Flow chart for the evaluation of potential tests for CJD for access to rare samples (such as plasma) from vCJD patients.

Only a small number of samples from vCJD patients are available so that they can only be used to assess detection methods for which there is a high probability of success. A process has therefore been developed by the CJD Resource Centre Oversight Committee to evaluate the suitability of methods in a stepwise manner. The process only covers access to samples from clinically affected vCJD patients; other samples available from the preclinical incubation period are currently outside the scope of this document as the significance of a negative result is not yet clear. It is recognised that the detection of preclinical infections is the object of the diagnostic methods to be evaluated.

The process described is based on assumptions on the nature of infectivity and markers in different tissues and species that are considered reasonable in the light of current scientific understanding. At all stages the assumptions may be wrong. For example it is assumed that the nature of the infectivity and associated markers in blood are in some sense similar to those in tissues of known infectivity, such as spleen or brain. It is possible, however, that an assay could detect a signal in blood but not in other tissues from patients. Similarly it is assumed that animal tses will in some sense resemble human tses in the distribution and development of markers and their detection by the assay, so that they provide a model. Nonetheless it is recognised that it is possible that an assay could work on human but not animal samples. A failure at any stage of the process to be described is therefore not necessarily fatal to an assay. However the reason for the failure should be scientifically justified by the developer. Moreover other data must be available that justify a high level of confidence in the reliability of the assay approach if access to the rare samples is to be allowed.

Applicants will be expected to be able to produce 5000 assays at some stage to allow further evaluation or use by interested parties such as blood transfusion services. While in the first instance the evaluation must be confidential on the parts of both the applicant and the oversight panel it is a condition of the process that the results will eventually be published under the auspices of the Oversight Committee. The applicants will have input into the publication.

**Stage 1: Background Data from Applicant**

The applicant should prepare a dossier to be evaluated by the CJD Resource Centre Oversight committee which should include the following:

1. A description of the scientific basis of the assay method. This should also cover consideration of any known or likely issues affecting assay performance, such as the nature of the material to be analysed, the effects of sample treatment including freeze/thawing or storage, the presence of specific anticoagulants, and any other relevant matters.

2. Data on the performance of the assay method including sensitivity, specificity and any matrix effects when it is applied to tissues of known infectivity such as brain, spleen, or other materials.
3. Species specificity if any and the reason for it (e.g. specificity of antibodies)

4. Data on the performance of the assay method on any blood samples tested from any species (including human) from both clinically affected and uninfected individuals; source, species and number of samples.

5. Data on the performance of the assay method on other specimens that could give false readings, such as other neurological diseases or other human tse.

6. Criteria for positivity: method of establishment of cut off and number of samples examined

**Stage 2 : CJD Resource Centre Oversight Committee evaluation**

**A. Blinded panels of plasma spiked with infected or uninfected human brain and spleen.**

Two panels with 48 samples each will be provided to the applicant who will be unaware of which pairs of specimens are duplicates. Each panel will consist of tenfold serial dilutions of tissues in pooled plasma and the Oversight Committee will evaluate the data returned, which will include, but not be confined to, the interpretation of the applicant. Raw data should be supplied to the Oversight Committee for analysis not just +/-.

Assays will be judged acceptable on the basis of:

1. **Limits of detection of infected materials in the blinded panels:** an unacceptable assay will fail to register a positive signal in a dilution of 10% infected brain homogenate equal to or greater than 1 to 10000, and in a dilution of 10% infected spleen homogenate equal to or greater than 1 to 10. The justification for these figures is given in Annex I.

2. **Matrix effects:** there should be either no signal above the cut off for any sample containing uninfected brain or spleen or, if there is a signal, there should be at least a one hundred fold difference in the dilutions giving a positive signal between infected and uninfected tissues.

3. **Reproducibility:** duplicates in the same blinded panel must give the same conclusion of positive or negative, the samples in the two blinded panels must give the same endpoints for dilutions scored positive.

**B. Blinded panels of plasma from sheep.**

If an assay is found satisfactory after phase A, a blinded panel of plasma samples from sheep will be supplied to the applicant. Of the order of fifty samples will be from clinical end stage cases of scrapie, and 200 from scrapie free animals. The clinical cases may include experimental as well as natural infections. The raw data should be supplied to the CJDRC for analysis as well as the applicant’s own interpretation.

It is difficult to be specific about the level of performance that will be considered acceptable. Ideally the samples from scrapie free animals should all be scored negative. For the limited number examined this will give a statistical range for specificity of 98.2-100%. If one is scored positive the range is 97.3-99.99%. There may be doubt over the status of the samples, although they are from a flock claimed to be scrapie free. The data will be assessed accordingly but they will give some estimate of specificity.

It is not yet known whether all the samples from clinical cases will be infectious or contain the marker to the same concentration. Comparison between different assays will therefore be of interest. Arbitrarily the minimum acceptable detection rate is currently set at greater
than 25% for statistical reasons; with the number of samples from scrapie affected animals included this means that an assay with a true detection rate of 50% would have a lower fiducial limit of 25%. The decision has thus been arbitrarily taken that at the moment the assay should have a target sensitivity of approximately 50% to be suitable for further evaluation. This may be re-evaluated in the light of submitted data. For example if the assay is designed for human samples and may require adaptation for animal blood (e.g. should an antibody need to be changed) or if there are factors related to the sample handling such as anticoagulant used or freeze/thawing that might affect assay performance, the required sensitivity might be amended but adequate justification should be given. In addition if it becomes clear at a later stage that the clinical scrapie materials held by the resource centre include some without any PrP or infectivity content, the sensitivity required will have to be modified.

In addition:
1. Variation between signals from the samples from uninfected animals should be low compared to the difference between the samples from scrapie affected and uninfected animals.
2. Ideally the samples from uninfected animals should all be scored negative consistently by the assay and consistent with other assays.
3. Samples from scrapie affected animals should be scored positive consistently by the assay and consistent with data from other assays.

C. **Blinded panel of uninfected human plasma including samples spiked at the acceptable limit of detection.**

Blinded panels of US sourced, presumed vCJD uninfected sera, will be distributed for a limited assessment of specificity if this is justified by the results obtained from the sheep panels. Sufficient will be supplied to allow repeat testing. The panels will consist of two sets of sera from 3000 donors, the panels being independently blinded. Thus a total of 6000 tests will be required, allowing the Committee to assess the initial reactive and repeat reactive rates independently, and to assess assay reproducibility in general. Samples spiked at the limit of detection based on the results of stage 2A above will be included as controls. Some samples will also be spiked with normal tissues to control for matrix effects. If all samples other than those spiked with infectious material are shown to be negative (if necessary after repeat testing) the specificity is statistically approximately 99.9%. In addition as for other stages both the applicant’s own interpretation and the raw data should be supplied for analysis to allow the CJDRC oversight committee to evaluate cut off.

1. All unspiked samples to be scored negative.
2. Variation between the readings for unspiked samples should be small compared to the difference between samples spiked with infectious materials and the unspiked samples.
3. Samples spiked with infectious material to be scored positive consistent with analytical sensitivity defined from the original spiking studies described in step A.

The samples described have been stored frozen and contain citrate as anti-coagulant. It is possible that the previous treatment of the samples could affect test performance; if this is the case and if supported by data it will also be possible to establish the test at NIBSC and test fresh samples from UK sources. Testing of the two sample sets can be carried out in parallel if justified.
D. **Supply of samples from vCJD patients**

If the oversight committee judges that the assay is acceptable when evaluated in phases A, B and C, the applicant will be given access to samples from vCJD cases distributed as part of a larger blinded collection of samples from uninfected individuals. As the patient samples are extremely rare the formulation of the panels to be sent out will depend on the results of the previous evaluations. For example if sensitivity is thought to be high, 5 samples might be sufficient, although even if all were scored positive the range of sensitivities statistically would be 48-100%. If sensitivity is thought to be low ten samples might be needed. The panels will consist of 100 samples made up from samples from patients, negative samples and samples spiked at the limit of sensitivity shown in the earlier evaluation. Raw data should be supplied for evaluation by the oversight committee as well as the applicant’s own interpretation.

1. Uninfected samples should be scored negative
2. Samples from clinical cases should be scored positive consistent with the results of earlier stages in the evaluation process.
3. Spiked samples must be scored positive consistent with the analytical sensitivity determined in step A.

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**Annex I**

**Analytical sensitivity required of an assay able to detect PrP<sup>se</sup> in blood**

Assume:

1. Blood contains 1-10 infectious units per ml.
2. The nature of the infectivity (i.e. ratio of PrP<sup>se</sup>: infectivity) in blood, brain and spleen is the same.
3. The infectivity in brain is between 10<sup>e6</sup> and 10<sup>e8</sup> per gram and therefore 10<sup>e5</sup> and 10<sup>e7</sup> per ml of 10% brain homogenate.

Then:

If there are 10 IU per ml in blood and 10<sup>e5</sup> IU per ml of 10% homogenate the assay must be able to detect a dilution greater than 10<sup>e4</sup> of brain homogenate.

If there is 1 IU per ml in blood and 10<sup>e7</sup> per ml of 10% brain homogenate the assay must be able to detect a dilution greater than 10<sup>e7</sup>.

The sensitivity required is therefore to detect a dilution of one in ten thousand or one in ten million depending on the assumptions, any of which may be wrong in either direction.
On the assumption that spleen contains 2 to 3 log lower levels of infectivity than brain (based on mouse titrations) the assay should detect a one to ten or one to one hundred dilution of 10% infected spleen.

These conclusions are reached on the basis of estimated levels of infectivity not abnormal PrP.