OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: TeSeE™ WESTERN BLOT
Manufacturer: Bio-Rad
OIE Approval number: 20090105
Date of Registration: May 2009

Disease: Prion associated diseases (Scrapie, Bovine Spongiform Encephalopathy, Chronical Waste Disease)

Pathogen Agent: Abnormal prion protein PrP^{Res}

Type of Assay: The TeSeE™ WESTERN BLOT kit is based on the Western blotting technique and allows detection of abnormal prion protein PrP^{Res} after protease digestion.

Purpose of Assay: Certified by the OIE in May 2009 as fit for the post-mortem detection of transmissible spongiform encephalopathies (TSEs) in cattle (bovine spongiform encephalopathy, BSE), in ovines and caprines (BSE and scrapie), and in cervids (chronic wasting disease, CWD), and for the following purposes:

1. To confirm TSE suspected positive samples detected at the screening laboratories in countries with active/passive surveillance programmes. Any sample with a negative result according to the TeSeE™ WESTERN BLOT assay interpretation criteria, following a positive rapid test result, should be tested with one of the other OIE certified confirmatory methods, Immunohistochemistry (IHC) or Scrapie-associated fibrils (SAF)-Immunoblot;
2. To confirm the prevalence of infection with one of the TSE associated diseases (BSE, scrapie, CWD) in the context of an epidemiological survey in a low prevalence country;
3. To estimate prevalence of infection to facilitate risk analysis (e.g. surveys, implementation of disease control measures) and to assist the demonstration of the efficiency of eradication policies.

Species and Specimen: Bovine (obex), Cervids (obex and lymph nodes), and ovine and caprine (obex)

1. Information on the kit

Information is available on Bio-Rad Website, at www.bio-rad.com

2. Summary of validation studies

Analytical characteristics

Calibration:

Since no reference standard is available, the TeSeE™ WESTERN BLOT assay was calibrated against the Bio-Rad TeSeE rapid test (TeSeE immunoassay: sandwich ELISA format), on a

panel of Immunohistochemistry (IHC) confirmed positive samples, including weak positive and atypical scrapie samples. The TeSeE™ WESTERN BLOT could detect all positive samples.

Repeatability:

Both within run and between run repeatability were evaluated.

Within run: The results were perfectly reproducible when testing 32 times within the same day, a panel of 1 negative and 3 positive samples. Within run repeatability was confirmed by the Reference Laboratory for European Union (CRL), VLA, Weybridge, UK, within assay study.

Between run: The results were perfectly reproducible when testing a panel of 1 negative and 3 positive samples, in duplicate, for 20 days.

Between serial repeatability is verified for every reagent batch produced through the final quality control procedure performed in the manufacturing site with a specifically designed Quality Control sample panel.

Analytical specificity:

As specimens collected on animals infected both prion and other pathogen are difficult to obtain, analytical specificity was not studied.

Analytical sensitivity:

The TeSeE™ WESTERN BLOT assay was demonstrating the same sensitivity than the Bio-Rad TeSeE S/G Rapid test (ELISA) and than the OIE scrapie-associated fibrils (SAF) blot method.

<u>Diagnostic characteristics</u>

Field samples from passive or active surveillance programs were used for the evaluation studies of the TeSeE™ WESTERN BLOT assay. The status (positive or negative) of these samples had been determined with one of the European Commission approved screening methods and/or IHC reference method. The ability of the TeSeE™ WESTERN BLOT assay to confirm their status was evaluated. The same evaluation approach was used in all evaluations performed.

Study I Internal evaluation at Bio-Rad, France
- Analytical sensitivity on ovine and bovine characterized samples from veterinary reference laboratories
- Diagnostic specificity on ovine and bovine samples from French slaughterhouses (animal without any clinical sign) classified as negative with a rapid screening tests (TeSeE™ ELISA)
- Diagnostic sensitivity on ovine samples from French flocks with natural cases of TSE tested with the TeSeE™ ELISA and IHC;

Study II External evaluation at CRL, VLA, Weybridge, UK
- Diagnostic specificity and sensitivity on bovine samples from UK passive surveillance
- Diagnostic specificity and sensitivity retrospective study on bovine samples from UK

- Diagnostic specificity and sensitivity on ovine samples from UK active and passive surveillance;

Study III External study at CODA-CERVA, Belgium: prospective study on bovine samples from routine screening and retrospective study on previously BSE positive samples from the laboratory routine;

Study IV External study at IZW Berlin, Germany: retrospective study on 104 cervid samples (deer and elk) from Germany, USA and Canada, characterized by IHC including 64 CWD positive samples;

Study V External study at AFSSA, France: retrospective study on scrapie positive ovine samples choosen randomly among the scrapie diagnosed cases between 2002 and 2004 in France (positive with the French reference AFSSA Western blot method and with the TeSeE™ Sheep/Goat ELISA);

Study VI External retrospective study at AFSSA, France: Arsac et al, Acta Neuropathol., July 2007: retrospective study on BSE positive obex collected in France during active and passive surveillance between 2001 and 2003 (confirmed positive with the AFSSA Western blot and analysed with IHC in case of discrepancy between rapid test and Western blot);

Study VII External retrospective study at AFSSA, France: Arsac et al, EID January 2007: retrospective study on 54 atypical scrapie positive samples detected by the rapid test TeSeE™ ELISA, collected in France during the scrapie surveillance program between 2002 and 2004;

Study VIII External retrospective study on BSE positive samples (including atypical BSE cases) at CRL, VLA, Weybridge: Terry et Al., Veterinary Record, June 2007: retrospective study on 5 BSE positive confirmed samples (including one French H type from AFSSA and one H type from UK) and 1 scrapie positive confirmed sample;

Study IX CFIA Ottawa (external study): retrospective study on 50 positive elk brain samples, 40 positive White Tailed Deer brain, 65 positive White Tailed Deer retropharyngeal lymph node samples;

Study X USDA Ames Iowa (external study): retrospective study on 53 positive deer retropharyngeal lymph node samples.

Threshold determination

The threshold was determined according to the following criteria:

Negative samples: They do not show any signal, since PrP^c was totally digested by proteinase K.

Positive samples:

• Classical positive samples: They show a typical 3 band pattern, demonstrating digestion of PrP^c and transformation of the disease specific prion protein into a proteinase resistant core fragment with reduced molecular mass following removal of N-terminus part of the protein. The two higher bands correspond to mono and di-glycosylated forms (27-30 kDa) while the lower band corresponds to the un-glycosylated form of the protein.

• Nor 98 positive samples: They show an atypical glycoprofile. A lower band is visible at approximately 12kDa, while other upper bands are not located at the same positions compare to classical scrapie cases.

• Other samples that should be repeated: The samples for which only the di-glycosylated band is detected and the samples for which only the di-glycosylated band and the mono-glycosylated band are detected.

Diagnostic sensitivity (DSn) and specificity (DSp) (with 95% confidence limits [CI])

TeSeE™ WESTERN BLOT		**Target Species : Bovine**
Diagnostic sensitivity	N DSn CI	*315* *99.0 %* *97.2 – 99.8*
Diagnostic specificity	N DSp CI	*282* *99.3 %* *97.5 – 99.9*

TeSeE™ WESTERN BLOT		**Target Species : Ovine**
Diagnostic sensitivity	N DSn CI	*306* *98.0 %* *95.7 – 99.3*
Diagnostic specificity	N DSp CI	*141* *100 %* *97.4 – 100*

TeSeE™ WESTERN BLOT		**Target Species : Cervid**
Diagnostic sensitivity	N DSn CI	*272* *100 %* *98.65 – 100*
Diagnostic specificity	N DSp CI	*40* *100 %* *91.2 – 100*

Agreement between tests

The TeSeE™ WESTERN BLOT assay was evaluated on different animal species (bovine, ovine, goat and cervids), in several National Reference laboratories, and in comparison with the confirmation methods currently in used in those laboratories.
Here are the performances of the TeSeE™ WESTERN BLOT assay:

TeSeE™ WESTERN BLOT versus VLA Hybrid WB		**Bovine**
Diagnostic sensitivity	N DSn CI	*223* *100 %* *98,4 % - 100 %*
Diagnostic specificity	N DSp CI	*50* *100 %* *92,9 % - 100 %*

TeSeE™ WESTERN BLOT versus AFSSA WB		**Bovine**
Diagnostic sensitivity	N DSn CI	*52* *100 %* *93,1 % - 100 %*
Diagnostic specificity	N DSp CI	*25* *84 %* *63,9 % - 95,5 %*

TeSeE™ WESTERN BLOT versus IHC		**Ovine**
Diagnostic sensitivity	N DSn CI	*211* *99 %* *96,4 % - 99,9 %*
Diagnostic specificity	N DSp CI	*71* *100 %* *94,9 % - 100 %*

TeSeE™ WESTERN BLOT versus VLA Hybrid WB		**Ovine**
Diagnostic sensitivity	N DSn CI	*73* *100 %* *95,1 % - 100 %*
Diagnostic specificity	N DSp CI	*61* *100 %* *94,1 % - 100 %*

TeSeE™ WESTERN BLOT versus SAF Immunoblot OIE		**Ovine**
Diagnostic sensitivity	N DSn CI	*0* *NC* *NC*
Diagnostic specificity	N DSp CI	*54 (*)* *0 %* *NC*

(*): the 54 discrepant samples were all confirmed positive by IHC (atypical scrapie)

TeSeE™ WESTERN BLOT versus AFSSA WB		**Ovine**
Diagnostic sensitivity	N DSn CI	*40* *100 %* *91,2 % - 100 %*
Diagnostic specificity	N DSp CI	*0* *NC* *NC*

TeSeE™ WESTERN BLOT versus IHC		**Cervids**
Diagnostic sensitivity	N DSn CI	*272* *100 %* *98.65 % - 100 %*
Diagnostic specificity	N DSp CI	*40* *100 %* *91,2 % - 100 %*

Reproductibility

The CRL regularly issues proficiency testing panels for a range of TSE tests. The samples consist of homogenised brain material presented as 50% tissue/water homogenates. The summarised results for confirmatory blotting rounds issued in 2007 are given below.

- CRL Scrapie confirmatory blotting Proficiency test round
A panel of 5 samples (1 strong positive, 2 weak positives [all classical scrapie] and 2 negatives) was issued to 23 European laboratories in November 2007.
10 sets of results were returned by laboratories using the BioRad TeSeE™ WESTERN BLOT. All of these 10 laboratories identified all samples correctly.

- CRL BSE confirmatory blotting Proficiency test round
A panel of 5 samples (1 strong positive, 1 medium positive, 1 weak positive [all classical BSE] and 2 negatives) was issued to 21 European laboratories in October 2007.
Eight sets of results were returned by laboratories using the BioRad TeSeE™ WESTERN BLOT. All of these 8 labs identified all samples correctly.

In addition, reproducibility of the TeSeE™ WESTERN BLOT was evaluated with the Bio-Rad control panel including 2 negative ovine samples, 1 negative bovine sample, 1 CWD positive sample, 3 scrapie positive samples and 1 BSE positive sample. The panel was provided to 3 TSE Reference laboratories: VLA in UK, CFIA in Canada and NVI in Norway. The results from the 3 sites were compared to the results obtained in the Bio-Rad facilities. All of these 3 laboratories identified all samples correctly (the BSE positive sample was not evaluated by the CFIA-ACIA laboratory in Ottawa since this laboratory is not a Reference laboratory for BSE in Canada).

Applications

The TeSeE™ WESTERN BLOT assay is only for use in National Reference Laboratories (NRLs) and Community Reference laboratories (CRLs). The TeSeE™ WESTERN BLOT assay can be used for different types of applications:

- **as a confirmatory assay** for the confirmation of TSE suspected cases, in cattle (BSE), small ruminants e.g. sheep/goat (BSE/Scrapie) and cervids (CWD). The diagnostic regimen includes several steps at both screening laboratories and Reference laboratories.

• at the screening laboratory: initial screening with one of the approved rapid test. If the sample turns positive (= initial reactive), the test needs to be repeated in duplicate with the same assay and with the same sample (sample homogenate). If one of the two duplicates or the two turn positive, the sample is then considered as “suspect”. All remaining material (sample homogenate + remaining tissues) are sent to the Reference laboratory for confirmation.

• at the reference laboratory: A negative result with the TeSeE™ WESTERN BLOT means that the test sample does not contain detectable PrPres by TeSeE™ WESTERN BLOT assay. However, as very low levels of PrPres may not be detected, such a result does not exclude the possibility of infection.
Any sample with a negative result according to the TeSeE™ WESTERN BLOT assay interpretation criteria, following a positive rapid test result, should be tested with one of the other OIE certified confirmatory methods, Immunohistochemistry (IHC) or SAF-Immunoblot.
Any sample with a reproducible positive result according to the test interpretation critera must be verified in accordance with current legal regulation.

- **as a rapid method** for the determination of the prevalence of infection with one of the TSE associated diseases (BSE, scrapie, CWD) in the context of an epidemiological survey in a low prevalence country.

REFERENCES

- J.N. Arsac, A.G Biacabe, J.Nicollo, A.Bencsik and T. Baron. Biochemical identification of bovine spongiform encephalopathies in cattle. Acta Neuropathologica 2007, vol 114:509-516
- J.N. Arsac, O. Andreoletti, J.M. Bilheude, C. Lacroux, S. L. Benestad and T. Baron. Similar Biochemical Signatures and Prion Protein Genotypes in Atypical Scrapie and Nor98

Cases, France and Norway. Emerging Infectious Diseases –Vol. 13, No. 1, January 2007, 58-65.

- Terry, L.A., Jenkins, R., Thorne, L., Everest, S.J., Chaplin, M.C., Davis, L.A. And Stack, M.J. (2007) First Case Of H-Type Bovine Spongiform Encephalopathy Identified In Great Britain. Veterinary Record 160: 873 – 874.
- Biacabe A.G, Morignat E, Vulin J, Calavas D, Baron TG.M; February 2008 Atypical Bovine Spongiform Encephalopathies, France, 2001-2007. Emerging Infectious Diseases Vol.14, n°2