TSE EURL
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin

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TSE EU REFERENCE LABORATORY 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

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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 1148/2014 (amended Regulation (EC) No 999/2001. Rapid Test approval is linked directly to the testing protocol which was used to generate the original validation data to support the use of the test.

If a test manufacturer wishes to vary this protocol in any way, approval must be obtained from the TSE European Union Reference Laboratory (EURL). The test manufacturer must have the agreement of the EURL that the quality systems used for manufacture are fit for purpose.

This document aims to provide guidelines within which EURL approval may be given to test kit manufacturers. 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, 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*.

*These noted changes above must be assessed by a full EU evaluation.

Please Note: Minor changes of homogenisation speeds (within 10% of the original quoted speed) will be considered by the EURL providing supporting validation data is provided. The EURL will also assess data which supports the use of a new or replacement machine, e.g. which merely increases or decreases the number of samples to be homogenised/prepared at a given time, but all other criteria remain the same, providing identical performance to the machine used in the original validation is demonstrated.

For further information on EU evaluation processes, 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 consists of up to 1,000 negative samples which are tested singularly and a small number of positive samples (not less than 20) which are tested 4 times each (as 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 (including atypical 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 by the EURL to assess whether the modifications produce an inferior test performance, this will include statistical analysis if it is considered necessary. 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, a sample of the correct weight from the right anatomical region provides the best chance of accurate diagnosis; 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 histopathological 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.

The EURL would expect to see a panel including a mixture of at least 20 positive (including atypical forms where applicable) 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 robustness, assays conducted on different days, assay variability and 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 (including atypical forms where applicable) and 20 negative homogenates, each tested on at least 4 plates in a randomised [] format comparing the approved version with the new version. In addition, any changes in specification to reagent containers or sample plates, etc., which 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 (including atypical forms where applicable) and 20 negative homogenates, each tested on at least 4 plates / combs / gels in a randomised [] 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 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.

3.5 Changes to the Method of Preparation of Reagents

3.5.1 Bovine rapid tests

If a manufacturer wishes to change reagent preparation methods or the supplier of a reagent, comparative data from 2 batches prepared by the approved vs. the new reagent must be provided. Following approval of the change, the next 3 EURL batch testing exercises must include EURL sample panel testing, the EURL will closely monitor the kit during these 3 EURL batch testing exercises to ensure that revised test continues to perform to the approved standard. Functionality should be assessed as in point 3.4.

3.5.2 Ovine rapid tests

If a manufacturer wishes to change reagent preparation methods or the supplier of a reagent, comparative data from 3 batches prepared by the approved vs. the new reagent must be provided. 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. This change would 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

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.

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

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.

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 comparative work to provide evidence that the change does not result in inferior test performance.

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 comparative 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 in English 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 with changes highlighted.
- 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 /External Packaging/ Reagent Labels

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. 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 ISO9001 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.

5. TSE EURL CONTACT DETAILS FOR RAPID TESTS

For issues related to rapid TSE tests please contact Daniela Meloni or Elena Bozzetta:
daniela.meloni@izsto.it
elena.bozzetta@izsto.it
For other issues please contact the TSE EURL general mailbox:
EURL.TSE@izsto.it

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.

This document is largely based on a previous one that originally was made available by APHA (UK) as EURL for TSEs. After the transition of the EURL to our consortium, in the documentation that we are making available, some minor changes were needed to update information (e.g. contacts).