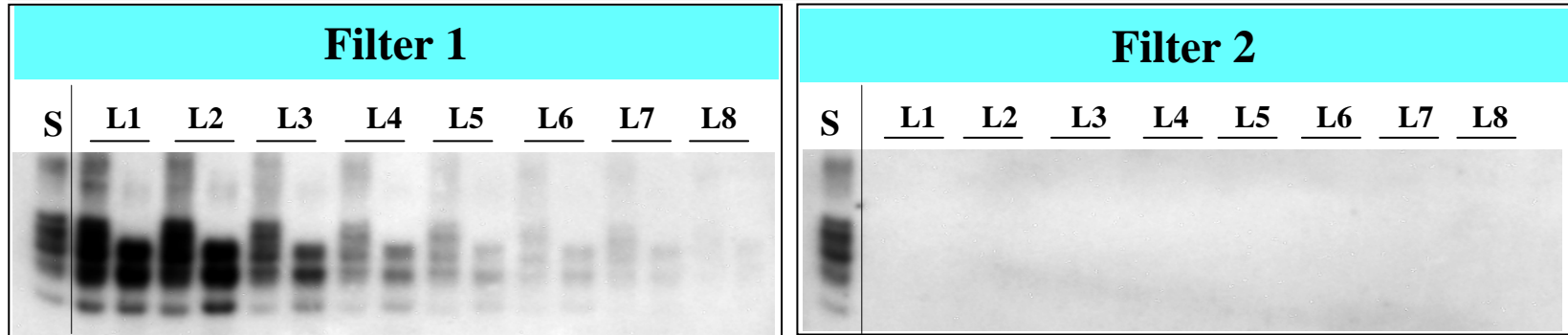


**0.02% sCJD HUMAN brain homogenate in human LR RBC**

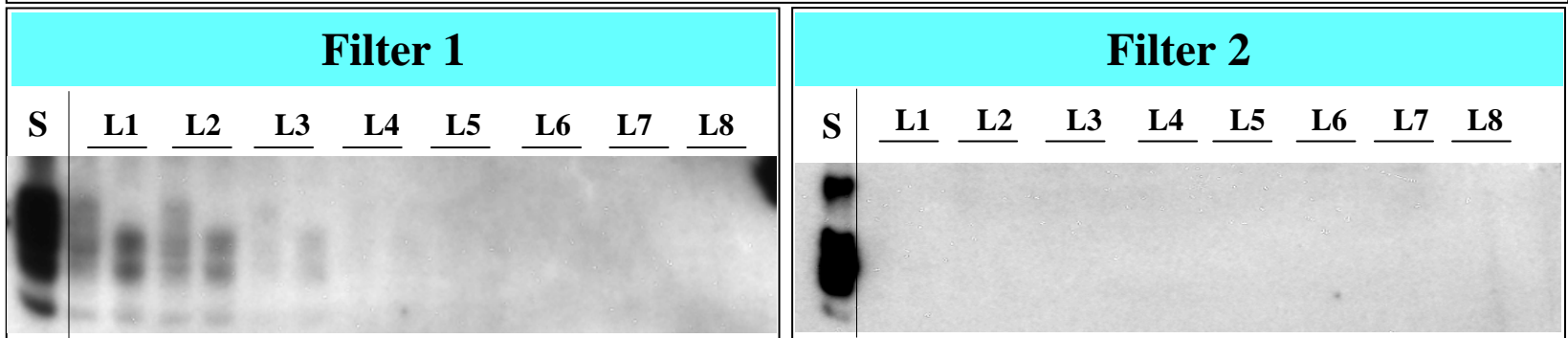


# Scrapie Squirrel Monkey

**0.01% SCRAPIE SQUIRREL MONKEY brain homog. in human LR RBC**

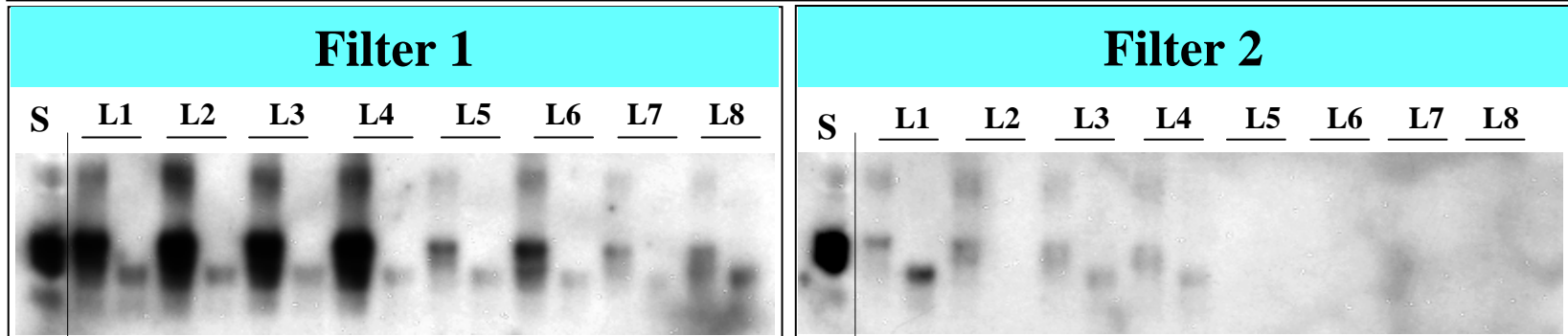


**0.005% SCRAPIE SQUIRREL MONKEY brain homog. in human LR RBC**

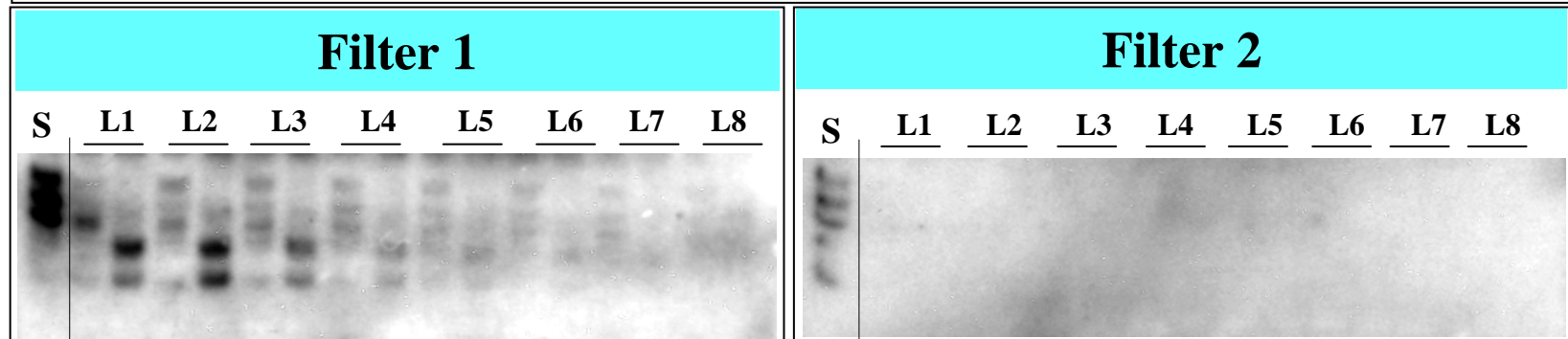


# Natural Scrapie in Sheep

## 0.33% SHEEP SCRAPIE brain homogenate in human LR RBC



## 0.05% SHEEP SCRAPIE brain homogenate in human LR RBC



# Conclusions

- **P-Capt filter behaves as a series of small chromatographic components**
- **Applicability to a variety of TSE strains, including vCJD and sCJD, and PrP<sup>TSE</sup> species**
- **Capacity is  $> 1.5 \times 10^7$  ID<sub>50</sub>/filter (hamster brain infectivity model)**
- **25,000-fold excess capacity for a unit of RBC**

# Acknowledgements

- **University of Maryland, BREF**
  - **Robert Rohwer, Director**
  - **Brian Lambert**
  - **Wei Zhou**
  - **BSL-3 Animal Facility Staff**
- **American Red Cross**
- **ProMetic BioSciences**
- **North Carolina State University**
- **Prolias LLC**
  - **Melanie Poncheri**
- **MacoPharma**
  - **Chryslain Sumian**
  - **Iwona Walicka**