[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).

F2CHAPTER A

[F2National reference laboratories

[F2. . . . . .

Textual Amendments

F2 Deleted by Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation) (Text with EEA relevance).

F2CHAPTER B

EU reference laboratory]

F2. . . . . .]

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 [F3]..., 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 [F4]....

Textual Amendments

F3 Words in Annex 10 Ch. C point 1 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(69)(a)**; 2020 c. 1, Sch. 5 para. 1(1)

F4 Words in Annex 10 Ch. C point 1 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(69)(b)**; 2020 c. 1, Sch. 5 para. 1(1)

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, [F5]... by using a second rapid test ’ , and provided that:

Textual Amendments

F5 Words in Annex 10 Ch. C point 3.1(a) omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(71)(a)**; 2020 c. 1, Sch. 5 para. 1(1)

(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, [F6]... by using a second rapid test, and provided that ' :

Textual Amendments

F6 Words in Annex 10 Ch. C point 3.1(b) omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(71)(b)**; 2020 c. 1, Sch. 5 para. 1(1)

(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, **[**[F7]where they must be further tested by a two-blot method for the provisional classification of bovine TSE isolates**]**.

Textual Amendments

F7 Words in Annex 10 Ch. C point 3.1(c) omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(71)(c)**; 2020 c. 1, Sch. 5 para. 1(1)

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.

[[F8]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.**]**

Textual Amendments

F8 Substituted by Commission Regulation (EU) 2020/1593 of 29 October 2020 amending Annex X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards further examination of positive cases of transmissible spongiform encephalopathies in ovine and caprine animals (Text with EEA relevance).

(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 **[**[F9]the relevant**]** 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.

Textual Amendments

F9 Words in Annex 10 Ch. C point 3.2(b) substituted (31.12.2020) by The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(72)(a)**; 2020 c. 1, Sch. 5 para. 1(1)

[[F8]If the result of one of the confirmatory examinations is positive, the animal shall be regarded as a positive TSE case.**]**

(c) *Further examination of positive TSE cases*

[[F10]Samples that, following the examinations referred to in points (a) or (b), are regarded as positive TSE cases, but which are not considered atypical cases, shall be examined to exclude the presence of BSE only when they come from an index case. Other cases, which display characteristics that, according to the testing laboratory, merit investigation, shall also be examined to exclude the presence of BSE.**]**

Textual Amendments

F10 Inserted by Commission Regulation (EU) 2020/1593 of 29 October 2020 amending Annex X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards further examination of positive cases of transmissible spongiform encephalopathies in ovine and caprine animals (Text with EEA relevance).

[[F8](i) Primary molecular testing with a discriminatory Western blotting method

For the exclusion of the presence of BSE, samples shall be examined by a discriminatory Western blotting method **[**[F11]approved by the national reference laboratory**]**.**]**

Textual Amendments

F11 Words in Annex 10 Ch. C point 3.2(c)(i) substituted (31.12.2020) by S.I. 2019/170, **reg. 2(72)(b)(i)** (as substituted by The Animals, Aquatic Animal Health, Invasive Alien Species, Plant Propagating Material and Seeds (Amendment) (EU Exit) Regulations 2020 (S.I. 2020/1388), regs. 1(2)(a), **20(2)(w)**)

[F12(ii) Secondary molecular testing with additional molecular testing methods

In TSE cases in which the presence of BSE cannot be excluded by the primary molecular testing referred to in point (i), the samples must 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. These additional tests will be carried out by the national reference laboratory.**]**

Textual Amendments

F12 Annex 10 Ch. C point 3.2(c)(ii) substituted (31.12.2020) by The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(72)(b)(ii)**; 2020 c. 1, Sch. 5 para. 1(1)

(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[F13]....

Textual Amendments

F13 Words in Annex 10 Ch. C point 3.2(c)(iii) omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(72)(b)(iii)(aa)**; 2020 c. 1, Sch. 5 para. 1(1)

F14...

Textual Amendments

F14 Words in Annex 10 Ch. C point 3.2(c)(iii) omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(72)(b)(iii)(bb)**; 2020 c. 1, Sch. 5 para. 1(1)

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.

F15 …

Textual Amendments

F15 Words in Annex 10 Ch. C point 3.3 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(73)**; 2020 c. 1, Sch. 5 para. 1(1)

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)),

— **F16** …

Textual Amendments

F16 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 [F17]... ensures that the test performance does not change. [F18]...

Textual Amendments

F17 Words in Annex 10 Ch. C point 4 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(74)(a)(i)**; 2020 c. 1, Sch. 5 para. 1(1)

F18 Words in Annex 10 Ch. C point 4 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(74)(a)(ii)**; 2020 c. 1, Sch. 5 para. 1(1)

Changes to rapid tests and to test protocols may only be made **[**[F19]if**]** the change does not alter the sensitivity, specificity or reliability of the rapid test. [F20]...

Textual Amendments

F19 Word in Annex 10 Ch. C point 4 substituted (31.12.2020) by The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(74)(b)(i)**; 2020 c. 1, Sch. 5 para. 1(1)

F20 Words in Annex 10 Ch. C point 4 omitted (31.12.2020) by virtue of The Transmissible Spongiform Encephalopathies and Animal By-Products (Amendment etc.) (EU Exit) Regulations 2019 (S.I. 2019/170), regs. 1, **2(74)(b)(ii)**; 2020 c. 1, Sch. 5 para. 1(1)

5. **Alternative tests**

(To be defined)**]**

Status:
Point in time view as at 31/12/2020.

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.