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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

[^{F1}CHAPTER 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

[^{F3}(a) Suspect cases

Samples from bovine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall immediately be subjected to confirmatory examinations using at least one of the following methods and protocols laid down in the latest edition of the Manual:

- (i) the immunohistochemical (IHC) method;
- (ii) SAF-immunoblot or OIE approved alternative;
- (iii) the demonstration of characteristic fibrils by electron microscopy;
- (iv) the histopathological examination;

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- (v) the combination of rapid tests as laid down in the third subparagraph.

In case the histopathological examination is inconclusive or negative, the tissues shall be submitted to a further examination by one of the other confirmatory methods and protocols.

Rapid tests may be used for both primary screening of suspect cases and, if inconclusive or positive, for subsequent confirmation, according to the guidelines from the Community reference laboratory and provided that:

- (i) the confirmation is carried out in a national reference laboratory for TSEs; and
- (ii) one of the two rapid tests is a Western blot; and
- (iii) the second rapid test used:
 - includes a negative tissue control and a bovine BSE sample as positive tissue control,
 - is of a different type than the test used for the primary screening; and
- (iv) if a rapid Western blot is used as the first test, the result of that test must be documented and submitted to the national reference laboratory for TSEs; and
- (v) where the result of the primary screening is not confirmed by the subsequent rapid test, the sample must be subjected to an examination by one of the other confirmatory methods; in case the histopathological examination is used for that purpose but proves to be inconclusive or negative, the tissues must be submitted to a further examination by one of the other confirmatory methods and protocols.

If the result of one of the confirmatory examinations referred to in points (i) to (v) of the first subparagraph is positive, the animals shall be regarded a positive BSE cases.

Textual Amendments

F3 Substituted by [Commission Regulation \(EC\) No 162/2009 of 26 February 2009 amending Annexes III 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 \(Text with EEA relevance\)](#).

- (b) BSE monitoring

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

When the result of the rapid test is inconclusive or positive, the sample shall immediately be subjected to confirmatory examinations using at least one of the following methods and protocols laid down in the latest edition of the Manual:

- (i) the immunohistochemical (IHC) method;
- (ii) SAF-immunoblot or OIE approved alternative;
- (iii) the demonstration of characteristic fibrils by electron microscopy;
- (iv) the histopathological examination;
- (v) the combination of rapid tests as laid down in the fourth subparagraph.

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In case the histopathological examination is inconclusive or negative, the tissues shall be submitted to a further examination by one of the other confirmatory methods and protocols.

Rapid tests may be used for both primary screening and, if inconclusive or positive, for subsequent confirmation, according to the guidelines from the Community reference laboratory and provided that:

- (i) the confirmation is carried out in a national reference laboratory for TSEs; and
- (ii) one of the two rapid tests is a Western blot; and
- (iii) the second rapid test used:
 - includes a negative tissue control and a bovine BSE sample as positive tissue control,
 - is of a different type than the test used for the primary screening; and
- (iv) if a rapid Western blot is used as the first test, the result of that test must be documented and submitted to the national reference laboratory for TSEs; and
- (v) where the result of the primary screening is not confirmed by the subsequent rapid test, the sample must be subjected to an examination by one of the other confirmatory methods; in case the histopathological examination is used for that purpose but proves to be inconclusive or negative, the tissues must be submitted to a further examination by one of the other confirmatory methods and protocols.

An animal shall be regarded a positive BSE case if the result of the rapid test is inconclusive or positive, and at least one of the confirmatory examinations referred to in points (i) to (v) of the first subparagraph is positive.]

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

[^{F3}(a) Suspect cases

Samples from ovine and caprine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall immediately be subjected to confirmatory examinations using at least one of the following methods and protocols laid down in the latest edition of the Manual:

- (i) the immunohistochemical (IHC) method;
- (ii) SAF-immunoblot or OIE approved alternative;
- (iii) the demonstration of characteristic fibrils by electron microscopy;
- (iv) the histopathological examination.

In case the histopathological examination is inconclusive or negative, the tissues shall be submitted to a further examination by one of the other confirmatory methods and protocols.

Rapid tests may be used for primary screening of suspect cases. Such tests may not be used for subsequent confirmation.

Where the result of the rapid test used for primary screening of suspect cases is positive or inconclusive, the sample shall be subjected to an examination by one of the confirmatory examinations referred to in points (i) to (iv) of the first subparagraph. In case the histopathological examination is used for that purpose but proves to be inconclusive or negative, the tissues shall be submitted to a further examination by one of the other confirmatory methods and protocols.

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If the result of one of the confirmatory examinations referred to in point (i) to (iv) of the first subparagraph is positive, the animals shall be regarded positive TSE cases and further examination as referred to in point (c) shall be performed.]

[^{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.]

[^{F3}(c) Further examination of positive TSE cases]

(i) [^{F3}Primary molecular testing with a discriminatory immuno-blotting

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 TSE cases but which are not atypical 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,
- 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.]

(ii) Ring trial with additional molecular testing methods

Samples from [^{F3}TSE] 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)

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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

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

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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.

[^F34. 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:

- the immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrP^{Res} (Prionics-Check Western test),
- the 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),
- the microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),
- the sandwich immunoassay for PrP^{Res} detection with the TeSeE SAP Detection kit carried out following denaturation and concentration steps with the TeSeE Purification kit (Bio-Rad TeSeE rapid test),
- the microplate-based immunoassay (ELISA) which detects Proteinase K-resistant PrP^{Res} with monoclonal antibodies (Prionics-Check LIA test),
- the 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),
- the lateral-flow immunoassay using two different monoclonal antibodies to detect Proteinase K-resistant PrP fractions (Prionics Check PrioSTRIP),
- the 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),
- the sandwich ELISA for the detection of Proteinase K-resistant PrP^{Sc} (Roche Applied Science PrionScreen).

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:

- the sandwich immunoassay for PrP^{Res} detection with the TeSeE SAP Detection kit carried out following denaturation and concentration steps with the TeSeE Purification kit (Bio-Rad TeSeE rapid test),
- the sandwich immunoassay for PrP^{Res} detection with the TeSeE Sheep/Goat Detection kit carried out following denaturation and concentration steps with the TeSeE Sheep/Goat Purification kit (Bio-Rad TeSeE Sheep/Goat rapid test),
- the chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer TSE Kit version 2.0),
- the microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),
- the 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),

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- the immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrP^{Res} (Prionics-Check Western Small Ruminant test),
- the 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 and ensures that the test performance does not change. Producers must provide the Community reference laboratory with the test protocols.

Changes to rapid tests and to test protocols may only be made after prior notification to the Community reference laboratory and provided that the Community reference laboratory 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.]

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 epidemic-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals \(Text with EEA relevance\).](#)

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