Status: *Point in time view as at 29/09/2008.*
Changes to legislation: *There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C. (See end of Document for details)*

ANNEX X

REFERENCE LABORATORIES, SAMPLING AND LABORATORY ANALYSIS METHODS

[F1CHAPTER C

Sampling and laboratory testing

[F2 1. Sampling

Any samples intended to be examined for the presence of a TSE shall be collected using the methods and protocols laid down in the latest edition of the Manual for diagnostic tests and vaccines for Terrestrial Animals of the International Office for Epizooties (IOE/OIE) (the Manual). 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 Community Reference Laboratory. In particular the competent authority shall collect the appropriate tissues, according to the available scientific advice and the guidelines of the Community Reference Laboratory, in order to ensure the detection of all known strains of TSE in small ruminants and shall 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 Community reference laboratory guidelines.

The samples shall be correctly marked as to the identity of the sampled animal.]

Textual Amendments

F2 Substituted by Commission Regulation (EC) No 727/2007 of 26 June 2007 amending Annexes I, III, VII and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (Text with EEA relevance).

2. Laboratories

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

3. Methods and protocols

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

(a) Suspect cases

Samples from bovine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall be subject to a histopathological examination as laid down in the latest edition of the Manual, except where the material is autolysed. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the tissues shall be subjected to an examination by one of the other diagnostic methods laid down in the Manual (immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy). However, rapid tests cannot be used for this purpose.

If the result of one of those examinations is positive, the animals shall be regarded a positive BSE case.

(b) BSE monitoring

Samples from bovine animals sent for laboratory testing pursuant to the provisions of Annex III, Chapter A, Part I (Monitoring in bovine animals) shall be examined by a rapid test.

When the result of the rapid test is inconclusive or positive, the sample shall immediately be subject to confirmatory examinations in an official laboratory. The confirmatory examination shall start by a histopathological examination of the brainstem as laid down in the latest edition of the Manual, except where the material is autolysed or otherwise not suitable for examination by histopathology. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the sample shall be subjected to an examination by one of the other diagnostic methods referred to in (a).

An animal shall be regarded a positive BSE case, if the result of the rapid test is positive or inconclusive, and either

— the result of the subsequent histopathological examination is positive, or

— the result of another diagnostic method referred to in (a) is positive.

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

(a) Suspect cases

Samples from ovine and caprine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall be subject to a histopathological examination as laid down in the latest edition of the Manual, except where the material is autolysed. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the sample shall be subjected to an examination by immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy, as laid down in the Manual. However, rapid tests cannot be used for this purpose.

If the result of one of those examinations is positive, the animal shall be regarded a positive scrapie case.

[[F2](b) TSE monitoring

Samples from ovine and caprine animals sent for laboratory testing pursuant to the provisions of Annex III, Chapter A, Part II (Monitoring in ovine and caprine animals) shall be examined by a rapid test using the appropriate methods and protocols, according to the available scientific advice and the guidelines of the Community Reference Laboratory, in order to ensure the detection of all known strains of TSE.

When the result of the rapid test is inconclusive or positive, the sampled tissues shall immediately be sent to an official laboratory for confirmatory examinations by immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy, as referred to in (a). If the result of the confirmatory examination is negative or inconclusive, additional confirmatory testing shall be carried out according the guidelines of the Community reference laboratory.

If the result of one of the confirmatory examination is positive, the animal shall be regarded a positive TSE case.**]**

(c) Further examination of positive scrapie cases

(i) Primary molecular testing with a discriminatory immuno-blotting

Status: *Point in time view as at 29/09/2008.*
Changes to legislation: *There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C. (See end of Document for details)*

Samples from clinical suspect cases and from animals tested in accordance with Annex III, Chapter A, Part II, points 2 and 3 which are regarded as positive scrapie cases following the examinations referred to in points (a) or (b), or which display characteristics which are deemed by the testing laboratory to merit investigation, shall be forwarded for further examination by a primary molecular typing method to:

— Agence Française de Sécurité Sanitaire des Aliments, Laboratoire de pathologie bovine, 31, avenue Tony Garnier, BP 7033, F-69342, Lyon Cedex, France, or

— Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom, or

— to a laboratory, appointed by the competent authority, which has participated successfully in proficiency testing organised by the Community Reference Laboratory for the use of a molecular typing method, or

— on a provisional basis until 1 May 2005, the laboratories approved for this purpose by the CRL panel of experts.

(ii) Ring trial with additional molecular testing methods

Samples from scrapie cases in which the presence of BSE cannot be excluded according to the guidelines issued by the Community Reference Laboratory by the primary molecular testing referred to in (i), shall be forwarded immediately to the laboratories listed in point (d) after consultation with the Community Reference Laboratory, and with all the relevant information available. They shall be submitted to a ring trial with at least:

— a second discriminatory immuno-blotting,

— a discriminatory immunocytochemistry, and

— a discriminatory ELISA (Enzyme linked ImmunoSorbent Assay)

carried out in the laboratories approved for the relevant method as listed in point (d). Where samples are unsuitable for immunocytochemistry, the Community Reference Laboratory will direct appropriate alternative testing within the ring trial.

The results shall be interpreted by the Community Reference Laboratory assisted by a panel of experts including a representative of the relevant National Reference Laboratory. The Commission shall be informed immediately about the outcome of that interpretation. Samples indicative for BSE by three different methods and samples inconclusive in the ring trial shall be further analysed by a mouse bioassay for final confirmation.

[[F2]Further testing of positive TSE samples detected in infected flocks on the same holding shall be carried out at least on the first two positive TSE cases detected every year following the index case.**]**

(d) Laboratories approved for performing further examination by molecular typing methods

The laboratories approved for further molecular typing are:

Agence Française de Sécurité Sanitaire des Aliments

Laboratoire de pathologie bovine

31, avenue Tony Garnier

BP 7033

Status: *Point in time view as at 29/09/2008.*
Changes to legislation: *There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C. (See end of Document for details)*

F-69342 Lyon Cedex

Centre CEA Fontenay-aux-Roses, BP 6

F-92265 Fontenay-aux-Roses Cedex

Service de Pharmacologie et d'Immunologie

Centre CEA Saclay, bâtiment 136

F-91191 Gif-sur-Yvette Cedex

Veterinary Laboratories Agency

Woodham Lane

New Haw

Addlestone

Surrey KT15 3NB

United Kingdom

3.3. Laboratory testing for the presence of TSEs in species other than those referred to in points 3.1. and 3.2.

Where methods and protocols are established for tests carried out to confirm the suspected presence of a TSE in a species other than bovine, ovine and caprine, they shall include at least a histopathological examination of brain tissue. The competent authority may also require laboratory tests such as immunocytochemistry, immuno-blotting, demonstration of characteristic fibrils by electron microscopy or other methods designed to detect the disease associated form of the prion protein. In any case at least one other laboratory examination shall be carried out if the initial histopathological examination is negative or inconclusive. At least three different examinations shall be carried out in the event of the first appearance of the disease.

In particular, where BSE is suspected in a species other than bovine animals, samples shall be submitted for strain-typing, where possible.

[F3 4. Rapid tests

For the purposes of carrying out the rapid tests in accordance with Articles 5(3) and 6(1), the following methods shall be used as rapid tests for the monitoring of BSE in bovine animals:

— immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrPRes (Prionics-Check Western test),

— chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer test & Enfer TSE Kit version 2.0, automated sample preparation),

— microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),

— sandwich immunoassay for PrPRes carried out following denaturation and concentration steps (Bio-Rad Te-SeE test),

— microplate-based immunoassay (ELISA) which detects Proteinase K-resistant PrPRes with monoclonal antibodies (Prionics-Check LIA test),

— conformation-dependent immunoassay, BSE antigen test kit (Beckman Coulter InPro CDI kit),

Status: *Point in time view as at 29/09/2008.*
Changes to legislation: *There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C. (See end of Document for details)*

— chemiluminescent ELISA for qualitative determination of PrP^{Sc} (CediTect BSE test),

— immunoassay using a chemical polymer for selective PrP^{Sc} capture and a monoclonal detection antibody directed against conserved regions of the PrP molecule (IDEXX HerdChek BSE Antigen Test Kit, EIA),

— lateral-flow immunoassay using two different monoclonal antibodies to detect Proteinase K-resistant PrP fractions (Prionics Check PrioSTRIP),

— two-sided immunoassay using two different monoclonal antibodies directed against two epitopes presented in a highly unfolded state of bovine PrP^{Sc} (Roboscreen Beta Prion BSE EIA Test Kit),

— sandwich ELISA for the detection of Proteinase K-resistant PrP^{Sc} (Roche Applied Science PrionScreen),

— antigen-capture ELISA using two different monoclonal antibodies to detect Proteinase K-resistant PrP fractions (Fujirebio FRELISA BSE post-mortem rapid BSE Test).

For the purposes of carrying out the rapid tests in accordance with Articles 5(3) and 6(1), the following methods shall be used as rapid tests for the monitoring of TSE in ovine and caprine animals:

— conformation-dependent immunoassay, BSE antigen test kit (Beckman Coulter InPro CDI kit),

— sandwich immunoassay for PrPRes carried out following denaturation and concentration steps (Bio-Rad Te-SeE test),

— sandwich immunoassay for PrPRes carried out following denaturation and concentration steps (Bio-Rad Te-SeE Sheep/Goat test),

— chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer TSE Kit version 2.0),

— microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),

— immunoassay using a chemical polymer for selective PrP^{Sc} capture and a monoclonal detection antibody directed against conserved regions of the PrP molecule (IDEXX HerdChek BSE-Scrapie Antigen Test Kit, EIA),

— microplate-based chemiluminiscent immunoassay for the detection of PrP^{Sc} in ovine tissues (POURQUIER'S-LIA Scrapie),

— immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrPRes (Prionics-Check Western Small Ruminant test),

— microplate-based chemiluminescent immunoassay for the detection of Proteinase K-resistant PrP^{Sc} (Prionics Check LIA Small Ruminants).

In all tests, sample tissue on which the test must be applied must comply with the manufacturer's instructions for use.

Producers of rapid tests must have a quality assurance system in place that has been approved by the Community Reference Laboratory (CRL) and ensures that the test performance does not change. Producers must provide the CRL with the test protocols.

Changes to rapid tests and to test protocols may only be made after prior notification to the CRL and provided that the CRL finds that the change does not alter the sensitivity, specificity or reliability of the rapid test. That finding shall be communicated to the Commission and to the national reference laboratories.**]**

Status: *Point in time view as at 29/09/2008.*
Changes to legislation: *There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C. (See end of Document for details)*

Textual Amendments

F3 Substituted by Commission Regulation (EC) No 315/2008 of 4 April 2008 amending Annex X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the lists of rapid tests (Text with EEA relevance).

5. Alternative tests

(To be defined)**]**

Textual Amendments

F1 Substituted by Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals (Text with EEA relevance).

Status:
Point in time view as at 29/09/2008.

Changes to legislation:
There are currently no known outstanding effects for the Regulation (EC) No 999/2001 of the European Parliament and of the Council, CHAPTER C.