Validation of a new rapid assay for BSE screening in cattle

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Abstract
Confirmatory diagnosis of BSE in cattle is done by histopathology and immunohistochemistry. In Europe three rapid tests, a Western Blot (Pionics) and two ELISA tests (BioRad, Enfer), were accepted for BSE screening in 2000. The EU defined the procedure how newly developed rapid tests have to be compared to accepted tests to ensure that they show no difference in accuracy (sensitivity and specificity). After allowing for single retesting of positive samples in a newly developed Prionics ELISA (LIA) there was 100% agreement between it and the comparison tests. The EU and Switzerland subsequently accepted the LIA for BSE screening.

Introduction
At current, the confirmatory diagnosis of transmissible spongiform encephalopathies (TSE) such as BSE in cattle and scrapie in small ruminants (sheep and goats) is primarily done by histopathology and immunohistochemistry (IHC). The detection of the protease-resistant form of the prion protein, PrP^{Sc}, in the brain stem (obex) of cattle is routinely used as the positive gold standard. The method has a very high specificity and, at least in clinically diseased cattle, a sensitivity that approaches 100%. However, the sensitivity quickly declines in animals in earlier stages of the incubation period. Testing data of 11'931 bovine brain samples from six different laboratories were used to compare the new Prionics LIA® ELISA with existing tests.

In the European Union in 2001 an active targeted screening program for BSE in cattle was started. Within this program, three EU-validated rapid tests have been used. Within the member states (excluding UK) between January 1, 2001 and Dec. 31, 2002, a total of 4'121 clinical BSE suspects, 1.7 Million risk animals (emergency slaughter, fallen stock), 16.6 Million adult healthy-slaughtered cattle and 0.1 Million culled cattle (BSE eradication) were tested with those rapid tests. In total, 1'970 BSE cases were detected in the rapid tests and subsequently confirmed by another methods (in most instances by IHC or and extended OIE Western blot).

The EU has defined the procedure under which newly developed rapid tests have to be compared to one of the three existing (validated) tests in order to ensure that the new test show little or no difference in accuracy (sensitivity and specificity) when compared to already validated and routinely applied rapid screening tests. In the context of this study, testing data of 11'931 bovine brain samples from six different testing sites were used to compare the new Prionics LIA® ELISA with existing tests (Prionics Check® Western Blot and BioRad Platelia® ELISA). The vast majority (96.4%) of the samples was reported to be of good quality (fresh), while 230 (1.9%) were autolytic. A total of 234 samples (2%) in the set were reference test positive, and

therefore considered to be true positive, while the remaining samples were considered to be gold standard negative.

Of the 238 samples that were initially positive (reactors) in the LIA, 10 came from reference test negative samples. The majority of those initially false-positive samples originated from one screening laboratory that only recently had implemented the LIA ELISA. After allowing for single retesting of the initial LIA-positive samples and classifying a retest-negative sample as negative there was 100% agreement between the LIA and the reference tests. Both the sensitivity and specificity point estimates were 100% with 5%iles (comparable to the lower limit of a 90% confidence interval) of at least 98.7% (SE) and 99.97% (SP).

Table 1 – Calculation of Prionics LIA® ELISA sensitivity and specificity in comparison with the results of a different EU-validated rapid test

Category	LIA result before retesting of initial reactors	First LIA test result and retesting result combined
True pos. (A)	228	228
False pos. (B)	10	0
False neg. (C)	0	0
True neg. (D)	11685	11695
Total	11923	11923
Overall agreement	99.92%	100%
KAPPA statistic	0.978	1.00
Sensitivity point est.	100	100
50%ile*	99.70	99.70
5%ile*	98.70	98.70
Specificity point est.	99.91	100
50%ile*	99.91	99.99
5%ile*	99.86	99.97

*The percentiles were calculated by using a probability function (with 10'000 iterations) of the type BETA(event + 1; total – event + 1) where the event is a true positive or a true negative test result, and the total is the number of gold-standard (reference test) positive resp. negative samples in the study. Of the 10’000 iterations to estimate the respective test sensitivity and specificity, half were above the given 50%ile value and only 5% below the given 5%ile value.

It was concluded that the LIA was at least comparable to the existing (validated) rapid tests, and the European Commission and the Swiss Federal Veterinary Office accepted the LIA in 2003 for BSE screening.

One of the remaining major problems of TSE testing is that only specific CNS regions can be used for diagnosis and that the sample preparation is very different for the different diagnostic methods (histology, immunohistochemistry, various rapid tests). One advantage in addition to increasing number of comparable tests available for BSE screening is that screening laboratories now have at least two tests (Prionics WB and Prionics LIA) that use the same CNS sample preparation (homogenisation) protocol.

Another observation is that there is a general expectation that BSE tests are 100% sensitive and specific, and therefore yield no false (positive and negative) results. This, for all practical purposes, is not possible with a serological test such as an ELISA. From the view of a reference laboratory we would expect a certain proportion of initial positive field test results that subsequently can not be confirmed with gold standard methods. If these initial reactive samples are not reported, we have to conclude that the test, in its application in the field, is operating with lower sensitivity than expected, and might miss some true positive samples.

References

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