Official Journal of the European Union

L 280

English edition

Legislation

Volume 59

18 October 2016

Contents

[1] Text with EEA relevance

EN

Acts whose titles are printed in light type are those relating to day-to-day management of agricultural matters, and are generally valid for a limited period.

The titles of all other acts are printed in bold type and preceded by an asterisk.

DECISIONS

[1] Text with EEA relevance

II

(Non-legislative acts)

INTERNATIONAL AGREEMENTS

COUNCIL DECISION (EU) 2016/1830

of 11 October 2016

on the conclusion, on behalf of the European Union, of the Amending Protocol to the Agreement between the European Community and the Principality of Monaco providing for measures equivalent to those laid down in Council Directive 2003/48/EC

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 115 in conjunction with Article 218(6)(b) and the second subparagraph of Article 218(8) thereof,

Having regard to the proposal from the European Commission,

Having regard to the opinion of the European Parliament ([1]),

Whereas:

(1) In accordance with Council Decision (EU) 2016/1392 ([2]), the Amending Protocol to the Agreement between the European Community and the Principality of Monaco providing for measures equivalent to those laid down in Council Directive 2003/48/EC ('the Amending Protocol') was signed on 12 July 2016, subject to its conclusion at a later date.

(2) The text of the Amending Protocol which is the result of the negotiations, duly reflects the negotiating directives issued by the Council as it aligns the Agreement between the European Community and the Principality of Monaco providing for measures equivalent to those laid down in Council Directive 2003/48/EC ([3]) (the 'Agreement') with the latest developments at international level concerning automatic exchange of information, namely, with the Global Standard for automatic exchange of financial account information in tax matters developed by the Organisation for Economic Cooperation and Development (OECD). The Union, the Member States and the Principality of Monaco have actively participated in the work of the Global Forum of the OECD to support the development and implementation of that Global Standard. The text of the Agreement, as amended by the Amending Protocol, is the legal basis for implementing the Global Standard in the relations between the Union and the Principality of Monaco.

(3) The European Data Protection Supervisor was consulted in accordance with Article 28(2) of Regulation (EC) No 45/2001 of the European Parliament and of the Council ([4]).

(4) The Amending Protocol should be approved,

([1]) Opinion of 23 June 2016 (not yet published in the Official Journal).
([2]) Council Decision (EU) 2016/1392 of 12 July 2016 on the signing, on behalf of the European Union, and provisional application, of the Amending Protocol to the Agreement between the European Community and the Principality of Monaco providing for measures equivalent to those laid down in Council Directive 2003/48/EC (OJ L 225, 19.8.2016, p. 1).
([3]) OJ L 19, 21.1.2005, p. 55.
([4]) Regulation (EC) No 45/2001 of the European Parliament and of the Council of 18 December 2000 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data (OJ L 8, 12.1.2001, p. 1).

HAS ADOPTED THIS DECISION:

Article 1

The Amending Protocol to the Agreement between the European Community and the Principality of Monaco providing for measures equivalent to those laid down in Council Directive 2003/48/EC is hereby approved on behalf of the Union ([1]).

Article 2

The President of the Council shall, on behalf of the Union, make the notification provided for in Article 2(1) of the Amending Protocol ([2]).

Article 3

This Decision shall enter into force on the day of its adoption.

Done at Luxembourg, 11 October 2016.

For the Council
The President
P. KAŽIMÍR

([1]) The text of the Amending Protocol has been published in OJ L 225, 19.8.2016, p. 3 together with the Decision regarding its signature and provisional application.

([2]) The date of entry into force of the Amending Protocol will be published in the *Official Journal of the European Union* by the General Secretariat of the Council.

REGULATIONS

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1831

of 14 October 2016

amending Council Regulation (EC) No 329/2007 concerning restrictive measures against the Democratic People's Republic of Korea

THE EUROPEAN COMMISSION,

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

Having regard to Council Regulation (EC) No 329/2007 of 27 March 2007 concerning restrictive measures against the Democratic People's Republic of Korea ([1]), and in particular Article 13(1)(g) thereof,

Whereas:

(1) Regulation (EC) No 329/2007 gives effect to measures provided for in Council Decision (CFSP) 2016/849 ([2]).

(2) On 2 March 2016, the United Nations Security Council adopted Resolution (UNSCR) 2270 (2016) providing for new measures against North Korea. In accordance with that Resolution, on 4 April 2016 the Sanctions Committee established pursuant to UNSCR 1718 (2006) published a list of additional goods to which prohibitions on the transfer, procurement and provision of technical assistance apply (the 'sensitive goods' list).

(3) Following the adoption of Resolution (UNSCR) 2270 (2016), on 4 August 2016 the Council adopted Decision (CFSP) 2016/1341 ([3]). Council Regulation (EU) 2016/1333 ([4]) amended Regulation (EC) No 329/2007 accordingly, adding Annex Ig.

(4) This proposal identifies the sensitive goods to be included in Annex Ig of Regulation (EC) No 329/2007 and allocates the reference numbers taken from the Combined Nomenclature as set out in Annex I to Council Regulation (EEC) No 2658/87 ([5]). Annex Ig of Regulation (EC) No 329/2007 should therefore be amended accordingly,

HAS ADOPTED THIS REGULATION:

Article 1

Annex Ig to Regulation (EC) No 329/2007 is amended in accordance with the Annex to this Regulation.

Article 2

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

([1]) OJ L 88 29.3.2007, p. 1.
([2]) Council Decision (CFSP) 2016/849 of 27 May 2016 concerning restrictive measures against the Democratic People's Republic of Korea and repealing Decision 2013/183/CFSP (OJ L 141, 28.5.2016, p. 79).
([3]) Council Decision (CFSP) 2016/1341 of 4 August 2016 amending Decision (CFSP) 2016/849 concerning restrictive measures against the Democratic People's Republic of Korea (OJ L 212, 5.8.2016, p. 116).
([4]) Council Regulation (EU) 2016/1333 of 4 August 2016 amending Regulation (EC) No 329/2007 concerning restrictive measures against the Democratic People's Republic of Korea (OJ L 212, 5.8.2016, p. 1).
([5]) Council Regulation (EEC) No 2658/87 of 23 July 1987 on the tariff and statistical nomenclature and on the Common Customs Tariff (OJ L 256, 7.9.1987, p. 1).

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

Done at Brussels, 14 October 2016.

For the Commission,
On behalf of the President,
Acting Head of the Service for Foreign Policy Instruments

ANNEX

Annex Ig to Council Regulation (EC) No 329/2007 is replaced by the following:

'ANNEX Ig

GOODS AND TECHNOLOGY REFERRED TO IN ARTICLES 2, 3 AND 6 [1]

Weapons of mass destruction-related items, materials, equipment, goods and technology identified and designated as sensitive goods, pursuant to paragraph 25 of UN Security Council Resolution 2270.

(a) **Nuclear- and/or Missile-usable Items**

(1) Ring Magnets

Permanent magnet materials having both the following characteristics:

i. Ring-shaped magnet with a relation between outer and inner diameter smaller or equal to 1.6:1; and

ii. Made of any of the following magnetic materials: aluminium-nickel-cobalt, ferrites, samarium-cobalt, or neodymium-iron-boron.

ex 8505 11 00

ex 8505 19 10

ex 8505 19 90

ex 8505 90 90

(2) Maraging Steel

Maraging steel having both the following characteristics:

i. "capable of" an ultimate tensile strength of 1 500 MPa or more at 293 K (20 °C).

ii. In bar or tube form, with an outer diameter of 75 mm or greater.

ex 7304 49 10

ex 7304 51 81

ex 7304 51 89

ex 7304 59 92

ex 7304 59 93

ex 7304 59 99

(3) Magnetic alloy materials in sheet or thin strip form having both of the following characteristics:

(a) Thickness of 0,05 mm or less; or height of 25 mm or less, and

(b) Made of any of the following magnetic alloy materials: iron-chromium-cobalt, iron-cobalt-vanadium, iron-chromium-cobalt-vanadium, or iron-chromium.

ex 7326 19 10

ex 7326 19 90

[1] The nomenclature codes are the ones applicable to the relevant products in the Combined Nomenclature as defined in Article 1(2) of Council Regulation (EEC) No 2658/87 and as set out in Annex I thereto.

ex 7326 90 92

ex 7326 90 94

ex 7326 90 96

ex 7326 90 98

(4) Frequency Changers (also known as converters or inverters)

Frequency changers, other than those specified in entries 0B001.b.13 or 3A225 of Annex 1, having all of the following characteristics, and specially designed software therefore:

i. Multiphase frequency output;

ii. Capable of providing a power of 40 W or greater; and

iii. Capable of operating anywhere (at any one point or more) within the frequency range of between 600 and 2 000 Hz.

Technical Notes:

(1) Frequency changers are also known as converters or inverters.

(2) The functionality specified above may be met by certain equipment described or marketed as electronic test equipment, AC power supplies, variable speed motor drives, or variable frequency drives.

ex 8504 40 84

ex 8504 40 88

ex 8504 40 90

ex 8537 10 99

ex 8537 20 91

ex 8537 20 99

(5) High-strength Aluminium Alloy

Aluminium alloys having both the following characteristics:

i. "capable of" an ultimate tensile of strength of 415 MPa or more at 293 K (20 °C) and

ii. In bar or tube form, with an outer diameter of 75 mm or greater.

Technical Note:

The phrase "capable of" encompasses aluminium alloy before or after heat treatment.

ex 7601 20 80

ex 7604 29 10

ex 7608 20 20

ex 7608 20 81

ex 7608 20 89

(6) Fibrous or Filamentary Materials

"Fibrous or filamentary materials" and prepregs, as follows:

i. Carbon, aramid, or glass "fibrous or filamentary materials" having both of the following characteristics:

(1) A "specific modulus" exceeding $3{,}18 \times 10^6$ m; and

(2) A "specific tensile strength" exceeding $76{,}2 \times 10^3$ m;

ii. Prepregs: Thermoset resin-impregnated continuous “yarns”, “rovings”, “tows” or “tapes” with a width of 30 mm or less, made from carbon, aramid, or glass “fibrous or filamentary materials” controlled in (a) above.

ex 3916 90 10
ex 3916 90 50
ex 3916 90 90
ex 3920 92 00
ex 3920 99 28
ex 3920 99 52
ex 3920 99 59
ex 3920 99 90
ex 3921 90 55
ex 3921 90 60
ex 3921 90 90
ex 3926 90 92
ex 3926 90 97
ex 5402 11 00
ex 5402 19 00
ex 5402 31 00
ex 5402 32 00
ex 5403 10 00
ex 5404 90 90
ex 5407 10 00
ex 5407 20 90
ex 5407 41 00
ex 5407 42 00
ex 5407 43 00
ex 5407 44 00
ex 5501 10 00
ex 5501 90 00
ex 5503 11 00
ex 5503 19 00
ex 5503 20 00
ex 5503 90 00
ex 5506 10 00
ex 5506 90 00
ex 5509 11 00
ex 5509 12 00
ex 5604 90 10
ex 5607 50 11
ex 5607 50 19
ex 5607 50 30
ex 5607 50 90
ex 5609 00 00
ex 5902 10 10
ex 5902 10 90
ex 5902 20 90
ex 5902 90 10
ex 5902 90 90
ex 5903 10 10
ex 5903 10 90
ex 5903 20 10
ex 5903 20 90
ex 5903 90 10
ex 5903 90 91
ex 5903 90 99
ex 6815 10 10
ex 6815 99 00
ex 7019 12 00
ex 7019 19 10
ex 7019 19 90
ex 7019 51 00
ex 7019 59 00
ex 7019 90 00

(7) Filament Winding Machines and Related Equipment

Filament winding machines and related equipment, as follows:

i. Filament winding machines having all of the following characteristics:

(1) Having motions for positioning, wrapping, and winding fibres coordinated and programmed in two or more axes;

(2) Specially designed to fabricate composite structures or laminates from "fibrous or filamentary materials"; and

(3) Capable of winding cylindrical tubes of diameter of 75 mm or greater;

ii. Coordinating and programming controls for filament winding machines specified in (a) above;

iii. Mandrels for filament winding machines specified in (a) above.

ex 8419 89 30
ex 8419 89 98
ex 8419 90 85
ex 8444 00 10
ex 8444 00 90
ex 8446 10 00
ex 8446 21 00
ex 8446 29 00
ex 8446 30 00
ex 8447 11 00
ex 8447 12 00
ex 8447 20 20
ex 8447 20 80
ex 8447 90 00
ex 8448 19 00
ex 8448 20 00
ex 8448 39 00
ex 8448 42 00
ex 8448 49 00
ex 8448 59 00
ex 8479 89 97
ex 8479 90 20
ex 8479 90 80
ex 8537 10 10
ex 8537 10 91
ex 8537 10 99
ex 8538 10 00
ex 9022 12 00
ex 9022 19 00
ex 9022 90 00
ex 9031 80 38
ex 9031 80 98
ex 9031 90 85

(8) Flow-forming Machines

As described in INFCIRC/254/Rev.9/Part2 and S/2014/253

ex 8463 90 00

ex 8466 94 00

(9) Laser welding equipment

ex 8515 80 10

ex 8515 80 90

ex 8515 90 00

(10) 4- and 5-axis CNC machine tools

ex 8457 10 10
ex 8457 10 90
ex 8457 20 00
ex 8457 30 10
ex 8457 30 90
ex 8458 11 20
ex 8458 11 41
ex 8458 11 49
ex 8458 11 80
ex 8458 19 00
ex 8458 91 20
ex 8458 91 80
ex 8458 99 00
ex 8459 10 00
ex 8459 21 00
ex 8459 31 00
ex 8459 40 10
ex 8459 51 00
ex 8459 61 10
ex 8459 61 90
ex 8460 11 00
ex 8460 21 11
ex 8460 21 15
ex 8460 21 19
ex 8460 21 90
ex 8460 31 00
ex 8460 40 10
ex 8460 90 10
ex 8460 90 90
ex 8461 20 00
ex 8461 30 10
ex 8461 40 11
ex 8461 40 31
ex 8461 40 71
ex 8461 40 90
ex 8461 90 00
ex 8464 20 11
ex 8464 20 19
ex 8464 20 80
ex 8464 90 00

(11) Plasma cutting equipment

ex 8456 10 00
ex 8456 90 80
ex 8515 31 00
ex 8515 39 90
ex 8515 80 10
ex 8515 80 90
ex 8515 90 00

(12) Metal hydrides such as, zirconium hydride

ex 2850 00 20

(b) **Chemical/Biological Weapons-usable Items**

(1) Additional chemicals suitable for the production of chemical warfare agents:

Product description		Cn code
Sodium metal (7440-23-5)		2805 11 00
Sulphur trioxide (7446-11-9)	ex	2811 29 10
Aluminium chloride (7446-70-0)		2827 32 00
Potassium Bromide (7758-02-3)		2827 51 00
Sodium bromide (7647-15-6)		2827 51 00
Dichloromethane (75-09-2)		2903 12 00
Isopropyl bromide (75-26-3)	ex	2903 39 19
Isopropyl ether (108-20-3)	ex	2909 19 90
Monoisopropylamine (75-31-0)	ex	2921 19 99
Trimethylamine (75-50-3)	ex	2921 11 00
Tributylamine (102-82-9)	ex	2921 19 99
Triethylamine (121-44-8)	ex	2921 19 99
N,N-Dimethylaniline (121-69-7)	ex	2921 42 00
Pyridine (110-86-1)	ex	2933 31 00

(2) Reaction vessels, reactors, agitators, heat exchangers, condensers, pumps, valves, storage tanks, containers, receivers, and distillation or absorption columns that meet performance parameters described in S/2006/853 and S/2006/853/corr.1

— Single-seal pumps with manufacturer's specified maximum flow-rate greater than 0,6 m^3/h and casings (pump bodies), preformed casing liners, impellers, rotors or jet pump nozzles designed for such pumps, in which all surfaces that come into direct contact with the chemical(s) being processed are made from any of the following materials:

(a) nickel or alloys with more than 40 % nickel by weight;

(b) alloys with more than 25 % nickel and 20 % chromium by weight;

(c) fluoropolymers (polymeric or elastomeric materials with more than 35 % fluorine by weight);

(d) glass or glass-lined (including vitrified or enamelled coating);

(e) graphite or carbon-graphite;

(f) tantalum or tantalum alloys;

(g) titanium or titanium alloys;

(h) zirconium or zirconium alloys;

(i) ceramics;

(j) ferrosilicon (high silicon iron alloys); or

(k) niobium (columbium) or niobium alloys.

ex 3925 10 00
ex 3925 90 80
ex 3926 90 92
ex 3926 90 97
ex 4009 21 00
ex 4009 22 00
ex 4009 41 00
ex 4009 42 00
ex 4016 93 00
ex 6909 11 00
ex 6909 12 00
ex 6909 19 00
ex 6909 90 00
ex 6914 90 00
ex 7020 00 10
ex 7020 00 30
ex 7020 00 80
ex 7304 41 00
ex 7304 49 93
ex 7304 49 95
ex 7304 49 99
ex 7304 51 81
ex 7304 51 89
ex 7304 59 92
ex 7304 59 93
ex 7304 59 99
ex 7306 40 20
ex 7306 40 80
ex 7306 50 20
ex 7306 50 80
ex 7306 69 10
ex 7306 69 90
ex 7306 90 00
ex 7309 00 10
ex 7309 00 30
ex 7309 00 51
ex 7309 00 59
ex 7309 00 90
ex 7310 10 00
ex 7310 29 10
ex 7310 29 90
ex 7311 00 00
ex 7326 90 92
ex 8414 80 11
ex 7326 90 94
ex 7326 90 96
ex 7326 90 98
ex 7507 11 00
ex 7507 12 00
ex 7507 20 00
ex 7508 90 00
ex 8103 90 90
ex 8108 90 50
ex 8108 90 60
ex 8108 90 90
ex 8109 90 00
ex 8112 99 30
ex 8401 20 00
ex 8401 40 00
ex 8401 10 00
ex 8412 90 20
ex 8413 50 40
ex 8413 60 39
ex 8413 60 61
ex 8413 60 69
ex 8413 60 70
ex 8413 60 80
ex 8413 70 21
ex 8413 70 29
ex 8413 70 45
ex 8413 70 51
ex 8413 70 59
ex 8413 70 65
ex 8413 70 75
ex 8413 70 81
ex 8413 70 89
ex 8413 81 00
ex 8413 82 00
ex 8413 91 00
ex 8414 10 25
ex 8414 10 81
ex 8414 10 89
ex 8414 40 10
ex 8414 40 90
ex 8414 59 20
ex 8414 59 40
ex 8414 59 80
ex 8418 99 10

ex 8414 80 19
ex 8414 80 59
ex 8414 80 73
ex 8414 80 75
ex 8414 80 78
ex 8414 80 80
ex 8414 90 00
ex 8417 80 30
ex 8417 80 50
ex 8417 80 70
ex 8418 69 00
ex 8419 40 00
ex 8419 50 00
ex 8419 89 10
ex 8419 89 30
ex 8419 89 98
ex 8419 90 85
ex 8477 80 93
ex 8477 80 99
ex 8479 82 00
ex 8479 89 97
ex 8479 90 80

(3) Conventional or turbulent air-flow clean-air rooms and self-contained fan-HEPA filter units that could be used for P3 or P4 (BSL 3, BSL 4, L3, L4) containment facilities.

ex 8414 51 00
ex 8414 59 00
ex 8414 60 00
ex 8414 80 80
ex 8421 39 20
ex 8479 89 97’

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1832

of 17 October 2016

amending the model certificates for imports into the Union of meat preparations, meat products and treated stomachs, bladders and intestines, as well as fresh meat of domestic solipeds set out in Decisions 2000/572/EC and 2007/777/EC and Regulation (EU) No 206/2010 as regards public health requirements for residues

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

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

Having regard to Council Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption ([1]), and in particular Article 9(2)(b) and Article 9(4) thereof,

Having regard to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin ([2]), and in particular Article 7(2)(a) thereof,

Whereas:

(1) Commission Decision 2000/572/EC ([3]) lays down the animal and public health veterinary certification conditions for the importation into the Union of consignments of certain meat preparations from third countries. It provides that such consignments are to be accompanied by an animal and public health certificate complying with the model set out in Annex II thereto ('the health certificate for meat preparations').

(2) Commission Decision 2007/777/EC ([4]) lays down the animal and public health conditions for imports into the Union of consignments of meat products and treated stomachs, bladders and intestines. It provides that only consignments complying with the requirements of the model animal health and public health certificate set out in Annex III thereto ('the health certificate for meat products and treated commodities') and accompanied by such a certificate are to be imported into the Union.

(3) Commission Regulation (EU) No 206/2010 ([5]) lays down the veterinary certification requirements for imports into the Union of consignments of fresh meat of equidae intended for human consumption. It provides that such consignments are only to be imported, if they are accompanied by a veterinary certificate drawn up in accordance with the model veterinary certificate 'EQU' for fresh meat, excluding minced meat, of domestic solipeds (*Equus caballus, Equus asinus* and their cross-breeds) set out in Part 2 of Annex II ('the EQU certificate') thereto.

(4) Council Directive 96/22/EC ([6]) prohibits, amongst others, the importation from third countries of meat or products intended for human consumption obtained from animals which have been administered certain substances, including beta-agonists. This Directive allows imports of animals intended for breeding, breeding animals at the end of their reproductive life, or meat therefrom, from third countries, which can afford

([1]) OJ L 18, 23.1.2003, p. 11.
([2]) OJ L 139, 30.4.2004, p. 55.
([3]) Commission Decision 2000/572/EC of 8 September 2000 laying down the animal and public health and veterinary certification conditions for imports of meat preparations into the Community from third countries (OJ L 240, 23.9.2000, p. 19).
([4]) Commission Decision 2007/777/EC of 29 November 2007 laying down the animal and public health conditions and model certificates for imports of certain meat products and treated stomachs, bladders and intestines for human consumption from third countries and repealing Decision 2005/432/EC (OJ L 312, 30.11.2007, p. 49).
([5]) Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements (OJ L 73, 20.3.2010, p. 1).
([6]) Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of ß-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC (OJ L 125, 23.5.1996, p. 3).

guarantees at least equivalent to those laid down in that Directive, which have been established for the purpose of giving effect to Chapter V of Council Directive 96/23/EC [1] describing the measures to be taken in the event of infringement.

(5) Directive 96/23/EC lays down measures for monitoring the presence of certain substances and groups of residues in live animals and animal products. It provides that imports of animals for slaughter and of products of animal origin intended for human consumption are only to be authorised from third countries whose monitoring plan has been approved by the Commission.

(6) Domestic solipeds are usually not raised solely for the production of meat and are only sent for slaughter at the end of their productive life. In the Union, animals of the equidae family are considered to be food-producing animals, unless they are irreversibly excluded from slaughter for human consumption in accordance with Directive 2001/82/EC of the European Parliament and of the Council [2].

(7) Following audit missions in certain third countries, where deficiencies had been detected, and in order to ensure compliance with the provisions of Directive 96/22/EC, it is necessary to reinforce the guarantees on imports of fresh meat of equidae intended for human consumption, meat preparations as well as meat products and treated stomachs, bladders and intestines produced therefrom as regards the monitoring of substances and groups of residues and substances referred to in Annex I to Directive 96/23/EC.

(8) Therefore, the health certificate for meat preparations, the health certificate for meat products and treated commodities and the EQU certificate should be amended so that they provide the necessary guarantees that the commodities covered by them, when they are produced from or contain meat of domestic solipeds, were produced from meat which meets the requirements set out for the imports of fresh meat of domestic solipeds.

(9) Decisions 2000/572/EC and 2007/777/EC and Regulation (EU) No 206/2010 should therefore be amended accordingly.

(10) To avoid any disruption of trade, imports into the Union of consignments of commodities accompanied by the health certificate for meat preparations, the health certificate for meat products and treated commodities and the EQU certificate issued in accordance with Decisions 2000/572/EC and 2007/777/EC and Regulation (EU) No 206/2010 before the amendments made by this Regulation should continue to be authorised for a transitional period.

(11) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

Article 1

Amendment to Decision 2000/572/EC

Annex II to Decision 2000/572/EC is amended in accordance with Annex I to this Regulation.

Article 2

Amendment to Decision 2007/777/EC

Annex III to Decision 2007/777/EC is amended in accordance with Annex II to this Regulation.

[1] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC (OJ L 125, 23.5.1996, p. 10).

[2] Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products (OJ L 311, 28.11.2001, p. 1).

Article 3

Amendment to Regulation (EU) No 206/2010

Part 2 of Annex II to Regulation (EU) No 206/2010 is amended in accordance with Annex III to this Regulation.

Article 4

Transitional provisions

1. For a transitional period until 31 March 2017, consignments of meat preparations accompanied by a health certificate for meat preparations issued in accordance with the model set out in Annex II to Decision 2000/572/EC before the amendments made by this Regulation, shall continue to be authorised for importation into the Union provided that the certificate was issued no later than 28 February 2017.

2. For a transitional period until 31 March 2017, consignments of meat products and treated stomachs, bladders and intestines, accompanied by a health certificate for meat products and treated commodities issued in accordance with the model set out in Annex III to Decision 2007/777/EC before the amendments made by this Regulation, shall continue to be authorised for importation into the Union provided that the certificate was issued no later than 28 February 2017.

3. For a transitional period until 31 March 2017, consignments of fresh meat of equidae intended for human consumption, accompanied by an EQU certificate issued in accordance with the model set out in Part 2 of Annex II to Regulation (EU) No 206/2010 before the amendments made by this Regulation, shall continue to be authorised for importation into the Union provided that the certificate was issued no later than 28 February 2017.

Article 5

Entry into force

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

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

Done at Brussels, 17 October 2016.

For the Commission
The President
Jean-Claude JUNCKER

ANNEX I

In Annex II to Decision 2000/572/EC, in the model animal and public health certificate for meat preparations intended for consignment to the European Union from third countries, the following point II.1.10 is added to the public health attestation in Part II:

'([2]) [II.1.10. if containing material from domestic solipeds, the fresh meat used in the preparation of the meat preparations:

either ([2]) [was obtained from domestic solipeds which immediately prior to slaughter had been kept for at least six months or since birth, if slaughtered at an age of less than six months, or since importation as food producing equidae from a Member State of the European Union, if imported less than six months prior to slaughter, in a third country:

(a) in which the administration to domestic solipeds:

(i) of thyrostatic substances, stilbenes, stilbene derivatives, their salts and esters, oestradiol 17β and its ester-like derivatives is prohibited;

(ii) of other substances having oestrogenic, androgenic or gestagenic action and of beta-agonists is only allowed for:

— therapeutic treatment as defined in Article 1(2)(b) of Directive 96/22/EC, where applied in conformity with Article 4(2) of that Directive, or

— zootechnical treatment as defined in Article 1(2)(c) of Directive 96/22/EC, where applied in conformity with Article 5 of that Directive; and

(b) which has had, at least during the six months prior to slaughter of the animals, a plan for the monitoring of the groups of residues and substances referred to in Annex I to Directive 96/23/EC which covers equidae born in and imported into the third country and was approved in accordance with the fourth subparagraph of Article 29(1) of Directive 96/23/EC;]]

and/or ([2]) [was imported from a Member State of the European Union.]]'

ANNEX II

In Annex III to Decision 2007/777/EC, in the model animal health and public health certificate for certain meat products and treated stomachs, bladders and intestines intended for consignment to the European Union from third countries, the following point II. 2.10. is added to the Public health attestation in Part II:

'([2]) II.2.10. if containing material from domestic equine animals, the fresh meat, stomachs, bladders or intestines used in the preparation of the meat products and/or treated stomachs, bladders and intestines

([2]) *either* [was/were obtained from domestic equine animals which immediately prior to slaughter had been kept for at least six months or since birth if slaughtered at an age of less than six months, or since importation as food producing equidae from a Member State of the European Union, if imported less than six months prior to slaughter, in a third country:

(a) in which the administration to domestic equine animals:

(i) of thyrostatic substances, stilbenes, stilbene derivatives, their salts and esters, oestradiol 17β and its ester-like derivatives is prohibited;

(ii) of other substances having oestrogenic, androgenic or gestagenic action and of beta-agonists is only allowed for:

— therapeutic treatment as defined in Article 1(2)(b) of Directive 96/22/EC, where applied in conformity with Article 4(2) of that Directive, or

— zootechnical treatment as defined in Article 1(2)(c) of Directive 96/22/EC, where applied in conformity with Article 5 of that Directive; and

(b) which has had, at least during the six months prior to slaughter of the animals, a plan for the monitoring of the groups of residues and substances referred to in Annex I to Directive 96/23/EC which covers equidae born in and imported into the third country and was approved in accordance with the fourth subparagraph of Article 29(1) of Directive 96/23/EC.]

([2]) *and/or* [was/were imported from a Member State of the European Union.]'

ANNEX III

In Part 2 of Annex II to Regulation (EU) No 206/2010, in the model veterinary certificate 'EQU' for fresh meat, excluding minced meat, of domestic solipeds (*Equus caballus, Equus asinus* and their cross-breeds), point II.1.7. of the Public Health Attestation in Part II is replaced by the following:

'II.1.7. the meat was obtained from domestic solipeds which immediately prior to slaughter had been kept for at least six months or since birth, if slaughtered at an age of less than six months, or since importation as food producing equidae from a Member State of the European Union, if imported less than six months prior to slaughter, in a third country:

(a) in which the administration to domestic solipeds:

(i) of thyrostatic substances, stilbenes, stilbene derivatives, their salts and esters, oestradiol 17β and its ester-like derivatives is prohibited;

(ii) of other substances having oestrogenic, androgenic or gestagenic action and of beta-agonists is only allowed for:

— therapeutic treatment, as defined in Article 1(2)(b) of Directive 96/22/EC, where applied in conformity with Article 4(2) of that Directive, or

— zootechnical treatment, as defined in Article 1(2)(c) of Directive 96/22/EC, where applied in conformity with Article 5 of that Directive; and

(b) which has had, at least during the six months prior to slaughter of the animals, a plan for the monitoring of the groups of residues and substances referred to in Annex I to Directive 96/23/EC which covers equidae born in and imported into the third country and was approved in accordance with the fourth subparagraph of Article 29(1) of Directive 96/23/EC;'

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1833

of 17 October 2016

concerning the authorisation of a preparation of kidney bean lectins (*Phaseolus vulgaris* lectins) as a feed additive for suckling piglets (holder of authorisation Biolek Sp. z o.o.)

(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 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition ([1]), and in particular Article 9(2) thereof,

Whereas:

(1) Regulation (EC) No 1831/2003 provides for the authorisation of additives for use in animal nutrition and for the grounds and procedures for granting such authorisation.

(2) In accordance with Article 7 of Regulation (EC) No 1831/2003 an application was submitted for the authorisation of a preparation of kidney bean lectins (*Phaseolus vulgaris* lectins). That application was accompanied by the particulars and documents required under Article 7(3) of Regulation (EC) No 1831/2003.

(3) That application concerns the authorisation of a preparation of kidney bean lectins (*Phaseolus vulgaris* lectins) as a feed additive for suckling piglets to be classified in the additive category 'zootechnical additives'.

(4) The European Food Safety Authority ('the Authority') concluded in its opinions of 29 October 2014 ([2]) and 22 October 2015 ([3]) that, under the proposed conditions of use, the preparation of kidney bean lectins (*Phaseolus vulgaris* lectins) does not have an adverse effect on animal health, human health or the environment. It further concluded that the additive should be considered as a respiratory sensitizer and there is a potential hazard by inhalation exposure. The Authority has also concluded that it can have some potential to improve the performance of the piglets during the post-weaning period. The Authority does not consider that there is a need for specific requirements of post-market monitoring. It also verified the report on the method of analysis of the feed additive in feed submitted by the Reference Laboratory set up by Regulation (EC) No 1831/2003.

(5) The assessment of the preparation of kidney bean lectins (*Phaseolus vulgaris* lectins) shows that the conditions for authorisation, as provided for in Article 5 of Regulation (EC) No 1831/2003, are satisfied. Accordingly, the use of that preparation should be authorised as specified in the Annex to this Regulation.

(6) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

Article 1

The preparation specified in the Annex, belonging to the additive category 'zootechnical additives' and to the functional group 'other zootechnical additives', is authorised as an additive in animal nutrition, subject to the conditions laid down in that Annex.

([1]) OJ L 268, 18.10.2003, p. 29.
([2]) EFSA Journal 2015;13(1):3903.
([3]) EFSA Journal 2015;13(11):4276.

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*.

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

Done at Brussels, 17 October 2016.

For the Commission

The President

Jean-Claude JUNCKER

ANNEX

Identification number of the additive	Name of the holder of authorisation	Additive	Composition, chemical formula, description, analytical method	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisation
						Units of activity animal/day			
Category of zootechnical additives. Functional group: other zootechnical additives (performance enhancer in weaned piglets)									
4d13	Biolek Sp. z o.o.	Kidney bean lectins	*Additive composition* Preparation of kidney bean lectins (*Phaseolus vulgaris* lectins), having a minimum of activity: 1 280 HAU/g (1) *Characterisation of the active substance* Mixture of phytohaemagglutinin (PHA) isoforms: PHA-E_4, PHA-E_3L, PHA-E_2L_2, PHA-EL_3, PHA-L_4 CAS (PHA-L) 9008-97-3 *Analytical methods* (2) For the quantification of the kidney bean lectin in the additive: Haemagglutination assay	Suckling piglets	14 days	220 HAU	660 HAU	1. In the directions for use of the additive and premixture, indicate the storage temperature and storage life. 2. The additive shall be fed only via a complementary feed to suckling piglets from 10th to 14th day of age with the maximum dose of: — 220 HAU/suckling piglet/day for 3 days or — 660 HAU/suckling piglet (in one day). 3. On the label of the additive, the instructions for use via complementary feed shall be indicated. 4. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from its use. Where those risks cannot be eliminated or reduced to a minimum by such procedures and measures, the additive and premixtures shall be used with personal protective equipment, including breathing protection.	7 November 2026

(1) 1 HAU (Haemagglutination Activity Units) is the amount of material (1 mg/ml) in the last dilution giving 50 % agglutination (clumping) of the red blood cells.
(2) Details of the analytical methods are available at the following address of the Reference Laboratory for Feed Additives: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1834

of 17 October 2016

amending Regulation (EU) No 37/2010 as regards the substance monepantel

(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 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and the Council ([1]), and in particular Article 14 in conjunction with Article 17 thereof,

Having regard to the opinion of the European Medicines Agency formulated by the Committee for Medicinal Products for Veterinary Use,

Whereas:

(1) Article 17 of Regulation (EC) No 470/2009 requires that the maximum residue limit (MRL) for pharmacologically active substances intended for use in the Union in veterinary medicinal products for food-producing animals or in biocidal products used in animal husbandry is established in a Regulation.

(2) Table 1 of the Annex to Commission Regulation (EU) No 37/2010 ([2]) sets out the pharmacologically active substances and their classification regarding maximum residue limits (MRLs) in foodstuffs of animal origin.

(3) Monepantel is currently included in that table as an allowed substance, for ovine and caprine species, applicable to muscle, fat, liver, kidney and milk.

(4) An application for the extension of the existing entry for monepantel to bovine species has been submitted to the European Medicines Agency (EMA).

(5) The EMA, based on the opinion of the Committee for Medicinal Products for Veterinary Use, has recommended the establishment of an MRL for monepantel in bovine tissues, excluding animals producing milk for human consumption.

(6) According to Article 5 of Regulation (EC) No 470/2009, the EMA is to consider using MRLs established for a pharmacologically active substance in a particular foodstuff for another foodstuff derived from the same species, or MRLs established for a pharmacologically active substance in one or more species for other species.

(7) The EMA has considered that the extrapolation of the MRL for monepantel from ovine and caprine milk to bovine milk is not appropriate at this time due to insufficient data.

(8) Regulation (EU) No 37/2010 should therefore be amended accordingly.

(9) It is appropriate to grant the stakeholders concerned a reasonable period of time to take measures that may be required to comply with the new MRL.

(10) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Veterinary Medicinal Products,

([1]) OJ L 152, 16.6.2009, p. 11.
([2]) Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (OJ L 15, 20.1.2010, p. 1).

HAS ADOPTED THIS REGULATION:

Article 1

The Annex to Regulation (EU) No 37/2010 is amended as set out in 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*.

It shall apply from 17 December 2016.

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

Done at Brussels, 17 October 2016.

For the Commission
The President
Jean-Claude JUNCKER

ANNEX

In Table 1 of the Annex to Regulation (EU) No 37/2010, the entry for the substance 'monepantel' is replaced by the following:

Pharmacologically active Substance	Marker residue	Animal Species	MRL	Target Tissues	Other Provisions (*according to Article 14(7) of Regulation (EC) No 470/2009*)	Therapeutic Classification
'Monepantel	Monepantel sulfone	Ovine, caprine	700 µg/kg 7 000 µg/kg 5 000 µg/kg 2 000 µg/kg 170 µg/kg	Muscle Fat Liver Kidney Milk	NO ENTRY	Antiparasitic agents/Agents (acting) against endoparasites'
		Bovine	300 µg/kg 7 000 µg/kg 2 000 µg/kg 1 000 µg/kg	Muscle Fat Liver Kidney	Not for use in animals producing milk for human consumption	

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1835

of 17 October 2016

establishing the standard import values for determining the entry price of certain fruit and vegetables

THE EUROPEAN COMMISSION,

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

Having regard to Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007 ([1]),

Having regard to Commission Implementing Regulation (EU) No 543/2011 of 7 June 2011 laying down detailed rules for the application of Council Regulation (EC) No 1234/2007 in respect of the fruit and vegetables and processed fruit and vegetables sectors ([2]), and in particular Article 136(1) thereof,

Whereas:

(1) Implementing Regulation (EU) No 543/2011 lays down, pursuant to the outcome of the Uruguay Round multilateral trade negotiations, the criteria whereby the Commission fixes the standard values for imports from third countries, in respect of the products and periods stipulated in Annex XVI, Part A thereto.

(2) The standard import value is calculated each working day, in accordance with Article 136(1) of Implementing Regulation (EU) No 543/2011, taking into account variable daily data. Therefore this Regulation should enter into force on the day of its publication in the *Official Journal of the European Union*,

HAS ADOPTED THIS REGULATION:

Article 1

The standard import values referred to in Article 136 of Implementing Regulation (EU) No 543/2011 are fixed in the Annex to this Regulation.

Article 2

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

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

Done at Brussels, 17 October 2016.

For the Commission,
On behalf of the President,
Jerzy PLEWA
Director-General for Agriculture and Rural Development

([1]) OJ L 347, 20.12.2013, p. 671.
([2]) OJ L 157, 15.6.2011, p. 1.

ANNEX

Standard import values for determining the entry price of certain fruit and vegetables

(EUR/100 kg)

CN code	Third country code (1)	Standard import value
0702 00 00	MA	132,6
	ZZ	132,6
0707 00 05	TR	145,2
	ZZ	145,2
0709 93 10	TR	138,5
	ZZ	138,5
0805 50 10	AR	87,8
	CL	82,2
	TR	89,3
	UY	51,6
	ZA	94,2
	ZZ	81,0
0806 10 10	BR	288,3
	EG	169,2
	TR	153,1
	ZZ	203,5
0808 10 80	AR	191,8
	AU	196,9
	BR	124,9
	CL	146,5
	NZ	137,9
	ZA	97,2
	ZZ	149,2
0808 30 90	CN	73,6
	TR	134,9
	ZZ	104,3

(1) Nomenclature of countries laid down by Commission Regulation (EU) No 1106/2012 of 27 November 2012 implementing Regulation (EC) No 471/2009 of the European Parliament and of the Council on Community statistics relating to external trade with non-member countries, as regards the update of the nomenclature of countries and territories (OJ L 328, 28.11.2012, p. 7). Code 'ZZ' stands for 'of other origin'.

DECISIONS

COUNCIL DECISION (EU) 2016/1836

of 10 October 2016

appointing an alternate member, proposed by the Republic of Austria, of the Committee of the Regions

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 305 thereof,

Having regard to the proposal of the Austrian Government,

Whereas:

(1) On 26 January 2015, 5 February 2015 and 23 June 2015, the Council adopted Decisions (EU) 2015/116 ([1]), (EU) 2015/190 ([2]) and (EU) 2015/994 ([3]) appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2015 to 25 January 2020. On 13 May 2016, by Council Decision (EU) 2016/814 ([4]), Ms Elisabeth VITOUCH was replaced by Ms Muna DUZDAR as an alternate member.

(2) An alternate member's seat on the Committee of the Regions has become vacant following the end of the term of office of Ms Muna DUZDAR,

HAS ADOPTED THIS DECISION:

Article 1

The following is hereby appointed as an alternate member of the Committee of the Regions for the remainder of the current term of office, which runs until 25 January 2020:

— Mr Peter FLORIANSCHÜTZ, *Abgeordneter zum Wiener Landtag und Mitglied des Gemeinderats der Stadt Wien*.

Article 2

This Decision shall enter into force on the date of its adoption.

Done at Brussels, 10 October 2016.

For the Council
The President
G. MATEČNÁ

([1]) Council Decision (EU) 2015/116 of 26 January 2015 appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2015 to 25 January 2020 (OJ L 20, 27.1.2015, p. 42).
([2]) Council Decision (EU) 2015/190 of 5 February 2015 appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2015 to 25 January 2020 (OJ L 31, 7.2.2015, p. 25).
([3]) Council Decision (EU) 2015/994 of 23 June 2015 appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2015 to 25 January 2020 (OJ L 159, 25.6.2015, p. 70).
([4]) Council Decision (EU) 2016/814 of 13 May 2016 appointing an alternate member, proposed by the Republic of Austria, of the Committee of the Regions (OJ L 133, 24.5.2016, p. 8).

COUNCIL IMPLEMENTING DECISION (EU) 2016/1837

of 11 October 2016

authorising the Republic of Poland to continue to apply measures derogating from point (a) of Article 26(1) and Article 168 of Directive 2006/112/EC on the common system of value added tax

THE COUNCIL OF THE EUROPEAN UNION,

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

Having regard to Council Directive 2006/112/EC of 28 November 2006 on the common system of value added tax ([1]), and in particular Article 395 thereof,

Having regard to the proposal from the European Commission,

Whereas:

(1) Article 168 of Directive 2006/112/EC establishes a taxable person's right to deduct value added tax (VAT) charged on supplies of goods and services received by him for the purposes of his taxed transactions. Point (a) of Article 26(1) of that Directive lays down a requirement to account for VAT when a business asset is put to use for the private purposes of the taxable person or his staff or, more generally, for purposes other than those of his business.

(2) By virtue of Council Implementing Decision 2013/805/EU ([2]), Poland was authorised, until 31 December 2016, to limit to 50 % the right to deduct VAT on the purchase, intra-Community acquisition, importation, hire, or leasing of certain motorised road vehicles and expenditure related thereto where such vehicle is not entirely used for business purposes, and to relieve the taxable person from accounting for VAT on the non-business use of vehicles covered by the restriction (the 'derogating measures').

(3) By letter registered with the Commission on 8 February 2016, Poland requested authorisation to continue to apply the derogating measures.

(4) In accordance with the second subparagraph of Article 395(2) of Directive 2006/112/EC, the Commission informed the other Member States, by letter dated 6 June 2016, of the request made by Poland. By letter dated 8 June 2016, the Commission notified Poland that it had all the information necessary to consider the request.

(5) In accordance with the Article 3(2) of Decision 2013/805/EU, Poland submitted, together with the extension request, a report to the Commission on the application of that Decision, including a review of the percentage limitation applied on the right of deduction. Based on currently available information, Poland believes that a rate of 50 % is still justifiable. At the same time, to avoid double taxation, the requirement for accounting for VAT on the non-business use of a motor vehicle should be suspended where it has been subject to that limitation. Those derogating measures can be justified by the need to simplify the procedure for charging VAT and to prevent evasion through incorrect record keeping and false tax declaration.

(6) The extension of these derogating measures should be limited in time to allow for an evaluation of their effectiveness and of the appropriate percentage; Poland should therefore be authorised to continue to apply the derogating measures until 31 December 2019.

(7) Where Poland considers that a further extension of the derogating measures beyond 2019 is necessary, a report on the application of the derogating measures, including a review of the percentage applied, should be submitted to the Commission, together with the extension request by no later than 1 April 2019.

([1]) OJ L 347, 11.12.2006, p. 1.

([2]) Council Implementing Decision 2013/805/EU of 17 December 2013 authorising the Republic of Poland to introduce measures derogating from point (a) of Article 26(1) and Article 168 of Directive 2006/112/EC on the common system of value added tax (OJ L 353, 28.12.2013, p. 51).

(8) The extension of the derogating measures will only have a negligible effect on the overall amount of tax collected at the stage of final consumption and will not adversely affect the Union's own resources accruing from VAT.

(9) Implementing Decision 2013/805/EU should be therefore amended accordingly,

HAS ADOPTED THIS DECISION:

Article 1

Article 3 of Implementing Decision 2013/805/EU is replaced by the following:

'*Article 3*

1. This Decision shall expire on 31 December 2019.

2. Any request for the extension of the derogating measures provided for in this Decision shall be submitted to the Commission by 1 April 2019. Such request shall be accompanied by a report including a review of the percentage restriction applied on the right to deduct VAT on the basis of this Decision.'.

Article 2

This Decision shall apply from 1 January 2017.

Article 3

This Decision is addressed to the Republic of Poland.

Done at Brussels, 11 October 2016.

For the Council
The President
P. KAŽIMÍR

COUNCIL DECISION (EU) 2016/1838

of 13 October 2016

on guidelines for the employment policies of the Member States for 2016

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 148(2) thereof,

Having regard to the proposal from the European Commission,

Having regard to the opinion of the European Parliament ([1]),

Having regard to the opinion of the European Economic and Social Committee ([2]),

After consulting the Committee of the Regions,

Having regard to the opinion of the Employment Committee ([3]),

Whereas:

(1) The Treaty on the Functioning of the European Union (TFEU) stipulates that Member States and the Union are to work towards developing a coordinated strategy for employment and particularly for promoting a skilled, trained and adaptable workforce as well as labour markets that are responsive to economic change with a view to achieving the objectives defined in Article 3 of the Treaty on European Union.

(2) The Europe 2020 strategy for smart, sustainable and inclusive growth ('Europe 2020 strategy') proposed by the Commission enables the Union to turn its economy towards smart, sustainable and inclusive growth, accompanied by high level employment, productivity and social cohesion. Five headline targets constitute shared objectives which guide the action of the Member States, and take into account their relative starting positions and national circumstances as well as the positions and circumstances of the Union. On 14 July 2015, the Council adopted Recommendation (EU) 2015/1184 ([4]) on broad guidelines for the economic policies of the Member States and of the Union. Furthermore, on 5 October 2015, the Council adopted Decision (EU) 2015/1848 ([5]) on guidelines for the employment policies of the Member States for 2015 ('employment guidelines'). Those two sets of guidelines form the integrated guidelines for implementing the Europe 2020 strategy ('Europe 2020 integrated guidelines'). The European Employment Strategy has the leading role in the implementation of the employment and labour market objectives of the Europe 2020 strategy.

(3) The Europe 2020 integrated guidelines are in line with the conclusions of the European Council of 17 and 18 March 2016 and with the Stability and Growth Pact. They give precise guidance to the Member States on defining their National Reform Programmes and on implementing reforms, while reflecting interdependence. The employment guidelines should form the basis for any country-specific recommendations the Council may address to the Member States under Article 148(4) TFEU, in parallel with the country-specific recommendations addressed to the Member States under Article 121(2) TFEU. The employment guidelines should also form the basis for the establishment of the Joint Employment Report sent annually by the Council and the Commission to the European Council.

(4) The examination of Member States' National Reform Programmes contained in the Joint Employment Report shows that Member States should make every effort to boost demand for labour, enhance labour supply, skills and competences, enhance the functioning of labour market, foster social inclusion, combat poverty and promote equal opportunities.

([1]) Opinion of 15 September 2016 (not yet published in the Official Journal).
([2]) OJ C 264, 20.7.2016, p. 134.
([3]) Opinion of 16 February 2016.
([4]) Council Recommendation (EU) 2015/1184 of 14 July 2015 on broad guidelines for the economic policies of the Member States and of the European Union (OJ L 192, 18.7.2015, p. 27).
([5]) Council Decision (EU) 2015/1848 of 5 October 2015 on guidelines for the employment policies of the Member States for 2015 (OJ L 268, 15.10.2015, p. 28).

(5) Member States should explore the use of the European Social Fund when implementing the employment guidelines.

(6) The employment guidelines should remain stable to ensure a focus on their implementation. Any updating of the employment guidelines should therefore remain strictly limited. In the light of an assessment of the developments of the labour markets and the social situation since the adoption of the employment guidelines in 2015, no update is necessary. The reasons for their adoption in 2015 remain valid, therefore those guidelines should be maintained,

HAS ADOPTED THIS DECISION:

Article 1

The guidelines for the employment policies of the Member States, as set out in the Annex to Decision (EU) 2015/1848, are maintained for 2016 and shall be taken into account by the Member States in their employment policies.

Article 2

This Decision is addressed to the Member States.

Done at Luxembourg, 13 October 2016.

For the Council
The President
J. RICHTER

COUNCIL DECISION (CFSP) 2016/1839

of 17 October 2016

amending Decision 2010/638/CFSP concerning restrictive measures against the Republic of Guinea

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on European Union, and in particular Article 29 thereof,

Having regard to the proposal from the High Representative of the Union for Foreign Affairs and Security Policy,

Whereas:

(1) On 25 October 2010, the Council adopted Decision 2010/638/CFSP [1] concerning restrictive measures against the Republic of Guinea.

(2) On the basis of a review of Decision 2010/638/CFSP, those restrictive measures should be extended until 27 October 2017.

(3) Decision 2010/638/CFSP should therefore be amended accordingly,

HAS ADOPTED THIS DECISION:

Article 1

Article 8(2) of Decision 2010/638/CFSP is replaced by the following:

'2. This Decision shall apply until 27 October 2017. It shall be kept under constant review. It shall be renewed or amended, as appropriate, if the Council deems that its objectives have not been met.'.

Article 2

This Decision shall enter into force on the date of its publication in the *Official Journal of the European Union*.

Done at Luxembourg, 17 October 2016.

For the Council
The President
F. MOGHERINI

[1] Council Decision 2010/638/CFSP of 25 October 2010 concerning restrictive measures against the Republic of Guinea (OJ L 280, 26.10.2010, p. 10).

COMMISSION IMPLEMENTING DECISION (EU) 2016/1840

of 14 October 2016

amending Annex IV to Council Directive 2009/156/EC as regards methods for African horse sickness diagnosis

(notified under document C(2016) 6509)

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

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

Having regard to Council Directive 2009/156/EC of 30 November 2009 on animal health conditions governing the movement and importation from third countries of equidae ([1]), and in particular Article 20 thereof,

Whereas:

(1) Annex IV to Directive 2009/156/EC sets out diagnostic methods for African horse sickness to be used, when necessary, for testing equidae prior to their movement within the Union or imports from non-EU countries.

(2) Since the adoption of Directive 2009/156/EC, laboratory capacities to carry out advanced, highly sensitive and efficient tests for the diagnosis of African horse sickness have developed. In parallel, the Chapter related to the diagnosis of African horse sickness in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (OIE) ([2]) has been amended to reflect that development.

(3) As part of their 2014 work programme, the European Union Reference Laboratory for African horse sickness ([3]) produced a report on the technical assessment of the diagnostic methods described in Annex IV to Directive 2009/156/EC. The assessment, which was presented to the Commission in May 2015, concluded that the competitive enzyme-linked immunosorbent assay (ELISA) is no longer available, indirect ELISA is not in common use but could be provided in 4-6 months after the request and that the blocking ELISA is commercially available and commonly used for analysis of samples during the Proficiency Test exercises organised by the European Union Reference Laboratory for African horse sickness.

(4) In addition, the report points out that the nucleic acid recognition by reverse-transcription polymerase chain reaction (RT-PCR) methods have advantages over serological diagnostic methods, because they allow for the detection of the disease at an early stage of infection. In addition, most of the national reference laboratories of the European Union Member States use real-time RT-PCR methods, including for the diagnosis of African horse sickness, which have proven to be fit for purpose in the annual Proficiency Test exercises performed from 2009 to 2014. The report also indicates that outside the Union there are a number of OIE reference laboratories and other laboratories with specific African horse sickness expertise that have implemented at least one of the real-time RT-PCR methods for the detection of African horse sickness genome.

(5) On the 24-25 November 2015, the Joined Workshop of African Horse Sickness/Bluetongue European Union Reference Laboratories together with national reference laboratories held in Ascot, United Kingdom, recommended the inclusion in Annex IV to Directive 2009/156/EC of real time reverse transcription (RRT)-polymerase chain reaction (PCR) methods for the detection of the African horse sickness virus.

([1]) OJ L 192, 23.7.2010, p. 1.
([2]) http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.05.01_AHS.pdf
([3]) Council Directive 92/35/EEC of 29 April 1992 laying down control rules and measures to combat African horse sickness (OJ L 157, 10.6.1992, p. 19).

(6) Although all available real-time RT-PCR methods for the African horse sickness genome detection are sufficiently sensitive, the procedure described by Agüero et al. (2008) ([4]) is the most widely used by laboratories. The method described by Guthrie et al. (2013) ([5]) was specifically designed to ensure that horses from areas at risk of African horse sickness can be transported safely after the minimum quarantine period required in accordance with the Terrestrial Animal Health Code ([6]) of the OIE.

(7) It is therefore appropriate to incorporate in Annex IV to Directive 2009/156/EC methods for agent identification and for the detection of antibody as complementary methods for a rapid diagnosis of African horse sickness.

(8) Annex IV to Directive 2009/156/EC should be therefore amended by deletion of the competitive ELISA test and by an updating the procedures for the indirect and blocking ELISA tests in accordance with Chapter 2.5.1. of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the OIE, Edition 2016 based on the Version adopted by the World Assembly of Delegates of the OIE in May 2012 ([7]). At the same time, real-time RT-PCR procedures as described by Agüero et al. (2008) as well as by Guthrie et al. (2013) should be included in that Annex to make those agent identification tests available for the purpose of pre-movement testing.

(9) Directive 2009/156/EC should therefore be amended accordingly.

(10) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS DECISION:

Article 1

Annex IV to Directive 2009/156/EC is replaced by the text set out in the Annex to this Decision.

Article 2

This Decision is addressed to the Member States.

Done at Brussels, 14 October 2016.

For the Commission
Vytenis ANDRIUKAITIS
Member of the Commission

([4]) Agüero M., Gomez-Tejedor C., Angeles Cubillo M., Rubio C., Romero E. and Jimenez-Clavero A. (2008). Real-time fluorogenic reverse transcription polymerase chain reaction assay for detection of African horse sickness virus. J. Vet. Diagn. Invest., 20, 325-328.
([5]) Guthrie AJ, MacLachlan NJ, Joone C, Lourens CW, Weyer CT, Quan M, Monyai MS, Gardner IA. Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus. Journal of Virological Methods. 2013;189 (1):30-35.
([6]) http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_ahs.pdf
([7]) See footnote 2.

ANNEX

'ANNEX IV

AFRICAN HORSE SICKNESS

DIAGNOSIS

PART A

Serological tests

The serological method described hereinafter are enzyme-linked immunosorbent assays (ELISA) based on point 2 of Section B in Chapter 2.5.1 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Edition 2016 as adopted by the World Assembly of Delegates of the OIE in May 2012.

The VP7 viral protein is an immuno-dominant major antigen of the African horse sickness virus (AHSV) and is conserved across the nine AHSV serotypes. Recombinant AHSV-VP7 proteins have been shown to be stable and innocuous and suitable to be used as antigens in ELISA procedures for determination of AHSV antibodies with a high degree of sensitivity and specificity (Laviada et al., 1992b ([1]); Maree and Paweska, 2005). The indirect ELISA and the blocking ELISA are the two AHS-VP7 ELISA tests suitable for serological diagnosis of African horse sickness (AHS).

1. **Indirect ELISA for the detection of antibodies to African horse sickness virus (AHSV)**

The conjugate used in this method is a horseradish peroxidase anti-horse gamma-globulin reacting with the serum of horses, mules and donkeys. The method described by Maree & Paweska (2005) ([2]) uses protein G as conjugate that also reacts with zebra serum.

The antigen may be provided by the Centro de Investigación en Sanidad Animal (CISA), Spain, within 4 to 6 months of request.

1.1. *Test procedure*

1.1.1. Solid phase

1.1.1.1. Coat ELISA plates with recombinant AHSV-4 VP7 diluted in carbonate/bicarbonate buffer, pH 9,6. Incubate plates overnight at 4 °C.

1.1.1.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.1.3. Block the plates with phosphate buffered saline (PBS) pH 7,2 + 5 % (w/v) skimmed milk (Nestlé Dry Skim Milk™), 200 µl/well, for 1 hour at 37 °C.

1.1.1.4. Remove the blocking solution and gently tap the plates onto absorbent material.

1.1.2. Test samples

1.1.2.1. Serum samples to be tested, and positive and negative control sera, are diluted 1 in 25 in PBS + 5 % (w/v) skimmed milk + 0,05 % (v/v) Tween 20, 100 µl per well. Incubate for 1 hour at 37 °C.

For titration, make a twofold dilution series from 1 in 25 (100 µl/well), one serum per plate column, and do the same with positive and negative controls. Incubate for 1 hour at 37 °C.

([1]) Laviada M.D., Roy P. and Sanchez-Vizcaino J.M (1992b). Adaptation and evaluation of an indirect ELISA and immunoblotting test for African horse sickness antibody detection. In: Bluetongue, African Horse Sickness and Related Orbiviruses: Proceedings of the Second International Symposium. Walton T.E. & Osburn B.I., Eds. CRC Press, Boca Raton, Florida, USA, 646-650.

([2]) Maree S. and Paweska J.T. (2005). Preparation of recombinant African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. J. Virol. Methods, 125 (1), 55-65.

1.1.2.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.3. Conjugate

1.1.3.1. Dispense 100 µl/well of horseradish-peroxidase (HRP) -conjugated anti-horse gamma-globulin diluted in PBS + 5 % milk + 0,05 % Tween 20, pH 7,2. Incubate for 1 hour at 37 °C.

1.1.3.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.4. Chromogen/Substrate

1.1.4.1. Add 200 µl/well of chromogen/substrate solution (10 ml of 80,6 mM DMAB (dimethyl aminobenzaldehyde) + 10 ml of 1,56 mM MBTH (3-methyl-2-benzo-thiazoline hydrazone hydrochlorid) + 5 µl H_2O_2).

Colour development is stopped by adding 50 µl of 3N H_2SO_4 after approximately 5 to 10 minutes (before the negative control begins to be coloured).

Other chromogens such as ABTS (2,2′-Azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]), TMB (tetramethyl benzidine), or OPD (ortho-phenyldiamine) can also be used.

1.1.4.2. Read the plates at 600 nm (or 620 nm).

1.2. *Interpretation of the results*

1.2.1. Calculate the cut-off value by adding 0,06 to the value of the negative control (0,06 is the standard deviation derived with a group of 30 negative sera).

1.2.2. Test samples giving absorbance values lower than the cut-off are regarded as negative.

1.2.3. Test samples giving absorbance values greater than the cut-off + 0,15 are regarded as positive.

1.2.4. Test samples giving intermediate absorbance values are considered to be inconclusive and a second technique must be employed to confirm the result.

2. **Blocking ELISA for the detection of antibodies to African horse sickness virus (AHSV)**

The competitive blocking ELISA is designed to detect specific AHSV antibodies in sera from animals of any equine species, i.e. horses, donkeys, zebra and their crosses, preventing the problem of specificity experienced occasionally using the indirect ELISAs.

The principle of the test is the blocking of the reaction between the recombinant VP7 protein absorbed to the ELISA plate and a conjugated AHS-VP7 specific monoclonal antibody (Mab). Antibody in the test sera will block the reaction between the antigen and the Mab resulting in a reduction in colour. Because the Mab is directed against the VP7, the assay will give a high level of sensitivity and specificity.

The competitive blocking ELISA is commercially available.

2.1. *Test procedure*

2.1.1. Solid Phase

2.1.1.1. Coat ELISA plates with 50-100 ng of recombinant AHSV-4 VP7 diluted in carbonate/bicarbonate buffer, pH 9,6. Incubate overnight at 4 °C.

2.1.1.2. Wash the plates three times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.2. Test samples and controls

2.1.2.1. Serum samples to be tested, and positive and negative control sera, are diluted 1 in 5 in diluent containing 0,35 M NaCl, 0,05 % (v/v) Tween 20 and 0,1 % Kathon, 100 µl per well. Incubate for 1 hour at 37 °C.

For titration, make a twofold dilution series of the test sera from 1 in 10 to 1 in 280 across 8 wells (100 µl/well), one serum per plate column, and do the same with positive and negative controls. Incubate for 1 hour at 37 °C.

2.1.2.2. Wash the plates five times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.3. Conjugate

2.1.3.1. Dispense 100 µl/well of horseradish peroxidase-conjugated Mab anti-VP7. In advance, this Mab has been diluted 1/5 000-1/15 000 in a 1/1 solution of StabiliZyme Select® Stabilizer (SurModics. Reference: SZ03) in distilled water. Incubate for 30 minutes at 37 °C.

2.1.3.2. Wash the plates five times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.4. Chromogen/Substrate

Add 100 µl/well chromogen/substrate solution, i.e. 1 ml of ABTS (2,2′-Azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]) 5 mg/ml + 9 ml of substrate buffer (0,1 M Phosphate-Citrate buffer of pH 4 containing 0,03 % H_2O_2), and incubate for 10 minutes at room temperature. Colour development is stopped by adding 100 µl/well of 2 % (w/v) SDS (sodium dodecyl sulphate).

2.1.5. Reading

Read at 405 nm in an ELISA reader.

2.2. *Interpretation of the results*

2.2.1. Determine the blocking percentage (BP) of each sample by applying the following formula, where "Abs" stands for antibodies:

$$BP = \frac{Abs(control^{-}) - Abs(sample)}{Abs(control^{-}) - Abs(control^{+})} \times 100$$

2.2.2. Samples showing a BP value higher than 50 % should be considered as positive for AHSV antibodies.

2.2.3. Samples showing a BP value lower than 45 % should be considered as negative for AHSV antibodies.

2.2.4. Samples showing a BP value between 45 % and 50 % should be considered as inconclusive and must be retested. If the result is again inconclusive, the animals should be retested on samples taken not earlier than two weeks after the sample which was considered to be inconclusive was taken.

PART B

Identification of the agent

Real-time Reverse-Transcription Polymerase Chain Reaction (rRT-PCR)

Agent identification tests based on nucleic acid methods must detect reference strains from the nine virus serotypes of the AHSV.

The method described in point 2.1 is based on point 1.2 of Section B in Chapter 2.5.1 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Edition 2016 as adopted by the World Assembly of Delegates of the OIE in May 2012.

Any RT-PCR detection method used for the testing of samples, either blood or spleen, in the context of Directive 2009/156/EC must perform equal to or exceed the sensitivity of the methodologies described in point 2.

Inactivated virus of serotypes 1 to 9 reference strains may be obtained from the European Union Reference Laboratory or the OIE Reference Laboratory for African horse sickness, Algete, Spain.

1. **Extraction of viral RNA**

To assure a good reaction it is necessary to extract from the sample an AHSV RNA of high quality. The extraction of nucleic acids from clinical samples can be performed by a variety of in-house and commercially available methods.

Commercial kits use different approaches for RNA isolation. Most are based on one of the following procedures:

— Phenol-chloroform extraction of nucleic acids;

— Adsorption of nucleic acids to filter system;

— Adsorption of nucleic acids to magnetic beads system.

An example of an in-house RNA extraction is given below:

1.1. 1 g of tissue sample is homogenised in 1 ml of denaturing solution (4 M guanidium thiocyanate, 25 mM sodium citrate, 0,1 M 2-mercaptoethanol, 0,5 % sarcosyl).

1.2. After centrifugation, 1 µg of yeast RNA, 0,1 ml of 2 M sodium acetate pH 4, 1 ml of phenol and 0,2 ml of chloroform/isoamyl alcohol mixture (49/1) are added to the supernatant.

1.3. The suspension is vigorously shaken and cooled on ice for 15 minutes.

1.4. After centrifugation, the RNA present in the aqueous phase is phenol extracted, ethanol precipitated and resuspended in sterile water.

2. **Real-time RT-PCR Procedure**

2.1. *Group-specific real-time RT-PCR by Agüero et al., 2008* ([1])

This group-specific real-time RT-PCR targets VP7 of the AHSV and is able to detect all known AHSV serotypes and strains currently circulating. It has been employed with very good results by the participating national reference laboratories of the European Union Member States in the proficiency tests annually organised by the European Union Reference Laboratory for the period 2009-2015. Moreover, in an international ring trial organised in 2015 in the framework of the OIE reference laboratories network this protocol was ranked very high amongst others.

Primer and probe sequences for the detection of AHSV species viruses:

— forward Primer 5′-CCA-GTA-GGC-CAG-ATC-AAC-AG-3′

— reverse Primer 5′-CTA-ATG-AAA-GCG-GTG-ACC-GT-3′

— MGB-TaqMan probe 5′-FAM-GCT-AGC-AGC-CTA-CCA-CTA-MGB-3′

2.1.1. Primer stock concentration is diluted to a working concentration of 8 µM (“primer working stock 8 µM”) whereas probe is diluted to a working concentration of 50 µM (“probe working stock 50 µM”). A test plate layout should be designed and loaded into the real time PCR machine software. Using the layout as a guide, 2,5 µl of each primer working stock 8 µM is added to each well that will contain RNA samples, positive and/or negative controls (final concentration of the primer will be 1 µM in the 20 µl RT-PCR mix). The plate is held on ice.

([1]) Agüero M., Gomez-Tejedor C., Angeles Cubillo M., Rubio C., Romero E. and Jimenez-Clavero A. (2008). Real-time fluorogenic reverse transcription polymerase chain reaction assay for detection of African horse sickness virus. J. Vet. Diagn. Invest., 20, 325-328.

2.1.2. 2 µl of isolated RNA (test samples and positive control), or 2 µl of RNAse-free water in negative reaction controls, is mixed with forward and reverse primers. This mixture is denatured by heating at 95 °C for 5 minutes, followed by rapid cooling on ice for at least 5 minutes.

2.1.3. An appropriate volume of real time one-step RT-PCR master mix for the number of samples to be tested is prepared following manufacturer's instructions. 0,1 µl of probe working stock 50 µM is added to each well containing RNA samples (final concentration of the probe will be 0,25 µM in each well containing RNA samples). 13 µl of real time one-step RT-PCR master mix is distributed in each well on the PCR plate containing the denatured primers and RNA.

2.1.4. The plate is placed in a real time thermal cycler programmed for reverse transcription and cDNA amplification/fluorescence detection. Amplification conditions consist of a first reverse-transcription step at 48 °C for 25 minutes, followed by 10 minutes at 95 °C ("hot start") and 40 cycles of 15 seconds at 95 °C, 35 seconds at 55 °C and 30 seconds at 72 °C (or 40 cycles at 97 °C for 2 seconds and 55 °C for 30 seconds if reagents and thermocycler allowing fast reactions are used). Fluorescence data are acquired at the end of the 55 °C step.

2.1.5. The assay is considered not valid if atypical amplification curves are obtained, and must be repeated.

Samples are considered positives, if the Ct value (cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold) is lower than or equal to the defined Ct threshold (35) within 40 PCR cycles (Ct ≤ 35).

Samples are considered inconclusive, if the Ct value is higher than the defined Ct threshold (35) within 40 PCR cycles (Ct > 35).

Samples are considered negative, if a horizontal amplification curve is obtained which does not cross the threshold line within 40 PCR cycles.

2.2. *Group-specific real-time RT-PCR by Guthrie et al., 2013* ([1])

Real-time RT-PCR using fluorescence resonance energy transfer (FRET) probes to detect nucleic acid of AHSV.

The AHSV RT-PCR assay described was designed using sequences from a wide variety of currently circulating field strains of AHSV (Quan et al., 2010 ([2])). It also incorporates a proprietary synthetic external control assay to verify proper functioning of the assay components.

Kits for the one-step real-time PCR are available commercially. Below are some basic steps as described by Guthrie et al. (2013), which can be modified depending upon local/case-specific requirements, kits used and equipment available.

Primer and probe sequences for the detection of AHSV species viruses:

— forward Primer 5′-AGA-GCT-CTT-GTG-CTA-GCA-GCC-T-3′

— reverse Primer 5′-GAA-CCG-ACG-CGA-CAC-TAA-TGA-3′

— MGB-TaqMan probe 5′-FAM-TGC-ACG-GTC-ACC-GCT-MGB-3′

2.2.1. Primer and probe mix stock solutions are made up in a 25× concentration at 5 µM for the forward and reverse primers and 3 µM for the probe. A test plate layout should be designed and loaded into the real-time PCR machine software. Using the layout as a guide, 5 µl of RNA samples, including test samples and positive and negative controls, are added to appropriate wells of the plate following the layout.

2.2.2. The RNA is denatured by heating at 95 °C for 5 minutes, followed by rapid cooling on ice for at least 3 minutes.

([1]) Guthrie AJ, MacLachlan NJ, Joone C, Lourens CW, Weyer CT, Quan M, Monyai MS, Gardner IA. Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus. Journal of Virological Methods. 2013;189 (1):30-5.

([2]) Quan, M., Lourens, C.W., MacLachlan, N.J., Gardner, I.A., Guthrie, A.J., 2010. Development and optimisation of a duplex real-time reverse transcription quantitative PCR assay targeting the VP7 and NS2 genes of African horse sickness virus. J. Virol. Methods 167, 45-52.

2.2.3. An appropriate volume of real-time one-step RT-PCR master mix for the number of samples to be tested is prepared, following the manufacturer's instructions. 1 µl of 25× primer probe mix stock solution (from point 2.2.1 above) is included in the master mix to give a final concentration in each well of 200 nM for each primer and 120 nM of the probe. 20 µl of the master mix is distributed in each well on the PCR plate containing the denatured RNA.

2.2.4. The plate is placed in a real-time thermal cycler programmed for reverse transcription and cDNA amplification/fluorescence detection as suggested by the manufacturers. Amplification conditions consist of, for example, a first reverse-transcription step at 48 °C for 10 minutes, followed by 10 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C and 45 seconds at 60 °C.

2.2.5. Samples are considered positives, if the normalised fluorescence for the AHSV RT-PCR assay exceeds a 0,1 threshold within 36 PCR cycles in all replicates of a sample.

Samples are considered inconclusive, if the normalised fluorescence for the AHSV RT-PCR assay exceeds a 0,1 threshold between 36 and 40 PCR cycles in any replicate of a sample.

Samples are considered negative, if the normalised fluorescence for the AHSV RT-PCR assay did not exceed a 0,1 threshold within 40 PCR cycles in all replicates of a sample and if the normalised fluorescence for the proprietary synthetic external control assay exceeded a 0,1 threshold within 33 PCR cycles.'

ISSN 1977-0677 (electronic edition)
ISSN 1725-2555 (paper edition)

Publications Office of the European Union
2985 Luxembourg
LUXEMBOURG

EN