Ref	Function
1.0 Co-ordination and harmonisation of confirmatory methods and reference materials	
1.1	With a view to establishing common practice, methodology and standards in diagnosis of TSEs, the EURL will organise proficiency tests for the interpretation of histopathology and immunohistochemistry (IHC) in 2012 (see section 2 for details).
1.2	Data on the strengths and weaknesses of existing, as well as new/emerging antibodies and protocols used in confirmatory diagnostic tests will be assessed, and protocols reflecting best practice placed on the website. This, and other information relating to assessment of antibody performance will be made available through the technical IHC QA round (see section 2.2), and at the annual NRL meetings.
1.3	Storage of infected tissues from suspects in the UK will continue in order to maintain (as far as possible) a supply of reference materials for National Laboratories (or at least sufficient to enable appropriate characterisation of internal reference material). This collection of tissues is managed by the VLA TSE Archive, and all release of tissues from this collection to the EURL (or any other user) is subject to the approval of Defra's Independent Archive Advisory Group (IAAG) and charges may be made (http://www.defra.gov.uk/corporate/vla/science/science-tse-arc-intro.htm). Standardised reference materials will also be prepared to facilitate batch testing activities (see section 6.1). Some reference materials, specifically for EURL use, will be generated through experimental challenge of animals (see sections 1.6, 1.7 and 1.8).
1.4	Positive and negative material necessary for annual QA purposes (see section 2 below) will be requested 'en bloc' from the TSE Archive, and reserved for EURL purposes. (This will require the continued maintenance of dedicated freezers. It was agreed in 2003 that the Commission would pay 1/5 depreciation costs each year.) We will request material in 2012, to prepare samples for EQA use in 2013. Homogenates will have been prepared in 2011 for QA testing in 2012. There will be requirement to prepare further supplies of homogenate in 2012 for use in 2013.
1.5	As discriminatory tests become more widely used, it will be necessary for the EURL to maintain stocks of ovine BSE for the provision of QA and QC material. To obviate the need for repeat requests to the UK Archive Group (with no guarantee of continued successful application), we challenged 4 ARQ/ARQ sheep with bovine BSE in 2005, 5 in 2006, 10 in 2007, 5 in 2009 and 5 in 2011. This provides material for test evaluation and sensitivity exercises, QA panels and species specific controls for STEG evaluations. However, some of these sheep did not succumb as expected, and were subsequently found to have the polymorphism T112, which has now been linked with resistance to BSE, so not all challenges resulted in positive material. One sheep from 2005 and 2 from 2006 remain alive and carry this polymorphism. They offer an excellent opportunity to establish whether this polymorphism confers absolute resistance, or merely prolongs incubation period, and it is proposed that they are kept alive to address this question. These three sheep, plus the 5 sheep challenged in 2011 will incur maintenance costs in 2012. As previously agreed with the COM, the need for

	future challenges will be reviewed annually, and challenges undertaken every other year if required. It is therefore not proposed to challenge further sheep in 2012, but tissue use will be monitored, and consideration will be given to the need for future challenges.
1.6	Experimental challenge of animals with 'atypical' scrapie was started in 2006, with further animals challenged subsequently, to provide a bank of material exclusively for EU QA purposes. To date, twelve animals have succumbed, and we have good evidence from parallel experiments that the anticipated attack rate in this model (AHQ/AHQ homologous challenges) will be 100%. The remaining six animals are currently healthy. Based on the incubation period of approximately 800 days observed in other research projects, it is predicted that these remaining sheep will succumb by mid 2012, The amount of tissue required to include a sample in a QA round is approximately 65g (equivalent to two sheep). It is therefore not proposed to challenge further sheep in 2012, but tissue use will be monitored, and consideration will be given to the need for future challenges.
1.7	The identification and characterisation of H and L type BSE raises the need for reference material from such cases. Global supplies are currently very limited, and predominantly prioritised for research. We have successfully challenged two cattle with H-type BSE and two with L-type BSE to generate a small bank of reference material for statutory testing purposes, and four further animals are scheduled to be challenged in 2011 to ensure that sufficient material will be available for discriminatory, rapid and confirmatory blot QAs as required in the future. Based on the observed incubation period in the first challenges it is anticipated that these four animals will incur maintenance costs throughout 2012. It is not proposed to challenge further cattle in 2012, but tissue use will be monitored, and consideration will be given to the need for future challenges.
2.0	**Proficiency testing (see appended timetable)**
2.1	The EURL will organise two proficiency tests for the interpretation of histopathology and immunohistochemistry (IHC) for BSE in bovines, and scrapie in sheep. The first of these will be in April 2012 and the second in October 2012. The format of these QA distributions will be based on a web-based QA system which enables timely completion of distributions, and greater flexibility to include examples of unusual cases, challenging artefacts, different IHC protocols (drawn from the technical QA round – see below). This system will be administered through our QA Unit as before, but the web images will be hosted by 'SlidePath', an external company which specialises in web-based imaging.
2.2	An additional technical IHC test will take the form of a comparative test on unstained sections supplied by the EURL. Following staining and initial interpretation by the National Laboratories, the stained sections will be read by the EURL pathologists. Follow-up of any sub-optimal staining or inappropriate interpretation, if required, will be individually tailored to each participating laboratory. The previous rounds have raised a number of issues in relation to method optimisation for different species and tissues, so it is intended to keep the round at its current size.

2.3	A proficiency test panel of ovine blood samples will be provided for the QA of laboratories undertaking genotyping for statutory purposes. Information will be requested about the methods used in each country. Reporting on 4 codons (136, 141, 154 and 171) of the ovine PrP gene will be required from all labs. Additionally, it is increasingly recognized that susceptibility to scrapie in goats is regulated very similarly to sheep through the PrP gene and its variations (polymorphisms). While, similar to the ovine gene, the caprine PrP gene is highly polymorphic, the polymorphisms are usually at different positions (codons) compared to sheep. Following our QA pilot scheme for the caprine PRP gene undertaken in 2011, we will make the outcome available at the EURL-meeting in June 2012. Possible further activities will wait until any regulations are put in place
2.4	The EURL will organise: • One proficiency test for rapid diagnostic methods to assess PrP detection in bovine brain tissue. • One proficiency test for confirmatory blotting methods to assess PrP detection in bovine brain tissue. Following successful transmission of H & L type BSE to cattle (see 1.7), and dependent on the demonstration of appropriate test sensitivity levels (see 3.4 in the 2011 workplan.report) it is proposed that this round in 2012 will include an H and/or an L type BSE sample in addition to the bovine classical BSE • One proficiency test for rapid diagnostic methods to assess PrP detection in ovine brainstem tissue. • One proficiency test for confirmatory blotting methods to assess PrP detection in ovine brain tissue. We intend to provide scrapie positive and negative goat samples in the rapid test round for 2012. • One proficiency test round for BSE/ scrapie discriminatory Western blots (ovine brain tissue only) in those NRLs which are operating such methods. Please note that atypical scapie, ovine BSE, bovine H & L type BSE and goat tissues are of limited availability and will not necessarily be included in subsequent years, unless challenged animals continue to succumb, and goat scrapie material can be sourced from outside the UK. The decision on whether to include this type of sample each year will depend partly on the performance of laboratories in receipt of the samples in the 2011 QA exercises, the outcome of which will not be known until near the end of 2011. Homogenates will have been prepared in 2011 for QA testing in 2012. There will be requirement to prepare further supplies of homogenate in 2012 for use in 2013.
2.5	The EURL will monitor proficiency testing practices to ensure that they remain relevant, through discussion at the EURL meeting. We will attempt to maintain up-to-date data from NRLs, regarding the methods currently in use, the NRL purposes of such tests (e.g. confirmatory, discriminatory, research, etc.) national QC and QA approaches etc. to enable the effective provision of relevant and targeted advice. As the EURL has not so far undertaken inspections of NRLs (see 6.1), this is necessary for maintaining some understanding of current practices. It will also advise on any necessary changes to the EURL proficiency testing programme,

	monitoring of trend data from routine testing or general QA advice as the need is identified. Rapid test sample sets for each QA round will contain a range of diluted reactor homogenates for assessment of laboratory and test performance. The EURL will maintain an up-to-date database of all relevant NRL contacts and contact details.
2.6	The Commission will continue to have direct access to all QA results on the web-based systems.
3.0 Provision of diagnostic and confirmatory testing and advice	
3.1	The demand for diagnostic testing will depend on individual countries. Most Member States have adequate arrangements and do not require significant help with routine diagnostic testing. However, confirmation of results may be an important task for EURL, which does not anticipate having to conduct many confirmatory tests but the service will be available on an ad hoc basis for difficult or perplexing cases (see also section 5). These tests will include HE sections, IHC sections and Western Blotting on unfixed material. The EURL will continue to attempt to collect data on cases which are in some way 'unusual', to enable comprehensive cross-referencing and collation of information on such cases for the Commission. Full instructions for sheep have been issued as part of the manual on Discriminatory Testing. The success of any such system is dependent on the willingness of MS to comply with such a request if our diagnostic opinion is not sought initially, and experience to date indicates that there are very differing views in the various MS on what and whether to refer.
3.2	The EURL will provide expert advice on the epidemiology and clinical manifestations of BSE and scrapie. In 2012 this will include clinical data generated from the Defra-funded experimental challenges of sheep with H- and L-type BSE. The EURL will also continue to provide scientific supervision of certain studies funded by the European Commission on request (see section 3.5).
3.3	All relevant information will be published on the EURL website, or TSE-LAB-NET, when appropriate. Discussion fora are possible on the password protected TSE-LAB-NET system. The system provides links to QA, batch release data (section 6.3) and workshop presentations, as well as closed discussion fora such as STEG.
3.4	Assistance and guidance will be provided to those laboratories experiencing difficulties. Specialist input to Commission *fora* on an *ad hoc* basis. Provision has been made in 2012 for two laboratory visits to assist with technical issues should the need arise.
4.0 Training and workshop	
4.1	A workshop for National experts will be arranged in the first half of 2012. This workshop will cover all aspects of NRL functions, with the exception of 4.3 below. Feedback will be provided and training needs identified following the outcome of

	the QA assessments outlined in 2.0.
4.2	Training in rapid diagnostic techniques will not be provided. All the evaluated tests are commercially available and it is assumed that the manufacturers will provide training/guidance on the use of the tests. Similarly, should problems be encountered then it is appropriate that the manufacturers address these directly with the test users. Feedback from the national laboratories will alert EURL to any problems and the EURL will liaise closely with the national laboratory and the test manufacturer. General advice and information will be posted (where relevant) on the website. Rapid test manufacturers are now invited to participate in a specific session at the EURL workshop (see 4.1) each year where issues can be discussed directly with NRL representatives.
5.0 Strain typing	
5.1	The EURL has established a working group of experts in the field of strain differentiation. It will continue to be responsible for the evaluation of any unusual results arising from TSE testing within Europe, and agree the criteria on which strains will be classified 'BSE-like' (and what that means). Advice will be provided on appropriate further investigation and interpretation, to enable the submitting NRL to appropriately and competently brief the relevant National authorities. The panel is drawn partly from experts within the EURL and NRLs, and partly from other sources. This group plans to meet once in 2012. Discussion will continue to focus on the validation/interpretation of the increasing range of Tg bioassay methodologies. Data emerging from the spiked pool study (5.4) will also be considered by the group. This group will coordinate the provision of material (see also 2.4 and 7.2) for the ring trial of any new potential discriminatory method not presented with sufficient supporting data to be approved by the group without further assessment. None are anticipated at present.
5.2	MS undertake the initial and discriminatory testing of sheep isolates at their own cost. However, if an unusual isolate is referred to the EURL strain typing group for subsequent investigation by ring-trial, the EURL will be liable for any laboratory costs incurred (see section 7.3).
5.3	Any isolate still considered BSE-like following ring trial (see section 7.3) will be forwarded for bioassay in mice. Historically, only conventional mice were sufficiently evaluated and defined for this purpose, and interpretation was based on a full panel (i.e. RIII, VM and C57Bl6). However, the choice of mouse strains is under continual active discussion and review (see section 5.1) including the selection of the most relevant Tg lines. Some have already been adopted by STEG for the bioassay of demanding referred samples. A major advantage of these Tg lines over the wild-type lines is their enhanced susceptibility to certain TSE isolates e.g. transgenic mouse lines are susceptible to atypical scrapie when conventional lines are not. The STEG referral bioassay panel therefore currently considers Tg338 (VRQ ovine), Tg110 (bovine) and TgShpXI (ARQ ovine) as possible options instead of or in conjunction with some wild-type mice.

	The cost estimates are based on the assumption that a maximum of 2 isolates will require further characterisation by bioassay.
5.4	A panel of samples representing mixtures of ovine BSE with various distinct scrapie sources (previously referred to as 'spiked pools') were subject to current discriminatory testing methods in 2010. These were used as a crude proxy for co-infected animals. Preliminary results show that while the discriminatory WB performs reasonably well in most mixtures for the detection of BSE, the ELISA is less successful. None of the tests identified all the samples containing BSE. Phase 2 of this study proposes the use of Tg338 and Tg110 mouse panels to determine whether increased discriminatory sensitivity can be achieved using this in vivo model, by examining 2 mixed samples from each combination, at the point at which the biochemical tests lost discriminatory potential. Bioassays are ongoing, and final analysis will be presented to STEG for peer review, and subsequent review of the confirmatory testing protocols.
6.0 Rapid diagnostic methods	
6.1	The EURL will contribute actively (on an *ad hoc* basis) to the continual assessment of existing rapid tests by contribution to relevant discussion fora, laboratory visits and comparative trials. The workload and costs for this component of EURL work cannot be readily predicted - if for example we have to undertake laboratory work to investigate a problem that arises in year. Additional costs may therefore arise in-year that would require additional funding, or a reassessment of EURL commitments to enable delivery within the agreed annual budget. The EURL has an ongoing commitment to assess changes to approved rapid test kits or sampling methods, which are proposed by manufacturers. This involves discussion with companies, input into protocol design, assessment of evaluation data and consideration of the impact of proposed changes. The proposals are then either accepted, further work requested or they are rejected. If proposals are accepted the company is required to update kit inserts or SOPs as appropriate. If changes are made to kit instructions, NRLs and the Commission are notified. If changes to production are necessary as a part of kit changes, Quality Control data may need to be provided by the manufacturer and assessed by the EURL to confirm adherence to the manufacturer's Quality System. An annual statement will be sent to the COM confirming which manufacturers continue to comply with these requirements, so that the listing in the regulation is kept up to date.
6.2	In 2004, a document was produced by a 'virtual working group' defining what 'minor test changes' are, and how such changes should be assessed. This information is now on the EURL website. The document will continue to be subject to annual review.
6.3	Batch testing of approved rapid tests for the detection of BSE in bovine samples was introduced in 2008. Nominated NRLs are responsible for testing to an agreed protocol and the EURL approves batches for release and communicates this information to NRLs for cascade to testing labs throughout the EU. Costs are

	based on current test usage, and an estimate of 40 batch releases per year. All documents are placed in the secure web areas within TSE-LAB-NET. Maintenance of this web area now incurs external contractor charges.
7.0 Discriminatory testing	
7.1	The Discriminatory Testing Handbook detailing precise methods for discriminatory blotting was produced in 2005, updated in 2007 and again in 2009. This Handbook will be reviewed annually, revised and updated as necessary to include additional information of relevance to the surveillance of TSE, and re-issued online (.http://www.defra.gov.uk/vla/science/docs/sci_tse_rl_handbookv2mar07.pdf) A protocol, as presented at the STEG meeting in April 2008, for the discrimination of H and L type BSE from C type has been made available on line.. Until initial QA or QC has been done, any testing performed in NRLs using this protocol will, by definition, remain 'out of control'. (A referral system has been recommended, but cannot be enforced).
7.2	There will be an annual QA round for discriminatory blotting (see section 2.4).
7.3	Any positive isolate which presents a discriminatory blot result which is BSE-like should be sent to the EURL. Such cases will be referred to the STEG (see section 5.1) and material distributed around ring-trial laboratories. Cost estimates have been based on the very low level (12 so far) of referrals to date, and assume that a maximum of 1 ring trial will be required. (see 5.3).

Appendix 1

PROVISIONAL TIMETABLE FOR TSE EURL QA EXERCISES IN 2012

Intended Start Date [i]	QA activity
February 2012	Ovine genotyping
March 2012	Immuno-histochemical technique
April 2012	Histopathology and immunohistochemistry interpretation (round 1)
October 2012	Histopathology and immunohistochemistry interpretation (round 2)
July 2012	Bovine rapid testing incorporating confirmatory blotting if appropriate
November 2012	Ovine rapid testing, incorporating confirmatory blotting if appropriate
September 2012	Ovine discriminatory Western blotting

[i] Some QA exercises (such as the technical and slide interpretation) take several weeks or months to complete. Any follow-up activities will also lengthen the duration. It is not therefore possible to accurately predict ***completion*** dates for these activities.