

# Guidelines for evaluation of changes to approved protocols for TSE rapid tests and details of information to be supplied by rapid test manufacturers to the **EURL**

Version 7.0 – October 2010

## 1 Introduction

Rapid tests are used for routine monitoring of BSE and scrapie within EU countries.

They are approved for use by the EU and listed in Regulation (EC) No 162/2009 (amending Regulation (EC) No 999/2001). The approval is linked to the testing protocol, which was used to generate the validation data. If a test manufacturer wishes to vary this protocol in any way, approval must be obtained from the TSE **European Union** Reference Laboratory (**EURL**). Additionally, the test manufacturer must have the agreement of the **EURL** that the quality system, batch testing and release arrangements used for manufacture are to a satisfactory standard.

This document aims to provide guidelines within which **EURL** approval may be given. The purpose of this document is to explain what types of changes may be made, how these changes may be made and to outline the types of supporting data required to validate such changes. This document is aimed primarily at the manufacturers of TSE rapid tests, but is also available to NRLs and testing labs so that they know what is required of kit manufacturers.

## 2 Types Of Changes Which Will Not Be Assessed By The **EURL**

There are two types of changes which will **not** be assessed by the **EURL**, and any changes of this type must be assessed by a full EU evaluation. These are:

- Changes to the primary antibody- either by addition of other antibodies or by replacement with a different antibody.
- Changes to the method of homogenisation (NB this means the mode of homogenisation, dimensions of homogenisation vessel, speeds used etc.)

*(Note The **EURL** will assess data which supports the use of a different machine, which merely increases or decreases the number of samples to be homogenised/prepared at a given time, but all other criteria remain the same, or where a new or replacement machine provides demonstrably identical performance to that used in the original validation )*

For further information on EU evaluation, please contact Directorate General Health & Consumer Protection of the European Commission 1049 Brussels.

## 3 Types Of Changes The **EURL** Will Consider

### 3.1 Post Homogenisation Changes to The Testing Procedure

Changes to stages of the test after the homogenisation stage will be assessed by comparative testing. This includes factors such as alteration of incubation times, temperatures or sequences (including treatment of initial positive reactors), post-homogenisation but pre-analysis.

A detailed protocol for the new method for which approval is sought should be lodged with the **EURL**. This method is then used to generate comparative data for assessing the proposed change.

A study protocol should be designed by the test manufacturer whereby a panel of samples is processed to the homogenisation stage, divided into aliquots and processed in parallel using the approved method and the new method for which approval is sought. It is necessary to agree the study protocol with the **EURL** before undertaking any laboratory work.

The panel usually consists of approximately 2,000 negative samples which are tested as normal and a small number of positive samples (not less than 20) which are tested 4 times each (replicates). Additionally these positive samples should also be tested as dilution series (fourfold dilutions), with each dilution tested in duplicate. The samples should be from the same host species for which the assay is routinely used. If the test is applicable to bovine BSE and scrapie in small ruminants, data corresponding to both of these uses is required, and the data and analysis of these two populations should be presented separately to the **EURL**.

The data will then be analysed to assess whether the modifications produce a statistically inferior test performance. Reduction of the number of samples may not be made without full justification and with the agreement of the **EURL**.

### **3.2 Changes To The Way The Sample Is Taken.**

The way the sample is obtained is important for two reasons:

Firstly, to obtain a sample of the correct weight from the right anatomical region provides the best chance of accurate diagnosis and these requirements are detailed in the protocols for each test

Secondly, the sampling method must not compromise the tissue remaining for confirmatory diagnosis.

Changes to the way the sample is taken must be discussed with the **EURL** and an assessment will be made on a case-by-case basis. It may be necessary for the manufacturer to undertake laboratory studies, provide validation data or samples for histo-pathological assessment, to aid this assessment. The sampling method is regarded as part of the testing protocol and changes may only be made with the written agreement of the **EURL**. It is a requirement that instructions for the use of sampling devices are part of the test kit instructions and not a “stand alone” document.

### **3.3 Changes To Storage Conditions For The Sample Or Sample Homogenate.**

Changes to storage temperatures or times will be considered. The sample in question (tissue or homogenate stages from the host species (cattle or small ruminants as appropriate for the test in question) should be divided into aliquots at the beginning of the study, stored for the specified times and then tested according to the standard protocol.

We would expect to see a panel including a mixture of at least 20 positive and 20 negative samples tested after storage under the original and proposed conditions. Consideration should be given when designing the experiment to ensure that confounding factors such as assays conducted on different days, assay variability, plate or sample orientation bias are accounted for in the study design. The data will

be analysed statistically and changes approved if the study provides evidence that the proposed storage conditions do not result in inferior performance.

### 3.4 Changes To Reagent Storage Conditions.

Changes to reagent storage conditions and times may be assessed using 20 positive and 20 negative homogenates, each tested on at least 4 plates in a randomised Latin square format comparing the approved version with the new version. In addition, any changes in specification to reagent containers or sample plates, etc., that could affect the performance of the test must be notified and validated.

The data will then be analysed to assess whether the modifications produce statistically inferior test performance.

#### 3.4.1 Changes To The Shelf Life For The Kit

If a manufacturer wishes to extend the shelf life approved for a kit, data from 3 batches tested in real time must be supplied to the [EURL](#). The data should extend for 3 months beyond the new shelf-life claim. i.e. to support a claim for 18 months shelf-life, stability must be demonstrated at 21 months.

Changes to shelf-life should be assessed either:

- a) using 20 positive and 20 negative homogenates, each tested on at least 4 plates in a randomised Latin square format comparing the entire time period at no more than 6 month intervals. At least 5 of the positive homogenates should be weak positives with a signal between the test cut-off and 20% of the maximum recordable value of the test or,
- b) using a dilution series of a pooled strong positive sample and a panel consisting of a high, medium and low positive samples. The dilution series should have a number (at least 4) of representative points and the sample should be diluted to extinction (no signal recorded). These samples should be tested in at least quadruplicate (4 replicates) on at least 4 different plates per batch, in a randomised Latin square format comparing the entire time period at no more than 6 month intervals. Additionally 20 negative samples should be tested as quadruplicates at the same time points on 4 plates per batch. Recombinant PrP at known concentrations (at least 2 different concentrations), including a concentration regarded as “weak” (see above for definition) can be used to support this data.

The data will then be analysed to assess whether the modifications produce statistically inferior test performance.

Manufacturers should discuss other equivalent approaches with the [EURL](#) and also requests for batch/lot specific release.

### 3.5 Changes To The Method Of Preparation Of Reagents

If a manufacturer wishes to change batch preparation methods, data from 3 batches prepared by the approved and three batches prepared by the new methods must be provided and the acceptance criteria for the reagents must be achieved using the new method. Functionality should be assessed as in point 3.4.

### 3.6 Changes To The Detection Method Of The Test

If a manufacturer wishes to change their detection method (e.g. from luminescent to colorimetric measurement, or from one colorimetric marker to another) the plan for

change and the acceptance criteria for the change needs to be discussed and agreed with the **EURL** before formal validation testing is initiated. The volume of data required will be related to the degree of change, but conversions to a different mechanism will be treated as a major change to the test and require extensive validation in terms of both specificity and sensitivity using a large number of positive and negative samples. All such tests must be in comparison with the currently approved test format. The **EURL** will require all related comparative results data before any decision on approval is made.

### **3.7 Changes to Interpretation of the Test**

Changes to the way the cut-off is calculated, use of controls or calculations must be submitted to the **EURL** with a reasoned case including the data upon which any proposal to change is based. This should include calculations for the approved and proposed systems for comparison. The effects on sensitivity and specificity of the test should be calculated.

### **3.8 Automation and Equipment**

**3.8.1** If the manufacturer wishes to market the test in an automated/semi automated/ manual format, which is different to the approved version they should supply data to the **EURL** comparing the approved and new versions as in point 3.1.

**3.8.2** Laboratories may only use the homogenisation system or systems, which are approved by the EU for each particular test.

**3.8.3** If a laboratory wishes to use a piece of equipment which is not recommended by the test manufacturer, they must provide supporting evidence to the appropriate NRL, that the piece of equipment does perform to any standards specified by the testing protocol (e.g. wavelength or volumes) and that it does not result in inferior test performance. Such equipment may include automated liquid handling systems, plate washers, plate readers etc.

**3.8.4** If a manufacturer wishes to change the way a piece of equipment (or consumable plastic ware) is used or reused in the test procedure, this must be agreed with the **EURL** prior to introduction. It is likely that the manufacturer will be asked to undertake some experimental work to provide evidence that the change does not result in inferior test performance.

**3.8.5** If a manufacturer wishes to change the specification of a piece of equipment (or consumable plastic ware which is specified in the kit instructions or supplied as part of the kit), such as a different source, this must be agreed with the **EURL** prior to introduction. It may be necessary for the manufacturer to provide information to satisfy the **EURL** that the new component is essentially **equivalent to** the original component and to undertake some experimental work to provide evidence that the change does not result in inferior test performance.

### **3.9 Changes To Wording In The Instructions For Use (IFU Or Kit Insert)**

All changes to the wording of the IFU must be submitted to the **EURL** for approval and only used after written approval has been granted. Such applications may be submitted because a change to the test method has been approved in one of the categories above, to comply with EU or national requirements or simply to clarify the text.

The documentation submitted must include:

- The current IFU
  - The new IFU
  - A change list, tabulating changes
- If this change list is not provided, the **EURL** may withhold approval until all changes on the new IFU can be identified by the manufacturer.
- Kit inserts must be clearly version controlled.

### 3.10 Other Documentation

If the manufacturer has any other documentation which is referred to in the IFU and contains information which is required for test performance, this should be included in the same version approval system described in 3.7

The **EURL** will consider these changes and seek advice on an *ad hoc* basis from experts in National Reference Laboratories.

## 4 Other Issues

If a National Reference Laboratory is aware of any problems or issues with test performance in their country, they must inform the **EURL**.

## 5 Quality Systems

The **EURL** is required to approve the quality systems of test manufacturers in order to provide reassurance that kits are manufactured to a high and consistent standard. The minimum requirements are:

- A third party quality certification, such as ISO9000 or equivalent.
- A quality manual which specifies:
  1. Overview of the quality system employed by the manufacturer, including strategic policies and how the manufacturer complies with the chosen quality standard for certification.
  2. Management responsibilities (an organigram is sufficient if the roles are identified) including management of resources and processes relevant to the specific kit under approval.
  3. Method of process documentation (e.g. document hierarchy such as Policies, SOPs, Forms, Records, etc.) with at least one example.
  4. Mechanism for management review of the quality system, including audit planning, performance and review.
  5. Monitoring and measurement processes, including quality control of products and methods of control of nonconforming product.
- Standard Operating procedures that describe production of all reagents which comprise the kits, testing and release of kits, with (if possible) a process flowchart to illustrate the overall production system.

The manufacturer must have an in house or contracted batch release procedure, to include:

1. A panel of high, medium, and low positive samples plus a negative sample with acceptance criteria (ranges) for the species for which the assay has approval.
2. A specificity panel of at least 600 samples

The **EURL** will require annual confirmation from test manufacturers that this remains in place and provision of supporting documentation as requested by the **EURL**.

## 6 European Batch Release

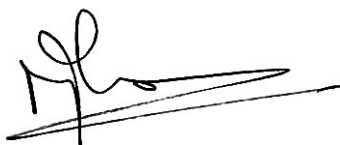
In 2008 the **EURL** implemented a system for EU batch release for TSE rapid kits. Batch release information is made available to NRLs and individual test kit manufacturers. Batch release information will be reported on TSE-LAB-NET [ ]. Any NRL or company needing to access this must apply through the **EURL** management system (either on-line or via M Simmons or P Bellerby, VLA Weybridge, UK).

### 6.1 TSE **EURL** contact details for rapid tests

For issues related to rapid TSE tests please contact Kath Webster  
[k.a.webster@vla.defra.gsi.gov.uk](mailto:k.a.webster@vla.defra.gsi.gov.uk)

For other issues please contact the TSE **EURL** general mailbox  
[tseeucl@vla.defra.gsi.gov.uk](mailto:tseeucl@vla.defra.gsi.gov.uk)

All the test data provided by rapid test kit manufacturers to support changes to protocols remains totally confidential and is not distributed beyond the **EURL**.



Signed :  
for the TSE EURL, VLA Weybridge, UK.

Date : **13<sup>th</sup> October 2010**