2017 First consultation

1 July – 30 September 2017

Compiled comments for Draft revision of DP 2: *Plum pox virus* (2016-007)

Summary of comments

Name	Summary
Cameroon	Exame achevé
Cuba	No hay sugerencias al Protocolo de Diagnóstico de Plum Pox Virus, estamos de acuerdo con el mismo.
EPPO Σ	Finalised by the EPPO Secretariat on behalf of its 51 Member Countries.
European Union	Finalised by the European Commission on behalf of the EU and its 28 Member States on 29/09/2017.
Samoa	no further comments
South Africa	No comments from the National Plant Protection Organisation of South Africa.
Turkey	Plum pox virus -TR

#	Para	Text	Comment
1	G	(General Comment)	**Cameroon** Les préoccupations que nous avons sont ccelles relatives à l'infrastructure et le niveau technique requis pour conduire de tels tests. Les formations et le développement de kits de diagnostic rapide pourraient aider à combler ces lacune pour les pays de notre région en gérénal. *Category : TECHNICAL*
2	G	(General Comment)	**Costa Rica** We do not have comments on this document, we agree *Category : TECHNICAL*
3	G	(General Comment)	**Myanmar** This disease absent in Myanmar.Myanmar has no comment, *Category : SUBSTANTIVE*
4	G	(General Comment)	**Peru** We agree with the Draft revision of DP 2: Plum pox virus (2016-007) *Category : TECHNICAL*
5	G	(General Comment)	**United States of America** Perhaps it would be more valuable if the protocol had input from researchers from a variety of regions to present more of a global expertise. *Category : SUBSTANTIVE*
6	G	(General Comment)	**Canada** Canada supports the draft diagnostic protocol on Plum pox virus (2016-007). *Category : SUBSTANTIVE*

#	Para	Text	Comment
7	G	(General Comment)	**European Union** Finally, it is suggested to improve the structure of the protocol by presenting more clearly which step of the diagnostic process is involved, e.g. sampling (most likely similar for all ELISA tests and all molecular tests, respectively); sample preparation; detection of PPV (all strains); identification of PPV (universal) and identification of individual strains (including overview table as suggested before). *Category : TECHNICAL*
8	G	(General Comment)	**Panama** Panama has no comments on this document. *Category : EDITORIAL*
9	G	(General Comment)	**Saint Vincent and The Grenadines** No additional comments. This standard is highly technical and would be difficult to be implemented by St. Vincent and the Grenadines *Category : SUBSTANTIVE*
10	G	(General Comment)	**Barbados** Barbados has no comments to make on this document. *Category : SUBSTANTIVE*
11	G	(General Comment)	**Viet Nam** Vietnam would like to request providing the protocol for detection and identification of Plum pox virus using RT-LAMP (Reverse transcription - Loop-mediated isothermal amplification) method and providing the method for preserving the suspectedly infested-PPV samples/ plant sap (after extraction). Because LAMP/ RT-LAMP is rapid, accurate and cost effective for detection and identification of virus that is highly potential to apply in Vietnam, especially in Plant Quarantine Stations or In international trade, it's necessary to preserve the samples/plant sap (after extraction) after performing a diagnosis for further technical argument/discussion. *Category : SUBSTANTIVE*
12	G	(General Comment)	**Tajikistan** I support the document as it is and I have no comments *Category : SUBSTANTIVE*
13	G	(General Comment)	**New Zealand** Have no comments on the draft. *Category : SUBSTANTIVE*
14	G	(General Comment)	**Bahamas** There is a need for strict plant quarantine and procedures for testing of imported nursery stock to prevent the introduction of PPV to the region. The Bahamas therefore support this diagnostic protocol. *Category : SUBSTANTIVE*
15	G	(General Comment)	**Thailand** agree with the proposed draft DP for Plum pox vivus *Category : SUBSTANTIVE*
16	G	(General Comment)	**Lao People's Democratic Republic** Lao PDR agreed with this draft revision. *Category : SUBSTANTIVE*
17	G	(General Comment)	**Honduras** HONDURAS NO TIENE COMENTARIOS *Category : TECHNICAL*

#	Para	Text	Comment
18	G	(General Comment)	**Algeria** No comment *Category : TECHNICAL*
19	G	(General Comment)	**Nicaragua** Nicaragua considera que este protocolo es una herramienta de gran importancia para los países, que por el comportamiento de la enfermedad tienen afectaciones del virus. El estudio de las diferentes cepas del virus permitirá contar la información actualizada para emitir un diagnóstico acertado y de manera especifica sobre las diferentes cepas del virus. *Category : EDITORIAL*
20	G	(General Comment)	**Colombia** No obstante, teniendo en cuenta las características variables de los síntomas que causa Plum Pox Virus –PPV-, se solicita mantener e incluir fotografías de los síntomas más relevantes, las cuales serían de gran utilidad para países que no registran esta plaga. *Category : TECHNICAL*
21	33	**Main discussion points during development of the diagnostic protocol**	**EPPO** *Category : TECHNICAL*
22	52	~~Sharka (plum pox)~~ *Plum pox virus* is one of the most serious diseases of stone fruit. The disease, caused by *Plum pox virus* (PPV), affects plants of the genus *Prunus*. It is particularly detrimental in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina* because it reduces quality and causes premature fruit drop. It is estimated that the costs of managing sharka worldwide since the 1970s exceed 10 000 million euros (Cambra *et al*., 2006b).	**Viet Nam** Do not use an other name *Category : TECHNICAL*
23	52	Sharka (plum pox) is one of the most serious viral diseases of stone fruit. The disease, caused by *Plum pox virus* (PPV), affects plants of the genus ~~*Prunus*~~*Prunus (family Rosacea). Sharka was first reported in P. domestica in Bulgaria in 1917–1918, and was described as a viral disease in 1932. Since then, the virus has spread progressively to a large part of Europe, around the Mediterranean basin and the Near East. It has been found with a restricted distribution in South and North America and Asia (EPPO, 2006; CABI, 2016)*. ~~It is particularly detrimental in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina* because it reduces quality and causes premature fruit drop. It is estimated that the costs of managing sharka worldwide since the 1970s exceed 10 000 million euros (Cambra *et al*., 2006b).~~	**Kenya** *Category : SUBSTANTIVE*
24	52	Sharka (plum pox) is one of the most serious diseases of stone fruit. The disease, caused by *Plum pox virus* (PPV), affects plants of the genus *Prunus*. It is particularly detrimental in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina* because it reduces quality and causes ~~premature~~ early fruit drop. It is estimated that the costs of managing sharka worldwide since the 1970s exceed 10 000 million euros (Cambra *et al*., 2006b).	**PPPO** replace premature with early *Category : SUBSTANTIVE*
25	53	~~Sharka~~ *Plum pox virus* was first reported in *P. domestica* in Bulgaria in 1917–1918, and was described as a viral disease in 1932. Since then, the virus has spread progressively	**Viet Nam** Should be use name of pest as title *Category : TECHNICAL*

#	Para	Text	Comment
		to a large part of Europe, around the Mediterranean basin and the Near East. It has been found with a restricted distribution in South and North America and Asia (EPPO, 2006; CABI, 2016).	
26	53	The disease~~Sharka was first reported~~ is particularly detrimental in P. armeniaca, P. domestica, P. persica and P. salicina because it reduces quality and causes premature fruit drop. It is estimated that the costs of managing sharka worldwide since the 1970s exceed 10 000 million euros (Cambra et al., 2006b). ~~*P. domestica* in Bulgaria in 1917–1918, and was described as a viral disease in 1932. Since then, the virus has spread progressively to a large part of Europe, around the Mediterranean basin and the Near East. It has been found with a restricted distribution in South and North America and Asia (EPPO, 2006; CABI, 2016).~~	**Kenya** *Category : SUBSTANTIVE*
27	54	*Plum pox virus* is a member of the genus *Potyvirus* in the family *Potyviridae*. The virus particles are flexuous rods of approximately 700 nm × 11 nm, and are composed of a single-stranded RNA molecule consisting of almost 10 000 nucleotides coated by up to 2 000 subunits of a single coat protein (García and Cambra, 2007). PPV is transmitted in the field by aphids in a non-persistent manner, but movement of infected propagative plant material is the main way in which PPV is spread over long distances.Transmission via seed and pollen have not been reported (Pasquini and Barba, 2006; Ilardi and Tavazza, 2015). Under experimental conditions, PPV can be transmitted mechanically to numerous Prunus spp. and several herbaceous plants e.g Arabidopsis thaliana, Chenopodium foetidum, Nicotiana benthamiana, N. clevelandii, N. glutinosa and Pisum sativum.	**Kenya** *Category : TECHNICAL*
28	54	*Plum pox virus* is a member of the genus *Potyvirus* in the family *Potyviridae*. The virus particles are flexuous rods of approximately 700 nm × 11 nm, and are composed of a single-stranded positive sense RNA molecule consisting of almost 10 000 nucleotides coated by up to 2 000 subunits of a single coat protein (García and Cambra, 2007). ~~PPV~~ ;Ilardi and Tavazza, 2015). Thegenomic RNA carries a virus-encoded protein(VPg) at its 5′ end and poly(A)tail at its 3′ end. It possess a long open reading frame which is ~~transmitted in the field by aphids in~~ translated into a ~~non-persistent manner~~large polyprotein precursor from the second AUG codon (García et al., ~~but movement of infected propagative plant material~~ 2014). The polyprotein is ~~the main way in which~~ later used to produce mature proteins. As an obligate intracellular pathogen with a limited genome capacity, PPV is ~~spread over long distances~~unable to accomplish its infection cycle. Therefore it relies on the multifunction properties of its proteins and host factors for its infectivity process(García et al., 2014) Transmission:	**Kenya** *Category : SUBSTANTIVE*

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		PPV is transmitted in the field by aphids in a non-persistent manner, but movement of infected propagative plant material is the main way in which PPV is spread over long distances.	
29	55	*Plum pox virus* isolates can be classified currently into nine strains: D (Dideron), M (Marcus), C (Cherry), EA (El Amar), W (Winona), Rec (Recombinant), T (Turkish), CR (Cherry Russian) and An (Ancestor Marcus) (James *et al*., 2013;). Most PPV isolates belong to the D and M strains. PPV D and M strains can easily infect *P. armeniaca* and *P. domestica* but differ in their ability to infect *P. persica* cultivars. The strains vary in their pathogenicity; for example M isolates generally cause faster epidemics and more severe symptoms than D isolates in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina*. EA isolates are geographically restricted to Egypt and little information is available about their epidemiology and biological properties. PPV isolates infecting *P. avium* and *P. cerasus* have been identified in several European countries. These isolates form two distinct strains that have been defined as PPV-C and PPV-CR. An atypical PPV was detected in *P. domestica* in Canada (PPV-W) representing a distinct PPV strain. PPV W has since been detected in several countries in Europe (James *et al.*, 2013). In addition, natural recombinants between the D and M strains of PPV have been described as PPV-Rec, these showing an epidemiological behaviour similar to the D strain. A second type of recombinant strain has been reported in Turkey (T strain, Ulubaş Sercçe *et al*., 2009). A single isolate of PPV An has been described and it has been proposed as a potential ancestor of PPV M (Palmisano *et al*., 2012). A novel sour cherry-adapted Tat strain, neither C nor CR, has also been proposed (Chirkov *et al*., 2016). Under natural conditions, PPV readily infects fruit trees of the genus Prunus used as commercial varieties or rootstocks: P. armeniaca, P. cerasifera, P. davidiana, P. domestica, P. mahaleb, P. marianna, P. mume, P. persica, P. salicina, and interspecific hybrids between these species. Prunus avium, P. cerasus and P. dulcis may be infected occasionally. The virus also infects many wild and ornamental Prunus species such as P. besseyi, P. cistena, P. glandulosa, P. insititia, P. laurocerasus, P. spinosa, P. tomentosa and P. triloba. Under experimental conditions, PPV can be transmitted mechanically to numerous Prunus spp. and several herbaceous plants (Arabidopsis thaliana, Chenopodium foetidum, Nicotiana benthamiana, N. clevelandii, N. glutinosa and Pisum sativum).	**Viet Nam** para 63 move to before para 56 *Category : EDITORIAL*
30	55	*Plum pox virus* isolates can be classified currently into nine monophyletic strains: D (Dideron), M (Marcus), C (Cherry), EA (El Amar), W (Winona), Rec (Recombinant), T (Turkish), CR (Cherry Russian) and An (Ancestor Marcus) (James *et al*., 2013;). Most PPV isolates belong to the D and M strains. PPV D and M strains can easily infect	**European Union** *Category : TECHNICAL*

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		P. armeniaca and *P. domestica* but differ in their ability to infect *P. persica* cultivars. The strains vary in their pathogenicity; for example M isolates generally cause faster epidemics and more severe symptoms than D isolates in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina*. EA isolates are geographically restricted to Egypt and little information is available about their epidemiology and biological properties. PPV isolates infecting *P. avium* and *P. cerasus* have been identified in several European countries. These isolates form two distinct strains that have been defined as PPV-C and PPV-CR. An atypical PPV was detected in *P. domestica* in Canada (PPV-W) representing a distinct PPV strain. PPV W has since been detected in several countries in Europe (James *et al.*, 2013). In addition, natural recombinants between the D and M strains of PPV have been described as PPV-Rec, these showing an epidemiological behaviour similar to the D strain. A second type of recombinant strain has been reported in Turkey (T strain, Ulubaş Sercçe *et al*., 2009). A single isolate of PPV An has been described and it has been proposed as a potential ancestor of PPV M (Palmisano *et al*., 2012). A novel sour cherry-adapted ~~Tat strain~~putative strain (Tat), neither C nor CR, has also been proposed (Chirkov *et al*., 2016).	
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32	55	~~*Plum pox virus*~~*PPV Isolates/Strains*	**Kenya** *Category : SUBSTANTIVE*

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35	55	*Plum pox virus* isolates can be classified currently into nine monophyletic strains: D (Dideron), M (Marcus), C (Cherry), EA (El Amar), W (Winona), Rec (Recombinant), T (Turkish), CR (Cherry Russian) and An (Ancestor Marcus) (James *et al*., 2013;). Most PPV isolates belong to the D and M strains. PPV D and M strains can easily infect *P. armeniaca* and *P. domestica* but differ in their ability to infect *P. persica* cultivars. The strains vary in their pathogenicity; for example M isolates generally cause faster epidemics and more severe symptoms than D isolates in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina*. EA isolates are geographically restricted to Egypt and little information is available about their epidemiology and biological properties. PPV isolates infecting *P. avium* and *P. cerasus* have been identified in several European countries. These isolates form two distinct strains that have been defined as PPV-C and PPV-CR. An atypical PPV was detected in *P. domestica* in Canada (PPV-W) representing a distinct PPV strain. PPV W has since been detected in several countries in Europe (James *et al.*, 2013). In addition, natural recombinants between the D and M strains of PPV have been described as PPV-Rec, these showing an epidemiological	**Turkey** *Category : TECHNICAL*

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		behaviour similar to the D strain. A second type of recombinant strain has been reported in Turkey (T strain, Ulubaş Sercçe *et al*., 2009). A single isolate of PPV An has been described and it has been proposed as a potential ancestor of PPV M (Palmisano *et al*., 2012). A novel sour cherry-adapted Tat strain, neither C nor CR, has also been proposed (Chirkov *et al*., 2016).	
36	55	*Plum pox virus* isolates can be classified currently into nine strains: D (Dideron), M (Marcus), C (Cherry), EA (El Amar), W (Winona), Rec (Recombinant), T (Turkish), CR (Cherry Russian) and An (Ancestor Marcus) (James *et al*., ~~2013;~~2013~~)~~). Most PPV isolates belong to the D and M strains. PPV D and M strains can easily infect *P. armeniaca* and *P. domestica* but differ in their ability to infect *P. persica* cultivars. The strains vary in their pathogenicity~~; for example~~ . The M isolates generally cause faster epidemics and more severe symptoms than D isolates in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina*. EA isolates are geographically restricted to Egypt and little information is available about their epidemiology and biological properties. PPV isolates infecting *P. avium* and *P. cerasus* have been identified in several European countries. These isolates form two distinct strains that have been defined as PPV-C and PPV-CR. An atypical PPV was detected in *P. domestica* in Canada (PPV-W) representing a distinct PPV strain. PPV W has since been detected in several countries in Europe (James *et al.*, 2013). In addition, natural recombinants between the D and M strains of PPV have been described as PPV-Rec, these showing an epidemiological behaviour similar to the D strain. A second type of recombinant strain has been reported in Turkey ~~(T strain, Ulubaş~~ as T strain (Ulubaş Sercçe *et al*., 2009). A single isolate of PPV An has been described and it has been proposed as a potential ancestor of PPV M (Palmisano *et al*., 2012). A novel sour cherry-adapted Tat strain, neither C nor CR, has also been proposed (Chirkov *et al*., 2016).	**Philippines** not appropriate *Category : EDITORIAL*
37	59	~~**Synonym:** Sharka virus~~	**Viet Nam** Which references to make this synonym? *Category : EDITORIAL*
38	62	**3. Detection and Identification** **Host range**	**Kenya** *Category : TECHNICAL*
39	63	~~Under natural conditions, PPV readily infects fruit trees of the genus *Prunus* used as commercial varieties or rootstocks: *P. armeniaca*, *P. cerasifera*, *P. davidiana*, *P. domestica*, *P. mahaleb*, *P. marianna*, *P. mume*, *P. persica*, *P. salicina*, and interspecific hybrids between these species. *Prunus avium*, *P. cerasus* and *P. dulcis* may be infected occasionally. The virus also infects many wild and ornamental *Prunus* species such as *P. besseyi*, *P. cistena*, *P. glandulosa*, *P. insititia*, *P. laurocerasus*, *P. spinosa*, *P. tomentosa* and *P. triloba*. Under experimental conditions, PPV can be~~	**Viet Nam** para 63 move to before para 56 *Category : EDITORIAL*

#	Para	Text	Comment
		~~transmitted mechanically to numerous *Prunus* spp. and several herbaceous plants (*Arabidopsis thaliana*, *Chenopodium foetidum*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa* and *Pisum sativum*).~~	
40	63	Under natural conditions, PPV readily infects fruit trees of the genus *Prunus* used as commercial varieties or rootstocks: *P. armeniaca*, *P. cerasifera*, *P. davidiana*, *P. domestica*, *P. mahaleb*, *P. marianna*, *P. mume*, *P. persica*, *P. salicina*, and interspecific hybrids between these species. *Prunus avium*, *P. cerasus* and *P. dulcis* may be infected occasionally. The virus also infects many wild and ornamental *Prunus* species such as *P. besseyi*, *P. cistena*, *P. glandulosa*, *P. insititia*, *P. laurocerasus*, *P. spinosa*, *P. tomentosa* and *P. triloba*. ~~Under experimental conditions, PPV can be transmitted mechanically to numerous *Prunus* spp. and several herbaceous plants (*Arabidopsis thaliana*, *Chenopodium foetidum*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa* and *Pisum sativum*).~~	**Kenya** *Category : TECHNICAL*
41	63	Under natural conditions, PPV readily infects fruit trees of the genus *Prunus* ~~-~~(family Rosacea) used as commercial varieties or rootstocks: Major hosts include, ~~*P.*~~ *P. armeniaca*, *P. cerasifera*, *P. davidiana*, *P. domestica*, *P. mahaleb*, *P. marianna*, *P. mume*, *P. persica*, *P. salicina*, and interspecific hybrids between these species. Occasonally, *Prunus avium*, *P. cerasus* and *P. dulcis* may be infected ~~occasionally~~. The virus also infects many wild and ornamental *Prunus* species such as *P. besseyi*, *P. cistena*, *P. glandulosa*, *P. insititia*, *P. laurocerasus*, *P. spinosa*, *P. tomentosa* and *P. triloba*. Under experimental conditions, PPV can be transmitted mechanically to numerous *Prunus* spp. and several herbaceous plants (*Arabidopsis thaliana*, *Chenopodium foetidum*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa* and *Pisum sativum*).	**Kenya** *Category : TECHNICAL*
42	64	~~Sharka~~ *Plum pox virus* symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*. *Prunus cerasifera* cv. GF 31 shows rusty-brown corking and cracking of the bark. Flower symptoms can occur on petals (discoloration) of some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of	**Viet Nam** *Category : EDITORIAL*

#	Para	Text	Comment
		P. armeniaca show typical pale rings or spots. The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour. Symptom development and intensity depend strongly on the host plant and climatic conditions; for example the virus may be latent for several years in cold climates.	
43	64	Sharka symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*. *Prunus cerasifera* cv. GF 31 shows rusty-brown corking and cracking of the bark. ~~Flower symptoms can occur on petals (discoloration) of some~~ Flower symptoms can occur on petals (discoloration) of some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. ~~Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of~~ Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of *P. armeniaca* show typical pale rings or spots. ~~The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour.~~ Symptom development and intensity depend strongly on the host plant and climatic conditions; for example the virus may be latent for several years in cold climates.The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour.	**Kenya** *Category : EDITORIAL*
44	64	Sharka ~~symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or~~	**Kenya** *Category : EDITORIAL*

#	Para	Text	Comment
		~~leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as~~ disease symptoms Sharka symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*. *Prunus cerasifera* cv. GF 31 shows rusty-brown corking and cracking of the bark. Flower symptoms can occur on petals (discoloration) of some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of *P. armeniaca* show typical pale rings or spots. The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour. Symptom development and intensity depend strongly on the host plant and climatic conditions; for example the virus may be latent for several years in cold climates.	
45	64	Sharka symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*. *Prunus cerasifera* cv. GF 31 shows rusty-brown corking and cracking of the bark. Flower symptoms can occur on petals (discoloration) of some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of	**PPPO** Need more explanation on flower symptoms occurring on petals. Not very clear. *Category : EDITORIAL*

#	Para	Text	Comment
		P. armeniaca show typical pale rings or spots. The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour. Symptom development and intensity depend strongly on the host plant and climatic conditions; for example the virus may be latent for several years in cold climates.	
46	64	Sharka symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of ~~these leaf~~ these symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*~~.~~ , *Prunus cerasifera* cv. GF 31 shows rusty-brown corking and cracking of the bark. Flower ~~symptoms~~ discoloration can occur on petals ~~(discoloration)~~ of some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of *P. armeniaca* show typical pale rings or spots. The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour. Symptom development and ~~intensity~~ severity depend strongly on the host plant and climatic conditions~~; for example the~~ . The virus may be latent for several years in cold climates.	**Philippines** *Category : SUBSTANTIVE*
47	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature	**European Union** Remark: In summer shoots without leaves are shipped as grafting material, therefore only phloem tissue or buds can be used for testing. *Category : SUBSTANTIVE*

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		fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	
48	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**European Union** Reliability of the detection of PPV may depend on the method used. Molecular tools such as real-time RT-PCR should be able to detect low virus concentration even in young shoots. Suggestion is added in the paragraph. *Category : SUBSTANTIVE*
49	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from grafted shoots that are at least ~~one year old~~ one-year-old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots	**European Union** SharCo : Containment of Sharka virus in view of EU-expansion, 2010. DE.3.2 : Procedures for sampling in nursery blocks and validated tools and protocols for reliable and accurate PPV detection. *Category : SUBSTANTIVE*

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		with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Dormant buds can also be used in summer, especially for testing graftwood. RT-PCR or real time PCR should be used for detection of PPV in dormant buds and preferably also in mature leaves in summer, especially for detection of latent infections. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, ~~shoots,~~ shoots or branches, or complete ~~spurs~~ spurs, can be selected.	
50	65	~~General guidance on~~ Sampling for analysis General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**Kenya** *Category : EDITORIAL*
51	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of	**Ghana** *Category : SUBSTANTIVE*

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		each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In ~~springtime~~springtime or during the rainy season, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and ~~autumn~~autumn or dry or the hot climate, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In ~~winter~~winter or cold season, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	
52	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn or dry season are less reliable than tests done on samples collected earlier in the ~~spring~~spring or in the rainy season. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**Ghana** *Category : SUBSTANTIVE*
53	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing	**EPPO** SharCo : Containment of Sharka virus in view of EU-expansion, 2010. DE.3.2 : Procedures for sampling in nursery blocks and validated tools and protocols for reliable and accurate PPV detection *Category : SUBSTANTIVE*

#	Para	Text	Comment
		symptoms. In symptomless plants, samples should be taken from grafted shoots that are at least ~~one year old~~ one-year-old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Dormant buds can also be used in summer, especially for testing graftwood. RT-PCR or real time PCR should be used for detection of PPV in dormant buds and preferably also in mature leaves in summer, especially for detection of latent infections. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, ~~shoots,~~ shoots or branches, or complete ~~spurs~~ spurs, can be selected.	
54	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**EPPO** *Category : SUBSTANTIVE*

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55	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**EPPO** Remark: In summer shoots without leaves are shipped as grafting material, therefore only phloem tissue or buds can be used for testing. *Category : SUBSTANTIVE*
56	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least ~~one year old~~ one-year-old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In	**Turkey** *Category : EDITORIAL*

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		winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	
57	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). For field sampling, a~~ppropriate~~ ppropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	**Philippines** *Category : EDITORIAL*
58	65	General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, samples should be collected of flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be done during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests done on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis.	**Slovenia** *Category : SUBSTANTIVE*

#	Para	Text	Comment
		Dormant buds can also be used in summer, especially for testing graftwood. RT-PCR or real time PCR must be used for detection of PPV in dormant buds and preferably also in mature leaves in summer, especially for detection of latent infections. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots, or branches, or complete spurs can be selected.	
59	66	Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, ~~subsequent tests~~ the confirmation should ~~use serological methods and vice versa~~be performed preferably with a test with a higher analytical sensitivity that the one used for initial identification.. It is possible to confirm the results of a molecular test by another molecular test targeting a different genome region or sequence analysis. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	**European Union** *Category : SUBSTANTIVE*
60	66	Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, subsequent tests should use serological methods and vice versa. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	**European Union** SharCo : Containment of Sharka virus in view of EU-expansion, 2010. DE.3.2 : Procedures for sampling in nursery blocks and validated tools and protocols for reliable and accurate PPV detection. *Category : SUBSTANTIVE*
61	66	~~Detection of~~ PPV ~~can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection~~ detection	**Kenya** *Category : EDITORIAL*

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		Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, subsequent tests should use serological methods and vice versa. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	
62	66	Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, subsequent tests should use serological methods and vice versa. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	**EPPO** *Category : SUBSTANTIVE*
63	66	Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, ~~subsequent tests~~ the confirmation should ~~use serological methods and vice versa~~be performed preferably with a test with a higher analytical sensitivity that the one used for initial identification.. It is possible to confirm the results of a molecular test by another molecular test targeting a different genome region or sequence analysis. Further tests may also be done to identify the strain of PPV present.	**EPPO** The confirmation should preferably be done with a more sensitive test than the test used for the initial identification. *Category : SUBSTANTIVE*

#	Para	Text	Comment
		In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	
64	66	Detection of PPV can be achieved using a biological, serological or molecular ~~test;~~ test while identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV ~~(e.g.~~ especially during routine diagnosis of a pest widely established in a ~~country)~~country. In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be done. Where the initial identification was done using a molecular method, subsequent tests should use serological methods and vice versa. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	**Philippines** *Category : EDITORIAL*
65	66	Detection of PPV can be achieved using a biological, serological or molecular test; identification requires either a serological or molecular test. A serological or molecular test is the minimum requirement to detect and identify PPV (e.g. during routine diagnosis of a pest widely established in a country). In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be ~~done. Where the initial identification was~~ done using a ~~molecular method~~different, ~~subsequent tests should use serological methods and vice versa~~preferably more sensitive method than the one used for initial identification. Further tests may also be done to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.	**Slovenia** Molecular methods for detection of PPV are more sensitive that serological ones therefore the result obtained by molecular method cannot always be confirmed by the use of serological analysis. *Category : TECHNICAL*
66	68	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and 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.	**European Union** *Category : EDITORIAL*
67	68	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and 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.	**EPPO** We note that this is an IPPC editorial policy *Category : EDITORIAL*

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68	68	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and 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.	**Uruguay** Text deleted to avoid repetition with text in the footnote *Category : TECHNICAL*
69	74	For sample processing, approximately 0.2–0.5 g of fresh plant material is cut into small pieces and placed in a suitable tube or plastic bag. The sample is homogenized in approximately 4–10 ml (1:20 w/v) of extraction buffer using an electrical tissue homogenizer, or a manual roller, hammer or similar tool. The extraction buffer is phosphate-buffered saline (PBS) pH 7.2–7.4, containing 2% polyvinylpyrrolidone and 0.2% sodium diethyl dithiocarbamate (Cambra *et al.*, 1994), or an alternative suitably validated buffer. Plant material should be homogenized thoroughly and used fresh.	**United States of America** Second sentence: Or 1:10 as recommended by the ELISA kit manufacturer *Category : TECHNICAL*
70	74	For sample processing, approximately 0.2–0.5 g of fresh plant material is cut into small pieces and placed in a suitable tube or plastic bag. The sample is homogenized in approximately 4–10 ml (1:20 w/v) of extraction buffer using an electrical tissue homogenizer, or a manual roller, hammer or similar tool. The extraction buffer is phosphate-buffered saline (PBS) pH 7.2–7.4, containing 2% polyvinylpyrrolidone and 0.2% sodium diethyl dithiocarbamate (Cambra *et al.*, 1994), or an alternative ~~suitably validated~~ suitable buffer. Plant material should be homogenized thoroughly and used fresh.	**European Union** It is unclear what 'a validated buffer' means. *Category : TECHNICAL*
71	74	For sample processing, approximately 0.2–0.5 g of fresh plant material is cut into small pieces and placed in a suitable tube or plastic bag. The sample is homogenized in approximately 4–10 ml (1:20 w/v) of extraction buffer using an electrical tissue homogenizer, or a manual roller, hammer or similar tool. The extraction buffer is phosphate-buffered saline (PBS) pH 7.2–7.4, containing 2% polyvinylpyrrolidone and 0.2% sodium diethyl dithiocarbamate (Cambra *et al.*, 1994), or an alternative suitably ~~validated~~ buffer. Plant material should be homogenized thoroughly and used fresh.	**EPPO** It is unclear what 'a validated buffer' means *Category : TECHNICAL*
72	76	Double-antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA), ~~also called triple-antibody sandwich (TAS)-ELISA,~~ should be performed according to Cambra *et al*. (1994) using a specific monoclonal antibody such as 5B-IVIA, following the manufacturer's instructions.	**China** DASI-ELISA and TAS-ELIS are different in their operating procedures. *Category : SUBSTANTIVE*
73	76	Double-antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA), also called triple-antibody sandwich (TAS)-ELISA, should be performed according to Cambra *et al*. (1994) using a specific monoclonal ~~antibody such as 5B-IVIA, following the manufacturer's instructions~~antibody.	**China** The serological methods cannot be established based on monoclonal antibody 5B-IVIA. Most people do not have any clues how to get the Mabs. *Category : SUBSTANTIVE*
74	77	The only monoclonal antibody currently demonstrated to detect all strains of PPV with high reliability, specificity and sensitivity is 5B-IVIA (Cambra *et al.*, 2006a). Optimal detection of isolates of strain CR requires adjustment of the extraction buffer to pH 6.0	**European Union** Furthermore, it is desirable to include additional ELISA tests using other antisera. Many labs still prefer the use of polyclonal antisera, and it can be expected that these polyclonal antisera would be

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		(Chirkov *et al.*, 2013; Glasa *et al.*, 2013). In a DIAGPRO[1] ring-test conducted by 17 laboratories using a panel of 10 samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using the 5B-IVIA monoclonal antibody was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR) which was 94% accurate (Olmos *et al*., 2007; Cambra *et al*., 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using the 5B-IVIA monoclonal antibody was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote *et al.* (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using the 5B-IVIA monoclonal antibody was a true positive.	suitable for universal PPV detection as well. It is suggested to make an inventory of available antisera and their performance based on data provided by producers and/or available from tests performance studies and proficiency tests on representative isolates of all relevant strains. However, it should be noted that the quality of specific antisera might exhibit batch-to-batch variation and, therefore, needs verification of the performance. *Category : TECHNICAL*
75	77	~~The only~~ This monoclonal antibody ~~currently~~ has demonstrated to detect all strains of PPV with high reliability, specificity and sensitivity ~~is 5B-IVIA~~ (Cambra *et al.*, 2006a). Optimal detection of isolates of strain CR requires adjustment of the extraction buffer to pH 6.0 (Chirkov *et al.*, 2013; Glasa *et al.*, 2013). In a DIAGPRO[1] ring-test conducted by 17 laboratories using a panel of 10 samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using the 5B-IVIA monoclonal antibody was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR) which was 94% accurate (Olmos *et al*., 2007; Cambra *et al*., 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using the 5B-IVIA monoclonal antibody was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote *et al.* (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using the 5B-IVIA monoclonal antibody was a true positive.	**Kenya** *Category : EDITORIAL*
76	77	The only monoclonal antibody currently demonstrated to detect all strains of PPV with high reliability, specificity and sensitivity is 5B-IVIA (Cambra *et al.*, 2006a). Optimal detection of isolates of strain CR requires adjustment of the extraction buffer to pH 6.0 (Chirkov *et al.*, 2013; Glasa *et al.*, 2013). In a DIAGPRO[1] ring-test conducted by 17 laboratories using a panel of 10 samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using the	**EPPO** *Category : TECHNICAL*

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		5B-IVIA monoclonal antibody was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR) which was 94% accurate (Olmos *et al*., 2007; Cambra *et al*., 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using the 5B-IVIA monoclonal antibody was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote *et al.* (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using the 5B-IVIA monoclonal antibody was a true positive.	
77	77	The only monoclonal antibody currently demonstrated to detect all strains of PPV with high reliability, specificity and sensitivity is 5B-IVIA (Cambra *et al.*, 2006a). Optimal detection of isolates of strain CR requires adjustment of the extraction buffer to pH 6.0 (Chirkov *et al.*, 2013; Glasa *et al.*, 2013). In a DIAGPRO[1] ring-test conducted by 17 laboratories using a panel of 10 samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using the 5B-IVIA monoclonal antibody was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR) which was 94% accurate (Olmos *et al*., 2007; Cambra *et al*., 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using the 5B-IVIA monoclonal antibody was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote *et al.* (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using the 5B-IVIA monoclonal antibody was a true positive.	**Philippines** Would this protocol/procedure be available online for free once this Annex is approved *Category : SUBSTANTIVE*
78	80	The conventional or biotin–streptavidin system of double-antibody sandwich (DAS)-ELISA ~~should be performed using~~ utilize kits based on the specific monoclonal antibody 5B-IVIA or on polyclonal antibodies that have been demonstrated to detect all strains of PPV without cross-reacting with other viruses or healthy plant material (Cambra *et al*., 2006a; Capote *et al*., 2009). The test should be done according to the manufacturer's instructions.	**Philippines** *Category : EDITORIAL*
79	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in	**United States of America** There is a citation for two papers but these paper do not list Agdia's kit and just briefly mentioned the Bioreba kit/antibody for DAS ELISA. Both kits/antibodies have been successfully used for the PPV

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		situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	Eradication program in USA. The Agdia kit antibodies were developed by a Canadian scientist Dr. Ann Rochon. *Category : TECHNICAL*
80	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**European Union** Regarding the second subsection, the referred papers do not provide the data substantiating the statements on the lack of sensitivity and specificity. *Category : TECHNICAL*
81	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**European Union** http://www.bioreba.ch/popup.php?docFile=http://www.bioreba.ch/files/Product_Info/ELISA_Reagents/PPV_DAS_ELISA.pdf. *Category : SUBSTANTIVE*
82	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**EPPO** *Category : SUBSTANTIVE*
83	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**EPPO** Regarding the second subsection, the referred papers do not provide the data substantiating the statements on the lack of sensitivity and specificity. *Category : TECHNICAL*
84	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**Philippines** If this will be adopted as a standard diagnostic protocol, recommended protocol should include only those with high sensitivity, reliability and accuracy to optimize resources available. *Category : SUBSTANTIVE*
85	81	Whereas the 5B-IVIA monoclonal antibody detects all PPV strains specifically, sensitively and reliably, some polyclonal antibodies are not specific and have limited sensitivity (Cambra *et al.*, 1994; Cambra *et al.*, 2006a). The use of additional methods is therefore recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.	**Slovenia** *Category : SUBSTANTIVE*
86	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker ~~with~~ and less ~~opportunity for~~ prone to contamination (with the target DNA) than conventional PCR.	**United States of America** For clarity *Category : EDITORIAL*
87	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for	**European Union** The general introduction needs to be updated, since molecular tests are commonly used for routine and large-scale testing nowadays.

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		large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker with less opportunity for contamination (with the target DNA) than conventional PCR.	Moreover, it is desirable to make an inventory of the currently used molecular tests, since the described tests have been designed long ago and new formats, such as LAMP, have been developed since then (e.g. Ion et al., 2016; https://ijair.org/administrator/components/com_jresearch/files/publications/36_IJAIR_1842_Final.pdf). It is desirable to provide data and/or references substantiating that all strains, including the recently described strains, will be detected by the respective tests. Furthermore, guidance on using RT-PCR and sequence analysis for detection and identification of PPV should be included. *Category : TECHNICAL*
88	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker with less opportunity for contamination (with the target DNA) than conventional PCR.	**European Union** Recently, researchers from Naktuinbouw (NL) designed a new real-time RT-PCR (Taqman) for universal PPV detection, based on currently available sequence data from GenBank. Preliminary results are promising and validation is in progress. If the performance characteristics fulfil the requirements, Naktuinbouw is willing to provide the details so that the test can be included in both the IPPC and EPPO protocols. *Category : TECHNICAL*
89	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker with less opportunity for contamination (with the target DNA) than conventional PCR.	**EPPO** *Category : TECHNICAL*
90	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker with less opportunity for contamination (with the target DNA) than conventional PCR.	**EPPO** The general introduction needs to be updated, since molecular tests are commonly used for routine and large-scale testing nowadays. Moreover, it is desirable to make an inventory of the currently used molecular tests, since the described tests have been designed long ago and new formats, such as LAMP, have been developed since then (e.g. Ion et al., 2016; https://ijair.org/administrator/components/com_jresearch/files/publications/36_IJAIR_1842_Final.pdf). It is desirable to provide data and/or references substantiating that all strains, including the recently described strains, will be detected by the respective tests. Furthermore, guidance on using RT-PCR and sequence analysis for detection and identification of PPV should be included. *Category : TECHNICAL*
91	83	Molecular methods using reverse transcription-polymerase chain reaction (RT-PCR) may be more expensive or time consuming than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is	**Philippines** However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. *Category : SUBSTANTIVE*

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		therefore quicker with less opportunity for contamination (with the target DNA) than conventional PCR.	
92	84	With the exception of IC-RT-PCR (for which RNA isolation is not required), RNA extraction should be conducted using appropriately validated protocols. The samples should be placed in individual plastic bags to avoid cross-contamination during extraction. Alternatively, for real-time RT-PCR, spotted plant extracts, printed tissue sections or squashes of plant material can be immobilized on blotting paper or nylon membranes and analysed by real-time RT-PCR (Olmos *et al.*, 2005; Osman and Rowhani, 2006; Capote *et al.*, 2009). It is not recommended that spotted or tissue-printed samples be used in conventional PCR because of the lower sensitivity compared with real-time RT-PCR.	**Philippines** RNA extraction should be conducted using appropriately validated protocols. (Please include RNA Extraction Protocol as attachment of this Annex) *Category : SUBSTANTIVE*
93	85	Each of the following methods describes the volume of extracted sample that should be used as a template. Depending on the sensitivity of the method, the minimum concentration of template required to detect PPV varies as follows: RT-PCR, 100 fg RNA template/ml; Co-RT-PCR, 1 fg RNA template/ml; and real-time RT-PCR, 2 fg RNA template/ml.	**European Union** The information does not seem very useful when testing field samples. It should be indicated, how much plant material is needed to obtain 100 fg of RNA. This will depend on the applied extraction protocols. However, if there are any data available to give an Information on the quantity of plant material it should be stated. *Category : TECHNICAL*
94	85	Each of the following methods describes the volume of extracted sample that should be used as a template. Depending on the sensitivity of the method, the minimum concentration of template required to detect PPV varies as follows: RT-PCR, 100 fg RNA template/ml; Co-RT-PCR, 1 fg RNA template/ml; and real-time RT-PCR, 2 fg RNA template/ml.	**EPPO** *Category : TECHNICAL*
95	85	Each of the following methods describes the volume of extracted sample that should be used as a template. Depending on the sensitivity of the method, the minimum concentration of template required to detect PPV varies as follows: RT-PCR, 100 fg RNA template/ml; Co-RT-PCR, 1 fg RNA template/ml; and real-time RT-PCR, 2 fg RNA template/ml.	**Philippines** We recommend deletion of this paragraph because we have to be certain on the sensitivity of the method if this will be adopted as standard diagnostic protocol. *Category : SUBSTANTIVE*
96	87	The RT-PCR primers used in this method are either the primers of Wetzel *et al.* (1991):	**European Union** Please provide more details on the performance of these tests, i.e. the sensitivity expressed in relative infection rate; specificity in terms of strains as provided in the paper of Wetzel et al. (1992) instead of isolates from different countries. Moreover, information on the sample preparation (RNA extraction) is not provided. *Category : TECHNICAL*
97	87	The RT-PCR primers used in this method are either the primers of Wetzel *et al.* (1991):	**EPPO** Please provide more details on the performance of these tests, i.e. the sensitivity expressed in relative infection rate; specificity in terms of strains as provided in the paper of Wetzel et al. (1992) instead of isolates from different countries. Moreover, information on the sample preparation (RNA extraction) is not provided. *Category : TECHNICAL*
98	93	The 25 µl reaction mixture is composed as follows: 1 µM of each primer (P1 and P2, or the 3′NCR primer pair), 250 µM dNTPs, 1 unit *Avian myeloblastosis virus* (AMV) reverse transcriptase, 0.5 units Taq DNA polymerase, 2.5 µl 10× Taq polymerase buffer, 1.5 mM MgCl2, 0.3% Triton X-100 and 5 µl RNA template. The reaction is performed under the following thermocycling conditions: 45 min at 42 °C, 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at either 60 °C (P1 and P2 primers) or 62 °C (3′NCR primers), and 1 min at 72 °C, followed	**European Union** In Austria 10 µl and a commercial one step kit is used. This may be considered in an additional PCR protocol. *Category : TECHNICAL*

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		by a final extension for 10 min at 72 °C. The PCR products are analysed by gel electrophoresis. The P1/P2 pair of primers produces a 243 base pair (bp) amplicon and the 3′NCR primers produce a 220 bp amplicon.	
99	93	The 25 µl reaction mixture is composed as follows: 1 µM of each primer (P1 and P2, or the 3′NCR primer pair), 250 µM dNTPs, 1 unit *Avian myeloblastosis virus* (AMV) reverse transcriptase, 0.5 units Taq DNA polymerase, 2.5 µl 10× Taq polymerase buffer, 1.5 mM MgCl2, 0.3% Triton X-100 and 5 µl RNA template. The reaction is performed under the following thermocycling conditions: 45 min at 42 °C, 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at either 60 °C (P1 and P2 primers) or 62 °C (3′NCR primers), and 1 min at 72 °C, followed by a final extension for 10 min at 72 °C. The PCR products are analysed by gel electrophoresis. The P1/P2 pair of primers produces a 243 base pair (bp) amplicon and the 3′NCR primers produce a 220 bp amplicon.	**EPPO** In Austria 10 µl and a commercial one step kit is used. This may be considered in an additional PCR protocol *Category : TECHNICAL*
100	96	The immunocapture phase should be performed according to Wetzel *et al.* (1992), using plant sap extracted as in section 3.2 using individual tubes or plastic bags to avoid contamination.	**European Union** This test uses the same primers as the test described in 3.3.1. The difference concerns the sample preparation, i.e. immunocapture using not further specified polyclonal antibodies or 5B-IVIA. It should be noted that the antiserum of Sanofi, used for the original test described by Wetzel et al. (1992) is no longer available. Furthermore, immunocapture appears sensitive to cross contamination compared to direct RNA extraction (e.g.RNeasy), especially when positive samples are present. Finally, it is not clear if the pH of the extraction buffer should be adapted 6.0 (see 3.2.1). Therefore, we have doubts whether this test should be recommended. *Category : TECHNICAL*
101	96	The immunocapture phase should be performed according to Wetzel *et al.* (1992), using plant sap extracted as in section 3.2 using individual tubes or plastic bags to avoid contamination.	**EPPO** This test uses the same primers as the test described in 3.3.1. The difference concerns the sample preparation, i.e. immunocapture using not further specified polyclonal antibodies or 5B-IVIA. It should be noted that the antiserum of Sanofi, used for the original test described by Wetzel et al. (1992) is no longer available. Furthermore, immunocapture appears sensitive to cross contamination compared to direct RNA extraction (e.g.RNeasy), especially when positive samples are present. Finally, it is not clear if the pH of the extraction buffer should be adapted 6.0 (see 3.2.1). Therefore, we have doubts whether this test should be recommended. *Category : TECHNICAL*
102	97	A dilution (1 µg/ml) is prepared of polyclonal antibodies or PPV-specific monoclonal antibody (5B-IVIA) in carbonate buffer pH 9.6. Aliquots of 100 µl diluted antibody are dispensed into PCR tubes and incubated at 37 °C for 3 h. The tubes are then washed twice with 150 µl sterile PBS-Tween (washing buffer), and rinsed twice with RNase-free water. Plant extract (100 µl; see section 3.2) is clarified by centrifugation (5 min at 15 500 *g*), and the supernatant added to the coated PCR tubes. The tubes are incubated for 2 h on ice or at 37 °C, and then washed three times with 150 µl sterile PBS-Tween. The RT-PCR reaction mixture is prepared as described in section 3.3.1 using the primers of Wetzel *et al.* (1992), and added directly to the coated PCR tubes. The amplification is performed as described in section 3.3.1.	**Philippines** It was mentioned that some Polyclonal Antibodies are not specific and have limited sensitivity, why recommend this? *Category : SUBSTANTIVE*

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103	100	The RT-PCR primers used in this co-operational (Co)-RT-PCR are the primers of ~~Olmos~~ Wetzel *et al.* ~~(2002)~~(1991):	**European Union** *Category : TECHNICAL*
104	100	The RT-PCR primers used in this co-operational (Co)-RT-PCR are the primers of Olmos *et al.* (2002):	**EPPO** Is this still a commonly used method/test? No information is provided on the specificity. *Category : TECHNICAL*
105	109	Real-time RT-PCR can be performed using either TaqMan or SYBR® Green I. Two TaqMan methods have been described for universal detection of PPV (Schneider *et al.*, 2004; Olmos *et al.*, 2005). The primers and TaqMan probe used in the first method are those reported by Schneider *et al.* (2004):	**European Union** The test described by Varga and James (2005) presents another test for universal detection and identification of D and M strains. How does this test perform in comparison the previous one? The fact that it is a two-step protocol is a disadvantage because of the risk of cross contamination. Furthermore , the same remarks apply as for the previous tests. Since the nad5 primers have been designed by Menzel et al (2002), this reference should be included. *Category : TECHNICAL*
106	109	Real-time RT-PCR can be performed using either TaqMan or SYBR® Green I. Two TaqMan methods have been described for universal detection of PPV (Schneider *et al.*, 2004; Olmos *et al.*, 2005). The primers and TaqMan probe used in the first method are those reported by Schneider *et al.* (2004):	**EPPO** *Category : TECHNICAL*
107	109	3.3.4.1 Real-time RT-PCR can be performed using either TaqMan or SYBR® Green I. Two TaqMan methods have been described for universal detection of PPV (Schneider *et al.*, 2004; Olmos *et al.*, 2005). The primers and TaqMan probe used in the first method are those reported by Schneider *et al.* (2004):	**Philippines** *Category : EDITORIAL*
108	113	The 25 µl reaction mixture is composed as follows: 1× reaction mix (0.2 mM of each dNTP and 1.2 mM MgSO4), 200 nM each of forward and reverse primers, 100 nM TaqMan probe, 4.8 mM MgSO4, 0.5 µl RT/Platinum® Taq mix (Superscript® One-Step RT-PCR with Platinum® Taq DNA polymerase; Invitrogen)[2] and 5 µl RNA template. The RT-PCR is performed under the following thermocycling conditions: 15 min at 52 °C, 5 min at 95 °C, 60 cycles of 15 s at 95 °C, and 30 s at 60 °C. The PCR products are analysed in real-time according to the equipment manufacturer's instructions.	**European Union** In Austria 10 µl and a commercial one step kit is used. This may be considered in an additional PCR protocol. *Category : TECHNICAL*
109	113	The 25 µl reaction mixture is composed as follows: 1× reaction mix (0.2 mM of each dNTP and 1.2 mM MgSO4), 200 nM each of forward and reverse primers, 100 nM TaqMan probe, 4.8 mM MgSO4, 0.5 µl RT/Platinum® Taq mix (Superscript® One-Step RT-PCR with Platinum® Taq DNA polymerase; Invitrogen)[2] and 5 µl RNA template. The RT-PCR is performed under the following thermocycling conditions: 15 min at 52 °C, 5 min at 95 °C, 60 cycles of 15 s at 95 °C, and 30 s at 60 °C. The PCR products are analysed in real-time according to the equipment manufacturer's instructions.	**EPPO** In Austria 10 µl and a commercial one step kit is used. This may be considered in an additional PCR protocol *Category : TECHNICAL*
110	114	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and 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~~ This information is given for the ~~protocols~~ convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and or	**Uruguay** Text deleted to avoid repetition with paragraph 68. Text added according to text agreed for footnotes *Category : TECHNICAL*

#	Para	Text	Comment
		equipment named. Equivalent products may be ~~adjusted~~ used if they can be shown to lead to the ~~standards of individual laboratories, provided that these are adequately validated~~same results.	
111	116	3.3.4.2 The primers and TaqMan probe used in the second method are those reported by Olmos *et al.* (2005):	**Philippines** *Category : EDITORIAL*
112	123	3.3.4.3 Varga and James (2005) described a SYBR® Green I method for the simultaneous detection of PPV and identification of D and M strains:	**Philippines** *Category : EDITORIAL*
113	139	This section describes additional methods (using DASI-ELISA, RT-PCR, Co-RT-PCR and real-time RT-PCR) for identification of PPV strains (see Figure 1). Strain identification is not an essential component of PPV identification but an NPPO may wish to determine the identity of the strain to assist in predicting its epidemiological behaviour.	**European Union** It is confusing that this introduction refers to RT-PCR sequencing as the method for identification of strains, whereas only semi-specific molecular tests are described in this section. See also general comment. Furthermore, no information is included on the identification of the T, An and TAT strains mentioned in section 1. How should these strains be identified? Moreover, for sequence analysis, information should be provided on the regions to be used for identification as well as the database to be used for reference. *Category : TECHNICAL*
114	139	This section describes additional methods (using DASI-ELISA, RT-PCR, Co-RT-PCR and real-time RT-PCR) for identification of PPV strains (see Figure 1). Strain identification is not an essential component of PPV identification but an NPPO may wish to determine the identity of the strain to assist in predicting its epidemiological behaviour.	**EPPO** It is confusing that this introduction refers to RT-PCR sequencing as the method for identification of strains, whereas only semi-specific molecular tests are described in this section. See also general comment. Furthermore, no information is included on the identification of the T, An and TAT strains mentioned in section 1. How should these strains be identified? Moreover, for sequence analysis, information should be provided on the regions to be used for identification as well as the database to be used for reference. *Category : TECHNICAL*
115	139	This section describes additional ~~methods~~ steps for identification of PPV Strains (using DASI-ELISA, RT-PCR, Co-RT-PCR and real-time ~~RT-PCR) for identification of PPV strains~~ RT-PCR) (see Figure 1). Strain identification is not an essential component of PPV identification but an NPPO may wish to determine the identity of the strain to assist in predicting its epidemiological behaviour.	**Philippines** *Category : SUBSTANTIVE*
116	141		**United States of America** Part of this table is confusing. For the box on the lower right, under "negative". Suggest it say instead "Plum pox virus present: atypical isolate of known strain (An, C, CR...) present, OR other undescribed strain" *Category : TECHNICAL*
117	141	Delete "atypical strain An, C, CR, D, EA, M, Rec, T or W present, or". Suggest sequencing to confirm the first findings.	**China** For PPV strains or other undescribed strains should be identified through sequencing and blasting to avoid false positive results. *Category : SUBSTANTIVE*
118	141		**Philippines** Figure 1. Steps in the Identification of Strains of PPV *Category : SUBSTANTIVE*
119	144	**~~4.1 Serological identification of strains~~**	**China** Firstly, it is very difficult for most people to get the Mabs. Secondly,

#	Para	Text	Comment
			sometimes, it is not very accurate when detecting with some antibodies, and maybe it confuses to determinate the results. *Category : SUBSTANTIVE*
120	145	~~DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra *et al.* (1994), using D- and M-specific monoclonal antibodies (Cambra *et al.*, 1994; Boscia *et al.*, 1997), according to the manufacturer's instructions.~~	**European Union** Consider removing this section, since the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
121	145	~~DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra *et al.* (1994), using D- and M-specific monoclonal antibodies (Cambra *et al.*, 1994; Boscia *et al.*, 1997), according to the manufacturer's instructions.~~	**EPPO** Consider removing this section, since it the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
122	145	~~DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra *et al.* (1994), using D- and M-specific monoclonal antibodies (Cambra *et al.*, 1994; Boscia *et al.*, 1997), according to the manufacturer's instructions.~~	**China** Firstly, it is very difficult for most people to get the Mabs. Secondly, sometimes, it is not very accurate when detecting with some antibodies, and maybe it confuses to determinate the results. *Category : SUBSTANTIVE*
123	145	DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra *et al.* (1994), using D- and M-specific monoclonal antibodies (Cambra *et al.*, 1994; Boscia *et al.*, 1997), according to the manufacturer's instructions.	**Philippines** provide protocol as attachment to this Annex *Category : SUBSTANTIVE*
124	146	~~This method has been validated in the DIAGPRO ring-test, showing an accuracy of 84% for PPV-D detection and 89% for PPV-M detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). The 4D monoclonal antibody is PPV-D specific but does not react with all PPV-D isolates. Furthermore, the AL monoclonal antibody used for PPV-M detection reacts with isolates belonging to strains M, Rec and T because these groups share the same coat protein sequence. A molecular test is therefore required to differentiate between M, Rec and T strains detected using an M-specific monoclonal antibody.~~	**European Union** Consider removing this section, since the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
125	146	~~This method has been validated in the DIAGPRO ring-test, showing an accuracy of 84% for PPV-D detection and 89% for PPV-M detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). The 4D monoclonal antibody is PPV-D specific but does not react with all PPV-D isolates. Furthermore, the AL monoclonal antibody used for PPV-M detection reacts with isolates belonging to strains M, Rec and T because these groups share the same coat protein sequence. A molecular test is therefore required to differentiate between M, Rec and T strains detected using an M-specific monoclonal antibody.~~	**EPPO** Consider removing this section, since it the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
126	146	This method has been validated in the DIAGPRO ring-test, showing an accuracy of 84% for PPV-D detection and 89% for PPV-M detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). The 4D monoclonal antibody is PPV-D specific but does not react with all PPV-D isolates. Furthermore, the AL monoclonal antibody used for PPV-M detection reacts with isolates belonging to strains M, Rec and T because these groups share the	**China** Firstly, it is very difficult for most people to get the Mabs. Secondly, sometimes, it is not very accurate when detecting with some antibodies, and maybe it confuses to determinate the results. *Category : SUBSTANTIVE*

#	Para	Text	Comment
		same coat protein sequence. A molecular test is therefore required to differentiate between M, Rec and T strains detected using an M-specific monoclonal antibody.	
127	146	~~This method has been validated in the DIAGPRO ring-test, showing an accuracy of 84% for PPV-D detection and 89% for PPV-M detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). The 4D monoclonal antibody is PPV-D specific but does not react with all PPV-D isolates. Furthermore, the AL monoclonal antibody used for PPV-M detection reacts with isolates belonging to strains M, Rec and T because these groups share the same coat protein sequence. A molecular test is therefore required to differentiate between M, Rec and T strains detected using an M-specific monoclonal antibody.~~	**China** Firstly, it is very difficult for most people to get the Mabs. Secondly, sometimes, it is not very accurate when detecting with some antibodies, and maybe it confuses to determinate the results. *Category : SUBSTANTIVE*
128	147	~~Serological identification of PPV isolates from EA and C groups may be done by DASI-ELISA using the EA- or the C-specific monoclonal antibodies described by Myrta *et al.* (1998, 2000). However, these tests need to be validated.~~	**European Union** Consider removing this section, since the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
129	147	~~Serological identification of PPV isolates from EA and C groups may be done by DASI-ELISA using the EA- or the C-specific monoclonal antibodies described by Myrta *et al.* (1998, 2000). However, these tests need to be validated.~~	**EPPO** Consider removing this section, since it the described tests are already known not to detect certain isolates of target strains and are not validated. *Category : TECHNICAL*
130	147	~~Serological identification of PPV isolates from EA and C groups may be done by DASI-ELISA using the EA- or the C-specific monoclonal antibodies described by Myrta *et al.* (1998, 2000). However, these tests need to be validated.~~	**China** Firstly, it is very difficult for most people to get the Mabs. Secondly, sometimes, it is not very accurate when detecting with some antibodies, and maybe it confuses to determinate the results. *Category : SUBSTANTIVE*
131	148	**4.2 Molecular identification of strains**	**United States of America** Listing all of these assays appears excessive. At the same time primers developed by Nemchinov et all for PPV-SoC detection are not listed. Nowadays people mostly use sequencing for this purposes, especially in cases of trade disputes. Even in developing countries conventional sequencing is not such a hurdle anymore. *Category : SUBSTANTIVE*
132	148	**4.2 Molecular identification of strains**	**European Union** Varga and James (2006): C, EA, W *Category : TECHNICAL*
133	148	**4.2 Molecular identification of strains**	**EPPO** Varga and James (2006): C, EA, W *Category : TECHNICAL*
134	149	***4.2.1 Reverse transcription-polymerase chain reaction***	**European Union** Add the sequence of the PM primer that is missing in section 4.2.1. Reverse transcription-polymerase chain reaction. The sequence of primer PM should be added just below the PD primer sequence. *Category : TECHNICAL*
135	149	***4.2.1 Reverse transcription-polymerase chain reaction***	**EPPO** add the sequence of the PM primer that is missing in section 4.2.1. Reverse transcription-polymerase chain reaction. The sequence of primer PM should be added just below the PD primer sequence. *Category : TECHNICAL*

#	Para	Text	Comment
136	150	4.2.1.1 PPV-D and PPV-M are identified using the primers described by Olmos *et al.* (1997):	**Philippines** *Category : EDITORIAL*
137	153	The 25 µl reaction mixture is composed as follows: 1 µM P1 primer, 1 µM of either PD or PM primer, 250 µM dNTPs, 1 unit AMV reverse transcriptase (10 units/µl), 0.5 units Taq DNA polymerase (5 units/µl), 2.5 µl 10× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.3% Triton X-100, 2% formamide and 5 µl RNA template. The RT-PCR is performed under the following thermocycling conditions: 45 min at 42 °C, 2 min at 94 °C, ~~40~~ 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and ~~1 min~~ 30 s at 72 °C, followed by a final extension for 10 min at 72 °C. The PCR products are analysed by gel electrophoresis. The P1/PD pair of primers, and the P1/PM pair of primers, both produce a 198 bp amplicon. Olmos *et al.* (1997) evaluated their method using six isolates of PPV-D and four PPV-M isolates.	**China** 30 seconds is enough for extension when PCR is performed using the primer pairs (P1 and P2, or the 3'NCR primers). Also, we think 40 cycles are too many, 35 cycles are enough for PCR in general. *Category : TECHNICAL*
138	154	4.2.1.2 The real-time reverse transcription-polymerase chain reaction with SYBR® Green I by Varga and James (2005) described in detail above in section 3.3.4 is also suitable for the identification of D and M strains of PPV.	**Philippines** *Category : EDITORIAL*
139	155	PPV-Rec is identified using the mD5 and mM3 Rec-specific primers described by Šubr *et al.* (2004):	**European Union** Missing PCR conditions. *Category : TECHNICAL*
140	155	PPV-Rec is identified using the mD5 and mM3 Rec-specific primers described by Šubr *et al.* (2004):	**EPPO** Missing PCR conditions *Category : TECHNICAL*
141	155	4.2.1.3 PPV-Rec is identified using the mD5 and mM3 Rec-specific primers described by Šubr *et al.* (2004):	**Philippines** *Category : EDITORIAL*
142	159	4.2.1.4 PPV-CR is identified using the CR8597F and CR9023R primers described by Glasa *et al.* (2013):	**Philippines** *Category : EDITORIAL*
143	162	A two-step RT-PCR protocol is used for specific detection of PPV-CR isolates (Glasa *et al*., 2013). Complementary (c)DNA is synthesized from total RNA extracts (NucleoSpin® RNA Plant Kit, Macherey-Nagel~~[2]~~) using random hexamer primers and AMV reverse transcriptase. An aliquot of cDNA is then added to the PCR reaction mix containing EmeraldAmp GT PCR Master Mix (TaKaRa Bio Inc.~~[2]~~). The PCR is performed under the following thermocycling conditions: 1 min at 98 °C, 35 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR products are analysed by gel electrophoresis. The CR-specific primers amplify a product 427 bp in size, targeting the 5′ terminal CP coding region. The specificity of the CR primers was validated using isolates of PPV strains D, M, Rec, T, W, EA and C (Glasa *et al*., 2013).	**European Union** *Category : EDITORIAL*
144	162	A two-step RT-PCR protocol is used for specific detection of PPV-CR isolates (Glasa *et al*., 2013). Complementary (c)DNA is synthesized from total RNA extracts (NucleoSpin® RNA Plant Kit, Macherey-Nagel[1]) using random hexamer primers and	**Japan** Add information on master mix composition for two-step RT-PCR. It is necessary information in molecular methods. *Category : TECHNICAL*

#	Para	Text	Comment
		AMV reverse transcriptase. An aliquot of cDNA is then added to the PCR reaction mix containing EmeraldAmp GT PCR Master Mix (TaKaRa Bio Inc.[1]). The PCR is performed under the following thermocycling conditions: 1 min at 98 °C, 35 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR products are analysed by gel electrophoresis. The CR-specific primers amplify a product 427 bp in size, targeting the 5′ terminal CP coding region. The specificity of the CR primers was validated using isolates of PPV strains D, M, Rec, T, W, EA and C (Glasa *et al*., 2013).	
145	162	A two-step RT-PCR protocol is used for specific detection of PPV-CR isolates (Glasa *et al*., 2013). Complementary (c)DNA is synthesized from total RNA extracts (NucleoSpin® RNA Plant Kit, Macherey-Nagel[12]) using random hexamer primers and AMV reverse transcriptase. An aliquot of cDNA is then added to the PCR reaction mix containing EmeraldAmp GT PCR Master Mix (TaKaRa Bio Inc.[12]). The PCR is performed under the following thermocycling conditions: 1 min at 98 °C, 35 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR products are analysed by gel electrophoresis. The CR-specific primers amplify a product 427 bp in size, targeting the 5′ terminal CP coding region. The specificity of the CR primers was validated using isolates of PPV strains D, M, Rec, T, W, EA and C (Glasa *et al*., 2013).	**EPPO** We note that this is IPPC editorial policy. *Category : EDITORIAL*
146	171	4.2.4.1 PPV-D and PPV-M are specifically identified using either SYBR® Green I chemistry according to the method of Varga and James (2005) (see section 3.3.4) or the TaqMan method described by Capote *et al.* (2006).	**Philippines** *Category : EDITORIAL*
147	178	4.2.4.2 PPV-C, PPV-EA and PPV-W are specifically identified using SYBR® Green I chemistry according to the method of Varga and James (2006). The primers used in this method are:	**Philippines** *Category : EDITORIAL*
148	179	~~P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′)~~	**China** One-step RT-SYBR Green was used for the detection of PPV-C, -EA,-W but P1 primer was not used here, and I suggest deleting the P1 primer. *Category : SUBSTANTIVE*
149	192	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction) and, in RT-PCR, the amplification. ~~Pre-prepared (stored)~~ Total plant or viral RNA or PPV-infected plant ~~material~~ material, including printed on a membrane may be used. The stored RNA or PPV preparations should be verified periodically to determine the quality of the control with increased storage time.	**United States of America** Plasmid DNA controls also could be used if PPV infected tissue or total plant/virus RNA is not available. *Category : TECHNICAL*
150	192	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction) and, in RT-PCR, the amplification. Pre-prepared (stored) RNA or PPV-infected plant material printed on a membrane may be used. The stored RNA or PPV preparations should be verified periodically to determine the quality of the control with increased storage ~~time.~~time plus d'éclaircissement sur l'utilité de	**Algeria** *Category : TECHNICAL*

#	Para	Text	Comment
		l'emploi du terme healthy plant (plant sain) dans le cas du positive extraction control sachant que ce dernier ne peut pas être extrait d'un healthy plant ;	
151	192	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction) and, in RT-PCR, the amplification. Pre-prepared (stored) RNA or PPV-infected plant material printed on a membrane may be used. The stored RNA or PPV preparations should be verified periodically to determine the quality of the control with increased storage time.	**Philippines** we suggest that this para will recommend the use of Certified Reference Material, which is important in case of dispute in diagnosis arise bet NPPO. *Category : SUBSTANTIVE*
152	193	**Internal control.** For the ~~real-time~~ RT-PCR, mRNA of the mitochondrial gene *NADH dehydrogenase* 5 (*nad5*) could be incorporated into the RT-PCR protocol as an internal control to eliminate the possibility of RT-PCR false negatives due to nucleic acid extraction failure or degradation or the presence of RT-PCR inhibitors.	**United States of America** Menzel, W., Jelkmann, W., Maiss, E., 2002. Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. J. Virol. Methods 99, 81–92. *Category : TECHNICAL*
153	195	**Positive extraction control.** This control is used to ensure that the target nucleic acid extracted is of sufficient quantity and quality for RT-PCR and that the target virus is detectable. Nucleic acid is extracted from PPV infected host tissue, or healthy plant or insect tissues that have been spiked with PPV.	**United States of America** For clarity *Category : EDITORIAL*
154	197	**Negative extraction control.** This control is used to monitor contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. It is recommended that multiple controls be included in random order when large numbers of positive samples are expected.	**United States of America** For accuracy *Category : TECHNICAL*
155	198	In the case of immunocapture RT-PCR where no nucleic extraction is performed, plant sap from a known PPV positive should be used as a positive control, and plant sap from a healthy plant should be used as a negative control. A negative amplification control may also be included. The latter control is used to rule out false positives due to contamination during the preparation of the reaction mixture. RNase-free PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage for use as a negative amplification control.	**United States of America** Negative amplification control described in 194 still may be needed. *Category : TECHNICAL*
156	201	In instances where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance and where the virus is found in an area for the first time, the following additional material should be kept:	**United States of America** This section should give some guidance on duration of time to keep/preserve the samples. As mentioned, different labs follow different protocols. *Category : TECHNICAL*
157	201	In instances where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance and where the virus is found in an area for the first time, the following additional material should be ~~kept~~kept for at least one year:	**European Union** Important precision to be given (duration consistent with other DPs). *Category : SUBSTANTIVE*
158	201	In instances where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance and where the virus is found in an area for the first time, the following additional material should be ~~kept~~kept for at least one year:	**EPPO** Important precision to be given (duration consistent with other DPs). *Category : SUBSTANTIVE*

#	Para	Text	Comment
159	202	The original sample (labelled appropriately for traceability) should be kept ~~frozen~~ frozen, if possible, at − 80 °C or freeze-dried and kept at room temperature.	**United States of America** See United States comment in paragraph 201 *Category : TECHNICAL*
160	203	If relevant, RNA ~~extractions~~ extracts should be kept at − 80 °C and spotted plant extracts or printed tissue sections (paper on paper or nylon membranes) should be kept at room temperature.	**European Union** *Category : EDITORIAL*
161	203	If relevant, RNA ~~extractions~~ extracts should be kept at − 80 °C and spotted plant extracts or printed tissue sections (paper on paper or nylon membranes) should be kept at room temperature.	**EPPO** *Category : EDITORIAL*
162	205	~~**6. Contact Points for Further Information**~~	**Viet Nam** This section move to Appendix 1 *Category : EDITORIAL*
163	206	~~United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Registrations Identifications Permits and Plant Safeguarding (RIPPS), Molecular Diagnostic Laboratory, BARC Building 580, Powder Mill Road, Beltsville, Maryland 20705, United States of America (Ms Laurene Levy, e-mail: Laurene.Levy@aphis.usda.gov; tel.: +1 3015045700; fax: +1 3015046124).~~	**Viet Nam** para 206 move to Appendix 1 *Category : EDITORIAL*
164	206	United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), ~~Registrations Identifications Permits~~ Science and ~~Plant Safeguarding (RIPPS), Molecular Diagnostic~~ Technology Beltsville Laboratory, ~~BARC Building~~ Bldg. 580, BARC-East, Powder Mill ~~Road~~Rd., Beltsville, ~~Maryland~~ MD 20705, ~~United States of America~~ USA (Ms ~~Laurene Levy~~Vessela Mavrodieva, e-mail: vessela.a.mavrodieva@aphis.usda.gov, tel: +1 3013139208; fax: +1 3023139232).~~Laurene.Levy@aphis.usda.gov; tel.: +1 3015045700; fax: +1 3015046124).~~	**United States of America** Updated the USDA laboratory contact information. *Category : EDITORIAL*
165	207	~~Equipe de Virologie Institut National de la Recherche Agronomique (INRA), Centre de Bordeaux, UMR GD2P, IBVM, BP 81, F-33883 Villenave d'Ornon Cedex, France (Mr Thierry Candresse, e-mail: tc@bordeaux.inra.fr; tel.: +33 557122389; fax: +33 557122384).~~	**Viet Nam** para 207 move to Appendix 1 *Category : EDITORIAL*
166	208	~~Faculty of Horticultural Science, Department of Plant Pathology, Corvinus University, Villányi út 29-43, H-1118 Budapest, Hungary (Mr Laszlo Palkovics, e-mail: laszlo.palkovics@uni-corvinus.hu; tel.: +36 14825438; fax: +36 14825023).~~	**Viet Nam** para 208 move to Appendix 1 *Category : EDITORIAL*
167	209	~~Institute of Virology, Slovak Academy of Sciences, Dúbravská, 84505 Bratislava, Slovakia (Mr Miroslav Glasa, e-mail: virumig@savba.sk; tel.: +421 259302447; fax: +421 254774284).~~	**Viet Nam** para 2096 move to Appendix 1 *Category : EDITORIAL*

#	Para	Text	Comment
168	210	~~Instituto Valenciano de Investigaciones Agrarias (IVIA), Plant Protection and Biotechnology Centre, Carretera Moncada-Náquera km 5, 46113 Moncada (Valencia), Spain (Mr Antonio Olmos, e-mail: aolmos@ivia.es; tel.: +34 963424000; fax: +34 963424001).~~	**Viet Nam** para 210 move to Appendix 1 *Category : EDITORIAL*
169	211	~~Istituto di Virologia Vegetale del CNR, sezione di Bari, via Amendola 165/A, I-70126 Bari, Italy (Mr Donato Boscia, e-mail: d.boscia@ba.ivv.cnr.it; tel.: +39 0805443067; fax: +39 0805442911).~~	**Viet Nam** para 211 move to Appendix 1 *Category : EDITORIAL*
170	212	~~Sidney Laboratory, Canadian Food Inspection Agency (CFIA), British Columbia, V8L 1H3 Sidney, Canada (Mr Delano James, e-mail: Delano.James@inspection.gc.ca; tel.: +1 250 3636650; fax: +1 250 3636661).~~	**Viet Nam** para 212 move to Appendix 1 *Category : EDITORIAL*
171	213	~~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), who will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).~~	**Viet Nam** para 213 move to Appendix 1 *Category : EDITORIAL*
172	214	~~**7. Acknowledgements**~~	**Viet Nam** This section move to Appendix 2 *Category : EDITORIAL*
173	215	~~This diagnostic protocol was drafted by Mr M. Cambra, Mr A. Olmos and N. Capote, IVIA (see preceding section); Mr N.L. Africander, Department of Agriculture, Forestry and Fisheries, Private Bag X 5015, Stellenbosch, 75999, South Africa; Ms L. Levy, USDA, United States of America (see preceding section); Mr S.L. Lenardon, Instituto de Fitopatologia y Fisiologia Vegetal - Instituto Nacional de Tecnologia Agropecuaria (IFFIVE-INTA), Cno. 60 Cuadras Km 51/2, Córdoba X5020ICA, Argentina; Mr G. Clover, Plant Health & Environment Laboratory, Ministry of Agriculture and Forestry, PO Box 2095, Auckland 1140, New Zealand; and Ms D. Wright, Plant Health Group, Central Science Laboratory, Sand Hutton, York YO41 1LZ, United Kingdom.~~	**Viet Nam** para 215 move to Appendix 2 *Category : EDITORIAL*
174	235	**García, J.A. & Cambra, M.** 2007. *Plum pox virus* and sharka disease. *Plant Viruses*, 1: 69–79. García,J.A.,Glasa,M.,Cambra,M.,andCandresse,T.(2014). Plum pox virus and sharka:a model potyvirus and a major disease. Mol.PlantPathol. 15, 226–241. doi:10.1111/mpp.12083	**Kenya** *Category : TECHNICAL*
175	238	**~~James~~Ilardi,V &Tarazza, ~~D., Varga, A~~M. ~~& Sanderson~~2015. Biotechnological strategies and tools for plum pox reistance; teans-,intra-, ~~D.~~cis-genesis and beyond. Frontiers in plant science,6:1-16**	**Kenya** *Category : TECHNICAL*

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#	Para	Text	Comment
		James, D., Varga, A. & Sanderson, D. 2013. Genetic diversity of *Plum pox virus*: Strains, disease and related challenges for control. *Canadian Journal of Plant Pathology*, 35: 431–441.	
176	240	**Levy, L. & Hadidi, A.** 1994. A simple and rapid method for processing tissue infected with *Plum pox potyvirus* for use with specific 3′ non-coding region RT-PCR assays. *EPPO Bulletin*, 24: 595–604. **Menzel W, Jelkmann W & Maiss E**. 2002. Detection of four apple viruses by multiplex RT-PCR tests with co-amplification of plant mRNA as internal control. Journal of Virological Methods 99, 81–92.	**European Union** Add the following reference (see comment on section 3.3.4). *Category : TECHNICAL*
177	240	**Levy, L. & Hadidi, A.** 1994. A simple and rapid method for processing tissue infected with *Plum pox potyvirus* for use with specific 3′ non-coding region RT-PCR assays. *EPPO Bulletin*, 24: 595–604. Menzel W, Jelkmann W & Maiss E (2002) Detection of four apple viruses by multiplex RT-PCR tests with co-amplification of plant mRNA as internal control. Journal of Virological Methods 99, 81–92.	**EPPO** add the following reference (see comment on section 3.3.4 *Category : TECHNICAL*
178	251	**Šubr, Z., Pittnerova, S. & Glasa, M.** 2004. A simplified RT-PCR-based detection of recombinant *Plum pox virus* isolates. *Acta Virologica*, 48: 173–176. Teshale, 2014. Evaluation of Molecular and Serological diagnostic techniques for a large scale detection of plum pox virus. Research in Plant sciences, 2:33-41	**Kenya** *Category : TECHNICAL*