



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**

Version 01 – March 2019

INDEX OF CONTENTS

1. Purpose.	2
2. Background-the current position	2
3. Aim- Unification of batch release throughout the EU.	3
4. Approach.	3
5. Manufacturer's batch release data.	3
6. NRL Batch testing.	4
6.1 BSE rapid test kits	5
6.2 EURL Reference control sample set	5
6.2.1 EURL Reference negative control sample	5
6.2.2 EURL Reference positive control samples	6
6.3 Initial levels agreement.....	6
7. QC batch testing protocol	6
7.1 Review Process only	6
7.2 Reference samples from EURL	6
7.3 Reference samples from manufacturer	7
7.4 Reference Batches	7
7.5 Analytical Timescale.....	7
7.6 Production of analytical data report	8
7.6.1 Reports that recommend non-release.....	8
7.7 Data Retention	8
8. Option for inspection of manufacturer's facilities	9
9. Contingencies	9

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



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Appendix 1: Actions for Manufacturers	10
Appendix 2: Actions for the nominated NRLs	11
Appendix 3: Reporting Spreadsheet Pages.....	12

1. PURPOSE

This procedural document details the approval process by which bovine 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 samples by:

- a) Assessment of batch release data provided by the manufacturer, and if required
- b) Production of additional QC data within a 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 authorised 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 Quality Control 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.

Initially, all kit batches were tested by the responsible NRL, but a risk-based approach is now adopted and, if evidence shows that paper based kit approval is sufficient, this is considered by the European Union Reference Laboratory (EURL) to be acceptable, as agreed by participating Member State (MS) NRLs at the EURL Conference in June 2010. In these circumstances, the right to test any or all kit batches will be retained by the responsible NRL. Manufacturers should continue to supply representative kits from each new batch.

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



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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

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

All NRLs involved must hold ISO17025 accreditation for the test for which they are responsible, to confirm their competency to perform the testing. If the NRL does not hold ISO17025 accreditation they can nominate an approved test laboratory in their country to carry out testing on their behalf. This laboratory must undergo regular proficiency testing (PT) to confirm their competency to perform the test. The NRL must have access to these PT results and must also be informed if any test related issues occur in the designated testing laboratory. NRLs may not control and approve tests 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 appropriate NRL:

1. An analytical batch release report. This 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). These have been approved by the EURL as part of quality system approval- manufacturers shall confirm that these are current on an annual basis.
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 taken. In the case of uncoated plates, evidence that the plates are homogeneous.



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

If the responsible NRL requires specific equipment to undertake batch testing this shall be provided, at no extra cost, by the manufacturer.

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

6. NRL BATCH TESTING

NRL batch testing can take 4 forms:

1. A small amount of analytical testing may be undertaken by the NRL. As standard, 2 sets of material should be used, with one set provided by the EURL and one by the manufacturer (ideally their current batch release panel). Each set shall consist of not more than 3 positives and at least 1 negative homogenate.
2. The kit manufacturer may elect to solely use the sample panel prepared by the EURL.
3. In exceptional circumstances, the kit manufacturer may elect not to use the sample panel prepared by the EURL. If the manufacturer makes this choice, they are responsible for:
 - i) Providing the EURL with a full explanation of why the EURL panel is unsuitable for the use as a batch testing panel for a particular test, including precise details of how a suitable sample panel should be prepared and stored.
 - ii) Providing an alternative sample set which is acceptable to the EURL (this is in addition to the current batch release panel). The manufacturer must provide the EURL with proper documentation attesting that the samples comply with the conditions in (i) above and detailing their origin and history.
 - iii) The manufacturer must prepare a suitable panel according to the above criteria and provide it to the EURL in sufficient quantity to be used as a stock for EC batch testing over a period of time. The manufacturer must provide the EURL with a copy of the protocol used to make the panel. The EURL will use an alternative test method on aliquots from all panel samples to quantify and accept them as appropriate.
 - iv) The sample set will be held by the EURL, provided to the responsible NRL for use and described as the "EURL approved panel".
4. Following consultation and agreement with the EURL, an NRL may choose to restrict their approval process to a review of the batch release QC data provided by the manufacturer (as described in 5.0).

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



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

6.1 BSE rapid test kits

These 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 nominated 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.

6.2 EURL Reference control sample set

These have been produced by the EURL and are pre-tested by rapid test and Western blot for level of activity. They are initially provided 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 NRL for parameter setting (6.3) and subsequent batch testing. Sufficient homogenate is supplied to all the relevant NRLs to undertake the required batch release analyses. All EURL reference control samples are provided free of charge.

6.2.1 EURL Reference negative control sample

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

6.2.2 EURL Reference positive control samples

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

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



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6.3 Initial levels agreement

The reference samples are to be used by the 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 4 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 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 an NRL chooses to approve by data review alone, a report should be produced which states this, as described in 7.6. The 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

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 homogenised) shall go through routine homogenisation/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.

Each of the 4 reference samples shall be tested in duplicate on each of 3 separate test plates. A mean result will be determined for each reference sample, and checked against the range. If all mean 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 both the remaining 2 plates, strips, etc. Kit reference samples are also to be used to confirm plate control reference results. Interpretation of this is up to the NRL but the EURL will review such results. The EURL reserves the right to make the final decision over interpretation of results.

7.3 Reference samples from manufacturer

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



TSE EURL

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Manufacturers are responsible for informing NRLs of the usage conditions for their own samples- in some cases samples are ready to put directly onto assay plates as per manufacturers routine QC procedures.

A manufacturer shall provide, for each batch, the reference samples used for sensitivity checks by the company, together with the acceptable range. These will be run on the same plates as the EURL reference samples and so should provide cross-checks on test performance. There should be three positive samples of different reactivity and one negative sample. They shall be tested in duplicate as for the EURL reference samples. If any of these fail to meet acceptance ranges provided by the manufacturer, the exercise shall be repeated in duplicate on the remaining 2 plates, strips, etc.

7.4 Reference Batches

The manufacturer shall also provide 3 plates from their current reference batch to the NRL. These shall be tested in parallel with the new batch, using both manufacturer's and 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 relevant NRL. The 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 10 working days of receipt of kits. This allows the NRL at least 5 days to undertake the testing and report to the EURL, and the EURL 5 days to complete analysis and report to the manufacturer and all EU NRLs. If a problem occurs, the appropriate NRL shall notify the EURL and the manufacturer promptly.

7.6 Production of analytical data report

The NRL shall prepare a report containing:

- An analysis of the batch release data produced by the NRL if performed and that provided by the manufacturer. Any NRL original data should be appended as an annex.

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



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

Istituto Superiore di Sanità - Rome

- Comment upon whether the manufacturers batch release criteria have been achieved.
- An analysis of the analytical results produced by the 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 EURL001v1.0** (Appendix 3). Reports should be sent by e-mail to: EURL.TSE.batchtesting@izsto.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.

7.6.1 Reports that recommend non-release

These 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 testing NRL shall treat all such information as confidential. In such a situation, it is imperative that the 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 to carry out trouble shooting and provide an anomaly report to 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 appropriate NRL and on EURL registered files.

8. OPTION FOR INSPECTION OF MANUFACTURER'S FACILITIES

The responsible 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

Batch Testing Protocol for Batch Release of TSE rapid test kits
TSE EURL Reviewed: March 2019 Version 01



TSE EURL

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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 appropriate NRL shall contact the EURL via the general email address (elena.bozzetta@izsto.it or daniela.meloni@izsto.it).

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).



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APPENDIX 1: ACTIONS FOR MANUFACTURERS

1. On production of a new batch of TSE kits intended for bovine use, an assessment of the batch release data for bovine performance must be provided to the NRL.
2. At least three plates from the current reference batch must be supplied to the nominated NRL for testing to ensure validity of measurement is achieved, for each batch release set.
3. Constituents (plates, reagents, buffers, etc.) sufficient for retesting at least 3 plates of each kit batch must be archived for reference purposes.
4. Representative samples of the production batch with appropriate controls and batch QC sensitivity reference samples, are to be supplied to the responsible NRL free of charge.
5. Any relevant equipment which may be required by the said NRL is to be provided at no further charge by the manufacturer.
6. Each delegated analyst for the kit testing at the nominated NRL must be certified by the kit manufacturer to undertake the QC testing as required.
7. An analytical batch release report must be supplied to the nominated NRL, to include sensitivity, specificity and intra and inter-plate controls.
8. The QC panel used by the manufacturer for batch release must be supplied to the appropriate NRL. Batch testing will follow one of 4 options as decided by the testing NRL and EURL.

Following this, options 1 to 4 will be considered by the EURL (*order changed*):-

1. Paper review only, if satisfied with manufacturer's data.
2. Occasional re-test with EURL panel, plus perhaps manuf. panel.
3. Continue testing with EURL panel only.
4. Keep testing with both panels (especially if issues have arisen).

If the NRL decide to approve without bench testing, the manufacturer will be notified immediately, but the manufacturer shall continue to supply all batch and QC materials as appropriate, to facilitate testing of the batch should it be required following data review by the NRL.

9. Reagents shall be used and appropriately labelled (as per section 6.1).



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APPENDIX 2: ACTIONS FOR THE NOMINATED 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.
5. Ensure that all relevant kit controls are utilised 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 programme, prepare the required short analytical report, in English, and supply to the EURL by e-mail to: EURL.TSE.batchtesting@izsto.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 NRL. 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 nominated 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 :			Kit Manufacturer :			
Test Kit Batch Number or ID :			Reference Kit Batch Number :			
Date Received at NRL :						
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 & 2	Interpretation	Test Plate/Strip 2 values 1 & 2	Interpretation	Test Plate/Strip 3 values 1 & 2	Interpretation
EURL Negative 1						
EURL Positive 1						
EURL Positive 2						
EURL Positive 3						
Manuf. Negative 1						
Manuf. Positive 1						
Manuf. Positive 2						
Manuf. Positive 3						
Reference batch results: These are reference plates provided by the manufacturer for comparison testing with new batches						
Reference Sample:	Ref. Plate/Strip 1 values 1 & 2	Interpretation	Ref. Plate/Strip 2 values 1 & 2	Interpretation		
EURL Negative 1						
EURL Positive 1						
EURL Positive 2						
EURL Positive 3						
Manuf. Negative 1						
Manuf. Positive 1						
Manuf. Positive 2						
Manuf. Positive 3						
Date of Testing :						
Comments :						
Accept Batch (Y/N) :			Date of Report :			
Reported by (name) :						



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Repeat Test Values (If Repeats Performed)			
Name of Testing NRL :			
TSE Kit Name :			Kit Manufacturer :
Test Kit Batch Number or ID :			Reference Kit Batch No :
Kit Batch Results: * These are new plates sent as representation of a newly produced batch for acceptance of the entire batch			
Reference Samples :	Test Plate/Strip 4 values 1 & 2	Interpretation	Test Plate/Strip 5 values 1 & 2
EURL Negative 1			
EURL Positive 1			
EURL Positive 2			
EURL Positive 3			
Manuf. Negative 1			
Manuf. Positive 1			
Manuf. Positive 2			
Manuf. Positive 3			
Reference batch results: * These are reference plates provided by the manufacturer for comparison testing with new batches			
Reference Samples :	Test Plate/Strip 4 values 1 & 2	Interpretation	
EURL Negative 1			
EURL Positive 1			
EURL Positive 2			
EURL Positive 3			
Manuf. Negative 1			
Manuf. Positive 1			
Manuf. Positive 2			
Manuf. Positive 3			
Date of Testing :			
Comments :			
Reported by (name)		Date of Report	
		Accept Batch (Y/N)	



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Batch Release Documentation Check Sheet			
TSE Kit Name :		Kit Manufacturer :	
Test Kit Batch Number or ID :		Reference Kit Batch No :	
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) :			