TSE EURL
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BATCH TESTING PROTOCOL FOR BATCH RELEASE OF TSE RAPID TEST KITS

To be used by all participating EU TSE National Reference Laboratories and TSE rapid test kit manufacturers

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1. PURPOSE

This procedural document details the approval process by which bovine and small ruminants TSE rapid test kit batches can be released onto the European market. The approval process provides reassurance that commercially available TSE rapid tests kits in the European Union (EU) are fit for the purpose of testing bovine and small ruminants samples by:

a) Assessment of batch release data provided by the manufacturer;

b) Production of additional Quality Control (QC) data within a designated National Reference Laboratory (NRL) for an individual rapid test kit batch.

This batch release assessment will be available to all NRLs within the EU and can replace any formal batch release testing conducted by individual member states (MS).

2. BACKGROUND-THE CURRENT POSITION

Each TSE rapid test kit that is released onto the European market must be authorized for statutory use within the European Union and listed in the TSE Regulation 999/2001. The approval is linked to the particular test protocol used during the evaluation study which led to EU approval being granted. Any modifications to the protocol are approved by the EURL on the basis of evidence submitted by the manufacturer.

Every test kit manufacturer carries out internal QC testing of kit batches before they are distributed to customers. Additionally, varying amounts of batch release testing and /or approval have previously been carried out by different MSs. This ranged from full release of all batches to acceptance of the manufacturer's release procedure.

The right to test any or all kit batches was retained by the designated NRL. Manufacturers should continue to supply representative kits from each new batch.

3. AIM - UNIFICATION OF BATCH RELEASE THROUGHOUT THE EU

The aim of this protocol is to provide a single batch release testing procedure which is acceptable to all NRLs. This document describes the current system in place.

4. APPROACH

Designated NRLs (as agreed between themselves and the EURL) are responsible for the batch release of a particular BSE and Scrapie rapid test kit. The responsible person at the designated NRL (plus deputy) must have received training and be certified by the kit manufacturer as competent to perform the test specified.

All designated NRLs involved must hold ISO17025 accreditation for the test for which they are responsible, to confirm their competency to perform the testing. If the designated NRL does not hold ISO17025 accreditation, it can nominate, in agreement with the TSE EURL and the manufacturer, a local official laboratory accredited for TSE rapid testing to carry out testing on its behalf. This official laboratory must undergo regular proficiency testing (PT) to confirm its competency to perform the test. The designated NRL must have access to these PT results and must also be informed if any test related issues occur in the nominated official laboratory. Designated NRLs may not control and approve test batches manufactured in their own country.

5. MANUFACTURER'S BATCH RELEASE DATA

When a kit batch is ready and the manufacturer is satisfied that the batch is suitable for release, the manufacturer shall provide to the designated NRL:

1. an analytical batch release report. This report shall contain analytical data, sensitivity, specificity and intra and inter-plate control. The batch release protocol for each manufacturer is defined in their own Quality System standard operating procedures (SOPs), which have been approved by the EURL as part of quality system approval.
2. Additionally, the manufacturers shall provide evidence of consistent plate coating by testing at least 3 plates, taken from the beginning, middle and end of each batch using appropriate kit controls throughout the plate. In the case of strips/ gels/ other layouts, appropriate variation in sampling locations should be applied. In the case of uncoated plates, evidence that the plates are homogeneous should be provided.

Reports should be sent by e-mail to the designated NRL.

Specific equipment to undertake batch testing shall be provided to the designated NRL, at no extra cost, by the manufacturer.

A summary of actions for Manufacturers is available in Appendix 1.

6. NRL BATCH TESTING

6.1 Batch testing process

Designated NRLs carry out the batch testing using the EURL set of material prepared for BSE and Scrapie.

Each set consists of not more than two positives and at least one negative homogenate constituted by CNS tissue diluted in water at a 50% concentration (w/v).

It is the responsibility of NRLs to properly store the reference materials.

NB: In case the EURL decides to include atypical scrapie samples a dedicated protocol of preparation will be applied using chopped tissue instead of homogenate.

A summary of actions for designated NRLs carrying out batch testing is available in Appendix 2.

6.2 Emergency situations

If the designated NRL is unavailable to carry out the full batch testing protocol, due to emergency situations (e.g., Covid-19) in which the laboratory is unable to carry out its routine activity, it will be possible, in agreement with TSE EURL, to restrict its approval process by reviewing the batch release QC data provided by the manufacturer.

6.3 BSE and Scrapie rapid test kits

BSE and Scrapie rapid test kits shall be provided by the manufacturers free of charge. The kits must be representative of products supplied to customers, i.e., kit reagents and consumables must be labelled and sealed, and the test kit Instructions for Use and outer packaging must be the versions currently approved by the EURL.

A representative selection of plates from the batch, shall be provided to the designated NRL by the manufacturer.

As: a single kit (as long as it contains at least 5 plates/gels/combs, etc.)

or as: 5 plates from batch plus reagents,

or as: sufficient strips and reagents to provide the equivalent of 5 plates for testing purposes.

Reagents should be supplied unused and labelled in the same manner as those reagents supplied in commercially available kits.

6.4 EURL Reference control sample sets

The manufacturers provide to the EURL the reference materials needed to carry out the batch testing and kits QC. The control sample sets are produced by the EURL and are pre-tested by rapid test and Western blot for level of activity. They are initially provided by the EURL to all manufacturers for pre-assessment testing to ensure that the range of reactions is appropriate and relevant to the test kit in question before being distributed to the designated NRL for parameter setting (6.5) and subsequent batch testing. Sufficient homogenate is supplied to all the designated NRLs to undertake the required batch release analyses. All reference control samples prepared by the EURL are provided to designated NRLs free of charge.

6.4.1 EURL Reference negative control sample

Pools of certified TSE negative bovine and small ruminants brainstem homogenate are produced using EURL standard methods.

6.4.2 EURL Reference TSE positive control samples

Pools of certified TSE positive bovine and small ruminants brainstem homogenate are produced using EURL standard methods. This material is supplied as two discreet pools, providing samples of two levels of positivity in terms of signal produced (these have already been diluted with TSE negative homogenate and are ready to use).

6.5 Initial levels agreement

The reference samples are to be used by the designated NRL in consultation with the manufacturer to determine the upper and lower acceptable ranges for the kit they are responsible for. The ranges are to be set by testing plates from representative batches using the standard control material in 4 replicate wells on each kit batch. The assays should be repeated on 3 successive days to allow for variation of the assays. The acceptable range for each of the 3 reference samples should be calculated from the mean and within an upper and lower limit (e.g. +/- 3SD) of these values and must be negotiated between the designated NRL and manufacturer, before being agreed with the EURL, prior to undertaking the actual batch release analyses.

7. QC BATCH TESTING PROTOCOL

7.1 Review Process only

If a designated NRL approves the batch by data review alone, in accordance with point 6.2, a report should be produced which states this, as described in 7.6. The designated NRL must continue to retain sufficient samples, reference and test kits in case there is a requirement to carry out batch testing.

7.2 Reference samples from EURL and reference batches

All test samples will be treated, as far as possible within the instructions for each particular kit, as if they were real samples and assayed accordingly. This means that EURL samples (although homogenized) shall go through routine homogenization/grinding/maceration etc. as described within kit instructions, including temperature and storage conditions as required. They shall be treated as whole tissue, with no compensation for homogenate diluent in the original sample.

7.3 Batch evaluation protocol

Each of the reference samples shall be tested in duplicate on each of three separate test plates. A result will be determined for each reference sample and checked against the range. If all values for the sample set are within range the kit is considered to have passed. If the results are out of range the testing shall be repeated in duplicate on the remaining two plates, strips, etc.

If the results are confirmed out of range, the kit has to be considered non-compliant and thus it cannot be released on the market.

7.4 Reference Batches

The manufacturer shall also provide three plates from their current reference batch to the designated NRL. These shall be tested in parallel with the new batch using EURL panel samples. The value ranges previously achieved for the reference batch shall be quoted by the manufacturer and used to ensure the kit under evaluation performs as well as, or better than, the reference kit.

If the kit reference batch is replaced, the manufacturer is obliged to provide details to both the EURL and designated NRL. The designated NRL must undertake a full batch test using the new reference batch in conjunction with the original reference batch to ensure that continued validity of measurement is maintained during testing.

7.5 Analytical Timescale

Wherever possible, the entire testing and reporting protocol shall be completed within 20 working days of receipt of kits. This allows the designated NRL at least 10 days to undertake the testing and report to the EURL, and the EURL 10 days to complete analysis and report to the manufacturer and all EU NRLs. If a problem occurs, the designated NRL shall notify the EURL and the manufacturer promptly.

7.6 Production of analytical data report

7.6.1 Reporting process

The designated NRL shall prepare a report containing:

- An analysis of the batch release data produced by the designated NRL, if performed, and that provided by the manufacturer. Any designated NRL original data should be appended as an annex.
- Comment upon whether the manufacturers batch release criteria have been achieved.
- An analysis of the analytical results produced by the designated NRL, if performed.
- Recommendations for kit release onto the European market.

This shall be provided to the EURL, in English, **as a short report completed on form** EURL001v3.0 (Appendix 3). Reports should be sent by e-mail to: eurl.tse.batchtesting@izsplv.it. The EURL will review the report and confirm approval. It will then co-ordinate the release of the approval statement and send alert e-mails to all the NRLs and to the appropriate manufacturer.

7.6.2 Unfavourable reports

Reports that recommend non-release shall only be sent by e-mail to the manufacturer from the EURL. It is then the manufacturer's responsibility to halt release of the batch and provide an alternative or re-worked batch for assessment. No other NRLs shall be notified, as no units of that batch should ever reach testing laboratories. The designated NRL shall treat all such information as confidential. In such a situation, it is imperative that the designated NRL performs actual kit testing prior to reporting batch failure and not restrict their approval solely to review of manufacturer's data. The manufacturer is in charge to carry out trouble shooting and provide an anomaly report to the designated NRL and the EURL explaining how the failed batch reached this phase in the release process.

7.7 Data Retention

The EURL will retain all manufacturer's analytical data for the appropriate batch release for a minimum of two years after the batch expiry date and then archive to EURL secure electronic storage for a minimum of five years**.**

Batch release paperwork is also to be kept as a hard copy at the designated NRL and on EURL registered files.

8 OPTION FOR INSPECTION OF MANUFACTURER'S FACILITIES

The designated NRL shall retain an option (but not a requirement) to visit the manufacturing facility to perform an inspection and provide a report to the EURL. This could be an initial visit, periodic visit, or visits if a problem occurs. Alternatively, the EURL shall perform the inspection.

9 CONTINGENCIES

In the event of a test failure, an inability to test (illness, facilities, workload crisis, etc.) or other such problem associated with batch testing within the 10 working day deadline, the designated NRL shall contact the EURL via the general email address (elena.bozzetta@izsplv.it or maria.mazza@izsplv.it).

APPENDIX 1: ACTIONS FOR MANUFACTURERS

1. On production of a new batch of TSE kits intended for bovine and small ruminants use, an assessment of the batch release data for bovine and small ruminants performance must be provided to the designated NRL
2. Provide to the EURL the reference material needed to carry out the batch testing and kits QC
3. At least three plates from the current reference batch must be supplied to the designated NRL for testing to ensure validity of measurement is achieved, for each batch release set.
4. Constituents (plates, reagents, buffers, etc.) sufficient for retesting at least 3 plates of each kit batch must be archived for reference purposes.
5. A Kit of the reference batch must be provided for each batch testing (as per section 7.5).
6. Any relevant equipment which may be required by the designated NRL is to be provided at no further charge by the manufacturer.
7. Each delegated analyst for the kit testing at the designated NRL must be certified by the kit manufacturer to undertake the QC testing as required.
8. An analytical batch release report must be supplied to the designated NRL, to include sensitivity, specificity and intra and inter-plate controls.
9. Reagents shall be used and appropriately labelled (as per section 6.3).

Following this, options 1 to 2 will be considered by the EURL *(order changed)*:

1. testing with EURL panel
2. paper review only, if satisfied with manufacturer's data

If the designated NRL approves without batch testing, the manufacturer will be notified immediately, but the manufacturer shall continue to supply all batch as appropriate, to facilitate testing of the batch should it be required following data review by the designated NRL.

APPENDIX 2: ACTIONS FOR THE DESIGNATED NRLs

1. Ensure that all required equipment is in place prior to commencement of QC testing. If not, liaise with kit manufacturer to supply (notifying the EURL).
2. Ensure that both the delegated analyst and the deputy entrusted to undertake the analysis are trained and certified as competent to undertake the task prior to commencement, within the ISO17025 accreditation system.
3. Check that all required kit constituents and reference control materials are in place for analysis.
4. Check that there is sufficient of each of the EURL certified control materials prior to each round of batch testing, if it is to be performed, and contact EURL for further supply if necessary. It is strongly recommended to request new reference materials from the EURL a long time in advance (at least two months)
5. Ensure that all relevant kit controls are utilized as specified to ensure kit validity is maintained, and check that the current IFU is employed for all batch testing, if performed.
6. On completion of the QC testing program, prepare the required short analytical report, in English, fill in the reporting spreadsheets (Appendix 3), and supply to the EURL by e-mail to: eurl.tse.batchtesting@izsplv.it. Additionally, supply the EURL with an electronic copy of the full manufacturer's release report including raw data if this has not been already uploaded by the manufacturer; archive a copy at the designated NRL. In case of unavailability to carry out the full evaluation, review the batch release QC data provided by the manufacturer.
Finally comment on whether the batch release criteria have been met.
7. Make a recommendation to the EURL as to whether the kit should be released onto the European market, on the analytical data report. Notify the EURL by email that this report is available.
8. As the delegated representative of the EURL, the designated NRL may retain an option, but not a requirement, to make an inspection visit to the manufacturer's facility and prepare a report for the EURL. Alternatively, the EURL may take on this visit.

APPENDIX 3: REPORTING SPREADSHEET PAGES

Batch Test Result Recording Form

Name of Testing NRL	
TSE Kit Name	
it Batch Release Number or ID	
Expiry	
IFU Version	
Date Received at NRL	

Manufacturer	
Kit Batch Number	
Reference Kit Batch (	

Initial Agreement		
Reference Sample	Range –	Range +
EURL BSE Negative 1		
EURL BSE Positive 1		
EURL BSE Positive 2		

Initial Agreement		
Reference Sample	Range –	Range +
EURL Scrapie Negative 1		
EURL Scrapie Positive 1		
EURL Scrapie Positive 2		

Interpretation Options – Positive, Negative, Inconclusive or No Result

Kit Batch Results * These are new plates sent as representation of a newly produced batch for acceptance of the entire batch

Reference Sample	Test Plate/Strip 1 values 1		Interpretation	Test Plate/Strip 2 values 1 & 2		Interpretation	Test Plate/Strip 3 values 1 & 2		Interpretation
	CUT-OFF:			CUT-OFF:			CUT-OFF:		
EURL BSE Negative 1									
EURL BSE Positive 1									
EURL BSE Positive 2									

Reference Sample	Test Plate/Strip 1 values 1		Interpretation	Test Plate/Strip 2 values 1 & 2		Interpretation	Test Plate/Strip 2 values 1 & 2		Interpretation
	CUT-OFF:			CUT-OFF:			CUT-OFF:		
EURL Scrapie Negative 1									
EURL Scrapie Positive 1									
EURL Scrapie Positive 2									

Reference batch results * These are reference plates provided by the manufacturer for comparison testing with new batches

Reference Sample	ef. Plate/Strip 1 values 1 &		Interpretation	Ref. Plate/Strip 2 values 1 & 2		Interpretation
	CUT-OFF:			CUT-OFF:		
EURL BSE Negative 1						
EURL BSE Positive 1						
EURL BSE Positive 2						

Reference Sample	ef. Plate/Strip 1 values 1 &		Interpretation	Ref. Plate/Strip 2 values 1 & 2		Interpretation
	CUT-OFF:			CUT-OFF:		
EURL Scrapie Negative 1						
EURL Scrapie Positive 1						
EURL Scrapie Positive 2						

Comments			
Accept Batch (Y/N)			
Reported by (name)		Date of Report	

Repeat Test Values (If Repeats Performed)

Name of Testing NRL	
TSE Kit Name	
Test Kit Batch Release Number or ID	
Expiry	
IFU Version	
Date Received at NRL	

Manufacturer	
Kit Batch Number	
Reference Kit Batch (Y	

Initial Agreement		
Reference Sample	Range -	Range +
EURL BSE Negative 1		
EURL BSE Positive 1		
EURL BSE Positive 2		

Initial Agreement		
Reference Sample	Range -	Range +
EURL Scrapie Negative 1		
EURL Scrapie Positive 1		
EURL Scrapie Positive 2		

Kit Batch Results * These are new plates sent as representation of a newly produced batch for acceptance of the entire batch

Reference Sample	Test Plate/Strip 4 values 1 & 2 CUT-OFF:		Interpretation	Test Plate/Strip 5 values 1 & 2 CUT-OFF:		Interpretation
EURL BSE Negative 1						
EURL BSE Positive 1						
EURL BSE Positive 2						

Reference Sample	Test Plate/Strip 4 values 1 & 2 CUT-OFF:		Interpretation	Test Plate/Strip 5 values 1 & 2 CUT-OFF:		Interpretation
EURL Scrapie Negative 1						
EURL Scrapie Positive 1						
EURL Scrapie Positive 2						

Reference batch results * These are reference plates provided by the manufacturer for comparison testing with new batches

Reference Sample	Ref. Plate/Strip 3 values 1 & 2 CUT-OFF:		Interpretation
EURL BSE Negative 1			
EURL BSE Positive 1			
EURL BSE Positive 2			

Reference Sample	Ref. Plate/Strip 3 values 1 & 2 CUT-OFF:		Interpretation
EURL Scrapie Negative 1			
EURL Scrapie Positive 1			
EURL Scrapie Positive 2			

Date of Testing	
Comments	
Reported by (name)	

Date of Report	
Accept Batch (Y/N)	

EURL001v3.0

Batch Release Documentation Check Sheet

TSE Kit Name :	
Test Kit Batch Number or ID :	
Expiry:	
IFU Version:	
Date Received at NRL :	

Manufacturer	
Kit Batch Number	
Reference Kit Batch (Y/	

Manufacturer's Batch Release Data	
Batch Report Received (Y/N)	
Batch Report Accepted (Y/N)	

Manufacturer's Plate-Coating Data (if Applicable)	
Coating Report Received (Y/N)	
Coating Report Accepted (Y/N)	

Further Manufacturer's Correspondence received?	
Documents Received (Y/N)	
Details (brief description)	
Batch Report Accepted (Y/N)	

Comments

Name of Testing NRL	

Reported by (name) :	
Date of Report:	

Proposal to Accept Batch (Y/N)	

EURL001v3.0