

# PRDT Prion Reduction Technology

## The Risk of vCJD: Transmission in Blood and Plasma products

- Variant Creutzfeldt-Jakob disease (vCJD) is a fatal degenerative brain disease characterised by the accumulation of abnormal prion protein (PrP<sup>sc</sup>/ PrP<sup>res</sup>) in the central nervous system. To date, there has been a total of 204 reported cases worldwide.
- Initially transmitted to humans by the consumption of BSE contaminated meat, a secondary route of transmission by the transfusion of blood units from asymptomatic individuals threatens to increase the prevalence of the fatal disease and the risk to the blood supply. Plasma and plasma products may also be at risk.
- Four confirmed cases of transmission of vCJD by transfusion.
- Currently there is no test for screening blood/plasma donations for vCJD.
- The prevalence of the disease within the general population is unknown. A “silent carrier” state is possible.
- Scientific research estimates that there may be 3,800 asymptomatic vCJD carriers in the UK and the 2006 National CJD Surveillance Unit report stated that “*the incidence of vCJD may increase again, particularly if different genetic subgroups are found but with longer incubation periods*”. The number of secondary transmissions could increase as well.
- Genotype studies confirm that all the population is at risk of vCJD.
- Regulatory bodies have increasingly adopted the precautionary principle with respect to both transfused blood/blood components and plasma products and recommend measures to reduce the potential risk of infectivity where possible.

- A removal strategy avoids all issues of notification, verification and management of false positives that are inevitably associated with screening tests for donor deferral.

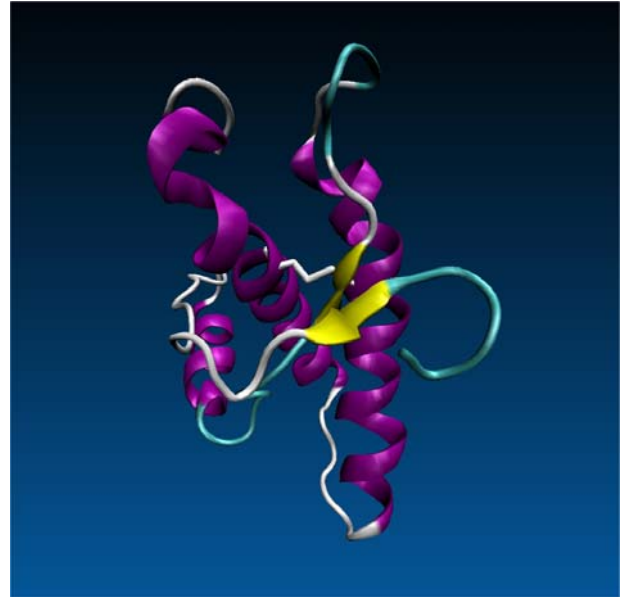


Figure 1: 3-Dimensional image of mammalian PrP.

## PRDT Prion Reduction Technology

PRDT (Pathogen Removal and Diagnostic Technologies Inc.; a joint venture between ProMetic Biosciences and the American Red Cross) has worked to apply its proven affinity technology to the design and development of a panel of affinity adsorbents that enable the highly effective reduction of abnormal prion infectivity from a range of biological materials.

- **Fast, Simple Single Step Clearance:**  
A range of ligands have been engineered which are highly specific for prions that threaten human and animal health.

#### ■ **Robust and Reliable:**

Removal of  $> 7 \log_{10}ID_{50}$  per ml of resin has been demonstrated in exogenous infectivity studies. The removal of endogenous infectivity from infected blood has also been validated to the detection limit of the bioassay.

#### ■ **Implementation for either raw materials or end products:**

The PRDT adsorbents have a broad operating range. Highly effective prion removal is achieved at flow rates ranging from a few cm/hr (applicable to prion removal from blood) up to several hundred cm/hr (applicable to downstream processing applications).

#### ■ **High Yields and Product Quality:**

The highly stable synthetic prion-binding ligands are immobilised on inert resin supports. Operating conditions are optimised for use in a variety of different applications. Purity of the bulk product is unaffected as a result of prion removal and yields are typically high. Minimal/no impact on the therapeutic application of the product.

#### ■ **Regulatory Compliance:**

PRDT products are manufactured to ISO9001:2000 for use in the pharmaceutical and biomedical industry. Safety profiles are available as part of Regulatory Support Files and PRDT prion reduction resin has been approved as a component of a CE mark approved medical device (P-CAPT™).

#### ■ **Multiple formats for ease-of-use:**

PRDT adsorbents are available in a variety of formats ranging from small columns for ligand screening and method development, to bulk quantities of resin for use in commercial scale applications.

#### ■ **Areas of specific application include:**

- vCJD removal from RBC, whole blood, plasma, for transfusion and plasma-products
- Removal of abnormal prions (in down stream processing mode) from biologics, including plasma proteins.
- Prion removal from raw materials for pharmaceutical, biologics, and biotechnology manufacturing.
- Prion removal from cell culture media and media additives.
- Medical devices for prion reduction (P-CAPT).

## **Technical Approach to the Design of Prion Reduction resins**

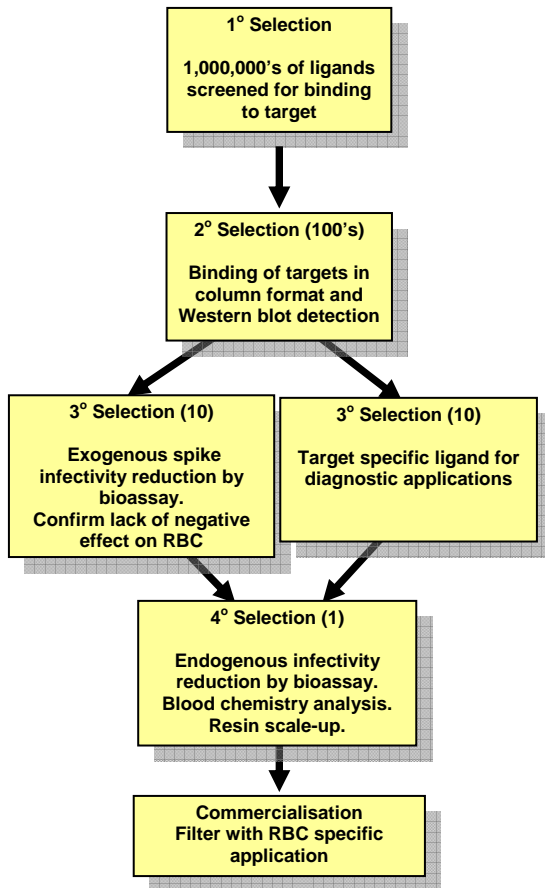
### **Advantages of Prion Reduction with Affinity Ligands:**

- Compatible with existing technologies.
- High efficacy with targeted ligand design and optimisation.
- Broad applicability.

### **Combinatorial approach to Affinity Ligand Selection:**

- Specific affinity ligands have been designed and identified. These ligands are highly specific for  $PrP^{Sc}/PrP^{res}$ .
- Target ( $PrP^{res}$ ) specific ligands were identified from millions of structures evaluated using proprietary selection techniques.
- Ligands immobilized on inert supports for easy use with chromatographic columns. Screening of resins to select a group of strong candidates.

- Selection of best candidate through a series of screenings terminating with demonstration of infectivity reduction from RBC (exogenous spike).
- Demonstration of endogenous infectivity reduction.



**Figure 2: Flow Chart of the various stages of the PRDT ligands screening program. All studies performed according to validated protocols / SOP's within ISO 9001:2000 Quality System.**

variant CJD, mouse-adapted familial CJD (fCJD) and BSE, hamster-adapted scrapie and BSE and natural sheep scrapie.

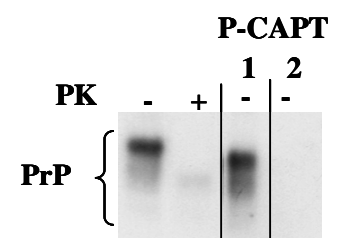
- In exogenous infectivity studies, the resin removed  $>3 \log_{10}$  infectivity which equates to  $10^7$  ID<sub>50</sub> per ml resin.
- In endogenous infectivity studies with leukoreduced scrapie infected hamster blood, the resin demonstrated removal of infectivity to below the limit of detection of the bioassay or  $>1.22 \log$  ID.
- The P-CAPT filter challenged with a unit of leukoreduced RBC spiked with 0.005% scrapie brain homogenate removed  $10^7$  ID<sub>50</sub>. This resin capacity is 4 to 5 orders of magnitude greater than the level of infectivity expected in a unit of leukoreduced RBC contaminated with vCJD.
- Analysis of the filter demonstrated that each layer (1-8) performed as an individual chromatographic component and that one filter has excess layers and excess resin capacity.

### P-CAPT™ Product profile:

- Product manufactured and marketed by MacoPharma SA using PRDT prion adsorbent resin.
- Sterile single use prion-reduction device.
- Incorporates selected prion-binding affinity adsorbent.
- Stand-alone filter or integrated into blood collection units.
- Product targeted to RBC.
- Validated brain spike and endogenous infectivity removal to meet blood agency required specifications.
- Device gained European approval (CE Mark) in September 2006.

### Removal of Prion Infectivity from Blood:

- Demonstrated selective binding of the chosen resin to spiked PrP<sup>res</sup> from humans, hamsters and other species in the presence of blood and blood components.
- Demonstrated strong binding to various prion strains: human sporadic CJD and



## Prion Reduction in Plasma Products

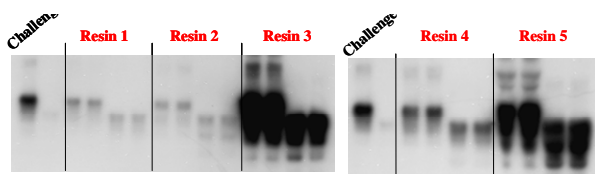
### Removal of Prions from Plasma Products:

The PRDT affinity resins can be used for removal of prion protein from plasma products in downstream processing mode.

#### ■ Removal of prions from plasma:

A large panel of PRDT resins available for screening with brain-derived PrP<sup>res</sup> spiked in human plasma. Strong binding to PrP<sup>res</sup> was shown by a group of these resins.

#### Binding of PrP<sup>res</sup> spiked in human plasma

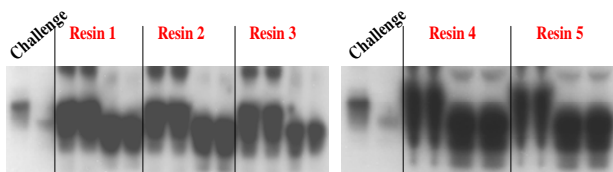


**Figure 3:** An example of 5 resins from the PRDT resins panel showing resin-bound brain PrP<sup>res</sup> removed from human plasma.

#### ■ Removal of prions from commercial human serum albumin:

The PRDT resins panel was screened with different formulations of commercial 25% human serum albumin. Several resins showed strong binding to PrP<sup>res</sup> in 25% human serum albumin and provided more than 95% protein recovery.

#### Binding of PrP<sup>res</sup> spiked in human serum albumin

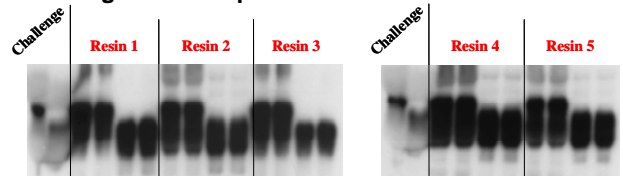


**Figure 4:** The same PRDT resins in Figure 3 showing resin-bound brain PrP<sup>res</sup> removed from 25% human serum albumin.

#### ■ Removal of prions from commercial human immunoglobulin (IVIG):

The PRDT resins panel was screened with a commercial formulation of 3% immunoglobulin (IVIG). Several resins showed strong binding to PrP<sup>res</sup> in human immunoglobulin and provided more than 85% protein recovery.

#### Binding of PrP<sup>res</sup> spiked in human IVIG



**Figure 5:** The same PRDT resins in Figure 3 showing resin-bound brain PrP<sup>res</sup> removed from 3% human immunoglobulin.

#### ■ PRDT Prion Reduction Resins:

- Manufactured at large scale according to validated processes.
- Industrial scale applicability in the form of chromatography columns /filters or medical devices.
- Non-Toxic (toxicological studies are available).
- No appreciable leachate (leachate studies are available).
- Highly robust and stable products (stability studies are available).

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