

safer blood and plasma products through innovative prion removal

PrioClear™ Prion Binding Affinity Adsorbents



Variant Creutzfeldt-Jakob disease (vCJD) is a fatal degenerative brain disease characterised by the accumulation of abnormal prion protein (PrP^{sc}/PrP^{res}) in the brain and central nervous system. To date there have been over 200 cases of vCJD reported worldwide.

Initially transmitted to humans by the consumption of BSE-contaminated meat, the greatest threat to humans is now secondary transmission by blood and blood-products derived from asymptomatic donors who are incubating vCJD and whose blood is infectious.

It is estimated that the number of people incubating vCJD in the general population is anywhere between 1 in 4,000 and 1 in 20,000.

Five confirmed cases of transmission of vCJD by blood and blood-products.

Currently there is no test for screening blood/plasma donations for vCJD.

KEY INFORMATION

Scientific research estimates that there may be at least 3,800 asymptomatic vCJD carriers in the UK and in 2006 the 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 recent confirmation of a vCJD victim of MV genotype suggests that this is now a real possibility.

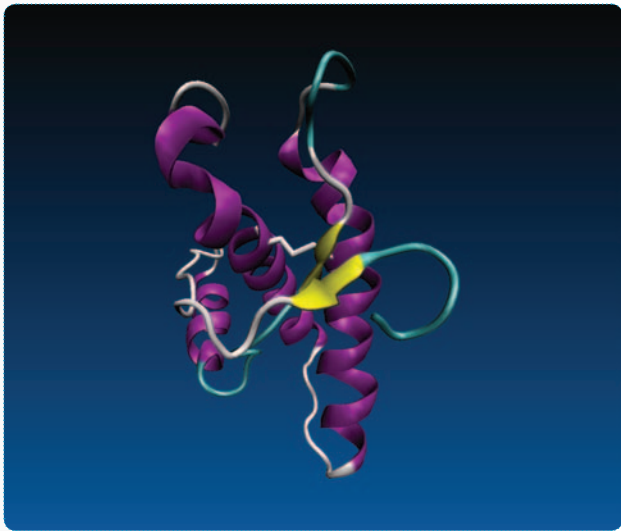
Genotype studies confirm that all the population is at risk of vCJD.

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



TECHNOLOGY

The PrioClear™ range of prion binding affinity resins was developed through Pathogen Reduction and Diagnostic Technologies, Inc. (PRDT), initiated as a collaborative venture with the American Red Cross and now majority owned by ProMetic BioSciences Ltd (PBL). PRDT has applied its proven affinity technologies to the design and development of a panel of affinity adsorbents that enable the highly effective capture of prion from a range of biological materials. The PrioClear™ resins are used commercially to increase the safety of blood and blood-derived products.

FAST, SIMPLE, SINGLE-STEP CLEARANCE

PrioClear™ adsorbents encompass a range of affinity ligands which are highly specific for prions which threaten human and animal health.

EFFECTIVE PRION REMOVAL

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

IMPLEMENTATION FOR RAW MATERIALS OR FINAL PRODUCTS

PrioClear™ adsorbents have a broad operating range and are simple to use. 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 PRODUCT RECOVERY AND 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

PrioClear™ resins are manufactured in a controlled environment under an ISO 9001 accredited quality system for use in the pharmaceutical and biomedical industry. Regulated PrioClear™ applications include use as a component of a CE mark approved medical device, the P-Capt® filter, and as a purification step in the manufacture of a licensed plasma product, OctaplasLG®.

MULTIPLE FORMATS FOR EASE-OF-USE

PrioClear™ resins 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. For further information please contact us at sales@prometicbiosciences.com.

AREAS OF SPECIFIC APPLICATION INCLUDE

- vCJD removal from red blood cell concentrates (RBC), whole blood, plasma for transfusion, and plasma-products.
- Removal of abnormal prions 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®).

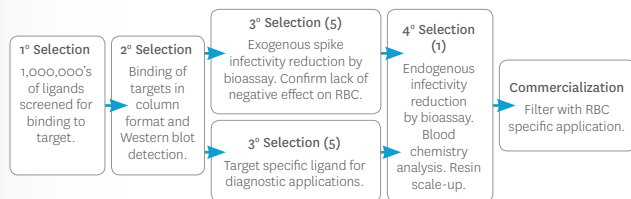
DEVELOPMENT

COMBINATORIAL APPROACH TO AFFINITY LIGAND SELECTION

- Specific affinity ligands were 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 PrioClear™ development program. All studies performed according to validated protocols/SOP's.



REMOVAL OF PRION INFECTIVITY FROM BLOOD WITH PRIOCLEAR™ ADSORBENTS

- Selective binding of prion infectivity demonstrated with spiked PrP^{Sc} from humans, hamsters and other species in the presence of blood and blood components.
- Strong binding to various prion strains including human sporadic CJD and variant CJD, mouse-adapted familial CJD (fCJD) and BSE, hamster-adapted scrapie and BSE and natural sheep scrapie.
- In exogenous infectivity studies, a PrioClear™ resin removed >3 log₁₀ infectivity which equated to 10⁷ ID₅₀ 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 (>1.22 log).
- The P-Capt® filter challenged with a unit of leukoreduced RBC spiked with 0.005% scrapie brain homogenate removed 10⁷ ID₅₀. The PrioClear™ 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 the filter has excess layers and excess resin capacity.

P-CAPT® PRODUCT PROFILE

- Product manufactured and marketed by MacoPharma SA.
- Sterile single use prion-reduction device.
- Incorporates a PrioClear™ 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 UK blood agency required specifications.
- Device gained European approval (CE Mark) in September 2006.
- Recommended by UK blood safety advisory board SaBTO.

FIGURE 3

P-Capt® filter



APPLICATION OF PRIOCLEAR™ TO PLASMA PRODUCTS

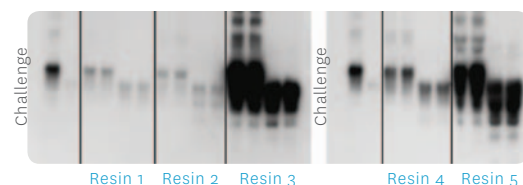
PrioClear™ resins can be used for removal of prion protein from plasma products in downstream processing mode.

REMOVAL OF PRIONS FROM PLASMA

A panel of five PrioClear™ resins were screened with brain-derived PrP^{Res} spiked into human plasma. Strong binding of PrP^{Res} was shown by two of these resins.

FIGURE 4

Capture of PrP^{Res} from human plasma with PrioClear™ affinity resins.



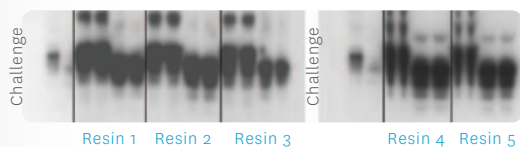
APPLICATION OF PRIOCLEAR™ (CONT'D)

REMOVAL OF PRIONS FROM COMMERCIAL HUMAN SERUM ALBUMIN

The PrioClear™ resin panel was screened with different formulations of commercial 25% human serum albumin. All resins showed strong binding to PrP^{res} in 25% human serum albumin and provided more than 95% protein recovery.

FIGURE 5

Binding of PrP^{res} spiked into 25% human serum albumin.

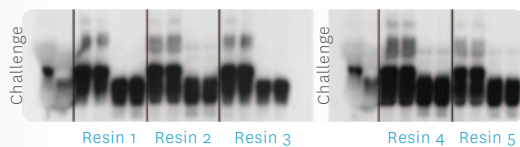


REMOVAL OF PRIONS FROM COMMERCIAL HUMAN IMMUNOGLOBULIN (IVIG)

The PrioClear™ resin panel was screened with a commercial formulation of 3% immunoglobulin (IVIG). All resins showed strong binding to PrP^{res} in human immunoglobulin and provided more than 85% protein recovery.

FIGURE 6

Capture of PrP^{res} from 3% human immunoglobulin.



PRIOCLEAR™ 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).

REFERENCES

- Gregori L. *et al.* (2006) *Transfusion* 46, 1152-61.
Gregori L. *et al.* (2006) *Lancet* 368, 2232-2236.
Lathrop JT *et al.* (2007) *Anal Biochem* 365, 91-101.
Lathrop, JT and Hammond D. (2007) *Nat Protoc* 2, 3102-10.

P-Capt® is a registered trademark of MacoPharma SA. OctaplasLG® is a registered trademark of Octapharma SA. PrioClear™, ProMetic BioSciences Ltd and the PBL logo are trademarks of ProMetic Biosciences Ltd. PRDT is a trademark of Pathogen Removal and Diagnostic Technologies, Inc.

©ProMetic Biosciences Ltd, 2010- Issue 190710 (V4)

CONTACT US

At www.prometicbiosciences.com.

For sales questions, contact: sales@prometicbiosciences.com.

For tech support questions, contact: techsupport@prometicbiosciences.com.

TEL +44 (0) 1223 420300/FAX +44 (0) 1223 420270