DRAFT

**Joint meeting**

**ACDP TSE Risk Assessment Sub Group**
**&**
**UK Blood Services Prion Working Group**

**was held on 25th October 2012 in Skipton House, Department of Health, London**

**Present:**

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| **Chairman:** | **Prof George Griffin** |
| **TSE RA Members:** | **Prof Jean Manson** |
| | **Prof James Ironside** |
| | **Prof Malcolm Bennett** |
| | **Prof Graham Medley** |
| | **Dr Roland Salmon** |
| | **Dr Simon Mead** |
| **PWG Members:** | **Prof Marc Turner** |
| | **Dr Gary Mallinson** |
| | **Dr Phil Minor** |
| | **Dr Pat Hewitt** |
| | **Mr Gordon Nicholson** |
| | **Ms Pauline Gowdy** |
| | **Mr Graham Rowe** |
| | **Dr Sheila MacLennan** |
| | **Dr Mark Head** |
| | **Dr Jonathan Wadsworth** |
| | **Dr Lorna Williamson** |
| | **Prof Richard Tedder** |
| | **Mr Willie Hughes** |
| | **Dr Neil Almond** |

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| **Observers and Officials:** | **Prof Noel Gill** | **HPA** |
| | **Mrs Ruth Parry** | **DH** |
| | **Dr Katy Sinka** | **HPA** |
| | **Dr Heather Elliott** | **DH** |
| | **Mrs Tina Lee** | **DH** |
| | **Dr Maren Daraktchiev** | **DH** |
| | **Mr Stephen Dobra** | **DH** |
| | **Dr Irene Hill** | **FSA** |
| | **Dr Joan O'Reardon** | **Irish Blood Service** |
| | **Dr Hans Zaaijer** | **Sanquin** |
| | **Dr Joliette Coste** | **EFS, France** |
| **Presenters:** | **Dr Mike Jones** | **SNBTS** |
| | **Dr Alex Raeber** | **Prionics** |
| | **Dr Graham Jackson** | **MRC Prion Unit** |
| **Secretariat:** | **Dr Julia Granerod** | **TSE RA SG** |
| | **Dr Stephen Thomas** | **PWG** |

**AGENDA ITEM 1 - Welcome, introductions and apologies**

1.1 The Chairman welcomed everyone to the first joint meeting of the ACDP TSE Risk Assessment Sub Group (TSE RA SG) and the Prion Working Group (PWG). Apologies had been received from Mark Noterman, Angela Mclean, Richard Knight, Andrew Riley, Elizabeth Mitchell, Michelle Ashford, Azra Ghani, and Helen Gillan.

1.2 The Chair advised the attendees that the information under consideration at this meeting was 'Commercial in Confidence.' In view of the number of people who attended this meeting, a confidentiality agreement was not practical. Instead, to safeguard as far as possible the confidential nature of the information presented, members formally endorsed the ACDP Code of Practice. The Chair advised that the minutes of the meeting would be circulated in draft for approval from the Developers who presented 'Commercial in Confidence' information so any confidential/proprietary

information could be removed. Two members, Dr Mark Head and Dr Jonathan Wadsworth, declared their interests.

**AGENDA ITEM 2 - Objectives**

2.1. A paper setting out questions for consideration had been sent out to attendees ahead of the meeting. These questions were discussed as noted below.

2.2. What scientific questions could be answered through an anonymised survey?

- It was highlighted that an anonymised versus a non-anonymised survey would answer very different questions.
- A positive test signal was suggested to mean little if the signal could not be characterised. It was suggested that further studies/assays could be used to understand what a positive signal meant.

2.3. How would the results of such a study be interpreted, in different possible scenarios for the percentage of "positive" samples?

- Members reported difficulty in answering the questions posed in the circulated paper until a discussion on sensitivity/specificity had taken place and "confirmatory testing" had been established.
- Members highlighted the resource value in identifying a group of individuals who have tested 'positive' in blood, even though their true status with respect to prion infection and infectivity would not be known with certainty. ~~It would be impossible to interpret a positive blood test at present~~. However, this resource would only be valuable if adequate amounts of sample were available for those who need to use them. It was suggested that provision be made up front regarding this.
- Each of the four blood tests is likely to be measuring something different, and it is not completely understood exactly what is being measured in each case. A loose analogy was suggested between this and the different reactivities seen with the four antibodies used in the appendix study. ~~A confirmatory assay~~

**Comment [s1]:** "impossible to interpret" is far too strong I have suggested an alternative – I don't recall the statement as minuted and would have challenged that - there would obviously be some interpretation that could be done about samples which test positive with several independent assays.

~~was not yet available and there was concern about what further steps to take if a 'positive' result was generated.~~

- If a sample was to be reactive in an assay it would not be clear whether the individual was infected or not, as the tests detect surrogate markers. To further understand this, it was proposed to consider prevalence in an exposed versus an unexposed population; a difference in these two populations might suggest evidence of infection. Members commented that this should be considered in revised power calculations (see final bullet below).

2.4. If any such test were also to be used to test asymptomatic people notified as being at increased risk, what would be the implications of negative and positive results?

- It was suggested that use of such a test would help with general management of 'at risk' patients, but there was no role for such a test yet in discussions with individual patients.
- The difference between *anonymity*, *traceability* and *imputability* was highlighted.
- The difference in ethical balance for the clinician versus the blood service was noted. It was thought that a blood test for patient purposes was a long way away. The implication for blood services was that if a donor tested 'positive,' this individual would have to be deferred from further donations and thus notified.
- Finding an alternative source of blood samples for a future prevalence study was recommended as sourcing from the blood service would add a whole level of complexity to the process.
- The importance of any future blood prevalence study being unlinked anonymous (like the appendix survey) was emphasized. The 'unlinked anonymous techniques' would also account for any imputability issue.
- It was suggested that the assumptions underlying the power calculations (presented in a paper circulated to members) be challenged.

**Comment [s2]:** I think these sentences are not clear or helpful (even if they were said, which I don't recall) because they seem to refer to the use of tests by the Blood Service and are not relevant comments for a prevalence survey

2.5. How could this affect the assessment of measures to reduce the risks of secondary vCJD transmission?

- The availability of a blood test would likely result in a reduction in the risk of onward transmission; however, some idea of the sensitivity and specificity of the test used would be needed.
- A decrease in the budget to fund CJD research was noted, as was the lack of interest from commercial companies to carry on with CJD-related work. A blood prevalence study would be a sensible step that would contribute to the evidence base.

2.6. What would be the acceptable test sensitivity and specificity for a prevalence study, as compared to the criteria set out in Annex IIa for a blood screening test?

- Members agreed that a test with high specificity (rather than sensitivity) would be required for a prevalence study, whereas the opposite would apply (i.e. high sensitivity) for use in an individual patient.

2.7. Would more than one test need to be available?

- There was general support that more than one test would need to be available for a blood prevalence study. Ideally, one test would be used for primary testing and this would be supported by one or two further confirmatory tests.
- It was noted that the use of a confirmatory test could only reduce the measured prevalence, as some of the primary test results would be 'false positive.' However, once again it was emphasized that it is still not known what exactly these tests are detecting.

**AGENDA ITEM 3 - Blood test 1 – MRC Prion Unit Assay – presented by Graham Jackson**

3.1. Dr Jackson gave a presentation, summarising the information given in paper ACDP_ TSERA_PWG _P3.1.

**Action: Secretariat to circulate slides following the meeting.**

3.2. The following points were raised in the discussion:

- Eight microlitres of whole blood was used for each test
- Why was the algorithm for a confirmed positive only based upon reactivity in two of two test runs? This is in contrast with other algorithms for viral marker testing. UK Blood Services screen on a single run, and retest twice any reactive samples. If samples are reactive in one or both repeat assays the donation is quarantined and the samples are sent for confirmatory testing. If both repeat assays are negative, the donation is allowed through to clinical stock.
- The 71% sensitivity of this assay could be due to absent prionaemia in some samples. It had not been possible to test repeat samples from the same donors.
- It is possible that the assay can be adapted, through minor changes, to detect other forms of CJD such as sporadic CJD.
- The presentation of data (in the distribution of sample signals chart) should be modified. The signal to cut off ratio for each assay plate should be standardised, and the inclusion of multiple results from single samples was misleading. Raw data was requested for both the first and second runs.
- The successful use of the steel capture matrix suggested there may be more prion available in the blood than previously thought (hence the 8 microlitre sample was sufficient). However the matrix to blood to buffer ratio was critical, and one theory may be that this enables some degree of disaggregation of the prions allowing monomer formation.
- Genotype information of the tested samples was not presented.
- ~~The samples in the published paper had not been tested in any other assay as it was not thought that a confirmatory test had been validated to test on human vCJD.~~
- The assay did not perform particularly well with ~~other~~ some animal models of TSE, although it did perform well with mouse RML prion infection.

**Comment [s3]:** I don't remember this being said, and it doesn't in any case make any sense. I commented at one point that the reason we can't have a single panel of samples for all assays is that many assays have different requirements eg. Whole blood, plasma, anticoagulant etc. We don't have the full range of types or volume required for the precious vCJD samples

- It was not entirely clear what is being detected, which is a common unknown across all tests.

**AGENDA ITEM 4 - Blood test 2 – NHSBT QuIC Assay – presented by Gary Mallinson**

4.1. Dr Mallinson gave a presentation, summarising the information given in paper ACDP_ TSERA_PWG _P4.1.
**Action: Secretariat to circulate slides following the meeting.**

4.2. In the discussion the following points were noted:

- If the antibody was not used in the capture stage the results were less clear.
- Whole blood is the preferred analyte but plasma can be used although it reduces sensitivity.
- There is some effect of anticoagulant. EDTA is the preferred anticoagulant; the background is higher with citrate.
- A number of antibodies have been used (including 15B3 as IgG and IgM, 6H4, 3F4) but no antibody mixing (competition) studies have been attempted.
- The test is repeated four times to avoid the risk of sampling missing the infectious agent (due to stochastic distribution in the primary sample) and sometimes the amplification just does not work.
- It was noted that this in an amplification assay and therefore has a considerable risk of contamination. The operators were aware of this and cautious.
- It was not known whether infectivity was being amplified, but similar assays that have been performed elsewhere have been shown not to produce an infectious product.
- The assay will probably never be quantitative. The aim is to get a screening test available for use in blood donors, or a cohort of blood donors, that could compliment another assay such as the MRC Prion Unit assay. This will take time and effort to achieve.

- The sequence of events will be to optimise the assay, finalise the protocol, and assess sensitivity (using the NIBSC panel) and specificity (using US blood samples). Due to the time and money involved it would be desirable to do this only once. It may also be appropriate to test clinical samples if available.

**AGENDA ITEM 5 - Blood test 3 – SNBTS PMCA assay – presented by Mike Jones**

5.1. Dr Jones gave a presentation, summarising the information given in paper ACDP_ TSERA_PWG _P5.1.

**Action: Secretariat to circulate slides following the meeting.**

5.2. In the discussion the following points were noted:

- The donor whose sample was highly reactive had been deferred from further donation. It was unlikely that this 'positive' result was contamination but it was impossible to completely rule this out as the donor had declined to give any further samples.
- The UK sourced platelets used as the substrate for the assay raised the concern that an infection present in the platelets could be amplified. However, each batch is checked and validated before use.
- The genotype of the substrate appeared to be critical to the amplification, which may introduce difficulties. No work had been done on looking at a mix of platelets of different genotypes as a substrate.
- This was unlikely to be a screening assay but detection of infection in the QuIC assay, for example, could be followed by confirmation of infection using the PMCA assay.
- It was noted that the mice used to check the infectivity in the amplified samples over-express prion protein.

**AGENDA ITEM 6 - Blood test 4 – Prionics – presented by Alex Raeber**

6.1. Dr Raeber gave a presentation, summarising the information given in paper ACDP_ TSERA_PWG _P6.1.

**Action: Secretariat to circulate slides following the meeting.**

6.2. In the discussion the following points were noted:

- The Prionics vCJD check 3.2 kit had been developed and was available to purchase.
- However, the kit was found to have low sensitivity when tested with clinical samples and therefore development had been stopped.
- Prionics are working on the eQuIC assay, and development of recombinant PrP for use in real time QuIC and eQuIC assays.
- Collaboration is ongoing with numerous groups including NHSBT and others, and it is likely to be 6 to 12 months before a prototype assay has been developed

**AGENDA ITEM 7 - General discussion**

7.1. It was highlighted that a different sample set had been used for test development in all four presentations; there was no commonality amongst the samples used. This raised the question of the availability of samples and their comparability. It was suggested that samples (e.g. brain, sheep blood, human blood samples) stored at NIBSC could be provided blindly for test development purposes ensuring developers were working on the same sample set. The volume required would need to be adapted to each individual assay. A member suggested pursuing comparability of detection before a prevalence study was carried out.

7.2. Comments **blood test 1 (MRC Prion Unit)** –

- The group agreed that this was the most promising of the four tests presented but that some further development would be required before this test could be in a blood prevalence study.
- It was still not known exactly how this test works and the group were keen to 1) see the data presented in a more robust and open manner, and 2) see more information on specificity, especially with regards to detection of other prion diseases apart from vCJD. It was mentioned

**Comment [s4]:** I think it is critical to say what is needed…I cannot recollect anything specific other than the presentation of the cut off to be modified. I do remember George referring to test needing "polish", and Richard T discussing the presentational aspects. But we can't leave in the minutes simply as "some further development"!

that the sensitivity of the test was very low in sCJD but that the test ~~would~~ might indeed detect some other prion diseases. This might not be surprising as in some cases of sCJD very low levels of abnormal prion protein can be detected outside the brain, for example in the spleen and skeletal muscle.

- As there is great variation in peripheral PrP between patients with vCJD, the working assumption ~~for this blood test~~ is that there are also very variable levels of prionaemia in vCJD.
- A member raised concern regarding the sensitivity of the test; it was suggested that a prevalence study would not be informative if the sensitivity was not high enough. It was also noted that it may be possible to modify the way the results are read in order to focus on specificity or sensitivity depending on the objective.
- The use of a cut off for signal versus noise on each individual plate is a valid method but this could be removed in future. For example, some assays use a 3 log difference from a normal result as a threshold for testing in a confirmatory assay.
- It was suggested that if a blood test can detect 3 of 4 cases of vCJD (as is the case for the MRC Prion Unit blood test) then it should be used for a prevalence study, but caution should be exercised in extrapolating the results.
- **SUMMARY** – It was agreed that this test needs further development but has the potential for use in a blood prevalence study.

7.3. Comments **blood test 2 (NHSBT)** –

- The group agreed that this test looked promising and had good sensitivity.
- The next steps in development should include sensitivity testing using vCJD samples, and specificity testing of 5000 samples. This may take 6 to 12 months.
- It was noted that there are samples available from very few vCJD patients (n=12) for test development purposes. Given the scarcity of samples for sensitivity testing, it may be prudent to perform specificity

**Comment [s5]:** This was my speculation only, that other (non-vCJD) prion diseases might be associated with prionemia, and could be left out of the minutes

**Comment [s6]:** Comment is not specific to the Prion Unit test

**Comment [s7]:** What development was needed, other than the presentational matters? Essential to minute this. Same comment above.

testing first. A sample committee had been set up to help decide on the best use of these samples.

- French colleagues have an archive of samples from their 27 vCJD cases. There are about 10-15 different types of sample available per patient but the samples volumes are small. They are developing a PMCA assay but would find it very laborious to test 5000 samples given that it takes 10 days to test 30 samples.
- A possible collaboration was suggested.
- **SUMMARY** – This test looks promising but needs more development with human samples.

7.4. Comments **blood test 3 (SNBTS)** –

- It was agreed that although promising, this test was not yet ready for consideration for a prevalence study.
- The assay is a lengthy process and therefore it might only be used as a confirmatory assay.
- Another practical problem was the need to consider containment as this test includes an amplification step, and the product has been shown to be infectious. Category 3 containment would therefore be necessary.
- **SUMMARY** – This test has potential to be used as a confirmatory test in the future but further work is required to get to that stage.

7.5. Comments **blood test 4 (Prionics)** –

- The first test presented (Prionics® – Check vCJD 3.2) had been developed into kit format but had been shelved as the test failed to detect two vCJD cases.
- The second test (Enhanced quaking-induced conversion assay - eQuIC) is similar to the NHSBT assay, but is still in early development and would take some time to be ready.
- The important contribution of Prionics was noted as they collaborate with the different research groups and are aware of the many issues in vCJD blood test development.
- **SUMMARY** – This test requires further work.

**AGENDA ITEM 8 - Summary and future steps**

8.1. The group concluded that the MRC Prion Unit test was the only test of the four presented that could be considered for a blood prevalence study in the near future.

8.2. It was noted that the criteria for a confirmatory assay was quite different to primary testing assay – i.e. NHSBT test would not need to test 5000 blood donors.

8.3. The group agreed that a blood prevalence study was worth doing but the techniques were not ~~quite~~ there yet. However, the planning ~~in terms of funding etc~~ could be started at all levels as it would be some years before a study could be undertaken. ~~as this was likely to take a long time.~~

8.4. Members were informed that the DH R&D budget for CJD-related research was capped at £5.5 million per year, and was to focus on surveillance and decontamination. ~~It was suggested that~~The ~~the~~ two further appendix studies (pre 1980 and post 1996 cohorts) should be given priority over a new blood prevalence study if funding was not sufficient to cover all three.

8.5. Development of the assays and a study protocol could continue whilst the appendix studies were performed, with a view to funding/beginning the prevalence study after the conclusion of the appendix studies should such a study address DH Policy's needs then.

~~8.5.~~8.6. Any test considered for use in a blood prevalence study would need to be subject to independent evaluation. All tests subject to evaluation should utilise the same panel where possible. ~~.~~

~~8.6.~~8.7. The group debated whether the appendix study and a new blood prevalence study would be addressing similar or different questions. One view was that the new appendix study would address whether it is indeed the case that 1 in 2000 individuals may have a vCJD infection in their peripheral tissues. If the "non- exposed" (pre 1980 and post 1996) groups were negative it would be assumed that this does represent vCJD in the periphery but it

**Comment [s8]:** See emailed comments most important correction in the document

**Comment [s9]:** These paragraphs need clarification, it was commented that DH is not the only source of funding for a prevalence study therefore the way forward is not restricted by DH's decisions to prioritise in the way it has done.

**Formatted:** Bullets and Numbering

**Comment [s10]:** This was not agreed at the meeting and should not be in the minutes. It's a perfectly reasonable discussion to have, but I don't have it in my notes at all

would still not be known whether this was infectious. If the "non-exposed" also had positive cases then it would be assumed that this represented other forms of misfolded prion protein which may or may not be infectious. The appendix study would take this issue no further as there would be no tissue resource to follow up. In addition, the sensitivity of the IHC test used in the appendix study is not known. Blood prevalence on the other hand could also address this issue and there is some idea of the sensitivity at clinical disease stage. It was suggested to test the post 1996 cohort and if there was any positivity further tests could be conducted to determine infectivity.

~~8.7.~~8.8. In the design of the study it would be important to make sure a large volume of blood was collected with proper informed consent. The samples would have to be unlinked in an appropriate way and the un-imputability of the sample would need to be sufficient.

~~8.8.~~8.9. It was suggested to test 40,000-50,000 blood samples and that an archive should be created as this would take a long time. Pooling of samples was not recommended.

8.10. All possible sources of blood should be explored, beyond that of the Blood and Tissue services. The Biobank was suggested as a possible source.

~~8.9.~~8.11. The group discussed ethical issues that could arise due to 'positive' tests.

~~8.10.~~8.12. It was suggested to hold another joint meeting in 6-9 months time.

**Secretariat**

**November 2012**

DRAFT

**Papers for the joint TSE RA SG & PWG meeting on 25th October 2012**

***For agenda item 2***

- **ACDP_TSERA_PWG_P2.1** – Questions for consideration
- **ACDP_TSERA_PWG_P2.2** – Annex IIa
- **ACDP_TSERA_PWG_P2.3** – HPA conference poster "Proposal for a study of the prevalence in the British population of abnormal prions in blood"

***For agenda item 3***

- **ACDP_ TSERA_PWG _P3.1** – MRC Prion Unit test details

***For agenda item 4***

- **ACDP_ TSERA_PWG _P4.1** – NHSBT 'Enhanced quaking-induced conversion assay' test details

***For agenda item 5***

- **ACDP_ TSERA_PWG _P5.1** – SNBTS 'PMCA/CDI' test details

***For agenda item 6***

- **ACDP_ TSERA_PWG _P6.1** – 'Prionics®-Check vCJD 3.2' test details
- **ACDP_ TSERA_PWG _P6.2** – 'Prionics®-Check vCJD 3.2' package insert
- **ACDP_ TSERA_PWG _P6.3** – Prionics 'Enhanced quaking-induced conversion assay' test details

***For agenda item 9***

- **ACDP_ TSERA_PWG _P9.1a** – Andreoletti *et al* paper
- **ACDP_ TSERA_PWG _P9.1b** – Gregori *et al* paper
- **ACDP_ TSERA_PWG _P9.2** – Tissue risk assessment paper