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)

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)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies[(1)], and in particular the first paragraph of Article 23 thereof,

Whereas:

(1) Regulation (EC) No 999/2001 lays down rules for the prevention, control and eradication of transmissible spongiform encephalopathies (TSEs) in bovine, ovine and caprine animals. It applies to the production and placing on the market of live animals and products of animal origin and in certain specific cases to exports thereof.

(2) Annex II to Regulation (EC) No 999/2001 lays down rules governing the determination of the bovine spongiform encephalopathy (BSE) status of Member States or third countries or regions thereof. These rules are based on the international standard established by the World Organisation for Animal Health (OIE) in the Terrestrial Animal Health Code (the Code). In the BSE chapter of the 2013 version of the Code, the expression ‘release assessment’ has been replaced by ‘entry assessment’, and the table providing the points targets for a country or region has been significantly amended to better meet the needs of countries with a small or very small cattle population. These amendments should be reflected in Annex II.

(3) Point 2.2.1 of Chapter B of Annex VII to Regulation (EC) No 999/2001 refers to the methods and protocols set out in Annex X. The wording of this point should be amended to reflect amendments to Annex X brought by this act.

(4) Chapter A of Annex VIII to Regulation (EC) No 999/2001 lays down rules governing intra-Union trade in live animals, semen and embryos, including the exemption of homozygous ovine ARR embryos from any other classical scrapie related requirement

in intra-Union trade. On 24 January 2013, the European Food Safety Authority (EFSA) adopted a scientific opinion on the risk of transmission of classical scrapie via in vivo derived embryo transfer in ovine animals[(2)], where it concluded that the risk of transmitting classical scrapie by the implantation of homozygous or heterozygous ovine ARR embryos could be considered negligible providing that the OIE recommendations and procedures relating to embryo transfer are adhered to. The relevant provisions of Annex VIII should therefore be amended to also exempt intra-Union trade of heterozygous ovine ARR embryos from any other classical scrapie related requirement.

(5) In certain language versions of Regulation (EC) No 999/2001, there is a terminology incoherence between Points 1.2. and 1.3 of Section A of Chapter A of Annex VIII to that Regulation and the rest of the text. For the sake of coherence, the same term should be used in the language versions concerned.

(6) Point 2 of Section A of Chapter A of Annex VIII to Regulation (EC) No 999/2001 lays down the rules governing the approval of the negligible risk status for classical scrapie of a Member State or zone of a Member State. On 4 July 2013, Austria submitted to the Commission the appropriate supporting documentation. Given the favourable outcome of the assessment of this application by the Commission, Austria should be listed as a Member State with a negligible risk of classical scrapie.

(7) Point 3.2 of Section A of Chapter A of Annex VIII to Regulation (EC) No 999/2001 lists Member States with an approved national control programme for classical scrapie. Considering that Austria should be listed as a Member State with a negligible risk of classical scrapie, it should simultaneously be deleted from the list of Member States with an approved national control programme for classical scrapie, as this status offers guarantees over and above those offered in the control programme.

(8) Chapter H of Annex IX to Regulation (EC) No 999/2001 lays down rules for the import in the Union of ovine and caprine semen and embryos. These import rules should be updated to reflect amendments to Annex VIII brought by this act.

(9) Annex X to Regulation (EC) No 999/2001 lays down the methods of analysis applicable to TSE testing in bovine, ovine and caprine animals. This Annex should be reviewed to update the information on the designated laboratories, adjust the reference to various guidelines, harmonize some technical terms, and clarify the discriminatory testing process in case of positive TSE cases in ovine and caprine animals, in accordance with the latest scientific knowledge and current practices in the Union.

(10) Point 4 of Chapter C of Annex X to Regulation (EC) No 999/2001 sets out the lists of rapid tests approved for the monitoring of TSEs in bovine, ovine and caprine animals. On 18 September 2013, IDEXX made an application in order that the name of the test IDEXX HerdChek BSE-Scrapie Antigen Test Kit, EIA be changed to HerdChek BSE-Scrapie Antigen (IDEXX Laboratories). The new package insert for this test has been approved by the European Union Reference laboratory for TSEs on 2 May 2013. Furthermore, on 6 December 2013, the Enfer Group informed that it has ceased the manufacture of the Enfer Version 3 TSE diagnostic kit and requested the deletion of this kit from the list of approved BSE rapid tests in bovine animals. The lists in point 4 of Chapter C of Annex X should therefore be adapted accordingly.

(11) In order to offer sufficient time to Member States to align their scrapie-related certification procedures for ovine embryos, certain amendments introduced by this Regulation should apply from 1 January 2015.

(12) Regulation (EC) No 999/2001 should therefore be amended accordingly.

(13) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1

Annexes II, VII, VIII, IX and X to Regulation (EC) No 999/2001 are amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

Paragraphs (a), (b) and (e) of point 3 and point 4 of the Annex shall apply from 1 January 2015.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 28 October 2014.

For the Commission

The President

José Manuel BARROSO

ANNEX

Annexes II, VII, VIII, IX and X to Regulation (EC) No 999/2001 are amended as follows:

(1) Annex II is amended as follows:

(a) Points 1 and 2 of Chapter B are replaced by the following:

1. **Structure of the risk analysis**

The risk analyses shall comprise an entry assessment and an exposure assessment.

2. **Entry assessment (external challenge)**

2.1. The entry assessment shall consist of assessing the likelihood that the BSE agent has either been introduced into the country or region via commodities potentially contaminated with a BSE agent, or is already present in the country or region.

The following risk factors shall be taken into account:

(a) the presence or absence of the BSE agent in the country or region and, if the agent is present, its prevalence based on the outcome of surveillance activities;

(b) the production of meat-and-bone meal or greaves from the BSE indigenous ruminant population;

(c) imported meat-and-bone meal or greaves;

(d) imported bovine and ovine and caprine animals;

(e) imported animal feed and feed ingredients;

(f) imported products of ruminant origin for human consumption, which may have contained tissues listed in point 1 of Annex V and may have been fed to bovine animals;

(g) imported products of ruminant origin for *in vivo* use in bovine animals.

2.2. Special eradication schemes, surveillance and other epidemiological investigations (especially surveillance for BSE conducted on the bovine animals population) relevant to the risk factors listed in point 2.1 should be taken into ac-count in carrying out the entry assessment.

(b) In point 3 of Chapter D, Table 2 is replaced by the following:

TABLE 2

Points targets for different adult bovine animals population sizes in a country or region

Points targets for country or region		
Adult bovine animals population	**Type A surveillance**	**Type B surveillance**

size(24 months and older)		
> 1 000 000	300 000	150 000
900 001-1 000 000	214 600	107 300
800 001-900 000	190 700	95 350
700 001-800 000	166 900	83 450
600 001-700 000	143 000	71 500
500 001-600 000	119 200	59 600
400 001-500 000	95 400	47 700
300 001-400 000	71 500	35 750
200 001-300 000	47 700	23 850
100 001-200 000	22 100	11 500
90 001-100 000	19 900	9 950
80 001-90 000	17 700	8 850
70 001-80 000	15 500	7 750
60 001-70 000	13 000	6 650
50 001-60 000	11 000	5 500
40 001-50 000	8 800	4 400
30 001-40 000	6 600	3 300
20 001-30 000	4 400	2 200
10 001-20 000	2 100	1 050
9 001-10 000	1 900	950
8 001-9 000	1 600	800
7 001-8 000	1 400	700
6 001-7 000	1 200	600
5 001-6 000	1 000	500
4 001-5 000	800	400
3 001-4 000	600	300
2 001-3 000	400	200
1 001-2 000	200	100

(2) In Annex VII, the first paragraph in point 2.2.1 of Chapter B is replaced by the following:

If BSE cannot be excluded after the results of the secondary molecular testing carried out in accordance with the methods and protocols set out in Annex X, Chapter C, point 3.2(c) (ii), the killing and complete destruction, without delay, of

all animals, embryos and ova identified by the inquiry referred to in the second to fifth indents of point 1(b).

(3) In Annex VIII, Section A of Chapter A is amended as follows:

(a) In point 1.2, paragraph (g) is replaced by the following:

(g) only the following ovine and caprine embryos/ova may be introduced:

(i) embryos/ova from donor animals which have been kept since birth in a Member State with a negligible risk of classical scrapie, or in a holding with a negligible or a controlled risk of classical scrapie, or which meet the following requirements:

— they are permanently identified to enable trace back to their holding of birth

— they have been kept since birth in holdings in which no case of classical scrapie has been confirmed during their residency

— they showed no clinical sign of classical scrapie at the time of embryo/ova collection;

(ii) ovine embryos/ova carrying at least one ARR allele.

(b) In point 1.3, paragraph (g) is replaced by the following:

(g) only the following ovine and caprine embryos/ova may be introduced:

(i) embryos/ova from donor animals which have been kept since birth in a Member State with a negligible risk of classical scrapie, or in a holding with a negligible or a controlled risk of classical scrapie, or which meet the following requirements:

— they are permanently identified to enable trace back to their holding of birth

— they have been kept since birth in holdings in which no case of classical scrapie has been confirmed during their residency

— they showed no clinical sign of classical scrapie at the time of embryo/ova collection;

(ii) ovine embryos/ova carrying at least one ARR allele.

(c) In point 2, the following point 3 is added:

2.3. The Member States or zone of the Member State with a negligible risk for classical scrapie are the following:

— Austria.

(d) Point 3.2 is replaced by the following:

3.2. The national scrapie control programmes of the following Member States are hereby approved:

— Denmark

— Finland

— Sweden.

(e) In point 4.2, paragraph (e) is replaced by the following:

(e) in the case of ovine embryos, be carrying at least one ARR allele.

(4) In Annex IX, point (ii) of point 2 of Chapter H is replaced by the following:

(ii) in the case of ovine embryos, the embryos carry at least one ARR allele.

(5) Annex X is replaced by the following:

ANNEX X

REFERENCE LABORATORIES, SAMPLING AND LABORATORY ANALYSIS METHODS

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) Institut für veterinärmedizinische Untersuchungen Robert Koch Gasse 17 A-2340 Mödling
Belgium:	CERVA-CODA-VAR Centre d'Étude et de Recherches Vétérinaires et Agrochimiques, Centrum voor Onderzoek in Diergeneeskunde en Agrochemie, Veterinary and Agrochemical Research Centre Groeselenberg 99 B-1180 Bruxelles
Bulgaria:	Национален диагностичен научноизследователски ветеринарномедицински институт ‘Проф. Д-р Георги Павлов’ Национална референтна лаборатория ‘Трансмисивни спонгиформни енцефалопатии’ бул. ‘Пенчо Славейков’ 15 София 1606 (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, Savska Cesta 143 10000 Zagreb
Cyprus:	State Veterinary Laboratories Veterinary Services CY-1417 Athalassa Nicosia
Czech Republic:	Státní veterinární ústav Jihlava (State Veterinary Institute Jihlava)

	National Reference Laboratory for BSE and Animal TSEs Rantířovská 93 586 05 Jihlava
Denmark:	Veterinærinstituttet Danmarks Tekniske Universitet Bülowsvej 27 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) Kreutzwaldi 30 Tartu 51006
Finland:	Finnish Food Safety Authority Evira Research and Laboratory Department Veterinary Virology Research Unit-TSEs Mustialankatu 3 FI-00790 Helsinki
France:	ANSES-Lyon, Unité MND 31, avenue Tony Garnier 69 364 LYON Cedex 07
Germany:	Friedrich-Loeffler-Institut Institute for Novel and Emerging Infectious Diseases at the Friederich-Loeffler-Institut Federal Research Institute for Animal Health Suedufer 10 D-17493 Greifswald Insel Riems
Greece:	Ministry of Agriculture — Veterinary Laboratory of Larissa 6th km of Larissa — Trikala Highway GR-41110 Larissa
Hungary:	Veterinary Diagnostic Directorate, National Food Chain Safety Office (VDD NFCSO) Tábornok u. 2 1143 Budapest
Ireland:	Central Veterinary Research Laboratory

	Department of Agriculture, Food and the Marine Backweston Campus Celbridge Co. Kildare
Italy:	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta — CEA Via Bologna, 148 I-10154 Torino
Latvia:	Institute of Food Safety, Animal Health and Environment (BIOR) Lejupes Str. 3 Riga LV 1076
Lithuania:	National Food and Veterinary Risk Assessment Institute J. Kairiūkščio str. 10 LT-08409 Vilnius
Luxembourg:	CERVA-CODA-VAR Centre d'Étude et de Recherches Vétérinaires et Agrochimiques, Centrum voor Onderzoek in Diergeneeskunde en Agrochemie, Veterinary and Agrochemical Research Centre Groeselenberg 99 B-1180 Bruxelles
Malta:	Veterinary Diagnostic Laboratory Department of Food Health and Diagnostics Veterinary Affairs and Fisheries Division Ministry for Rural Affairs and the Environment Albert Town Marsa
Netherlands:	Central Veterinary Instutute of Wageningen UR Edelhertweg 15 8219 PH Lelystad P.O. Box 2004 NL-8203 AA Lelystad
Poland:	Państwowy Instytut Weterynaryjny (PIWet) 24-100 Puławy al. Partyzantów 57
Portugal:	Setor diagnóstico EET Laboratório de Patologia

	Unidade Estratégica de Investigação e Serviços de Produção e Saúde Animal Instituto Nacional de Investigação Agrária e Veterinária Rua General Morais Sarmento 1500-311 Lisboa
Romania:	Institutul de Diagnostic și Sănătate Animală (Institute for Diagnosis and Animal Health) Department of Morphology Strada Dr Staicovici nr. 63, 5 București 050557
Slovakia:	State Veterinary Institute Zvolen Pod dráhami 918 SK-960 86, Zvolen
Slovenia:	University of Ljubljana, Veterinary faculty National Veterinary Institute Gerbičeva 60 SI-1000 Ljubljana
Spain:	Laboratorio Central de Veterinaria (Algete) Ctra. M-106 pk 1,4 28110 Algete (Madrid)
Sweden:	National Veterinary Institute S-751 89 Uppsala
United Kingdom:	Animal Health and Veterinary Laboratories Agency Woodham Lane 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)),

— the lateral-flow immunoassay using two different monoclonal antibodies to detect Proteinase K-resistant PrP fractions (Prionics — Check PrioSTRIP SR, visual reading protocol).

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)

(**1**) OJ L 147, 31.5.2001, p. 1.

(**2**) EFSA Journal 2013; 11(2):3080.

Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EU) No 1148/2014.