European Commission

Better Training for Safer Food

Initiative

Laboratory confirmation and characterisation of TSE cases in bovine and small ruminants

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Consumers, Health And Food Executive Agency

European Commission

TOPICS

- *Documents important for laboratory confirmation of BSE/TSE*
- *Diagnostic methods and protocols for active and passive surveillance:*
  - Sampling and samples for active and passive surveillance
  - Rapid tests and interpretation of results
  - Confirmatory testing and interpretation of results
  - BSE subtyping
  - BSE/TSE discriminatory testing and interpretation of results
- *Diagnostic laboratories*

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Documents important for laboratory diagnostics of BSE/TSE

- **Annex X to the Regulation (EC) No 999/2001 as last amended**
- **OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

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Documents important for laboratory diagnostics of BSE/TSE

- **Annex X to the Regulation (EC) No 999/2001 as last amended**
  - Annex X is periodically updated and chronological list of amendments is available at: http://ec.europa.eu/food/food/biosafety/tse_bse/chronological_list_tse_en.htm

Documents important for laboratory diagnostics of BSE/TSE

- **OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**
  - Bovine spongiform encephalopathy. Chapter 2.4.6. Last version adopted by the World Assembly of Delegates of the OIE in May 2010.
  - Scrapie. Chapter 2.7.13. Last version adopted by the World Assembly of Delegates of the OIE in May 2009.
    - available from OIE home page

Annex X to the Regulation 999/2001

- Annex X regulates:
  - Chapter A. National Reference Laboratories
    - NRLs for TSE
  - Chapter B. European Union Reference Laboratory
    - EURL for TSE
  - Chapter C, 1. Sampling for the presence of a TSE
    - with the reference to OIE Manual and guidelines issued by EURL for TSE
  - Chapter C, 2. Laboratories for TSE examinations

Annex X to the Regulation 999/2001

- Annex X regulates:
  - Chapter C, 3.1 to 3.3. Methods and protocols for the laboratory testing for the presence of BSE in bovine animals, for the presence of TSE in ovine and caprine animals and for the presence of TSE in other species
    - What we use and do in suspect cases and for monitoring, and
    - What to do in case of negative, inconclusive or positive test results of rapid test and/or applied confirmatory test
  - Chapter C, 4. Rapid tests for BSE and TSE

Active and passive surveillance for BSE/TSE and sampling

- Bovines
  - BSE
- Small ruminants (sheep, goats)
  - TSE and BSE
- Other species
  - TSE and BSE

- The same principles of sampling for TSEs are used for all species

Annex X, Chapter C, 1.: Sampling

- The latest version of OIE Manual defines the methods and protocols for samples intended to be examined for the presence of a TSE and how they should be sampled.

- In addition, or in the absence, of OIE methods and protocols, and to ensure that sufficient material is available, the competent authority shall ensure the use of sampling methods and protocols in accordance with guidelines issued by the EURL.

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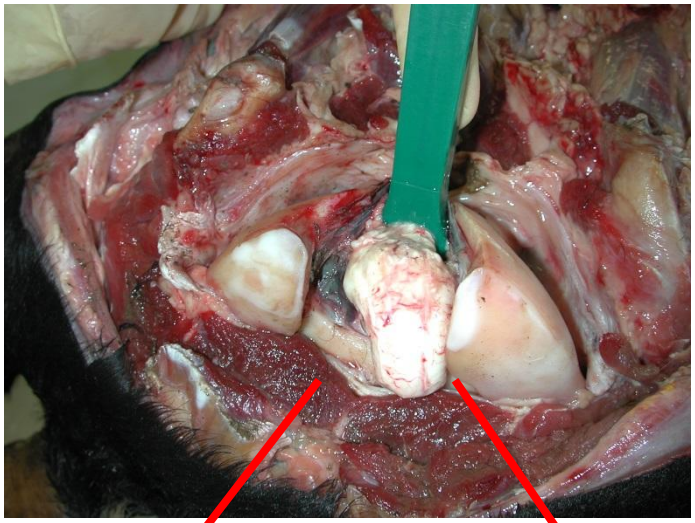
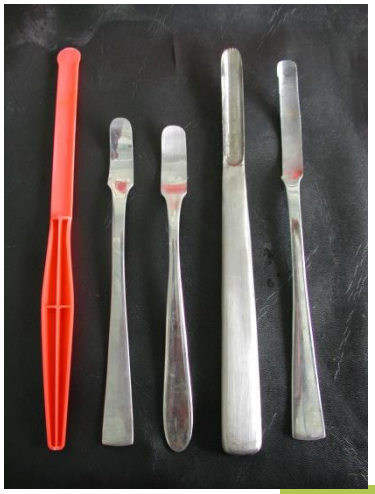
Annex X, Chapter C, 1.: Sampling

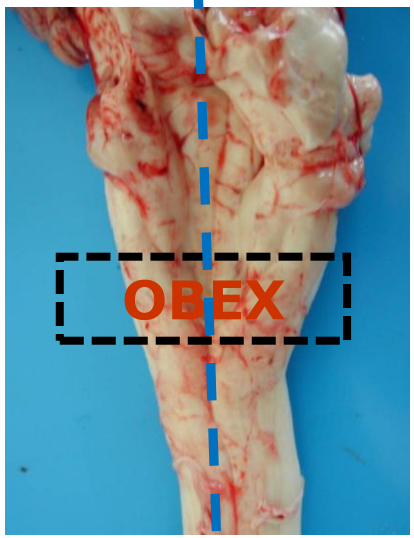
- It will keep at least half of the collected tissues fresh but not frozen until the result of the rapid test is negative.
- Where the result is positive or inconclusive the residual tissues must be processed in accordance with the EURL guidelines.
- The samples shall be correctly marked as to the identity of the sampled animal.

Sampling

- Tools (spoons) for occipital sampling for rapid testing
  - **Fixed and unfixed tissue (not frozen!)**

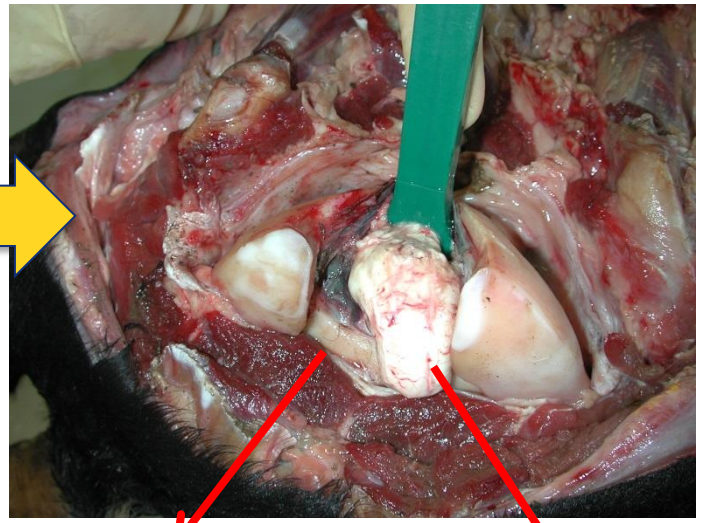


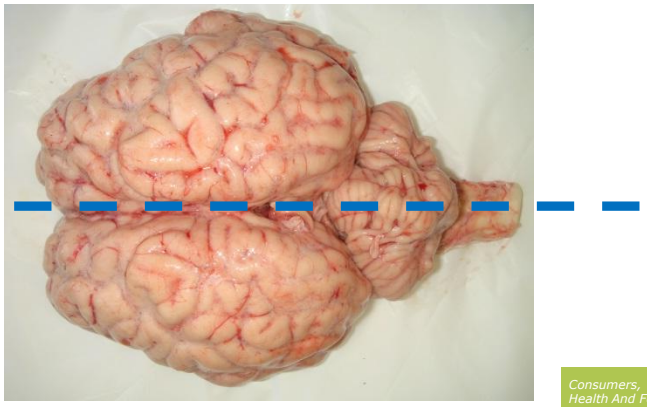


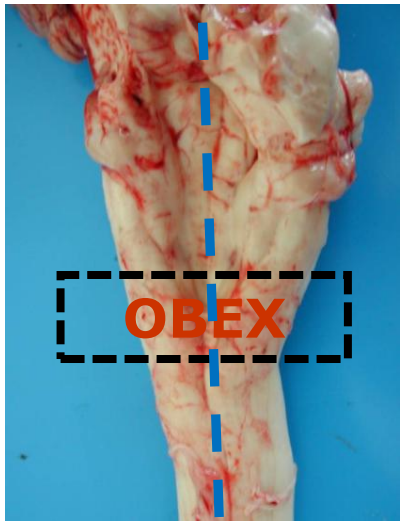


Sampling

- Brain stem for confirmatory methods – what is left from RT
  - Fixed and unfixed tissue (not frozen!)
- Brain extraction for whole brain examination and confirmatory methods
  - Fixed and unfixed tissue (not frozen!)







Annex X, Chapter C, 2.: Laboratories

Any laboratory examination for TSE shall be carried out in laboratories approved for that purpose by the competent authority.

Monitoring

- *Approved diagnostic laboratories*
  - Rapid tests

Suspect cases

- *NRL for TSEs*
  - Confirmatory methods*
  - Primary molecular testing with discriminatory immuno-blotting (DT BSE/TSE tests)*
  - BSE subtyping*

**If approved for the method, otherwise tests are made in EURL for TSE or other approved contracting NRL for TSE.*

Annex X, Chapter C, 3.: Methods *and protocols*

3.1. Laboratory testing for the presence of BSE in bovine animals

- (a) Suspect cases (according to provisions of Article 12(2))
- (b) BSE monitoring

3.2. Laboratory testing for the presence of TSE in ovine and caprine animals

- (a) Suspect cases (according to provisions of Article 12(2))
- (b) TSE monitoring
- (c) Further examination of positive TSE cases

3.3. Laboratory testing for the presence of TSEs in other species

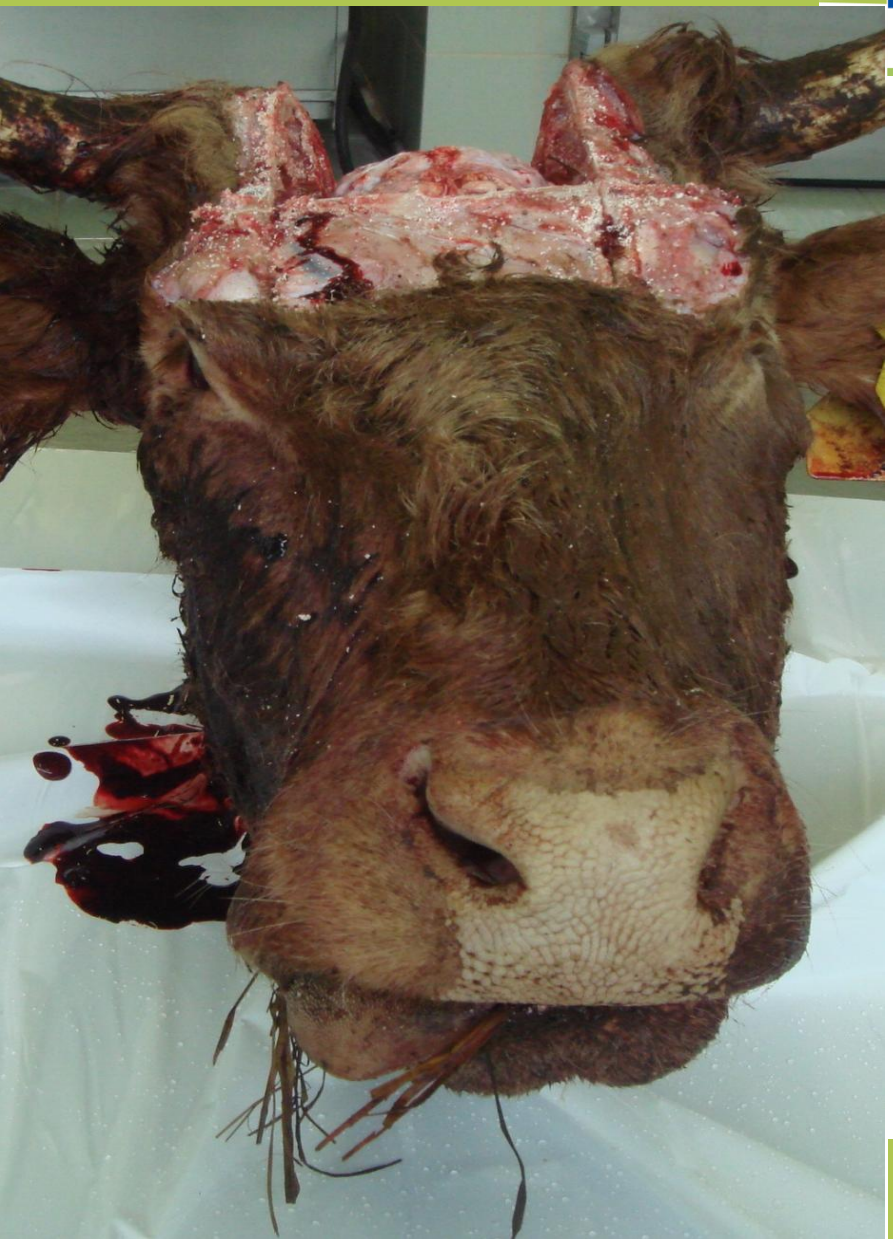
Annex X, Chapter C: Rapid tests

- We have no tests for the use in live animals, only post-mortem tests.
- Only approved tests from the list published in Annex X as last amended may be used as rapid-post mortem tests.
- Producers of rapid tests must have a quality assurance system in place that has been approved by the EU Reference Laboratory and must ensure that the test performance does not change.

Laboratory tests for active surveillance of BSE/TSE in the following streams:

- Healthy animals
- Animals with observations at ante-mortem inspection (Sick ante-mortem)
- Emergency slaughtered
- Fallen stock

Rapid post-mortem tests



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Laboratory tests for passive surveillance of BSE/TSE:

Animals showing suspect clinical symptoms

Rapid post-mortem tests

Additional methods if case suspected for BSE or TSE

Annex X, Chapter C: Methods and protocols – Bovine samples

Monitoring

- *Rapid tests*

Suspect cases

- *Immunohistochemistry (IHC)*
- *SAF immunoblot or OIE approved alternative*
- *Demonstration of characteristic fibrils by electron microscopy*
- *Histopathology*
- *The combination of rapid tests as laid down in Annex X, section 3.1.*

Annex X, Chapter C: Methods and protocols – Small ruminant samples

Monitoring

- *Rapid tests*

Suspect cases

- *Immunohistochemistry (IHC)*
- *SAF immunoblot or OIE approved alternative*
- *Demonstration of characteristic fibrils by electron microscopy*
- *Histopathology*

Annex X, Chapter C: Rapid tests

- Rapid tests for the monitoring of BSE in bovine animals
  - Initially (May 2001): 3 tests
  - 2006: 11 tests
  - 2008: 12 tests
  - 2009: 9 tests
  - 2013 (expected to decrease to): 7 tests (6)
- Rapid tests for the monitoring of TSE in small ruminants
  - Initially (May 2001): 3 tests
  - 2006: 5 tests
  - 2008: 9 tests
  - 2009: 4 tests
  - 2013 (expected to decrease to): 5 tests (4)

Annex X, Chapter C: Rapid tests - BO

2001 (3):

- Prionics Check test
- Enfer test
- Bio-Rad Platelia test

2006 (11):

- Prionics Check Western test
- Enfer TSE Ver 2.0
- Bio-Rad TeSeE test
- Prionics-Check LIA test
- InPro CDI-5 test
- CediTect BSE test
- IDEXX HerdChek BSE Antigen TSE kit, EIA
- Institut Pourquier Speed'it BSE
- Prionics Check PrioSTRIP
- Roboscreen Beta Prion BSE EIA test
- Roche Applied Science PrionScreen

2013 (7) – (*to be omitted in 2014)

- Prionics Check Western test
- (*Enfer TSE test Ver 3.0)
- Bio-Rad TeSeE SAP rapid test
- Prionics-Check LIA test
- IDEXX HerdChek BSE Antigen kit, EIA & IDEXX HerdChek BSE-Scrapie Antigen test kit
- Prionics Check PrioSTRIP
- Roboscreen Beta Prion BSE EIA

Annex X, Chapter C: Rapid tests - SR

2001 (3):

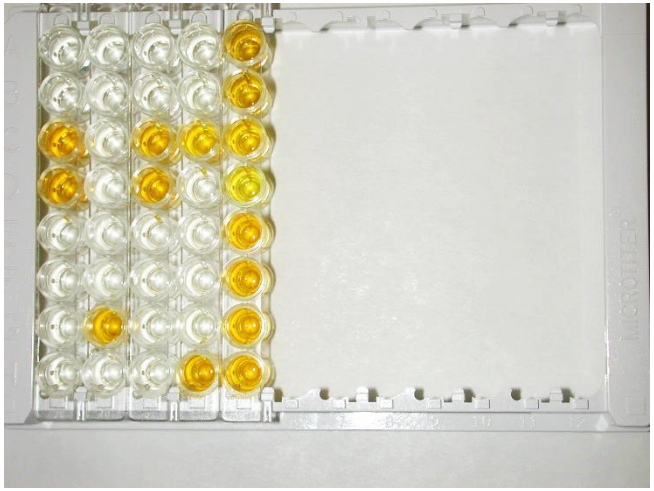
- Prionics Check test
- Enfer test
- Bio-Rad Platelia test

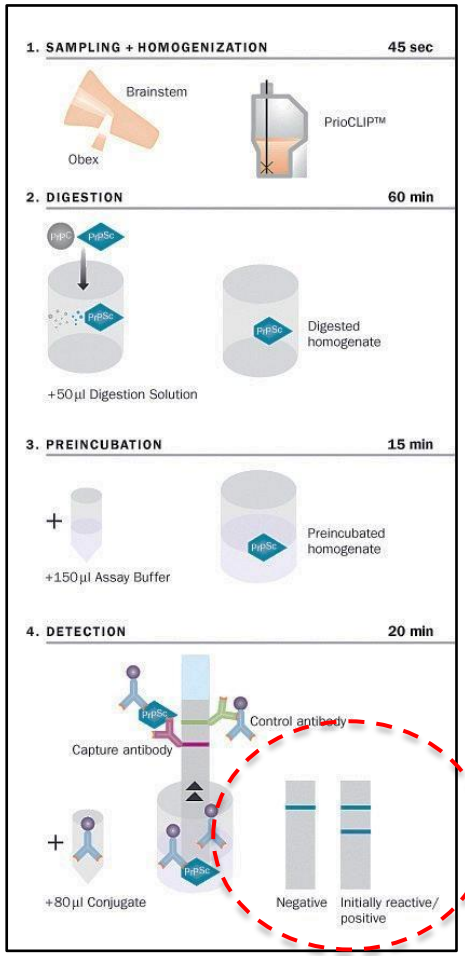
2006 (5):

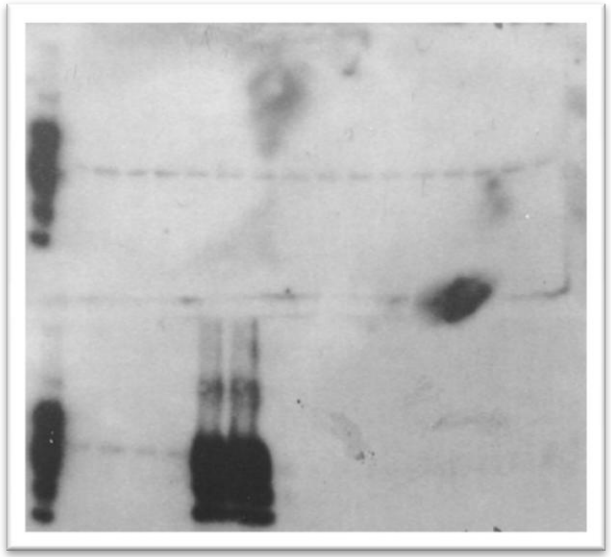
- Prionics Check Western test
- Enfer test
- Bio-Rad Platelia test
- Prionics Check LIA test
- InPro CDI-5 test

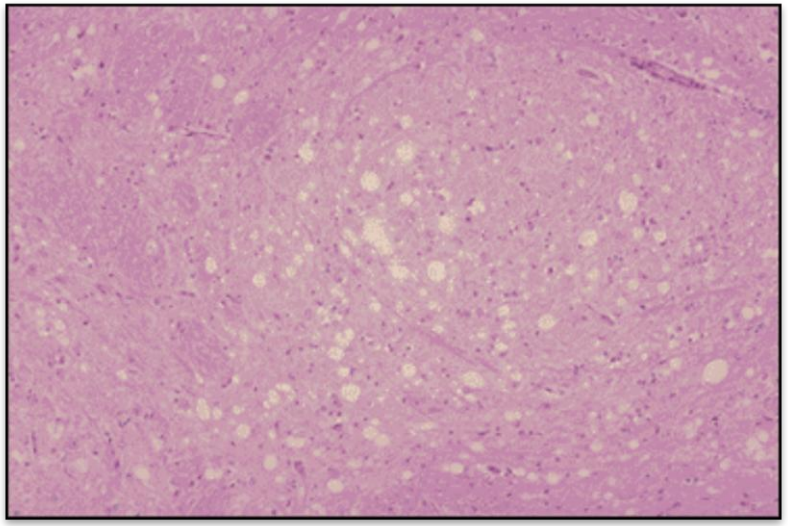
2013 (4): (*to be omitted in 2014)

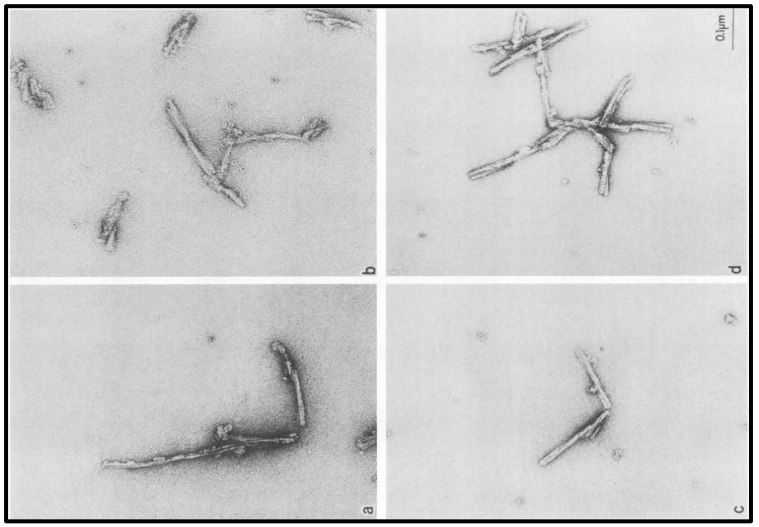
- Bio-Rad TeSeE SAP rapid test)
- Bio-Rad TeSeE Sheep/Goat rapid test
- HerdChek BSE-Scrapie Antigen Test (IDEXX)
- (*Prionics Check LIA Small Ruminants)
- Prionics Check PrioSTRIP SR



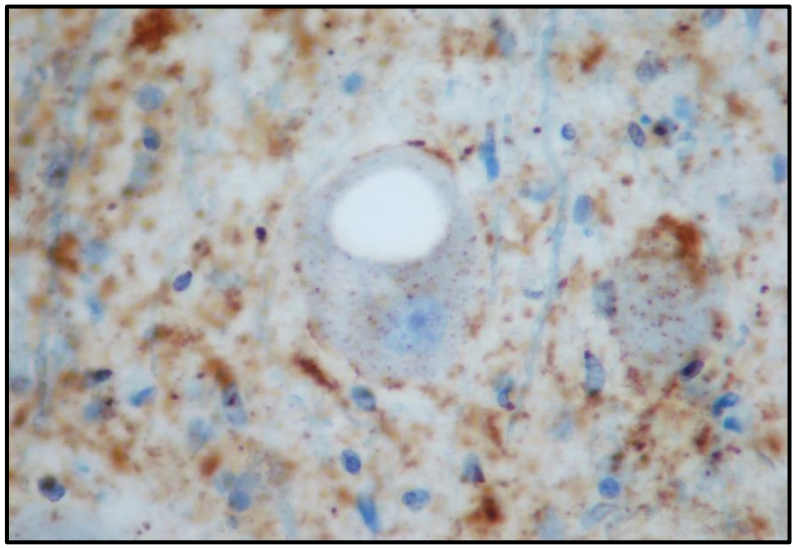

1. SAMPLING + HOMOGENIZATION
45 sec
Brainstem
Obex
PrioCLIP™
2. DIGESTION
60 min
PrPSc
Digested homogenate
+50µl Digestion Solution
3. PREINCUBATION
15 min
Preincubated homogenate
+150µl Assay Buffer
4. DETECTION
20 min
Control antibody
Capture antibody
+80µl Conjugate
Negative
Initially reactive/ positive

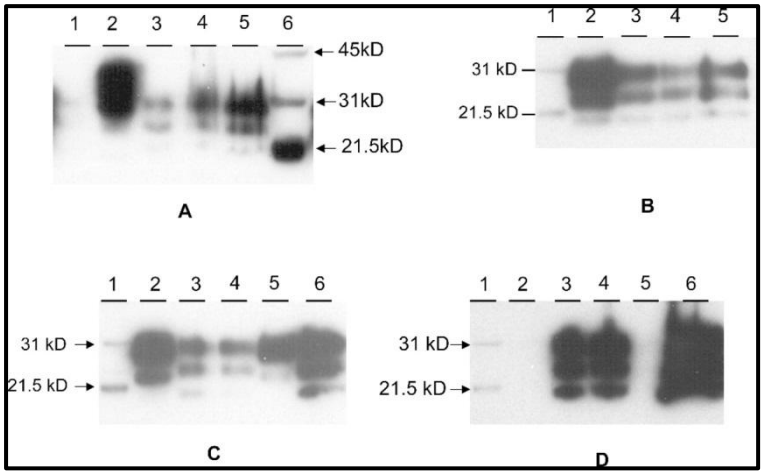






From: J Virol. Sep 1986; 59(3): 676–683.





Richt J A et al. J VET Diagn Invest 2007;19:142-154

Interpretation of test results for BSE

BSE monitoring
If RT is inconclusive or positive, samples are

BSE suspect
RT may be used for primary screening of suspect cases. If the results are inconclusive or positive, samples are

immediately subjected to confirmatory examinations using at least one of confirmatory methods and protocols from the latest edition of OIE Manual.

If HP is inconclusive or negative ⇨ further examinations are made by one of the other confirmatory methods and protocols (IHC, immunoblot, SAF fibrils with EM, secondary RT).

Sample is regarded positive for BSE if at least one of confirmatory tests is positive. If BSE is confirmed, BSE subtyping is required.

BSE subtyping

Mandatory from 1. July 2013 on by Commision Regulation No 600/2013

PURPOSE:

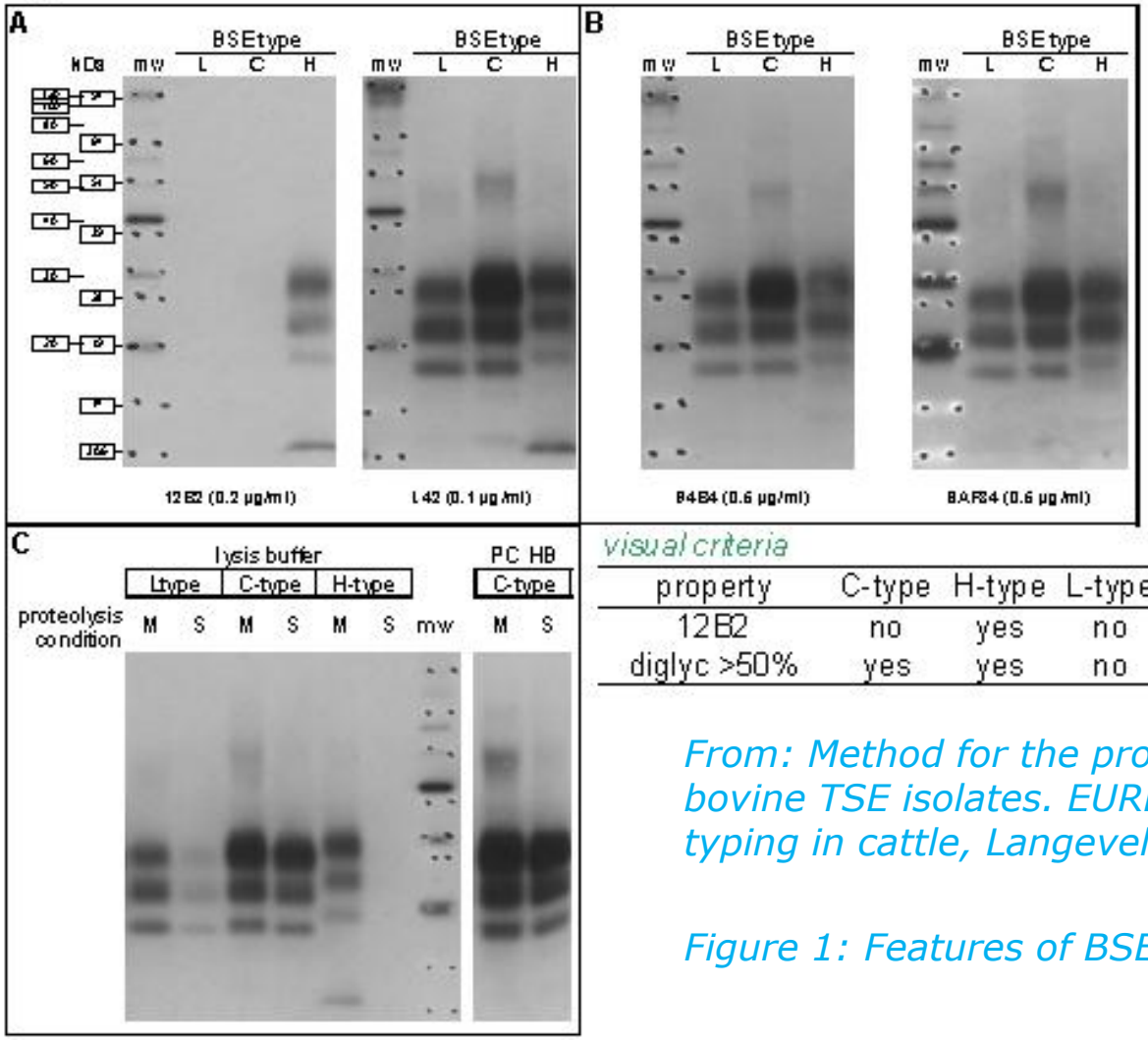
- Development of sensitive PrP^{res} immuno-detection diagnostic techniques has led to the detection of a small number of geographically wide spread sporadic cases of deviant types, predominantly in older animals.

- These isolates have now been confirmed in mice as distinct strains and have been defined as H- (highj) or L – low) type based on the molecular mass of the unglycosylated fragment of PK resistant PrP in Western blot.

Method for the provisional classification of the bovine TSE isolates. EURL for TSE (2-blot protocol for PrP typing in cattle, Langeveld (based on Jacobs et al 2007).

BSE subtyping

Figure 1



visual criteria

property	C-type	H-type	L-type
12B2	no	yes	no
diglyc >50%	yes	yes	no

From: Method for the provisional classification of the bovine TSE isolates. EURL for TSE (2-blot protocol for PrP typing in cattle, Langeveld (based on Jacobs et al 2007).

Figure 1: Features of BSE types.

BSE subtyping

Table I: Discrimination between BSE-types based on molecular properties of PrP^{res} (from: Jacobs et al., 2007)

BSE type	size difference[a] in kDa	binding to 12B2	glycoprofile[b] di-glyc (%)	deglycosylation with PNGase F[c] (163-242 epitopes)	proteolytic susceptibility pH 8/pH 6.5[d]
C	ref	no	>50	1 band	> 0.7
H	+1.4	yes	dual character[e]	2 bands	< 0.6
L	-0.3	no	<50	1 band	< 0.6

[a] Approximate difference value with C-type for the nonglycosylated band of the PrP^{res} population in 17-19 kDa region; tested with group B antibodies 9A2, L42 or 6H4.
[b] Percentages of diglycosylated fraction should be compared together with C-type.
[c] Two bands can only be observed with group C antibodies like 94B4 and SAF84 that bind to the C-terminal domain 163-242 of bovine PrP .
[d] Ratio calculation, see Jacobs et al., 2007.
[e] Depending on the use of antibodies of groups A & B or of group C (see Table II).

Interpretation of test results for TSE

TSE monitoring
If RT is inconclusive or positive, samples are

TSE suspect
RT may be used for primary screening of suspect cases. If the results are inconclusive or positive, samples are

immediately subjected to confirmatory examinations using at least one of confirmatory methods and protocols from the latest edition of OIE Manual.

If HP is inconclusive or negative ⇨ further examinations are made by one of the other confirmatory methods and protocols (IHC, immunoblot, SAF fibrils with EM).

Sample is regarded positive for TSE if at least one of confirmatory tests is positive. If TSE is confirmed, primary molecular testing with discriminatory immuno-blotting is required (Annex X, 3.2.(c)).

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TSE strain characterisation in small ruminants (BSE/TSE DT)

Mandatory from 12. January 2005 on by Commission Regulation (EC) No 36/2005.

PURPOSE:

- To support the statutory discriminatory testing of the different TSEs in small ruminants as laid down in Annex X
- To document properties which can be used to distinguish small ruminant TSE isolates requiring further investigation
- To provide sampling and testing strategies and protocols for discriminatory methods which have been approved by the EURL strain typing expert group
- To identify those isolates which have characteristics of the BSE agents

TSE Strain characterisation in small ruminants. A Technical handbook for national reference laboratories in the EU. (Guidelines prepared by EURL for TSE)

European Commission

TSE strain characterisation in small ruminants (BSE/TSE DT)

Obligatory for positive TSE cases which are not atypical scrapie cases

Primary molecular testing with a discriminatory WB

Carried out in NRL for TSE, which has successfully past proficiency testing for the use of molecular typing method

Ring trial with additional molecular testing methods

Samples in which BSE cannot be excluded are tested in EURL for TSE or other laboratory listed in Annex X

Tertiary testing strategy – Mouse bioassay

Strain typing expert group (STEG)

TSE strain characterisation in small ruminants (BSE/TSE DT)

Interpretation of the results

Primary molecular testing with a discriminatory WB

Interpretation made by the laboratory. Samples in which BSE cannot be excluded are send to secondary molecular testing.

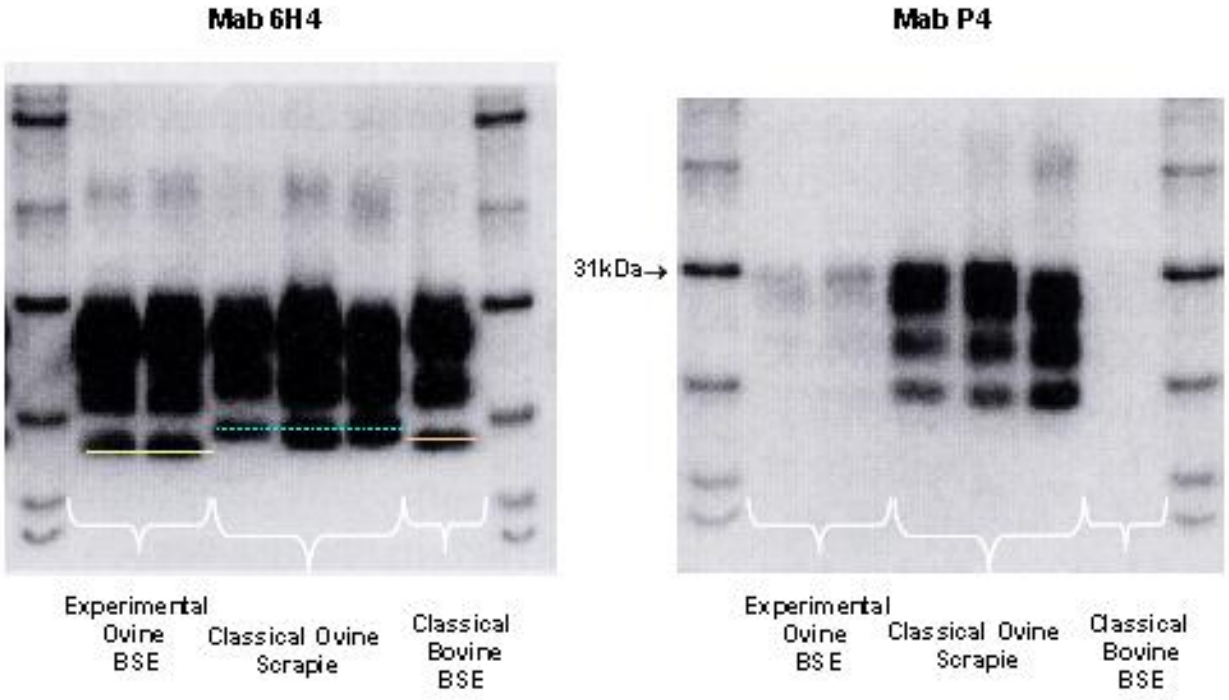
Ring trial with additional molecular testing methods

Interpretation made by EURL for TSE assisted by a panel of experts including a representative of the relevant NRL.
Commission would be informed immediately about the outcome of that interpretation.
Samples indicative for BSE by three different methods and samples inconclusive in the ring trial would be further analysed by a mouse bioassay for final confirmation.

Tertiary testing strategy – Mouse bioassay (STEG)

Figure 2

Image showing clear differential molecular mass migration with core antibody (6H4) and differential N-terminal antibody affinity for classical scrapie, classical BSE and experimental ovine BSE.



AHVLA Prionics Based Hybrid method (From: TSE strain characterisation in small ruminants. EURL TSE)

Diagnostic laboratories

- **Approved laboratories for rapid testing**
- **National Reference Laboratories (NRL TSE)**
- **European Union Reference Laboratory (EURL TSE)**
  - AHVLA, Weybridge, UK
- **Laboratories approved for performing further examination by molecular typing**
  - Agence Française de Sécurité Sanitaire des Aliments; Laboratoire de pathologie bovine, Lyon, France
  - Centre CEA Fontenay-aux-Roses, Gif-sur-Yvette Cedex, France
  - AHVLA, Weybridge, UK

- **OIE Reference laboratories for BSE and TSE**

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- *Diagnostic laboratories*

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Better Training for Safer Food
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