Advisory Committee
Blood Safety and Availability

Department of Health and Human Services

TWENTY- SIXTH MEETING

May 16 and 17, 2005
Bethesda North Marriott Hotel and Conference Center
5701 Marinelli Road
North Bethesda, MD 20852

Detection of Protein Conformational Disorders

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Progress towards a feasible TSE blood test

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Sculpture in the grounds of Des Moines Art Centre, Iowa

The problem

- Case 1. A blood recipient was identified with vCJD 6.5 years after receiving red cells donated by an individual 3.5 years before the donor developed vCJD.

- Case 2. Preclinical vCJD was found in a patient 5 years after receiving blood from a donor who subsequently developed vCJD. The patient was unusual in having heterozygote genes for the prion protein which had been believed to protect against the disease.

  Both cases reported in the Lancet 2004

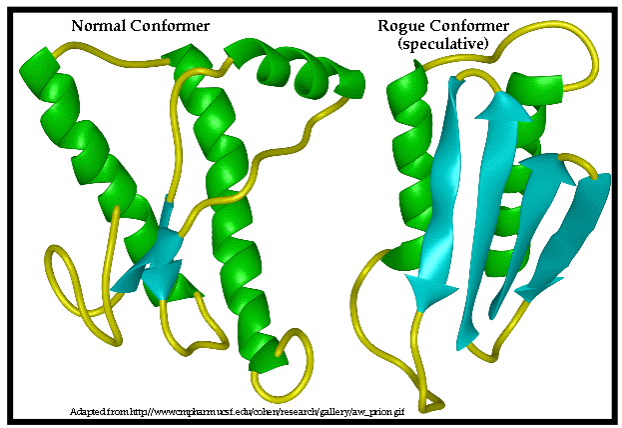
- The UK National Blood Service identified 9 donors between 1985 and 1999 who subsequently developed vCJD. These donors made a total of 23 blood donations, which are thought to have contributed to 200 batches of plasma products for use in blood products.

- If a blood screening test were available it would be implemented.

An Unique Problem

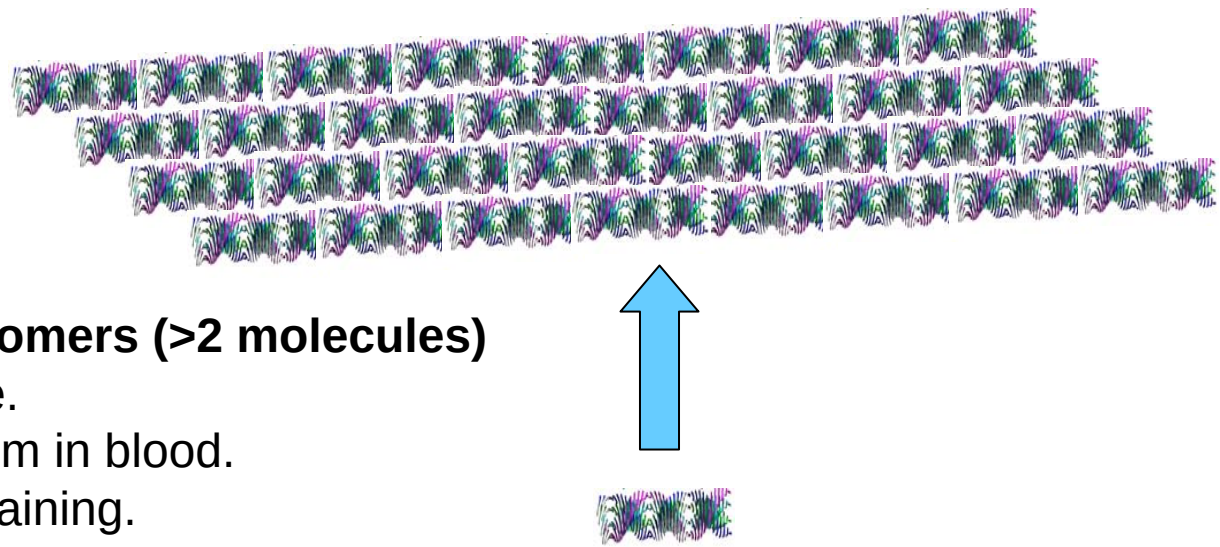
The unique problem of excluding vCJD from the blood supply

- It is a self-protein, not a virus
  - No genome or mRNA to detect
  - No non-self proteins to detect
  - No immune response
- There is not a lot of it mixed in with a vast excess of normal prion protein (PrPc)
  - Problems with sensitivity and specificity



From oligomers to fibrils

Amyloid fibrils (aggregates of thousands of molecules)
Large, insoluble.
Visible by staining.
Found deposited in tissues eg. brain and spleen. Readily available and used extensively for spiking studies.



Amyloid oligomers (>2 molecules)
Small, soluble.
Most likely form in blood.
Invisible by staining.

Post-mortem tests and the use of protease

The large aggregates of rogue prion protein in the brain are relatively resistant to protease allowing the normal prion protein (PrPc) to be digested away prior to testing.

But:

- Problems with standardisation – lab to lab; sample to sample; tissue to tissue leading to under-digestion and false positives or over-digestion and false negatives
- Problems with automation
- No guarantee that all rogue prion is protease resistant
  - Even post-mortem - atypical scrapie (nor98) and BSE
  - Ante-mortem - is rogue prion in blood resistant to protease?
  - Most post-mortem tests cannot easily be applied to ante-mortem testing

Innovative approaches are needed

- Reduce risk by exclusion
  - Donor exclusion. Incomplete.
  - Leucodepletion. Animal models demonstrate that 55% of infectivity remains (Rohwer, 2004)
  - Filtration. Pall Corp, PRDT. Not 100% efficient.
- Abrogate risk through blood testing
  - Surrogate markers. Lack of specificity.
  - Rogue-prion specific antibodies. Low affinity.
  - Rogue-prion specific ligands

Introduction to the Seprion ligand platform

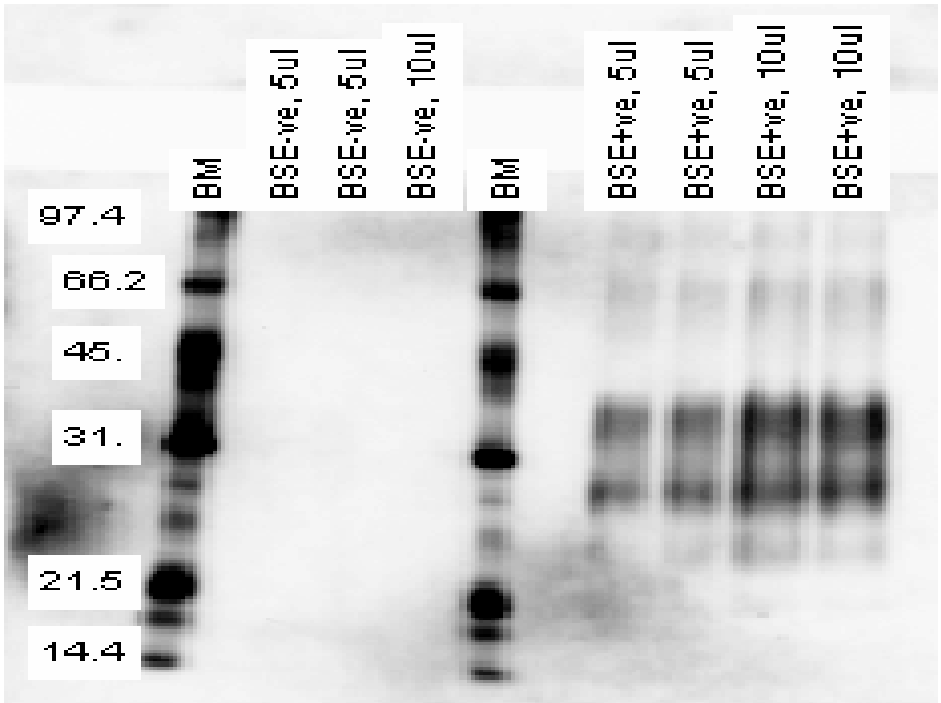
Technology background

There is a wealth of scientific literature demonstrating that polyionic polymers can bind to rogue prion protein: histopathological stains, curing infected cell lines, delaying or preventing disease
http://www.priondata.org

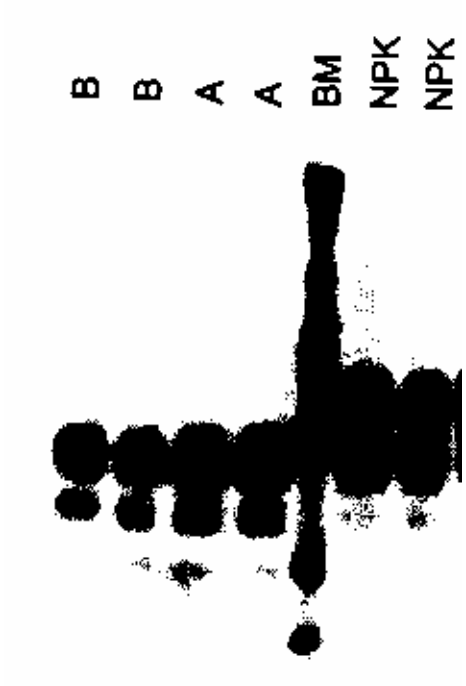
We have developed the use of polyionic polymers to specifically capture rogue prion protein and avoid the need for proteinase K. Normal PrPc does not bind to the ligand in the presence of the competing polyanionic surfactant.

Western analysis of Seprion-captured material from infected and uninfected brain

no protease used



Western analysis of Seprion-captured material – effect of Proteinase K



NPK: Seprion captured material without proteinase K treatment

A: Proteinase K treatment of captured material

B: Proteinase K treatment of material prior to capture

TSE post-mortem summary

- The Idexx BSE and CWD assay has 100% specificity and 100% sensitivity compared to existing EU approved tests on brain and lymph nodes

- USDA approval for BSE and CWD
- EU approval for BSE

Technology extensively validated

TSE ante-mortem
(towards a feasible blood test)

Protocol for 125 microliters whole blood

Blood lysis and DNAse treatment
(60 min)

Capture on Seprion coated magnetic beads
(60 min with shaking)

Washing of beads by magnetic capture
(10 min) (fits into standard automated magnetic bead handlers)

Elution and denaturation of captured prion
(5 min at 95^{o}C) (acid, alkali, salt elution alternatives)

ELISA detection
(2h 30 min – 3h 30 min) (standard automation)

Total time 4h 45 min – 5h 45 min for each sample run
– numbers determined by automated platform chosen

Protocol for 225 microliters plasma

Seprion capture using coated magnetic beads
(30 min with shaking)

Washing of beads by magnetic capture
(10 min) (fits into standard automated magnetic bead handlers)

Elution and denaturation of captured prion
(5 min at 95^{o}C) (acid, alkali, salt elution alternatives)

ELISA detection
(2h 30 min – 3h 30 min) (standard automation)

Total time 3h 15 min – 4h 15 min for each sample run
– numbers determined by automated platform chosen

Spiking as a model system

We know:

- There are about 10^7 IU of rogue prion per gm of BSE-infected bovine brain (figure accepted by U.S Food and Drug Administration) probably 100-fold less in spleen.

- Mouse adapted human TSE strain has a concentration of 20-100 IU/ml of buffy coat *(Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN (1999) Transfusion, vol 39, page1169; Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P.(2003)Transfusion vol 43, page1687)*.

Evaluation of the sensitivity of the Seprion plasma assay for spiked vCJD brain

Dilutions of positive human vCJD brain were prepared in negative control human brain (final brain quantity in all assays 1mg)

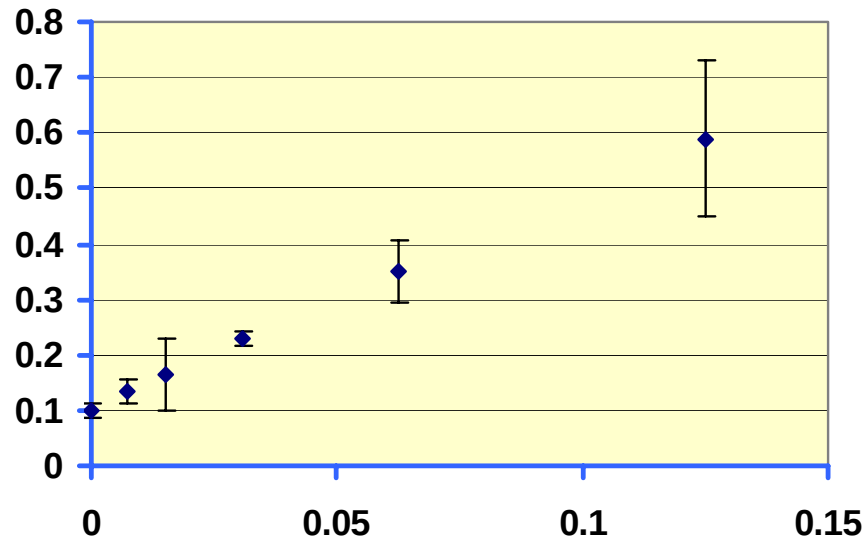
The brain dilutions were spiked into plasma

Rogue prion was captured using Seprion coated magnetic particles

Captured rogue prion was eluted and detected by ELISA

Signal shown is mean of duplicate assays

Detection limit : <0.03 mg vCJD brain, about 300 IU



Spiked recovery of vCJD spleen from plasma

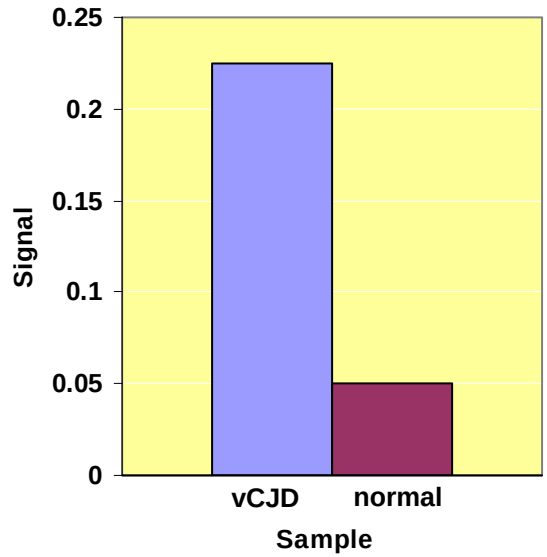
5 ml of plasma was spiked with 5µl of vCJD or normal spleen

Rogue prion was captured using Seprion coated magnetic particles

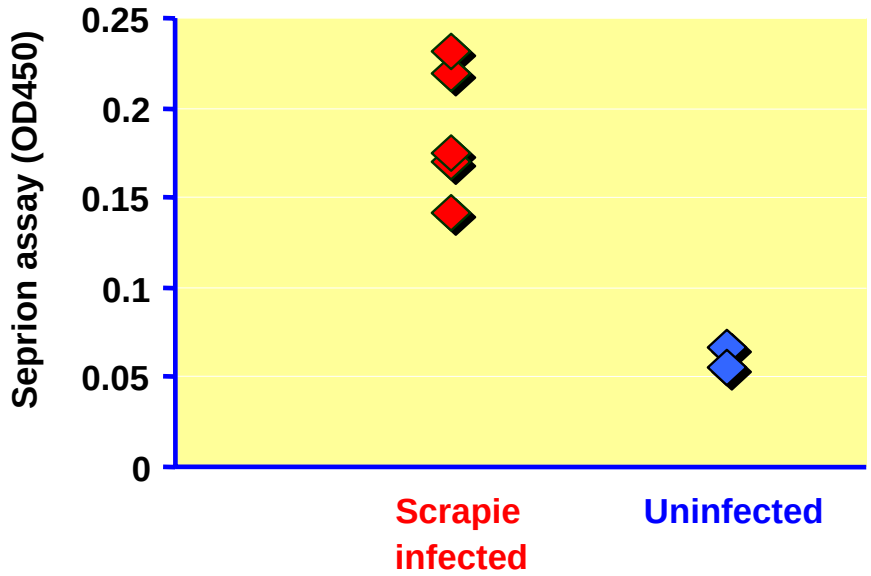
Captured rogue prion was eluted and detected by ELISA

Signal shown is mean of duplicate assays

Detection limit : 500 IU per sample of plasma or 100 IU/ml plasma



Detection of PrP^{Sc} in sheep exposed to scrapie (symptomatic)



Non-red cell fraction was prepared from 5 ml blood

Rogue prion was captured using Seprion coated magnetic particles

Captured rogue prion was detected by direct assay on the bead

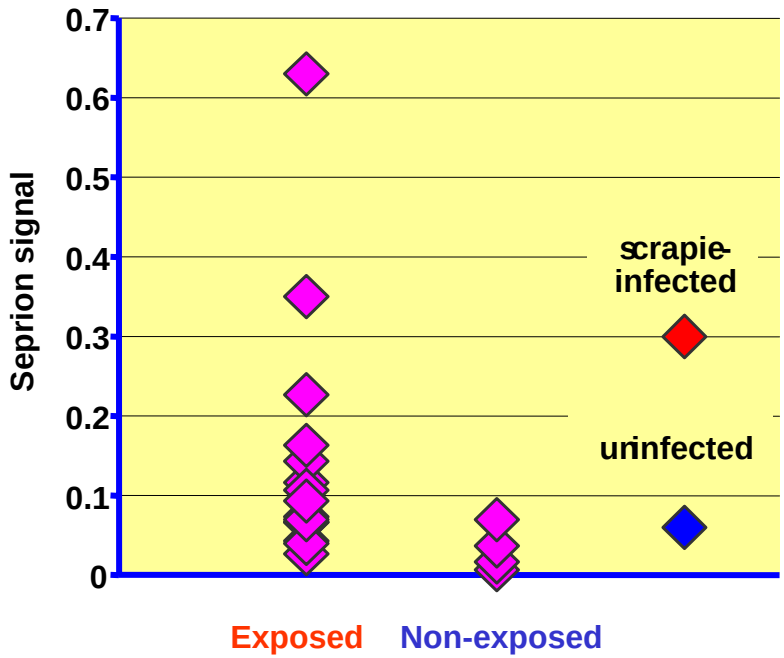
Results on the blind panel using the revised protocol

		SEPRION ASSAY		
		Positive	Negative	*Total*
VLA designation by Western blot	Positive	**2**	**0**	*2*
	Negative	**1***	**26**	*27*
	Total	*3*	*26*	

Sample from a suspect from the same farm as three previously confirmed positives ie. from a farm with endemic scrapie

Sensitivity **100%**
Specificity **96** %

Detection of PrP^{Sc} in sheep exposed to scrapie (asymptomatic)

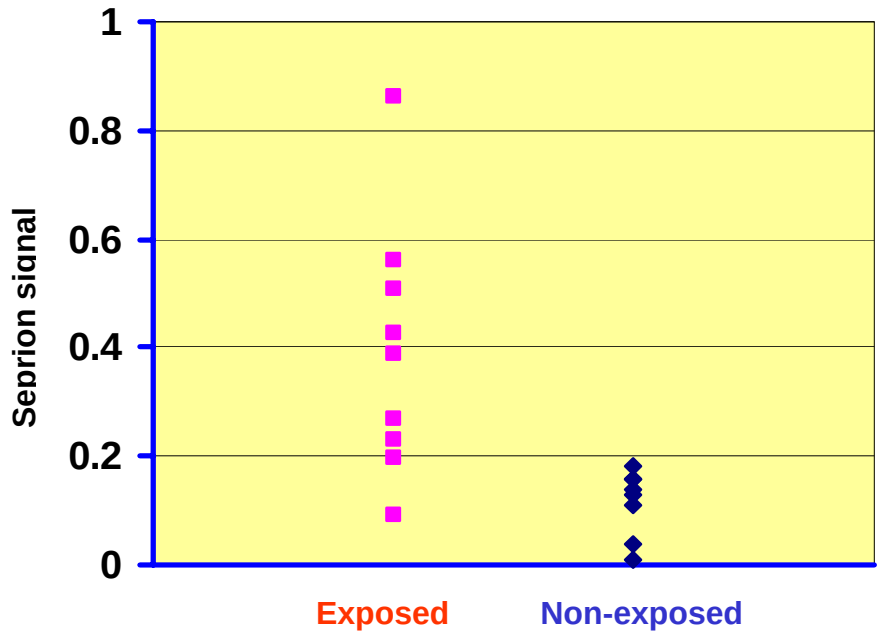


Non-red cell fraction was prepared from 5 ml blood

Rogue prion was captured using Seprion coated magnetic particles

Captured rogue prion was detected by direct assay on the bead

Detection of PrPSc in sheep exposed to scrapie (asymptomatic)

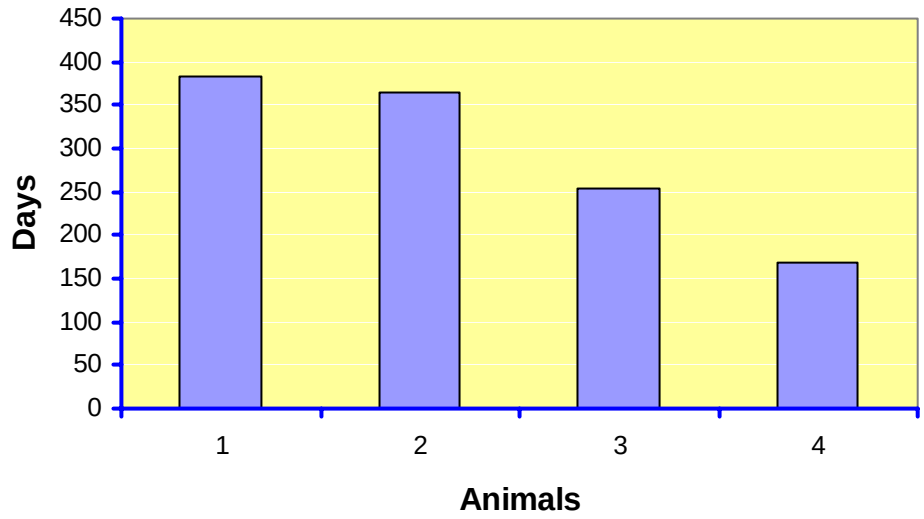


Non-red cell fraction was prepared from 5 ml blood

Rogue prion was captured using Seprion coated magnetic particles

Captured rogue prion was eluted and detected by ELISA

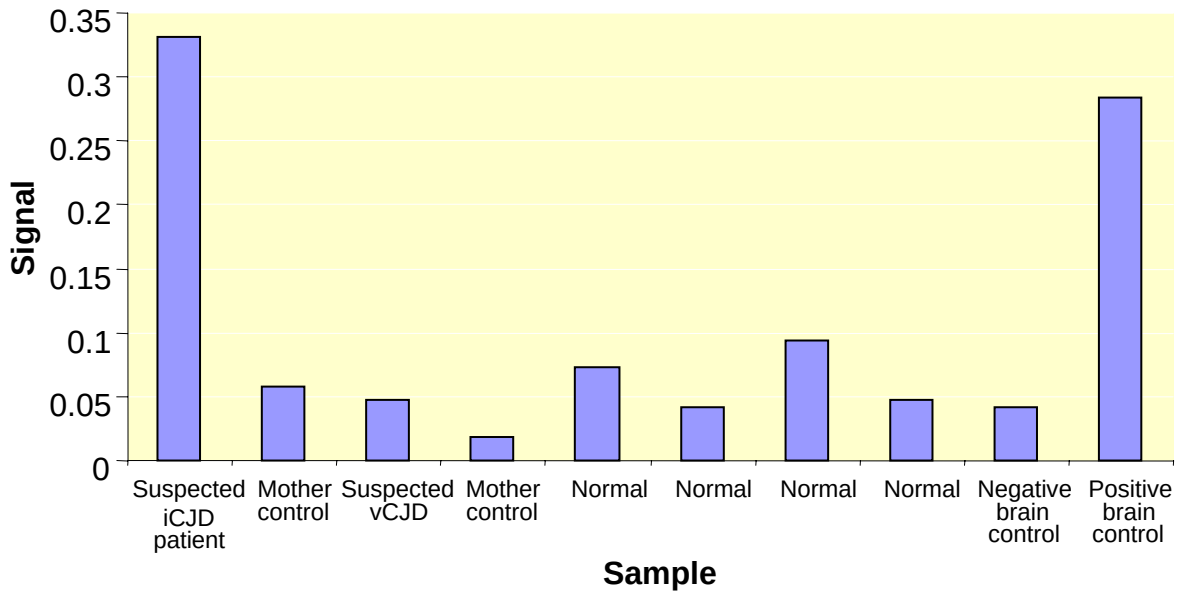
Time from Seprion assay positive to clinical signs for four scrapie infected sheep



Investigation of suspected CJD blood using the Seprion assay

We already known that the assay works on human post-mortem sCJD and vCJD brain samples. It also works on vCJD spleen and vCJD spleen spiked into plasma.

Non-red cell fraction was prepared from 10 ml blood
Rogue prion was captured using Seprion coated magnetic particles
Captured rogue prion was eluted and detected by ELISA



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Total time 3h 15 min – 4h 15 min for each sample run
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Independent evaluation of the Seprion technology on a blind panel

Proof of principle for a TSE ante-mortem test

We could detect rogue prion in the blood of symptomatic and asymptomatic scrapie infected sheep and in the blood of an iatrogenic CJD patient.

Protocols for small volume whole blood, cellular preparations and plasma have been developed and independently evaluated.

Feasibility for blood screening

The protocols fit with existing automated high-throughput platforms.