Para	Text	Comment
G	(General Comment)	**Algeria submitted a track-changed verison of the draft (30 September) to the Secretariat. This will be forwarded to the TPDP for their consideration.**
G	(General Comment)	*Category : TECHNICAL* **(488) Venezuela (1 Oct 2016 2:52 AM)** El grupo de Venezuela no tiene por ahora.
G	(General Comment)	*Category : SUBSTANTIVE* **(487) Congo, DR (1 Oct 2016 2:01 AM)** Les méthodes décrites dans ce protocole sont sophistiqués pour les niveaux d' équipement de nos laboratoires.Il serait intéressant que des études études évoluent vers le développement de kits d'analyse rapide plus à utiliser par les services d' inspection aux frontières , Les niveaux de spécificité et de sensibilité de ces techniques devraient être fournies pour permettre la comparaison avec d' autres techniques de diagnostic (paragraphe 232))
G	(General Comment)	Category : TECHNICAL **Burkina Faso** Lev specificity waters and-Sensitivity ity of these techniques should be fou r ned to allow comparison with other techn ical with diag No. stic (paragraph 232).
G	(General Comment)	*Category : TECHNICAL* **(486) Mexico (30 Sep 2016 11:55 PM)** Please review the final volumen concentration of all PCR´s because these missmatch. You can only indicate the final concentration and reagents .
G	(General Comment)	*Category : TECHNICAL* **(485) Zambia (30 Sep 2016 11:08 PM)** Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods (Paragraphs 60 and 77).
G	(General Comment)	*Category : TECHNICAL* **(484) Zambia (30 Sep 2016 11:05 PM)** REFERENCES Paragraph 499 and 500 should be interchanged to reflect the sequence in year of publication i.e., 2002 before 2007. Paragraph 516-519 should be rearranged to follow sequence in year of publication and number of authors. The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in Africa countries. Studies should be continued to come out with quick diagnostic kits ready to be used by inspection services at the border points.
G	(General Comment)	*Category : TECHNICAL* **(483) Zambia (30 Sep 2016 10:57 PM)** Paragraph 83-84 moved to underneath Paragraph 112

		Paragraph 158-159 number of cycles for the PCR cycling parameters is confusing, see Paragraph 166 also. Same for paragraph 213-219, 435-455 i.e., number of cycles for denaturation; annealing and elongation steps should be placed appropriately.
G	(General Comment)	*Category : SUBSTANTIVE* **(481) Canada (30 Sep 2016 9:23 PM)** Canada supports the Draft Annex to ISPM 27 – Phytophthora ramorum (2004-013)
G	(General Comment)	*Category : SUBSTANTIVE* **(478) Guyana (30 Sep 2016 6:41 PM)** We accept the contents of the document.
G	(General Comment)	*Category : TECHNICAL* **(469) Bolivia (30 Sep 2016 2:10 AM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.
G	(General Comment)	*Category : TECHNICAL* **(456) Peru (29 Sep 2016 7:51 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.
G	(General Comment)	*Category : TECHNICAL* **(455) Nigeria (29 Sep 2016 4:56 PM)** The diagnostic methods described are sophisticated and beyond the capacity of many national laboratories and inspectors. scientists should come up with simpler and quicker diagnostic kits ready to be used at the national borders.
G	(General Comment)	*Category : SUBSTANTIVE* **(454) Barbados (29 Sep 2016 4:41 PM)** In general the draft protocol is well written and is a good guide for the diagnosis of the pathogen P.ramorum.
G	(General Comment)	*Category : TECHNICAL* **(443) Brazil (29 Sep 2016 4:09 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.
G	(General Comment)	*Category : TECHNICAL* **(432) EPPO (29 Sep 2016 12:14 PM)** It is recommended to use consistent terminology through out the protocol: e.g. in the current text PCR methods/tests/assay are used.
G	(General Comment)	*Category : SUBSTANTIVE* **(334) China (29 Sep 2016 11:30 AM)** Add the host information.The hosts of this pest is wide, and difference host has difference symptoms .
G	(General Comment)	*Category : SUBSTANTIVE* **(333) China (29 Sep 2016 11:29 AM)** Add the section of tests in this draft.The related molecular detection methods are all cited from the documents, so they should be verified by tests to ensure their accuracy.

G	(General Comment)	*Category : TECHNICAL* **(313) Argentina (29 Sep 2016 1:52 AM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC
G	(General Comment)	*Category : TECHNICAL* **(223) Chile (28 Sep 2016 5:28 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.
G	(General Comment)	*Category : TECHNICAL* **(196) Iraq (28 Sep 2016 11:00 AM)** No comment
G	(General Comment)	*Category : TECHNICAL* **(195) Burundi (28 Sep 2016 9:00 AM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. Studies should be continued to come out with quick diagnostic kits ready to be used by inspection services at the border points. Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods(Paragraphs 60 and 77).
G	(General Comment)	*Category : TECHNICAL* **(194) Burundi (28 Sep 2016 8:59 AM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. Studies should be continued to come out with quick diagnostic kits ready to be used by inspection services at the border points. Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods(Paragraphs 60 and 77).
G	(General Comment)	*Category : TECHNICAL* **(187) Viet Nam (26 Sep 2016 12:16 PM)** Section 4.2.1 Pathogenicity test, should complement the necessary and sufficient conditions for pathogenicity to host plants Koch cycle
G	(General Comment)	*Category : EDITORIAL* **(186) PPPO (25 Sep 2016 10:51 PM)** PPPO Has no comment on the draft ISPM
G	(General Comment)	*Category : TECHNICAL* **(185) United States of America (23 Sep 2016 10:07 PM)** The United States has no comments on this diagnostic protocol.
G	(General Comment)	*Category : SUBSTANTIVE* **(183) Thailand (21 Sep 2016 6:01 AM)** agree with this diagnostic prorocol.
G	(General Comment)	*Category : TECHNICAL* **(182) Samoa (20 Sep 2016 3:29 AM)** no further comment
G	(General Comment)	*Category : TECHNICAL*

		(169) Uruguay (15 Sep 2016 8:34 PM) We request the TPDP to revise the use of footnotes associated to brand names for consistency and modifications are suggested as commented in other consultation periods according to footnote text previously agreed by the SC.
G	(General Comment)	*Category : SUBSTANTIVE* **(168) Zambia (8 Sep 2016 6:19 AM)** This Annex as it is , is well drafted and understood and if implemented based on the reasons and guidance given, I don't foresee any negative implementation issues.
G	(General Comment)	*Category : SUBSTANTIVE* **(133) Tajikistan (22 Aug 2016 12:13 PM)** I support the document as it is and I have no comments
G	(General Comment)	*Category : TECHNICAL* **(121) COSAVE (11 Aug 2016 10:44 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.
G	(General Comment)	*Category : SUBSTANTIVE* **(118) New Zealand (11 Aug 2016 6:10 AM)** The proposed molecular method (Hughes et al (2006) TaqMan real-time PCR) has been demonstrated to be highly efficacious at detecting symptomatic material and material with high concentrations of P. ramorum DNA. For international trade, it would be beneficial if the diagnostic protocol could also be used to show that material was absent of the pathogen. In order to do this, the protocol would need to include consideration of the reliability of testing of latent infections (asymptomatic plant material) with a focus on the effects of season on testing efficacy, and effect of variability or susceptibility of colonisation of P. ramorum in different tissues of an infected host and/or different hosts.
G	(General Comment)	*Category : TECHNICAL* **(1) Sri Lanka (22 Jul 2016 10:44 AM)** entire content is accepted
1	**Draft Annex to ISPM 27 – *Phytophthora ramorum* (2004-013)**	*Category : SUBSTANTIVE* **(482) Belize (30 Sep 2016 10:09 PM)** We have no comments on this standard.
49	*Phytophthora ramorum* Werres, de Cock & Man in't Veld (Werres *et al*., 2001) is an oomycete pathogen of unknown origin (Brasier *et al.*, 2004). It is considered to have been introduced into western North America and western Europe in the late twentieth century by the ornamental plant trade (Prospero *et al.*, 2007; Mascheretti *et al.*, 2008; Goss *et al.*, 2011; Grünwald *et al.*, 2012; Van Poucke *et al.*, 2012). *P. ramorum* attacks a wide range of trees and shrubs in nurseries and in the field, causing leaf blight, stem cankers, bleeding stem lesions and dieback,Leaf Spots, Blotches, and Scorches (Rizzo et al., 2002b; Garbelotto et al., 2003).	*Category : SUBSTANTIVE* **(357) Kenya (29 Sep 2016 11:58 AM)**

50	In North America the pathogen was found in the early 1990s causing mortality of *Quercus* (oak) trees and *Lithocarpus densiflorus* (tanoaks), mainly in California and Oregon (Rizzo *et al*., ~~2002~~2002). Named "Sudden Oak Death" (SOD), the disease has reached epidemic proportions in North America at present. The pathogen was originally considered a woodland disease but since 2003 nursery plants in several states of the United States have been affected. The disease has also been found in Canada.	*Category : EDITORIAL* **(360) EPPO (29 Sep 2016 12:13 PM)** One bracket is missing.
50	In North America the pathogen was found in the early 1990s causing mortality of *Quercus* (oak) trees and *Lithocarpus densiflorus* (tanoaks), mainly in California and Oregon (Rizzo *et al*., 2002. Named "Sudden Oak Death" (SOD), the disease has reached epidemic proportions in North America at present. The pathogen was originally considered a woodland disease but since 2003 nursery plants in several states of the United States have been affected. The disease has also been found in Canada. What is the source of he information? Provide reference.	*Category : TECHNICAL* **(358) Kenya (29 Sep 2016 12:00 PM)**
50	In North America the pathogen was found in the early 1990s causing mortality of *Quercus* (oak) trees and *Lithocarpus densiflorus* (tanoaks), mainly in California and Oregon (Rizzo *et al*., ~~2002~~2002). Named "Sudden Oak Death" (SOD), the disease has reached epidemic proportions in North America at present. The pathogen was originally considered a woodland disease but since 2003 nursery plants in several states of the United States have been affected. The disease has also been found in Canada.	*Category : EDITORIAL* **(197) European Union (28 Sep 2016 4:19 PM)** A bracket was missing.
50	In North America the pathogen was found in the early 1990s causing mortality of *Quercus* (oak) trees and *Lithocarpus densiflorus* (tanoaks), mainly in California and Oregon (Rizzo *et al*., ~~2002~~2002). Named "Sudden Oak Death" (SOD), the disease has reached epidemic proportions in North America at present. The pathogen was originally considered a woodland disease but since 2003 nursery plants in several states of the United States have been affected. The disease has also been found in Canada.	*Category : EDITORIAL* **(2) France (5 Aug 2016 5:06 PM)** One bracket is missing.
53	*P. ramorum* has a complex life cycle and is adapted to cool temperatures, with 20 ºC being optimal. Although *P. ramorum* is soil-borne, deciduous, asexually produced sporangia are formed (these symptoms are not produced on all hosts) on the surface of infected leaves or twigs and, depending on environmental conditions, are locally splash-dispersed or spread over long distances by wind and wind-driven rain (Davidson *et al.*, 2005). Rivers, streams and other waterways can also carry the sporangia and thus spread the pathogen (Defra, 2007). Sporangia that land on suitable hosts germinate to produce hyphae. In the presence of water, sporangia will release motile zoospores that encyst on the host surface, germinate and	*Category : TECHNICAL* **(361) EPPO (29 Sep 2016 12:13 PM)** Clarify that this is not on all hosts

	penetrate the host tissue, forming a colony from which more sporangia are produced. These sporangia repeat the cycle and with enough repetitions, under the right environmental conditions, an epidemic can ensue. Different asexual spores, chlamydospores, are produced in abundance within infected plant tissue and allow *P. ramorum* to survive adverse conditions in infected stems and leaves on the plant, in plant debris on the soil surface, or in the soil (Grünwald *et. al*., 2012).	
53	*P. ramorum* has a complex life cycle and is adapted to cool temperatures, with 20 ºC being optimal. Although *P. ramorum* is soil-borne, deciduous, asexually produced sporangia are formed on the surface of infected leaves or twigs and, depending on environmental conditions, are locally splash-dispersed or spread over long distances by wind and wind-driven rain (Davidson *et al.*, 2005). Rivers, streams and other waterways can also carry the sporangia and thus spread the pathogen (Defra, 2007). Sporangia that land on suitable hosts germinate to produce hyphae. In the presence of water, sporangia will release motile zoospores that encyst on the host surface, germinate and penetrate the host tissue, forming a colony from which more sporangia are produced. These sporangia repeat the cycle and with enough repetitions~~,~~ ,(How Many are enough repetitions) under the right environmental conditions, an epidemic can ensue. Different asexual spores, chlamydospores, are produced in abundance within infected plant tissue and allow *P. ramorum* to survive adverse conditions in infected stems and leaves on the plant, in plant debris on the soil surface, or in the soil (Grünwald *et. al*., 2012).	*Category : TECHNICAL* **(359) Kenya (29 Sep 2016 12:06 PM)**
53	*P. ramorum* has a complex life cycle and is adapted to cool temperatures, with 20 ºC being optimal. Although *P. ramorum* is soil-borne, deciduous, asexually produced sporangia are formed (these symptoms are not produced on all hosts) on the surface of infected leaves or twigs and, depending on environmental conditions, are locally splash-dispersed or spread over long distances by wind and wind-driven rain (Davidson *et al.*, 2005). Rivers, streams and other waterways can also carry the sporangia and thus spread the pathogen (Defra, 2007). Sporangia that land on suitable hosts germinate to produce hyphae. In the presence of water, sporangia will release motile zoospores that encyst on the host surface, germinate and penetrate the host tissue, forming a colony from which more sporangia are produced. These sporangia repeat the cycle and with enough repetitions, under the right environmental conditions, an epidemic can ensue. Different asexual spores, chlamydospores, are produced in abundance within infected plant tissue and allow *P. ramorum* to survive adverse conditions in infected stems and leaves on the plant, in plant debris on the soil surface, or in the soil (Grünwald *et. al*., 2012).	*Category : TECHNICAL* **(198) European Union (28 Sep 2016 4:22 PM)** Clarify that this is not on all hosts.
53	*P. ramorum* has a complex life cycle and is adapted to cool temperatures, with 20 ºC being optimal. Although *P. ramorum* is soil-borne, deciduous, asexually produced sporangia are formed on the surface of infected leaves or twigs and,	*Category : TECHNICAL* **(184) South Africa (23 Sep 2016 2:08 PM)** Propose replacement of the word: “right” with “favorable” because “favorable” has a more specific meaning in this context than “right”, and would convey a more

	depending on environmental conditions, are locally splash-dispersed or spread over long distances by wind and wind-driven rain (Davidson *et al.*, 2005). Rivers, streams and other waterways can also carry the sporangia and thus spread the pathogen (Defra, 2007). Sporangia that land on suitable hosts germinate to produce hyphae. In the presence of water, sporangia will release motile zoospores that encyst on the host surface, germinate and penetrate the host tissue, forming a colony from which more sporangia are produced. These sporangia repeat the cycle and with enough repetitions, under the ~~right~~ favorable environmental conditions, an epidemic can ensue. Different asexual spores, chlamydospores, are produced in abundance within infected plant tissue and allow *P. ramorum* to survive adverse conditions in infected stems and leaves on the plant, in plant debris on the soil surface, or in the soil (Grünwald *et. al.*, 2012).	appropriate intended plant pathological meaning.
54	*P. ramorum* is a heterothallic species and may produce sexual oospores, but this requires both mating types. No evidence exists that natural crossing of these mating types has occurred in nature although crossing has been achieved in the laboratory (Brasier and Kirk, 2004). Currently, mating type A1 is the predominant type in Europe while A2 is the predominant type in North America (Werres and Kaminski, 2005). There are four clonal lineages known, with the first three designated as: NA1 (mating type: A2; distribution: North America; environment: forest and nurseries); NA2 (mating type: A2; distribution: North America; environment: nurseries); and EU1 (mating type: predominantly A1, rarely A2; distribution: Europe and North America; environment: nurseries and gardens) (Grünwald *et al.*, 2009). The fourth, a new lineage designated as EU2, was discovered recently in Northern Ireland and western Scotland and is associated with four host plants, ~~including~~ [which are the host plants?]including *L. kaempferi* (Van Poucke *et al.*, 2012).	*Category : TECHNICAL* **(434) Kenya (29 Sep 2016 1:05 PM)**
54	*P. ramorum* is a heterothallic species and may produce sexual oospores, but this requires both mating types. No evidence exists that natural crossing of these mating types has occurred in nature although crossing has been achieved in the laboratory (Brasier and Kirk, 2004). Currently, mating type A1 is the predominant type in Europe while A2 is the predominant type in North America (Werres and Kaminski, 2005). There are four clonal lineages known, with the first three designated as: NA1 (mating type: A2; distribution: North America; environment: forest and nurseries); NA2 (mating type: A2; distribution: North America; environment: nurseries); and EU1 (mating type: predominantly A1, rarely A2; distribution: Europe and North America; environment: nurseries and gardens) (Grünwald *et al.*, 2009). The fourth, a new lineage designated as EU2, was discovered recently in Northern Ireland	*Category : TECHNICAL* **(362) EPPO (29 Sep 2016 12:13 PM)** 'four host plants' Avoid exact number? already five hosts now known (larch, Vaccinium myrtillus, Pieris sp, Magnolia sp., Rhododendron sp and there is bound to be a steady increase. It is suggested to replace this text by The fourth, a new lineage designated as EU2, was discovered recently in Northern Ireland and western Scotland and is associated in particular with L. kaempferi (Van Poucke et al., 2012).

	and western Scotland and is associated with four host plants, including *L. kaempferi* (Van Poucke *et al.,* 2012).	
54	*P. ramorum* is a heterothallic species and may produce sexual oospores, but this requires both mating types. No evidence exists that natural crossing of these mating types has occurred in nature although crossing has been achieved in the laboratory (Brasier and Kirk, 2004). Currently, mating type A1 is the predominant type in Europe while A2 is the predominant type in North America (Werres and Kaminski, 2005). There are four clonal lineages known, with the first three designated as: NA1 (mating type: A2; distribution: North America; environment: forest and nurseries); NA2 (mating type: A2; distribution: North America; environment: nurseries); and EU1 (mating type: predominantly A1, rarely A2; distribution: Europe and North America; environment: nurseries and gardens) (Grünwald *et al.*, 2009). The fourth, a new lineage designated as EU2, was discovered recently in Northern Ireland and western Scotland and is associated with ~~four host plants~~*four host plants*, including *L. kaempferi* (Van Poucke *et al.*, 2012).	*Category : TECHNICAL* **(199) European Union (28 Sep 2016 4:25 PM)** ...'four host plants': Avoid exact number? already five hosts now known (larch, Vaccinium myrtillus, Pieris sp, Magnolia sp., Rhododendron sp and there is bound to be a steady increase. It is suggested to replace this text by: The fourth, a new lineage designated as EU2, was discovered recently in Northern Ireland and western Scotland and is associated in particular with L. kaempferi (Van Poucke et al., 2012).
58	**Taxonomic position:** Chromista, Oomycota, Oomycetes, ~~Pythiales~~Peronosporales, ~~Pythiaceae~~Peronosporales (http://www.cabi.org/isc/datasheet/40991)	*Category : TECHNICAL* **(480) Algeria (30 Sep 2016 8:28 PM)**
58	**Taxonomic position:** Chromista, Oomycota, Oomycetes, Pythiales, ~~Pythiaceae~~Pythiaceae [Order: Peronosporales, Family: Peronosporaceae. What about this classification by CABI, 2016	*Category : TECHNICAL* **(435) Kenya (29 Sep 2016 1:07 PM)**
58	**Taxonomic position:** ~~Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae~~ *Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae*	*Category : EDITORIAL* **(363) EPPO (29 Sep 2016 12:13 PM)**
58	**Taxonomic position:** ~~Chromista, Oomycota,~~ Chromista, Oomycota, Peronosporea, Peronosporales, Peonosporaceae~~Oomycetes, Pythiales, Pythiaceae~~	*Category : SUBSTANTIVE* **(335) China (29 Sep 2016 11:31 AM)** The new classification system described according to the index fungorum.
58	**Taxonomic position:** ~~Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae~~ *Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae*	*Category : EDITORIAL* **(200) European Union (28 Sep 2016 4:25 PM)**
60	**Reference:** Mycobank MB474485	*Category : TECHNICAL* **(467) Ghana (29 Sep 2016 11:37 PM)** Levels of sensitivity and specificity of the proposed method should be indicated in order to compare these methods with other methods
60	**Reference:** Mycobank ~~MB474485~~MB474485 Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods.	*Category : TECHNICAL* **(440) Kenya (29 Sep 2016 2:08 PM)**
62	Laboratory studies have shown that the time between foliage infection and visible disease expression is typically between 3 and 14 days, depending on host and	*Category : EDITORIAL* **(365) EPPO (29 Sep 2016 12:13 PM)** Created by merging other changes together

	temperature. However, the period may be longer in the field and on different plant parts (Defra, 2007). Leaves selected at random can be checked for surface contamination or latent infection by baiting (section 3.4.2.) or molecular methods (section 3.6). The use of fungicides can make it more difficult to detect infected plant material by ~~culture~~ isolation on agar media (Hamelin *et al.*, 2000; Shishkoff, 2014). Fungicides may suppress symptom development as well as the viability of the pathogen, which may lead to false negative test results.	
62	Laboratory studies have shown that the time between foliage infection and visible disease expression is typically between 3 and 14 days, depending on host and temperature. However, the period may be longer in the field and on different plant parts (Defra, 2007). Leaves selected at random can be checked for surface contamination or latent infection by baiting or molecular methods (section 3.6). The use of fungicides can make it more difficult to detect infected plant material by culture (Hamelin *et al.*, 2000; Shishkoff, 2014). Fungicides may suppress symptom development as well as the viability of the pathogen, which may lead to false negative test results.	*Category : TECHNICAL* **(364) EPPO (29 Sep 2016 12:13 PM)** The preferred sample is leaves with symptoms and that should be clarified. Samples without symptoms should be selected according to statistic rules, otherwise negative results will be without any meaning.
62	Laboratory studies have shown that the time between foliage infection and visible disease expression is typically between 3 and 14 days, depending on host and temperature. However, the period may be longer in the field and on different plant parts (Defra, 2007). Leaves selected at random can be checked for surface contamination or latent infection by baiting or molecular methods (section 3.6). The use of fungicides can make it more difficult to detect infected plant material by culture (Hamelin *et al.*, 2000; Shishkoff, 2014). ~~Fungicides may suppress symptom development as well as the viability of the pathogen, which may lead to false negative test results.~~	*Category : SUBSTANTIVE* **(336) China (29 Sep 2016 11:32 AM)** The content described by the sentence is not the operating procedure of the standard.
62	Laboratory studies have shown that the time between foliage infection and visible disease expression is typically between 3 and 14 days, depending on host and temperature. However, the period may be longer in the field and on different plant parts (Defra, 2007). Leaves selected at random can be checked for surface contamination or latent infection by baiting (section 3.4.2.) or molecular methods (section 3.6). The use of fungicides can make it more difficult to detect infected plant material by ~~culture~~ isolation on agar media (Hamelin *et al.*, 2000; Shishkoff, 2014). Fungicides may suppress symptom development as well as the viability of the pathogen, which may lead to false negative test results.	*Category : TECHNICAL* **(201) European Union (28 Sep 2016 4:27 PM)** 1. The preferred sample is leaves with symptoms and that should be clarified. Samples without symptoms should be selected according to statistic rules, otherwise negative results will be without any meaning. 2. (EDIT.) Clarify by being more specific and include additional reference..
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be	*Category : TECHNICAL* **(366) EPPO (29 Sep 2016 12:13 PM)** 'can be' better reflects what happens

	achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods ~~are often~~ can be used first as a screening test, for the presence of *Phytophthora* spp but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or ~~polymerase chain reaction (PCR) methods. PCR~~ molecular methods ~~can also be used for detection but~~ according to the ~~presence of some other~~ flow diagram described in Figure 1. ~~*Phytophthora* species may lead to false positive results. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by sequencing or by isolation for morphological identification.~~ If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	As mentioned later in this protocol, the flow diagram should be revised to take account of the lack of specificity of the real-time PCR test of Hughes et al. It is suggested to make reference to figure 1 here, and not to go into details regarding the different detection methods used in this protocol. Some additional points are questionable in this text that also support a replacement Some available PCR tests proved to be specific and to show no cross-reactions up to now (e.g. Ioos et al. (2006), Schena et al. (2008)). The affirmation about 'false positive result should be moderated or illustrated. Howeer the suggestion is made to rather refer to Fig. 1 The confirmation of a result for a PCR targeting ITS region can be made with a PCR test targeting COX or intronic regions, for example.
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be achieved using the serological, biological and molecular methods shown in Figure 1. ~~Serological methods are often used first as a screening test, for the presence of~~ When a *Phytophthora* ~~spp but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora*~~ species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or polymerase chain reaction (PCR) methods. PCR methods can also be used for detection but the presence of some other *Phytophthora* species may lead to false positive results. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by sequencing or by isolation for morphological identification. If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	*Category : SUBSTANTIVE* **(339) China (29 Sep 2016 11:37 AM)** Serological method is not accuracy. And Culture-based and molecular method can replace it. So it's not necessary to use this method.
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods are often used first as a screening test, for the presence of *Phytophthora* spp but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or polymerase chain reaction (PCR) methods. PCR methods can also be used for detection but the presence of some other *Phytophthora* species may lead to false positive results. Therefore, detection by	*Category : SUBSTANTIVE* **(337) China (29 Sep 2016 11:34 AM)** Sequencing and morphology are the methods combining molecular and traditional methods and thus indispensable for accurate identification of "Phytophthora ramorum".

	serological methods can be confirmed by PCR, but the latter must be followed by sequencing ~~or~~ and by isolation for morphological identification. If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods are often used first as a screening test, for the presence of *Phytophthora* spp. but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or polymerase chain reaction (PCR) methods. PCR methods can also be used for detection but the presence of some other *Phytophthora* species may lead to false positive results. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by sequencing or by isolation for morphological identification. If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	*Category : EDITORIAL* **(250) COSAVE (28 Sep 2016 5:46 PM)**
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods ~~are often~~ can be used first as a screening test, for the presence of *Phytophthora* spp~~.~~. but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or ~~polymerase chain reaction (PCR) methods. PCR~~ molecular methods ~~can also be used for detection but~~ according to the ~~presence of some other~~ flow diagram described in Figure 1. ~~*Phytophthora* species may lead to false positive results. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by sequencing or by isolation for morphological identification.~~ If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	*Category : TECHNICAL* **(202) European Union (28 Sep 2016 4:40 PM)** 1. As mentioned later in this protocol, the flow diagram should be revised to take account of the lack of specificity of the real-time CR test of Hughes et al. I suggest to make reference to figure 1 here, and not to go into details regarding the different detection methods used in this protocol. 2. 'can be': clarity and better reflects what happens. 3. Some available PCR tests proved to be specific and to show no cross-reactions up to now (e.g. Ioos et al. (2006), Schena et al. (2008)). This affirmation should be moderated or illustrated. 4. The confirmation of a result for a PCR targeting ITS region can be confirmed by a PCR test targeting COX or intronic regions, for example. 5. A fullstop was mising.
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be	*Category : TECHNICAL* **(4) France (5 Aug 2016 5:13 PM)** The confirmation of a result for a PCR targeting ITS region can be confirmed by a PCR test targeting COX or intronic régions, for example.

	achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods are often used first as a screening test, for the presence of *Phytophthora* spp but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or polymerase chain reaction (PCR) methods. PCR methods can also be used for detection but the presence of some other *Phytophthora* species may lead to false positive results. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by ~~sequencing or~~ sequencing, by isolation for morphological ~~identification~~identification or by another PCR test targeting a different locus. If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	
63	This diagnostic protocol describes well-established methods for the detection and identification of *P. ramorum.* It is not a comprehensive review of all methods available for the diagnosis of *P. ramorum*. Detection of *P. ramorum* can be achieved using the serological, biological and molecular methods shown in Figure 1. Serological methods are often used first as a screening test, for the presence of *Phytophthora* spp but may yield false negative or false positive results (Kox *et al.*, 2007). When a *Phytophthora* species has been detected by a serological method, the identity of the species must be confirmed by isolation and morphological identification or polymerase chain reaction (PCR) methods. PCR methods can also be used for detection but the presence of some other *Phytophthora* species may lead to false positive ~~results~~results [include examples?]. Therefore, detection by serological methods can be confirmed by PCR, but the latter must be followed by sequencing or by isolation for morphological identification. If identification of *P. ramorum* represents the first finding for a country, the laboratory may wish to have the diagnosis confirmed by another laboratory.	*Category : TECHNICAL* **(3) France (5 Aug 2016 5:10 PM)** Some available PCR tests proved to be specific and to show no cross-reactions up to now (e.g. Ioos et al. (2006), Schena et al. (2008)). This affirmation should be moderated or illustrated.
64	**3.1 Symptoms** **Propose to add the description of symptoms.**	*Category : SUBSTANTIVE* **(340) China (29 Sep 2016 11:37 AM)** Propose to add the description of symptoms.
66	**3.1.~~1~~ ~~Tree dieback~~1 Bleeding canker**	*Category : SUBSTANTIVE* **(341) China (29 Sep 2016 11:38 AM)**
70	**3.1.3 Leaf blight**	*Category : TECHNICAL* **(367) EPPO (29 Sep 2016 12:13 PM)** It would be useful to put a picture of leaves with small spots caused by P. ramorum as this symptom is not so typical
70	**3.1.3 Leaf blight**	*Category : TECHNICAL* **(203) European Union (28 Sep 2016 4:45 PM)** It would be useful to put a picture of leaves with small spots caused by P. ramorum

		as this symptom is not so typical.
71	On *Rhododendron*, *Camellia*, *Kalmia* and *Pieris* species black–brown lesions occur on leaves, usually at the tip but often at the petiole end. Disease develops across infected leaves often following the midrib, eventually leading to premature leaf fall. On *Magnolia* spp. and *Rhododendron* spp. multiple small spots can also be observed, eventually merging into larger necrotic areas.	*Category : TECHNICAL* **(368) EPPO (29 Sep 2016 12:13 PM)** The symptoms described for Magnolia can also be seen on rhododendron at the beginning of symptom expression (drops with zoospores falling down on the leaves causing round, dark spots)
71	On *Rhododendron, Camellia, Kalmia* and *Pieris* species black–brown lesions occur on leaves, usually at the tip but often at the petiole end. Disease develops across infected leaves often following the midrib, eventually leading to premature leaf fall. On *Magnolia* spp. multiple small spots can also be observed, eventually merging into larger necrotic areas.	*Category : TECHNICAL* **(204) European Union (28 Sep 2016 4:46 PM)** Last sentence The symptoms described for Magnolia can also be seen on rhododendron at the beginning of symptom expression (drops with zoospores falling down on the leaves causing round, dark spots) Suggested change On Magnolia spp. and Rhododendron spp. multiple small spots can also be observed, eventually merging into larger necrotic areas.
72	**3.1.4 Symptoms on Coniferae**	*Category : TECHNICAL* **(205) European Union (28 Sep 2016 4:47 PM)** It would be useful to introduce pictures of symptoms on larch (blackening of needles, bleeding canker, crown defoliation).
72	**3.1.4 ~~Symptoms on Coniferae~~Needlle blight**	*Category : EDITORIAL* **(134) New Zealand (30 Aug 2016 5:34 AM)** Since all the other subsections of 3.1 are given with symptom names, it would be better to rename that section for a uniform approach.
72	**3.1.4 Symptoms on Coniferae**	*Category : TECHNICAL* **(5) France (5 Aug 2016 5:14 PM)** Pictures of symptoms on conifers would be useful (larch for example).
73	*P. ramorum* causes needle blight and dieback of young shoots of the conifers *Pseudotsuga menziesii* (Douglas fir), *Sequoia sempervirens* (coastal ~~redwood)~~ redwood), Larix kaempferi (Japanese larch), Taxus baccata (English Yew) and *Abies grandis* (grand fir). Typical symptoms observed on larches are needle infections, shoot dieback, and branch and trunk cankers. Infected shoot tips wither and wilt and infected needles appear blackened. Early needle abscission of infected needles also occurs.	*Category : TECHNICAL* **(369) EPPO (29 Sep 2016 12:13 PM)** Hosts should be added
73	*P. ramorum* causes needle blight and dieback of young shoots of the conifers *Pseudotsuga menziesii* (Douglas fir), *Sequoia sempervirens* (coastal ~~redwood)~~ redwood), *Taxus baccata* (English yew), *Larix kaempferi* (Japanese larch) and *Abies grandis* (grand fir). Typical symptoms observed on larches are needle infections, shoot dieback, and branch and trunk cankers. Infected shoot tips wither and wilt and infected needles appear blackened. Early needle abscission of infected needles also occurs.	*Category : TECHNICAL* **(206) European Union (28 Sep 2016 4:50 PM)** Hosts should be added.
75	Different techniques for sampling and sample preparation as described below are	*Category : TECHNICAL*

	recommended depending on the material being tested. Samples should be kept cool and sent to the diagnostic laboratory in strong closed plastic bags or containers, or double-bagged for next day isolation, as prolonged transit times or raised temperatures can reduce the likelihood of successful isolation and detection. Placing a small amount of damp tissue with the plant material will reduce sample desiccation and may increase the chance of isolation. However, in sealed self-closing plastic bags, excessive moisture can hasten tissue degradation and saprophytic activity. Storage at ~~4~~ 2-8 °C is highly recommended to prolong sample life but storage for longer than seven days reduces the ease of isolation.	**(370) EPPO (29 Sep 2016 12:13 PM)** this range is appropriate.
75	Different techniques for sampling and sample preparation as described below are recommended depending on the material being tested. Samples should be kept cool and sent to the diagnostic laboratory in strong closed plastic bags or containers, or double-bagged for next day isolation, as prolonged transit times or raised temperatures can reduce the likelihood of successful isolation and detection. Placing a small amount of damp tissue with the plant material will reduce sample desiccation and may increase the chance of isolation. However, in sealed self-closing plastic bags, excessive moisture can hasten tissue degradation and saprophytic activity. Storage at ~~4~~ 2-8 °C is highly recommended to prolong sample life but storage for longer than seven days reduces the ease of isolation.	*Category : TECHNICAL* **(207) European Union (28 Sep 2016 4:51 PM)** This range is appropriate for storage.
77	When sampling bleeding cankers from trees, the outer bark around the canker should be removed to reveal the inner bark and the margin of necrosis. Pieces of phloem and xylem can then be excised from across the leading edge and sent for testing. Symptomatic shoots and twig samples approximately 15 cm long, spanning the leading edge of an infection, should be taken while for leaves, several, showing a range of typical symptoms, should be taken.	*Category : TECHNICAL* **(468) Ghana (29 Sep 2016 11:39 PM)** We propose that levels of sensitivity and specificity of the proposed method should be indicated in order to compare these methods with other methods.
77	When sampling bleeding cankers from trees, the outer bark around the canker should be removed to reveal the inner bark and the margin of necrosis. Pieces of phloem and xylem can then be excised from across the leading edge and sent for testing. Symptomatic shoots and twig samples approximately 15 cm long, spanning the leading edge of an infection, should be taken while for leaves, several, showing a range of typical symptoms, should be taken. Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods.	*Category : TECHNICAL* **(441) Kenya (29 Sep 2016 2:10 PM)**
77	When sampling bleeding cankers from trees, the outer bark around the canker should be removed to reveal the inner bark and the margin of necrosis. Pieces of phloem and xylem can then be excised from across the leading edge (junction between healthy and necrotic tissue) and sent for testing. Symptomatic shoots and twig samples approximately 15 cm long, spanning the leading edge of an infection,	*Category : TECHNICAL* **(371) EPPO (29 Sep 2016 12:13 PM)** more detailed description

	should be taken while for leaves, several, showing a range of typical symptoms, should be taken.	
77	When sampling bleeding cankers from trees, the outer bark around the canker should be removed to reveal the inner bark and the margin of necrosis. Pieces of phloem and xylem can then be excised from across the leading edge (junction between healthy and necrotic tissue) and sent for testing. Symptomatic shoots and twig samples approximately 15 cm long, spanning the leading edge of an infection, should be taken while for leaves, several, showing a range of typical symptoms, should be taken.	*Category : TECHNICAL* **(208) European Union (28 Sep 2016 4:53 PM)** More detailed description.
79	**3.2.2 Water** **Add the test for rainwater.**	*Category : SUBSTANTIVE* **(342) China (29 Sep 2016 11:39 AM)** Add the test for rainwater.Because rainwater is an important spread way of P.ramorum.
80	Water samples should be at least 1 litre in volume and be taken from the surface of the area being tested, preferably where the water is flowing and is not below 4 °C or deeper than 15 cm. The water samples should be kept cool (5–20 °C) during storage and transport and tested within 48 h of collection. Water bait bags sometimes called "bobs" (muslin bags containing leaves for baiting) are an alternative, very effective method of on-site testing for water (Defra, 2007; USDA-APHIS, 2014b). They consist of ~~cut~~ whole leaves of rhododendron (*Rhododendron. catawbiense* "Grandiflorum", *R* "Cunningham's White" or *R. ponticum*) in muslin bags containing polystyrene to aid flotation. They have been used extensively in field situations to check water sources, including streams and irrigation ponds, for *P. ramorum* (Defra, 2007). Bait bags are best deployed where the water is flowing, however slow, rather than still. Bait bags can be used when the water to be tested is above 4 °C (Defra, 2007).	*Category : TECHNICAL* **(372) EPPO (29 Sep 2016 12:13 PM)** Whole leaves work better than cut leaves as baits because of less contamination particularly with Pythium (see Ghimire et al, 2009, Plant Pathology 58, 577-583)
80	Water samples should be at least 1 litre in volume and be taken from the surface of the area being tested, preferably where the water is flowing and is not below 4 °C or deeper than 15 cm. The water samples should be kept cool (5–20 °C) during storage and transport and tested within 48 h of collection. Water bait bags sometimes called "bobs" (muslin bags containing leaves for ~~baiting)~~ baiting- At least four 1 cm2 pieces of healthy rhododendron leaf that has not been treated with fungicide are placed on the water surface. Alternatively, small young rhododendron leaves that have been cut several times on the leaf margin with a sterile scalpel can be used. Rhododendron "Cunningham's White" is recommended because it is highly susceptible to P. ramorum; however, many other rhododendron species are as susceptible (De Dobbelaere et al., 2010).) are an alternative, very effective method of on-site testing for water (Defra, 2007; USDA-APHIS, 2014b). They	*Category : SUBSTANTIVE* **(324) Philippines (29 Sep 2016 8:46 AM)** description of the preparation of leaf baits was extracted from 3.4.2 and included here

	consist of cut leaves of rhododendron (*Rhododendron. catawbiense* "Grandiflorum", *R* "Cunningham's White" or *R. ponticum*) in muslin bags containing polystyrene to aid flotation. They have been used extensively in field situations to check water sources, including streams and irrigation ponds, for *P. ramorum* (Defra, 2007). Bait bags are best deployed where the water is flowing, however slow, rather than still. Bait bags can be used when the water to be tested is above 4 °C (Defra, 2007).	
80	Water samples should be at least 1 litre in volume and be taken from the surface of the area being tested, preferably where the water is flowing and is not below 4 °C or deeper than 15 cm. The water samples should be kept cool (5–20 °C) during storage and transport and tested within 48 h of collection. Water bait bags sometimes called "bobs" (muslin bags containing leaves for baiting) are an alternative, very effective method of on-site testing for water (Defra, 2007; USDA-APHIS, 2014b). They consist of whole~~cut~~ leaves of rhododendron (*Rhododendron. catawbiense* "Grandiflorum", *R* "Cunningham's White" or *R. ponticum*) in muslin bags containing polystyrene to aid flotation. They have been used extensively in field situations to check water sources, including streams and irrigation ponds, for *P. ramorum* (Defra, 2007). Bait bags are best deployed where the water is flowing, however slow, rather than still. Bait bags can be used when the water to be tested is above 4 °C (Defra, 2007).	*Category : TECHNICAL* **(209) European Union (28 Sep 2016 4:54 PM)** Whole leaves work better than cut leaves as baits because of less contamination particularly with Pythium (see Ghimire et al, 2009, Plant Pathology 58, 577-583).
83	**3.3 Detection by serological methods**	*Category : EDITORIAL* **(298) Ghana (28 Sep 2016 6:29 PM)** This topic 3.3 'Detection by serological methods' should be moved from here to be placed under 3.6 'Detection by molecular methods'
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use. The use of names of reagents, chemicals or equipment in these diadnostic protocol implies no approval of them to the exlusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results	*Category : TECHNICAL* **(470) Bolivia (30 Sep 2016 2:22 AM)** See general comments

84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : TECHNICAL* **(457) Peru (29 Sep 2016 7:53 PM)** See general comments
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : TECHNICAL* **(444) Brazil (29 Sep 2016 4:10 PM)** See general comments
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use.	*Category : TECHNICAL* **(314) Argentina (29 Sep 2016 1:55 AM)**

84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.,* 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use.	*Category : EDITORIAL* **(300) Ghana (28 Sep 2016 6:31 PM)** The sentence starting with 'Sserological methods may be..... will remain moist' should be moved from here to be placed under 3.6 'Detection by molecular methods'
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(225) Chile (28 Sep 2016 5:30 PM)** see general comments
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adquately validated.	*Category : TECHNICAL* **(170) Uruguay (15 Sep 2016 8:38 PM)** See general comment
84	Serological methods may be used only to pre-screen samples for the presence of *Phytophthora* spp. A low level of false negative and false positive results may occur (Kox *et al.*, 2007). Different formats are available, including lateral flow devices (Forsite Diagnostics[1]) and ImmunoStrip Tests (Agdia[2]), which are both suitable for field use, primarily to screen out negative samples. Larger format enzyme-linked immunosorbent assays (ELISA) are also available (from Neogen[3], Lexington[4] or Agdia[5]), and are more suitable for laboratory use.	*Category : TECHNICAL* **(122) COSAVE (11 Aug 2016 10:45 PM)** See general comments

	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	
85	-The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~	*Category : TECHNICAL* **(246) Chile (28 Sep 2016 5:42 PM)** see general coments
85	-The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not consitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead the same results.~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~	*Category : TECHNICAL* **(171) Uruguay (15 Sep 2016 8:43 PM)** See general comment
85	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : TECHNICAL* **(123) COSAVE (11 Aug 2016 10:46 PM)** See general comments
93	Where no semi-selective medium is used, surface sterilization is recommended. The 1 cm^2 pieces ~~are~~ can for example be dipped in an aqueous solution of bleach (1% active sodium hypochlorite) for 2–5 min depending on the thickness of the	*Category : TECHNICAL* **(373) EPPO (29 Sep 2016 12:13 PM)** As other sterilization methods are in use, it should be presented as an option.

	material (e.g. if they are leaves or stems) or 70% ethanol for 30 s, then rinsed in sterile distilled water and dried. The stem sections are split lengthwise before plating to aid culture growth.	
93	Change the method in this draft to surface sterilization used with selective medium. And that can improve the isolation. Where no semi-selective medium is used, surface sterilization is recommended. The 1 cm^2 pieces are dipped in an aqueous solution of bleach (1% active sodium hypochlorite) for 2–5 min depending on the thickness of the material (e.g. if they are leaves or stems) or 70% ethanol for 30 s, then rinsed in sterile distilled water and dried. The stem sections are split lengthwise before plating to aid culture growth.	*Category : TECHNICAL* **(343) China (29 Sep 2016 11:39 AM)** Change the method in this draft to surface sterilization used with selective medium. And that can improve the isolation.That can improve the isolation.
93	Where no semi-selective medium is used, surface sterilization is recommended. The 1 cm^2 pieces ~~are~~ can for example be dipped in an aqueous solution of bleach (1% active sodium hypochlorite) for 2–5 min depending on the thickness of the material (e.g. if they are leaves or stems) or 70% ethanol for 30 s, then rinsed in sterile distilled water and dried. The stem sections are split lengthwise before plating to aid culture growth.	*Category : TECHNICAL* **(210) European Union (28 Sep 2016 4:56 PM)** As other sterilization methods are in use, it should be presented as an option.
93	Where no semi-selective medium is used, surface sterilization is recommended. The 1 cm^2 pieces ~~are~~ can for example be dipped in an aqueous solution of bleach (1% active sodium hypochlorite) for 2–5 min depending on the thickness of the material (e.g. if they are leaves or stems) or 70% ethanol for 30 s, then rinsed in sterile distilled water and dried. The stem sections are split lengthwise before plating to aid culture growth.	*Category : TECHNICAL* **(6) France (5 Aug 2016 5:15 PM)** As other sterilization methods are in use, it should be presented as an option.
96	In the laboratory, water samples are placed in ~~a 1 litre sterilised~~ an appropriate sterilized container with a large surface area (such as a Ziploc[6] 946 ml square disposable plastic box wiped with 50% ethanol and dried before use). To promote infection from zoospores, a sterilized metal screen or cheese cloth may be used in the box to keep floating debris from touching the leaf baits. At least four 1 cm^2 pieces of healthy rhododendron leaf that has not been treated with fungicide are placed on the water surface. Alternatively, ~~small young~~ fully developed rhododendron leaves that have been cut several times on the leaf margin with a sterile scalpel can be used. *Rhododendron* "Cunningham's ~~White" is~~ White", *R. catawbiense* "Grandiflorum" and *R. ponticum* are recommended because it is highly susceptible to *P. ramorum*; however, many other rhododendron species are as susceptible (De Dobbelaere *et al.*, 2010).	*Category : TECHNICAL* **(374) EPPO (29 Sep 2016 12:13 PM)** In section 3.2.2, the volume of the water sample is at least 1 liter. So a container of 1 liter may be too small. The size of the container should be adapted to the volume of the water sample. The original paper on the rhododendron bait test from Werres and Themann 1999 stresses the importance of using "well developed leaves" –not young leaves-with a "well developed cuticula" to have less contamination Add R. catawbiense "Grandiflorum" and R. ponticum to keep it the same as under 3.2.2
96	In the laboratory, water samples are placed in a 1 litre sterilised container with a large surface area (such as a Ziploc[6] 946 ml square disposable plastic box wiped with 50% ethanol and dried before use). To promote infection from zoospores, a sterilized metal screen or cheese cloth may be used in the box to keep floating	*Category : SUBSTANTIVE* **(325) Philippines (29 Sep 2016 8:48 AM)** transferred to 3.2.2

	debris from touching the leaf baits. ~~At least four 1 cm² pieces of healthy rhododendron leaf that has not been treated with fungicide are placed on the water surface. Alternatively, small young rhododendron leaves that have been cut several times on the leaf margin with a sterile scalpel can be used. *Rhododendron* "Cunningham's White" is recommended because it is highly susceptible to *P. ramorum*; however, many other rhododendron species are as susceptible (De Dobbelaere *et al.*, 2010).~~	
96	In the laboratory, water samples are placed in ~~a 1 litre sterilised~~ an appropriate sterilized container with a large surface area (such as a Ziploc® 946 ml square disposable plastic box wiped with 50% ethanol and dried before use). To promote infection from zoospores, a sterilized metal screen or cheese cloth may be used in the box to keep floating debris from touching the leaf baits. At least four 1 cm² pieces of healthy rhododendron leaf that has not been treated with fungicide are placed on the water surface. Alternatively, ~~small young~~ fully developed rhododendron leaves that have been cut several times on the leaf margin with a sterile scalpel can be used. *Rhododendron* "Cunningham's ~~White" is~~ White", *R. catawbiense* "Grandiflorum"and *R. ponticum* are recommended because ~~it is~~ they are highly susceptible to *P. ramorum*; however, many other rhododendron species are as susceptible (De Dobbelaere *et al.*, 2010).	*Category : TECHNICAL* **(211) European Union (28 Sep 2016 4:57 PM)** 1. In section 3.2.2, the volume of the water sample is at least 1 litre. So a container of 1 litre may be too small. The size of the container should be adapted to the volume of the water sample. 2. The original paper on the rhododendron bait test from Werres and Themann 1999 stresses the importance of using "well developed leaves" –not young leaves-with a "well developed cuticula" to have less contamination. Also add R. catawbiense "Grandiflorum" and R. ponticum to keep it the same as under 3.2.2 3. (EDIT.) "sterilised" to be replaced with "sterilized" (with a "z").
96	In the laboratory, water samples are placed in ~~a 1 litre sterilised container~~ an appropriate sterilised container with a large surface area (such as a Ziploc® 946 ml square disposable plastic box wiped with 50% ethanol and dried before use). To promote infection from zoospores, a sterilized metal screen or cheese cloth may be used in the box to keep floating debris from touching the leaf baits. At least four 1 cm² pieces of healthy rhododendron leaf that has not been treated with fungicide are placed on the water surface. Alternatively, small young rhododendron leaves that have been cut several times on the leaf margin with a sterile scalpel can be used. *Rhododendron* "Cunningham's White" is recommended because it is highly susceptible to *P. ramorum*; however, many other rhododendron species are as susceptible (De Dobbelaere *et al.*, 2010).	*Category : TECHNICAL* **(7) France (5 Aug 2016 5:18 PM)** In paragraph 3.2.2, the volume of the water sample is at least 1 liter. So a container of 1 liter may be too small. The size of the container should be adapted to the volume of the water sample.
98	The box is sealed and incubated on the laboratory bench at room temperature (18–25°C). Within three to seven days symptoms of *P. ramorum* infection usually develop if the pathogen is present; however, the lack of symptoms is not conclusive that *P. ramorum* is absent. The bait leaves should be plated as described in section 3.5 or used directly for DNA extraction. Alternatively, whole or partial leaf baits can be slipped under the selective media with the aid of a sterile spatula to help discourage bacterial contamination and allow the suspect *Phytophthora* to grow through the media. It can then be excised from the surface and transferred to	*Category : TECHNICAL* **(375) EPPO (29 Sep 2016 12:13 PM)** Additional information

	a non-selective medium. Where bait bags have been used, the rhododendron leaves are retrieved after three to seven days, and washed and plated (section 3.4.1) or used directly for DNA extraction. If no symptoms appear, baiting can be extended to 10 days, after which plating on agar media should be done, regardless of the fact that symptoms are present or not.	
98	The box is sealed and incubated on the laboratory bench at room temperature (18–25°C). Within three to seven days symptoms of *P. ramorum* infection usually develop if the pathogen is present; however, the lack of symptoms is not conclusive that *P. ramorum* is absent. The bait leaves should be plated as described in section 3.5 or used directly for DNA extraction. Alternatively, whole or partial leaf baits can be slipped under the selective media with the aid of a sterile spatula to help discourage bacterial contamination and allow the suspect *Phytophthora* to grow through the media. It can then be excised from the surface and transferred to a non-selective medium. Where bait bags have been used, the rhododendron leaves are retrieved after three to seven days, and washed and plated (section 3.4.1) or used directly for DNA extraction. If no symptoms appear, baiting can be extended to 10 days, after which plating on agar media should be done, regardless of the fact that symptoms are present or not.	*Category : TECHNICAL* **(212) European Union (28 Sep 2016 5:10 PM)** Additional useful information.
99	Baiting with rhododendron ~~can~~ has been demonstrated to detect *P. ramorum* at sporangial concentrations of 1 to 40 000 or more per litre of water (Defra, 2007). Other baiting substrates have been described, such as *Pyrus communis* (pear fruit) (Themann *et al.*, 2002), but rhododendron leaves have been used most commonly, work very well and are easy to handle.	*Category : TECHNICAL* **(376) EPPO (29 Sep 2016 12:13 PM)** Surely there is no upper limit?
99	Baiting with rhododendron ~~can~~ has been demonstrated to detect *P. ramorum* at sporangial concentrations of 1 to 40 000 or more per litre of water (Defra, 2007). Other baiting substrates have been described, such as *Pyrus communis* (pear fruit) (Themann *et al.*, 2002), but rhododendron leaves have been used most commonly, work very well and are easy to handle.	*Category : TECHNICAL* **(213) European Union (28 Sep 2016 5:11 PM)** Surely there is no upper limit?
100	Baiting is not specific to *P. ramorum* and may pick up other *Phytophthora* species, as well as *Pythium* species. Using selective media when plating out helps reduce the growth of other ~~fungi~~organisms, making morphological identification of *P. ramorum* easier.	*Category : TECHNICAL* **(377) EPPO (29 Sep 2016 12:13 PM)** Not only fungi may grow on rhododendron leaves.
100	Baiting is not specific to *P. ramorum* and may pick up other *Phytophthora* species, as well as *Pythium* species. Using selective media when plating out helps reduce the growth of other ~~fungi~~organisms, making morphological identification of *P. ramorum* easier.	*Category : TECHNICAL* **(214) European Union (28 Sep 2016 5:13 PM)** Not only fungi may grow on rhododendron leaves.
100	Baiting is not specific to *P. ramorum* and may pick up other *Phytophthora* species, as well as *Pythium* species. Using selective media when plating out helps reduce	*Category : TECHNICAL* **(8) France (5 Aug 2016 5:20 PM)** Not only fungies may grow on rhododendron leaves.

	the growth of other ~~fungi~~organisms, making morphological identification of *P. ramorum* easier.	
102	Approximately 250 g soil to be tested is placed in a large plastic box, covered with about 500 ml Petri's mineral solution (1 litre distilled water with $CaNO_3$ 0.4 g; $MgSO_4.7H_2O$ 0.15 g; KH_2PO_4 0.15 g; and KCl 0.06 ~~g)~~g, or sterile demineralized water), and ~~cut~~ whole rhododendron leaves are placed as baits on the surface of the solution, as described in section 3.4.2. Plant debris can be treated in the same manner. The box is incubated for three to seven days then the sample is checked for the presence of *P. ramorum* by plating or molecular methods (section 3.6). Where bait bags have been used, these are treated as for water samples.	*Category : TECHNICAL* **(378) EPPO (29 Sep 2016 12:13 PM)** Sterile, demineralized water can also be used See previous comments on the recommendation to use whole leaves What is meant by ' Buried bait bags are treated the same way as water bait bags? 'Where bait bags have been used, these are treated as for water samples'' Does the last sentence 'Where bait bags have been used, these are treated as for water samples.' Mean Buried bait bags are treated the same way as water bait bags.
102	Approximately 250 g soil to be tested is placed in a large plastic box, covered with about 500 ml Petri's mineral solution (1 litre distilled water with $CaNO_3$ 0.4 g; $MgSO_4.7H_2O$ 0.15 g; KH_2PO_4 0.15 g; and KCl 0.06 ~~g)~~g, or sterile demineralized water), and cut rhododendron leaves are placed as baits on the surface of the solution, as described in section 3.4.2. Plant debris can be treated in the same manner. The box is incubated for three to seven days then the sample is checked for the presence of *P. ramorum* by plating or molecular methods (section 3.6). Where bait bags have been used, these are treated as for water samples.	*Category : TECHNICAL* **(215) European Union (28 Sep 2016 5:19 PM)** 1. Petri's mineral solution can be usefully replaced by distilled, demineralized or osmozed water with good results. 2. Does the last sentence 'Where bait bags have been used, these are treated as for water samples.' mean 'Buried bait bags are treated the same way as water bait bags.' ? 3. Also, see previous comments on the recommendation to use whole leaves.
102	Approximately 250 g soil to be tested is placed in a large plastic box, covered with about 500 ml Petri's mineral solution (1 litre distilled water with $CaNO_3$ 0.4 g; $MgSO_4.7H_2O$ 0.15 g; KH_2PO_4 0.15 g; and KCl 0.06 g), and cut rhododendron leaves are placed as baits on the surface of the solution, as described in section 3.4.2. Plant debris can be treated in the same manner. The box is incubated for three to seven days then the sample is checked for the presence of *P. ramorum* by plating or molecular methods (section 3.6). Where bait bags have been used, these are treated as for water samples.	*Category : TECHNICAL* **(9) France (5 Aug 2016 5:23 PM)** Petri's mineral solution can be usefully replaced by distilled, demineralized or osmozed water with good results.
103	**3.5 Isolation media** **Propose to add the description of efficiency of different isolation media.**	*Category : SUBSTANTIVE* **(344) China (29 Sep 2016 11:40 AM)** Propose to add the description of efficiency of different isolation media.
105	P5ARP(H) medium is made by adding 17 g cornmeal agar to 1 litre distilled water, stirring thoroughly, then autoclaving at 121 °C for 15 min before cooling to 50 °C in a water bath (EPPO, 2012, validation 2009-03-31–F16_S08). Additions, where necessary, are prepared by suspending them in 10 ml sterile distilled water or	*Category : TECHNICAL* **(379) EPPO (29 Sep 2016 12:14 PM)** This range is appropriate

	dissolving them in ethanol before adding to the medium. For 1 litre P5ARP(H) medium, 5 mg pimaricin, 250 mg ampicillin (sodium salt), 10 mg rifampicin (dissolved in 1 ml of 95% ethanol), 100 mg pentachloronitrobenzene and 75 mg (final concentration: 22.5 parts per million (p.p.m.)) hymexazol (30% active substance) are added to the cooled (50 °C) medium, stirred thoroughly and poured onto plates. The plates should be stored at ~~4~~ 2-8 °C in the dark (rifampicin is inactivated by light) and used before five to seven days have elapsed since they were made (Jeffers and Martin, 1986).	
105	P5ARP(H) medium is made by adding 17 g cornmeal agar to 1 litre distilled water, stirring thoroughly, then autoclaving at 121 °C for 15 min before cooling to 50 °C in a water bath (EPPO, 2012, validation 2009-03-31–F16_S08). Additions, where necessary, are prepared by suspending them in 10 ml sterile distilled water or dissolving them in ethanol before adding to the medium. For 1 litre P5ARP(H) medium, 5 mg pimaricin, 250 mg ampicillin (sodium salt), 10 mg rifampicin (dissolved in 1 ml of 95% ethanol), 100 mg pentachloronitrobenzene and 75 mg (final concentration: 22.5 parts per million (p.p.m.)) hymexazol (30% active substance) are added to the cooled (50 °C) medium, stirred thoroughly and poured onto plates. The plates should be stored at ~~4~~ 2-8 °C in the dark (rifampicin is inactivated by light) and used before five to seven days have elapsed since they were made (Jeffers and Martin, 1986).	*Category : TECHNICAL* **(216) European Union (28 Sep 2016 5:20 PM)** This range is appropriate.
107	Other media that can be used include cherry decoction agar (CHA). Cherry juice is made by boiling 1 kg cherries, free of stones and petioles, in 1 litre tap water for approximately 2 h. The juice is filtered through muslin or cheesecloth, poured into bottles, sterilized at 110 °C for 30 min, adjusted to pH 4.5 with 1 N KOH or 1 N HCl, and stored until use. In a bottle containing 0.8 litre distilled water, 20 g Technical Agar No. 3 is added and the mixture is sterilized at 121 °C for 15 min. Immediately after sterilization, 0.2 litre sterilized cherry extract is added, mixed well and sterilized at 102 °C for 5 min (Gams *et al*., 1998).	*Category : TECHNICAL* **(381) EPPO (29 Sep 2016 12:14 PM)**
107	Other media that can be used include cherry decoction agar (CHA). Cherry juice is made by boiling 1 kg cherries, free of stones and petioles, in 1 litre tap water for approximately 2 h. The juice is filtered through cheesecloth, poured into bottles, sterilized at 110 °C for 30 min, adjusted to pH 4.5 with 1 N KOH or 1 N HCl, and stored until use. In a bottle containing 0.8 litre distilled water, 20 g Technical Agar No. 3 is added and the mixture is sterilized at 121 °C for 15 min. Immediately after sterilization, 0.2 litre sterilized cherry extract is added, mixed well and sterilized at 102 °C for 5 min (Gams *et al.*, 1998).	*Category : TECHNICAL* **(380) EPPO (29 Sep 2016 12:14 PM)** It is not clear for which purpose the other media may be used (to transfer and to study the clean isolated?). It should be clarified.
107	Other media that can be used include cherry decoction agar (CHA). Cherry juice is made by boiling 1 kg cherries, free of stones and petioles, in 1 litre tap water for	*Category : TECHNICAL* **(217) European Union (28 Sep 2016 5:22 PM)**

	approximately 2 h. The juice is filtered through muslin or cheesecloth, poured into bottles, sterilized at 110 °C for 30 min, adjusted to pH 4.5 with 1 N KOH or 1 N HCl, and stored until use. In a bottle containing 0.8 litre distilled water, 20 g Technical Agar No. 3 is added and the mixture is sterilized at 121 °C for 15 min. Immediately after sterilization, 0.2 litre sterilized cherry extract is added, mixed well and sterilized at 102 °C for 5 min (Gams *et al*., 1998).	1. Addition of another filtration possibility. 2. It is not clear for which purpose the other media may be used (to transfer and to study the clean isolated?). It should be clarified.
107	Other media that can be used include cherry decoction agar (CHA). Cherry juice is made by boiling 1 kg cherries, free of stones and petioles, in 1 litre tap water for approximately 2 h. The juice is filtered through cheesecloth, poured into bottles, sterilized at 110 °C for 30 min, adjusted to pH 4.5 with 1 N KOH or 1 N HCl, and stored until use. In a bottle containing 0.8 litre distilled water, 20 g Technical Agar No. 3 is added and the mixture is sterilized at 121 °C for 15 min. Immediately after sterilization, 0.2 litre sterilized cherry extract is added, mixed well and sterilized at 102 °C for 5 min (Gams *et al*., 1998).	*Category : TECHNICAL* **(10) France (5 Aug 2016 5:25 PM)** It is not clear for which purpose the other media may be used (to transfer and to study the clean isolated?). It should be clarified.
108	The plant material to be tested should be decontaminated as described in section 3.4.1, then at least four small pieces (about 2 cm^2) of tissue from each sample should be aseptically transferred onto either of the semi-selective media. As much of each section as practically possible should be slid under the media to force any *Phytophthora* present to grow through the media. A maximum of ten leaf sections should be placed on each plate. Leaf sections from different sites (e.g. nurseries) or different subsamples (hosts or locations) within a site should be placed on different plates. Sporangia are formed more readily on unsealed plates (T. Giltrap, personal communication, 2014). The plates are incubated in daylight or in the dark (the dark favours chlamydospore production) between 18 and 25°C, and examined for *Phytophthora* growth after three to seven days. Samples plated onto media containing rifampicin should be incubated in the dark because rifampicin is inactivated by light. Growth should occur within ten days but morphological features can be seen after three days in some cases.	*Category : EDITORIAL* **(382) EPPO (29 Sep 2016 12:14 PM)** This paragraph, except the sentence "Samples ... light", would be better placed under section 3.4 because section 3.5 is on isolation media.
108	The plant material to be tested should be decontaminated as described in section 3.4.1, then at least four small pieces (about 2 cm^2) of tissue from each sample should be aseptically transferred onto either of the semi-selective media. As much of each section as practically possible should be slid under the media to force any *Phytophthora* present to grow through the media. A maximum of ten leaf sections should be placed on each plate. Leaf sections from different sites (e.g. nurseries) or different subsamples (hosts or	*Category : EDITORIAL* **(218) European Union (28 Sep 2016 5:23 PM)** This paragraph, except the sentence "Samples ... light", would be better placed under section 3.4 because section 3.5 is on isolation media.

	locations) within a site should be placed on different plates. Sporangia are formed more readily on unsealed plates (T. Giltrap, personal communication, 2014). The plates are incubated in daylight or in the dark (the dark favours chlamydospore production) between 18 and 25°C, and examined for *Phytophthora* growth after three to seven days. Samples plated onto media containing rifampicin should be incubated in the dark because rifampicin is inactivated by light. Growth should occur within ten days but morphological features can be seen after three days in some cases.	
108	The plant material to be tested should be decontaminated as described in section 3.4.1, then at least four small pieces (about 2 cm^2) of tissue from each sample should be aseptically transferred onto either of the semi-selective media. As much of each section as practically possible should be slid under the media to force any *Phytophthora* present to grow through the media. A maximum of ten leaf sections should be placed on each plate. Leaf sections from different sites (e.g. nurseries) or different subsamples (hosts or locations) within a site should be placed on different plates. Sporangia are formed more readily on unsealed plates (T. Giltrap, personal communication, 2014). The plates are incubated in daylight or in the dark (the dark favours chlamydospore production) between 18 and 25°C, and examined for *Phytophthora* growth after three to seven days. Samples plated onto media containing rifampicin should be incubated in the dark because rifampicin is inactivated by light. Growth should occur within ten days but morphological features can be seen after three days in some cases.	*Category : TECHNICAL* **(135) New Zealand (30 Aug 2016 5:36 AM)** This section does not fit under “Isolation media” because it is covering how to plate out plant material for isolations. It would be more suitable to incorporate that text un section 3.4.1.
108	The plant material to be tested should be decontaminated as described in section 3.4.1, then at least four small pieces (about 2 cm^2) of tissue from each sample should be aseptically transferred onto either of the semi-selective media. As much of each section as practically possible should be slid under the media to force any *Phytophthora* present to grow through the media. A maximum of ten leaf sections should be placed on each plate. Leaf sections from different sites (e.g. nurseries) or different subsamples (hosts or locations) within a site should be placed on different plates. Sporangia are formed more readily on unsealed plates (T. Giltrap, personal communication, 2014). The plates are incubated in daylight or in the dark (the dark favours chlamydospore production) between 18 and 25°C, and examined for *Phytophthora* growth after three to seven days. Samples plated onto media containing rifampicin should be incubated in the dark because rifampicin is inactivated by light. Growth should occur within ten days but morphological	*Category : EDITORIAL* **(11) France (5 Aug 2016 5:27 PM)** This paragraph, except the sentence "Samples ... light" would be better placed Under paragraph 3.5 on isolation media.

	features can be seen after three days in some cases.	
110	It should be noted that *P. ramorum* isolation from woody tissue is difficult and can lead to false negative results. Therefore, for woody tissue, more than one method of detection is advisable. Isolation is as for soil or plant debris (section 3.4.3) – covering the woody material in Petri's mineral solution and using cut rhododendron leaves as bait, which are then plated or tested by molecular methods.	*Category : EDITORIAL* **(383) EPPO (29 Sep 2016 12:14 PM)** The first two sentences would be better placed under section 3, before section 3.1. The last sentence would be better placed at the end of section 3.4.3., but as it adds no additional information to section 3.4.3 it could just be deleted.
110	It should be noted that *P. ramorum* isolation from woody tissue is difficult and can lead to false negative results. Therefore, for woody tissue, more than one method of detection is advisable. Isolation is as for soil or plant debris (section 3.4.3) – covering the woody material in Petri's mineral solution and using cut rhododendron leaves as bait, which are then plated or tested by molecular methods.	*Category : EDITORIAL* **(219) European Union (28 Sep 2016 5:23 PM)** The first two sentences would be better placed under section 3, before section 3.1. The last sentence would be better placed at the end of section 3.4.3., but as it adds no additional information to section 3.4.3 it could just be deleted.
110	It should be noted that *P. ramorum* isolation from woody tissue is difficult and can lead to false negative results. Therefore, for woody tissue, more than one method of detection is advisable. Isolation is as for soil or plant debris (section 3.4.3) – covering the woody material in Petri's mineral solution and using cut rhododendron leaves as bait, which are then plated or tested by molecular methods.	*Category : TECHNICAL* **(136) New Zealand (30 Aug 2016 5:38 AM)** This section covers instructions how to isolate P. ramorum from woody material. Therefore, it is not appropriate to be included under section "Isolation media". Suggest to create a separate subsection for this information, i.e. "3.4.4. Isolation from woody tissue".
110	It should be noted that *P. ramorum* isolation from woody tissue is difficult and can lead to false negative results. Therefore, for woody tissue, more than one method of detection is advisable. Isolation is as for soil or plant debris (section 3.4.3) – covering the woody material in Petri's mineral solution and using cut rhododendron leaves as bait, which are then plated or tested by molecular methods.	*Category : EDITORIAL* **(12) France (5 Aug 2016 5:29 PM)** The first two sentences would be better placed under paragraph 3, before section 3.1. The last sentence would be better placed at the end of paragraph 3.4.3.
111	~~Molecular tests can be used directly on plant material. The methods for these tests are described in section 3.6.~~	*Category : EDITORIAL* **(137) New Zealand (30 Aug 2016 5:40 AM)** Remove this section. The text does not fit under section "Isolation media" and does not add any value since the information is given in the following paragraph.
112	**3.6 Detection by molecular ~~methods~~ methods** **Add the method of LAMP.**	*Category : SUBSTANTIVE* **(345) China (29 Sep 2016 11:41 AM)** Add the method of LAMP.LAMP is a new method and easy for field test.
113	Molecular tests have been developed to identify *P. ramorum* from culture or *in planta* using conventional or real-time PCR. Many of these methods were compared by Kox *et al.* (2007) and Martin *et al.* (2009). Four methods are	*Category : SUBSTANTIVE* **(384) EPPO (29 Sep 2016 12:14 PM)** BE same comment as France. The advantage of Schena et al. 2006 is that no cross reaction with Phytophthora

	described below and were selected because of the experience obtained by laboratories with them and the availability of validation data. However, other PCR methods can be used. PCR-based methods will detect non-viable *P. ramorum* in infected plant material, which would not be detected by isolation and culture (Bilodeau *et al.*, 2007). Real-time PCR may be preferred for high throughput, routine testing as the closed-tube format reduces the risk of carrying over contamination due to processing of amplification products (e.g. for nested PCR or gel electrophoresis).	lateralis is observed
113	Molecular tests have been developed to identify *P. ramorum* from culture or *in planta* using conventional or real-time PCR. Many of these methods were compared by Kox *et al*. (2007) and Martin *et al*. (2009). Four methods are described below and were selected because of the experience obtained by laboratories with them and the availability of validation data. However, other PCR methods can be used. PCR-based methods will detect non-viable *P. ramorum* in infected plant material, which would not be detected by isolation and culture ~~(Bilodeau~~ (Detection of mRNA by reverse-transcription PCR as an indicator of viability in Phytophthora ramorum~~*et al.*, 2007)~~). Real-time PCR may be preferred for high throughput, routine testing as the closed-tube format reduces the risk of carrying over contamination due to processing of amplification products (e.g. for nested PCR or gel electrophoresis).	*Category : SUBSTANTIVE* **(346) China (29 Sep 2016 11:42 AM)** Propose to adopt the newest reference(Detection of mRNA by reverse-transcription PCR as an indicator of viability in Phytophthora ramorum).
113	Molecular tests have been developed to identify *P. ramorum* from culture or *in planta* using conventional or real-time PCR. Many of these methods were compared by Kox *et al*. (2007) and Martin *et al.* (2009). Four methods are described below and were selected because of the experience obtained by laboratories with them and the availability of validation data. However, other PCR methods can be used. PCR-based methods will detect non-viable *P. ramorum* in infected plant material, which would not be detected by isolation and culture (Bilodeau *et al.*, 2007). Real-time PCR may be preferred for high throughput, routine testing as the closed-tube format reduces the risk of carrying over contamination due to processing of amplification products (e.g. for nested PCR or gel electrophoresis).	*Category : TECHNICAL* **(220) European Union (28 Sep 2016 5:24 PM)** Even if several PCR tests are aleady described in the protocol, the inclusion and full description of the Schena et al. (2006) PCR test would be very relevant as it provides complete validation data and does not show any cross-reaction (Schena, L., Hughes, K. J. D. & Cooke, D. E. L. 2006. Detection and quantification of Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology, 7(5): 365-379).
113	Molecular tests have been developed to identify *P. ramorum* from culture or *in planta* using conventional or real-time PCR. Many of these methods were compared by Kox *et al*. (2007) and Martin *et al.* (2009). Four methods are described below and were selected because of the experience obtained by laboratories with them and the availability of validation data. However, other PCR methods can be used. PCR-based methods will detect non-viable	*Category : TECHNICAL* **(13) France (5 Aug 2016 5:32 PM)** Even if several PCR tests are aleady described in the protocol, the inclusion and full description of the Schena et al. (2006) PCR test would be very relevant as it provides complete validation data and does not show any cross-reaction (Schena, L., Hughes, K. J. D. & Cooke, D. E. L. 2006. Detection and quantification of Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology, 7(5): 365-379).

	P. ramorum in infected plant material, which would not be detected by isolation and culture (Bilodeau *et al.,* 2007). Real-time PCR may be preferred for high throughput, routine testing as the closed-tube format reduces the risk of carrying over contamination due to processing of amplification products (e.g. for nested PCR or gel electrophoresis).	
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, ~~or the Homex grinder (Bioreba~~tissuelyser (Qiagen[1]) or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	*Category : TECHNICAL* **(471) Bolivia (30 Sep 2016 2:41 AM)** An other alternative
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, tissuelyser (Qiagen1) or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	*Category : TECHNICAL* **(458) Peru (29 Sep 2016 7:59 PM)** An other alternative
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, tissuelyser (Qiagen) or the Homex grinder (Bioreba[7]) (for	*Category : TECHNICAL* **(445) Brazil (29 Sep 2016 4:17 PM)** An other alternative

	cultures and tough woody tissue).	
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, tissuelyser (Qiagen[1]), or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	*Category : TECHNICAL* **(315) Argentina (29 Sep 2016 2:04 AM)** Another alternative
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills~~,~~ ,tissuelyser (Qiagen[1]) or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	*Category : TECHNICAL* **(228) Chile (28 Sep 2016 5:32 PM)** an other alternative
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, ~~or the Homex grinder (Bioreba~~tissuelyser (Quiagen[1]) or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	*Category : TECHNICAL* **(173) Uruguay (15 Sep 2016 8:50 PM)** Another alternative
115	DNA for PCR can be extracted from plant material or from cultures using various commercially available kits and other methods. When testing symptomatic plant material it may be beneficial to sample from the leading edge of the lesion. Depending on the sample matrix (leaves or stems or soil), different methods may be used for homogenization or disruption of the tissue. Plant tissue (from leaves) or	*Category : TECHNICAL* **(124) COSAVE (11 Aug 2016 10:52 PM)** An other alternative

	mycelium (from cultures) may be disrupted using a tissue pulverizer or bead beater. Pre-freezing in liquid nitrogen can be beneficial for disruption. Various grinding methods can be used, providing they produce a homogenously ground sample; for example, mortar and pestle with liquid nitrogen (for leaves and cut stems), bead mills, tissuelyser (Qiagen[1]) or the Homex grinder (Bioreba[7]) (for cultures and tough woody tissue).	
117	**3.6.2 DNA extraction** **Propose to add the difference methods of DNA extraction, e.g soil DNA extraction and some DNA extraction(reference: New technologies to detect and monitor Phytophthora ramorum in plant, soil, and water samples).**	*Category : SUBSTANTIVE* **(347) China (29 Sep 2016 11:43 AM)** Propose to add the difference methods of DNA extraction, e.g soil DNA extraction and some DNA extraction(reference: New technologies to detect and monitor Phytophthora ramorum in plant, soil, and water samples).
118	DNA extraction can be performed using commercial kits (e.g. the NucleoSpin Plant II Extraction Kit (Macherey-Nagel[8]) or the DNeasy Plant Mini Kit (Qiagen[9]) following the manufacturers' instructions. For DNA extraction from cultured isolates, the same kits can be used. DNA should be stored at –20 °C until use. Refer to the source papers in the following sections for the extraction methods originally used; however, laboratories may find that alternative extraction techniques work equally well. 3.6.2.1 Controls for DNA Extraction Positive extraction control. This control is used to ensure that target nucleic acid extracted is of sufficient quantity and quality for PCR and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target can be used if suitable infected material is not available. It is recommended that plant samples be tested at a minimum of two concentrations – out of undiluted, 1:5 and 1:10 – to overcome effects caused by inhibitors, which can occur with many of the host plant species that infect P. ramorum. Negative extraction control. This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positives are expected. Alternatively, extraction blanks can be processed with the samples to be tested if sufficient uninfected host tissue is not available. This will allow contamination of extraction reagents and/or cross-contamination between samples to be identified (but will not confirm the efficacy of nucleic acid extraction).	*Category : EDITORIAL* **(330) Philippines (29 Sep 2016 9:00 AM)** extracted from 3.6.5.1
118	DNA extraction can be performed using commercial kits (e.g. the NucleoSpin	*Category : EDITORIAL*

	Plant II Extraction Kit (Macherey-Nagel[8]) or the DNeasy Plant Mini Kit (Qiagen[9]) following the manufacturers' instructions. For DNA extraction from cultured isolates, the same kits can be used. DNA should be stored at –20 °C until use. Refer to the source papers in the following sections for the extraction methods originally used; however, laboratories may find that alternative extraction techniques work equally well.	**(308) Ghana (28 Sep 2016 6:40 PM)** These should be described in more details and or references cited.
122	There are several conventional PCR methods described in the literature. Two of these are described below. Propose to add the description of efficiency of two methods. It's for benificial to use.	*Category : EDITORIAL* **(348) China (29 Sep 2016 11:43 AM)** Propose to add the description of efficiency of two methods. It's for benificial to use.
122	There are several *P. ramorum* specific conventional PCR methods described in the literature. Two of these are described below.	*Category : EDITORIAL* **(138) New Zealand (30 Aug 2016 5:42 AM)** It should be specified that these sections talk about species specific PCR assays.
123	**3.6.3.1 Conventional PCR of Kox *et al.* ~~(2002)~~(2002) targeting P. ramorum**	*Category : EDITORIAL* **(139) New Zealand (30 Aug 2016 5:45 AM)** It should be specified that this is a species specific test, following the format used for qPCR assays in section s3.6.4.1 and 3.6.4.2
127	**Table 1.** Master mix composition, cycling parameters and amplicons for conventional PCR with primers Phyto 1 and Phyto 4	*Category : TECHNICAL* **(385) EPPO (29 Sep 2016 12:14 PM)** the tables should be consistent regarding the information provided. The concentration is not mentioned in this table but is mentioned in Table 3.
127	**Table 1.** Master mix composition, cycling parameters and amplicons for conventional PCR with primers Phyto 1 and Phyto 4	*Category : TECHNICAL* **(221) European Union (28 Sep 2016 5:26 PM)** The tables should be consistent in the information provided. The concentration is not mentioned in this table but is mentioned in Table 3. It should be added. (EPPO will provide the corrections to the IPPC Secretariat)
130	~~Master mix for 25 µl final reaction volume (µl)~~	*Category : TECHNICAL* **(258) Chile (28 Sep 2016 5:53 PM)** Depends on the initial concentration. Suggest only show the final concentration and the final reaction volume (25uL
133	~~17.70~~	*Category : TECHNICAL* **(260) Chile (28 Sep 2016 5:54 PM)** see comments p.130
136	~~2.50~~	*Category : TECHNICAL* **(261) Chile (28 Sep 2016 5:55 PM)** see comments p.130
139	~~0.75~~	*Category : TECHNICAL* **(262) Chile (28 Sep 2016 5:55 PM)** see comments p.130
142	~~0.50~~	*Category : TECHNICAL* **(264) Chile (28 Sep 2016 5:55 PM)** see comments p.130
145	~~0.50~~	*Category : TECHNICAL* **(265) Chile (28 Sep 2016 5:56 PM)** see comments p.130

148	~~0.50~~	*Category : TECHNICAL* **(266) Chile (28 Sep 2016 5:56 PM)** see comments p.130
151	~~0.05~~	*Category : TECHNICAL* **(267) Chile (28 Sep 2016 5:57 PM)** see comments p.130
153	5 µ~~l~~l Concentration in ng?	*Category : TECHNICAL* **(472) Bolivia (30 Sep 2016 4:19 AM)** To check concentration in ng
153	5 ~~µ~~µl Concentration in ng?~~l~~	*Category : TECHNICAL* **(459) Peru (29 Sep 2016 8:02 PM)** To check concentration in ng
153	5 µ~~l~~l concentration in ng?	*Category : TECHNICAL* **(446) Brazil (29 Sep 2016 4:18 PM)** Confirm the concentration
153	5 µ~~l~~l Concentration in ng?	*Category : TECHNICAL* **(316) Argentina (29 Sep 2016 2:05 AM)** To check concentration in ng
153	5 µ~~l~~l ¿Concentration in ng?	*Category : TECHNICAL* **(229) Chile (28 Sep 2016 5:33 PM)** to check concentration in ng
153	5 µ~~l~~l (Concentration in ng??)	*Category : TECHNICAL* **(174) Uruguay (15 Sep 2016 8:52 PM)** Concentration in ng should be added. This should be revised
153	5 µ~~l~~l Concentration in ng?	*Category : TECHNICAL* **(125) COSAVE (11 Aug 2016 10:56 PM)** To check concentration in ng
154	~~2.50~~	*Category : TECHNICAL* **(268) Chile (28 Sep 2016 5:57 PM)** see comments p.130
158	Number of ~~cycles~~cycles [Not clear need clarification]	*Category : TECHNICAL* **(438) Kenya (29 Sep 2016 1:31 PM)**
159	~~35~~35 [Not clear need clarification]	*Category : TECHNICAL* **(439) Kenya (29 Sep 2016 1:32 PM)**
161	94 °C for 15 s	*Category : TECHNICAL* **(386) EPPO (29 Sep 2016 12:14 PM)** The duration of denaturation is rather short. It would be useful to add a paragraph dealing with the kit used by the authors (fast cycling kits or classical cycling kit?)
161	94 °C for 15 s	*Category : TECHNICAL* **(222) European Union (28 Sep 2016 5:28 PM)** The duration of denaturation is rather short. It would be useful to add a paragraph dealing with the kit used by the authors (fast cycling kits or classical cycling kit?).
166	~~Repeat 35~~	*Category : TECHNICAL* **(140) New Zealand (30 Aug 2016 5:48 AM)** Remove that line as the number of cycles is already given on section 159.
169	~~4 °C pause~~	*Category : TECHNICAL*

		(141) New Zealand (30 Aug 2016 5:50 AM) Remove that line. The temperature for final hold depends on laboratory practices and is not essential to be specified. Since the other protocol does not provide that value, it is recommended to remove it for this assay as well.
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(473) Bolivia (30 Sep 2016 4:22 AM)**
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(460) Peru (29 Sep 2016 8:04 PM)**
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(447) Brazil (29 Sep 2016 4:19 PM)**
172	687 base ~~pairs~~pairs (bp)	*Category : TECHNICAL* **(317) Argentina (29 Sep 2016 2:07 AM)**
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(231) Chile (28 Sep 2016 5:34 PM)**
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(175) Uruguay (15 Sep 2016 8:54 PM)** Editorial
172	687 base ~~pairs~~pairs (bp)	*Category : EDITORIAL* **(126) COSAVE (11 Aug 2016 10:57 PM)**
173	**3.6.3.2 Conventional PCR of Ioos *et al.* ~~(2006)~~(2006) targeting P. ramorum**	*Category : EDITORIAL* **(142) New Zealand (30 Aug 2016 5:51 AM)** As for section 123.
174	This PCR is based on the amplification of DNA from intronic regions using two pairs of specific primers: GPA-PRAM-F (forward) and GPA-PRAM-R (reverse) from intron GPA1, and TRP-PRAM-F (forward) and TRP-PRAM-R (reverse) from intron TRP1. The primers TRP-PRAM-F/R can be used for detection and GPAPRAM-F/R for confirmation, and have been fully validated and characterized (Ioos *et al.*, 2006). They are listed below, and the details for the PCR are in Table 2.	*Category : EDITORIAL* **(143) New Zealand (30 Aug 2016 5:52 AM)** The primer names TRP-PRAM-F/R and GPAPRAM-F/R appear in a different font style.
179	**Table 2.** Master mix composition, cycling parameters and amplicons for conventional PCR with primers GPA-PRAM-F/GPA-PRAM-R and TRP-PRAM-F/TRP-PRAM-R	*Category : TECHNICAL* **(387) EPPO (29 Sep 2016 12:14 PM)** The tables should be consistent regarding the information provided. The concentration is not mentioned in this table but is mentioned in Table 3.
179	**Table 2.** Master mix composition, cycling parameters and amplicons for conventional PCR with primers GPA-PRAM-F/GPA-PRAM-R and TRP-PRAM-F/TRP-PRAM-R	*Category : TECHNICAL* **(224) European Union (28 Sep 2016 5:29 PM)** The tables should be consistent in the information provided. The concentration is not mentioned in this table but is mentioned in Table 3. It should be added. (EPPO will provide the corrections to the IPPC Secretariat)
182	**Master mix for ~~25~~ 20 µl final reaction volume (µl)**	*Category : TECHNICAL*

		(388) EPPO (29 Sep 2016 12:14 PM) The original paper mentions a final volume of 20 µL. The volumes included in the table should be corrected accordingly.
182	~~**Master mix for 25 µl final reaction volume (µl)**~~	*Category : TECHNICAL* **(269) Chile (28 Sep 2016 5:58 PM)** see comments p.130
182	**Master mix for ~~25~~ 20 µl final reaction volume (µl)**	*Category : TECHNICAL* **(226) European Union (28 Sep 2016 5:30 PM)** The original paper mentions a final volume of 20 µL. The volumes included in the table should be corrected accordingly (EPPO will provide the corrections to the IPPC Secretariat).
182	**Master mix for ~~25~~ 20 µl final reaction volume (µl)**	*Category : TECHNICAL* **(14) France (5 Aug 2016 5:33 PM)** The original paper mentions a final volume of 20 µL. The volumes included in the table should be corrected accordingly.
185	~~13.20~~	*Category : TECHNICAL* **(270) Chile (28 Sep 2016 5:58 PM)** see comments p.130
188	~~2.50~~	*Category : TECHNICAL* **(271) Chile (28 Sep 2016 5:59 PM)** see comments p.130
191	~~1.00~~	*Category : TECHNICAL* **(273) Chile (28 Sep 2016 5:59 PM)** see comments p.130
194	~~0.30~~	*Category : TECHNICAL* **(274) Chile (28 Sep 2016 6:00 PM)** see comments p.130
197	~~2.25~~	*Category : TECHNICAL* **(276) Chile (28 Sep 2016 6:02 PM)** see comments p.130
197	~~2~~0.~~25~~5	*Category : TECHNICAL* **(188) Japan (27 Sep 2016 8:05 AM)** It may be misprint.
200	~~2.25~~	*Category : TECHNICAL* **(278) Chile (28 Sep 2016 6:02 PM)** see comments p.130
203	~~0.50~~	*Category : TECHNICAL* **(279) Chile (28 Sep 2016 6:02 PM)** see comments p.130
203	~~0~~2.~~50~~25	*Category : TECHNICAL* **(189) Japan (27 Sep 2016 8:05 AM)** It may be misprint. The volumes and final concentrations in primer sets for PCR are usually the same in forward and reverse.
206	~~1.00~~	*Category : TECHNICAL* **(280) Chile (28 Sep 2016 6:03 PM)** see comments p.130

209	~~2.00~~	*Category : TECHNICAL* **(282) Chile (28 Sep 2016 6:03 PM)** see comments p.130
229	There are several real-time PCR methods described in the literature. Two of these are described below. Propose to add the description of efficiency of two methods. It's for benificial to use.	*Category : SUBSTANTIVE* **(349) China (29 Sep 2016 11:44 AM)** Propose to add the description of efficiency of two methods. It's for benificial to use.
229	There are several *P. ramorum* specific real-time PCR methods described in the literature. Two of these are described below.	*Category : EDITORIAL* **(144) New Zealand (30 Aug 2016 5:58 AM)** As for section 123.
230	**3.6.4.1 Real-time PCR of Hughes *et al.* (2006) targeting *P. ramorum***	*Category : SUBSTANTIVE* **(389) EPPO (29 Sep 2016 12:14 PM)** With the test of Hughes et al., there is a risk of cross-reaction with P. lateralis (according to the authors). As P. ramorum and P. lateralis can be found on Chamaecyparis lawsoniana (see Brasier and Webber (2012). New Disease Reports 25, 26), it would be useful to add the real-time PCR developed by Schena et al. to the protocol (as a confirmation for the test of Hughes in case of positive results on plants susceptible to P. ramorum and P. lateralis) or as a screening method in all cases.
230	**3.6.4.1 Real-time PCR of Hughes *et al.* (2006) targeting *P. ramorum***	*Category : SUBSTANTIVE* **(227) European Union (28 Sep 2016 5:31 PM)** With the test of Hughes et al., there is a risk of cross-reaction with P. lateralis (according to the authors). As P. ramorum and P. lateralis can be found on Chamaecyparis lawsoniana (see Brasier and Webber (2012). New Disease Reports 25, 26), it would be useful to add the qPCR method developed by Schena et al. to the protocol (as a confirmation method for the test of Hughes in case of positive results on plants susceptible to P. ramorum and P. lateralis) or as a screening method in all cases.
231	The primers and probe described by Hughes *et al*. (2006) target the ITS-1 region of the nuclear ribosomal (nr) RNA gene. Primer and probe sets have been developed that target other genes such as for cytochrome oxidase subunit I (COXI) (Tooley *et al*., 2006), beta-tubulin and elicitin (Bilodeau *et al*., 2007) and *Ypt*1 (Schena *et al*., ~~2008)~~2006).	*Category : TECHNICAL* **(390) EPPO (29 Sep 2016 12:14 PM)** This section deals with real time PCR. But the reference Schena et al. (2008) refers to a conventional PCR which includes the primers cited but the probe. For an appropriate reference to Schena and a complete description of primers and not the probe, Schena et al. (2006) should replace Schena et al. (2008).
231	The primers and probe described by Hughes *et al*. (2006) target the ITS-1 region of the nuclear ribosomal (nr) RNA gene. Primer and probe sets have been developed that target other genes such as for cytochrome oxidase subunit I (COXI) (Tooley *et al*., 2006), beta-tubulin and elicitin (Bilodeau *et al*., 2007) and *Ypt*1 (Schena *et al*., ~~2008)~~2006).	*Category : TECHNICAL* **(230) European Union (28 Sep 2016 5:34 PM)** The reference Schena et al. (2008) refers to a conventional PCR which includes the primers cited but not the probe. For an appropriate reference to the real-time PCR targeting Ypt1 and a complete description of primers and probe, refer to Schena et al. (2006) and not Schena et al. (2008). Reference: Schena et al. 2006. Molecular Plant Pathology 7(5), 365-379
231	The primers and probe described by Hughes *et al*. (2006) target the ITS-1 region of the nuclear ribosomal (nr) RNA gene. Primer and probe sets have been developed that target other genes such as for cytochrome oxidase subunit I (COXI) (Tooley *et al*., 2006), beta-tubulin and elicitin (Bilodeau *et al*., 2007) and *Ypt*1 (Schena	*Category : TECHNICAL* **(15) France (5 Aug 2016 5:37 PM)** This section deals with real time PCR. But the reference Schena et al. (2008) refers to a conventional PCR which includes the primers cited but the probe. For an appropriate reference to Schena and a complete description of primers and probe, Schena et al.

	et al., ~~2008)~~2006).	(2006) should replace Schena et al. (2008).
232	Hughes *et al*. (2006) reported a limit of detection of 10 pg genomic DNA, and no cross-reactivity with 29 species of non-target *Phytophthora*, with the exception of *P. lateralis*, which was detected at or above concentrations of approximately 10 ng per 25 µl reaction. For a full list of species used for the assessment of specificity, see Hughes *et al*. (2006).	*Category : TECHNICAL* **(391) EPPO (29 Sep 2016 12:14 PM)** Another reason to include the test from Shena et al 2006 is that The LOD is 100 fg.
232	Hughes *et al*. (2006) reported a limit of detection of 10 pg genomic DNA, and no cross-reactivity with 29 species of non-target *Phytophthora*, with the exception of *P. lateralis*, which was detected at or above concentrations of approximately 10 ng per 25 µl reaction. For a full list of species used for the assessment of specificity, see Hughes *et al*. (2006).	*Category : TECHNICAL* **(232) European Union (28 Sep 2016 5:35 PM)** Another reason to include the test from Shena et al 2006 is that The LOD is 100 fg.
234	~~Pram 114 FC: 5′ TCA TGG CGA GCG CTG GA 3′~~Pram 114-Fc: 5′-TCATGGCGAGCGCTGGA-3′	*Category : EDITORIAL* **(145) New Zealand (30 Aug 2016 6:01 AM)** The original primer name has a small “c”. Also, remove spaces in the sequence to keep consistency in formatting.
235	~~Pram 190R: 5′ AGT ATA TTC AGT ATT TAG GAA TGG GTT TAA AAA GT-3′~~Pram 190R: 5′-AGTATATTCAGTATTTAGGAATGGGTT TAAAAAGT-3′	*Category : EDITORIAL* **(146) New Zealand (30 Aug 2016 6:02 AM)** Remove spaces in the sequence to keep consistency in formatting
236	~~Probe Pram 134 T: 6 FAM 5′ TTC GGG TCT GAG CTA GTA G 3′ TAMRA~~Pram Probe: 6-FAM 5′-TTCGGGTCTGAGCTAGTAG-3′ TAMRA	*Category : EDITORIAL* **(147) New Zealand (30 Aug 2016 6:05 AM)** The original primer name as per Hughes et al 2006, is “Pram Probe”. Also, remove spaces in the sequence to keep consistency in formatting.
240	~~**Master mix for 25 µl final reaction volume (µl)**~~	*Category : TECHNICAL* **(283) Chile (28 Sep 2016 6:04 PM)** see comments p.130
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(461) Peru (29 Sep 2016 8:06 PM)** Also write the initial concentrtion in master mix for conventional PCR (above) or no final volume (in µl) for none
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(448) Brazil (29 Sep 2016 4:22 PM)** Also write the initial concentrtion in master mix for conventional PCR (above) or no final volume (in µl) for none
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(318) Argentina (29 Sep 2016 2:08 AM)** Also write the initial concentrtion in master mix for conventional PCR (above) or no final volume (in µl) for none
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(233) Chile (28 Sep 2016 5:35 PM)** Also write the initial concentrtion in master mix for conventional PCR (above) or no final volume (in µl) for none
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(128) COSAVE (11 Aug 2016 11:01 PM)**

		Also write the initial concentration in master mix for conventional PCR (above) or no final volume (in µl) for none
247	$MgCl_2$ (50 mM)	*Category : TECHNICAL* **(176) Uruguay (15 Sep 2016 9:00 PM)** Also write the initial concentration in master mix for conventional PCR (above) or no final volume (in µl) for none
259	Probe Pram 134-T	*Category : TECHNICAL* **(392) EPPO (29 Sep 2016 12:14 PM)** The concentration of the solution is missing: 2 µM ?
259	Probe Pram 134-T	*Category : TECHNICAL* **(234) European Union (28 Sep 2016 5:37 PM)** The concentration of the solution is missing: 2 µM ?
262	DNA ~~polymerase~~polymerase (5U/µl)	*Category : TECHNICAL* **(393) EPPO (29 Sep 2016 12:14 PM)** concentration missing
262	DNA ~~polymerase~~polymerase (5 U/µl)	*Category : TECHNICAL* **(237) European Union (28 Sep 2016 5:38 PM)** concentration missing
263	5 U/µl	*Category : TECHNICAL* **(394) EPPO (29 Sep 2016 12:14 PM)** This concentration (5 U/µl) represents the concentration of the reagent (expressed in U/µl) and not the final concentration in the PCR (normally expressed in U). Based on the volume taken (0.2 µl), the final concentration in the PCR reaction should be 1U. This should be checked.
263	5 U/µl	*Category : TECHNICAL* **(239) European Union (28 Sep 2016 5:39 PM)** This concentration (5 U/µl) represents the concentration of the reagent (expressed in U/µl) and not the final concentration in the PCR (normally expressed in U). Based on the volume taken (0.2 µl), the final concentration in the PCR reaction should be 1U. This should be checked.
266	1 µl?(20–100 ng)	*Category : EDITORIAL* **(474) Bolivia (30 Sep 2016 4:38 AM)** To check
266	1 µl ?(20–100 ng)	*Category : EDITORIAL* **(462) Peru (29 Sep 2016 8:08 PM)** To check
266	1 µl? (20–100 ng)	*Category : EDITORIAL* **(449) Brazil (29 Sep 2016 4:23 PM)** To check.
266	1 µl? (20–100 ng)	*Category : TECHNICAL* **(319) Argentina (29 Sep 2016 2:09 AM)** To check
266	~~1~~ 1µl?((20–100 ng)	*Category : EDITORIAL* **(235) Chile (28 Sep 2016 5:37 PM)** to check
266	1 µl? (20–100 ng)	*Category : EDITORIAL* **(177) Uruguay (15 Sep 2016 9:01 PM)** To check
266	1 µl?(20–100 ng)	*Category : EDITORIAL*

		(127) COSAVE (11 Aug 2016 10:59 PM) To check
270	50 °C for 2 min	*Category : TECHNICAL* **(395) EPPO (29 Sep 2016 12:14 PM)** This step does not represent the initial denaturation but the activation of the enzyme UNG (Uracyl-N-Glycosylase) before the PCR in order to eliminate carry over PCR products
270	50 °C for 2 min	*Category : TECHNICAL* **(241) European Union (28 Sep 2016 5:40 PM)** This step does not represent the initial denaturation but the activation of the enzyme UNG (Uracyl-N-Glycosylase) before the PCR in order to eliminate carry over PCR products.
271	95 °C for 10 min	*Category : TECHNICAL* **(396) EPPO (29 Sep 2016 12:14 PM)** This step represents the initial denaturation
271	95 °C for 10 min	*Category : TECHNICAL* **(242) European Union (28 Sep 2016 5:40 PM)** This step represents the initial denaturation.
272	Number of cycles	*Category : EDITORIAL* **(310) Ghana (28 Sep 2016 6:48 PM)** Paragraph 158-159 thus under 'Cycling parameters' and NUMBER OF CYCLES for the PCR cycling parameters is confusing. Also see Paragraph 166. Same for Paragraph 213-219, 435-455 i.e. NUMBER OF CYCLES FOR DENATURATION; ANNEALING AND ELONGATION STEPS, these should be placed appropriately for they are confusing.
278	Elongation	*Category : TECHNICAL* **(397) EPPO (29 Sep 2016 12:14 PM)** It should be more correct to put annealing and elongation on the same line as both steps are carried out at 60°C
278	Elongation	*Category : TECHNICAL* **(244) European Union (28 Sep 2016 5:41 PM)** It should be more correct to put annealing and elongation on the same line as both steps are carried out at 60°C.
280	~~Final elongation~~	*Category : TECHNICAL* **(398) EPPO (29 Sep 2016 12:14 PM)** delete as there is none
280	~~Final elongation~~	*Category : TECHNICAL* **(245) European Union (28 Sep 2016 5:42 PM)** Delete as there is none.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]),	*Category : EDITORIAL* **(475) Bolivia (30 Sep 2016 4:39 AM)** Suggestion: uniform format like a Table

	using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units)~~.~~.	
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : EDITORIAL* **(463) Peru (29 Sep 2016 8:09 PM)** Suggestion: uniform format like a Table.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : EDITORIAL* **(450) Brazil (29 Sep 2016 4:23 PM)** Suggestion: uniform format like a Table.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : TECHNICAL* **(399) EPPO (29 Sep 2016 12:14 PM)** Why are the concentrations of primers and probes different compared to Table 3? It should be standardized. Part of the information included in this paragraph is already present in table 3. The table 3 should be completed where appropriate (master mix provider for example) and the duplicated information deleted from the paragraph 285.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR	*Category : EDITORIAL* **(320) Argentina (29 Sep 2016 2:11 AM)** Suggestion: uniform format like a Table.

	Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : TECHNICAL* **(247) European Union (28 Sep 2016 5:43 PM)** Why are the concentrations of primers and probes different compared to Table 3? It should be standardized. Part of the information included in this paragraph is already present in table 3. The table 3 should be completed where appropriate (master mix provider for example) and the duplicated information deleted from the paragraph 285.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : EDITORIAL* **(236) Chile (28 Sep 2016 5:37 PM)** Suggestion: uniform format like a Table.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System	*Category : EDITORIAL* **(178) Uruguay (15 Sep 2016 9:02 PM)** We suggest to present this paragraph as a Table

	(Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : EDITORIAL* **(129) COSAVE (11 Aug 2016 11:03 PM)** Suggestion: uniform format like a Table.
285	Real-time PCR carried out by Hughes *et al.* (2006) used TaqMan Universal PCR Master Mix (Applied Biosytems[10]). The Real-time PCR was carried out in 96 well plates in 25 µl reactions containing 1 x Master Mix, 300 nM each primer, 100 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems[11]), using real-time data collection. The cycle threshold (Ct) value was assessed using a default threshold setting of 0.2 ΔRn (fluorescence units).	*Category : EDITORIAL* **(16) France (5 Aug 2016 5:40 PM)** Part of the information included in this paragraph is already present in table 3. The table 3 should be completed where appropriate (master mix provider for example) and the duplicated information deleted from the paragraph 285.
288	Under the Hughes *et al*. (2006) conditions, samples with Ct values less than 36 may be considered positive for *P. ramorum*. Ct values between 36 and 40 may be a result of aerosol contamination or cross-reaction with non-target DNA at high concentrations (e.g. *P. foliorum* or *P. lateralis*, see section 4). Samples giving these results should be re-sampled or re-tested and if the result is still in doubt, the presence of *P. ramorum* confirmed by another means. Samples with Ct values of 40 or more are considered negative.	*Category : TECHNICAL* **(400) EPPO (29 Sep 2016 12:14 PM)** The test developed by Schena et al. 2006 could be used as a confirmation method.
288	Under the Hughes *et al*. (2006) conditions, samples with Ct values less than 36 may be considered positive for *P. ramorum*. Ct values between 36 and 40 may be a result of aerosol contamination or cross-reaction with non-target DNA at high concentrations (e.g. *P. foliorum* or *P. lateralis*, see section 4). Samples giving these results should be re-sampled or re-tested and if the result is still	*Category : TECHNICAL* **(248) European Union (28 Sep 2016 5:44 PM)** The test developed by Schena et al. 2006 could be used as a confirmation method.

	in doubt, the presence of *P. ramorum* confirmed by another means. Samples with Ct values of 40 or more are considered negative.	
288	Under the Hughes *et al*. (2006) conditions, samples with Ct values less than 36 may be considered positive for *P. ramorum*. Ct values between 36 and 40 may be a result of aerosol contamination or cross-reaction with non-target DNA at high concentrations (e.g. *P. foliorum* or *P. lateralis*, see section 4). Samples giving these results should be re-sampled or re-tested and if the result is still in doubt, the presence of *P. ramorum* confirmed by ~~another~~ other means. Samples with Ct values of 40 or more are considered negative.	*Category : EDITORIAL* **(148) New Zealand (30 Aug 2016 6:07 AM)** Typo
289	**3.6.4.2 Real-time PCR of Hughes *et al.* (2006) targeting *P. ramorum* and host**	*Category : TECHNICAL* **(401) EPPO (29 Sep 2016 12:14 PM)** I would suggest to combine section 3.6.4.1 and 3.6.4.2. into one section with both tests as they have to be carried out together. The test described in section 3.6.4.2. does not represent an additional separate real-time PCR for the detection of P. ramorum. It is only an internal positive control for the test of Hughes et al. 2006.
289	**3.6.4.2 Real-time PCR of Hughes *et al.* (2006) targeting *P. ramorum* and host**	*Category : TECHNICAL* **(249) European Union (28 Sep 2016 5:45 PM)** We would suggest to combine section 3.6.4.1 and 3.6.4.2. into one section with both tests as they have to be carried out together. The test described in section 3.6.4.2. does not represent an additional separate real-time PCR for the detection of P. ramorum. It is only an internal positive control for the test of Hughes et al. 2006.
289	**3.6.4.2 Real-time PCR of Hughes *et al.* (2006) targeting *P. ramorum* and host**	*Category : SUBSTANTIVE* **(149) New Zealand (30 Aug 2016 6:10 AM)** This section presents a duplex protocol where internal control is added to the assay described in 3.6.4.1. Since this does not represent a different test to detect P. ramorum, I would suggest to include it as a subsection 3.6.4.1.1. If this change is incorporated, please ensure that the text in 229 is changed to reflect that only one pathogen specific real-time PCR test is given here.
291	~~COX F~~COX F: ~~5′-CGTCGCATTCCAGATTATCCA-3′~~5′-CGTCGCATTCCAGATTATCCA-3″	*Category : EDITORIAL* **(150) New Zealand (30 Aug 2016 6:11 AM)** Adjusted the primer name to match the original description.
292	~~COX RW: 5′-CAACTACGGATATATAAGRRCCRRAACTG-3′~~COX RW: 5′-CAACTACGGATATATAAGRRCCRRAACTG-3′	*Category : EDITORIAL* **(151) New Zealand (30 Aug 2016 6:13 AM)** As for section 291.
293	~~Probe COX P: VIC 5′-AGGGCATTCCATCCAGCGTAAGCA-3′ TAMRA~~COX probe: VIC 5′-AGGGCATTCCATCCAGCGTAAGCA-3′ TAMRA	*Category : EDITORIAL* **(152) New Zealand (30 Aug 2016 6:14 AM)** As for section 291.
294	**Table 4.** Master mix composition, cycling parameters and amplicons for real-time PCR with primers Pram 114-FC/Pram 190R and probe Pram 134-T and primers COX-F/COX-RW and probe COX-P	*Category : TECHNICAL* **(442) Kenya (29 Sep 2016 2:21 PM)** The Diagnostic methods described in his protocol are sophisticated, compared to the level of equipment s of Laboratories in African Countries. Studies should be continued to come up with quick diagnostic kits ready to be used by the inspection services at the border points.
297	~~Master mix for 25 µl final reaction volume (µl)~~	*Category : TECHNICAL* **(285) Chile (28 Sep 2016 6:04 PM)**

		see comments p.130
325	Platinum Taq (5 U/µl) (Invitrogen[12])	*Category : TECHNICAL* **(402) EPPO (29 Sep 2016 12:14 PM)** Why is the type of Taq described here and not in the previous test (section 3.6.4.1) if both tests have to be done together. The commercial kit used should not be described in these "general tables" to enable people to use their own kits if they have correct validation data
325	Platinum Taq (5 U/µl) (Invitrogen[12])	*Category : TECHNICAL* **(251) European Union (28 Sep 2016 5:47 PM)** Why is the type of Taq described here and not in the previous protocol (section 3.6.4.1) if both tests have to be done together. The commercial kit used should not be described in these "general tables" to enable people to use their own kits if they have correct validation data.
330	1 µl?(20–100 ng)	*Category : EDITORIAL* **(476) Bolivia (30 Sep 2016 4:40 AM)** To check
330	1 µl? (20–100 ng)	*Category : EDITORIAL* **(464) Peru (29 Sep 2016 8:10 PM)**
330	1 µl? (20–100 ng)	*Category : EDITORIAL* **(451) Brazil (29 Sep 2016 4:24 PM)** To check
330	1 µl? (20–100 ng)	*Category : TECHNICAL* **(321) Argentina (29 Sep 2016 2:11 AM)** To check
330	1 µl?(20–100 ng)	*Category : EDITORIAL* **(238) Chile (28 Sep 2016 5:38 PM)** to check
330	~~1~~ 1µl?? (20–100 ng)	*Category : EDITORIAL* **(179) Uruguay (15 Sep 2016 9:03 PM)** To check
330	1 µl? (20–100 ng)	*Category : EDITORIAL* **(130) COSAVE (11 Aug 2016 11:04 PM)** to check
332	**Cycling parameters (Cepheid SmartCycler[13] II settings)**	*Category : TECHNICAL* **(403) EPPO (29 Sep 2016 12:14 PM)** If the test is intended to be used in combination with the primers described in section 3.6.4.1, it would be useful to have the same cycling conditions
332	**Cycling parameters (Cepheid SmartCycler[13] II settings)**	*Category : TECHNICAL* **(252) European Union (28 Sep 2016 5:47 PM)** If the test is intended to be used in combination with the primers described in section 3.6.4.1, it would be useful to have the same cycling conditions.
337	Optics OFF	*Category : TECHNICAL* **(404) EPPO (29 Sep 2016 12:14 PM)** This information is probably linked to the thermocycler used. What happens if people don't use the same thermocycler ? The thermal protocol should be standardized to be used by any operator working with real-time PCR technology
337	Optics OFF	*Category : TECHNICAL* **(253) European Union (28 Sep 2016 5:48 PM)**

		This information is probably linked to the thermocycler used. What happens if people don't use the same thermocycler ? The themal protocol should be standardized to be used by any operator working with qPCR technology.
342	Optics OFF	*Category : TECHNICAL* **(405) EPPO (29 Sep 2016 12:14 PM)** This information is probably linked to the thermocycler used. What happens if people don't use the same thermocycler ? The themal protocol should be standardized to be used by any operator working with real-time PCR technology
342	Optics OFF	*Category : TECHNICAL* **(254) European Union (28 Sep 2016 5:49 PM)** This information is probably linked to the thermocycler used. What happens if people don't use the same thermocycler ? The themal protocol should be standardized to be used by any operator working with qPCR technology.
345	Elongation	*Category : TECHNICAL* **(406) EPPO (29 Sep 2016 12:14 PM)** Annealing and elongation should be placed on the same line as both steps are carried out at the same temperature.
345	Elongation	*Category : TECHNICAL* **(255) European Union (28 Sep 2016 5:50 PM)** Annealing and elongation should be placed on the same line as both steps are carried out at the same temperature.
348	~~Final elongation~~	*Category : TECHNICAL* **(407) EPPO (29 Sep 2016 12:14 PM)** delete as there is none
348	~~Final elongation~~	*Category : TECHNICAL* **(256) European Union (28 Sep 2016 5:50 PM)** Delete as there is none.
354	**3.6.5 Controls for molecular tests**	*Category : SUBSTANTIVE* **(408) EPPO (29 Sep 2016 12:14 PM)** Before this section is is suggested to add the text from Shena et al 2006 A text is suggested below 3.6.5 Controls for molecular tests4.3. Real-time PCR of Schena et al. (2006) targeting P. ramorum Schena et al. (2006) developed a multiplex real-time PCR based on the ras-related protein gene (Ypt1) to detect Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in infected plant material. Regarding P. ramorum, the authors report a limit of detection of 100 fg per PCR reaction (in singleplex) and there is no cross reaction with Phytophthora lateralis, The primers and probe are listed below, and the details for the PCR are in Table 5. Yram4F: 5'- TTTGTCAGTGACCTCTCTCTCTCTC-3' Yram3R: 5'-GCATAAGTATAAGTCAGCAAGCCTGT-3' YramP: 6-FAM 5'-AGAACACGATCCCCTCGTCAGCAGTC-3' BHQ Table 5. Master mix composition, cycling parameters and amplicons for real-time PCR with primers Yram4F, Yram3R and probe YramP Reagent Final concentration Master mix for 25 µl final reaction volume (µl) PCR-grade water – 13.45 10× PCR buffer 1× 2.50

		MgCl2 (50 mM) 5.0 mM 2.50 dNTPs (10 mM each) 200 µM 0.50 Primer Yram4F (5 µM) 330 nM 1.65 Primer Yram3R (5 µM) 330 nM 1.65 Probe YramP (5 µM) 130 nM 0.65 DNA polymerase (5 U/µl) 0.5 U 0.10 DNA (quantity/volume) 1 (10-100 ng) 2.00 [Cycling parameters UNG activation Initial denaturation 50 °C for 2 min 95 °C for 10 min Number of cycles 40 Denaturation 95 °C for 20 s Annealing/Elongation 62.5 °C for 20 s Real-time PCR performed by Schena et al. (2006) used qPCR Core kit . The Real-time PCR was carried out in 15 µl reactions containing 1 x Master Mix, 330 nM each primer, 130 nM probe and 1µl sample or control DNA. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 20 s at 95 °C and 20 s at 62.5 °C). Amplifications were performed using a Chromo 4TM Detector and data acquisition and analysis realized using the supplied Opticon MonitorTM software version 2.03 (MJ Research). A cycle cut off set at 36 (corresponding to the detection of 100 fg of target DNA) was obtained with the equipment, material and chemistry described by Schena et al. (2006). The cycle cut off needs to be verified in each lab when implementing the test for the first time
354	**3.6.5 Controls for molecular tests**	*Category : SUBSTANTIVE* **(257) European Union (28 Sep 2016 5:52 PM)** Before this section it is suggested to add the text from Shena et al 2006. The text is suggested below: 3.6.5 Controls for molecular tests4.3. Real-time PCR of Schena et al. (2006) targeting P. ramorum Schena et al. (2006) developed a multiplex real-time PCR based on the ras-related protein gene (Ypt1) to detect Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in infected plant material. Regarding P. ramorum, the authors report a limit of detection of 100 fg per PCR reaction (in singleplex) and there is no cross reaction with Phytophthora lateralis, The primers and probe are listed below, and the details for the PCR are in Table 5. Yram4F: 5'- TTTGTCAGTGACCTCTCTCTCTC-3' Yram3R: 5'-GCATAAGTATAAGTCAGCAAGCCTGT-3' YramP: 6-FAM 5'-AGAACACGATCCCCTCGTCAGCAGTC-3' BHQ Table 5. Master mix composition, cycling parameters and amplicons for real-time PCR with primers Yram4F, Yram3R and probe YramP Reagent Final concentration Master mix for 25 µl final reaction volume (µl) PCR-grade water – 13.45 10× PCR buffer 1× 2.50 MgCl2 (50 mM) 5.0 mM 2.50 dNTPs (10 mM each) 200 µM 0.50

		Primer Yram4F (5 µM) 330 nM 1.65 Primer Yram3R (5 µM) 330 nM 1.65 Probe YramP (5 µM) 130 nM 0.65 DNA polymerase (5 U/µl) 0.5 U 0.10 DNA (quantity/volume) 1 (10-100 ng) 2.00 [Cycling parameters UNG activation 50 °C for 2 min Initial denaturation 95 °C for 10 min Number of cycles 40 Denaturation 95 °C for 20 s Annealing/Elongation 62.5 °C for 20 s Real-time PCR performed by Schena et al. (2006) used qPCR Core kit . The Real-time PCR was carried out in 15 µl reactions. Reactions were subjected to generic thermal cycling conditions (10 min at 95 °C and 40 cycles of 20 s at 95 °C and 20 s at 62.5 °C). Amplifications were performed using a Chromo 4TM Detector and data acquisition and analysis realized using the supplied Opticon MonitorTM software version 2.03 (MJ Research). A cycle cut off set at 36 (corresponding to the detection of 100 fg of target DNA) was obtained with the equipment, material and chemistry described by Schena et al. (2006). The cycle cut off needs to be verified in each lab when implementing the test for the first time.
355	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. The use of an internal control assay for the detection of host plant DNA, to be used in multiplex with the pathogen-specific assay or in parallel singleplex reactions, or in parallel tests for conventional and real-time PCR, can assist in the interpretation of P. ~~ramorum-negative~~ *ramorum*-negative results. The use of an internal control plant is highly recommended to confirm the quality of the extracted DNA, especially for the test described in section 3.6.4.2.	*Category : EDITORIAL* **(436) Kenya (29 Sep 2016 1:09 PM)**
355	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. The use of an internal control assay for the detection of host plant DNA, to be used in multiplex with the pathogen-specific assay or in parallel singleplex reactions, or in parallel tests for conventional and real-time PCR, can assist in the interpretation of P. ramorum-negative results. ~~The use of an internal control plant is highly recommended to confirm the quality of the extracted DNA, especially for~~	*Category : TECHNICAL* **(409) EPPO (29 Sep 2016 12:14 PM)** The internal control should not be recommended all the time. It should be considered as an additional control.

	~~the test described in section 3.6.4.2~~.	
355	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. The use of an internal control assay for the detection of host plant DNA, to be used in multiplex with the pathogen-specific assay or in parallel singleplex reactions, or in parallel tests for conventional and real-time PCR, can assist in the interpretation of P. ramorum-negative results. ~~The use of an internal control plant is highly recommended to confirm the quality of the extracted DNA, especially for the test described in section 3.6.4.2.~~	*Category : TECHNICAL* **(259) European Union (28 Sep 2016 5:54 PM)** The internal control should not be recommended all the time. It should be considered as an additional control.
355	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. The use of an internal control assay for the detection of host plant DNA, to be used in multiplex with the pathogen-specific assay or in parallel singleplex reactions, or in parallel tests for conventional and real-time PCR, can assist in the interpretation of ~~P. ramorum-negative~~ *P. ramorum*-negative results. The use of ~~an~~ a plant internal control ~~plant~~ is highly recommended to confirm the quality of the extracted DNA, especially for the test described in section 3.6.4.2.	*Category : EDITORIAL* **(153) New Zealand (31 Aug 2016 6:15 AM)** typos
356	*Positive nucleic acid control.* This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product) may be used. A good positive control for *P. ramorum* is DNA extracted from a host plant (e.g. *Rhododendron*) infected with *P. ramorum*. ~~with 20.00 ≤ FAM Ct ≤ 23 and 20.00 ≤ TxR Ct (COX) ≤ 25. These are suggested Ct values (Z.G. Abad, personal communication, 2015).~~	*Category : TECHNICAL* **(410) EPPO (29 Sep 2016 12:14 PM)** Suggestion to remove reference to Ct values as it will depend on a lot of parameters (real-time PCR testused, equipment, kits…). Moreover, it is better to use positive PCR control near the LOD.
356	*Positive nucleic acid control.* This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product) may be used. A good positive control for *P. ramorum* is DNA extracted from a host plant (e.g. *Rhododendron*) infected with *P. ramorum* ~~with .20.00 ≤ FAM Ct ≤ 23 and 20.00 ≤ TxR Ct (COX) ≤ 25. These are suggested Ct values (Z.G. Abad, personal communication, 2015).~~	*Category : TECHNICAL* **(263) European Union (28 Sep 2016 5:55 PM)** Suggestion to remove reference to Ct values as it will depend on a lot of parameters (qPCR method used, equipment, kits…). Moreover, it is better to use positive PCR control near the LOD.
358	*Internal control.* ~~For testing plant samples by real-time PCR~~To eliminate the	*Category : SUBSTANTIVE* **(411) EPPO (29 Sep 2016 12:14 PM)**

	possibility of PCR false negatives due to DNA extraction failure, nucleic acid degradation or the presence of PCR inhibitors, primers and probe targeting plant internal controls DNA (e.g. COXI as used by Hughes et al. (2006)) can be incorporated into the protocol. ~~COXI as used by Hughes *et al.* (2006)) should be incorporated into the protocol to eliminate the possibility of PCR false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors.~~	1) The use of an internal control is not required provided that other controls (positive isolation control and inhibition control for instance) are used to evaluate the quality of the DNA. 2) The ultimate goal of the test is not to test plant samples but to evaluate the quality of the DNA (yield and purity). That's the reason why it is proposed to change the sentence. 3) As the only test described for the amplification of plant DNA is a real-time PCR in this protocol, it is important to mention that this test is not mandatory as some labs only use
358	*Internal control*. ~~For testing plant samples by real-time PCR~~To eliminate the possibility of PCR false negatives due to DNA extraction failure, nucleic acid degradation or the presence of PCR inhibitors, primers and probe targeting plant internal controls DNA (e.g. COXI as used by Hughes *et al*. (2006)) ~~should~~ can be incorporated into the ~~protocol to eliminate the possibility of PCR false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors~~protocol.	*Category : SUBSTANTIVE* **(272) European Union (28 Sep 2016 5:59 PM)** 1. The use of an internal control is not required provided that other controls (positive isolation control and inhibition control for instance) are used to evaluate the quality of the DNA. 2. The ultimate goal of the test is not to test plant samples but to evaluate the quality of the DNA (yield and purity). That's the reason why it is proposed to change the sentence. 3. As the only test described for the amplification of plant DNA is a qPCR in this protocol, it is important to mention that this test is not mandatory as some labs only use conventional PCR.
359	The internal control primers can be used in a multiplex reaction with the pathogen-specific primers or they can be used in parallel singleplex reactions. Performing the reactions in singleplex may help to avoid a reduction in the sensitivity of detection of *P. ramorum*. Laboratories may choose to establish a cut-off Ct value to be used to identify samples for which extraction or amplification has not failed but was suboptimal (which could lead to false negative results). The appropriate cut-off may need to be determined for each sample type (host, tissue, etc.). Samples with failed internal controls should be plated onto selective media to try to derive a culture for DNA extraction and subsequent PCR. A dilution (e.g. 1:10) of the DNA extract can also help to overcome a problem due to the presence of inhibitors.	*Category : TECHNICAL* **(412) EPPO (29 Sep 2016 12:14 PM)** It is suggested to include the possible use of dilution of DNA extract to overcome inhibitors effect.
359	The internal control primers can be used in a multiplex reaction with the pathogen-specific primers or they can be used in parallel singleplex reactions. Performing the reactions in singleplex may help to avoid a reduction in the sensitivity of detection of *P. ramorum*. Laboratories may choose to establish a cut-off Ct value to be used to identify samples for which extraction or amplification has not failed but was suboptimal (which could lead to false negative results). The appropriate cut-off may need to be determined for each sample type (host, tissue, etc.). Samples with failed internal controls should be plated onto selective media to try to derive a culture for DNA extraction and subsequent PCR. A dilution (e.g. 1:10) of the DNA	*Category : TECHNICAL* **(277) European Union (28 Sep 2016 6:02 PM)** It is suggested to include the possible use of dilution of DNA extract to overcome inhibitors effect.

	extract can also help to overcome a problem due to the presence of inhibitors.	
359	The internal control primers can be used in a multiplex reaction with the pathogen-specific primers or they can be used in parallel singleplex reactions. Performing the reactions in singleplex may help to avoid a reduction in the sensitivity of detection of *P. ramorum*. Laboratories may choose to establish a cut-off Ct value to be used to identify samples for which extraction or amplification has not failed but was suboptimal (which could lead to false negative results). The appropriate cut-off values may need to be determined for each sample type (host, tissue, etc.). Samples with failed internal controls should be plated onto selective media to try to derive a culture for DNA extraction and subsequent PCR.	*Category : EDITORIAL* **(154) New Zealand (31 Aug 2016 6:17 AM)** missing word
359	The internal control primers can be used in a multiplex reaction with the pathogen-specific primers or they can be used in parallel singleplex reactions. Performing the reactions in singleplex may help to avoid a reduction in the sensitivity of detection of *P. ramorum*. Laboratories may choose to establish a cut-off Ct value to be used to identify samples for which extraction or amplification has not failed but was suboptimal (which could lead to false negative results). The appropriate cut-off may need to be determined for each sample type (host, tissue, etc.). Samples with failed internal controls should be plated onto selective media to try to derive a culture for DNA extraction and subsequent PCR. A dilution (e.g. 1:10) of the DNA extract can also help to overcome a problem due to the presence of inhibitors.	*Category : TECHNICAL* **(17) France (5 Aug 2016 5:42 PM)** It is suggested to include the possible use of dilution of DNA extract to overcome inhibitors effect.
361	~~**3.6.5.1 Additional controls**~~	*Category : EDITORIAL* **(326) Philippines (29 Sep 2016 8:56 AM)** transffered to 3.6.2 because these are controls for DNA extraction
362	*Positive extraction control.* This control is used to ensure that target nucleic acid extracted is of sufficient quantity and quality for PCR and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target can be used if suitable infected material is not available. It is recommended that plant samples be tested at a minimum of two concentrations – out of undiluted, 1:5 and 1:10 – to overcome effects caused by inhibitors, which can occur with many of the host plant species that infect *P. ramorum*.	*Category : TECHNICAL* **(413) EPPO (29 Sep 2016 12:14 PM)** Normally, validation should be done to select the best DNA extraction method (to limit PCR inhibitors) for the different host plants. I would delete this sentence
362	~~*Positive extraction control.* This control is used to ensure that target nucleic acid extracted is of sufficient quantity and quality for PCR and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target can be used if suitable infected material is not available. It is recommended that plant samples be tested at a minimum of two concentrations – out of undiluted, 1:5 and 1:10 – to overcome effects caused by~~	*Category : EDITORIAL* **(327) Philippines (29 Sep 2016 8:57 AM)**

	~~inhibitors, which can occur with many of the host plant species that infect *P. ramorum*.~~	
362	*Positive extraction control.* This control is used to ensure that target nucleic acid extracted is of sufficient quantity and quality for PCR and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target can be used if suitable infected material is not available. It is recommended that plant samples be tested at a minimum of two concentrations – out of undiluted, 1:5 and 1:10 – to overcome effects caused by inhibitors, which can occur with many of the host plant species that infect *P. ramorum*.	*Category : TECHNICAL* **(281) European Union (28 Sep 2016 6:03 PM)** Normally, validation should be done to select the best DNA extraction method (to limit PCR inhibitors) for the different host plants. We suggest to delete this sentence.
363	*Negative extraction control.* This control is used to monitor contamination during nucleic acid ~~extraction and/or cross-reaction with the host tissue~~extraction. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positives are expected.	*Category : TECHNICAL* **(414) EPPO (29 Sep 2016 12:14 PM)** Again, if the primer are correctly designed and if a specificity test has been done correctly, the risk of cross-reaction with the host tissue should not exist. I would delete this part of the sentence.
363	~~*Negative extraction control.* This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positives are expected.~~	*Category : EDITORIAL* **(328) Philippines (29 Sep 2016 8:57 AM)**
363	*Negative extraction control.* This control is used to monitor contamination during nucleic acid extraction ~~and/or cross-reaction with the host tissue~~. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positives are expected.	*Category : TECHNICAL* **(287) European Union (28 Sep 2016 6:06 PM)** Again, if the primer are correctly designed and if a specificity test has been done correctly, the risk of cross-reaction with the host tissue should not exist. I would delete this part of the sentence.
364	Alternatively, extraction blanks (sterile water) can be processed with the samples to be tested if sufficient uninfected host tissue is not available. This will allow contamination of extraction reagents and/or cross-contamination between samples to be identified (but will not confirm the efficacy of nucleic acid extraction).	*Category : TECHNICAL* **(415) EPPO (29 Sep 2016 12:14 PM)** The text in brackets (but will not confirm the efficacy of nucleic acid extraction) should be deleted. The efficacy of nucleic acid extraction is monitored via the positive DNA extraction control and not by the negative DNA extraction control
364	~~Alternatively, extraction blanks can be processed with the samples to be tested if sufficient uninfected host tissue is not available. This will allow contamination of extraction reagents and/or cross-contamination between samples to be identified (but will not confirm the efficacy of nucleic acid extraction).~~	*Category : EDITORIAL* **(329) Philippines (29 Sep 2016 8:57 AM)**
364	Alternatively, extraction blanks (sterile water) can be processed with the samples to be tested if sufficient uninfected host tissue is not available. This will allow	*Category : TECHNICAL* **(288) European Union (28 Sep 2016 6:07 PM)** Text added to be more specific.

	contamination of extraction reagents and/or cross-contamination between samples to be ~~identified (but will not confirm the efficacy of nucleic acid extraction)~~identified.	The text in brackets, '(but will not confirm the efficacy of nucleic acid extraction)' should be deleted. The efficacy of nucleic acid extraction is monitored via the positive DNA extraction control and not by the negative DNA extraction control.
366	*P. ramorum* may be identified either by its growth characteristics and morphology in culture ~~or~~ and by sequence analysis.	*Category : SUBSTANTIVE* **(350) China (29 Sep 2016 11:45 AM)** Culturing growth characteristics, morphology and sequence analysis are the methods combining molecular and traditional methods and thus indispensable for accurate identification of "Phytophthora ramorum".
367	Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora* while *P. lateralis* and *P. foliorum* may give a cross-reaction in a PCR test (section 4.3).	*Category : TECHNICAL* **(416) EPPO (29 Sep 2016 12:14 PM)** This is only if the test of Hughes et al. 2006 is used. Based on in silico analysis, there is no risk of cross-reaction with P. foliorum and P. hibernalis if the test of Schena et al. 2006 is used. Moreover, as already mentioned, there is no cross-reaction with P. lateralis (checked by in silico analysis and also by specificity test carried out by the authors)
367	Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora* while *P. lateralis* and *P. foliorum* may give a cross-reaction in a PCR test (section 4.3)~~.~~. Propose to add the description for morphology and cultural characteristics of three pest.	*Category : SUBSTANTIVE* **(351) China (29 Sep 2016 11:45 AM)** It's more easy to identify.
367	Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora* while *P. lateralis* and *P. foliorum* may give a cross-reaction in a PCR test (section 4.3).	*Category : TECHNICAL* **(290) European Union (28 Sep 2016 6:10 PM)** This is only if the test of Hughes et al. 2006 is used. Based on in silico analysis, there is no risk of cross-reaction with P. foliorum and P. hibernalis if the test of Schena et al. 2006 is used. Moreover, as already mentioned, there is no cross-reaction with P. lateralis (checked by in silico analysis and also by specificity test carried out by the authors).
369	A very low percentage of cross reactivity has been observed with ITS real-time PCR when *P. foliorum* or *P. hibernalis* are present in very high concentration. The Ct values are usually over 36 Ct and for those cases morphological or sequencing studies of pure cultures are needed for a final identification.	*Category : TECHNICAL* **(417) EPPO (29 Sep 2016 12:14 PM)** If the test of Hughes et al. 2006 is used. Based on in silico analysis, there is no risk of cross-reaction with P. foliorum and P. hibernalis if the test of Schena et al. 2006 is used.
369	A very low percentage of cross reactivity has been observed with ITS real-time PCR when *P. foliorum* or *P. hibernalis* are present in very high concentration. The Ct values are usually over 36 Ct and for those cases morphological or sequencing studies of pure cultures are needed for a final identification.	*Category : TECHNICAL* **(291) European Union (28 Sep 2016 6:11 PM)** If the test of Hughes et al. 2006 is used. Based on in silico analysis, there is no risk of cross-reaction with P. foliorum and P. hibernalis if the test of Schena et al. 2006 is used.
369	A very low percentage of cross reactivity has been observed with ITS real-time PCR when *P. foliorum* or *P. hibernalis* are present in very high concentration. The Ct values are usually over 36 Ct and for those cases morphological or	*Category : TECHNICAL* **(155) New Zealand (31 Aug 2016 6:20 AM)** It should be specified here, which real-time PCR protocol this refers to. Is this for Hughes et al. 2006?

	sequencing studies of pure cultures are needed for a final identification.	
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : EDITORIAL* **(465) Peru (29 Sep 2016 8:12 PM)** to check
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : EDITORIAL* **(452) Brazil (29 Sep 2016 4:25 PM)** To check.
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters;	*Category : EDITORIAL* **(418) EPPO (29 Sep 2016 12:14 PM)** From experience the chlamydospore can be brown

	chlamydospores are numerous, thin-walled, globose, hyaline to ~~pale~~ brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : EDITORIAL* **(322) Argentina (29 Sep 2016 2:12 AM)** To check
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to ~~pale~~ brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : TECHNICAL* **(292) European Union (28 Sep 2016 6:12 PM)** Delete 'pale': from experience, it can reach brown colour. In addition, editorial.
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On	*Category : EDITORIAL* **(243) Chile (28 Sep 2016 5:41 PM)** to check

	CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : EDITORIAL* **(180) Uruguay (15 Sep 2016 9:05 PM)** Editorial correction
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : EDITORIAL* **(156) New Zealand (31 Aug 2016 6:22 AM)** Typo
372	The growth characteristics and morphological features of *P. ramorum* on agar,	*Category : EDITORIAL*

	described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	**(131) COSAVE (11 Aug 2016 11:05 PM)** to check
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with short a pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to ~~pale~~ brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	*Category : TECHNICAL* **(19) France (5 Aug 2016 5:45 PM)** From experience, it can reach brown colour.
372	The growth characteristics and morphological features of *P. ramorum* on agar, described in Werres *et al*. (2001), can be affected by the type of agar, substrate or host plant (T. Giltrap, personal communication, 2014). Colonies on carrot piece agar, PARP-V8 agar and cornmeal agar are submerged, showing pronounced (PARP-V8 agar) or weak (carrot piece and cornmeal agar) concentric rings. On CHA, colonies have an appressed aerial mycelium with weak rosette-like patterns. Sporangia are ellipsoid, elongate-ovoid, caducous, often with ~~short~~ a short pedicel, semipapillate, hyaline, 45.6–65 × 21–28.3 µm, single but in clusters; chlamydospores are numerous, thin-walled, globose, hyaline to pale brown, mostly 46–60 µm, terminal or intercalary. Generally, characteristic chlamydospores allow accurate identification of *P. ramorum* in culture. Possible confusion in morphology and cultural characteristics is most likely to occur with *P. palmivora*. The key	*Category : EDITORIAL* **(18) France (5 Aug 2016 5:43 PM)** editorial

	characteristics are illustrated in Figures 7, 8, 9 and 10. The most essential features formed on examples of selective and non-selective media are given in Table 5.	
374	**Table 5.** Growth characteristics of *Phytophthora ramorum* on selective and non-selective media Add the description of morphological features of oospores in Table 5.	*Category : SUBSTANTIVE* **(352) China (29 Sep 2016 11:46 AM)** the morphological features of oospores is the main features for identification of Phytophthora ramorum, but there is no description for morphological features of oospores in table 5.
385	Produced abundantly on the agar surface, semipapillate, caducous with short (5 µm) or no stalk. Size: 20–32 × 40–80 µm, average 24 × 52 µm; average length/width ratio: 2.16.	*Category : EDITORIAL* **(331) Philippines (29 Sep 2016 9:08 AM)** is this applicable to both columns? i yes a line should be drawn below the sentence
385	Produced abundantly on the agar surface, semipapillate, caducous with short (5 µm) or no stalk. Size: ~~20~~40-80×20-32µm~~–~~, average 24 ~~32~~ ×~~ 40–80 µm, average 24 ×~~ 52 µm; average length/width ratio: 2.16.	*Category : EDITORIAL* **(192) Japan (27 Sep 2016 9:29 AM)** editorial
387	Ellipsoid, frequently in small clusters and relatively narrow, initial sporangium commonly producing secondary, smaller sporangia. When growing out of plant material, can appear papillate wh~~ee~~n about to germinate. Sporangia with constrictions (central or at pedicel end) have been observed[3], particularly when growing out of plant material.	*Category : EDITORIAL* **(419) EPPO (29 Sep 2016 12:14 PM)** The "e" shouldn't be in talics in the word "when".
387	Ellipsoid, frequently in small clusters and relatively narrow, initial sporangium commonly producing secondary, smaller sporangia. When growing out of plant material, can appear papillate ~~wh~~when~~en~~ about to germinate. Sporangia with constrictions (central or at pedicel end) have been observed[3], particularly when growing out of plant material.	*Category : EDITORIAL* **(293) European Union (28 Sep 2016 6:15 PM)** The "e" shouldn't be in italics in the word "when".
394	[2] Sexual structures can be observed on carrot piece agar after pairing with an opposite mating type; for example, *Phytophthora cryptogea* (Werres and Kaminski, 2005). A *P. ramorum* × *P. ramorum* pairing is also possible *in vitro* (not with all isolates) (Brasier and Kirk, 2004) and in rhododendron twigs (Werres and Zielke, 2003).	*Category : TECHNICAL* **(193) Japan (27 Sep 2016 9:38 AM)** Morphological characteristics (e.g. shape, size and position of antheidium (whether amphigynous or paragynous)) of sexual reproductive organ (antheridium, oogonium and oospore) should be added to this para because these characteristics are useful to identify Phytophthora ramorum.
399	Koch's postulates have to be performed if *P. ramorum* has been found on a new host species.	*Category : EDITORIAL* **(420) EPPO (29 Sep 2016 12:14 PM)** This sentence is very general and should be placed earlier in the protocol. Or this sentence implies specific action for a biological identification and it should be clarified.
399	Koch's postulates have to be performed if *P. ramorum* has been found on a new host species~~.~~. The method of "Pathogenicity tests"should be described in "3.Detection".	*Category : SUBSTANTIVE* **(353) China (29 Sep 2016 11:47 AM)** "Koch's postulates have to be performed if P. ramorum has been found on a new host species.", but there is no any method description for "Pathogenicity tests" in"3.Detection".
399	Koch's postulates have to be performed if *P. ramorum* has been found on a new host species.	*Category : EDITORIAL* **(294) European Union (28 Sep 2016 6:16 PM)** This sentence is very general and should be placed earlier in the protocol. Or this sentence implies specific action for a biological identification and it should be clarified.
399	Koch's postulates have to be performed if *P. ramorum* has been found on a new host species.	*Category : EDITORIAL* **(20) France (5 Aug 2016 5:47 PM)** This sentence is very general and should be placed earlier in the protocol. Or this sentence implies specific action for a biological identification and it should be clarified.
400	**4.3 Molecular identification** **Propose to add the newest methods of molecular idendification.**	*Category : SUBSTANTIVE* **(354) China (29 Sep 2016 11:47 AM)**

401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis, P. hibernalis* and *P. foliorum*, is possible at high DNA concentrations. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level identification.	*Category : TECHNICAL* **(422) EPPO (29 Sep 2016 12:14 PM)** We suggest to use the conventional PCR methods or the real-time PCR method developed by Schena et al. 2006 on pure culture.
401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis*, *P. hibernalis* and *P. foliorum*, is possible at high DNA ~~concentrations~~concentrations with some methods. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level identification: other loci (e.g. COX) may also be used for this purpose.	*Category : TECHNICAL* **(421) EPPO (29 Sep 2016 12:14 PM)** The possible cross reactions with non target species occur especially with primers targetting ITS regions. But all tests described in section 3.6 don't target this region (e.g. Ioos et al. (2006). Suggestion for addition at the end of the paragraph to give more options.
401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis*, *P. hibernalis* and *P. foliorum*, is possible at high DNA ~~concentrations~~concentrations with some methods. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level identification: other loci (e.g. COX) may also be used for this purpose.	*Category : TECHNICAL* **(296) European Union (28 Sep 2016 6:20 PM)** 1. The possible cross reactions with non target species occur especially with primers targetting ITS regions. But all tests described in section 3.6 don't target this region (e.g. Ioos et al. (2006). Suggestion for addition at the end of the paragraph to give more options. 2. We would suggest to use the conventional PCR methods or the real-time PCR method developed by Schena et al. 2006 on pure culture.

401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis*, *P. hibernalis* and *P. foliorum*, is possible at high DNA concentrations. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level identification. The sequence can be performed also for other genes like COXI and II (Martin et al. 2003, 2004) and Ypt1 (Schena et al. 2008).	*Category : TECHNICAL* **(289) Chile (28 Sep 2016 6:09 PM)** an other alternative
401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis*, *P. hibernalis* and *P. foliorum*, is possible at high DNA concentrations. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level ~~identification~~identification of *Phytophthora* isolates..	*Category : TECHNICAL* **(157) New Zealand (31 Aug 2016 6:25 AM)** Words added to clarify that this method is suitable for identifying pure cultures, and not when plant material is tested.
401	The following tests are recommended for identification of species, including *P. ramorum*, from clean cultures. The conventional PCR and real-time PCR methods described in section 3.6 for *in planta* detection of *P. ramorum* are species-specific and are used for detection of the pathogen in infected material or in cultures. Molecular diagnostic tests detect DNA, not the viable organism, and cross-reaction with closely related species, including *P. lateralis*, *P. hibernalis* and *P. foliorum*, is possible at high DNA ~~concentrations~~concentrations with some methods. In addition, environmental samples (infected samples) that have very low titre can yield negative results, so care should be taken in the interpretation of results when testing DNA extracts from cultures, which may be at a higher concentration than extracts from plant material. ITS sequencing as described in section 4.3.1 may be used for species level identification: other loci (e.g. COX) may also be used for this purpose.	*Category : TECHNICAL* **(21) France (5 Aug 2016 5:55 PM)** The possible cross reactions with non target species occur especially with primers targetting ITS régions. But all tests dscribed in section 3.6 don't target this région (e.g. Ioos et al. (2006). Suggestion for addition at the end of the paragraph to give more options.

403	The identity of *P. ramorum* isolated in culture can be confirmed by sequencing the ~~ITS-1~~ ITS-1, 5.8S and ITS-2 ~~regions~~ region of the nrRNA gene as described below and in Table 6. These primers can be used to generate amplification products for sequencing from all species of *Phytophthora*.	*Category : TECHNICAL* **(158) New Zealand (31 Aug 2016 6:31 AM)** The amplified region contains ITS1, 5.8S and ITS2, not just the two ITS regions. Therefore, the text is edited the sentence to be correct.
404	~~ITS-5 F: 5′ GGA AGT AAA AGT CGT AAC AAG G 3′~~ITS5: 5′-GGAAGTAAAAGT CGTAACAAGG-3′	*Category : TECHNICAL* **(159) New Zealand (31 Aug 2016 6:32 AM)** The original primer name as per White et al 1990, is “ITS5”. Also, remove spaces in the sequence to keep consistency in formatting.
405	~~ITS-4 R: 5′ TCC TCC GCT TAT TGA TAT GC 3′~~ ITS4: 5′-TCCTCCGCTTATTGATATGC-3′	*Category : TECHNICAL* **(160) New Zealand (31 Aug 2016 6:34 AM)** The original primer name as per White et al 1990, is “ITS4”. Also, remove spaces in the sequence to keep consistency in formatting.
409	~~Master mix for 25 µl final reaction volume (µl)~~	*Category : TECHNICAL* **(286) Chile (28 Sep 2016 6:05 PM)** see comments p.130
418	1.25	*Category : TECHNICAL* **(423) EPPO (29 Sep 2016 12:14 PM)** If the final concentration in the PCR is 1.5 mM, then the volume taken should be 0.75 µl – to be checked
418	1.25	*Category : TECHNICAL* **(297) European Union (28 Sep 2016 6:21 PM)** If the final concentration in the PCR is 1.5 mM, then the volume taken should be 0.75 µl – to be checked.
424	2.50	*Category : TECHNICAL* **(299) European Union (28 Sep 2016 6:31 PM)** If the final concentration in the PCR is 0.2 µM, then the volume taken should be 1 µl – to be checked.
427	2.50	*Category : TECHNICAL* **(424) EPPO (29 Sep 2016 12:14 PM)** If the final concentration in the PCR is 0.2 µM, then the volume taken should be 1 µl – to be checked
427	2.50	*Category : TECHNICAL* **(301) European Union (28 Sep 2016 6:31 PM)** If the final concentration in the PCR is 0.2 µM, then the volume taken should be 1 µl – to be checked.
428	DNA polymerase	*Category : TECHNICAL* **(425) EPPO (29 Sep 2016 12:14 PM)** If the volume taken is 0.25 µl and the final concentration is 0.5 U, it means that the concentration in the reagent is 2 U/µl (not usual…normally 5 U/µl– to be checked
428	DNA polymerase	*Category : TECHNICAL* **(302) European Union (28 Sep 2016 6:32 PM)** If the volume taken is 0.25 µl and the final concentration is 0.5 U, it means that the concentration in the reagent is 2 U/µl (not usual…normally 5 U/µl– to be checked.
432	1 µl? (500-50 pg)	*Category : EDITORIAL* **(477) Bolivia (30 Sep 2016 4:42 AM)** To check
432	1 µl?(500-50 pg)	*Category : EDITORIAL* **(466) Peru (29 Sep 2016 8:13 PM)**

		to check
432	1 µl? (500-50 pg)	*Category : TECHNICAL* **(453) Brazil (29 Sep 2016 4:26 PM)** To check
432	1 µl? (500-50 pg)	*Category : EDITORIAL* **(323) Argentina (29 Sep 2016 2:15 AM)** To check
432	~~1~~ 1µl?((500-50 pg)	*Category : EDITORIAL* **(240) Chile (28 Sep 2016 5:40 PM)** to check
432	1 µl?? (500-50 pg)	*Category : EDITORIAL* **(181) Uruguay (15 Sep 2016 9:07 PM)** To check
432	1 µl?(500-50 pg)	*Category : EDITORIAL* **(132) COSAVE (11 Aug 2016 11:12 PM)** to check
436	95 °C for 1 min 25 s	*Category : TECHNICAL* **(426) EPPO (29 Sep 2016 12:14 PM)** Not frequent! normally, 3 or 5 minutes OK – to be checked
436	95 °C for 1 min 25 s	*Category : TECHNICAL* **(303) European Union (28 Sep 2016 6:33 PM)** Not frequent! Normally, 3 or 5 minutes OK – to be checked.
447	~~4 °C pause~~	*Category : TECHNICAL* **(427) EPPO (29 Sep 2016 12:14 PM)** Delete not needed
447	~~4 °C pause~~	*Category : TECHNICAL* **(304) European Union (28 Sep 2016 6:34 PM)** Not needed - can be deleted.
447	~~4 °C pause~~	*Category : TECHNICAL* **(161) New Zealand (31 Aug 2016 6:39 AM)** As for section 169.
451	Amplification products may be visualized by agarose gel electrophoresis: a single amplicon of 800–900 base pairs (bp) is produced by DNA from *Phytophthora* spp. The remaining amplification product can be purified using a suitable PCR purification kit following the manufacturer's instructions and the purified amplicon can be two-way sequenced with ITS-5 F (forward) and ITS-4 R (reverse) primers. The quality of the resulting sequence should be checked by visual assessment of the electropherograms. Consensus sequences may be built from the forward and reverse reads and compared with published sequences using the Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information (NCBI), United States; http://www.ncbi.nlm.nih.gov/). In order to make a correct identification of the generated sequences to *Phytophthora* species level, use of the GenBank accession number that corresponds to the ex-type of *P. ramorum* P10103 (WPC) ~~NCBI~~ is recommended, which is FJ801269.	*Category : EDITORIAL* **(162) New Zealand (31 Aug 2016 6:42 AM)** Remove NCBI from the sentence as that does not add any value

452	The following steps are suggested for processing sequences by BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq Select "Align two or more sequences using BLAST" (under Specialized BLAST)	*Category : SUBSTANTIVE* **(163) New Zealand (31 Aug 2016 6:44 AM)** Sentence " Select "Align two or more sequences using BLAST" (under Specialized BLAST)" should be given in a separate section as it is a step for blast search.
453	Paste the ~~FASTA~~ obtained sequence in a FASTA format in the first box	*Category : TECHNICAL* **(164) New Zealand (31 Aug 2016 6:46 AM)** Added a clarification that this sequence should be the one obtained by the user via sequencing.
456	~~Click in BLAST.~~Click on BLAST – or - Click BLAST	*Category : EDITORIAL* **(165) New Zealand (31 Aug 2016 6:48 AM)** Typo
462	Cultures of *P. ramorum* can be stored on carrot piece or oatmeal agar slopes at room temperature or in sterile distilled water at 5 ºC. DNA can be stored at –80 °C or -20 °C.	*Category : TECHNICAL* **(428) EPPO (29 Sep 2016 12:14 PM)** Storage of DNA extracts at -20°C is also approriate and allows more laboratories to access to such storage.
462	Cultures of *P. ramorum* can be stored on carrot piece or oatmeal agar slopes at room temperature or in sterile distilled water at 5 ºC. DNA can be stored at –80 ~~ºC.~~°C or -20 °C.	*Category : TECHNICAL* **(305) European Union (28 Sep 2016 6:35 PM)** Storage of DNA extracts at -20°C is also appropriate and allows more laboratories to access to such storage.
462	Cultures of *P. ramorum* can be stored on carrot piece or oatmeal agar slopes at room temperature or in sterile distilled water at 5 ºC. DNA can be stored at –80 ~~ºC~~°C or -20°C.	*Category : TECHNICAL* **(22) France (5 Aug 2016 5:59 PM)** Storage of DNA extracts at -20°C is also approriate and allows more laboratories to access to such storage.
466	A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).	*Category : SUBSTANTIVE* **(167) New Zealand (31 Aug 2016 7:05 AM)** for the following sections 253/259; 310/316/319/322; 422/425; and in text in 390 and 451 Change the primer names in the PCR mix instructions to reflect the names given by original authors and corrected above.
478	**CABI.** n.d. Crop Protection Compendium. Wallingford, UK, CABI. Available at http://www.cabi.org/cpc/datasheet/40991 (last accessed ~~6~~ 6th August 2014).	*Category : EDITORIAL* **(437) Kenya (29 Sep 2016 1:10 PM)**
497	**Ioos, R., Laugustin, L., Rose, S., Schenck, N., Husson, C. & Frey, P.** 2006. Usefulness of single copy genes containing introns in ~~Phytophthora~~ *Phytophthora* for the development of detection tools for the regulated species *P. ramorum* and *P. fragariae*. *European Journal of Plant Pathology*, 116: 171–176.	*Category : EDITORIAL* **(429) EPPO (29 Sep 2016 12:14 PM)** Italics for Latin names.
497	**Ioos, R., Laugustin, L., Rose, S., Schenck, N., Husson, C. & Frey, P.** 2006. Usefulness of single copy genes containing introns in ~~Phytophthora~~ *Phytophthora* for the development of detection tools for the regulated species *P. ramorum* and *P. fragariae*. *European Journal of Plant Pathology*, 116: 171–176.	*Category : EDITORIAL* **(306) European Union (28 Sep 2016 6:36 PM)** Italics for Latin names.
497	**Ioos, R., Laugustin, L., Rose, S., Schenck, N., Husson, C. & Frey, P.** 2006. Usefulness of single copy genes containing introns in ~~Phytophthora~~ *Phytophthora*	*Category : EDITORIAL* **(23) France (5 Aug 2016 6:00 PM)** editorial italic for latin name.

	for the development of detection tools for the regulated species *P. ramorum* and *P. fragariae*. *European Journal of Plant Pathology*, 116: 171–176.	
500	**Kox, L.F.F., De Gruyter, J., Garbelotto, M., Van Brouwershaven, I., Admiraal, J. & Baayen, R.P.** 2002. Validation of a PCR method for detection and identification of *Phytophthora ramorum* (Abstract) pp. 57–58. *In.* Sudden Oak Death, a Science Symposium. The State of our Knowledge. Monterey, CA, USA 15–18 December, 2002, 96 pp.	*Category : EDITORIAL* **(311) Ghana (28 Sep 2016 6:55 PM)** Kox, L.F.F., De Gruyter, J., Garbelotto, M., Van Brouwershaven, I., Admiraal, J. & Baayen, R.P. 2002 should come before the Kox, L.F.F., Van Brouwershaven, I.R., Van de Vossenberg, B.T.L.H., van den Beld, H.E., Bonants, P.J.M. & De Gruyter, J. 2007. to reflect the sequence in year of publication i.e 2002 before 2007
507	**Schena, L., Duncan, J.M. & Cooke, D.E.L.** ~~2008~~2006. ~~Development~~ Detection and ~~application~~ quantification of ~~a PCR-based "molecular tool box" for the identification of~~ Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology 7(5): 365-379~~*Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology*, 57: 64–75.~~	*Category : TECHNICAL* **(430) EPPO (29 Sep 2016 12:14 PM)** SHould be changed to Schena, L., Hughes K.J.D., Cooke, D.E.L. 2006. Detection and quantification of Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology 7(5): 365-379
507	**Schena, L., Duncan, J.M. & Cooke, D.E.L.** ~~2008~~2006. ~~Development and application of a PCR-based "molecular tool box" for the identification of~~ Detection and application quantification of a PCR-based "molecular tool box" for the identification of *Phytophthora ramorum, P. kernoviae*~~species damaging forests and natural ecosystems. ,*Plant Pathology*~~ *P. citricola* ,and *P. quercina* ~~57: 64–75.~~in symptomatic leaves by multiplex real-time PCR. *Molecular Plant Pathology*, 7(5): 365-379.	*Category : TECHNICAL* **(307) European Union (28 Sep 2016 6:39 PM)** Amend with Schena, L., Hughes K.J.D., Cooke, D.E.L. 2006. Detection and quantification of Phytophthora ramorum, P. kernoviae, P. citricola and P. quercina in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology 7(5): 365-379
516	**Werres, S. & Kaminski, K.** 2005. Characterisation of European and North American *Phytophthora ramorum* isolates due to their morphology and mating behaviour *in vitro* with heterothallic *Phytophthora* species. *Mycological Research*, 109: 860–871.	*Category : EDITORIAL* **(312) Ghana (28 Sep 2016 7:01 PM)** The references of 'Werres, S. & Marwitz, R. 1997 to Werres, S. & Kaminski, K. 2005 should be rearranged to follow sequence in year of publication and number of authors.

522		*Category : SUBSTANTIVE* **(431) EPPO (29 Sep 2016 12:14 PM)** If the qPCR test developed by Schena et al. 2006 is used, it is not necessary to confirm the result by isolation or sequencing. - If the test of Hughes et al. 2006 is used there is a risk of false positive results on pure culture (P. lateralis, P. foliorum, P. hibernalis). The flow diagram should be modified accordingly. Positive: include the possiblity to use other loci for confirmation of positive result, to be consistent with previous comment. Suggestion to add at the end of the paragraph 526: "- PCR test on another locus".

522		*Category : SUBSTANTIVE* **(332) Philippines (29 Sep 2016 9:14 AM)** is figure 9 based on the figure published on http://.padil.gov.au/pbt/files/uall/SOD_Figure_16.jpg? on the published figure by Australia Real time PCR is seperated from conventional PCR because Real time PCR result is conclusive while Conventional PCR needs further verification.. therefor we suggest that on this diagram we separate Real time PCR from Conventional PCR Procedure.

522		*Category : SUBSTANTIVE* **(309) European Union (28 Sep 2016 6:43 PM)** 1. If the qPCR test developed by Schena et al. 2006 is used, it is not necessary to confirm the result by isolation or sequencing. - If the test of Hughes et al. 2006 is used there is a risk of false positive results on pure culture (P. lateralis, P. foliorum, P. hibernalis). The flow diagram should be modified accordingly. 2. (TECH.) Paragraph 523 - Positive: include the possiblity to use other loci for confirmation of positive result, to be consistent with previous comment. Suggestion to add at the end of the paragraph 526: "- PCR test on another locus".

522		*Category : SUBSTANTIVE* **(166) New Zealand (31 Aug 2016 7:02 AM)** for 549 - The text box needs to be extended to show the word in full length. Also, the arrow leading to 545, should come out of this box, not from the arrow leading to 550. For 550 Change to Serological test ...In the text, several serological methods are mentioned (not only ELISA), therefore a more general approach should also be taken on the Figure. For 546 ...change to P. ramorum specific real-time PCR or....Since species specific and generic PCR protocols are given in the main document, it should be specified here what kind of PCR this refers to. For 541 change to Amplification and sequencing of the ITS region with generic primers....The original text does not specify that this means the use of generic primers for ITS. For 542 change to Species specific real-time or conventional PCR followed by sequencing ...The original text does not specify that this means the use of specific PCR assay. for 523.....Please reconsider the conclusions resulting from this text box. As it reads right now, then the following scenario would be possible: Positive reaction from serological test, positive result from specific PCR, no pathogen successfully isolated, the decision is made that no P. ramorum is present. That would not be justified because the result could be false-negative (many reasons why pathogen might not be isolated when present). Changes into diagram are needed to eliminate this confusing factor. For 525 ...change to ...Sequencing the amplified PCR product..To clarify that the obtained product will be sequenced.

522		*Category : TECHNICAL* **(24) France (5 Aug 2016 6:03 PM)** paragraph 523 - positive: include the possiblity to use other loci for confirmation of positive result, to be consistent with previous comment. Suggestion to add at the end of the paragraph 523 "-PCR test on another locus"

575	add morphological features of oospores.	*Category : SUBSTANTIVE* **(356) China (29 Sep 2016 11:49 AM)** the morphological features of oospores is the main features for identification of Phytophthora ramorum, but there is no morphological features of oospores and its notes in [575].
575	add scale bar in fig.7 .	*Category : EDITORIAL* **(355) China (29 Sep 2016 11:49 AM)**