2004-019: Draft Annex to ISPM 27 – Tomato spotted wilt virus, Impatiens necrotic spot virus and Watermelon silver mottle virus

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
1.	*G*	Editorial	We support the contents of the diagnostic protocol. However, since there are errors in the references in the text, these should by corrected. They should be carefully checked when creating a final draft.	.	Japan
2.	*G*	Substantive	I support the document as it is and I have no comments		Guyana, Congo, Mexico
3.	*G*	Substantive	Include guidance on equivalence and look at scope for including not just surveillance.	Equivalence: While it is necessary and beneficial to have sufficiently detailed diagnostic protocols for practical usefulness, this could also be perceived as quite prescriptive and potentially restrictive. It would be advisable to also include a guidance/framework for proposing, evaluating, validating and implemented equivalent diagnostic protocols. Scope: The proposed annex appears focused on surveillance. ISPM 27 scope does primarily cover surveillance diagnostic protocols, but it also covers pest identification associated with imported consignments. The authors appear to be considering the draft chiefly within the narrower scope of surveillance. The draft should acknowledge this limited scope and that additional consideration may be required for the application of diagnostic protocols in the broader context of phytosanitary measures. Alternatively, expand the scope of the annex.	Australia
4.	*G*	Substantive	Footnotes related to the use of commercial brands should be included in the draft DP	The draft protocol includes at least 6 footnotes (Nº 1 to 6) but the text of the footnotes is missing. Commercial brands are mentioned in paragraphs 52, 60 and 79. The same number of footnote should be associated to the brands mentioned in these paragraphs. The footnote should read: "The use of the brands .....,....in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the	Uruguay, Argentina, Chile

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				convenience of users of this protocol and does not constitute and endorsment by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results"	
5.	*G*	Technical		Although it is acknowledged that the current protocol is concerned with the three most economically important tospoviruses more information is now available and a broader scope could be considered in a future revision. Of the tests described in this protocol, only bioassay and maybe the generic RT-PCR enable a broad detection. For the latter test, which has been designed already many years ago, crucial validation data on specificity are missing. We would like to let you know that recently, a generic RT-PCR test for detection of tospoviruses, has been designed which could be a valuable addition to a broader scope of the protocol (Hassani-Meharaban et al, 2015, manuscript in preparation NPPO-NL). This RT-PCR test additionally can be used for (preliminary) identification of the species. The other specific tests described in this protocol, have their value for specific screening purposes. However, also for this application, more validation data on specificity are desirable. In this case it is important to know if all variants of a species would be detected. Although it is acknowledge that the scope of the protocol is not generic detection, the generic tests should be better highlighted in the protocol. Since the test performance studies were conducted on some tests additional tospovirus species have been described and not information is available on the analytical specificity of these tests regarding these new viruses. Terminology In general in protocols, when sensitivity and specificity are referred to it should be specified if this is analytical or diagnostic . When preparing the EPPO Standard on interlaboratory comparison Quality Assurance experts (from outside plant pest diagnostics) commented that the term ring test should	European Union

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				not be used any longer but test performance study should be used instead. This terminology has now been adopted in EPPO Standards. We suggest replacing ring test by test performance study Although it is acknowledged that the current protocol is concerned with the three most economically important tospoviruses more information is now available and a broader scope could be considered in a future revision. Of the tests described in this protocol, only bioassay and maybe the generic RT-PCR enable a broad detection. For the latter test, which has been designed already many years ago, crucial validation data on specificity are missing. We would like to let you know that recently, a generic RT-PCR test for detection of tospoviruses, has been designed which could be a valuable addition to a broader scope of the protocol (Hassani-Meharaban et al, 2015, manuscript in preparation NPPO-NL). This RT-PCR test additionally can be used for (preliminary) identification of the species. The other specific tests described in this protocol, have their value for specific screening purposes. However, also for this application, more validation data on specificity are desirable. In this case it is important to know if all variants of a species would be detected. Although it is acknowledge that the scope of the protocol is not generic detection, the generic tests should be better highlighted in the protocol. Since the test performance studies were conducted on some tests additional tospovirus species have been described and not information is available on the analytical specificity of these tests regarding these new viruses. In general in protocols, when sensitivity and specificity are referred to it should be specified if this is analytical or diagnostic . When preparing the EPPO Standard on interlaboratory comparison Quality Assurance experts (from outside plant pest diagnostics) commented that the term ring test should not be used any longer but test performance study	

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				should be used instead. This terminology has now been adopted in EPPO Standards. We suggest replacing ring test by test performance study	
6.	*G*	Technical	rewording on biological indexing	It appears that the draft standard proposes that biological indexing is adequate alone for verification of virus absence, with positives requiring secondary serological/molecular confirmation. Additionally the standard appears to suggest that biological indexing is the preferred primary screening method. It is understandable that a country may not want to conduct a secondary confirmation test for a negative result for efficiency and economy in surveillance to focus resources on positives, however this may not be appropriate in all situations. Additionally, it appears restrictive that biological indexing is being proposed as the sole primary screening method. Depending on circumstances, it may be more appropriate to use serological/molecular techniques in this role. Is it possible that this section be revised to allow greater flexibility, acknowledging of course that these are minimum requirements.	Australia
7.	*8*	Editorial	The genus *Tospovirus* includes the plant-pathogenic, thrips-transmitted members of the family Bunyaviridae. Tospoviruses are transmitted exclusively by thrips belonging to the family Thripidae, subfamily Thripinae (Riley *et al.*, 2011). There are eight definite members of the genus *Tospovirus*, of which *Tomato spotted wilt virus* (TSWV) is the type species, and at least 15 tentative members (King *et al.*, 2012). Tospoviruses have been classified according to serological differences but more recent classifications are based on molecular data (de Avila *et al.*, 1993). Viruses in the family Bunyaviridae have genomes composed of three negative or ambisense single-stranded RNAs that occur as ribonucleoprotein complexes (RNPs). Characteristic pleomorphic virus particles are formed by enclosure of RNPs in a host-derived membrane studded with surface projections composed of virally encoded glycoproteins. The viruses of this family are quasi-	King et al. (2012) should be listed in “8. References”. King et al. (2012) is cited in the main text. However, the article is not listed in “8. References”.	Japan

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			spherical, enveloped plant viruses 70–110 nm in diameter (EPPO, 1999a; Mumford *et al*., 1996b).		
8.	*8*	Technical	The genus *Tospovirus* includes the plant-pathogenic, thrips-transmitted members of the family Bunyaviridae. Tospoviruses are transmitted exclusively by thrips belonging to the family Thripidae, subfamily Thripinae (Riley *et al.*, 2011). There are eight definite members of the genus *Tospovirus*, of which *Tomato spotted wilt virus* (TSWV) is the type species, and at least 15 tentative members (King *et al.*, 2012). For latest data consult ICTV online: http://ictvonline.org/ . Tospoviruses have been classified according to serological differences but more recent classifications are based on molecular data (de Avila *et al.*, 1993). Viruses in the family Bunyaviridae have genomes composed of three negative or ambisense single-stranded RNAs that occur as ribonucleoprotein complexes (RNPs). Characteristic pleomorphic virus particles are formed by enclosure of RNPs in a host-derived membrane studded with surface projections composed of virally encoded glycoproteins. The viruses of this family are quasi-spherical, enveloped plant viruses 70–110 nm in diameter (EPPO, 1999a; Mumford *et al*., 1996b).	Information should be provided on where to find the most up todate information as well. It is suggested to add the following sentence For latest data consult ICTV online: http://ictvonline.org/.	European Union
9.	*8*	Technical	Add a statement to indicate the genus Tospvirus is the only plant infecting virus, others being animal viruses.The genus *Tospovirus* includes the plant-pathogenic, thrips-transmitted members of the family Bunyaviridae. Tospoviruses are transmitted exclusively by thrips belonging to the family Thripidae, subfamily Thripinae (Riley *et al.*, 2011). There are Eleven-International committees of taxonomy 0 viruses, 2014 Report. eight definite members of the genus *Tospovirus*, of which *Tomato spotted wilt virus* (TSWV) is the type species, and at least increase tentative number to 18 international committee of taxonomy viruses 15 tentative members (King *et al.*, 2012). Tospoviruses have been classified according to serological differences but more recent classifications	The small RNA segment nucleoprotein is the one generally used for identification. Pest information needs to be enhanced to provide comprehensive biology that reflects on the special nature of the pest. Example 1.) The vector acquires the virus at the larval stage. 2.) The virus replicates in the thrips before it can be transmitteed by the adults 3). There is no transvarian transmission not passed from adults to the larvae.	Kenya

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			are based on molecular data (de Avila *et al.*, 1993). Viruses in the family Bunyaviridae have genomes composed of three negative or ambisense single-stranded RNAs that occur as ribonucleoprotein complexes (RNPs). Characteristic pleomorphic virus particles are formed by enclosure of RNPs in a host-derived membrane studded with surface projections composed of virally encoded glycoproteins. The viruses of this family are quasi-spherical, enveloped plant viruses 70–110 nm in diameter (EPPO, 1999a; Mumford *et al*., 1996b).		
10.	*9*	Editorial	Tospoviruses cause devastating crop losses because of their wide distribution, broad host range (approximately 1000 plant species) and the circulative replicative relationship between the virus and its thrips vector. This diagnostic protocol covers the three most economically important tospoviruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). Examples of economically important hosts for TSWV are *Arachis hypogea* (peanut), *Capsicum annuum* (sweet pepper), *Carica papaya* (papaya), *Lactuca sativa* (lettuce), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* *(Lycopersicon esculentum)* (tomato) and *Solanum tuberosum* (potato) (EPPO, 1999a). INSV also causes significant damage in vegetable crops as well as in ornamental plants, including *Ageratum* spp., *Begonia* spp., *Chrysanthemum* spp. and *Impatiens* spp. (EPPO, 1999b). WSMoV is a pathogen of cucurbits, the principal hosts being *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) (EPPO, 1999c). Spread or movement of all three of the viruses and their vectors on infected nursery stock is common, making detection and removal of infected material crucial.	To ensure consistency in the binomial name of tomato i.e. either Solanum lycopersicum or its synonyms -Lycopersicon lycopersicum or L. esculentum. Currently, in this DP, tomato is referred to as Solanum lycopersicum in para 9 but Lycopersicon esculentum is used in table 1 (para 43) under the indicator hosts for the viruses.	Singapore
11.	*9*	Editorial	Tospoviruses cause devastating crop losses because of their wide distribution, broad host range (approximately 1000 plant species) and the circulative replicative relationship between the virus and its thrips vector. This diagnostic protocol covers the three most	Corrigendum	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			economically important tospoviruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). Examples of economically important hosts for TSWV are *Arachis hypogaea* (peanut), *Capsicum annuum* (sweet pepper), *Carica papaya* (papaya), *Lactuca sativa* (lettuce), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) (EPPO, 1999a). INSV also causes significant damage in vegetable crops as well as in ornamental plants, including *Ageratum* spp., *Begonia* spp., *Chrysanthemum* spp. and *Impatiens* spp. (EPPO, 1999b). WSMoV is a pathogen of cucurbits, the principal hosts being *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) (EPPO, 1999c). Spread or movement of all three of the viruses and their vectors on infected nursery stock is common, making detection and removal of infected material crucial.		
12.	*9*	Substantive	Tospoviruses cause devastating crop losses because of their wide distribution, broad host range (approximately 1000 plant species) and the circulative replicative relationship between the virus and its thrips vector. This diagnostic protocol covers the three most economically important tospoviruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). Examples of economically important hosts for TSWV are *Arachis hypogea* (peanut), *Capsicum annuum* (sweet pepper), *Carica papaya* (papaya), *Lactuca sativa* (lettuce), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) (EPPO, 1999a). INSV also causes significant damage in vegetable crops as well as in ornamental plants, including *Ageratum* spp., *Begonia* spp., *Chrysanthemum* spp. and *Impatiens* spp. (EPPO, 1999b). WSMoV is a pathogen of cucurbits, the principal hosts being *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) (EPPO, 1999c). Spread or movement of all three of the viruses and their vectors	In case the imported plant products (which are hosts to the Tospoviruses) are infected by thrips, What kind of tests that can be carried out on thrips taking into consideration the relationship between the virus and its vector?	Bahrain

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			on infected nursery stock is common, making detection and removal of infected material crucial.		
13.	*9*	Substantive	Tospoviruses cause devastating crop losses because of their wide distribution, broad host range (approximately 1000 plant species) and the circulative replicative relationship between the virus and its thrips vector. This diagnostic protocol covers the three most economically important tospoviruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). Examples of economically important hosts for TSWV are *Arachis hypogea* (peanut), *Capsicum annuum* (sweet pepper), *Carica papaya* (papaya), *Lactuca sativa* (lettuce), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) (EPPO, 1999a). INSV also causes significant damage in vegetable crops as well as in ornamental plants, including ~~*Ageratum* spp.,~~ *Begonia* spp., ~~*Chrysanthemum* spp.~~ and *Impatiens* spp. (EPPO, 1999b). WSMoV is a pathogen of cucurbits, the principal hosts being *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) (EPPO, 1999c). Spread or movement of all three of the viruses and their vectors on infected nursery stock is common, making detection and removal of infected material crucial.	Ageratum spp. and Chrysanthemum spp. are not described as the host of INSV in EPPO (1999b) .	Japan
14.	*9*	Technical	Tospoviruses cause ~~devastating~~ crop losses because of their wide distribution, broad host range (approximately 1000 plant species) and the circulative replicative relationship between the virus and its thrips vector. This diagnostic protocol covers the three most economically important tospoviruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). Examples of ~~economically important~~ hosts for TSWV are *Arachis hypogea* (peanut), *Capsicum annuum* (sweet pepper), *Carica papaya* (papaya), *Lactuca sativa* (lettuce), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato)	place value on the level of losses as a result e.g e.g upto 95% losses	Kenya

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			(EPPO, 1999a). add ornamentals. INSV also causes ~~significant damage~~ in vegetable crops as well as in ornamental plants, including *Ageratum* spp., *Begonia* spp., *Chrysanthemum* spp. and *Impatiens* spp. (EPPO, 1999b). WSMoV is a pathogen of cucurbits, the principal hosts being *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) (EPPO, 1999c). Spread or movement of all three of the viruses and their vectors on infected nursery stock is common, making detection and removal of infected material crucial.		
15.	*10*	Editorial	TSWV is one of the most widespread plant viruses and occurs in countries of ~~Europe, Asia,~~ Africa, Asia, Central America and the Caribbean, Europe, North America, ~~Central America and the Caribbean,~~ Oceania and South America ~~and Oceania~~ (EPPO, 1999b). INSV has a more restricted geographic distribution than TSWV, occurring within Europe, Asia, North America, Central America and the Caribbean, Africa and Australasia (EPPO, 1999b). WSMoV is currently restricted to Asia and possibly parts of South America (EPPO, 1999c).	Change the listing of areas to an alphabetical order.	European Union
16.	*10*	Editorial	TSWV is one of the most widespread plant viruses and occurs in countries of Europe, Asia, Africa, North America, Central America and the Caribbean, South America and Oceania (EPPO, 1999a~~b~~). INSV has a more restricted geographic distribution than TSWV, occurring within Europe, Asia, North America, Central America and the Caribbean, Africa and Australasia (EPPO, 1999b). WSMoV is currently restricted to Asia and possibly parts of South America (EPPO, 1999c).	The reference that described TSWV is EPPO (1999a).	Japan
17.	*10*	Substantive	TSWV is one of the most widespread plant viruses and occurs in countries of Europe, Asia, Africa, North America, Central America and the Caribbean, South America and Oceania (EPPO, 1999b). INSV has a more restricted geographic distribution than TSWV, occurring within Europe, Asia, North America, Central America and the Caribbean, Africa and Australasia but this may partly reflect the fact that the two viruses have only rather recently been distinguished (EPPO, 1999b).	The next sentence is described in EPPO (1999b). "but this may partly reflect the fact that the two viruses have only rather recently been distinguished." This should be added as reference information, because of the uncertain distribution of these viruses.	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			WSMoV is currently restricted to Asia and possibly parts of South America (EPPO, 1999c).		
18.	*11*	Substantive	It should be verified whether seed transmission can cause disease spread.The three viruses are all transmitted and spread in nature by thrips (*Frankliniella* spp. and *Thrips* spp.), which acquire virus during the larval stages and transmit it via the adults. The viruses are not reported to be seed- or pollen-transmitted or mechanically transmitted by contact between plants.However, experimentally, they may be transmitted mechanically or by grafting (EPPO, 1999a, 1999b, 1999c).	It has been confirmed that the virus is present in the seed. But it should be verified whether seed transmission can cause disease spread.	China
19.	*14*	Substantive	**Synonyms:** ~~None~~Pineapple yellow spot virus(EPPO,1999a)	This is described in EPPO (1999a).	Japan
20.	*22*	Substantive	**Synonyms:** ~~None~~Watermelon silvery mottle virus,Watermelon tospovirus,TSWV-W(EPPO,1999b)	This is described in EPPO (1999b)	Japan
21.	*26*	Substantive	Add the detection of hosts seeds.All plant parts of infected hosts, except seeds and pollen, can potentially harbour the viruses. Lists of hosts of TSWV, INSV and WSMoV hosts are provided in EPPO (1999b), (1999a) and (1999c), respectively.	China have detected and identified TSWV many times in the imported hosts seeds.	China
22.	*26*	Technical	All plant parts of infected hosts, except seeds and pollen, can potentially harbour the viruses. Lists of hosts of TSWV, INSV and WSMoV hosts are provided in EPPO (1999b), (1999a) and (1999c), respectively.	Regarding the statement "all plant parts of infected hosts, except seeds and pollen, can potentially harbour the viruses" : a reference should be added if possible.	European Union
23.	*27*	Editorial	Tospoviruses generally induce symptoms that include leaf necrosis, chlorosis, ring patterns, mottling, silvering, local lesions and stunting. Symptoms depend upon the strain of the virus, the host plant, and the environmental conditions at the time of infection and plant growth. However, in combination with other information such as the presence of thrips, symptoms can be an indicator for the presence of a tospovirus. More detailed symptom descriptions for TSWV, INSV and WSMoV are given below and have been described also in publications by Cho et *al*~~al~~. (1987, Lisa *et al.* (1990), Yeh *et al*. (1992), Daughtrey (1996) and Chatzivassiliou *et al.* (2000).	italics missing.	European Union

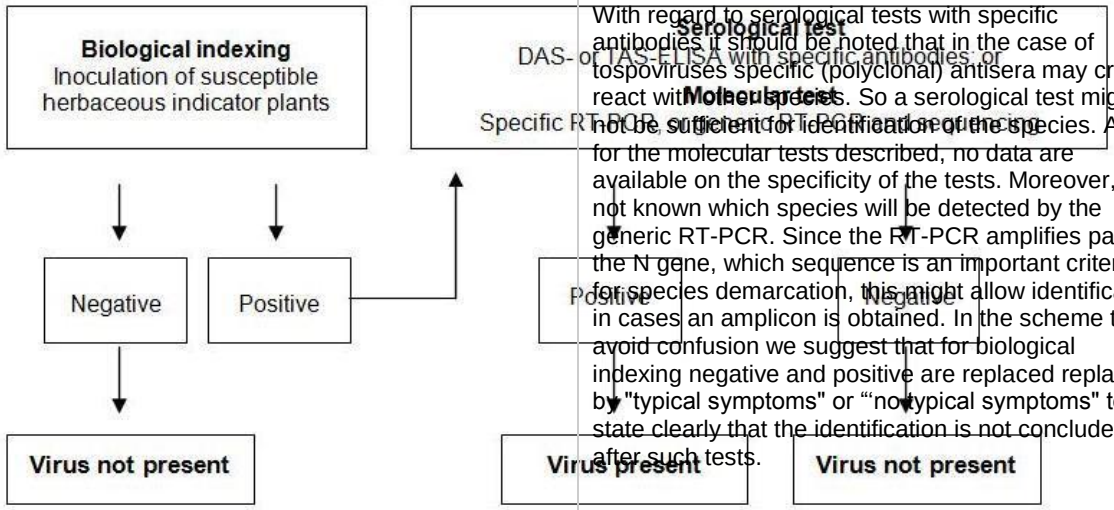
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
24.	*27*	Editorial	Tospoviruses generally induce symptoms that include leaf necrosis, chlorosis, ring patterns, mottling, silvering, local lesions and stunting. Symptoms depend upon the strain of the virus, the host plant, and the environmental conditions at the time of infection and plant growth. However, in combination with other information such as the presence of thrips, symptoms can be an indicator for the presence of a tospovirus. More detailed symptom descriptions for TSWV, INSV and WSMoV are given below and have been described also in publications by Cho et al. (1987), Lisa *et al.* (1990), Yeh *et al*. (1992), Daughtrey (1996) and Chatzivassiliou *et al.* (2000).	Corrigendum	Japan
25.	*28*	Substantive	TSWV symptoms on tomato include leaf bronzing, curling, necrotic spots, necrotic streaks and stunting of the plants. Fruit symptoms are usually either irregular yellow–orange flecks and occasionally rings on red fruits, or necrotic lesions or rings on other fruits. Ripe fruits of affected plants have paler red or yellow skin. Affected plants may have severe necrosis and sometimes die prematurely. On *C. annuum*, the first symptom is vein yellowing, which is usually followed by chlorosis, stunting and yellowing of the plant, chlorotic line patterns or mosaics with necrotic spots on leaves, and necrotic streaks on stems extending to terminal shoots. Yellow spots or necrotic streaks may be observed on ripe fruits. On *L. sativa*, the main symptom is the appearance of numerous necrotic lesions; other symptoms include leaf discoloration and one-sided growth. On *N. tabacum*, necrotic lesions, necrotic rings and chlorotic rings are observed. ~~On *Solanum melongena* (aubergine) and *Vicia faba* (fava bean), symptoms include necrotic lesions on the leaves (Cho *et al*., 1987; Daughtrey *et al*., 1997)~~.	The corresponding description is not described in Cho et al. (1987) and Daughtrey et al. (1997).	Japan
26.	*28*	Translation	TSWV symptoms on tomato include leaf bronzing, curling, necrotic spots, necrotic streaks andstunting of the plants.(add plants turn purple as an additional symptom) Fruit symptoms are usually either irregular yellow–orange flecks and occasionally rings on red and	make it clear on wheather other the statement is refering to tomato fruit when stating other fruits - scope	Kenya

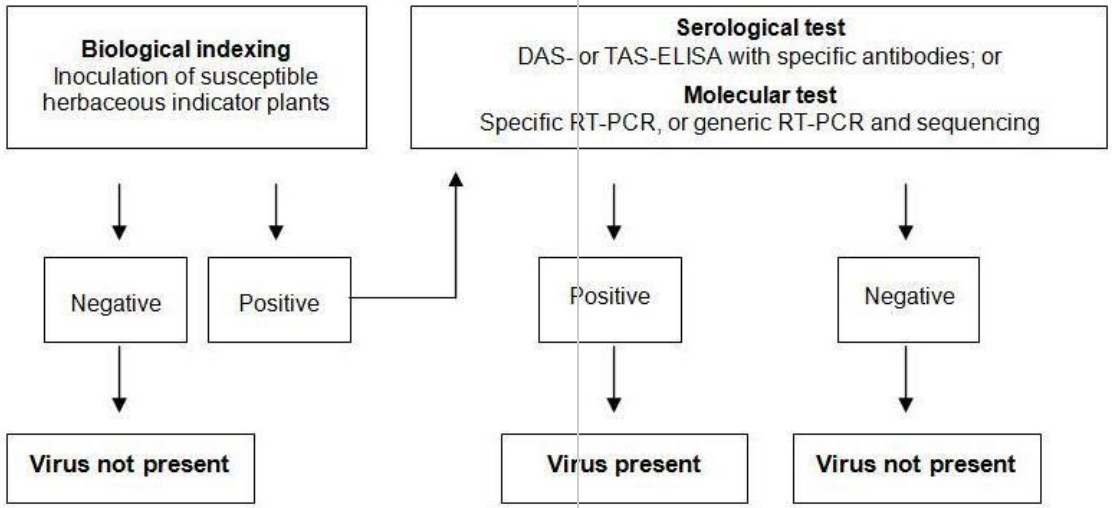
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			green fruits, or necrotic lesions or rings (on other fruits). Ripe fruits of affected plants have paler red or yellow skin. Affected plants may have severe necrosis and sometimes die prematurely. On *C. annuum*, the first symptom is vein yellowing, which is usually followed by chlorosis, stunting and yellowing of the plant, chlorotic line patterns or mosaics with necrotic spots on leaves, and necrotic streaks on stems extending to terminal shoots. Yellow spots or necrotic streaks may be observed on ripe fruits. On *L. sativa*, the main symptom is the appearance of numerous necrotic lesions; other symptoms include leaf discoloration and one-sided growth. On *N. tabacum*, necrotic lesions, necrotic rings and chlorotic rings are observed on leaves. On *Solanum melongena* (aubergine) and *Vicia faba* (fava bean), symptoms include necrotic lesions on the leaves (Cho *et al*., 1987; Daughtrey *et al*., 1997).		
27.	*29*	Editorial	INSV symptoms on New Guinea impatienshybrids include stunting, leaf spots and black discoloration at the leaf bases. A range of symptoms occurs on ornamental plant hosts such as *Alstroemeria* spp., *Gladiolus* spp. and *Lobelia* spp., and on vegetable crops such as *C. annuum*, *Cichorium endivia* (endive), *Cucumis sativus* (cucumber), and *L. sativa* (Cho *et al*., 1987; Daughtrey *et al*., 1997).	the name is not correct	European Union
28.	*29*	Editorial	INSV symptoms on New Guinea impatienshybrids include stunting, leaf spots and black discoloration at the leaf bases. A range of symptoms occurs on ornamental plant hosts such as *Alstromeria* spp., *Gladiolus* spp. and *Lobelia* spp., and on vegetable crops such as *C. annuum*, *Cichorium endivia* (endive), *Cucumis sativus* (cucumber), and *L. sativa* (~~Cho *et al*., 1987; Daughtrey *et al*., 1997~~EPPO, 1999b).	Cho et al. (1987) and Daughtrey et al. (1997) are cited in the main text as references. However, the information cited in the main text cannot be found in these papers.	Japan
29.	*30*	Editorial	WSMoV symptoms on *Citrullus lanatus* include foliar mottling, crinkling, yellow spotting and narrowing of leaf laminae as well as the growth of small, malformed fruits with necrotic spots or silver mottling, a reduced fruit set, upright growth of branches and tip necrosis. On *Cucumis melo*, foliar mottling, stunting, upright growth	Cho et al. (1987) and Daughtrey et al. (1997) are cited in the main text as references. However, the information cited in the main text cannot be found in these papers.	Japan

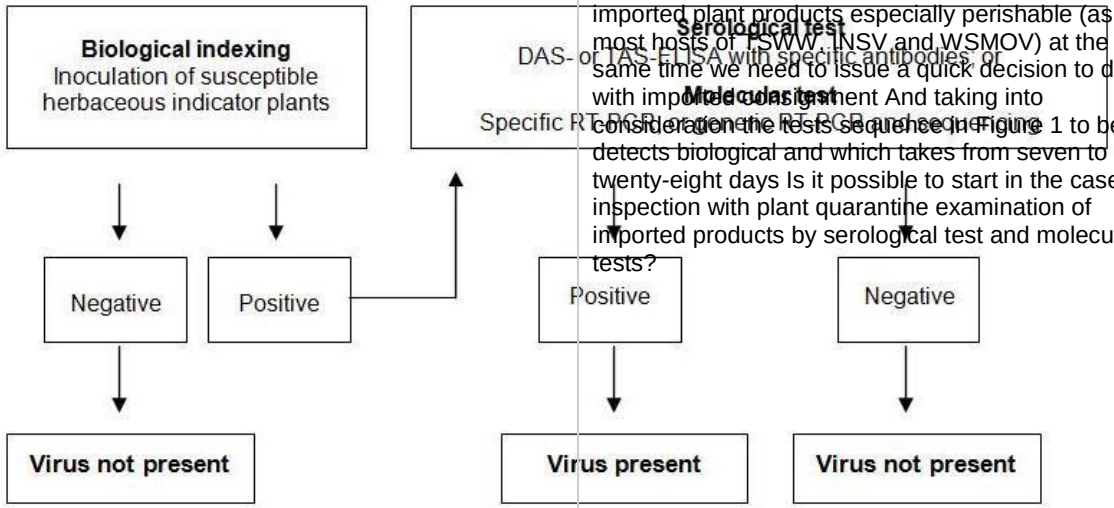
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			of branches and tip blight are observed (~~Cho *et al.*, 1987; Daughtrey *et al.*, 1997~~Yeh et al., 1992; EPPO, 1999c).		
30.	*30*	Substantive	WSMoV symptoms on *Citrullus lanatus* include foliar mottling, crinkling, yellow spotting and narrowing of leaf laminae as well as the growth of small, malformed fruits with necrotic spots or silver mottling, a reduced fruit set severly stunted, shorted internods, upright growth of branches and tip necrosis. On *Cucumis melo*, foliar mottling, stunting, upright growth of branches and tip blight are observed (Cho *et al*., 1987; Daughtrey *et al*., 1997).	This is described in Yeh et al. (1992) and EPPO (1999c), and this is effective information as a guide for symptoms.	Japan
31.	*31*	Substantive	It is better to give the sampling methods, such as fruit, stalk, petals and Thrips.Appropriate sample selection is important for the detection of tospoviruses because they can be unevenly distributed in naturally infected hosts. Virus titre is likely to be low in hosts that have been infected recently by viruliferous thrips, depending on environmental conditions and on the host species or cultivar. Symptomatic leaves (or parts of symptomatic leaves, for example around necrotic lesions) should be used when available. It is recommended that newly expanded leaves should be selected rather than senescing material. Leaves should be stored at 4 °C for no more than seven days before processing.	Because of the system infection, many parts of infected hosts and Thrips can potentially harbour the viruses.	China
32.	*31*	Technical	Appropriate sample selection is important for the detection of tospoviruses because they can be unevenly distributed in naturally infected hosts. Virus titre is likely to be low in hosts that have been infected recently by viruliferous thrips, depending on environmental conditions and on the host species or cultivar. Symptomatic leaves (or parts of symptomatic leaves, for example around necrotic lesions) should be used when available. It is recommended that newly expanded leaves should be selected rather than senescing material. Leaves should be stored at 4 °C for no more than seven days before processing. Alternatively leaves can be stored as prepared/sampled at minimum -80°C for longer periods.	Storage at -80, should also be recommended as an alternative option at the end of the paragraph.	European Union

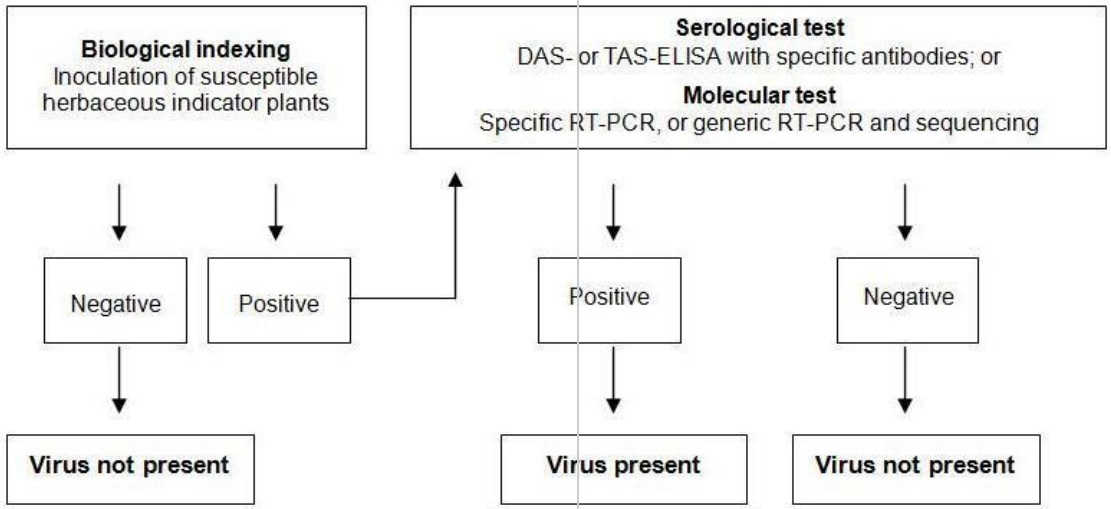
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
33.	*31*	Technical	Appropriate sample selection is important for the detection of tospoviruses because they can be unevenly distributed in naturally infected hosts. Virus titre is likely to be low in hosts that have been infected recently by viruliferous thrips, depending on environmental conditions and on the host species or cultivar. Symptomatic leaves (or parts of symptomatic leaves, for example around necrotic lesions) should be used when available. It is recommended that newly expanded leaves should be selected rather than senescing material. Leaves should be stored at (4 °C for no more than seven days before processing.)	Alternative storage conditions e.g lower temperatures of -80degrees to avoid RNA degradation clarify the temperature condition provision	Kenya
34.	*32*	Substantive	Detection and identification of TSWV, INSV and WSMoV can be achieved using biological, serological or molecular tests following the flow diagram shown in Figure 1. Lateral flow tests may be used as a preliminary screening tool for virus detection in symptomatic material	A clearer distinction should be made in the protocol between tests that can allow the generic detection of tospoviruses and those that allow specific identification. Are the tests described in Figure 1 able to discriminate between the different tospovirus species? The monoclonal antibodies have been produced more than ten years ago. Have these been tested for specificity against all tospovirus species described since then? The same holds true for the molecular tests described.	European Union
35.	*33*	Substantive	The tests described in Figure 1 are the minimum requirements to detect and identify the three viruses (e.g. during routine diagnosis of a pest widely established in a country), but further tests may be required where the national plant protection organization (NPPO) requires additional confidence in the identification (e.g. detection in an area where the virus is not known to occur). For example, sequencing of amplicons generated using molecular tests may be done. When a virus is suspected to be present in a new region or host it is recommended that both a serological test and a molecular test be used for detection.	Regarding the 'detect and identify' in sentence 1, see comment on paragraph 32.	European Union
36.	*35*	Editorial	In this diagnostic protocol, ~~methods~~ tests(including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of products of commercial brands in this diagnostic	harmonsation of terminology	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			protocol implies no approval of them to the exclusion of others that may be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalen products may be used if they can be shown to lead to the same results.		
37.	*35*	Editorial	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of products of commercial brands in this diagnostic protocol implies no approval of them to the exclusion of others that may be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	Typo	Australia
38.	*35*	Editorial	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of products of commercial brands in this diagnostic protocol implies no approval of them to the exclusion of others that may be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	Corrigendum	Japan
39.	*35*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. ~~The use of products of commercial brands in this diagnostic protocol implies no approval of them to the exclusion of others that may be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalen products may be~~	Text deleted as per general comment. Text added for consistency with other DP.	Uruguay, Argentina

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			~~used if they can be shown to lead to the same results~~. Laboratory procedures persented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated		
40.	*36*	Substantive	 **Figure 1.** Minimum requirements for the detection and identification of *Impatiens necrotic spot virus, Tomato spotted wilt virus* and *Watermelon silver mottle virus* (e.g. for the routine diagnosis of a pest widely established in a country).	Also in realtion to comment made on paragraph 32. With regard to serological tests with specific antibodies it should be noted that in the case of tospoviruses specific (polyclonal) antisera may cross react with other species. So a serological test might not be sufficient for identification of the species. Also for the molecular tests described, no data are available on the specificity of the tests. Moreover, it is not known which species will be detected by the generic RT-PCR. Since the RT-PCR amplifies part of the N gene, which sequence is an important criterion for species demarcation, this might allow identification in cases an amplicon is obtained. In the scheme to avoid confusion we suggest that for biological indexing negative and positive are replaced replace by "typical symptoms" or "'non typical symptoms" to state clearly that the identification is not concluded after such tests.	European Union
41.	*36*	Substantive	The flow diagram should be revised. The flow should be started from suspected or symptomic host. Biological ,Serological and molecular tests should betaken as recommended methods. Only the data of two results of three methods are positive indicated the virus present, and two results of three methods are negative indicated the virus is not present.	More reliable and practicable.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			 **Figure 1.** Minimum requirements for the detection and identification of *Impatiens necrotic spot virus, Tomato spotted wilt virus* and *Watermelon silver mottle virus* (e.g. for the routine diagnosis of a pest widely established in a country).		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
42.	*36*	Substantive	 **Figure 1.** Minimum requirements for the detection and identification of *Impatiens necrotic spot virus, Tomato spotted wilt virus* and *Watermelon silver mottle virus* (e.g. for the routine diagnosis of a pest widely established in a country).	Taking into consideration sensitivity of dealing with imported plant products especially perishable (as most hosts of TSWV, INSV and WSMOV) at the same time we need to issue a quick decision to deal with imported consignment And taking into consideration the tests sequence in Figure 1 to begin detects biological and which takes from seven to twenty-eight days Is it possible to start in the case of inspection with plant quarantine examination of imported products by serological test and molecular tests?	Bahrain
43.	*36*	Translation	Improve the table by indicate serological AND/OR molecular instead of OR	Diagram Diagram is simplified/basic. need to show a little detail on the flow Improve. when the serological tests are negative it is not conclusive to confirm that the virus is not present, thus it will require to move to molecular test.	Kenya

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			 **Figure 1.** Minimum requirements for the detection and identification of *Impatiens necrotic spot virus, Tomato spotted wilt virus* and *Watermelon silver mottle virus* (e.g. for the routine diagnosis of a pest widely established in a country).		
44.	*40*	Editorial	Indicator plants should be propagated from seed, planted in a well-drained soil mixture and maintained in an insect-proof facility at approximately 20–25 °C. Indicator plants should be kept in the dark for 24 h before inoculation to enhance susceptibility. ~~Infected p~~Plant material to be tested should be macerated with chilled inoculation buffer (0.01 M phosphate buffer, pH 7.0, containing 1% sodium sulphite) using a chilled mortar and pestle. Tospoviruses are very labile therefore buffers should be kept ice-cold and inoculum used as soon as possible after preparation. Apply sap extract to the leaves of young plants with a small	Replace 'infected plant material' by plant material to be tested; it is not known if plant material is infested at the start of the process.	European Union

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			amount of celite (mixed with sap) or carborundum powder (applied lightly to leaves). Using a gloved finger, gently rub the sap down the top surface of the lamina away from the plant stem. Wash plants carefully to remove any residual abrasive powder. Following inoculation, maintain the indicator plants at either approximately 20 °C (for INSV and TSWV) or approximately 20–25 °C (for WSMoV). Symptoms usually develop within 7 to 28 days.		
45.	*40*	Substantive	Indicator plants should be propagated from seed, planted in a well-drained soil mixture and maintained in an insect-proof facility at approximately 20–25 °C. Indicator plants should be kept in the dark for 24 h before inoculation to enhance susceptibility. Infected plant material should be macerated with chilled inoculation buffer (0.01 M phosphate buffer, pH 7.0, containing 1% sodium sulphite) using a chilled mortar and pestle. Tospoviruses are very labile therefore buffers should be kept ice-cold and inoculum used as soon as possible after preparation. Apply sap extract to the leaves of young plants with a small amount of celite (mixed with sap) or carborundum powder (applied lightly to leaves). Using a gloved finger, gently rub the sap down the top surface of the lamina away from the plant stem. Wash plants carefully to remove any residual abrasive powder. Following inoculation, maintain the indicator plants at either approximately 20 °C (for INSV and TSWV) or approximately 20–25 °C (for WSMoV). Symptoms usually develop within 7 to 28 days.	Taking into consideration sensitivity of dealing with imported plant products especially perishable (as most hosts of TSWW, INSV and WSMOV) at the same time we need to issue a quick decision to deal with imported consignment And taking into consideration the tests sequence in Figure 1 to begin detects biological and which takes from seven to twenty-eight days Is it possible to start in the case of inspection with plant quarantine examination of imported products by serological test and molecular tests?	Bahrain
46.	*40*	Technical	Indicator plants should be propagated from seed, planted in a well-drained soil mixture and maintained in an insect-proof facility at approximately 20–25 °C. Indicator plants should be kept in the dark for 24 h before inoculation to enhance susceptibility. Infected plant material should be macerated with chilled inoculation buffer (0.01 M phosphate buffer, pH 7.0, containing 1% sodium sulphite) using a chilled mortar	1. Second sentence : experts have commented that they never keep the plants in the dark and it is not necessary for successful inoculation. Could the authors consider this point and delete in the dark? 2. it is important to add the recommended ration a proposal is made. 3. Regarding the sentence starting with 'Wash plants carefully' : Normally you would allow the inoculum to sit on the leave for a minute	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			and pestle (ration 1g in 4 to 10mL of buffer). Tospoviruses are very labile therefore buffers should be kept ice-cold and inoculum used as soon as possible after preparation. Apply sap extract to the leaves of young plants with a small amount of celite (mixed with sap) or carborundum powder (applied lightly to leaves). Using a gloved finger, gently rub the sap down the top surface of the lamina away from the plant stem. Wash plants carefully to remove any residual abrasive powder. Following inoculation, maintain the indicator plants at either approximately 20 °C (for INSV and TSWV) or approximately 20–25 °C (for WSMoV). Symptoms usually develop within 7 to 28 days.	before washing off. Could this recommendation be added.	
47.	*40*	Technical	Indicator plants should be propagated from seed, planted in a well-drained soil mixture and maintained in an insect-proof facility at approximately 20–25 °C. Indicator plants should be kept in the dark for 24 h before inoculation to enhance susceptibility. Infected plant material should be macerated with chilled inoculation buffer (0.01 M phosphate buffer, pH 7.0, containing 1% sodium sulphite) using a chilled mortar and pestle. Tospoviruses are very labile therefore buffers should be kept ice-cold and inoculum used as soon as possible after preparation. Apply sap extract to the leaves of young plants with a small amount of celite (mixed with sap) or carborundum powder (applied lightly to leaves). Using a gloved finger, gently rub the sap down the top surface of the lamina away from the plant stem. Wash plants carefully to remove any residual abrasive powder. Following inoculation, maintain the indicator plants at either approximately 20 °C (for INSV and TSWV) or approximately 20–25 °C (for WSMoV). Symptoms usually develop within 7 to 28 days.depending on the indicator plant response	Response varry	Kenya
48.	*41*	Editorial	Herbaceous indexing is considered to be a reliable and sensitive method of detection, but there are no quantitative data published on its specificity, sensitivity or reliability. It is not a rapid test (symptom development requires at least seven days after inoculation), it	Why usiong agent and not pest?	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			requires dedicated facilities (such as temperature-controlled greenhouse space) and the symptoms may be confused with those of other pests ~~agents~~ (in particular other tospoviruses). However, virus concentration is often greater in infected herbaceous indicator species than in the natural host plants and therefore TSWV, INSV and WSMoV can be detected more reliably in herbaceous indicator plants.		
49.	*41*	Technical	Herbaceous indexing is considered to be a reliable and sensitive method of detection, but there are no quantitative data published on its specificity, sensitivity or reliability. It is not a rapid test (symptom development requires at least seven days after inoculation), it requires dedicated facilities (such as temperature-controlled greenhouse space) and the symptoms may be confused with those of other ~~agents~~ pests (in particular other tospoviruses). However, virus concentration is often greater in infected herbaceous indicator species than in the natural host plants and therefore TSWV, INSV and WSMoV can be detected more reliably in herbaceous indicator plants.	1. At present, biological testing offers the most generic test for tospoviruses, esp. inoculation onto Nicotiana benthamiana. This is an important feature of the test, since for none of the other tests it is know whether they will be able to detect all tospovirus species. In addition information on possible inhibition of transfer from some host species by host plant components should be mentioned. As suggested in the general comment, the generic detection tests should be better highlighted although we accept that this is not the main focus of the protocol.. 3. Regarding the last sentence : We assume it should be “TSWV, INSV and WSMoV can be detected more reliably by other assays described in this standard by testing inoculated herbaceous indicator plants” Is that what was meant?	European Union
50.	*48*	Editorial	Lateral flow tests are commercially available for TSWV and INSV and may be used to rapidly detect these viruses. No tests are currently available for WSMoV. The tests are designed for use with symptomatic material. Different formats are available from Agdia[1], Forsite Diagnostics[1] and Neogen[1], and the tests should be done according to these manufacturers’ instructions. There is no positive or negative control; rather, there is an internal control to verify the test has performed as it should.	There is no foot note attached to any of the 1. same for 52 60 79.	European Union
51.	*51*	Substantive	Samples should be tested in duplicate using two wells on the microtitre plate, and with appropriate controls run alongside. Positive controls can be infected tissue or virus maintained in indicator plants (frozen at −80 °C or lyophilized). Negative controls ~~can~~ should preferably be	Although it is recognized that this is a difficult issue and that negative controls of the same species is not always available it should be recommended as the preferred option. Replacement suggested.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			healthy ~~leaves~~ plant material from the same species as that being tested as well as extraction buffer. A healthy negative control is important as certain plant extracts, for example *Fuchsia*,may give false positive results (Louro, 1996).		
52.	*51*	Substantive	1.Add the method of sample and increase the number of sample. 2.Add buffer control in DAS-ELISA and TAS-ELISA.Samples should be tested in duplicate using two wells on the microtitre plate, and with appropriate controls run alongside. Positive controls can be infected tissue or virus maintained in indicator plants (frozen at −80 °C or lyophilized). Negative controls can be healthy leaves from the same species as that being tested as well as extraction buffer. A healthy negative control is important as certain plant extracts, for example *Fuchsia*,may give false positive results (Louro, 1996).	1.Because the viruses usually distribute unevenly in the plants. 2.Buffer control is also important when make DAS-ELISA and TAS-ELISA besides positive and negative control.	China
53.	*52*	Editorial	The ELISA methodologies, including reagents, were validated in a European Union DIAGPRO ringtest (SMT 4-CT98-2252 (EPPO, 2004)) with all laboratories accurately detecting TSWV and INSV (antisera source: Neogen-Adgen[2]) and WSMoV (antiserum source: DSMZ[3]). The respective antisera reacted only with the homologous virus species.	terminology issue (see geenral comment) There is no foot note attached to 2 or 3.	European Union
54.	*52*	Substantive	The ELISA methodologies, including reagents, were validated in a European Union DIAGPRO ringtest (SMT 4-CT98-2252 (EPPO, 2004)) with all laboratories accurately detecting TSWV and INSV (antisera source: Neogen-Adgen[2]) and WSMoV (antiserum source: DSMZ[3]). The respective antisera reacted only with the homologous virus species.	Regarding the last sentence, the question is is this still the case? Since the test performance study additional tospovirus species have been described. Consider adding more data.	European Union
55.	*53*	Technical	**3.2.3 Interpretation of results**	Unlike the protocol on CTV there is no guidance on the interpretation of the ELISA test apart from a correct reading of the controls. There should be some guidance provided. As mentioned in the comments provided on the draft IPPC protocol on CTV, an EPPO Standard on ELISA is under development that proposes options, however the standard is under consultation so no specific proposal can be made at	European Union

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				this stage. As soon as the Standard is adopted, the EPPO Secretariat will communicate elements for the TPDP to consider.	
56.	*55*	Editorial	• the positive controls included in the ~~assay~~ test produce the expected colour/colorimetric response	Harmonize terminology.	European Union
57.	*58*	Editorial	Molecular ~~test~~ methods may be more expensive and/or time-consuming than serological ~~techniques~~methods, especially for large-scale testing. However, molecular methods are generally more sensitive than serological techniques (see, for example, Chu *et al*. (2001)). The reverse transcription (RT)-polymerase chain reaction (PCR) method described in this diagnostic protocol enables the detection of TSWV, INSV or WSMoV using species-specific primers, or tospovirus species (including *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) as well as TSWV, INSV and WSMoV) using genus-specific primers. Liu *et al*. (2009) described primers for RT-PCR detection of INSV that target the nucleoprotein gene and generate an amplicon approximately 364 bp in size, but no data were provided on reaction conditions or specificity. The protocols described below give some indication of specificity.	Harmonize terminology.	European Union
58.	*59*	Technical	Real-time RT-PCR methods have been published for TSWV but not for INSV or WSMoV. However, the specificity of the TSWV method published by Dietzgen *et al*. (2005) and Robert et *al*. (2000). has not been reported, while that of Boonham *et al*. (2002) cross-reacts with GRSV and TCSV. Detection of a tospovirus using real-time RT-PCR may result in an inability to confirm the identity of the virus using other methods because of the inherent sensitivity of real-time RT-PCR. If it is used as a confirmatory test then the issue of the lack of specificity of the real-time RT-PCR may not be a concern. For monitoring the presence of viruliferous thrips, the real-time RT-PCR method described by	Add : Roberts et al. (2000) which is also mentioned later in 107.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Boonham *et al*. (2002) has been used for virus detection even in individual thrips.		
59.	*60*	Substantive	It is better to give the sampling methods, such as fruit, stalk, petals and Thrips.For molecular tests, plant extracts that are fresh or frozen (stored between −20 and −80 °C for periods of up to one year) can be used. Extraction of RNA should be done using the RNeasy Plant Mini Kit (Qiagen[4]), SV Total RNA Isolation System (Promega[5]) or any other appropriately validated protocol, according to the manufacturer's instructions.	Because of the system infection, many parts of infected hosts and Thrips can potentially harbour the viruses.	China
60.	*62*	Technical	Three sets of primers may be used for the RT-PCR test, as follows.	Consider adding an alternative primer set from Chen et al. (2012).	European Union
61.	*63*	Substantive	*Add "degenerate primer pairs gM410/gM870c".Generic primers of Mumford* et al. *(1996a) for tospoviruses:*	Literature: "Genomic characterization of Calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses, Plant Pathology (2012) 61, 183–194.	China
62.	*74*	Technical	The RT reaction is done in a microfuge tube containing 10 µl reaction mixture composed of: 0.2 µM reverse primer (S2 UNIV-R, L1 TSWV-R, S2 INSV-R or WSMoV-NR), 1 mM dNTPs, 2 µl of 5× M-MLV buffer, 100 U M-MLV reverse transcriptase, 0.5 U RNase inhibitor and 1 µl RNA sample. The reaction conditions are: 42 °C for 15 min, 99 °C for 5 min and 5 °C for 5 min.	Regarding the quantities of the reaction mix : Can they be confirmed?	European Union
63.	*76*	Technical	The S1/S2 INSV and L1/L2 TSWV primers produce a 602 base pair (bp) and a 276 bp amplicon with INSV and TSWV, respectively. The WSMoV-NR/NF primers produce a 700 bp amplicon with WSMoV. The generic S1/S2 UNIV primers produce a 871 bp amplicon with TSWV, INSV and other tospoviruses, or a 933 bp amplicon with WSMoV.	Is the obtention of a prodcut of a correct size sufficient for identification?	European Union
64.	*77*	Editorial	In the DIAGPRO test performance study ~~ringtest,~~ laboratories detected TSWV, INSV and WSMoV accurately, but there were insufficient molecular data to compare detection with the serological tests. The specificity of the molecular tests has been evaluated by Mumford *et al.* (1996a) and Chu *et al*. (2001). Mumford *et al*. (1996a) showed that the primers S1 and S2 were	harmonize terminology (see general comment)	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			specific for INSV and did not cross-react with TSWV, TCSV or GRSV. The degenerate broad spectrum primers described by Chu *et al*. (2001) were able to detect isolates of TSWV, INSV, WSMoV and other tospoviruses. Species identification was possible by restriction fragment length polymorphism (RFLP) analysis or sequence analysis of the amplicon.		
65.	*77*	Technical	In the DIAGPRO ~~ringtest~~test performance study, laboratories detected TSWV, INSV and WSMoV accurately, but there were insufficient molecular data to compare detection with the serological tests. The specificity of the molecular tests has been evaluated by Mumford *et al.* (1996a) and Chu *et al*. (2001). Mumford *et al*. (1996a) showed that the primers S1 and S2 were specific for INSV and did not cross-react with TSWV, TCSV or GRSV. The degenerate broad spectrum primers described by Chu *et al*. (2001) were able to detect isolates of TSWV, INSV, WSMoV and other tospoviruses. Species identification was possible by restriction fragment length polymorphism (RFLP) analysis or sequence analysis of the amplicon.	Is "Mumford et al. (1996a) showed that the primers S1 and S2 were specific for INSV and did not cross-react with TSWV, TCSV or GRSV" sufficient to state that these primers are specific?	European Union
66.	*79*	Substantive	If real-time RT-PCR result is positive, Specific primers of TSWV or tospoviruses primer binding sequence analysis should be done in further.The real-time RT-PCR described by Boonham *et al*. (2002) was used to detect all isolates of TSWV included in the analysis. Positive results were observed also with the tospoviruses TCSV and GRSV, but no reactions were observed with INSV, WSMoV, *Iris yellow spot virus* (IYSV) or *Chrysanthemum stem necrosis virus* (CSNV). The total volume of the reaction was 25 µl, and reactions were carried out in 96-well reaction plates using the TaqMan® EZ RT-PCR Kit (PE Biosystems[6]), but with the addition of 25 U M-MLV reverse transcriptase (Mumford *et al*., 2000). After cetyl trimethylammonium bromide (CTAB) extraction and re-suspension of the final pellet in 50 µl diethylpyrocarbonate (DEPC)-treated water, 1 µl RNA was used to prepare the final volume of 25 µl for the	The positive results of TSWV real-time RT-PCR were observed also with the tospoviruses TCSV and GRSV.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			reaction (Mumford *et al*., 2000). Plates were cycled at 48 °C for 30 min, 95 °C for 10 min, and 40 cycles of 60 °C for 1 min and 95 °C for 15 s. Using suitable positive and negative controls each laboratory or user should validate the cycle threshold (Ct) values that represent a positive result.		
67.	*79*	Technical	The real-time RT-PCR described by Boonham *et al*. (2002) was used to detect all isolates of TSWV included in the analysis. Positive results were observed also with the tospoviruses TCSV and GRSV, but no reactions were observed with INSV, WSMoV, *Iris yellow spot virus* (IYSV) or *Chrysanthemum stem necrosis virus* (CSNV). The total volume of the reaction was 25 µl, and reactions were carried out in 96-well reaction plates using the TaqMan® EZ RT-PCR Kit (PE Biosystems[6]), but with the addition of 25 U M-MLV reverse transcriptase (Mumford *et al*., 2000). After cetyl trimethylammonium bromide (CTAB) extraction and re-suspension of the final pellet in 50 µl diethylpyrocarbonate (DEPC)-treated water, 1 µl RNA was used to prepare the final volume of 25 µl for the reaction (Mumford *et al*., 2000). Plates were cycled at 48 °C for 30 min, 95 °C for 10 min, and 40 cycles of 60 °C for 1 min and 95 °C for 15 s. Using suitable positive and negative controls each laboratory or user should validate the cycle threshold (Ct) values that represent a positive result.	The buffer composition but also the description of the extraction procedure should be provided as requested in the instructions to authors.	European Union
68.	*88*	Technical	**Internal control.** For conventional and real-time PCR, plant internal controls (e.g. a housekeeping gene (HKG) such as *nad5*, or the ribosomal RNA gene) should be incorporated into the protocol to eliminate the possibility of PCR false negatives due to nucleic acid extraction failure or degradation or the presence of PCR inhibitors. The internal control primers should preferably be used in a duplex reaction with the target virus primers. However, because this may be difficult to achieve without reducing the sensitivity of the test, it is recommended, where practical, to run a duplex reaction	Regarding the following sentence : "However, because this may be difficult to achieve without reducing the sensitivity of the test, it is recommended, where practical, to run a duplex reaction of the virus primers with the HKG primers and also a simplex reaction with only the virus primers." : Another option is to add the option of two simplex reaction (one for the plant marker and one for the virus targeted).	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			of the virus primers with the HKG primers and also a simplex reaction with only the virus primers. An RT-PCR using internal control primers (primers designed to detect a sequence conserved in plants such as the 5S ribosomal RNA gene (Kolchinsky *et al.* (1991)) may be used to confirm that RNA of sufficient quality for amplification has been extracted.		
69.	*89*	Editorial	The *nad5* mitochondrial *NADH dehydrogenase* 5 gene fragment has been shown to be a reliable indicator of the performance of the extraction procedure and RT step for conventional RT-PCR (Menzel *et al*., 2002). It has been tested against many plant species, including *S. tuberosum* and other *Solanum* species (*S. bonariensis*, *S. dulcamara*, *S. jasminoides*, *S. nigrum*, *S. pseudocapsicum*, *S. rantonnetii*, *S. sisymbrifolium*), *Acnistus arborescens*, *Atropa belladonna*, *Brugmansia* spp., *Capsicum* spp., *Cestrum* spp., ~~L~~*l*ochroma *cyanea*, *Nicotiana* spp. and *Physalis* spp. (Seigner *et al*., 2008).	Corrigendum	Japan
70.	*94*	Technical	For RT-PCR, care needs to be taken to avoid cross-contamination due to aerosols from the positive control or from positive samples. The positive control used in the laboratory should be sequenced so that this sequence can be readily compared with sequence obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence that, again, can be compared with PCR amplicons of the correct size.	This paragraph deals with possible cross-contamination due to aerosol from the positive control. It is not explicit, but the protocol recommend to compare the sequence of the positive control and the one obtained for any positive sample. But if the sequences are identical, it cannot be concluded that there was a contamination. There is a possibility that the same strain is present in the control and in the sample. Could it be clarified that if sequences compared are identical there could be a doubt that cross contamination has occured (but no definite proof).	European Union
71.	*95*	Technical	**Negative extraction control.** This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positive samples are expected.	To monitor the contamination during nucleic acid extraction only clean extraction buffer may be used for extraction (and subsequent amplification) instead of healthy host tissue". Healthy plant tissue is not always available... If cross-reaction with the host tissue has been excluded during the validation of the method, it is not needed to include this type of control	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				in each run (although in each run it is necessary to monitor the contamination). see also comment on paragraph 51	
72.	*100*	Editorial	• the negative extraction control and the negative amplification control do not produce ~~the correct size~~a product of the correct size for the virus.	Better wording.	European Union
73.	*101*	Substantive	Add the primer sequence of *nad5* internal control.If the *nad*5internal control primers are used, then the negative extraction control, the positive extraction control (if used) and each of the test samples must produce a 181 bp band (*nad*5). Failure of the samples to amplify with the internal control primers suggests, for example, that the RNA extraction has failed, the nucleic acid has not been included in the reaction mixture, the RT step has failed, compounds inhibitory to PCR are present in the RNA extract, or the RNA or DNA has degraded.	Helpful for practice.	China
74.	*101*	Technical	If the *nad*5internal control primers are used, then the negative extraction control, the positive extraction control (if used) and each of the test samples must produce a 181 bp band (*nad*5). Failure of the samples to amplify with the internal control primers suggests, for example, that the RNA extraction has failed, the nucleic acid has not been included in the reaction mixture, the RT step has failed, compounds inhibitory to PCR are present in the RNA extract, or the RNA or DNA has degraded.	Regarding the first sentence of the paragraph : There may not be a 181bp band in a really strong positive control as the nad5 may be out competed.	European Union
75.	*103*	Technical	**Real-time RT-PCR**	The same information as in 98-102 should be provided	European Union
76.	*105*	Substantive	**4. Identification**	According to paragraph 32 'Detection and identification of TSWV, INSV and WSMoV can be achieved using biological, serological or molecular tests following the flow diagram shown in Figure 1' so ELISA is included as a possible test for identification. In this part ELISA is not mentioned. the paragraph 107 and 108 naming the tests considered appropriate	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				or not do not mention ELISA. However, we believe that it should be clearer in the protocol which are the tests used for detection and identification. However as already stated in other comments we have concerns about the use of ELISA.	
77.	*105*	Technical	Add the result description of "3.1 Biological detection"**4. Identification**	Biological detection is one of recommended identification methods.	China
78.	*108*	Substantive	However, the real-time RT-PCR of Boonham *et al*. (2002) can be used as a method for confirmation of TSWV in positive samples.	1. Can this test be used for confirmation when cross reactions with GRSV and TCSV occur? Also in ELISA cross reactions of the TSWV antiserum against these viruses have been reported (Hasani-Mehraban et al., 2005, A New tomato-infecting tospovirus from Iran, Phytopathology 95:852-858). This means that the presence of one of these tospoviruses can be confirmed but not its identity. 2. The wording may need clarification in particular on what is meant by "positive samples" (ie GRSV and TCSV have been ruled out in another test???)	European Union