NHS
Blood and Transplant

Head Office
Oak House
Reeds Crescent
Watford
Hertfordshire
WD24 4QN

Tel: 01923 366804
www.nhsbt.nhs.uk

By email:

Mr Bernard Jolles
Box 118
Institute of Neurology
National Hospital for Neurology
& Neurosurgery
Queen Square
London
WC1N 3BG

11 March 2015

Dear Bernard

At a meeting with Professor Collinge in December last year we explored ways in which the UK Blood Services may be able to help the further development of your prototype vCJD blood test to support a UK blood study. We are now formally requesting that NHS Blood & Transplant (NHSBT) be granted permission to undertake further development work in this regard.

We propose that Dr Gary Mallinson leads on the further development of this assay as outlined in the attached draft protocol which aims to increase assay throughput and sample handling to the point where the assay could be used for a population study. Additionally, we would seek to address the ACDP TSE Sub Group concerns regarding the ability of the assay to detect prion disease in preclinical, asymptomatic individuals by testing bloods from BSE sheep and vCJD macaques taken throughout the incubation period. This aspect would use protocols developed by NIBSC and be under the aegis of the CJD Research and Resource Centre Oversight Committee. This further development can only be supported if the assay is transferred to our Prion Diagnostics Laboratory in NHSBT Filton. As part of this transfer, we would call upon the existing expertise and preliminary work of MRC Prion Unit scientists. Such a transfer would be covered under an MTA which would also cover data sharing, intellectual property and how we would work in partnership with the MRC Prion Unit. We understand that D-Gen would seek to protect background know-how, intellectual property and materials. If you agree, a draft MTA will be prepared by Dr Nicholas Watkins, Assistant Director – Research & Development, NHSBT.

We are keen to progress this quickly, as funding is only available for a period of 7 months from March to the end of September 2015. If the evaluation takes a little longer, we would hope to obtain additional UK Blood Service funding to complete the work. No funding has yet been allocated to conduct a population study until the assay evaluation is complete and signed off by CJD Research and Resource Centre Oversight Committee and the ACDP TSE Sub Group.

We look forward to an early response regarding this proposal.

Yours sincerely

GRO C

Dr Lorna Williamson
Medical & Research Director

GRO C

Dr Philip Minor
Deputy Director, NIBSC

Business Disability Forum
Building disability-smart organisations

POSITIVE ABOUT DISABLED PEOPLE

Evaluation of the D-Gen direct binding assay for a UK population study for variant Creutzfeldt-Jakob Disease

Summary

Following recommendations by the ACDP TSE Sub Group NIBSC and NHSBT have been asked to evaluate the MRC Prion Unit/D-Gen prototype blood based vCJD assay for suitability for use in a blood study. The D-Gen assay is the only assay potentially capable of being used for such a medium/large scale study at the current time. However, the current assay is performed manually and is too low throughput to carry out a study within a reasonable time period. Also, it is currently unknown whether the assay is able to detect any asymptomatic abnormal prion infections in pre-clinical individuals. The only practical way to address these questions is to test blood samples from animal models of prion disease. There are limited data on the performance of the D-Gen assay on animal blood samples.

NHSBT are proposing to carry out an appraisal of the vCJD assay to determine if practical measures to increase throughput can be made without impact on assay sensitivity and specificity. In parallel, with the co-operation with D-Gen scientists and under supervision by NIBSC, NHSBT will test appropriate animal bloods to determine if the assay can detect pre-clinical prion disease. The proposed evaluation process will take 7 months from March to the end of September 2015 in three discrete stages:

Stage 1: Transfer of the vCJD assay from the MRC Prion Unit to the NHSBT laboratories at Filton. The assay will be validated by blinded testing of vCJD brain homogenate spiked human blood and clinical vCJD blood samples (potentially including samples already tested by the Prion Unit and reported in the 2011 *Lancet* paper).

Stage 2: Assessment of pre-clinical samples: Support and collaboration will be solicited from MRC Prion Unit scientists to identify a suitable animal model to detect prion in pre-clinical blood samples. Potential animal samples are included in the protocol. Work on stage 2 will take place throughout the 7 month evaluation period and will involve blinded panels prepared by NIBSC.

Stage 3: Development of the assay protocol to increase sample throughput to 500 samples per week. At the end of the development period the assay will be revalidated to ensure the sensitivity and specificity are not affected. Revalidation would include testing an

extended number of vCJD blood samples provided by the MRC Prion Unit and the NCJDRSU.

Funding

NHSBT will provide funding for the evaluation protocol outlined below until 30th September 2015. Funding to support further development/evaluation of the assay will be sought from the UK Blood Services from October to March 2016. Funding for a UK population study for vCJD is not currently in the UK Blood Services budget.

Stage 1: Establishing the D-Gen assay in the NHSBT Filton laboratory.

The establishment of the blood based vCJD assay at NHSBT will require a transfer agreement with D-Gen. Since the published data are insufficiently detailed for either NHSBT or an independent body to ensure that the transferred assay will work at an equivalent level as the original we would expect D-Gen to provide full disclosure of the assay protocol and data.

The transfer, establishment and validation of the assay in the NHSBT Filton laboratory will be an incremental process that will involve an initial NIBSC blinded assessment of sensitivity and specificity using blood spiked with brain or spleen from vCJD patients and samples from normal human donors and will include the WHO vCJD brain homogenate used as a comparator in all tests evaluated by the CJD Research & Resource Centre/NIBSC.

The size of the panel and the assessment plan need to be determined, by the CJD Research & Resource Centre Oversight Committee, but should be sufficient to demonstrate that the assay has been transferred appropriately and has equivalent sensitivity to the published data. The samples to be tested would include:

a) Blinded panel of spikes of the WHO vCJD brain provided by NIBSC and control brain homogenates serially diluted into whole blood of normal American Red Cross blood donors (a minimum of 20 samples) with dilution ranges based on published detection limits. Other samples should include vCJD brain homogenates provided by D-Gen which have an established end-point titre with the vCJD assay for performance comparison.

b) vCJD clinical samples. A minimum of 6-8 samples previously tested positive by the vCJD assay would be required. Access to these samples would depend on the successful testing of spiked samples (a).

Depending on the sensitivity of the assay to other parameters such as the anticoagulant in blood samples it may be necessary to repeat the assessment of specificity determined from individual normal donor samples provided by the American Red Cross (ARC) with blood from other sources. This will ensure that ARC whole blood EDTA samples supplied by NIBSC give a comparable signal distribution to that previously tested by Prion Unit.
Further blinded materials may be supplied by NIBSC at other stages in the evaluation protocol as necessary. Suitable positive "run" controls will need to be defined for use throughout the stage 3 evaluation. These are likely to include both exogenous human samples and endogenous animal bloods.

Estimated time for stage 1 validation 1-2 months.

Stage 2: Assessment of prion detection in pre-clinical samples.

Since it is essential that the assay demonstrates detection of prion in preclinical samples known to be infectious, the assay will also be adapted to a format suitable to testing animal tissues such as blood from sheep or primates. We will seek advice and guidance from MRC Prion Unit scientists, through D-Gen, regarding changes to the assay protocol required to test these samples. Samples which could be used to assess pre-clinical detection of prion in blood include:

a) BSE challenged sheep whole blood collected into EDTA from the Roslin Institute:

Orally challenged animals from which blood donations were taken and that were confirmed BSE positive and infectious by transfusion to recipient sheep, and appropriate control samples.

Blood samples from sheep infected after transfusion of blood, buffy coat or red cell concentrates from the orally challenged animals above, plus associated transfused but uninfected controls. Whole blood samples that have been shown to be infectious by onward transfusion. Samples from sheep of these secondary transfusions.

Whole blood samples from individual TSE-free sheep (to be provided by APHA).

b) vCJD infected macaque samples (to be provided by FDA).

Whole blood panel of samples from vCJD infected macaques throughout the incubation period, early preclinical, preclinical and

clinical and matched controls from animals challenged with control brain.

Whole blood samples from [unifected?] individual macaques.

c) Whole blood from mice infected with RML scrapie, and/or FDA hamster 263K blood.

Estimated time for stage 2 development work, 3-4 months (to run concurrently with the stage 3 work).

Stage 3: Investigate possible changes to the test protocol to increase throughput to 500 samples per week

To conduct a study of the UK population an assumption is made that the test must be capable of testing 50,000 samples over a reasonable time frame, say 2 years. This will require testing of at least 500 samples per week. The published protocol involves extensive manually processing samples in tubes in batches of 24 (17 samples, 6 negative controls and 1 positive) with a final division of analyte into triplicates on a 96 well microtitre plate prior to readout. The 6 negatives are used to establish the baseline to calculate the assay cut off. Manually processing of 500 samples per week would not be tenable by this protocol.

Some modifications to the assay protocol could have a significant effect on throughput. Outlined below are changes which will be investigated. The protocol steps referenced are listed in table 1.

The use of deep well microtitre plates for sample handling would allow more samples to be simultaneously processed with little increase in manual work and a reduction in the number of negative controls as a ratio of samples processed. Additionally, if it is assumed that the vast majority of the samples will be negative it should be possible to use the mean of the sample signals on the read plate to establish the assay baseline. Cut off would be calculated from this figure. Reformatting the plate in this way would permit analysis of 29 samples per plate with 2 positive controls (1 high, 1 low) and 1 negative/blank control. Upper and lower limits for baseline/cut off would need to be defined to ensure poorly performing plates are repeated. Analysis of 500 samples would require 18 read plates. This would be feasible with 250 samples being processed at one time taking 2 working days. Additionally, using deep well plates to process samples, up to 87 samples could be processed at one time (steps 3-13) requiring only 3 plates per batch run and 9 final read plates.

With the suggested modifications testing 500 samples per week would be possible using entirely manual processes with 2-3 laboratory staff. However, the number and duration of washing steps is concerning (tedious, requiring sustained concentration, physically fatiguing with extensive, repetitive pipetting using multichannel pipettes) and it is unlikely that testing 500 samples per week manually, even with these modifications, would be desirable over a sustained period. However, wash steps could be readily automated using a deep well plate magnetic plate washer and this would have a high impact on staff time and workflow. Other areas which would benefit from automation would be sample handling at start up and final readout. Automation in these areas would have the added advantage of improving sample traceability by using barcodes and reporting of results by automatic generation and population of worksheets.

a) Use of deep well microtitre plate (steps 3-13): Deep well plates will be required as the initial sample volume is 800µl. Use of microtitre plates will speed up many laborious steps by allowing use of multichannel pipettes. Magnets suitable for bead isolation on microplates are available. If suitable, the wash steps could be semi-automated by use of a plate washer. There is a possibility of further automation using robotics and plate stackers. Avoidance of cross well contamination during multiple steps will be paramount.

b) Dilution of samples and addition of samples to plates (steps 2 & 3): With the anticipated throughput this area will be a 'pinch point' being both time consuming, labour intensive and prone to operator error if performed manually. Protocols need to be established to minimise these errors.

c) Assessment of wash steps on day 2 (steps 5, 9 &12): The number and duration of these wash steps will cause a bottleneck in throughput and plate washes will have to be staged carefully to ensure reproducibility. Improvements to manual throughput could be made by reducing the number of washes and/or the standing time between each individual washes. Assay performance would be monitored by using standard dilutions of vCJD in human blood and/or animal blood controls. Washes could be readily automated using a magnetic deep well plate washer e.g., Biotek ELX405.

d) Splitting samples at step 14: This step requires splitting the analyte into 3 equal aliquots and placing each aliquot onto a microtitre plate. The position of the aliquot will not correspond to the position of the sample on the original plate. This is not an easy step to perform manually. The analyte will be a suspension of beads in substrate and will be difficult to dispense accurately. The change layout from process plate to read plate will require sustained concentration to avoid error.

Estimated time 3 months to evaluate changes to increase throughput (a-d)

e) Evaluation of automation and impact on throughput: Three key areas have been identified where automation could have a significant impact on throughput. Wash steps may be easy to automate at relatively low cost using a plate washer. The sample handling steps 2 & 3 and redistribution of samples at step 14 are steps where automation would increase accuracy, speed and reproducibility by parallel processing and elimination of operator error. Furthermore, barcode reading of samples (positive identification) could generate a worksheet linked to results reporting at step 14 and eliminate reporting errors. An assessment of sampling robots with these capabilities will be made. It may be possible to use the same robot to manipulate samples at steps 2 & 14. The entire assay process will be further examined to see if automation steps could be added and/or linked without significant extra cost, e.g., using plate stackers.

Estimated time 3 months. This would take in parallel to the evaluation steps (a-d) above

Establish protocols for plate assessment, calculating assay cut-offs and reporting of results

The performance of the assay as changes are made to the protocol needs to be closely monitored to ensure that assay performance is not affected. Performance monitoring would include assay controls values, baselines values and cut offs. Results reporting and reconciliation protocols need to be developed with consideration to quality assurance compliance

Estimated time 3 months. This would be done in parallel to the other evaluation steps (a-d) above

Assay revalidation

Once the evaluation process is finalised the assay will require revalidation. This will involve retesting NIBSC blinded panels provided at the technology transfer stage. Additional testing of more elaborate panels is likely to be required by NIBSC at this stage to satisfy the ACDP TSE Sub Group and the CJD Research & Resource Centre Oversight Committee requirements. This would include retesting of human blood samples from vCJD affected individuals (a minimum of 6-8 known positives) with additional vCJD samples which could include samples previously tested as negative and/or untested samples. Samples would be provided by the MRC Prion Unit and the NCJDRSU. Additionally, at least

200 whole blood ARC samples would be tested to assess specificity. NHSBT can provide an additional 700 whole blood samples in EDTA.

Estimated time for assay revalidation 1-2 months

Trial runs

Any improvements in assay throughput need to be measured against the original objective of testing at least 500 samples per week. The assay would be carried out on 250 samples over 2 working days to confirm this output is achievable. Trial runs would be repeated as required to develop robust protocols and SOPs for managing this level of throughput.

Estimated time for trial runs 1 month

Dr Lorna Williamson
Medical and Research Director
NHS Blood & Transplant

Dr Philip Minor
On behalf of the
CJD Research & Resource Centre Oversight Committee
NIBSC

2nd March 2015

Table 1: MRC Prion Unit/D-Den blood vCJD assay protocol

Timings shown are estimates to process and test 24 samples using manual techniques

	Step	Time
1.	Preparation of steel beads	See separate protocol, 6hrs
2.	Dilution of samples. 8µl blood sample into 800 µl capture matrix	30 mins
3.	Adding beads to samples. Measure 23mgs beads into capture matrix, aliquot into plate	Approx 30 mins
4.	Incubation 18°C with agitation	Overnight/18hrs
5.	Wash 5 times with 1ml PBST	Manual wash, 10 mins per wash, 60 mins
6.	Heat 110°C, 5 mins	5 mins
7.	Add 50µl biotinylated ICSM18 in PBST	2 mins per plate
8.	Incubate 37°C.	60 mins
9.	Wash 5 times with 1ml PBST	Manual wash 10 mins washes, total 60 mins
10.	Neutravidin-HRP 1:100K in PBST	2 mins per plate
11.	Incubate 45 mins	45 mins
12.	Wash 3 times with 1ml PBST	Manual wash 5 mins per wash 15 mins
13.	Add 60µl chemiluminescence substrate	2 mins per plate
14.	Divide into 3 aliquots of 20µl on black microplate	Full plate 20mins
15.	Add 80µl chemiluminescence substrate to each well	2 mins per plate
16.	Read in luminometer	10 mins per plate