The Marker

Abnormal form of PrP (PrP^{tse}, PrP^{res}, PrP*)

(Not well defined. Is it oligomers, proteinase resistant materials? If so what form? Are all forms infectious?)

At present it is a correlate of infection not a quantitative measure of infectivity.

An assay must:

Have a very high specificity
Have a significant sensitivity
Be practical and robust

Types of assay

Direct detection of PrPsc by antibody or other method with or without a concentration step (Amorfix, Prionics 1.0)

Amplification of existing small amounts of PrPsc by conversion of normal PrP (PMCA, QUIC)

Prion Unit assay

Importance of independent blinded assessment

- Carefully designed blinded studies provide convincing evidence of test performance
  - The use of tissue spikes allows direct comparison of test sensitivities
- With very limited amounts of vCJD plasma blinded studies provide a fair way to determine appropriate access to these rare samples
- Experience has shown that laboratories do much better when they know the answer than when testing blinded panels

Direct detection: Amorfix assessment
(Epitope protection assay)

1. Sensitivity to spikes
2. Sheep blood
3. Specificity with the inclusion of blinded spikes
4. Specificity real time in France
5. Real test, new kits, spikes etc.

Results of CJD plasma testing- 2 individual samples were tested

Sample	Raw data	Scoring (positive/negative)
vCJD brain 10^{-4}	34.64	Positive
vCJD brain 10^{-4}	33.44	Positive
vCJD brain 10^{-5}	2.13	Negative
vCJD brain 10^{-5}	1.57	Negative
Pooled plasma	1.11	Negative
Pooled plasma	0.81	Negative
Pooled plasma	0.78	Negative
Sporadic CJD 1	0.88	Negative
Sporadic CJD 2	0.77	Negative
Sporadic CJD 3	0.89	Negative
Variant CJD 1	0.86	Negative
Variant CJD 2	1.04	Negative

Calculated assay cut off 1.38 (average+3SD) or 2.0 assigned by developer

Another company: vCJD plasma compared to normals (raw data)

585		ind154
589		vCJD plasma 2
651		ind125
670		ind165
720		ind164
743		ind128
743		ind166
774		ind147
782		ind138
792		ind133
867		ind137
945		ind141
1001		vCJD plasma 1
1126		ind130
1187		ind134
1226		ind167

Calculated assay cut-off (av+3SD) =1193

1 normal plasma would have been falsely scored positive

Sensitivities assessed by spike panels.

Group	Sensitivity with brain	Matrix effect	Sensitivity with spleen	Matrix effect
1	10^{-3} (1/3)	No	10^{-1}	No
2	10^{-4}	No	10^{-2}	No
3	10^{-4}(2/6)	Yes	10^{-2}	Yes
4	10^{-2}	No	nd *	No
5	10^{-4}	No	10^{-3} (1/3)	No
6	10^{-5}	Yes	10^{-3} (2/3)	Yes

Tests that are still there.

1. PMCA
2. QUIC
3. Prion Unit
4. Others?

PMCA

- PMCA (protein mis-folding cyclic amplification)
  - Detects infectivity in hamster scrapie blood (clinical and pre-clinical) and natural scrapie (clinical)
  - Assay takes several days to complete-unsuitable for individual donor screening
  - Problems with specificity-unacceptable false positive scoring rate
  - Has not been independently evaluated-studies are however underway
  - Sensitivity:8 log dilution of infected brain

  - Under investigation by SNBTS and French Transfusion service.
  - A ‘normal donor’ positive at SNBTS caused major issues.
  - Questions remain.

QUIC (Quaking induced conversion) after immunoprecipitation with 15B3

- Detects markers of infectivity in scrapie infected hamster plasma (263K) at both clinical and preclinical stages of infection (even very early post infection)
- 3 step assay:
  - Immunoprecipitation using 15B3, incubation overnight
  - Amplification of PrP^{Sc} after IP using recombinant PrP^{C} as a substrate (2 to 3 days)
  - Detection using Thioflavin T (fluorescence is enhanced by binding protein aggregates)
  - Sensitivity: 14 log dilution of infected brain (non blinded)

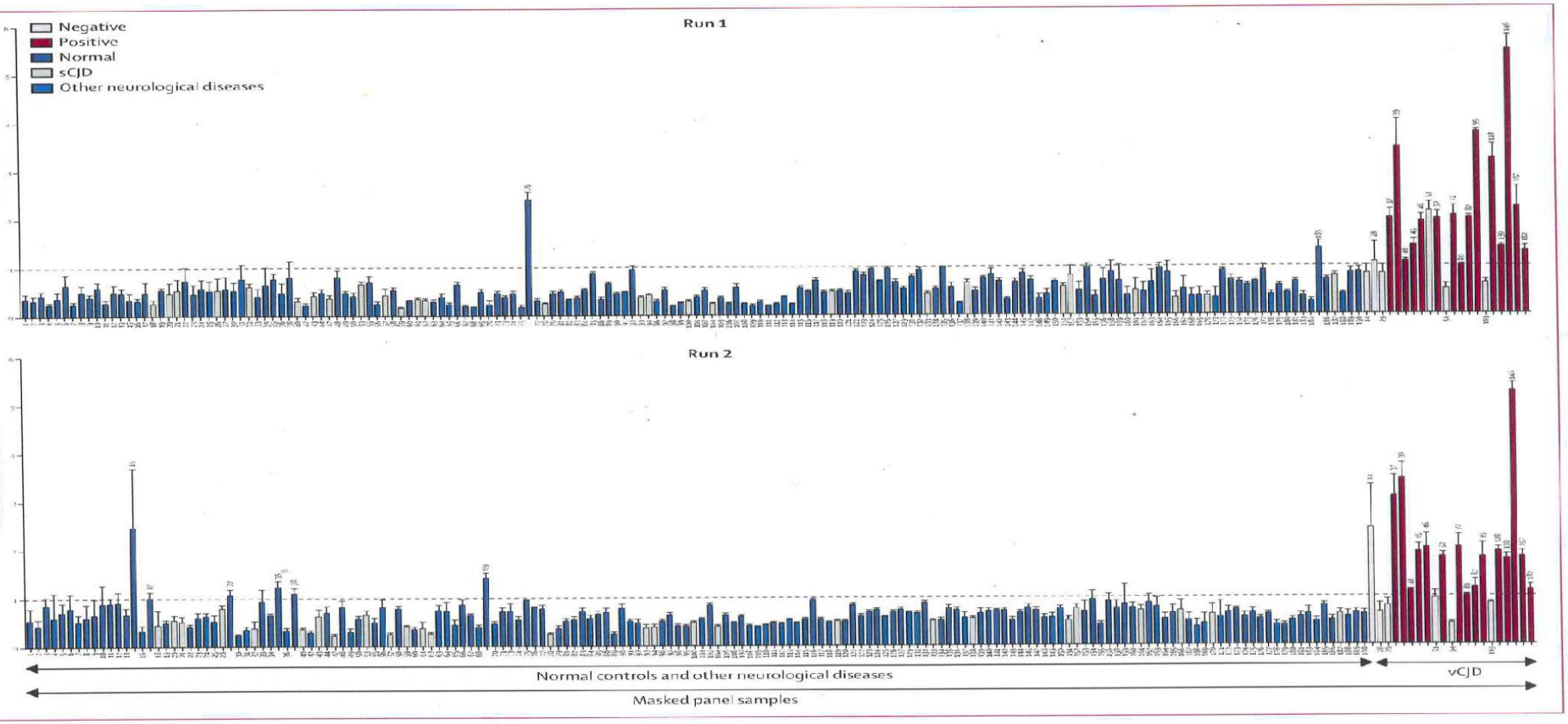
Under investigation by Rocky Mountain laboratory and Bristol NHSBT.

At Bristol, convincingly detects positives in unblinded blood (plasma) samples.

Questions remain.

Prion Unit test

1. Reported analytical sensitivity of 10 log dilution of brain.
2. Analyte is EDTA treated whole blood (eight microlitres)
3. Specificity: 100 normals no repeat reactives.
4. 69 other neurological diseases gave no repeat reactives
5. Sensitivity: 15/21 vCJD patients were positive. (71.4%)



ure 3: Two independent assay runs for a masked panel of 190 blood samples
masked panel consisted of blood samples from 21 patients infected with vCJD, 100 healthy normal controls (dark blue bars), 27 patients with sCJD (green bars), and 42 controls with other rological diseases (turquoise bars). Data are shown as the chemiluminescent signal ratio relative to a cutoff established as the mean of the signal for normal controls plus three SDs. Samples shown rey are from patients infected with vCJD that had a ratio of less than 1 in one or both assays and were therefore scored as negative. Red bars show all samples that had a ratio greater than 1 relative utoff in both assays and were scored as positive. vCJD=variant Creutzfeldt-Jakob disease. sCJD=sporadic Creutzfeldt-Jakob disease.

spikes of vCJD-infected brain homogenate could clearly be distinguished from normal brain homogenate, even at a 10^{10}-fold dilution (figure 1), a sensitivity more than four orders of magnitude higher than previously achieved for immunoassay of vCJD tissue.[26] A chemiluminescent signal of mean $1{\cdot}3{\times}10^{5}$ (SD $1{\cdot}1{\times}10^{4}$) was obtained with 10^{-10} dilution of vCJD-infected brain versus $9{\cdot}9{\times}10^{4}$ (SD $4{\cdot}5{\times}10^{3}$) for a 10^{-6} dilution of normal control brain, corresponding to a ratio relative to the cutoff threshold of 1·2 (SD 0·1). The difference was highly significant ($p<0{\cdot}0001$). The highest dilution of

sufficient to detect infection in blood samples from patients with vCJD, on the basis of estimates of titre obtained from rodent models.[24,25] However, the biochemical nature of infectivity and abnormal PrP associated with blood is unknown. To ensure that this level of discrimination could be achieved with endogenous blood samples, we tested a subset of blood samples from 14 patients with confirmed vCJD from whom larger volumes of blood were available, and compared these with normal control blood samples (figure 2). The samples were analysed as groups and had mean

Prion Unit test

Currently being evaluated by Prion Unit and HPA (Colindale) on normal US donors to determine specificity.