# [F1ANNEX X

## REFERENCE LABORATORIES, SAMPLING AND LABORATORY ANALYSIS METHODS

**Textual Amendments**

**F1** Substituted by Commission Regulation (EU) No 1148/2014 of 28 October 2014 amending Annexes II, VII, VIII, IX 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).

### CHAPTER A

### National reference laboratories

1. The designated national reference laboratory is to:

   (a) have at its disposal facilities and expert personnel enabling it to show at all times, and especially when the disease in question first appears, the type and strain of the agent of TSE, and to confirm results obtained by official diagnostic laboratories. Where it is not capable of identifying the strain-type of the agent, it shall set up a procedure to ensure that the identification of the strain is referred to the EU reference laboratory;

   (b) verify diagnostic methods used in official diagnostic laboratories;

   (c) be responsible for coordination of diagnostic standards and methods within the Member State. To this end, it:
   - may provide diagnostic reagents to official diagnostic laboratories;
   - is to control the quality of all diagnostic reagents used in the Member State
   - is to periodically arrange comparative tests
   - is to hold isolates of the agents of the disease in question, or corresponding tissues containing such agents, coming from cases confirmed in the Member State
   - is to ensure confirmation of results obtained in diagnostic laboratories;

   (d) is to cooperate with the EU reference laboratory, which includes the participation in the periodic comparative tests organised by the EU reference laboratory. Should a national reference laboratory fail in a comparative test organised by the EU reference laboratory, it shall take immediately all the corrective actions to remedy the situation and successfully pass the repeat comparative test or the next comparative test organised by the EU reference laboratory.

2. However, by way of derogation from point 1, Member States which do not have a national reference laboratory shall use the services of the EU reference laboratory or of national reference laboratories located in other Member States or European Free Trade Association (EFTA) Members.

3. The national reference laboratories are:

| | |
|---|---|
| Austria: | Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES)<br>Institut für veterinärmedizinische Untersuchungen<br>Robert Koch Gasse 17<br>A-2340 Mödling |
| Belgium: | CERVA-CODA-VAR<br>Centre d'Étude et de Recherches Vétérinaires et Agrochimiques, Centrum voor Onderzoek in Diergeneeskunde en Agrochemie, Veterinary and Agrochemical Research Centre<br>Groeselenberg 99<br>B-1180 Bruxelles |
| Bulgaria: | Национален диагностичен научноизследователски ветеринарномедицински институт ‘Проф. Д-р Георги Павлов’<br>Национална референтна лаборатория ‘Трансмисивни спонгиформни енцефалопатии’<br>бул. ‘Пенчо Славейков’ 15<br>София 1606<br>(National Diagnostic Veterinary Research Institute ‘Prof. Dr Georgi Pavlov’, National Reference Laboratory for Transmissible Spongiform Encephalopathies, 15 Pencho Slaveykov Blvd., 1606 Sofia) |
| Croatia: | Hrvatski veterinarski institut,<br>Savska Cesta 143<br>10000 Zagreb |
| Cyprus: | State Veterinary Laboratories<br>Veterinary Services<br>CY-1417 Athalassa<br>Nicosia |
| Czech Republic: | Státní veterinární ústav Jihlava (State Veterinary Institute Jihlava)<br>National Reference Laboratory for BSE and Animal TSEs<br>Rantířovská 93<br>586 05 Jihlava |
| Denmark: | Veterinærinstituttet<br>Danmarks Tekniske Universitet<br>Bülowsvej 27<br>DK-1870 Frederiksberg C |

| | |
|---|---|
| | (National Veterinary Institute, Technical University of Denmark, 27, Bülowsvej, DK — 1870 Frederiksberg C) |
| Estonia: | Veterinaar- ja Toidulaboratoorium (Estonian Veterinary and Food Laboratory)<br>Kreutzwaldi 30<br>Tartu 51006 |
| Finland: | Finnish Food Safety Authority Evira<br>Research and Laboratory Department<br>Veterinary Virology Research Unit-TSEs<br>Mustialankatu 3<br>FI-00790 Helsinki |
| France: | ANSES-Lyon, Unité MND<br>31, avenue Tony Garnier<br>69 364 LYON Cedex 07 |
| Germany: | Friedrich-Loeffler-Institut<br>Institute for Novel and Emerging Infectious Diseases at the Friederich-Loeffler-Institut<br>Federal Research Institute for Animal Health<br>Suedufer 10<br>D-17493 Greifswald Insel Riems |
| Greece: | Ministry of Agriculture — Veterinary Laboratory of Larissa<br>6th km of Larissa — Trikala Highway<br>GR-41110 Larissa |
| Hungary: | Veterinary Diagnostic Directorate, National Food Chain Safety Office (VDD NFCSO)<br>Tábornok u. 2<br>1143 Budapest |
| Ireland: | Central Veterinary Research Laboratory<br>Department of Agriculture, Food and the Marine<br>Backweston Campus<br>Celbridge<br>Co. Kildare |
| Italy: | Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta — CEA<br>Via Bologna, 148<br>I-10154 Torino |
| Latvia: | Institute of Food Safety, Animal Health and Environment (BIOR)<br>Lejupes Str. 3 |

| | |
|---|---|
| | Riga LV 1076 |
| Lithuania: | National Food and Veterinary Risk Assessment Institute<br>J. Kairiūkščio str. 10<br>LT-08409 Vilnius |
| Luxembourg: | CERVA-CODA-VAR<br>Centre d'Étude et de Recherches Vétérinaires et Agrochimiques, Centrum voor Onderzoek in Diergeneeskunde en Agrochemie, Veterinary and Agrochemical Research Centre<br>Groeselenberg 99<br>B-1180 Bruxelles |
| Malta: | Veterinary Diagnostic Laboratory<br>Department of Food Health and Diagnostics<br>Veterinary Affairs and Fisheries Division<br>Ministry for Rural Affairs and the Environment<br>Albert Town Marsa |
| Netherlands: | Central Veterinary Instutute of Wageningen UR<br>Edelhertweg 15<br>8219 PH Lelystad<br>P.O. Box 2004<br>NL-8203 AA Lelystad |
| Poland: | Państwowy Instytut Weterynaryjny (PIWet)<br>24-100 Puławy<br>al. Partyzantów 57 |
| Portugal: | Setor diagnóstico EET<br>Laboratório de Patologia<br>Unidade Estratégica de Investigação e Serviços de Produção e Saúde Animal<br>Instituto Nacional de Investigação Agrária e Veterinária Rua General Morais Sarmento<br>1500-311 Lisboa |
| Romania: | Institutul de Diagnostic și Sănătate Animală (Institute for Diagnosis and Animal Health)<br>Department of Morphology<br>Strada Dr Staicovici nr. 63, 5<br>București 050557 |
| Slovakia: | State Veterinary Institute Zvolen<br>Pod dráhami 918<br>SK-960 86, Zvolen |

| | |
|---|---|
| Slovenia: | University of Ljubljana, Veterinary faculty<br>National Veterinary Institute<br>Gerbičeva 60<br>SI-1000 Ljubljana |
| Spain: | Laboratorio Central de Veterinaria (Algete)<br>Ctra. M-106 pk 1,4<br>28110 Algete (Madrid) |
| Sweden: | National Veterinary Institute<br>S-751 89 Uppsala |
| United Kingdom: | Animal Health and Veterinary Laboratories Agency<br>Woodham Lane<br>New Haw, Addlestone, Surrey KT15 3NB |

CHAPTER B

**EU reference laboratory**

1. The EU reference laboratory for TSEs is:

   The Animal Health and Veterinary Laboratories Agency

   Woodham Lane

   New Haw

   Addlestone

   Surrey KT15 3NB

   United Kingdom

2. The functions and duties of the EU reference laboratory are:

   (a) to coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing TSEs and the determination of the prion protein genotype in ovine animals, specifically by:

   — storing and supplying corresponding tissues containing the TSE agents, for the development or production of the relevant diagnostic tests or for typing strains of the TSE agents

   — supplying standard sera and other reference reagents to the national reference laboratories in order to standardise the tests and reagents used in the Member States

   — building up and retaining a collection of corresponding tissues containing the agents and strains of TSEs

   — organising periodic comparative tests for the procedures for the diagnosis of TSEs and for the determination of the prion protein genotype in ovine animals at EU level

— collecting and collating data and information on the methods of diagnosis used and the results of tests carried out in the EU
— characterising isolates of the TSE agent by the most up-to-date methods to allow greater understanding of the epidemiology of the disease
— keeping abreast of trends in surveillance, epidemiology and prevention of TSEs throughout the world
— maintaining expertise on prion diseases to enable rapid differential diagnosis
— acquiring a thorough knowledge of the preparation and use of diagnostic methods used to control and eradicate TSEs;

(b) to assist actively in the diagnosis of outbreaks of TSEs in Member States by studying samples from TSE-infected animals sent for confirmatory diagnosis, characterisation and epidemiological studies;

(c) to facilitate the training or retraining of experts in laboratory diagnosis with a view to the harmonisation of diagnostic techniques throughout the EU.

## CHAPTER C

## Sampling and laboratory testing

### 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 World Organisation for Animal Health (OIE) (the Manual). In addition to, 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 EU reference laboratory.

In particular the competent authority shall collect the appropriate tissues, according to the available scientific advice and the guidelines of the EU 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 subject to confirmatory testing, and be processed subsequently in accordance with the EU reference laboratory guidelines on discriminatory testing and classification — ‘TSE strain characterisation in small ruminants: A technical handbook for National Reference Laboratories in the EU’.

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

### 2. Laboratories

Any laboratory examination for TSE shall be carried out in official diagnostic laboratories designated 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 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) Western blot;

(iii) the demonstration of characteristic fibrils by electron microscopy;

(iv) histopathological examination;

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

If 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 EU reference laboratory —'OIE rules for the official confirmation of BSE in bovines (based on an initial reactive result in an approved rapid test) by using a second rapid test', 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 the blot image 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; where 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 animal shall be regarded as 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 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) Western blot;

(iii) the demonstration of characteristic fibrils by electron microscopy;

(iv) histopathological examination;

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

Where 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 EU reference laboratory — 'OIE rules for the official confirmation of BSE in bovines (based on an initial reactive result in an approved rapid test) by using a second rapid test, 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 the blot image 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; where 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 second subparagraph is positive.

(c) *Further examination of positive BSE cases*

Samples from all positive BSE cases shall be forwarded to a laboratory, appointed by the competent authority, which has participated successfully in the latest proficiency testing organised by the EU reference laboratory for discriminatory testing of confirmed BSE cases, where they shall be further tested in accordance with the methods and protocols laid down in the EU reference laboratory's method for the classification of bovine TSE isolates (a two-blot method for the provisional classification of bovine TSE isolates).

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 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) Western blot;

(iii) the demonstration of characteristic fibrils by electron microscopy;

(iv) 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. Where 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.

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

(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, 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 histopathology, immunohistochemistry, Western blotting or demonstration of characteristic fibrils by electron microscopy, as referred to in point (a). If the result of the confirmatory examination is negative or inconclusive, the tissues shall be submitted to a further examination by immunohistochemistry or Western blotting.

If the result of one of the confirmatory examinations is positive, the animal shall be regarded as a positive TSE case and further examination as referred to in point (c) shall be performed.

(c) *Further examination of positive TSE cases*

(i) Primary molecular testing with a discriminatory Western blotting method

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 examined using a discriminatory Western blotting method listed in the guidelines of the EU reference laboratory by an official diagnostic laboratory designated by the competent authority, which has participated successfully in the latest proficiency testing organised by the EU reference laboratory for the use of such a method.

(ii) Secondary molecular testing with additional molecular testing methods

TSE cases in which the presence of BSE cannot be excluded according to the guidelines issued by the EU reference laboratory by the primary molecular testing referred to in point (i), shall be referred immediately to the EU reference laboratory, with all the relevant information available. The samples shall be submitted to further investigation and confirmation by at least one alternative method, differing immunochemically from the original primary molecular method, depending on the volume and nature of the referred material, as described in the guidelines of the EU reference laboratory. These additional tests will be carried out in the following laboratories approved for the relevant method:

Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail

31, avenue Tony Garnier

BP 7033

F-69342 Lyon Cedex

Commissariat à l'Energie Atomique

18, route du Panorama

BP 6

F-92265 Fontenay-aux-Roses Cedex

Animal Health and Veterinary Laboratories Agency

Woodham Lane

New Haw

Addlestone

Surrey KT15 3NB

United Kingdom

The results shall be interpreted by the EU reference laboratory assisted by a panel of experts referred to as the Strain Typing Expert Group (STEG), including a representative of the relevant national reference laboratory. The Commission shall be informed immediately about the outcome of that interpretation.

(iii) Mouse bioassay

Samples indicative of BSE or inconclusive for BSE, following secondary molecular testing, shall be further analysed by mouse bioassay for final confirmation. The nature or quantity of available material may influence the bioassay design, which will be approved by the EU reference laboratory assisted by the STEG on a case by case basis. Bioassays will be performed by the EU reference laboratory, or by laboratories designated by the EU reference laboratory.

The results shall be interpreted by the EU reference laboratory assisted by the STEG. The Commission shall be informed immediately about the outcome of that interpretation.

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 immunohistochemistry, Western 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 with positive results 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, the cases shall be referred to the EU reference laboratory assisted by the STEG for further characterisation.

## 4. Rapid tests

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

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

— the sandwich immunoassay for PrPRes detection (short assay protocol) carried out following denaturation and concentration steps (Bio-Rad TeSeE SAP rapid test),

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

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

— 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 PrPSc (Roboscreen Beta Prion BSE EIA Test Kit).

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

— the sandwich immunoassay for PrPRes detection (short assay protocol) carried out following denaturation and concentration steps (Bio-Rad TeSeE SAP rapid test),

— the sandwich immunoassay for PrPRes 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 immunoassay using a chemical polymer for selective PrPSc capture and a monoclonal detection antibody directed against conserved regions of the PrP molecule (HerdChek BSE-Scrapie Antigen (IDEXX Laboratories)),

**Textual Amendments**

**F2** Deleted by Commission Regulation (EU) 2017/110 of 23 January 2017 amending Annexes IV 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).

In all rapid 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 EU reference laboratory and ensures that the test performance does not change. Producers must provide the EU reference laboratory with the test protocols.

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

**Status:**
Point in time view as at 13/02/2017.

**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, ANNEX X.