



A **New Sensitive Test** for the Detection of Ovine and Caprine PrP^{Sc}: **TeSeE™ Sheep/Goat Assay**

M. Feyssaguet, J.-L. Morize, A. Compoin, G. Nespoulous, J.-M. Bilheude, J.-P. Bourgeois, and J. Grassi

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Abstract

Scrapie is a slow degenerative disease of the central nervous system of sheep and goats induced by an abnormal prion. A new rapid assay, developed by Bio-Rad Laboratories, for the *post-mortem* diagnosis of TSEs in small ruminants is presented.

This new assay is based on the same assay procedure as the current TSE kit with a combination of monoclonal antibodies selected for their high affinity and specificity to sheep and goat PrP^{Sc}. The assay involves purification and concentration of PrP^{Sc} followed by detection by an immunoenzymatic technique (sandwich format). Internal and external evaluations conducted in nervous tissues (obex, brainstem, spinal cord) and in peripheral tissues (lymph node, tonsil, spleen, ileum) from naturally or experimentally infected sheep and goats clearly demonstrate the very high sensitivity of this new test. In an analytical sensitivity dilution series experiment on various genotypes of sheep and goats, the new assay was able to detect, in most cases, a 1/2,500 dilution of positive tissue. Tissue was standardized in homogenized tissue that tested negative for TSEs. Specificity studies conducted on the semi-automated platform with 838 fresh samples of nervous or peripheral tissues collected from slaughterhouses show that the assay is well adapted for screening large numbers of samples. The semi-automated system allows the user to process 800-1,000 samples per day with full sample traceability. Twenty-eight autolysed samples were tested and the performance of the assay was not affected when tissue was held at 25°C for up to 9 days.

The TeSeE Sheep/Goat assay represents a promising tool for determination of the prevalence of scrapie in surveillance and routine testing programs.

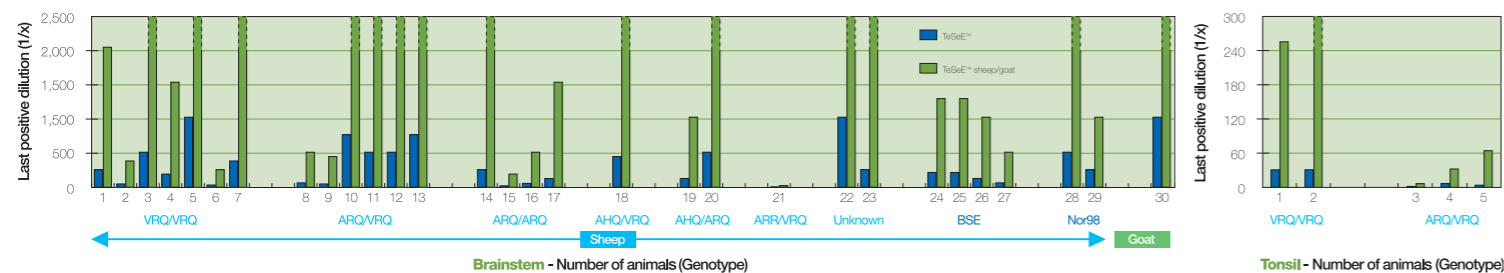
Introduction

TeSeE sheep/goat is a new rapid assay for the *post-mortem* diagnosis of TSEs in small ruminants (sheep and goat). This new Bio-Rad ELISA kit is based on the same assay procedure than the TeSeE assay actively used in most laboratories for the detection of BSE in cattle. The new TeSeE sheep/goat assay has the capacity to detect PrP^{Sc} in different types of tissues from infected animals. The presented data were obtained when comparing the performances of the two TeSeE and TeSeE sheep/goat assays, with different ovine and goat tissue samples (central nervous system tissues, lymph nodes tissues, ...). Assays were performed either manually or semi automatically with the TeSeE NSP system.

Performances

Analytical sensitivity

Comparative performances of TeSeE sheep/goat and current TeSeE assays on different types of animals (sheep/goats), tissues (brainstem/tonsil) and genotypes



Clinical sensitivity

Comparative performances of TeSeE sheep/goat and current TeSeE assays on different tissues from a flock with TSE infected sheep

Brainstem					Tonsil					All tissues (1)				
TeSeE Product Range					TeSeE Product Range					TeSeE Product Range				
TeSeE sheep/goat Assay	+	d	-	Total	TeSeE sheep/goat Assay	+	d	-	Total	TeSeE sheep/goat Assay	+	d	-	Total
+	225	0	20*	285	+	103	2*	76**	243	+	236*	0	18***	295
d	0	0	0		d	0	0	4***		d	0	0	0	
-	0	0	40		-	0	0	58		-	0	0	0	

*: 4 H/IHC negative, 2 H/IHC doubtful, 14 H/IHC positive
H: Histopathology, IHC: Immunohistochemistry

*: 2 H/IHC positive
**: 7 unknown, 2 H/IHC doubtful
***: 4 H/IHC positive

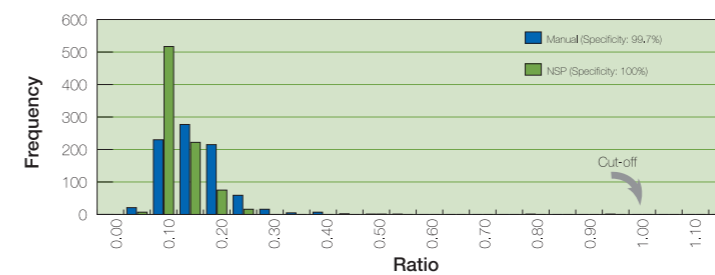
*: 2 H/IHC negative, 1 H/IHC doubtful, 1 H/IHC unknown
**: 1 H/IHC positive
***: 4 H/IHC negative, 2 H/IHC doubtful, 1 H/IHC unknown
(1) Brainstem / Tonsil / Mesenteric LN

Comparative performances of TeSeE sheep/goat assay and IHC method on animals from flocks with TSE

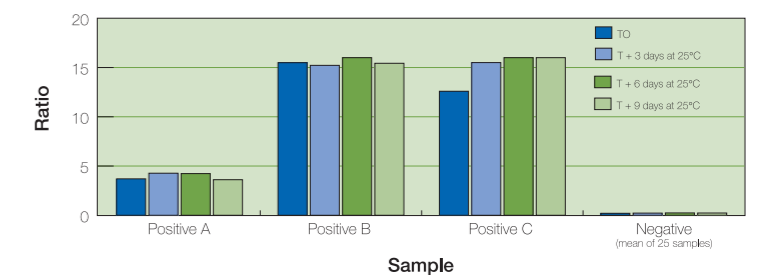
Sheep					Goat				
All tissues (1)					All tissues (1)				
Brainstem / Tonsil / Mes. LN					Brainstem / Tonsil / Mes. LN				
TeSeE sheep/goat Assay	+	d	-	Total	TeSeE sheep/goat Assay	+	d	-	Total
+	79	0	0	89	+	14	0	0	97
d	0	0	0		d	0	0	0	
-	0	0	10		-	0	0	83	

(1) general conclusion of the status of the sample taken on the basis of the positivity of at least one tissue.

Specificity on 838 fresh ovine obex



Autolysed samples



Conclusion:

With the same assay procedure for both sample processing and immunometric detection, TeSeE sheep/goat assay can be easily adapted to all TSEs screening laboratories. The assay sensitivity and specificity are improved by the specifically designed buffer A composition. Newly selected monoclonal antibodies enhance the specific recognition of ovine and caprine PrP^{Sc} in various tissues from infected animals, with a very low affinity with bovine PrP^{Sc}. The addition of a macro bead in the grinding tube makes the preparation of homogenates from various tissues easier that can be used in this test. For better adaptability of the test to the laboratory activity, the sample purification stage of the TeSeE sheep/goat assay has been adapted and validated on the TeSeE NSP System. Thus, assay reproducibility is improved even when large series of samples are tested. This test has been evaluated in February 2004 under the control of the European Community Directorate General Joint Research Center (IRMM) and has also been approved by the USDA, VLA and EU.

