

Application of the Seprion Ligand System for detection of PrP^{Sc} in ovine blood

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Background

We have developed the PrP^{Sc}-specific ligand, Seprion, into a very simple TSE post-mortem immunoassay which removes the need for sample preparation, including proteinase K. This Seprion Assay has 100% sensitivity and specificity compared to current gold standards (1) and can be applied to CJD, BSE, Scrapie and CWD. We present here further progress in developing the assay for detection of TSEs in blood with a view to developing the assay for human blood bank screening.

Method

Animals were asymptomatic, from an exposed flock with a high incidence of scrapie. Blood from a scrapie-free flock was used as negative controls. The red cell fraction of whole blood was lysed. The white cell fraction was collected by centrifugation and resuspended in Seprion Capture Buffer. PrP^{Sc} was assayed as described in (1). Briefly, PrP^{Sc} from the lysed cells was captured to immobilised Seprion ligand-coated paramagnetic beads and detected with an anti-prion conjugate.

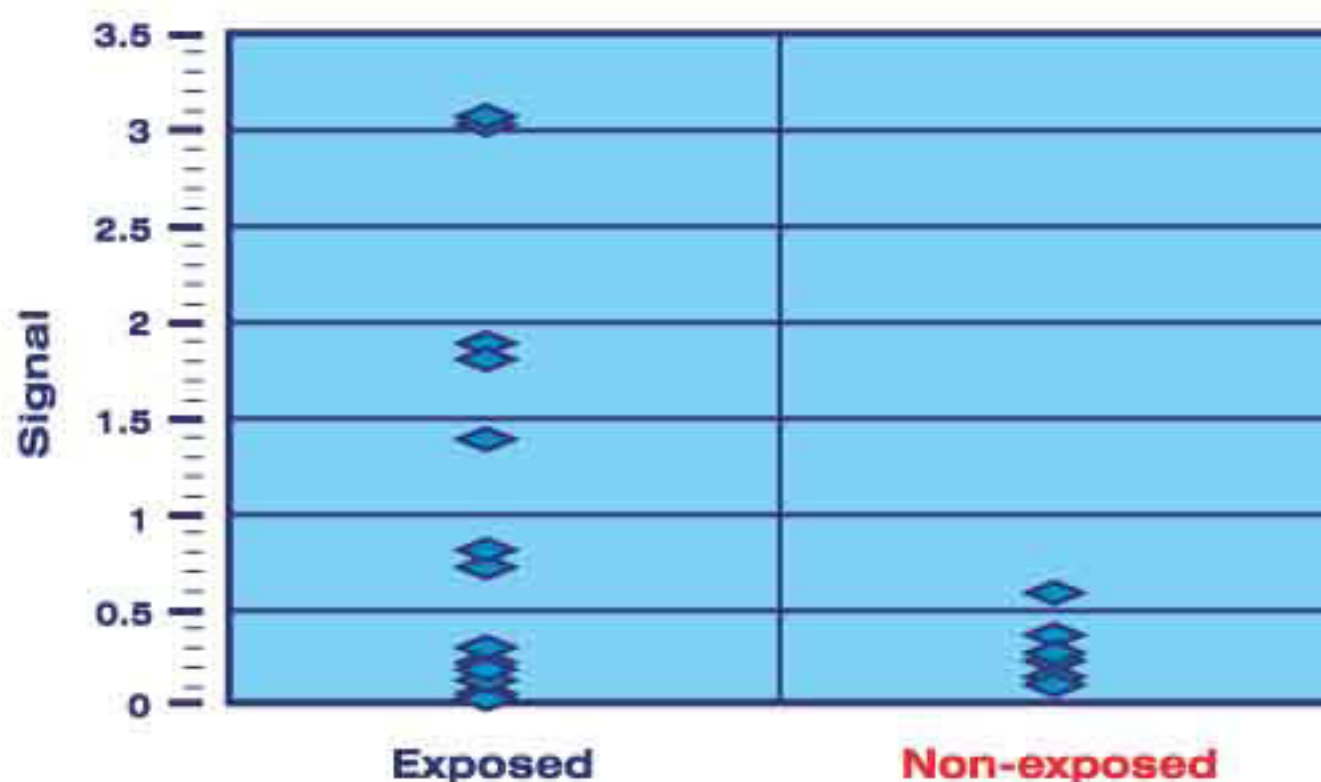
Results

The results from 20 animals investigated on two occasions are shown in Table 1. The results from exposed and non-exposed animals are compared in Graph 1.

Table 1.

Animal	Signal 19.05	Signal 18.09
1 exposed	0.034	
2 exposed	0.700	
3 exposed	0.018	
4 exposed	1.887	
5 exposed		1.818
6 exposed		0.194
7 exposed		0.297
8 exposed		3.015
9 exposed		0.812
10 exposed		1.815
11 exposed		0.146
12 exposed		1.379
13 exposed		0.186
14 exposed		3.046
15 non-exposed	0.180	
16 non-exposed	0.260	
17 non-exposed	0.607	
18 non-exposed	0.131	
19 non-exposed		0.400
20 non-exposed		0.304

Graph 1.



Data on the prion genotype and breed of sheep was available for the samples analyzed on the 18.09.03 (see Table 2). Control unexposed animals are shown in grey type and marked with *. The results from genotype and breed matched sheep are highlighted in red and blue.

Table 2.

Animal	Genotype	Breed	Signal
5 exposed	ARQ/VRQ	Swaledale	1.818
6 exposed	ARQ/VRQ	Swaledale	0.194
7 exposed	ARQ/VRQ	Welsh Mountain	0.297
8 exposed	VRQ/VRQ	Welsh Mountain	3.015
9 exposed	VRQ/VRQ	Swaledale	0.812
10 exposed	ARQ/VRQ	Swaledale	1.815
11 exposed	ARQ/VRQ	Cheviot	0.146
12 exposed	ARQ/VRQ	Cheviot	1.379
13 exposed	ARQ/VRQ	Swaledale	0.186
14 exposed	ARQ/VRQ	Swaledale	3.046
19 non-exposed*	ARR/ARR	Suffolk	0.400
20 non-exposed*	ARR/ARR	Suffolk	0.304

Discussion

The Seprion Ligand has been shown to bind selectively to PrP^{Sc} and not to PrP^C (see the handout 'Detection of Protein Aggregation Diseases with the Seprion Ligand System'). Using this ligand we were able to build an assay that demonstrates an increased signal in some of the blood from exposed animals. This increased signal was not dependent upon prion genotype or breed. The blood from non-exposed animals did not show the same response. In addition, analysis of a large volume of pooled blood from exposed animals by Seprion capture followed by elution, polyacrylamide gel electrophoresis and Western blotting has revealed a triplet of bands of the same molecular weight as recombinant prion. The study on the exposed flock is continuing and the results presented here will be correlated with subsequent development of symptomatic disease in these animals.

Reference.

1. Lane A, Stanley CJ, Dealler S, Wilson SM. (2003) Polymeric ligands with specificity for aggregated prion proteins. Clin. Chem.;49 (10):1774-5

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