OIE Procedure for Validation and Certification of Diagnostic Assays

Abstract sheet

Name of the diagnostic kit: Prionics AG-Check WESTERN
Manufacturer: Prionics AG
OIE Approval number: 20080102
Date of Registration: May 2008

Disease: Bovine Spongiform Encephalopathy

Pathogen Agent: BSE-related prion protein PrP^{Sc}

Type of Assay: The Prionics AG-Check WESTERN kit is based on the Western blotting technique and allows detection of the altered prion protein PrP^{Sc} after protease digestion, from brain tissue.

Purpose of Assay: Certified by the OIE in May 2008 as fit for the post-mortem diagnosis of bovine spongiform encephalopathy in cattle and for the following purposes:

1. To confirm diagnosis of suspect or clinical cases (includes confirmation of a positive screening test);
2. To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control) and to assist in the demonstration of the efficiency of control policies;
3. To confirm a non-negative test result obtained during active surveillance with a different type of test.

Species and Specimen: Cattle. PrP^{sc} is not homogeneously distributed in brain tissue. It is important to follow the sampling instructions provided in the kit precisely. It is recommended that labs use a method to monitor sampling accuracy - advice is available from OIE reference laboratories for TSE.

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at info@prionics.com Phone: +41 44 200 20 00; Fax: +41 44 200 20 10.

2. Summary of validation studies

STAGE 1 Validation

Analytical sensitivity

- Dilution series were tested in the course of the European Union validation study. Of 20 positive homogenates tested at a 10^{-1} dilution, 15 scored positive, two doubtful and three negative. At the $10^{-1.5}$ dilution three samples were scored doubtful and the remainder negative. Two samples were also scored doubtful at 10^{-2} and a single sample at $10^{-2.5}$ dilution. [Positive samples were supplied by the Central Veterinary Laboratory (CVL), Weybridge: brainstem and spinal

cord samples were selected from bovines showing clinical signs of BSE with confirmation by histological examination.]

Analytical specificity — - This has not been specifically examined. However some field studies using fallen stock samples (animals suffering from disorders other than BSE, e.g. encephalitis, brain oedema, rabies, listeriosis) have consistently shown that Prionics AG-Check WESTERN kit has a good analytical specificity [see Surveillance of BSE, D. Heim and al, Arch Virol, Suppl. 2000, (16):127-33)].

Repeatability data — - Tests carried out over a period 2002 to 2007 show that the kit could detect a BSE positive sample at a 1/10 dilution.

- The repeatability was also studied in comparative testing of three BSE tests (Enfer – Bio-Rad TeSeE – Prionics Check Western) conducted by the EU Community Reference Laboratory for TSE (VLA, United Kingdom). The consistency between duplicate samples (n=277) was high, except on two occasions when carryover from an adjacent positive well resulted in a small non-specific smudge in one of the adjacent wells of a negative sample. This was clearly different from positive results, which were of characteristic size, banding pattern and present in both duplicate wells.

STAGE 2 Validation

Three external evaluation studies have been described for the estimation of diagnostic sensitivity and specificity:

- Tests performed at the Swiss BSE reference laboratory (Neurocenter/University of Bern) in February 2003 on 38 positive samples (18 homogenates & 20 tissue samples) from the UK / 190 negative samples from the Swiss BSE surveillance program (tissues samples from cattle older than 30 months that had tested negative by histology/immunohistochemistry).

- Canadian field study, 2003. As a follow up to the detection of the index case in May, 2003, 2036 birth and feed cohorts were tested by immunohistochemistry (IHC) and the Check WESTERN kit. This work was carried out by Canadian Food Inspection Agency laboratories at Lethbridge, Winnipeg, Nepean and St-Hyacinthe.

- Evaluation of tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines for the European Commission, 1999. All samples were prepared by the EU Institute for Reference Materials and Measurements (IRMM) at Geed, Belgium and presented for testing in a coded ‘blind’ format to the different participants (Prionics AG-Check WESTERN kit was one of the four selected kits): A total of 300 positive samples (collected from cattle showing clinical signs of BSE and confirmed by histological examination - supplied by CVL Weybridge) and 1000 negative samples (collected in New Zealand from healthy adult cattle of at least 4 years of age and confirmed negative by histological examination).

- Threshold determination

This test does not give a quantitative reading. The response is qualitative and is based on the two criteria of the presence of the signal and its position. This allows easy discrimination between true positives and (potentially) false positives due to undigested normal PrP.

- Diagnostic sensitivity (DSn) and specificity (DSp) estimates with 95% confidence limits (CI)

Tests performed at the Swiss BSE reference laboratory:

	BSE positive by IHC	**BSE negative by IHC**
Test Positive by Prionics Check-WESTERN	38	0
Test Negative by Prionics Check-WESTERN	0	190
Diagnostic Sensitivity: 100%, CI [90.7 – 100.0%] Diagnostic Specificity: 100%, CI [98.1 – 100.0%]		

Canadian Field study:

	BSE positive by IHC	**BSE negative by IHC**
Test Positive by Prionics Check WESTERN	1	0
Test Negative by Prionics Check WESTERN	0	2036
Diagnostic Specificity: 100%, CI [99.8 – 100.0%]		

Evaluation of tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines for the European Commission

	BSE positive by histology	**BSE negative by histology**
Test Positive by Prionics Check-WESTERN	300	0
Test Negative by Prionics Check-WESTERN	0	1000
Diagnostic Sensitivity: 100%, CI [98.8 – 100%] Diagnostic Specificity: 100%, CI [99.6 – 100.0%]		

- Agreement between tests

The agreement of the Prionics AG-Check WESTERN with histological examination and IHC is high.

STAGE 3 Validation

During the EU field evaluation of 2004, samples were tested using the Prionics AG-Check WESTERN. These results were compared to the results achieved with other tests under evaluation. The samples were divided into three categories: positive samples, negative samples and samples of poor quality.

- A total of 335 BSE positive samples were tested in three labs (VLA, Newcastle, UK - Analytico Food BV, Heerenveen, Holland and Laboratorio Central de Veterinaria, Algete, Spain).

- A total of 24,534 BSE negative samples were tested in eight labs (Analytico Food BV, Heerenveen, Holland,; Institut für Veterinärmedizin, Mödling, Austria; Instituut voor Dierhouderij en Diergezondheid, Lelystad, Holland; Laboratorio EET, Leon, Spain; Labor Dr. Guenteert, Luzern, Switzerland; Instituto Zooprofilatiico Sperimentale del Piemonte, Turin, Italy; Irish Equine Centre, Kildare, Ireland; Arthus Biotech GmbH, Hamburg, Germany).

- An additional 423 samples of poor quality were tested in two labs (VLA, Newcastle, UK and NeuroCenter, Bern, Switzerland).

The inter-laboratory results of all samples tested were identical for the Prionics AG-Check WESTERN. Agreement with other tests under evaluation were very high with the exception of one weak positive sample detected in the Prionics AG-Check WESTERN that was not identified as positive when using the Ceditect.

Source: Report: The Field Trial of Seven New Rapid Post Mortem Test for the Diagnosis of Bovine Spongiform Encephalopathy in Bovines; IRMM; 12 November 2004

Stage 4 Validation

This is an ongoing process. Testing laboratories should participate in proficiency testing and laboratory training programmes organized by OIE Reference Laboratories.

Prionics AG-Check WESTERN is currently in use in both reference and routine laboratories throughout the world.

3. References:

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2. DOHERR MG, OESCH B, MOSER M, VANDEVELDE M, HEIM D.; Targeted surveillance for bovine spongiform encephalopathy; Veterinary Record 1999 Dec 4; 145:672.
3. SCHALLER O, FATZER R, STACK M, CLARK J, COOLEY W, BIFFIGER K, EGLI S, DOHERR M, VANDEVELDE M, HEIM D, OESCH B, MOSER M.; Validation of a Western immunoblotting procedure for bovine PrPSc detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE); Acta Neuropathol (Berl). 1999 Nov;98(5):437-43
4. CALAVAS D, DUCROT C, BARON T, MORIGNAT E, VINARD JL, BIACABE AG, MADEC JY, BENCSIK A, DEBEER S, ELIAZSEWICZ M.; Prevalence of BSE in western France by screening cattle at risk: preliminary results of a pilot study; Vet Rec 2001 Jul 14;149:55-56
5. D. HEIM, D AND WILESMITH, JW; Surveillance of BSE; Arch Virol, Suppl. 2000, (16):127-33
6. Chapter 2.4.6., Bovine Spongiform Encephalopathy; *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 2008, OIE