

The Application Of The Seprion Ligand System To Blood Screening For Rogue Prion Protein

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Introduction

In 2004 there were two reports of blood related transmission of vCJD in the UK. There is now a clear need for a technology that can be implemented as a blood screen to identify contaminated blood. However, it is difficult to test for vCJD because the infectious prion protein is present in the blood at low levels and there is a large excess of normal prion protein in the blood. Approaches that can be used in post-mortem screening have not been successfully demonstrated on blood. In this report we demonstrate that our rogue-prion specific ligand, Seprion, can be used to detect the infectious prion protein in the blood of scrapie infected sheep, an animal model for human vCJD, using a protocol that is feasible to implement in blood bank screening.

Development of the Seprion Ligand System

A large body of scientific evidence pointed the way to the development of the ligand. In particular, there is a large number of scientific publications that demonstrate that sulphated polyanionic compounds can bind to amyloid proteins (for a review see <http://www.priondata.org/>). We have utilised this property to develop the Seprion ligand which under optimised reaction conditions only binds to the aggregated amyloid protein and not to the normal unaggregated protein (Microsens patent application WO 03/073106; Lane A, Stanley, C, Dealler, S and Wilson S Clin. Chem. 2003;49:1773-1774).

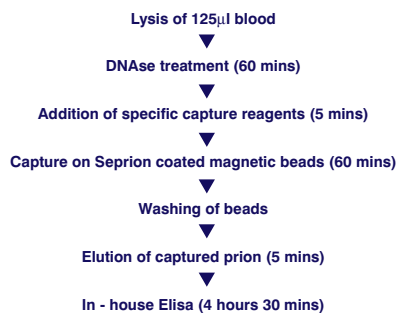
The Seprion Ligand System has been built into a simple post-mortem TSE assay

Compared to existing TSE tests which have a separate sample preparation requiring a critical protease step to remove normal prion, the Seprion assay is very simple with few steps and no protease or other sample preparation steps. Consequently, the Seprion assay is more robust, involves less hands on time and is easily automated. The application of the Seprion technology in the animal testing field has now been licensed and approval has been received from the EU and USDA for kits using the technology to be used for BSE testing.

The Seprion technology as a blood screening tool

As a model for human blood screening we have demonstrated detection of pathological prion protein in scrapie infected sheep blood.

In August 2004 blinded and coded blood was received frozen from the Veterinary Laboratories Agency TSE Archive (Weybridge UK). The panel included blood from negative control animals from New Zealand derived scrapie-free flocks and blood from scrapie suspects with clinical signs. The blood was processed as outlined in the figure below.



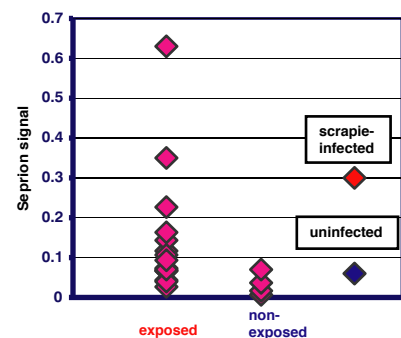
The results of the Seprion assay were returned to the VLA and subsequently the codes were broken by the VLA and the status of the samples revealed to us. The table below summarizes the results compared to the Western blot for rogue prion protein.

		Seprion Assay result		
		Positive	Negative	Total
Western Blot result	Positive	2	0	2
	Negative	1*	26	27
Total		3	26	29

Compared to Western blot the assay has a sensitivity of 100% and a specificity of 96%. The one animal that was picked up by the Seprion assay but not by Western blot was from a farm known to have endemic scrapie.

The Seprion ligand assay can also be used to detect pathological prion protein in the blood of asymptomatic sheep that are incubating the infection.

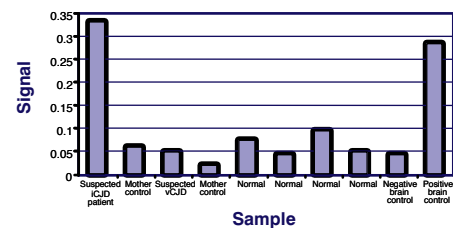
The results of the Seprion ligand assay performed on asymptomatic but experimentally exposed sheep blood is shown on the below. Some of the exposed animals demonstrated an increase in signal that was not observed in the non-exposed animals. This increased signal was at the same level as the signal observed in a scrapie-infected terminal control animal (red diamond in the graph) and was not breed or prion genotype specific. Similarly, the signals from the non-exposed animals were at the same level as that from an uninfected animal from a different source (blue diamond in the graph). All exposed animals subsequently developed disease 6-8 months later.



The ability of the Seprion ligand system to directly detect pathological prion protein in the blood of asymptomatic animals has implications for the human blood screening service in which contaminated blood from people without overt disease but incubating the disease could be excluded from the blood supply.

We have developed the Seprion Ligand System for detection of pathological prion in human blood.

Blood was received from a suspected, fully diagnosed iCJD patient (from growth hormone supplements), a suspected vCJD patient, their mothers and other control patients. The Seprion ligand assay was used to demonstrate pathological prion protein in the blood of the iCJD patient and not in the blood of the mother or other control human blood (see graph to the below). The suspected vCJD patient was negative and was subsequently shown not to have vCJD. This is the first demonstration of pathological prion protein in the blood of a patient with TSE.



Summary

- The Seprion ligand system is specific for the rogue form of prion protein
- Applications in the animal testing field have been licensed and approval has been received from the EU and USDA for BSE kit sales and USDA for CWD kit sales
- The Seprion ligand system has been used to detect pathological prion in the blood of symptomatic and pre-symptomatic sheep
- The Seprion system has been used to detect pathological prion in a human iCJD blood sample