

1. Background

Prion infectivity is present in blood and is transmissible by blood transfusion. We have developed a device, the P-Capt™ filter (manufactured by MacoPharma, Inc. using PRDT's affinity technology), to reduce endogenous infectivity from human leucoreduced red blood cells (RBC). The P-Capt™ filter is composed of eight membrane layers, each containing an embedded resin that adsorbs the prion protein and infectivity from brain-spiked RBC and from endogenously infected blood.

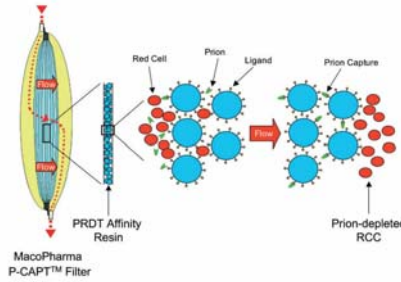
The resin was developed in column format using the hamster 263K scrapie model. The studies presented here were conducted to assess the P-Capt™ filter implementation for binding specificity for PrP from various animal models and prion strains. We have also investigated the prion binding pattern across the membrane layers of the filter.

2. Methods

Specificity was assayed by measuring PrP binding from brain homogenates of human vCJD and sCJD, uninfected macaque, mouse-adapted BSE, hamster-adapted BSE and scrapie, and natural sheep scrapie. Each brain homogenate was spiked into a unit of leucoreduced RBC and applied to a P-Capt™ filter. The filter was cut open and the resin from each membrane layer was analyzed by Western blot of the resin-bound proteins.

3. Filter Layout

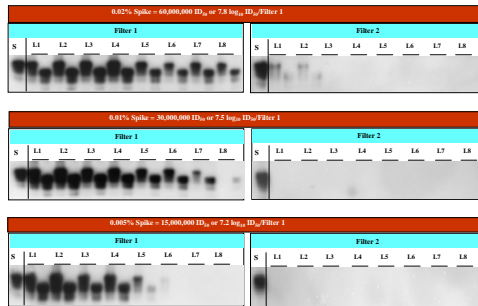
- Several layers of particle-impregnated membrane are stacked and fused together, forming a filtration device.
- The particles immobilized between the membrane layers contain a ligand specific to prions.
- Red blood cells pass through the device unharmed, while the particles capture prion infectivity.



Device does not damage Red Blood Cells while removing infectivity

4. P-Capt Filter Capacity

- Human leucoreduced RBC unit spiked with various concentrations of 263K hamster scrapie brain
- Application to two P-Capt™ filters in series
- Recovery of resin from each filter layers
- Elution of bound proteins, PK digestion
- Western blot of resin-bound proteins
- Stock hamster scrapie brain homogenate titer = 10E8 ID₅₀/ml



- Each layer works as a discreet component, maximizing removal
- At a spike concentration of 0.005%, device removed 10E7 ID₅₀, 4 to 5 orders of magnitude greater infectivity than expected in a unit of leucoreduced RBC

Device has excess layers and excess binding capacity

5. TSE specificity of P-Capt Filter

- Screening of the filter with brain infectivity from
 - 301V mouse
 - vCJD human
 - spCJD human
 - Squirrel monkey scrapie
 - Sheep scrapie
- Human leucoreduced RBC used for all spikes

Device is able to remove infectious prions from different TSE species

