

Polymeric ligands with specificity for aggregated prion proteins

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Introduction

The misfolding of proteins and their subsequent aggregation in the brain is thought to be the cause of a group of related neurological diseases including Parkinson's, Alzheimer's and the transmissible spongiform encephalopathies (TSEs) such as BSE, scrapie, vCJD and CVWD; the 'protein conformational disorders'. The development of highly sensitive and specific diagnostic assays for these diseases is a high priority.

We report on the development of an immunometric assay for abnormal, aggregated prion protein in brain tissue that uses an immobilised polymeric ligand with specificity for the rogue form of the protein, in combination with a specific antibody/enzyme conjugate.

Diagnostic assays for TSEs using polymeric ligands

The current, EU approved, ELISA assay kits available for the detection of BSE and scrapie infection in brain tissue use antibodies which cannot distinguish the normal prion protein (PrP^{Sc}) from the rogue, infectious and aggregated form (PrP^{Res}). Thus a proteinase K digestion step is used to remove the large excess of PrP^{Sc} present in the tissue homogenate prior to assay. Under- or over-digestion problems can lead to a compromised specificity and sensitivity performance in the ELISA.

As an alternative approach we have been screening synthetic polymeric ligands with the intention of identifying compounds with extremely high specificity for PrP^{Res}. Several compounds (the 'Seprion' range) have now been identified that, under the appropriate conditions, have the ability to bind the PrP^{Res} present in BSE and scrapie-infected bovine brain tissue with high selectivity. One compound (designated 'v2') was chosen for inclusion in an assay development programme.

The immunometric assay employs the Seprion ligand immobilised directly to the wells of a microplate (illustrated schematically in Figure 1). After addition of bovine or ovine brain homogenate and direct capture of PrP^{Res} the bound prion protein is then denatured *in situ* and detected with a commercially available prion protein-specific monoclonal antibody/peroxidase conjugate. The Seprion assay protocol is compared with that of the commercially available kits in Figure 2.

Results

Results were obtained with UK-derived bovine brain samples, confirmed as positive (BSE infected) or negative by histopathology. Figure 3 shows the performance of the Seprion assay and the EU approved assay with negative, non-infected brain samples, whilst Figure 4 shows a comparison of the two assay methodologies with both positive and negative brain samples.

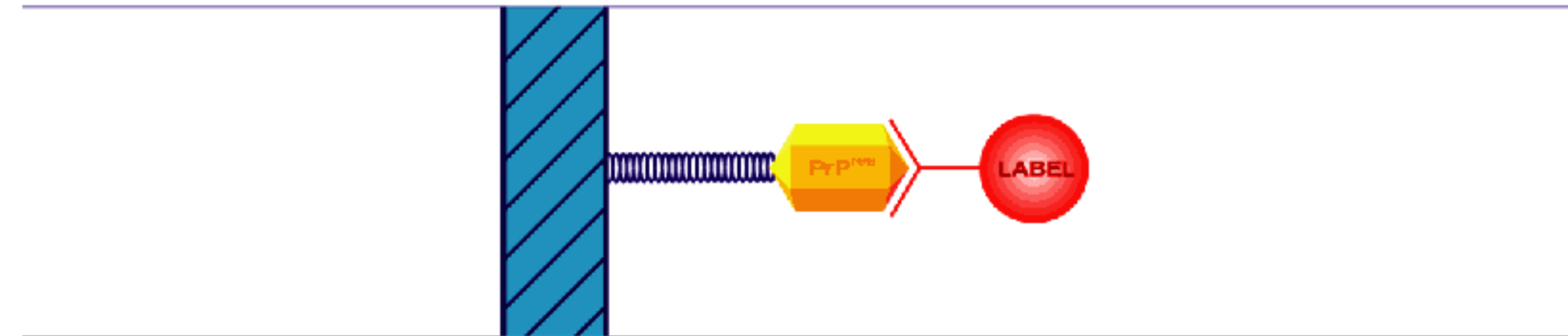


Figure 1. The principle of the Seprion immunometric assay for PrP^{Res}

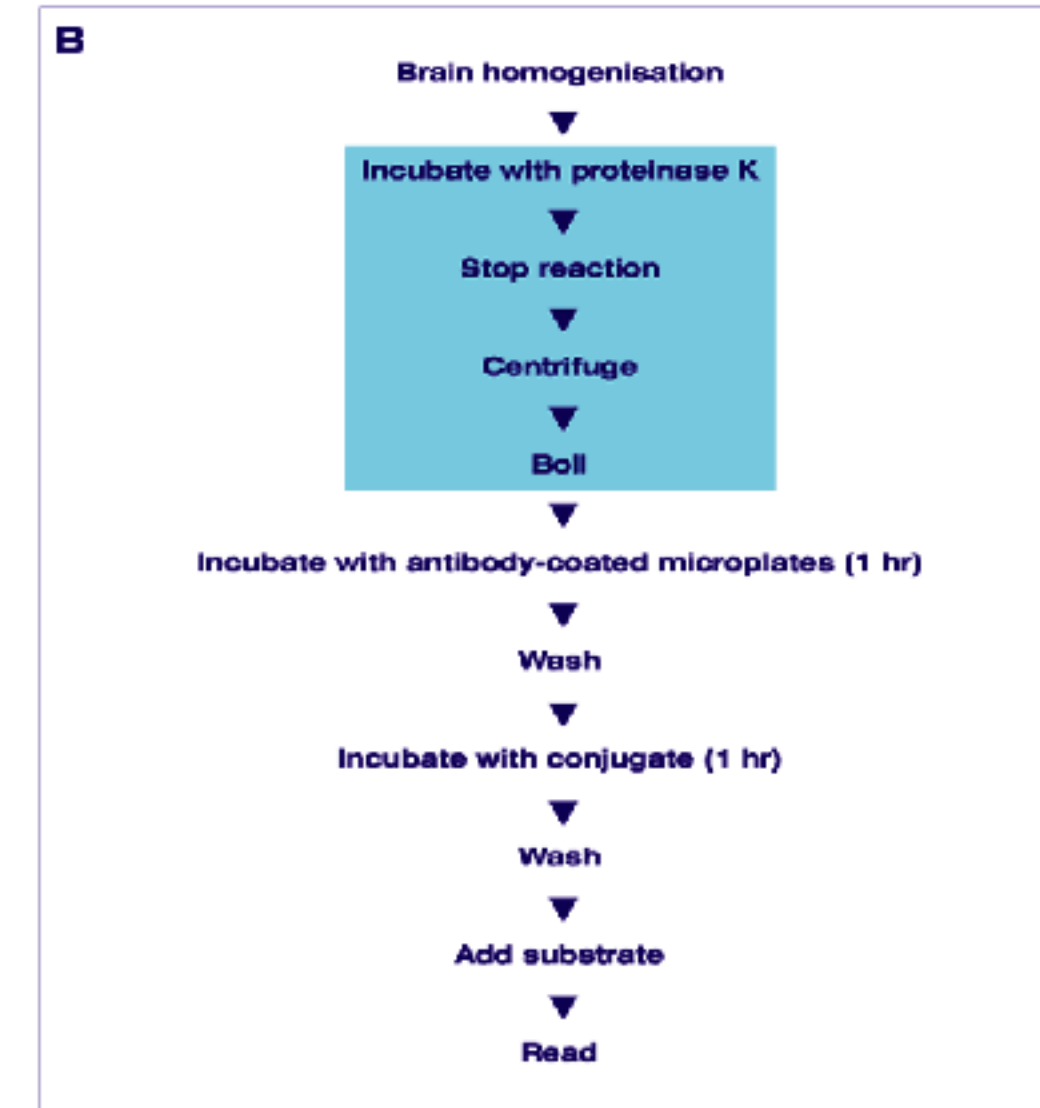
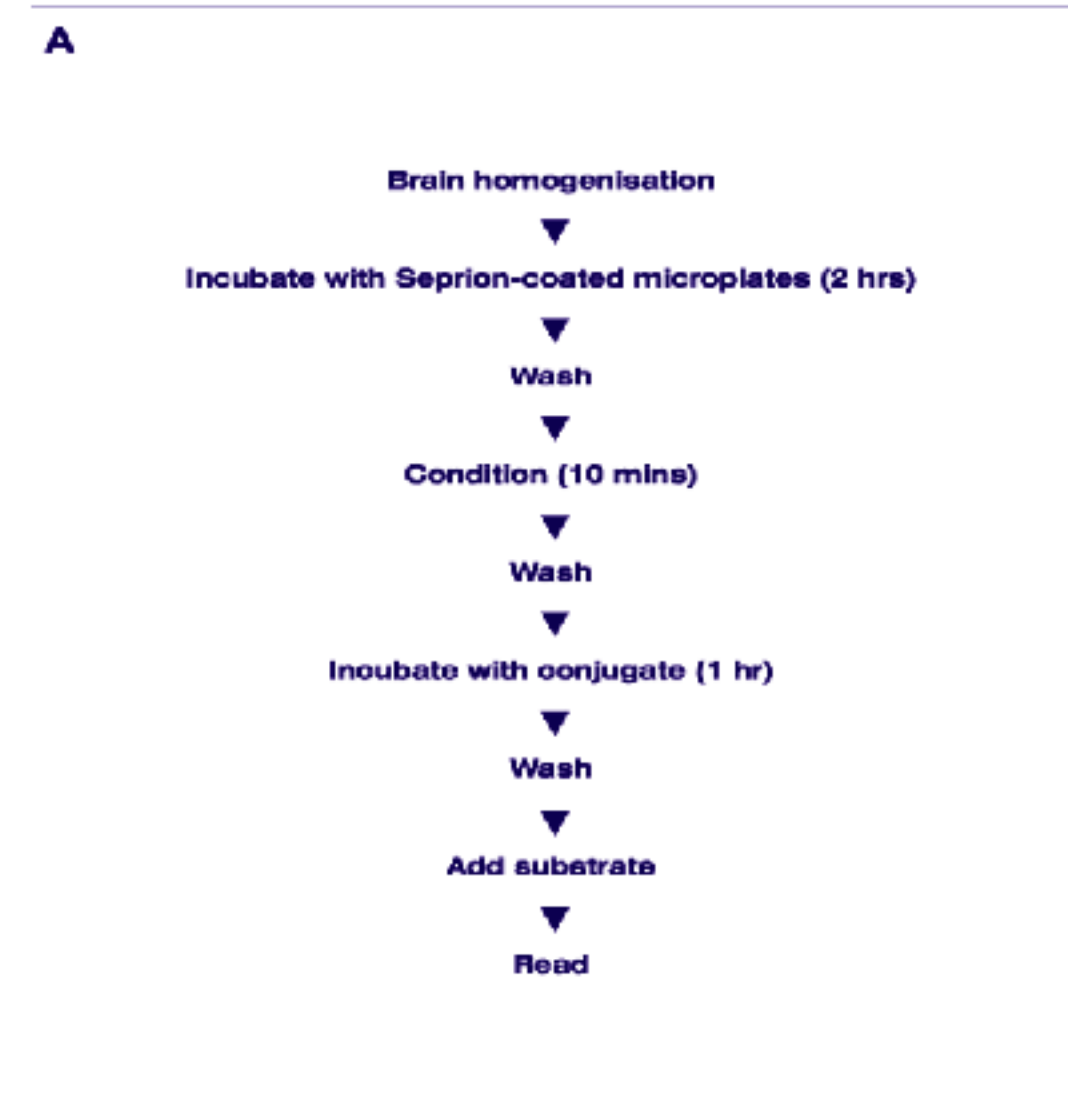


Figure 2. The Seprion assay protocol (A) compared with an EU approved ELISA for BSE detection (B) - the steps highlighted are not used in the Seprion-based assay.

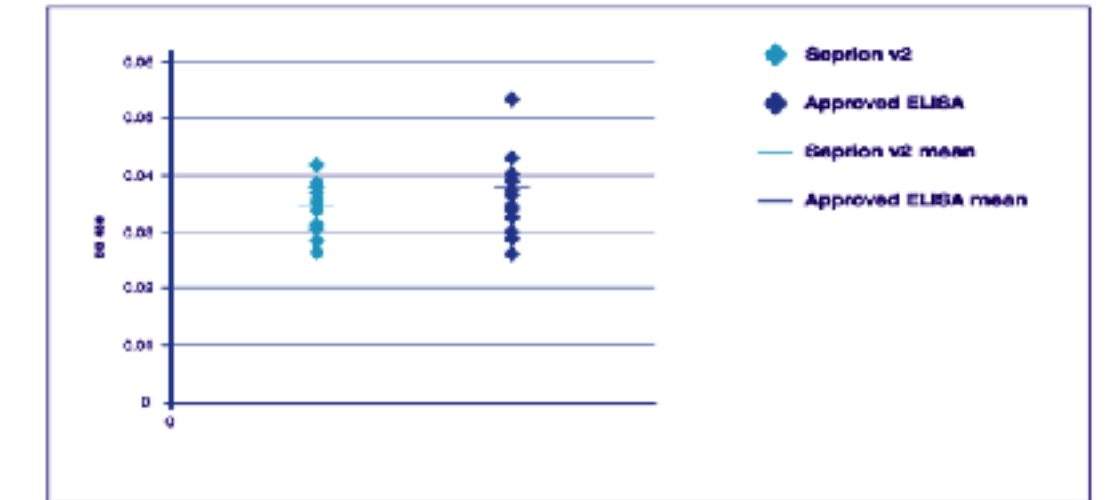


Figure 3. A comparison of the Seprion immunometric assay with an EU approved ELISA using negative, non-infected bovine brain sample

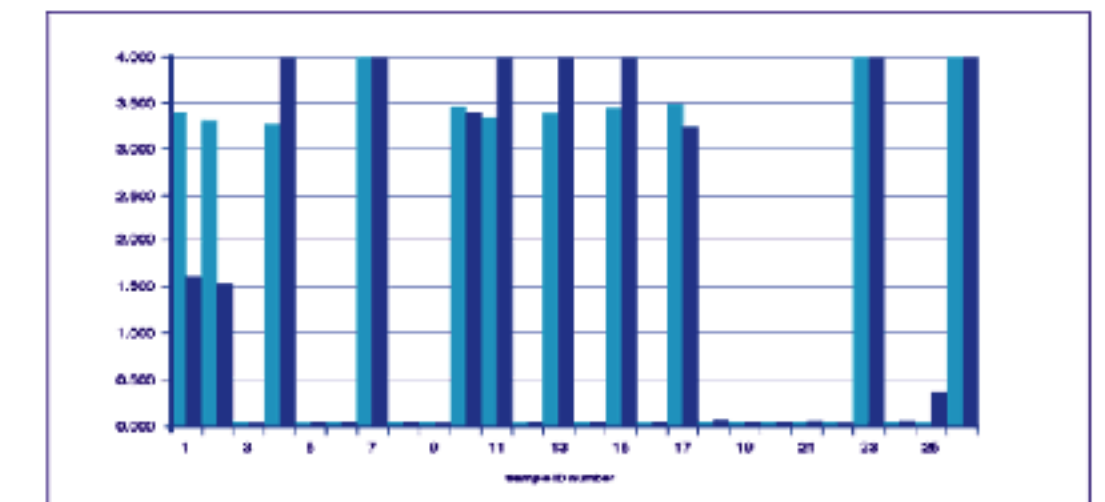


Figure 4. A comparison of the Seprion immunometric assay (■) with an EU approved ELISA (■) using a panel of BSE infected and non infected bovine brain samples.

Conclusions

The Seprion affinity ligand immunometric assay correctly identified all the BSE infected animals. The assay exhibits a consistently low background signal with negative, non-infected brain tissue.

The Seprion assay procedure is greatly simplified compared to the proteinase K digestion methods and is suitable for automation on an ELISA processor. Affinity-ligand coated magnetic microparticles have now been prepared and studies are underway on the capture of the abnormal prion protein from larger quantities of brain homogenate, urine and blood fractions.