INTER-LABORATORY COMPARISONS

The provision of proficiency testing for TSE rapid tests by the APHA

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Abstract

Under Regulation (EC) No. 999/2001, the Animal and Plant Health Agency (APHA) in the UK, in its capacity as a European Union Reference Laboratory (EURL), is responsible for the provision of an annual programme of proficiency testing (PT) schemes for the assessment of diagnostic procedures for transmissible spongiform encephalopathies (TSEs) at both European and national levels. These ISO17043-certified schemes target European Union (EU)-approved commercial screening tests used to carry out surveillance of bovine spongiform encephalopathy (BSE) and scrapie. Reference materials are generated from whole tissues; all include the marker protein PrPres in a range of concentrations similar to those found in both clinical and preclinical animals. Participating laboratories must perform satisfactorily in these PT schemes to be authorised to carry out national surveillance testing. Root cause analysis indicates that PT failures are most likely to occur as a result of inadequate laboratory protocols or training. However, trend analysis of EU National Reference Laboratory (NRL) test data demonstrates that, despite variation in the quantitative performance of tests, standards are being maintained at a high level despite a declining prevalence in disease across Europe.

Keywords

- BSE
- Laboratory proficiency testing
- Rapid test
- Scrapie
- Statutory

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INTER-LABORATORY COMPARISONS

Introduction

Transmissible spongiform encephalopathies (TSEs), such as bovine spongiform encephalopathy (BSE) in cattle and scrapie in small ruminants, are a group of rare neurodegenerative disorders affecting the central nervous system of both humans and animals that can arise as genetic disorders, transmit directly from animal to animal or be transmitted through the presence of infectivity in feed or food [Mastrianni, 2010; Bruce *et al.*, 1997; Wilesmith *et al.*, 1988]. These disorders are also referred to as Prion diseases and are characterised by the accumulation of an abnormal isoform of a host-encoded protein called prion protein (PrP) [Collinge and Clarke, 2007]. This abnormal prion protein (PrPres) is disease-specific, protease-resistant and is currently the most consistent marker for disease. PrPres can only be reliably detected on post-mortem examination.

In order to safeguard public and animal health, the European Commission introduced Regulation (EC) No. 999/2001 laying down rules for the prevention, control and eradication of certain TSEs [EU, 2001], and each Member State was required to nominate a National Reference Laboratory (NRL) to implement the requirements of the regulation. An over-arching EU reference laboratory (EURL) was also nominated to oversee testing and related activities. First published in 2001, and updated on numerous occasions in line with updated scientific evidence, these regulations define the functions and responsibilities of the TSE EURL and each individual Member State NRL, along with rules for sampling and laboratory testing approaches. The Animal and Plant Health Agency (APHA) has been the UK NRL and the EURL since 2001.

Due to the effect of control policies introduced at a European level, the prevalence of naturally occurring cases of TSE in food animal species has declined. However, the statutory testing defined in the Regulation means that over 3 million cattle and almost 500,000 small ruminants were subjected to screening for TSEs in 2013 [EU, 2015].

TABLE 1 / TSE rapid tests approved for use in Europe and defined within Commission Regulation (EU) No 1148/2014 [EU, 2014].

Rapid Tests	BSE Monitoring	Scrapie Monitoring
Bio-Rad TeSeE® SAP	✓	✓
Bio-Rad TeSeE® Sheep/Goat	×	✓
IDEXX HerdChek® BSE-Scrapie Antigen EIA	✓	✓
Prionics®-Check LIA	✓	×
Prionics®-Check Western	✓	×
Prionics®-Check PrioSTRIP	✓	×
Prionics®-Check PrioSTRIP SR, visual reading protocol	×	✓
AJ Roboscreen BetaPrion® BSE EIA	✓	×

The EU legislation covering TSEs clearly defines the commercial screening tests that have been approved by the Commission for use in the statutory surveillance of TSEs (Table 1). Prior to approval, these tests were subject to independent evaluation of test performance for specific forms of TSE [EFSA, 2009; EFSA, 2012]. Commonly known as 'rapid tests', they all use the principle of immunocapture to detect the presence of PrPres. The test platforms include an immunoblot and a variety of different immunoassays, including sandwich, microplate-based, chemical polymer and lateral flow immunoassays. The common feature of all of these tests is that they enable high throughput testing which is fast and reliable.

INTER-LABORATORY COMPARISONS

Overview of TSE proficiency testing

Proficiency testing (PT) allows samples of known but undisclosed provenance to be introduced into laboratories for the purpose of providing independent and confidential verification of the testing process. This is essential for assuring the European Commission, the EURL, NRLs and other testing laboratories of the continued competence of laboratory staff and ensuring the maintenance of technical expertise.

Under the TSE legislation, the APHA has responsibilities under both its TSE EURL and NRL remit to provide periodic PT to assess diagnostic procedures at the EU and national levels. This service is also provided commercially to TSE screening laboratories worldwide.

All EURL PT testing for TSE rapid tests in cattle and small ruminants is distributed through the APHA Quality Assurance Unit 'Vetqas', an internationally recognised market leader in the provision of PT schemes for veterinary laboratories that currently offers a range of veterinary schemes (http://ahvla.defra.gov.uk/apha-scientific/services/vetqas/index.htm).

■ Reference materials

Prion protein cannot be propagated in vitro in quantities sufficient for use in PT schemes. Reference material therefore has to be created from whole tissues from a variety of sources. Where possible, these are natural field cases of TSEs, identified through both active and passive surveillance routes in the UK. Additionally, the TSE EURL receives funding directly from the EC to generate infected tissue from animals experimentally inoculated with less prevalent strains of TSE, which cannot be sourced in sufficient quantity (if at all) from natural cases. These experimental tissues are generated specifically for use in PT schemes and test evaluation exercises.

All source materials used in the manufacture of TSE reference samples are managed by the APHA TSE Biological Archive, the largest and most comprehensive repository of TSE material worldwide (http://www.tse-lab-net.eu/biological-archive/index.html).

■ Preparation and storage of PT samples

All samples distributed within TSE rapid test PT schemes are brain homogenates or macerates which have been prepared at APHA using protocols developed by the TSE EURL so that they are suitable for use by all EU-approved rapid tests. All tissues are initially cleaned to remove connective tissue and other debris. To prepare tissue homogenates, samples are combined with an equal volume of purified water and then homogenised using a household hand blender for three bursts of 30 seconds. The addition of purified water ensures the creation of a homogeneous product which can be easily diluted using negative tissue homogenates from the same species in order to provide a range of PT samples (classified as strong, medium or weak positive) to mimic the range of PrPres signals that may be expected in preclinical animals [Arnold *et al.*, 2007]. The only exception to the above protocol is for atypical scrapie samples, which are finely diced with no addition of purified water, resulting in a fine macerate. This approach was developed following anecdotal data from NRLs, and EURL observations that atypical scrapie PrPres signal stability could be compromised by sample homogenisation.

Homogenates and macerates are generally subdivided into aliquots of approximately 1.3 g. This provides sufficient material for each participant to carry out up to three different tests, depending on the methods in use within their laboratory.

Prior to their use as PT samples, 4% of the total number of aliquots from each batch are pre-tested by an EU-approved rapid test, and a single sample is tested by Western blot. This establishes both consistency across aliquots (as required by ISO/IEC 17043 Conformity As-

INTER-LABORATORY COMPARISONS

sessment – Guidelines for Proficiency Testing) and a baseline value (the intended result) for assessing samples as strong, medium or weak positive.

Following preparation and initial suitability testing, samples are returned to dedicated PT sample storage at -80°C until required for use. A specialised database is used to trace the history, storage location and destination of each sample used in PT distributions.

■ TSE PT Schemes

APHA currently provides ISO/IEC 17043-accredited PT schemes for both bovine and small ruminant rapid tests. TSE testing laboratories working to ISO/IEC 17025 or equivalent (as required by EC Regulation 882/2004), can use successful participation in these schemes as evidence of competence for third party accreditation bodies.

The TSE EURL currently holds multiple aliquots from 17 bovine and 27 small ruminant sources representing all currently identified TSE field strains. This allows the delivery of targeted rounds, which may include all the TSE types known to infect each species. These schemes are distributed 1-3 times per year and comprise a combination of 5 to 7 positive or negative samples (Table 2). The positive samples are selected to represent a variety of target signal intensity, including those signal intensities that may challenge the detection threshold of approved rapid tests. Inclusion of samples from the same batch in multiple PT schemes enables monitoring of signal stability over time. In addition, duplicate samples may be included within a single distribution to further challenge test repeatability.

TABLE 2 / Summary of annual TSE rapid test PT schemes organised by APHA in 2015.

PT Distribution type	Target	Frequency (per year)	No of participating laboratories (2015)	No of samples in distribution
Bovine BSE Rapid Test	EU NRLs	1	26	7
	UK Diagnostic Laboratories	3	2	7
	Worldwide Commercial	2	9	5
Small Ruminant Scrapie Rapid Test	EU NRLs	1	27	7
	UK Diagnostic Laboratories	2	2	7
	Worldwide Commercial	2	8	5

Each PT distribution is dispatched in a frozen state as UN3373 Biological Substance Category B. Samples are blind labelled and provide enough tissue for 2-3 different tests to be carried out, enabling sufficient available material for testing labs to maintain proficiency using a number of different test methods or personnel, if required.

■ Reporting and analysis of results

Vetqas operates an on-line Proficiency Testing Laboratory Information Management System (PT LIMS). Participating laboratories are allocated a confidential laboratory ID number which

they use when entering their test results and supplementary supporting information such as test expiry dates, positive and negative control values and test cut-offs directly onto the PT LIMS.

Participants are encouraged to provide comments and raw data in support of their reported results. Such supporting information also provides assurance that laboratories are able to correctly interpret their testing outcomes and would take the correct action if an anomalous result occurred under routine testing conditions.

Once participants have entered their results, PT LIMS is used to produce tabulated reports which are reviewed and commented upon by the EURL. Reports receive a conformity check by Vetqas staff, and an e-mail is then sent from PT LIMS informing participants that tabulated results and commentary are available to view.

■ Follow-up of anomalous results

If any laboratory performing statutory testing fails to demonstrate their competence in EURL PT schemes, the Commission requires the EURL to suspend the testing activities of that laboratory. Such laboratories must submit an anomaly report to the EURL and refer testing activities to a competent laboratory until issues have been resolved.

In discussion with the affected laboratory, the EURL performs a root cause analysis based upon scrutiny of the original raw data and the submitted anomaly report. Root cause analysis has identified several reasons why participants fail to provide the expected diagnostic result. These have included transcription error, operator error, equipment or test kit failure, accidental or deliberate use of out of date kits, failure to follow instructions as stated within test kit inserts, possible sample misidentification, variations in signal stability across different combinations of TSE strains, and test kit format.

If the error identified through root cause analysis is not technically based, *e.g.* transcription error, and the laboratory has demonstrated that protocols have been reviewed and retraining has taken place, then the EURL can elect to allow the laboratory to resume testing without undertaking any follow-up PT.

When technical issues are identified, the laboratory must confirm that the appropriate corrective actions and retraining have been completed. Competence must then be demonstrated by successful participation in a follow-up PT scheme before statutory testing can be resumed.

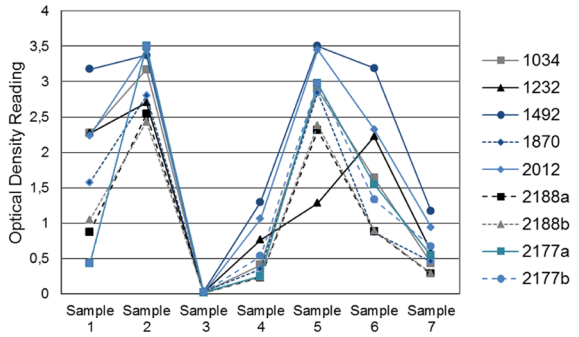
Trend Analysis of PT data

Regardless of the test being used, variability at a laboratory level may lead to differences in absolute test outputs. The data in figures 1, 2 and 3 illustrate a single representative PT distribution for three different bovine rapid tests. Each set of data was generated from laboratories that used the same test batch. It can be seen that, despite the differences between absolute values generated by laboratories, performance is generally consistent within any individual laboratory when individual replicate samples are compared, or where laboratories have carried out tests twice.

The ability to differentiate between strong, medium and weak positive samples varies depending on the test used. The absolute values generated by tests 1 and 3 reflect the intended sample strength (strong, medium and weak positive), however they differ in the range of data submitted, for example the range of data submitted for sample 1 using test one was 0.437-3.176 (upper limit of detection 3.5) and the range for test 3 for the same sample was

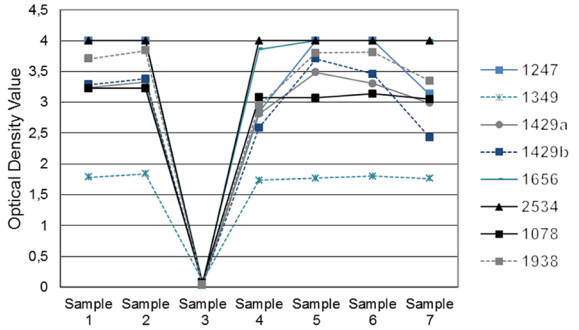
INTER-LABORATORY COMPARISONS

FIGURE 1 / Bovine rapid test 1 data from all laboratories using the same batch of a single test.



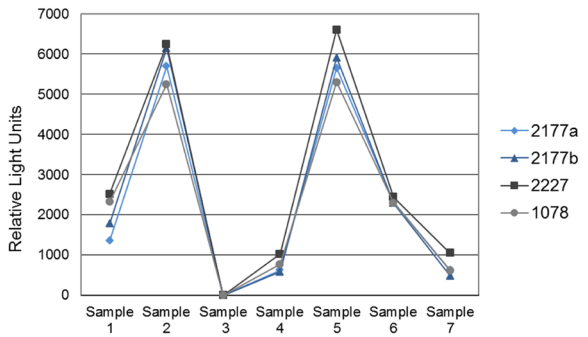
Each coloured line represents a single laboratory identified by its confidential laboratory ID number. Laboratories carrying out the tests twice are denoted by a or b. Samples 1 and 6 are medium positive replicates, samples 2 and 5 are strong positive replicates, samples 4 and 7 are weak positive replicates.

FIGURE 2 / Bovine rapid test 2 data from all laboratories using the same batch of a single test.



Each coloured line represents a single laboratory identified by its confidential laboratory ID number. Laboratories carrying out the tests twice are denoted by a or b. Samples 1 and 6 are medium positive replicates, samples 2 and 5 are strong positive replicates, samples 4 and 7 are weak positive replicates.

FIGURE 3 / Bovine rapid test 3 data from all laboratories using the same batch of a single test



Each coloured line represents a single laboratory identified by its confidential laboratory ID numberLaboratories carrying out the tests twice are denoted by a or b. Samples 1 and 6 are medium positive replicates, samples 2 and 5 are strong positive replicates, samples 4 and 7 are weak positive replicates.

1356-2519 (upper limit of detection >6,600), a much tighter distribution. Conversely, data from test 2 indicates that this sample panel does not appear to fully challenge the lower limit of detection threshold for this test.

However, the basic requirement of all screening tests is that they are able to distinguish between positive and negative samples under field conditions. EURL analysis of PT data clearly shows that the laboratories currently undertaking TSE surveillance testing in the EU are doing so competently, with only 0.224% (6 in 2675) PT test failures occurring between 2009 and 2015, attributable to operator errors.

Root cause analyses have shown that a small number of infrequent anomalies relate to human factors, such as sample handling errors. Consequently, the EURL is of the opinion that the key to sustaining test competency lies in the maintenance of effective laboratory protocols and operator training.

Despite the variation observed, the sample panels used are appropriate for assessing the performance of the tests at the qualitative level, which is the primary requirement of these exercises. Additionally, examination at a quantitative level helps the EURL to informally monitor test performance, both in terms of batch quality and of individual laboratory performance. Batch performance can be monitored at a single time point using data generated from within a single distribution, or within the same laboratories at multiple time points using data gathered from multiple distributions, allowing the EURL to raise any emerging issues with the manufacturers for investigation. Issues relating to dips or increased variability in individual laboratory performance may also be identified using single sample data gained at single/multiple time points from single/multiple distributions that can be investigated at a local level.

These data are therefore of value in the ongoing assessment of both test and laboratory performance, providing European laboratories with confidence in their ability to successfully maintain these tests in spite of the declining prevalence of TSE disease and reducing surveillance requirements.

INTER-LABORATORY COMPARISONS

Conclusion

TSE rapid screening tests are designed to differentiate between positive and negative samples. Through the provision of periodic proficiency testing using sample panels which are suitable for use on all EU-approved rapid tests, the TSE EURL and NRL assess the diagnostic capability of laboratories at both EU and national levels. Overall, the test data generated within these PT schemes supports the robustness of the tests currently in use.

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