1.0 Creation and maintenance of reference materials

1.1 ***Storage and release of reference tissues from UK field suspects***

Weighting (percentage) of this subactivity in the budget of the activity: approx 3%

Background for this subactivity

Storage of infected tissues from suspects in the UK will continue in order to maintain (as far as possible) a supply of reference materials, on request, to National Reference Laboratories (or at least sufficient to enable each NRL to undertake appropriate characterisation of internal reference material). This collection of frozen tissues is currently managed by the AHVLA Biological Archive Group (BAG)[1], 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 [2] (http://www.defra.gov.uk/corporate/vla/science/science-tse-arc-intro.htm).

Fixed material is not managed through the BAG, and is released on request by the EURL directly.

Deliverables for this subactivity

	Planned	Achieved
Number of requests for reference tissues	*15*[3]	

1.2 ***Production of reference panels for EQA and NRL batch testing.***

Weighting (percentage) of this subactivity in the budget of the activity: approx 10%

Background for this subactivity

Standardised reference panels are necessary to facilitate EQA and batch testing activities. The material needs to be obtained from AHVLA BAG or EURL tissue production activities, and the panels of samples created.

Deliverables for this subactivity

	Planned	Achieved
Number of reference panels for EQA	*5*	
Number of reference panels for batch testing	*2*	

1.3 ***Experimental production of atypical TSE material for reference and EQA purposes***

Weighting (percentage) of this subactivity in the budget of the activity: 0%

Background for this subactivity

Some reference materials, specifically for EURL use, need to be generated through

[1] This activity is currently marginally costed, with only the staff time required to assess requests and select the appropriate material. This is dependent on Defra's continuing support of the staffing and equipment infrastructure of the AHVLA BAG

[2] At present, the BAG does not charge the EURL for any materials provided, but it does charge commercial companies, and (depending on the request) other NRLs may be charged. The charging policy is regularly reviewed and may be subject to change.

[3] This assumes that not every NRL will need tissue in the forthcoming year, and if they do, they will only make one request.

experimental challenge of animals. Field case atypical material (both ovine and bovine) tends to be limited in quantity and frequently compromised in quality. Experimental challenge makes sufficient volume available for multiple aliquots of equivalent tissue to be included in EQA and test evaluation exercises.
At our current rate of usage, existing stock will meet our requirements for the foreseeable future (approx 8 years). This situation will be kept under review. No challenges are proposed for 2015[4].

Deliverables for this subactivity

	Planned	Achieved
Maintenance and clinical monitoring of animals	*0*	
Postmortem collection, confirmation of disease status and storage of material from animals reaching clinical disease	*0*	

1.4 ***Experimental production of small ruminant BSE material for reference and EQA purposes***

Weighting (percentage) of this subactivity in the budget of the activity: approx 86%

Background for this subactivity

In the absence of relevant field case material, and while the statutory requirement for discriminatory testing remains, It is necessary for the EURL to maintain stocks of small ruminant BSE for the provision of EQA and QC material, and positive reference material for any further investigation of unusual or suspicious isolates.

- Sheep:

At our current rate of usage, existing stock will meet our requirements for the foreseeable future (approx 8 years). No further challenges are proposed at this time.

Some of the first sheep challenged (2005/6) did not succumb as expected, and were subsequently found to have the polymorphism T112, which has now been linked with resistance to BSE. Three of these sheep remain alive. 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, and to provide material from a different genotype (for characterisation purposes - see above) in the event of them succumbing after a very prolonged incubation period.

- Goats:

We also have approx 250g of material remaining from the initial 5 experimentally-challenged goats, enough for at least 4 EQAs. Evidence from the Cypriot project indicates that caprine samples may be more difficult to classify than ovine samples using current methods, all of which were evaluated and approved for use in SR based entirely on ovine data. In anticipation of caprine samples having to be independently represented in future EQA panels for detection and discrimination, a further 5 goats were challenged in 2013 to replenish the EURL stock of positive control material. Two of these goats died as a result of intercurrent problems (urolithiasis; necrotic enteritis) in 2014. The remaining animals are currently alive, and are predicted to survive into 2015 (based on average incubation

[4] Should alternative presentation of disease occur in field cases, it will be necessary to review the adequacy of current stocks (based entirely on AHQ/AHQ sheep) if genotype or phenotype is shown to have a bearing on disease detection. Further caprine challenges may also be required in the future, if differences between testing efficiencies in sheep and goats are identified which might lead to separate testing panels

times for previously challenged goats).

Deliverables for this subactivity

	Planned	Achieved
Maintenance and clinical monitoring of animals previously challenged (3 goats for a full year, 2 sheep for a full year, and 1 sheep and 1 goat for at least part of the year)	*3 sheep 4 goats*	
Postmortem collection, confirmation of disease status and storage of material from animals reaching clinical disease (assuming one sheep and one goat in 2015)	*1 sheep and 1 goat*	

2.0 External Quality Assurance (EQA) (see also appended timetable)

2.1 ***Immunohistochemistry and histopathology EQA***

Weighting (percentage) of this subactivity in the budget of the activity: approx 34%

Background for this subactivity

The EURL will organise two EQA rounds for the interpretation of histopathology and immunohistochemistry (IHC), and one technical IHC EQA round, covering BSE in bovines, and scrapie in sheep.

- The 2 interpretation EQA rounds will be based on a web-based EQA system which enables timely completion of distributions, and greater flexibility to include examples of unusual cases, challenging artefacts and different IHC protocols (Some examples might be drawn from the technical EQA round). This system will be administered through a sub-contract with 'SlidePath', an external company which specialises in web-based imaging and the hosting of EQA.

- The technical IHC EQA will take the form of a comparative test on unstained sections supplied by the EURL. Following staining and initial interpretation by the National Reference Laboratories, the stained sections will be read by the EURL technical experts and pathologists[5]*.*

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, including bovine and ovine brain, and ovine lymphoid tissue.

Deliverables for this subactivity

	Planned	Achieved
Organisation of an EQA for the interpretation of histopathology and IHC	*2: March, October*	

[5] The technical EQA has to be sent out from the EURL laboratory in Weybridge directly to participants, and the sections returned to the EURL. It does not therefore come under the direct management of the VETQAS team at AHVLA Sutton Bonington like all the other EQA activities. However, the protocols and practices involved are subject to audit by UKAS when inspecting AHVLA for compliance with ISO17025.

	Organisation of a technical EQA for IHC	*1: March/April*	
	Provide a copy of the outcomes to the Commission as appendices to the annual EURL report (see 4.2)	*1*	

2.2 ***Rapid and confirmatory testing EQA for bovine, ovine and caprine samples***

Weighting (percentage) of this subactivity in the budget of the activity: approx 23%

Background for this subactivity

The EURL will organise both bovine and small ruminant EQA rounds for rapid screening and confirmatory Western blotting methods.

Overall, the panels will comprise ovine classical scrapie, caprine classical scrapie, ovine atypical scrapie and classical BSE of varying signal intensity (to assess local operational testing sensitivity), and some negative samples (to assess specificity). Some samples will be duplicates, to look at robustness/reproducibility of testing.

- Goat tissues are of limited availability in the UK, and will not necessarily be included routinely.

Deliverables for this subactivity

	Planned	Achieved
Organisation of an EQA for rapid diagnostic methods to assess PrP detection in bovine brain tissue	*1: November*	
Organisation of an EQA for confirmatory blotting methods to assess PrP detection in bovine brain tissue	*1: November*	
Organisation of an EQA for rapid diagnostic methods to assess PrP detection in ovine/caprine brain tissue	*1: May*	
Organisation of an EQA for confirmatory blotting methods to assess PrP detection in ovine/caprine brain tissue	*1: May*	
Provide an electronic copy of the outcomes to the Commission as rounds take place, and as appendices to the annual EURL report (see 4.2).	*5*	

2.3 ***TSE classification/discrimination EQA***

Weighting (percentage) of this subactivity in the budget of the activity: approx11%

Background for this subactivity

EU regulations require the classification/discrimination of small ruminant TSE as classical scrapie, atypical scrapie or BSE-like, with prescribed further investigation and analysis of any isolate which has BSE-like characteristics. This process starts with a discriminatory WB in the NRL of each MS, with referral of unusual samples for ring trial coordinated by the EURL. We undertake one EQA round each year to assess this discriminatory blotting procedure. Until 2012, bovine BSE was used as a proxy for ovine BSE (based on initial test evaluation ring trial data), but ovine BSE is now available in sufficient quantity, and is routinely included.

In recent years, two atypical forms of bovine TSE (H- and L-BSE) have been identified, and it is now a regulatory requirement for all bovine isolates to be classified. In 2013 of the 7 cases of BSE identified in the EU, 5 were atypical, these cases are very rare, with fewer than 50 cases of each variant so far identified globally, but appear to be forming a large proportion of current cases. Field case material is therefore unavailable for test evaluation or EQA purposes. Following successful transmission of H & L type BSE to cattle this has become a standard EQA round, restricted to laboratories selected on the basis of expressions of interest, with preference being given to those laboratories which had already demonstrated competence in identification of such variants. NRLs which do not anticipate any bovine positive submissions or have a very low throughput of TSE samples are encouraged to plan referral of any bovine samples which might require classification. This allows us to preserve the material we have available to support this exercise, and reduces the cost for the NRLs and for the EURL

Deliverables for this subactivity

	Planned	Achieved
Organisation of an EQA for BSE / scrapie discriminatory Western blots in small ruminants (ovine and caprine brain tissue) in those NRLs which are operating such methods	*1: April*	
Organisation of an EQA for H- L- and C-BSE classification	*1: November*	
Provide an electronic copy of the outcomes to the Commission as rounds take place, and as appendices to the annual EURL report (see 4.2).	*2*	

2.4 ***Atypical scrapie – testing sensitivity***

*Weighting (percentage) of this subactivity in the budget of the activity**:** approx 30%*

Background for this subactivity

It has become apparent in 2014 that there are some test sensitivity issues relating to atypical scrapie cases that the EURL cannot fully explain. The aim of this activity is to build on the information and data being collected in 2014, with test manufacturer and NRL involvement where necessary, and to coordinate a review of small ruminant test analytical sensitivity performance for classical (See section XXX) and atypical scrapie.

Deliverables for this subactivity

	Planned	Achieved
Design and carry out a laboratory based study to investigate the sensitivity of currently approved scrapie rapid tests.	*1*	
Provide a report detailing the issues relating to atypical scrapie and test sensitivity, and indicating possible future approaches to EQA, test evaluation and trend monitoring	*1*	

2.5 ***Genotyping EQA***

*Weighting (percentage) of this subactivity in the budget of the activity**:** approx 2%*

Background for this subactivity

A proficiency test panel of ovine blood samples will be provided for the QA of NRLs undertaking genotyping for statutory purposes (all Member States with the exception of Malta,). 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.

Deliverables for this subactivity

	Planned	Achieved
Organisation of an EQA for ovine 4 codon genotyping	*1: February*	
Provide an electronic copy of the outcomes to the Commission as rounds take place, and as appendices to the annual EURL report (see 4.2).	*2*	

3.0 Strain typing/discrimination

3.1 ***Strain Typing Expert Group***

Weighting (percentage) of this subactivity in the budget of the activity: approx 68%

Background for this subactivity

The EURL has established a working group of experts in the field of strain differentiation. It is responsible for:

- the evaluation of any unusual results arising from TSE testing in small ruminants within Europe,

- agreeing the criteria on which strains will be classified 'BSE-like' (and what that means).

- coordinating the provision of material 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.

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 a year. Discussion will continue to focus on the validation/interpretation of the increasing range of Tg bioassay methodologies, and how to interpret complex data. Definition of interpretational limits will also be a key topic for this group.

The group will review ring-trial and bioassay data for any isolate referred to this group under regulation 36/2005, and reach a final conclusion of BSE-like or non-BSE-like which will be reported to the Commission.

Deliverables for this subactivity

	Planned	Achieved

	Organisation of one STEG meeting	*1*	
	Organisation of a ring trial of any new potential discriminatory method needing further assessment	*0 in 2015*	
	Number of new referrals requiring ring trial/bioassay	*1*	
	Final reports on current STEG referral bioassay cases	*2 in 2015*	

3.2 ***Bioassay of unusual/BSE-like isolates***

Weighting (percentage) of this subactivity in the budget of the activity: approx 32%

Background for this subactivity

Any submitted isolate considered BSE-like following ring trial will be forwarded for bioassay in mice.

The choice of mouse strains has been reviewed, and the current STEG recommendation proposes Tg338 (VRQ ovine), Tg110 (bovine) and TgShpXI (ARQ ovine Tg110 as the most appropriate of the available well characterised lines. 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.

Deliverables for this subactivity

	Planned	Achieved
Number of ongoing bioassay studies on samples referred to STEG in previous years	*2 from 2014*[6]	
Number of new referrals requiring bioassay[7]	*0*	

4.0 Advice, training, assistance and communication

4.1 ***Annual workshop***

Weighting (percentage) of this subactivity in the budget of the activity: approx 50%

Background for this subactivity

A workshop for National experts will be arranged in the first half of 2015. This workshop will cover all aspects of NRL functions, and provide updates on areas of science relevant to TSE surveillance and testing. Feedback will be provided and training needs identified following the outcome of the QA assessments.

Deliverables for this subactivity

	Planned	Achieved

[6] This is correct at the time of writing. However, if a submission comes through STEG in the latter part of 2013, this will change, and follow-on costs for bioassay may be incurred in 2014. No costs have been put in at this point to reduce the amount of contingency planning, and on the understanding that there will be sufficient flexibility by pre-arrangement to accommodate this if the situation should change

[7] Budget request for 2015 does not include the mouse costs of undertaking a bioassay. If a new isolate required bioassay we would contact the commission to seek approval for the additional spend, which would be approx £5,400 over 2 years [at the current AHVLA rates, which are subject to change]

	Organisation of the annual workshop	*1: June*	
	Technical report and claim submitted to EC	*1: December*	

4.2 ***Communications***

Weighting (percentage) of this subactivity in the budget of the activity: approx 7%

Background for this subactivity

Formal communications between the EURL and NRLs and the COM take several forms. In addition to the information disseminated at the workshop and through EQA comment, the EURL maintained two websites, which in 2014 were amalgamated into a single site (TSE-LAB-NET) with both public and limited access areas. . The public, open access areas will continue to host reference material, links to relevant regulations, NRL contact details, protocols and STEG reports. The password protected limited access area (previously the only function of TSE-LAB-NET) will continue to host discussion fora, batch testing/ batch release data and presentations from the annual workshops. TSE-LAB-NET will also link to an area detailing services provided by the Biological Archive Group. All website content will be reviewed at least annually.

The EURL will monitor national quality assurance practices to ensure that they remain relevant, through discussion at the EURL meeting. We will attempt[8] to maintain an up-to-date internal database of information from NRLs, regarding the methods currently in use, the NRL applications of such tests (e.g. rapid, confirmatory, discriminatory, research, etc.) national QC and QA approaches etc. to enable the effective provision of relevant and targeted advice. As the EURL does not at present undertake inspections of NRLs, 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. A list of which NRLs are currently performing which tests will be maintained on the TSE-LAB-NET to facilitate referral of samples between NRLs in the event of specific testing being stopped, or temporarily suspended for technical reasons.

The EURL will maintain an up-to-date database of all relevant NRL principal contacts and contact details with access through the public website. The EURL will also maintain a database of current NRL contacts by activity for internal use, and to facilitate communications.

A formal annual report on EURL activities and financial summaries is sent to the COM each year.

Deliverables for this subactivity

	Planned	Achieved
All documents on the public website will be updated as required, with a baseline of one annual review within 2015,	*1: December*	
Maintenance of a table of current NRL testing competences on TSE LAB NET, updated after each EQA exercise	*1*	
A snapshot of the internal databases of NRL contacts and testing information will be provided to the COM as	*1*	

[8] The quality and completeness of this information relies totally on the responses we receive from the NRLs. Historically this varies considerably.

	appendices to the annual technical report (see below)		
	Provision of 2014 final technical and financial report to COM	*1: March*	
	Provision of full 2015 technical and financial workplan proposal to COM	*1: August*	
	Provide the automatic weblink for all VETQAS-coordinated EQA results for participating laboratories and COM		*Already in place for all relevant bodies*

4.3 ***Approval of minor testing kit changes and batch testing. Regular communication with manufacturers.***

Weighting (percentage) of this subactivity in the budget of the activity: approx 3%

Background for this subactivity

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. 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. 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. In addition, an annual statement will be sent to the COM confirming which manufacturers continue to comply with the requirement to keep the EURL advised of all relevant changes to their systems and products, so that the listing in the regulation is kept up to date.

Test manufacturers are also approached annually by the EURL to provide confirmation that all relevant quality systems are up to date.

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.

Batch testing of approved rapid tests for the detection of Scrapie in ovine and caprine samples is under debate. The main limiting factor for implementing batch testing of approved small ruminant tests is the relative paucity of atypical scrapie material. Discussion between the EURL and the Commission is planned within 2013. The outcomes of this discussion will inform further plans for 2014 and successive years.

Deliverables for this subactivity

	Planned	Achieved
Number of minor testing kit changes assessments (approval is sent to NRLs and COM, provision of annual summary to COM in final technical report)	*3*	
Number of batch testings (publication on TSE_LAB-NET of batch release authorisations)	*15*	

	Annual statement sent to the COM confirming details of IFU (Instructions for use/kit inserts) changes and which manufacturers continue to comply with requirements	*1: December*	
	Annual review of manufacturers' quality systems	*1: June*	

4.4 ***Referral diagnostics, EQA troubleshooting, provision of advice and training***

Weighting (percentage) of this subactivity in the budget of the activity: approx 40%

Background for this subactivity

EQA troubleshooting: Assistance and guidance will be provided to those laboratories experiencing difficulties. Initial support will be provided through telephone and/or email contact, and discussion with the COM and manufacturers may also be initiated if a more widespread problem is identified with any particular test.

Ad hoc requests for training: Provision of training at AHVLA may be offered if a need is identified (with relevant NRL bearing all travel and subsistence costs). Provision is made for one mission to provide local technical troubleshooting and training for NRLs with difficulties that are not due solely to resource issues. TSE-LAB-NET also offers the opportunity for NRLs to raise issues and solve problems through discussion fora.

2nd opinion referral cases: 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. 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. (The success of any such system is dependent on the willingness of MS to comply with 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.)

Specialist input to Commission fora, by request, on an ad hoc basis. Provision has been made in 2014 for four people to travel to Brussels.

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 EURL will provide expert advice on the clinical manifestations of BSE and scrapie. The EURL will also continue to provide epidemiological advice on an ad hoc basis. Provision of this advice will be dependent on the continued availability of specialist staff in other departments, who are supported by other sources of funding. If these key staff leave the organisation, the ability to provide up-to-the-minute advice will be compromised.

Please note that 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 invited to participate in a specific session at the EURL workshop each year where issues can be discussed directly with NRL representatives. This was well-supported initially, but all manufacturers have declined since 2012.

As testing throughput drops and individual MSs rationalise their testing capacity we are increasingly asked for advice on decommissioning and decontamination. Following discussion at the 2011 NRL workshop it was made clear by the COM representative that this falls outside the scope of 999/2001 and does not form a part of the EURL remit. We do not, therefore offer any specific support to NRLs or CAs in this regard. We continue to maintain that this should be addressed at a national level, taking a risk-based approach and considering the general principles of TSE waste decontamination and disposal, and the relevant local rules and National legislation for waste disposal.

<u>Deliverables for this subactivity</u>

	Planned	Achieved
Assistance missions to NRLs	*1*	
Provision of diagnostic reports on 2nd opinion referral cases as required	*10*	
EURL specialist input to Commission fora, travel to Brussels	*4*	
Number of significant follow-up/troubleshooting dossiers generated through EQA activities	*3*	
Number of significant follow-up/troubleshooting dossiers with test manufacturers, following NRL concerns	*3*	

PROVISIONAL TIMETABLE FOR TSE EURL QA EXERCISES IN 2015

Intended Start Date[9]	QA activity
February 2015	Ovine genotyping
March 2015	Immuno-histochemical technique
April 2015	TSE discriminatory Western blotting
April 2015	Histopathology and immunohistochemistry interpretation (round 1)
May 2015	Ovine rapid testing
May 2015	Ovine confirmatory blotting
October 2015	Histopathology and immunohistochemistry interpretation (round 2)
November 2015	Bovine rapid testing
November 2015	Bovine confirmatory blotting
November 2015	Bovine BSE classification

[9] 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.