2004-011: Draft Annex to ISPM 27:2006 – Xanthomonas citri subsp. citri

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
1.	*G*	Editorial	It is suggested to include a flowchart for detection	It is recommended to include it in paragraph 20, for consistency with other DP	English	Uruguay
2.	*G*	Editorial	It is suggested to include a flowchart for detection	It is recommended to include it in paragraph 20, for consistency with other DP	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
3.	*G*	Editorial	I support the document as it is and I have no comments		English	Malaysia
4.	*G*	Editorial	I support the document as it is and I have no comments		English	Canada
5.	*G*	Editorial	I support the document as it is and I have no comments		English	Lao People's Democratic Republic
6.	*G*	Editorial	I support the document as it is and I have no comments		English	Korea, Republic of
7.	*G*	Editorial	I support the document as it is and I have no comments		English	Guyana
8.	*G*	Editorial	I support the document as it is and I have no comments		English	Mexico
9.	*G*	Editorial	I support the document as it is and I have no comments		English	Barbados

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
10.	G	Editorial	I support the document as it is and I have no comments		English	New Zealand
11.	G	Editorial	I support the document as it is and I have no comments		English	Nepal
12.	G	Editorial	I support the document as it is and I have no comments		English	Congo
13.	G	Editorial	I support the document as it is and I have no comments		English	Lesotho
14.	G	Editorial	I support the document as it is and I have no comments		English	Costa Rica
15.	G	Substantive	Document well written however references were cited in the document which were not found in the reference section namely Gadriel et al., 1989 Timmer et al., 2000 Berman et al., 1981 The following references were included in the reference section but not stated in the document Kuo et al., 1994 Bradbury,J.F., 1986 Wu et al., 1993 and 1996	References cited in the document not in the reference section and references included in the reference section not in the document.	English	Jamaica

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
16.	*G*	Substantive	Suggest drafting this annex once again because the description of the pest information is not clear and confusion in logistic, the technical methods is not well-organized and part technical measures(3.2 in the annex) is difficult to operate.	1.The taxonomic information is not clear. And it's not easy to understand. 2.There is overlapping in the content of the third part and the forth part. 3.Isolation methods is not scientific for detection in asymptomatic plants. The methods more sensitivity such as PCR should be added in this part.	English	China
17.	*G*	Technical		The addition of a flow chart on detection in symptomatic and asymptomatic plant material could help the reader in deciding which tests to use.	English	EPPO
18.	*G*	Technical		The addition of a flow chart on detection in symptomatic and asymptomatic plant material could help the reader in deciding which tests to use.	English	European Union
19.	*3*	Editorial	**Date of this document**: 2013-04-04 **Document category**: Draft new annex to ISPM 27:2006 (*Diagnostic protocols for regulated pests)* **Current document stage**: Approved by SC e-decision for member consultation (MC) **Origin**: Work programme topic: Bacteria, CPM-1 (2006) Original subject: *Xanthomonas axonopodis* pv. *citri* (2004-011) **Major stages**: 2004-11 SC added topic to work program CPM-1 (2006) added topic to work program (2004-011) 2012-11 TPDP revised draft protocol 2013-04 SC approved by e-decision to member consultation (MC) (2013_eSC_May_12) 2013-07 Member consultation (MC)	Spanish spelling of i	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			Discipline leads history: 2006-07 SC Lum KENG-YEANG (MY) 2011-05 SC Robert TAYLOR (AU)			
			Consultation on technical level: The first draft of this protocol was written by: • Enrique VERDIER (General Direction of Agricultural Services, Biological Laboratories Department, Montevideo, Uruguay) • Rita LANFRANCHI (Plant Pests and Diseases Laboratory, National Service of Agrifood Health and Quality (SENASA), Capital Federal, Argentina) • María ~~Maria~~ M. LÓPEZ (Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain). The following expert also contributed to the preparation of the draft: • Jaime CUBERO (Instituto Nacional de Investigación y Tecnologia Agraria y Alimentaria (INIA), Spain).			
			Main discussion points during development of the diagnostic protocol: -			
			Notes: 2013-05-06 edited (AF)			
20.	*3*	Editorial	**Date of this document**: 2013-04-04 **Document category**: Draft new annex to ISPM 27:2006 (*Diagnostic protocols for regulated pests)* **Current document stage**: Approved by SC e-decision for member consultation (MC)	Spanish spelling of i	English	European Union

Comm. no.	Para. no.	Comment type	Comment		Explanation	Language	Country
			Origin	Work programme topic: Bacteria, CPM-1 (2006) Original subject: *Xanthomonas axonopodis* pv. *citri* (2004-011)			
			Major stages	2004-11 SC added topic to work program CPM-1 (2006) added topic to work program (2004-011) 2012-11 TPDP revised draft protocol 2013-04 SC approved by e-decision to member consultation (MC) (2013_eSC_May_12) 2013-07 Member consultation (MC)			
			Discipline leads history	2006-07 SC Lum KENG-YEANG (MY) 2011-05 SC Robert TAYLOR (AU)			
			Consultation on technical level	The first draft of this protocol was written by: • Enrique VERDIER (General Direction of Agricultural Services, Biological Laboratories Department, Montevideo, Uruguay) • Rita LANFRANCHI (Plant Pests and Diseases Laboratory, National Service of Agrifood Health and Quality (SENASA), Capital Federal, Argentina) • María ~~Maria~~ M. LÓPEZ (Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain). The following expert also contributed to the preparation of the draft: • Jaime CUBERO (Instituto Nacional de Investigación y Tecnologia			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			Agraria y Alimentaria (INIA), Spain). **Main discussion points during development of the diagnostic protocol**: - **Notes**: 2013-05-06 edited (AF)			
21.	5	Editorial	*Xanthomonas citri* subsp. *citri* (Xcc) is the causal agent of citrus bacterial canker. It causes severe damage to many cultivated species of Rutaceae~~*Rutaceae*~~ (EPPO, 1979) – primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. – grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, USA (CABI, 2006; EPPO, 2006). Atypical strains of Xcc with a restricted host range have been identified and are designated as strains A* and A^{w} (Sun *et al.*, 2004; Vernière *et al*., 1998). These strains affect only *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* Webster (Alemow) in Florida, USA (Cubero & Graham, 2002, 2004).	A word "Rutaceae" should not be italicized.	English	Thailand
22.	5	Substantive	*Xanthomonas citri* subsp. *citri* (Xcc) is the major causal agent of citrus bacterial canker. It causes severe damage to many cultivated species of *Rutaceae* (EPPO, 1979) – primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. – grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, USA (CABI, 2006; EPPO, 2006). Atypical strains of Xcc with a restricted host range have been identified and are designated as strains A* and A^{w} (Sun *et al.*, 2004; Vernière *et al*., 1998). These strains affect only *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* Webster (Alemow) in Florida, USA (Cubero & Graham, 2002, 2004).	There three species of genus Xanthomonas can cause citrus bacterial canker. Besides Xanthomonas citri subsp. citri, there are Xanthomonas fuscans subsp. aurantifolii Schaad et al. 2007, and Xanthomonas alfalfae subsp. citrumelonis Schaad et al. 2007. Add 'major' to make clear in text express.	English	China
23.	5	Substantive	*Xanthomonas citri* subsp. *citri* (Xcc) is the causal agent of citrus bacterial canker. It causes ~~severe~~ damage to many cultivated species of *Rutaceae* (EPPO, 1979) â€“ primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. â€“ grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, USA (CABI, 2006; EPPO, 2006). Atypical strains of Xcc with a restricted host range have been identified and are designated as strains A* and A^{w} (Sun *etÂ al.*, 2004; VerniÃ¨re *etÂ al*., 1998). These strains affect only *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* Webster (Alemow) in Florida, USA (Cubero & Graham, 2002, 2004).	Not appropriate to qualify damage in a diagnostic protocol	English	United States of America, Mexico

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
24.	5	Technical	*Xanthomonas citri* subsp. *citri* (Xcc) is the causal agent of citrus bacterial canker. It causes severe damage to many cultivated species of *Rutaceae* (EPPO, 1979) – primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. – grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, USA (CABI, 2006; EPPO, 2006). Atypical strains of Xcc with a restricted host range have been identified and are designated as strains A* and A^w (Sun *et al.*, 2004; Vernière *et al*., 1998). ~~These strains affect only *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* Webster (Alemow) in Florida, USA (Cubero & Graham, 2002, 2004).~~ Strains A* affect *Citrus aurantiifolia* (Mexican lime or Key lime) in natural conditions in Asia. Those of type A^w cause canker in *C. aurantiifolia* and *C. macrophylla* (alemow) in Florida, USA, in natural conditions (Cubero & Graham, 2002, 2004). Both strains may cause atypical lesions in other citrus species.	1. Xcc is also commonly used as an abbreviation for X. campestris pv. campestris. It is suggested to avoid Xcc and use X. citri subsp citri. 2. For clarification of the pathogenicity on different citrus species. See also Escalon et al. Mol Plant Pathol 2013 DOI: 10.1111/mpp.12019 3. The authority name for C macrophylla was removed; they are not used for hosts in DPs.	English	EPPO
25.	5	Technical	*Xanthomonas citri* subsp. *citri* (Xcc) is the causal agent of citrus bacterial canker. It causes severe damage to many cultivated species of *Rutaceae* (EPPO, 1979) – primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. – grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, USA (CABI, 2006; EPPO, 2006). Atypical strains of Xcc with a restricted host range have been identified and are designated as strains A* and A^w (Sun *et al.*, 2004; Vernière *et al*., 1998). ~~These strains affect only *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* Webster (Alemow) in Florida, USA (Cubero & Graham, 2002, 2004).~~ Strains A* affect *Citrus aurantiifolia* (Mexican lime or Key lime) in natural conditions in Asia. Those of type A^w cause canker in *C. aurantiifolia* and *C. macrophylla* (alemow) in Florida, USA, in natural conditions (Cubero & Graham, 2002, 2004). Both strains may cause atypical lesions in other citrus species.	1. Xcc is also commonly used as an abbreviation for X. campestris pv. campestris. It is suggested to avoid Xcc and use X. citri subsp citri. 2. For clarification of the pathogenicity on different citrus species. See also Escalon et al. Mol Plant Pathol 2013 DOI: 10.1111/mpp.12019 3. The authority name for C macrophylla was removed; they are not used for hosts in DPs.	English	European Union
26.	6	Editorial	Citrus bacterial canker typically occurs on seedlings and young trees in which there is a flush of actively growing shoots and leaves from late summer through to autumn. Canker lesions are formed on the leaves, shoots, twigs and fruits of susceptible hosts. Attacks of *Phyllocnistis citrella*, the citrus leaf miner, can increase the susceptibility of leaves to citrus canker (Hall *et al.*, 2010).	The content of this paragraph could be included in the Symptoms section (paragraph 24).	English	EPPO
27.	6	Editorial	Citrus bacterial canker typically occurs on seedlings and young trees in which there is a flush of actively growing shoots and leaves from late summer through to autumn. Canker lesions are formed on the leaves, shoots, twigs and fruits of susceptible hosts. Attacks of *Phyllocnistis citrella*, the citrus leaf miner, can increase the susceptibility of leaves to citrus canker (Hall *et al.*, 2010).	The content of this paragraph could be included in the Symptoms section (paragraph 24).	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
28.	6	Technical	Citrus bacterial canker typically occurs on seedlings, ~~and~~ young and adult trees of susceptible hosts in which there is a flush of actively growing shoots and leaves from late summer through to autumn. Canker lesions are formed on the leaves, shoots, twigs and fruits of susceptible hosts. Wounds caused by wind, thorns, insects, grove or nursery maintenance operations favour infection of mature tissues.Attacks of *Phyllocnistis citrella*, the citrus leaf miner, can increase the susceptibility of leaves to citrus canker (Hall *et al.*, 2010).	1. For clarification 2. Is it true that canker typically occurs from late summer through to autumn for all citrus growing areas? 3. For clarification	English	EPPO
29.	6	Technical	Citrus bacterial canker typically occurs on seedlings, ~~and~~ young and adult trees of susceptible hosts in which there is a flush of actively growing shoots and leaves from late summer through to autumn. Canker lesions are formed on the leaves, shoots, twigs and fruits of susceptible hosts. Wounds caused by wind, thorns, insects, grove or nursery maintenance operations favour infection of mature tissues.Attacks of *Phyllocnistis citrella*, the citrus leaf miner, can increase the susceptibility of leaves to citrus canker (Hall *et al.*, 2010).	1. For clarification 2. Is it true that canker typically occurs from late summer through to autumn for all citrus growing areas? 3. For clarification	English	European Union
30.	9	Technical	**Name:** *Xanthomonas citri* subsp. *citri* (ex Hasse 1915) Gabriel *et al.*, 1989, subsp. nov (Schaad et al., 2006)	This is the last nomenclature for the targeted pathogen X axonopodis pv. citri pathotype A (Systematic and Applied Microbiology 29: 690-695). The last accepted is from Ah-You et al 2009, but both are used in publications.	English	EPPO
31.	9	Technical	**Name:** *Xanthomonas citri* subsp. *citri* (ex Hasse 1915) Gabriel *et al.*, 1989, subsp. nov (Schaad et al., 2006)	This is the last nomenclature for the targeted pathogen X axonopodis pv. citri pathotype A (Systematic and Applied Microbiology 29: 690-695). The last accepted is from Ah-You et al 2009, but both are used in publications.	English	European Union
32.	10	Technical	**Synonyms:***Xanthomonasaxonopodis* pv. *citri* (Hasse) Vauterin *et al*., 1995 Xanthomonas citri pv. citri (Gabriel et al., 1989) Ah-You et al., 2009	1. The synonyms could be organized chronologically from the last to the first name according to the year of the name, or the opposite. 2. Additional synonym	English	EPPO
33.	10	Technical	**Synonyms:***Xanthomonasaxonopodis* pv. *citri* (Hasse) Vauterin *et al*., 1995 Xanthomonas citri pv. citri (Gabriel et al., 1989) Ah-You et al., 2009	1. The synonyms could be organized chronologically from the last to the first name according to the year of the name, or the opposite. 2. Additional synonym	English	European Union
34.	10	Technical	**Synonyms:***Xanthomonasaxonopodis* pv. *citri* (Hasse) Vauterin *et al*., 1995 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv.	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			Citrumelo(Gabriel,1989）”			
35.	*11*	Technical	*Pseudomonas citri* Hasse, 1915 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
36.	*12*	Technical	*Xanthomonas citri* (Hasse, 1915) Gabriel *et al.*, 1989 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
37.	*13*	Technical	*Xanthomonas citri* f.sp. *aurantifoliae* Namekata & Oliveira, 1972 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
38.	*14*	Technical	*Xanthomonas campestris* pv. *citri* (Hasse) Dye, 1978 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
39.	*15*	Technical	*Xanthomonas citri* (ex Hasse) nom. rev. Gabriel *et al.*, 1989 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
40.	*16*	Technical	*Xanthomonas campestris* pv. *aurantifolii* Gabriel *et al.*, 1989 Xanthomonas campestris pv. Citrumelo(Gabriel,1989） Synonyms should be in order according time and add one name “Xanthomonas campestris pv. Citrumelo(Gabriel,1989）”	The name “Xanthomonas campestris pv. Citrumelo (Gabriel,1989）” is an important synoym. It can be more logistic after revise.	English	China
41.	*18*	Technical	**Common names:** citrus canker, citrus bacterial canker, asiatic canker	Additional English common name	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
42.	*18*	Technical	**Common names:** citrus canker, citrus bacterial canker, asiatic canker	Additional English common name	English	European Union
43.	*19*	Substantive	**Note:** Xcc has been recently reclassified from the A pathotype *X. axonopodis* pv. *citri*. The nomenclature of Gabriel *et al.* (1989) has been reinstated and the accepted name for the citrus bacterial canker pathogen is now *X. citri* subsp. *citri* (Bull *et al*., 2010; Schaad *et al.*, 2006). The B and C pathotypes of *X. axonopodis* pv. *citri* have been reclassified as *X*. *fuscans* subsp. *aurantifolii* (Schaad *et al.*, 2006). The taxon of Citrus bacterial canker's causal agent has changed greatly. Five pathotypes, cankers 'A', 'B', 'C', 'D', and 'E' have been described. The canker 'A' is the most damaging for many Rutaceae species, including Citrus sinensis , C. reticulata , C. limetta, C. maxima, and Citrus x paradise. The cancrosis 'B' affecting primarily C. limon in Argentina, Paraguay, and Uruguay. The canker 'C' affecting only Mexican lime in Brazil. Cancrosis 'D' was described on Mexican lime in Mexico; the organism was reported differed pathologically by failing to cause symptoms on fruit. Cancrosis 'E' was originally described in Florida, presenting only in nursery stocks. The causal bacterium produces flat, sometimes sunken, water-soaked chlorotic lesions which become black, not the erupted canker lesions typical of the cankers 'A'. The accepted name of citrus bacterial canker pathogen is now X. citri subsp. citri (Bull et al., 2010; Schaad et al., 2006). Xcc has been recently reclassified from the A pathotype of X. axonopodis pv. citri. The B, C and D pathotypes of X. axonopodis pv. aurantifolii have been reclassified as X. fuscans subsp. aurantifolii (Schaad et al., 2006). The E. pathotype of X. axonopodis pv. citrumelo have been reclassified as Xanthomonas alfalfae subsp. citrumelonis (Schaad et al. 2006).	The classification of taxon for causal agent of citrus bacterial canker has changed greatly. There are other two pathotypes D, E which also cause citrus bacterial canker. It is necessary to introduce all of them in details.	English	China
44.	*19*	Substantive	**Note:** Xcc has been recently reclassified from ~~the A pathotype~~ *X. axonopodis* pv. *citri* (*X. campestris* pv. *citri* pathotype A). The nomenclature of Gabriel *et al.* (1989) has been reinstated and the accepted name for the citrus bacterial canker pathogen is now *X. citri* subsp. *citri* (Bull *et al*., 2010; Schaad *et al.*, 2006). The ~~B and C pathotypes of *X. axonopodis* pv. *citri*~~ other pathotypes of *X. campestris* pv. *citri* have been reclassified as *X*. *fuscans* subsp. *aurantifolii* (pathotype B, C and D) or *X. alfalfae* subsp. *citrumelonis* (pathotype E) (Schaad *et al.*, 2006).	These modificaitons are consistent with classification of Vauterin et al. (1995)*1 and Schaad et al.(2006)*2. *1 Para[176] :Vauterin et al. (1995) Reclassification of Xanthomonas. International Journal of Systematic Bacteriology, 45: 472–489. *2 Para[173] :Schaad et al. (2006). Emended classification of xanthomonad pathogens on citrus. Systematic and Applied Microbiology, 29: 690 -695.	English	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
45.	*19*	Technical	**Note:** Xcc has been recently reclassified from the A pathotype *X. axonopodis* pv. *citri*. The nomenclature of Gabriel *et al.* (1989) has been reinstated and the accepted name for the citrus bacterial canker pathogen is now *X. citri* subsp. *citri* (Bull *et al*., 2010; Schaad *et al.*, 2006). The B and C pathotypes of *X. axonopodis* pv. *citri* have been reclassified as *X*. *fuscans* subsp. *aurantifolii* (Schaad *et al.*, 2006) and a synonym has been proposed, Xanthomonas citri pv. aurantifolii (Ah-You et al., 2009.IJSEM 59 :306-318).	Additional clarification	English	EPPO
46.	*19*	Technical	**Note:** Xcc has been recently reclassified from the A pathotype *X. axonopodis* pv. *citri*. The nomenclature of Gabriel *et al.* (1989) has been reinstated and the accepted name for the citrus bacterial canker pathogen is now *X. citri* subsp. *citri* (Bull *et al*., 2010; Schaad *et al.*, 2006). The B and C pathotypes of *X. axonopodis* pv. *citri* have been reclassified as *X*. *fuscans* subsp. *aurantifolii* (Schaad *et al.*, 2006) and a synonym has been proposed, Xanthomonas citri pv. aurantifolii (Ah-You et al., 2009.IJSEM 59 :306-318).	Additional clarification	English	European Union
47.	*22*	Substantive	Diagnosis of citrus canker can be achieved by observing morphological characteristics of the colonies on nutrient media and by~~,~~ serological testing (by immunofluorescence (IF)), molecular testing (by polymerase chain reaction (PCR)) and~~,~~ bioassay of leaf discs or detached leaves~~, and pathogenicity testing~~. Positive and negative controls must be included for all tests (see section 4 for reference controls).	1. The tests are used in addition to colony morphology for detection. 2. Pathogenicity testing is not a detection test, so it should not be mentioned in detection but in identification.	English	EPPO
48.	*22*	Substantive	Diagnosis of citrus canker can be achieved by observing morphological characteristics of the colonies on nutrient media and by~~,~~ serological testing (by immunofluorescence (IF)), molecular testing (by polymerase chain reaction (PCR)) and~~,~~ bioassay of leaf discs or detached leaves~~, and pathogenicity testing~~. Positive and negative controls must be included for all tests (see section 4 for reference controls).	1. The tests are used in addition to colony morphology for detection. 2. Pathogenicity testing is not a detection test, so it should not be mentioned in detection but in identification.	English	European Union
49.	*26*	Technical	*Symptoms on branches.*In dry conditions, the canker spot is corky or spongy, is raised, and has a ruptured surface. In moist conditions, the lesion enlarges rapidly, and the surface remains unruptured and is oily at the margin. In the ~~more resi~~less susceptible~~stant~~ cultivars, a callus layer may form between the diseased and healthy tissues. The scar of a canker may be identified by scraping the rough surface with a knife to remove the outer corky layer, revealinglight to dark brown lesions in the healthy green bark tissues. The discoloured area can vary in shape and in size from 5 to 10 mm, depending on the susceptibility of the host plant.	'resistant' implies infection not possible but some level of infection is expected.	English	Australia
50.	*27*	Technical	*Symptoms on leaves.* Bright yellow spots are first apparent on the underside of leaves, followed by erumpent brownish lesions on both sides of the leaves, which become rough, cracked and corky. The canker may be surrounded by a water-soaked yellow or chlorotic halo margin.	Further clarification	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
51.	*27*	Technical	*Symptoms on leaves.* Bright yellow spots are first apparent on the underside of leaves, followed by erumpent brownish lesions on both sides of the leaves, which become rough, cracked and corky. The canker may be surrounded by a water-soaked yellow or chlorotic halo margin.	Further clarification	English	European Union
52.	*28*	Editorial	Confusion may occur between symptoms of citrus canker and scab or leaf spot-like symptoms caused by other ~~plant pathogenic~~ bacteria or~~and~~ fungi that infest citrus or by physiological disorders. Other bacteria ~~on citrus~~ that can cause citrus canker-like symptoms are *X. alfalfa*e subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both these bacteria have a limited host range, cause less aggressive symptoms~~,~~ and rarely produce lesions on fruit (Timmer *et al.*, 2000). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.	1. Suggestions to aid clarity 2. Typos - missing e, comma not needed.	English	EPPO
53.	*28*	Editorial	Confusion may occur between symptoms of citrus canker and scab or leaf spot-like symptoms caused by other ~~plant pathogenic~~ bacteria or~~and~~ fungi that infest citrus or by physiological disorders. Other bacteria ~~on citrus~~ that can cause citrus canker-like symptoms are *X. alfalfa*e subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both these bacteria have a limited host range, cause less aggressive symptoms~~,~~ and rarely produce lesions on fruit (Timmer *et al.*, 2000). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.	1. Suggestions to aid clarity 2. Typos - missing e, comma not needed.	English	European Union
54.	*28*	Editorial	Confusion may occur between citrus canker and scab or leaf spot-like symptoms caused by other plant pathogenic bacteria and fungi or by physiological disorders. Other bacteria on citrus that can cause citrus canker-like symptoms are *X. alfalfa* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both of these bacteria have a limited host range, cause less aggressive symptoms, and rarely produce lesions on fruit (Timmer *et al.*, 2000). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be	for clarity	English	Ghana

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			differentiated from citrus canker by the lack of bacterial ooze.			
55.	*28*	Substantive	Confusion may occur between citrus canker and scab or leaf spot-like symptoms caused by other plant pathogenic bacteria and fungi or by physiological disorders. Other bacteria on citrus that can cause citrus canker-like symptoms are *X. alfalfa* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both these bacteria have a limited host range, cause less aggressive symptoms, and rarely produce lesions on fruit (Timmer *et al.*, 2000). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.	A paper of Timmer et al., 2000 is missing in section 8.References.	English	Thailand
56.	*28*	Technical	Confusion may occur between citrus canker and scab or leaf spot-like symptoms caused by other plant pathogenic bacteria and fungi or by physiological disorders. Other bacteria on citrus that can cause citrus canker-like symptoms are *X. alfalfa* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both these bacteria have a limited host range, cause less aggressive symptoms, and rarely produce lesions on fruit (Timmer *et al.*, 2000; Schaad *et al.*, 2005 and 2006). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.	1. Please clarify whether the confusion refers to symptoms in fruit or also in leaves and branches. 2. Timmer et al., 2000 is not in the reference list. 3. Additional relevant references 4. In the last two sentences please clarify the statements regarding lack of halo and bacterial ooze - the ooze was described above in fruits and the halo in leaves.	English	EPPO
57.	*28*	Technical	Confusion may occur between citrus canker and scab or leaf spot-like symptoms caused by other plant pathogenic bacteria and fungi or by physiological disorders. Other bacteria on citrus that can cause citrus canker-like symptoms are *X. alfalfa* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both these bacteria have a limited host range, cause less aggressive symptoms, and rarely produce lesions on fruit (Timmer *et al.*, 2000; Schaad *et al.*, 2005 and 2006). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002; Timmer *et al.*, 2000), but in general, its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.	1. Please clarify whether the confusion refers to symptoms in fruit or also in leaves and branches. 2. Timmer et al., 2000 is not in the reference list. 3. Additional relevant references 4. In the last two sentences please clarify the statements regarding lack of halo and bacterial ooze - the ooze was described above in fruits and the halo in leaves.	English	European Union
58.	*30*	Editor	Freshly prepared sample extracts are essential for successful isolation of Xcc from symptomatic plant material. However, when symptoms are very advanced or when	For clarity	English	Ghana

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
		ial	environmental conditions are not favourable, the number of Xcc culturable cells can be very low and isolation can result in plates being overcrowded with competing saprophytic or antagonistic bacteria. Particular care should be taken ~~to~~ not to confuse Xcc colonies with *Pantoea agglomerans*, which is also commonly isolated from canker lesions and produces yellow colonies on standard bacteriological media.			
59.	*30*	Substantive	Freshly prepared sample extracts are essential for successful isolation of Xcc from symptomatic plant material. However, when symptoms are very advanced or when environmental conditions are not favourable, the number of Xcc culturable cells can be very low and isolation can result in plates being overcrowded with competing saprophytic or antagonistic bacteria. Particular care should be taken to not confuse Xcc colonies with *Pantoea agglomerans*, which is also commonly isolated from canker lesions and produces yellow colonies on standard bacteriological media. Pa is generally a brighter yellow than the pale Xcc, and is faster growing than Xcc.	this information may be helpful in reducing the confusion between Pantoea and Xcc.	English	Australia
60.	*30*	Technical	Freshly prepared sample extracts are essential for successful isolation of Xcc from symptomatic plant material. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC until processing. However, when symptoms are very advanced or when environmental conditions are not favourable, the number of Xcc culturable cells can be very low and isolation can result in plates being overcrowded with competing saprophytic or antagonistic bacteria. Particular care should be taken to not confuse Xcc colonies with *Pantoea agglomerans*, which is also commonly isolated from canker lesions and produces yellow colonies on standard bacteriological media.	Text moved from paragraph 35 to here and reference to 'up to two weeks' has been removed because it seems to contradict the first part of the sentence.	English	EPPO
61.	*30*	Technical	Freshly prepared sample extracts are essential for successful isolation of Xcc from symptomatic plant material. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC until processing. However, when symptoms are very advanced or when environmental conditions are not favourable, the number of Xcc culturable cells can be very low and isolation can result in plates being overcrowded with competing saprophytic or antagonistic bacteria. Particular care should be taken to not confuse Xcc colonies with *Pantoea agglomerans*, which is also commonly isolated from canker lesions and produces yellow colonies on standard bacteriological media.	Text moved from paragraph 35 to here and reference to 'up to two weeks' has been removed because it seems to contradict the first part of the sentence.	English	European Union
62.	*32*	Editorial	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar	Simpler language and cross reference to the relevant part of the text	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			(YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged, ~~and the colony is~~ mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may not be easily cultured~~have difficulty growing on the plates~~; therefore, longer ~~more~~ incubations ~~days~~ may be required or bioassays can be used to recover the bacteria from the samples as described in 3.1.6.2.			
63.	*32*	Editorial	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged, ~~and the colony is~~ mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may not be easily cultured~~have difficulty growing on the plates~~; therefore, longer ~~more~~ incubations ~~days~~ may be required or bioassays can be used to recover the bacteria from the samples as described in 3.1.6.2.	Simpler language and cross reference to the relevant part of the text	English	European Union
64.	*32*	Editorial	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and pulverised~~comminuted~~. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary after autoclaving the media. The colony morphology on all three media is round, convex and smooth-	Comminuted is not commonly used. Pulverised is much more frequently used and would increase ease of reading and understanding, and aid translation	English	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			edged and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may have difficulty growing on the plates; therefore, more incubation days may be required or bioassays can be used to recover the bacteria from the samples.			
65.	32	Substantive	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may have difficulty growing on the plates; therefore, more incubation days may be required or bioassays can be used to recover the bacteria from the samples.	Would it be simplier to disinfect and rinse the lesions first before grinding to reduce the risk of loosing samples in the drain?	English	Jamaica
66.	32	Technical	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be previously disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on ~~nutrient~~ media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary as a fungicide after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may have difficulty growing on the plates; therefore, more incubation days may be required or bioassays can be used to recover the bacteria from the samples.Integration of kasugamycin and cephalexin in the medium (semi selective KC or KCB medium) inhibits several saprophytic bacteria and facilitates the isolation of the pathogen (Graham et al.	Further clarifications References: Graham et al. (1989). Plant Dis. 73: 423-427 Pruvost et al..,2005 J. Appl. Microbiol. 99: 803-815	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			(1989).; Pruvost et al..,2005).			
67.	32	Technical	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be previously disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on ~~nutrient~~ media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary as a fungicide after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may have difficulty growing on the plates; therefore, more incubation days may be required or bioassays can be used to recover the bacteria from the samples.Integration of kasugamycin and cephalexin in the medium (semi selective KC or KCB medium) inhibits several saprophytic bacteria and facilitates the isolation of the pathogen (Graham et al. (1989).; Pruvost et al..,2005).	Further clarifications References: Graham et al. (1989). Plant Dis. 73: 423-427 Pruvost et al..,2005 J. Appl. Microbiol. 99: 803-815	English	European Union
68.	32	Technical	Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and comminuted. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto™ Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7) and Wakimoto medium : potato broth (250 ml; sucrose, 15 g; peptone, 5 g; $Na_2HPO_4.12H_2O$, 0.8 g; $Ca(NO_3)_2 \cdot 7\ H_2O$, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary after autoclaving the media. The colony morphology on all three media is round, convex and smooth-edged and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 ºC for three to five days. In commercial fruit samples, the bacteria can be stressed and may have difficulty growing on the plates; therefore, more incubation days may be required or bioassays can be used to recover the bacteria from the samples. Add the details of isolation. Change the sentence 1 and 2 into the follows: Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected with 1% NaClO for 1 min,or alcohol for	The causal agent bacteria of citri canker is difficultyt to isolation, as it is easy to be contaminated. It is necessary to clarify the method of isolation.	English	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			7-10 second. Small pieces of the water-soaked tissue at the lesion margin are excised with a sterilized scalpel or razor blade. rinsed three times with sterile distilled water, and comminuted. Three Sterile plates (dia. 90mm) are prepared, put 0.5 ml sterile distilled water in every plate. The tissue takes into one plate and is chopped or diced in the sterile distilled water. After 12 minutes, the resulting suspension is taken three times with sterile loop into one plate, full mixed. Then take three loop suspensions into another plate. An aliquot of the extract is streaked on nutrient media. Reference: 1. Chao Jin, Xiao Qiming, Tan Zhoujin, Xie Xinwen. Study of Xanthomonas Campestris Separation Hunan Agricultural Sciences. 2005(5) : 53—55			
69.	*33*	Substantive	**3.1.3 Serological detection – immunofluorescence**	Provide information on positive and negative controls in this section	English	EPPO
70.	*33*	Substantive	**3.1.3 Serological detection – immunofluorescence**	Provide information on positive and negative controls in this section	English	European Union
71.	*33*	Technical	**3.1.3 Serological detection – indirect immunofluorescence**	For clarity	English	EPPO
72.	*33*	Technical	**3.1.3 Serological detection – indirect immunofluorescence**	For clarity	English	European Union
73.	*34*	Editorial	For serological detection on bacterial cells, a loopful of fresh culture is collected from the plate and resuspended in 1 ml phosphate-buffered saline (PBS) (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) to make approximately 10^8 colony-forming units (cfu ~~c.f.u.~~)/ml. The suspension is centrifuged at 10 000 *g* for 2 min, and then the supernatant is discarded and the cells are resuspended in 100 ml coating buffer and applied to the serological test.	An abbreviation of colony forming unit is normally cfu.	English	Thailand
74.	*34*	Substantive	For serological detection ~~on~~for bacterial cells, a loopful of fresh culture is collected from the plate and resuspended in 1 ml phosphate-buffered saline (PBS) (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) to make approximately 10^8 colony-forming units (c.f.u.)/ml. The suspension is centrifuged at 10 000 *g* for 2 min, and then the supernatant is discarded and the cells are resuspended in 100 ml coating buffer and applied to the serological test.	The serological test 'for' (or 'of') the bacterial cells, not on them.	English	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
75.	*34*	Technical	For serological detection on bacterial cells, a loopful of fresh culture is collected from the plate and resuspended in 1 ml phosphate-buffered saline (PBS) (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) to make approximately 10^8 colony-forming units (c.f.u.)/ml. ~~The suspension is centrifuged at 10 000 *g* for 2 min, and then the supernatant is discarded and the cells are resuspended in 100 ml coating buffer and applied to the serological test.~~	Like PCR, IF can be used for detection and identification. If the same technique is used for both purposes it is better to use different antibodies (or primers) for detection and for identification. This should be stated in the protocol. Final sentence: This step is not necessary for IF. A reference to the IF details indicated in the EPPO standard should be added (OEPP/EPPO Bull. 39: 413-416.	English	EPPO
76.	*34*	Technical	For serological detection on bacterial cells, a loopful of fresh culture is collected from the plate and resuspended in 1 ml phosphate-buffered saline (PBS) (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) to make approximately 10^8 colony-forming units (c.f.u.)/ml. ~~The suspension is centrifuged at 10 000 *g* for 2 min, and then the supernatant is discarded and the cells are resuspended in 100 ml coating buffer and applied to the serological test.~~	Like PCR, IF can be used for detection and identification. If the same technique is used for both purposes it is better to use different antibodies (or primers) for detection and for identification. This should be stated in the protocol. Final sentence: This step is not necessary for IF. A reference to the IF details indicated in the EPPO standard should be added (OEPP/EPPO Bull. 39: 413-416.	English	European Union
77.	*35*	Editorial	For serological detection in~~on~~ plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing. The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2~~,~~) sterilized by filtration) or in PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.	Clearer	English	EPPO
78.	*35*	Editorial	For serological detection in~~on~~ plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing. The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2~~,~~) sterilized by filtration) or	Clearer	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			in PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.			
79.	*35*	Editorial	For serological detection ~~on~~ in plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing. The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) sterilized by filtration or PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.	the pathogen is within the plant tissues, not on them.	English	Australia
80.	*35*	Substantive	For serological detection on plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing. The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) sterilized by filtration or PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.	How much PBS is used for sterilzation of the buffer? Is there a protocol?	English	Jamaica
81.	*35*	Technical	For serological detection on plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. ~~Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing.~~ The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) sterilized by filtration or PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.	Sentence moved to paragraph 30 - more relevant to isolation.	English	EPPO
82.	*35*	Technical	For serological detection on plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. ~~Plant material should be~~	Sentence moved to paragraph 30 - more relevant to isolation.	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			~~analysed as soon as possible after collection; it may be stored at 4–8 ºC for up to two weeks until processing.~~ The samples should be processed following the general procedure recommended for the specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP-10), 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) sterilized by filtration or PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests.			
83.	*36*	Editorial	Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium or sample, and also for positive and negative controls as are used for enzyme-linked immunosorbent assay (ELISA).Commercially available antiserum is diluted with PBS (pH 7.2) and appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl goat anti-rabbit gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the dark at room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine (pH 7.6) with an anti-fading agent isadded to each window, which is then covered with a coverslip.	Consistency with earlier sentence	English	EPPO
84.	*36*	Editorial	Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium or sample, and also for positive and negative controls as are used for enzyme-linked immunosorbent assay (ELISA).Commercially available antiserum is diluted with PBS (pH 7.2) and appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl goat anti-rabbit gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the dark at room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine	Consistency with earlier sentence	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			(pH 7.6) with an anti-fading agent isadded to each window, which is then covered with a coverslip.			
85.	*36*	Editorial	Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium, and also for positive and negative controls as are used for enzyme-linked immunosorbent assay (ELISA).Commercially available antiserum is diluted with PBS (pH 7.2) and appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl goat anti-rabbit gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the darkat room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine (pH 7.6) with an anti-fading agent ~~is~~ added to each window, which is then covered with a coverslip.	for clarity	English	Ghana
86.	*36*	Technical	Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium, and also for positive and negative controls as are used for enzyme-linked immunosorbent assay (ELISA).Commercially available antiserum or monoclonal antibodies are~~is~~ diluted with PBS (pH 7.2) and 25 µl of appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl of the appropriate anti-species~~goat anti-rabbit~~ gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the darkat room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine (pH 7.6) with an anti-fading agent isadded to each window, which is then covered with a coverslip.	1. If the authors have comparative data on the usefulness of several commercial antisera or monoclonal antibodies for IF, it would be useful to have a comment on them added here. 2. Clarity 3. Clarification to allow for antibodies raised in other species	English	EPPO
87.	*36*	Technical	Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium, and also for positive and negative controls as are used for enzyme-linked immunosorbent assay (ELISA).Commercially available antiserum	1. If the authors have comparative data on the usefulness of several commercial antisera or monoclonal antibodies for IF, it would be useful to have a comment on them added here. 2. Clarity 3. Clarification to allow for antibodies raised in other	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			or monoclonal antibodies areis diluted with PBS (pH 7.2) and 25 µl of appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl of the appropriate anti-speciesgoat anti-rabbit gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the darkat room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine (pH 7.6) with an anti-fading agent isadded to each window, which is then covered with a coverslip.	species		
88.	*37*	Editorial	The slides are examined under immersion oil with a fluorescence microscope at 600× or 1 000× magnification. FITC fluoresces bright green under the ultraviolet light of the microscope. If the positive control with known bacterium shows fluorescent rod-shaped bacterial cells and the negative controls of normal serum and PBS do not, the sample windows are examined for bacterial cell wall fluorescence, looking for the cells with the size and form of Xcc. This method permits detection in the order of approximately 10^3 cells/ml.	1. Simpler language (2nd & 3rd sentences) 2. Last sentence: cells/ml here, c.f.u./ml in para 51; please ensure consistency of units	English	EPPO
89.	*37*	Editorial	The slides are examined under immersion oil with a fluorescence microscope at 600× or 1 000× magnification. FITC fluoresces bright green under the ultraviolet light of the microscope. If the positive control with known bacterium shows fluorescent rod-shaped bacterial cells and the negative controls of normal serum and PBS do not, the sample windows are examined for bacterial cell wall fluorescence, looking for the cells with the size and form of Xcc. This method permits detection in the order of approximately 10^3 cells/ml.	1. Simpler language (2nd & 3rd sentences) 2. Last sentence: cells/ml here, c.f.u./ml in para 51; please ensure consistency of units	English	European Union
90.	*43*	Technical	For conventional and real-time PCR, a plant housekeeping gene (HKG) such as COX (Weller *et al.*, 2000), GADPH (Mafra *et al.*, 2012) or 16S ribosomal (r)DNA (Weisberg *et al.,* 1991) should be incorporated into the PCR protocol as a control to eliminate the possibility of false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors.	According Mafra el al. the GADPH gene, used for internal control in citrus for PCR, presents better results than COX	English	Uruguay
91.	*43*	Technical	For conventional and real-time PCR, a plant housekeeping gene (HKG) such as COX (Weller *et al.*, 2000), GADPH (Mafra *et al.*, 2012) or 16S ribosomal (r)DNA (Weisberg *et al.,* 1991) should be incorporated into the PCR protocol as a control to eliminate the possibility of false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors.	According Mafra el al. the GADPH gene, used for internal control in citrus for PCR, presents better results than COX	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
92.	*43*	Technical	For conventional and real-time PCR, a plant housekeeping gene (HKG) such as COX (Weller *et al.*, 2000) or 16S ribosomal (r)DNA (Weisberg *et al.,* 1991) should be incorporated into the PCR protocol as a control to eliminate the possibility of false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors.	Weisberg et al lists several potential primers. Which are the preferred?	English	Australia
93.	*45*	Technical	**Positive extraction control** This control is used to ensure that nucleic acid from the target is of sufficient quantity and quality for PCR amplification and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target at the concentration considered the detection limit of the protocol.	This represents best practice. It could be qualified by adding 'preferably'.	English	EPPO
94.	*45*	Technical	**Positive extraction control** This control is used to ensure that nucleic acid from the target is of sufficient quantity and quality for PCR amplification and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target at the concentration considered the detection limit of the protocol.	This represents best practice. It could be qualified by adding 'preferably'.	English	European Union
95.	*49*	Editorial	DNA extraction from infected citrus tissue was originally performed by Hartung *et al.* (1993) with a hexadecyltrimethylammonium bromide (CTAB) protocol, but there are commercial methods and an isopropanol protocol (not requiring phenol) that have been extensively evaluated (Llop *et al.*, 1999). In the isopropanol protocol, lesions or plant material suspected to be infected are cut into small pieces, covered with PBS and shaken in a rotary shaker for 20 min at room temperature. The supernatant is filtered (to remove plant material) and then centrifuged for 20 min at 10 000 *g*. The pellet is resuspended in 1 ml PBS: 500 µl is saved for further analysis or for direct isolation on agar plates, and 500 µl is centrifuged at 10 000 *g* for 10 min. The pellet is resuspended in 500 µl extraction buffer (200 mM Tris-HCl, pH 7.5; 250 mM NaCl; 25 mM ethylenediaminetetraacetic (EDTA); 0.5% sodium dodecyl sulphate (SDS); 2% polyvinylpyrrolidone (PVP)), vortexed and left for 1 h at room temperature with continuous shaking. The suspension is then centrifuged at 5 000 *g* for 5 min, after which 450 µl of the supernatant is~~is~~ transferred to a new tube and mixed with 450 µl isopropanol. The suspension is mixed gently and left at room temperature for 1 h. Precipitation can be improved by the use of Pellet Paint® co-precipitant (Cubero *et al.,* 2001). The suspension is centrifuged at 13 000 *g* for 10 min, the supernatant is discarded, and the pellet is dried. The pellet is resuspended in 100 µl water. A 5 µl sample is used in a 50 µl PCR reaction. The conventional PCR method allows detection of 10^3 cfu ~~c.f.u.~~/ml (Hartung *et al.*, 1993).	- wrong word - An abbreviation of colony forming unit is normally cfu.	English	Thailand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
96.	49	Technical	DNA extraction from infected citrus tissue was originally performed by Hartung *et al.* (1993) with a hexadecyltrimethylammonium bromide (CTAB) protocol, but there are commercial methods and an isopropanol protocol (not requiring phenol) that have been extensively evaluated (Llop *et al.*, 1999). In the isopropanol protocol, lesions or plant material suspected to be infected are cut into small pieces, covered with PBS and shaken in a rotary shaker for 20 min at room temperature. The supernatant is filtered (to remove plant material) and then centrifuged for 20 min at 10 000 *g*. The pellet is resuspended in 1 ml PBS: 500 µl is saved for further analysis or for direct isolation on agar plates, and 500 µl is centrifuged at 10 000 *g* for 10 min. The pellet is resuspended in 500 µl extraction buffer (200 mM Tris-HCl, pH 7.5; 250 mM NaCl; 25 mM ethylenediaminetetraacetic (EDTA); 0.5% sodium dodecyl sulphate (SDS); 2% polyvinylpyrrolidone (PVP)), vortexed and left for 1 h at room temperature with continuous shaking. The suspension is then centrifuged at 5 000 *g* for 5 min, after which 450 µl of the supernatant isis transferred to a new tube and mixed with 450 µl isopropanol. The suspension is mixed gently and left at room temperature for 1 h. Precipitation can be improved by the use of Pellet Paint® co-precipitant (Cubero *et al.,* 2001). The suspension is centrifuged at 13 000 *g* for 10 min, the supernatant is discarded, and the pellet is dried. The pellet is resuspended in 100 µl water. A 5 µl sample is used in a 50 µl PCR reaction. The conventional PCR method allows detection of 10^3 c.f.u./ml (Hartung *et al.*, 1993).	Centrifuge speed should include revolution per minute (rpm) conversion for easy of application.	English	Nigeria
97.	49	Technical	DNA extraction from infected citrus tissue was originally performed by Hartung *et al.* (1993) with a hexadecyltrimethylammonium bromide (CTAB) protocol, but there are commercial methods and an isopropanol protocol (not requiring phenol) that have been extensively evaluated (Llop *et al.*, 1999). In the isopropanol protocol, lesions or plant material suspected to be infected are cut into small pieces, covered with PBS and shaken in a rotary shaker for 20 min at room temperature. The supernatant is filtered (to remove plant material) and then centrifuged for 20 min at 10 000 *g*. The pellet is resuspended in 1 ml PBS: 500 µl is saved for further analysis or for direct isolation on agar plates, and 500 µl is centrifuged at 10 000 *g* for 10 min. The pellet is resuspended in 500 µl extraction buffer (200 mM Tris-HCl, pH 7.5; 250 mM NaCl; 25 mM ethylenediaminetetraacetic (EDTA); 0.5% sodium dodecyl sulphate (SDS); 2% polyvinylpyrrolidone (PVP)), vortexed and left for 1 h at room temperature with continuous shaking. The suspension is then centrifuged at 5 000 *g* for 5 min, after which 450 µl of the supernatant isis transferred to a new tube and mixed with 450 µl isopropanol. The suspension is mixed gently and left at room temperature for 1 h. Precipitation can be improved by the use of Pellet Paint® co-precipitant (Cubero *et al.,* 2001). The suspension is centrifuged at 13 000 *g* for 10 min, the supernatant is discarded, and	1. Move last sentence to section 3.1.4.3 as it fits better there. 2. A sentence should be added at the end of this paragraph to refer to the existence of commercial kits with examples e.g. Promega Wizard Genomic DNA purification kit (Coletta-Filho et al. 2006. J. Appl. Microbiol.).	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			the pellet is dried. The pellet is resuspended in 100 µl water. A 5 µl sample is used in a 50 µl PCR reaction. ~~The conventional PCR method allows detection of 10^3 c.f.u./ml (Hartung *et al.*, 1993).~~			
98.	*49*	Technical	DNA extraction from infected citrus tissue was originally performed by Hartung *et al.* (1993) with a hexadecyltrimethylammonium bromide (CTAB) protocol, but there are commercial methods and an isopropanol protocol (not requiring phenol) that have been extensively evaluated (Llop *et al.*, 1999). In the isopropanol protocol, lesions or plant material suspected to be infected are cut into small pieces, covered with PBS and shaken in a rotary shaker for 20 min at room temperature. The supernatant is filtered (to remove plant material) and then centrifuged for 20 min at 10 000 *g*. The pellet is resuspended in 1 ml PBS: 500 µl is saved for further analysis or for direct isolation on agar plates, and 500 µl is centrifuged at 10 000 *g* for 10 min. The pellet is resuspended in 500 µl extraction buffer (200 mM Tris-HCl, pH 7.5; 250 mM NaCl; 25 mM ethylenediaminetetraacetic (EDTA); 0.5% sodium dodecyl sulphate (SDS); 2% polyvinylpyrrolidone (PVP)), vortexed and left for 1 h at room temperature with continuous shaking. The suspension is then centrifuged at 5 000 *g* for 5 min, after which 450 µl of the supernatant isis transferred to a new tube and mixed with 450 µl isopropanol. The suspension is mixed gently and left at room temperature for 1 h. Precipitation can be improved by the use of Pellet Paint® co-precipitant (Cubero *et al.,* 2001). The suspension is centrifuged at 13 000 *g* for 10 min, the supernatant is discarded, and the pellet is dried. The pellet is resuspended in 100 µl water. A 5 µl sample is used in a 50 µl PCR reaction. ~~The conventional PCR method allows detection of 10^3 c.f.u./ml (Hartung *et al.*, 1993).~~	1. Move last sentence to section 3.1.4.3 as it fits better there. 2. A sentence should be added at the end of this paragraph to refer to the existence of commercial kits with examples e.g. Promega Wizard Genomic DNA purification kit (Coletta-Filho et al. 2006. J. Appl. Microbiol.).	English	European Union
99.	*51*	Editorial	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A^* and A^w detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains A^* and A^w or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A^* and A^w are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime)	Typo (authority names not used)	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			and *C. macrophylla* ~~Webster (A~~alemow) – both primer sets should be used.			
100.	51	Editorial	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A^* and A^w detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains A^* and A^w or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A^* and A^w are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* ~~Webster (A~~alemow) – both primer sets should be used.	Typo (authority names not used)	English	European Union
101.	51	Editorial	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 cfu ~~c.f.u.~~/ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A^* and A^w detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 cfu ~~c.f.u.~~/ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains A^* and A^w or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A^* and A^w are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both primer sets should be used.	An abbreviation of colony forming unit is normally cfu.	English	Thailand
102.	51	Substantive	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp.	According to Cubero and Graham (2002) on which PCR protocol in this draft is based, the Hartung (1993) primers can detect Xcc strains A*.	English	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			aurantifolii (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A* and A^{w} detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains ~~A* and~~ A^{w} or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains ~~A* and~~ A^{w} are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both primer sets should be used.			
103.	51	Technical	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* ~~(formerly citrus canker pathotype strains B and C)~~ and the atypical Xcc strains A* and A^{w} detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the ~~atypical Xcc~~ Xcc- A^{W} and a few Xcc-A* strains ~~A* and A^{w}~~ or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A* and A^{w} are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both primer sets should be used. The detection limit of the conventional PCR protocol is approximately 10^3 c.f.u./ml (Hartung et al., 1993).	1. Already stated earlier 2. More precice 3. Sentence moved from paragraph 49 and modified for consistency.	English	EPPO
104.	51	Technical	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A* and A^{w} detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains A* and A^{w} or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A* and A^{w} are suspected – for example, where	Diagnosis for Xcc. using primers 2 and 3 need restriction enzyme digestion to identifie Xanthomonas citri subspecies. Others primers with the same sensibility present more specific results without using a restricition enzyme digestion to identify subspecies	English	Uruguay

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both primer sets should be used. Other primers with the same sensibility present more specific results without using a restriction enzyme digestion to identify subspecies (Coletta Filho et al, 2007)			
105.	51	Technical	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* (formerly citrus canker pathotype strains B and C) and the atypical Xcc strains A^* and A^w detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the atypical Xcc strains A^* and A^w or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A^* and A^w are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both primer sets should be used. Other primers with the same sensibility present more specific results without using a restriction enzyme digestion to identify subspecies (Coletta Filho et al, 2007)	Diagnosis for Xcc. using primers 2 and 3 need restriction enzyme digestion to identifie Xanthomonas citri subspecies. Others primers with the same sensibility present more specific results without using a restricition enzyme digestion to identify subspecies	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
106.	51	Technical	Several primer pairs are available for diagnosis of Xcc. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to Xcc and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10^2 c.f.u./ml). Primers J-pth1 and J-pth2 target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include Xcc, *X. fuscans* subsp. *aurantifolii* ~~(formerly citrus canker pathotype strains B and C)~~ and the atypical Xcc strains A^* and A^w detected in Florida (Cubero & Graham, 2002). The primers are universal, but they have lower sensitivity (10^4 c.f.u./ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the ~~atypical Xcc~~ Xcc- A^W and a few Xcc-A* strains ~~A^* and A^w~~ or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical Xcc strains A^* and A^w are suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* Webster (Alemow) – both	1. Already stated earlier 2. More precice 3. Sentence moved from paragraph 49 and modified for consistency.	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			primer sets should be used. The detection limit of the conventional PCR protocol is approximately 10^3 c.f.u./ml (Hartung et al., 1993).			
107.	56	Substantive	The PCR mixture is prepared in a sterile vial and consists of PCR buffer (50 mM Tris-HCl, pH 9; 20 mM NaCl; 1% Triton™ X-100; 0.1% gelatin; 3 mM $MgCl_2$), 1 µM of each primer 2 and 3, 0.2 mM of each deoxynucleotide triphosphate (dNTPs) and 1.25 U Taq DNA polymerase. Extracted DNA sample volume of 5 µl is added to 45 µl of the PCR mixture to give a total of 50 µl per reaction. The reaction conditions are an initial denaturation step of 95 ºC for 2 min followed by 35 cycles of 95 ºC for 60 s, 58 ºC for 70 s and 72 ºC for 75 s, and a final elongation step of 72 ºC for 10 min. The amplicon size is 222 bp.	The conditions specified for using Hartung 93 could be simplified for ease and speed. The buffer has high Mg2+ and a bit of a bother to make detergent plus gelatin. Reactions worked well in buffer supplied with Taq polymerase which is much easier Cycle step times are long at 60, 70, 75 seconds....	English	Australia
108.	56	Technical	The PCR mixture is prepared in a sterile ~~vial~~tube and consists of PCR buffer (50 mM Tris-HCl, pH 9; 20 mM NaCl; 1% Triton™ X-100; 0.1% gelatin; 3 mM $MgCl_2$), 1 µM of each primer 2 and 3, 0.2 mM of each deoxynucleotide triphosphate (dNTPs) and 1.25 U Taq DNA polymerase. Extracted DNA sample volume of 5 µl is added to 45 µl of the PCR mixture to give a total of 50 µl per reaction. The reaction conditions are an initial denaturation step of 95 ºC for 2 min followed by 35 cycles of 95 ºC for 60 s, 58 ºC for 70 s and 72 ºC for 75 s, and a final elongation step of 72 ºC for 10 min. Visualise the PCR products using agarose gel electrophoresis. The amplicon size is 222 bp.	From a molecular perspective, the word 'vial' is not used. It is a 'microcentrifuge tube' usually shortened to 'tube'. A 'vial' is more likely to be glass with a stopper or screw-cap lid. New sentence is missing or assumed from the protocol, but fits in with what follows regarding the observation of the amplicon.	English	Australia
109.	61	Technical	The PCR mixture is prepared in a sterile ~~vial~~ tube and consists of 1× Taq buffer, 3 mM $MgCl_2$, 1 µM of each primer *J-pth1* and *J-pth2*, 0.2 mM of each dNTPs and 1 U Taq DNA polymerase. Extracted DNA sample volume of 2.5 µl is added to 22.5 µl of the PCR mixture to give a total of 25 µl per reaction. The reaction conditions are an initial denaturation step of 94 ºC for 5 min followed by 40 cycles of 93 ºC for 30 s, 58 ºC for 30 s and 72 ºC for 45 s, and a final elongation step of 72 ºC for 10 min. The amplicon size is 197 bp.	From a molecular perspective, the word 'vial' is not used. It is a 'microcentrifuge tube' usually shortened to 'tube'. A 'vial' is more likely to be glass with a stopper or screw-cap lid.	English	Australia
110.	63	Editorial	**3.1.4.4 Real-time PCR**	add a space between section number and topic	English	Thailand
111.	65	Editorial	A set of primers, *J-pth3* (5'-ACC GTC CCC TAC TTC AAC TCA A-3') and *J-pth4* (5'-CGC ACC TCG AAC GAT TGC-3'), and the corresponding TaqMan® probe (*J-Taqpth2*) (5'-ATG CGC CCA GCC CAA CGC-3') labelled at the 5′ end with 6-carboxyfluorescein (FAM) and at the 3′ end with tetramethylrhodamine were designed based on sequences of the *pth* gene, a major virulence gene used in other studies specifically to detect Xcc strains (Cubero & Graham, 2005). These strains include Xcc, *X. fuscans* subsp. *aurantifolii*~~(formerly citrus canker pathotype strains B and C)~~ and the atypical Xcc strains A^* and A^w detected in Florida.	Already stated earlier	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
112.	65	Editorial	A set of primers, *J-pth3* (5'-ACC GTC CCC TAC TTC AAC TCA A-3') and *J-pth4* (5'-CGC ACC TCG AAC GAT TGC-3'), and the corresponding TaqMan® probe (*J-Taqpth2*) (5'-ATG CGC CCA GCC CAA CGC-3') labelled at the 5′ end with 6-carboxyfluorescein (FAM) and at the 3′ end with tetramethylrhodamine were designed based on sequences of the *pth* gene, a major virulence gene used in other studies specifically to detect Xcc strains (Cubero & Graham, 2005). These strains include Xcc, *X. fuscans* subsp. *aurantifolii*~~(formerly citrus canker pathotype strains B and C)~~ and the atypical Xcc strains A^* and A^w detected in Florida.	Already stated earlier	English	European Union
113.	66	Technical	Real-time PCR is carried out by adding 2 µl template DNA to a reaction mixture containing 12.5 µl QuantiMix Easy Kit, which comprises QuantiMix Easy Master Mix[1] and $MgCl_2$ (50 mM), 1 µl of 10 µM forward primer (*J-RTpth3*), 1 µl of 10 µM reverse primer (*J-RTpth4*) and 0.5 µl of 10 µM TaqMan® probe (*J-Taqpth2*) and made up to a final reaction volume of 25 µl with sterile distilled water. The protocol for real-time PCR has been developed using~~is completed in~~ an ABI[2] PRISM® 7000 Sequence Detection System. Other equipment has given similar results (Lopez, pers. comm. 2013). Amplification conditions for all primers and probes are an initial activation step of 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A complete real –time PCR kit based on this protocol and including master mix and enzyme is available commercially from Plant Print Diagnostics(www.plantprint.net).	1. For clarity and to indicate that other equipment also works using this protocol. 2. Further relevant information	English	EPPO
114.	66	Technical	Real-time PCR is carried out by adding 2 µl template DNA to a reaction mixture containing 12.5 µl QuantiMix Easy Kit, which comprises QuantiMix Easy Master Mix[1] and $MgCl_2$ (50 mM), 1 µl of 10 µM forward primer (*J-RTpth3*), 1 µl of 10 µM reverse primer (*J-RTpth4*) and 0.5 µl of 10 µM TaqMan® probe (*J-Taqpth2*) and made up to a final reaction volume of 25 µl with sterile distilled water. The protocol for real-time PCR has been developed using~~is completed in~~ an ABI[2] PRISM® 7000 Sequence Detection System. Other equipment has given similar results (Lopez, pers. comm. 2013). Amplification conditions for all primers and probes are an initial activation step of 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A complete real –time PCR kit based on this protocol and including master mix and enzyme is available commercially from Plant Print Diagnostics(www.plantprint.net).	1. For clarity and to indicate that other equipment also works using this protocol. 2. Further relevant information	English	European Union
115.	67	Editorial	The real-time PCR provides similar specificity to the *pth* gene primers used in the conventional PCR method (Cubero & Graham, 2002, 2005) and enables reliable detection of approximately 10 cfu ~~c.f.u.~~ of Xcc from diseased leaf lesions and from a dilution of cultured cells (Mavrodieva *et al.*, 2004). This method has recently been compared with standard and nested PCR (Golmohammadi *et al.*, 2007) and the sensitivity obtained is very good (10 cfu ~~c.f.u.~~/ml).	An abbreviation of colony forming unit is normally cfu.	English	Thailand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
116.	*67*	Technical	The real-time PCR provides similar specificity to the *pth* gene primers used in the conventional PCR method (Cubero & Graham, 2002, 2005) and enables reliable detection of approximately 10 c.f.u. of Xcc from diseased leaf lesions and from a dilution of cultured cells (Mavrodieva *et al.*, 2004). This method has recently been compared with standard and nested PCR (Golmohammadi *et al.*, 2007) and the reported sensitivity was ~~obtained is~~ very good (10 c.f.u./ml) in the analysis of fruit lesions.	Suggestion to clarify the information.	English	EPPO
117.	*67*	Technical	The real-time PCR provides similar specificity to the *pth* gene primers used in the conventional PCR method (Cubero & Graham, 2002, 2005) and enables reliable detection of approximately 10 c.f.u. of Xcc from diseased leaf lesions and from a dilution of cultured cells (Mavrodieva *et al.*, 2004). This method has recently been compared with standard and nested PCR (Golmohammadi *et al.*, 2007) and the reported sensitivity was ~~obtained is~~ very good (10 c.f.u./ml) in the analysis of fruit lesions.	Suggestion to clarify the information.	English	European Union
118.	*73*	Technical	If 16S rDNAinternal control primers are also used, then the negative (healthy plant tissue) control (if used), positive control, and each of the test samples must produce a 1.6 kilobase (kb) band (16S rDNA). Note that synthetic and plasmid positive controls will not produce a 1.6 kb band. Failure of the samples to amplify with the internal control primers suggests, for example, that the DNA extraction has failed, the nucleic acid has not been included in the reaction mixture, compounds inhibitory to PCR are present in the DNA extract, or the DNA has degraded.	Using the 16S rRNA gene for an internal control doesn't necessarily give a 1.6kb product. This depends on the primers used as most commonly-used 16S primers give shorter products. Unless it states amplifying the whole 16S rRNA gene with identified primers, it is not possible to say definitely 1.6 kb therefore question the use of 'must'	English	Australia
119.	*84*	Editorial	The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young *Citrus paradisi* var. Duncan (grapefruit) leaves are surface-disinfected for 1 min with 1% NAClO. The leaves are rinsed three times with sterile distilled water and then surface dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 96% ethanol), are placed back up in each well with the agar-water. Fifty microlitres of macerated citrus canker lesions (four replicates for each sample) are added.	query - should 96% ethanol be 95% (the more usual standard)?	English	Australia
120.	*84*	Technical	The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young *Citrus paradisi* var. Duncan (grapefruit) leaves (or other highly susceptible Xcc hosts) are surface-disinfected for 1 min with 1% NAClO. The leaves are rinsed three times with sterile distilled	1. Mexican lime (Citrus aurantifolia) should also be used to allow Xcc-Aw and Aw to produce canker, because grapefruit is not susceptible to those	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			water and then surface dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 96% ethanol), are placed adaxial surface down on the water agar ~~back up~~ in each well~~with the agar-water~~. Fifty microlitres of macerated citrus canker lesions (four replicated wells for eachplant sample) are added.	strains. 2. Clearer explanation		
121.	*84*	Technical	The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young *Citrus paradisi* var. Duncan (grapefruit) leaves (or other highly susceptible Xcc hosts) are surface-disinfected for 1 min with 1% NAClO. The leaves are rinsed three times with sterile distilled water and then surface dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 96% ethanol), are placed adaxial surface down on the water agar ~~back up~~ in each well~~with the agar-water~~. Fifty microlitres of macerated citrus canker lesions (four replicated wells for eachplant sample) are added.	1. Mexican lime (Citrus aurantifolia) should also be used to allow Xcc-Aw and Aw to produce canker, because grapefruit is not susceptible to those strains. 2. Clearer explanation	English	European Union
122.	*84*	Technical	The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young citrus leaves from *Citrus paradisi* var. Duncan (grapefruit) or *C. aurantifolia* (Mexican lime) ~~leaves~~ are surface-disinfected for 1 min with 1% NAClO. The leaves are rinsed three times with sterile distilled water and then surface dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 96% ethanol), are placed back up in each well with the agar-water. Fifty microlitres of macerated citrus canker lesions (four replicates for each sample) are added.	C. aurantifolia can be used as a host for bioassay.	English	Thailand
123.	*84*	Technical	The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young *Citrus paradisi* var. Duncan (grapefruit) leaves are surface-disinfected for 1 min with 1% NAClO. The leaves should be fully expanded but not mature and hard. They ~~leaves~~ are rinsed three times with sterile distilled water and then surface dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 96% ethanol), are placed back up in each well with the agar-water. Fifty microlitres of macerated citrus canker lesions (four replicates for each sample) are added.	It is important that the leaves are of the right age and stage. Note: in our experience, Poncirus tirifoliata is a more sensitive host than Duncan grapefruit in bioassays.	English	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
124.	*85*	Editorial	An Xcc suspension of 10^5 cfu ~~c.f.u.~~/ml is used as a positive control and sterile saline as a negative control (four replicates each). Plates are sealed with Parafilm®, achieving a relative humidity of almost 100%, and incubated at 28 ºC for 12 days under constant light. The formation of incipient whitish pustules in each of the leaf discs is evaluated from the third day using stereoscopic microscopy and isolation techniques for Xcc as described in section 3.1.2. The symptomless discs can be further analysed for the presence of living bacteria by isolation onto semi-selective media (Verdier *et al*., 2008). After 12 days, if Xcc is present, the bacterial cells have multiplied on the plant tissue and can be isolated onto media in higher numbers. This bioassay is a very specific and sensitive (10^2 cfu ~~c.f.u.~~/ml) diagnostic method (Verdier *et al.*, 2008).	An abbreviation of colony forming unit is normally cfu.	English	Thailand
125.	*85*	Technical	An Xcc suspension of 10^5 c.f.u./ml is used as a positive control and sterile saline as a negative control (four replicates each). Plates are sealed with Parafilm®, achieving a relative humidity of almost 100%, and incubated at 28 ºC for 12 days under constant light, but check progress regularly before then. The formation of incipient whitish pustules in each of the leaf discs is evaluated from the third day using stereoscopic microscopy and isolation techniques for Xcc as described in section 3.1.2. The symptomless discs can be further analysed for the presence of living bacteria by isolation onto semi-selective media (Verdier *et al*., 2008). After 12 days, if Xcc is present, the bacterial cells have multiplied on the plant tissue and can be isolated onto media in higher numbers. This bioassay is a very specific and sensitive (10^2 c.f.u./ml) diagnostic method (Verdier *et al.*, 2008).	Need to check progress earlier than 12 days due to the possible contamination by environmental organisms. If use Poncirus tirifoliata, will definitely show response much quicker.	English	Australia
126.	*87*	Editorial	Xcccan also be selectively enriched in wounded detached leaves of *Citrus paradisi* var. Duncan (grapefruit). Young terminal leaves from glasshouse-grown plants are washed for 10 min in running tap water, surface-disinfected in 1% NACIO for 1 min, and aseptically rinsed thoroughly with sterile distilled water. The lower surface of each leaf is aseptically wounded by puncturing it with a needle or by making small cuts with a scalpel, and the whole leaves are placed onto 1% agar in sterile water in the wells of ELISA plates with their lower surface up. Droplets of 10–20 µl of macerated citrus canker lesions are added. Positive and negative controls as for the leaf disc bioassay are used. After 7–12 days at 25 ºC in a lighted incubator, pustule development is evaluated and Xcc is isolated as described above (EPPO, 1998).	For clarity	English	Ghana
127.	*87*	Technical	Xcccan also be selectively enriched in wounded detached leaves of *Citrus paradisi* var. Duncan (grapefruit) (or other higly susceptible Xcc hosts). Young terminal leaves from glasshouse-grown plants are washed for 10 min in running tap water, surface-disinfected in 1% NACIO for 1 min, and aseptically rinsed thoroughly with sterile distilled water. The lower surface of each leaf is aseptically wounded by	1. See previous comment in relation to paragraph 84. 2. For clarity. 3. The enrichment in detached leaves is used as a bioassay to increase the number of viable and culturable cells. It is usually needed when analysing fruits with canker-like	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			puncturing it with a needle or by making small cuts with a scalpel, and the whole leaves are placed onto 1% agar in sterile water in the wells of ELISA plates with their lower surface up. Droplets of 10–20 µl of macerated citrus canker lesions are added to the wounds. Positive and negative controls as for the leaf disc bioassay are used. After 7–12 days at 25 ºC in a lighted incubator, pustule development is evaluated and if negative Xcc is isolated as above (EPPO, 1998).	lessions. If the PCR test is positive and isolation negative, the enrichment in a detached leaf can help to the multiplication of X. citri to numbers sufficient to give lesions (positive pustules) or if pustules do not appear, at least to give positive colonies in the isolation from the wounds where the inoculations for the enrichment were performed.		
128.	*87*	Technical	Xcccan also be selectively enriched in wounded detached leaves of *Citrus paradisi* var. Duncan (grapefruit) (or other higly susceptible Xcc hosts). Young terminal leaves from glasshouse-grown plants are washed for 10 min in running tap water, surface-disinfected in 1% NAClO for 1 min, and aseptically rinsed thoroughly with sterile distilled water. The lower surface of each leaf is aseptically wounded by puncturing it with a needle or by making small cuts with a scalpel, and the whole leaves are placed onto 1% agar in sterile water in the wells of ELISA plates with their lower surface up. Droplets of 10–20 µl of macerated citrus canker lesions are added to the wounds. Positive and negative controls as for the leaf disc bioassay are used. After 7–12 days at 25 ºC in a lighted incubator, pustule development is evaluated and if negative Xcc is isolated as above (EPPO, 1998).	1. See previous comment in relation to paragraph 84. 2. For clarity. 3. The enrichment in detached leaves is used as a bioassay to increase the number of viable and culturable cells. It is usually needed when analysing fruits with canker-like lessions. If the PCR test is positive and isolation negative, the enrichment in a detached leaf can help to the multiplication of X. citri to numbers sufficient to give lesions (positive pustules) or if pustules do not appear, at least to give positive colonies in the isolation from the wounds where the inoculations for the enrichment were performed.	English	European Union
129.	*87*	Technical	Xcccan also be selectively enriched in wounded detached leaves of Poncirus trifoliata (if available) or *Citrus paradisi* var. Duncan (grapefruit). Young terminal leaves from glasshouse-grown plants are washed for 10 min in running tap water, surface-disinfected in 1% NAClO for 1 min, and aseptically rinsed thoroughly with sterile distilled water. The lower surface of each leaf is aseptically wounded by puncturing it with a needle or by making small cuts with a scalpel, and the whole leaves are placed onto 1% agar in sterile water in the wells of ELISA plates with their lower surface up. Droplets of 10–20 µl of macerated citrus canker lesions are added. Positive and negative controls as for the leaf disc bioassay are used. After 4 days for P. tirifoliata; or 7~~7~~–12 days at 25 ºC in a lighted incubator, pustule development is evaluated and Xcc is isolatedas above (EPPO, 1998).	Need to check progress earlier than 7 days due to the possible contamination by environmental organisms. Poncirus tirifoliata will show positive response in 4 days.	English	Australia
130.	*88*	Technical	**3.2 Detection in asymptomatic plants** Adding other methods including serological detection and molecular detection.	For asymptomatic plants, we nearly can't got Xcc by islation. Suggest using other detection methods they are more sensitive than isolation.	English	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
131.	*89*	Substantive	Isolation of Xcc from asymptomatic plants on semi-selective media can be achieved by washing the leaf or fruit samples in peptone buffer, concentrating the supernatant, and then plating onto the media (Verdier *et al.*, 2008). Ten leaves or one fruit constitute a sample. Note: Apparently healthy mature Satsuma mandarin fruit is not the source of infection of Xanthomonas citri subsp. citri.	According to Shiotani et al. (2008)*1 and Shiotani et al. (2009)*2, apparently healthy mature Satsuma mandarin fruit is not the source of infection of Xanthomonas citri subsp. citri. *1 Shiotani et al. (2008) J. Gen. Plant Pathol. 74 (2) : 133-137 *2 Shiotani et al. (2009) Crop protection 28 (1) : 19-23	English	Japan
132.	*90*	Substantive	Samples are shaken for 20 min at room temperature in 50 ml peptone buffer (NaCl, 8.5 g; peptone, 1 g; Tween® 20, 250 µl; distilled water, 1 litre; pH 7.2). For bulked samples, 100 leaves in 200 ml peptone buffer can be used. Individual fruits are shaken for 20 min at room temperature in sterile bags containing 50 ml peptone buffer. Note: Apparently healthy mature Satsuma mandarin fruit is not the source of infection of Xanthomonas citri subsp. citri.	The same as paragraphs [89].	English	Japan
133.	*91*	Technical	The suspension is then centrifuged at 6 000 *g* for 20 min. The supernatant is decanted and the pellet resuspended in 10 ml of 0.85% saline. Aliquots (100 µl) of 1:100 and 1:1000 dilutions of each suspension are streaked in triplicate onto XOS semi-selective medium (sucrose, 20 g; peptone, 2 g; monosodium glutamate, 5 g; $Ca(NO_3)_2$, 0.3 g; K_2HPO_4, 2 g; EDTA-Fe, 1 mg; cycloheximide, 100 mg; cephalexine, 20 mg; kasugamycine, 20 mg; methyl violet 2B, 0.3 mg; Bacto™ Agar, 17 g; distilled water, 1 litre; pH 7.0) (Monier, 1992). After incubation at 28 ºC for 5–6 days, growth as well as colony type and morphology are evaluated (see section 3.1.2).	If molecular methods such as real-time PCR can be applied as an screening method for detection in asymptomatic plant parts, then this should be indicated.	English	EPPO
134.	*91*	Technical	The suspension is then centrifuged at 6 000 *g* for 20 min. The supernatant is decanted and the pellet resuspended in 10 ml of 0.85% saline. Aliquots (100 µl) of 1:100 and 1:1000 dilutions of each suspension are streaked in triplicate onto XOS semi-selective medium (sucrose, 20 g; peptone, 2 g; monosodium glutamate, 5 g; $Ca(NO_3)_2$, 0.3 g; K_2HPO_4, 2 g; EDTA-Fe, 1 mg; cycloheximide, 100 mg; cephalexine, 20 mg; kasugamycine, 20 mg; methyl violet 2B, 0.3 mg; Bacto™ Agar, 17 g; distilled water, 1 litre; pH 7.0) (Monier, 1992). After incubation at 28 ºC for 5–6 days, growth as well as colony type and morphology are evaluated (see section 3.1.2).	If molecular methods such as real-time PCR can be applied as an screening method for detection in asymptomatic plant parts, then this should be indicated.	English	European Union
135.	*93*	Editorial	Identification of presumptive Xcc colonies should be verified by several techniques because other species of *Xanthomonas*, such as *X. fuscans* subsp. *aurantifolii* and *X. alfalfa*e subsp. c~~C~~*itrumelonis*,can be isolated from citrus. Techniques include observing morphological characteristics on nutrient media, serological testing,	Missing e, typo and better English	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			molecular testing, bioassay on~~f~~ leaf discs or detached leaves, and pathogenicity testing.			
136.	*93*	Editorial	Identification of presumptive Xcc colonies should be verified by several techniques because other species of *Xanthomonas*, such as *X. fuscans* subsp. *aurantifolii* and *X. alfalfa*e subsp. c~~C~~*itrumelonis*,can be isolated from citrus. Techniques include observing morphological characteristics on nutrient media, serological testing, molecular testing, bioassay on~~f~~ leaf discs or detached leaves, and pathogenicity testing.	Missing e, typo and better English	English	European Union
137.	*93*	Editorial	Identification of presumptive Xcc colonies should be verified by several techniques because other species of *Xanthomonas*, such as *X. fuscans* subsp. *aurantifolii* and *X. alfalfa*subsp. c~~C~~*itrumelonis*, can be isolated from citrus. Techniques include observing morphological characteristics on nutrient media, serological testing, molecular testing, bioassay of leaf discs or detached leaves, and pathogenicity testing.	lower case	English	Australia
138.	*94*	Editorial	The minimum requirements for identification are isolation of the bacterium and a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see section~~s~~ 4.3 ~~and 3.1.5~~). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	Typo - incorrect reference to section 3.1.5.	English	EPPO
139.	*94*	Editorial	The minimum requirements for identification are isolation of the bacterium and a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see section~~s~~ 4.3 ~~and 3.1.5~~). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	Typo - incorrect reference to section 3.1.5.	English	European Union
140.	*94*	Substantive	The minimum requirements for identification are isolation of the bacterium and a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see sections 4.3 and 3.1.5). Additional tests (see sections 4.4	The sensitivity of isolation is lower than other methods. It is not appropriate to be the minimum requirement.	English	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections. The minimum requirements for identification are get two arbitrary positive result from the three techniques: (1) isolation of the bacterium ; (2) PCR using two sets of primers (see section 4.1); (3) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1)			
141.	*94*	Substantive	The minimum requirements for identification are isolation of the bacterium and a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see sections 4.3 and 3.1.6~~5~~). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	Section number 3.1.5 in sentense 1 should be changed to 3.1.6.	English	Thailand
142.	*94*	Substantive	The minimum requirements for identification are isolation of the bacterium and a positive result from either (1) PCR using two sets of primers(see section 4.1) and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates(see sections 4.3 and 3.1.6) or ~~each of the three techniques: (1) PCR using two sets of primers (see section 4.1);~~ (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1)~~,~~ and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see sections 4.3 and ~~3.1.5~~ 3.1.6). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	It is not necessary to conduct both (1) PCR and (2) ELISA for the minimum requirements for identification. Either conducting (1) PCR or (2) ELISA, and inoculation test are enough for the purpose. It may be appropriate that inoculation testing refers to section 3.1.6 "Bioassays", not section 3.1.5 "Interpretation of results from conventional and real-time PCR "	English	Japan
143.	*94*	Technical	The minimum requirements for identification of a pure culture are ~~isolation of the bacterium and~~ a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see sections 4.3 and 3.1.5). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	Clearer language	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
144.	*94*	Technical	The minimum requirements for identification of a pure culture are ~~isolation of the bacterium and~~ a positive result from each of the three techniques: (1) PCR using two sets of primers (see section 4.1); (2) double antibody sandwich (DAS)-ELISA or indirect ELISA using specific monoclonal antibodies (see sections 4.2 and 4.2.1); and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (see sections 4.3 and 3.1.5). Additional tests (see sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and negative controls must be included. The recommended techniques are described in the following sections.	Clearer language	English	European Union
145.	*97*	Technical	• CFPB 2911 from Collection Française de Bactéries Phytopathogènes, INRA Station Phytobactériologie, Angers, France (a Xcc-A* strain).	For clarity	English	EPPO
146.	*97*	Technical	• CFPB 2911 from Collection Française de Bactéries Phytopathogènes, INRA Station Phytobactériologie, Angers, France (a Xcc-A* strain).	For clarity	English	European Union
147.	*102*	Substantive	**4.1 PCR methods** It is recommended that in addition to the PCR protocol described in section 3.1.4.2, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.	Recently, the specificity of nine polymerase chain reaction primers previously designed for the identification of X. citri pv. citri or citrus bacterial canker strains (both pvs. citri and aurantifolii) was assayed on a large strain collection, including the two pathotypes of X. citri pv. citri, other genetic related or unrelated pathogenic xanthomonads, and saprophytic xanthomonads. (Delcourt et al., 2013. Plant Disease 97, 3, 373-378). The authors recommend to use in combination, the primer sets J-pth1/2 and XACF/R (Park et. al., 2006. Microbiological Research 161 145-149), which should react positively with all the strains of X. citri pathogenic to citrus listed on the EPPO list A1 and should discriminate pv. aurantifolii from pvs. citri and bilvae. As some positive reactions were obtained for some saprophytic strains isolated from citrus with the primer pair J-RXg/c2 and could lead to doubtful or false positive results it should be made clear that a combination of primer sets J-pth1/2 and XACF/R should be used. Paragraph 107 has therefore been moved to be the first	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				paragraph in this section.		
148.	102	Substantive	**4.1 PCR methods** It is recommended that in addition to the PCR protocol described in section 3.1.4.2, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.	Recently, the specificity of nine polymerase chain reaction primers previously designed for the identification of X. citri pv. citri or citrus bacterial canker strains (both pvs. citri and aurantifolii) was assayed on a large strain collection, including the two pathotypes of X. citri pv. citri, other genetic related or unrelated pathogenic xanthomonads, and saprophytic xanthomonads. (Delcourt et al., 2013. Plant Disease 97, 3, 373-378). The authors recommend to use in combination, the primer sets J-pth1/2 and XACF/R (Park et. al., 2006. Microbiological Research 161 145-149), which should react positively with all the strains of X. citri pathogenic to citrus listed on the EPPO list A1 and should discriminate pv. aurantifolii from pvs. citri and bilvae. As some positive reactions were obtained for some saprophytic strains isolated from citrus with the primer pair J-RXg/c2 and could lead to doubtful or false positive results it should be made clear that a combination of primer sets J-pth1/2 and XACF/R should be used. Paragraph 107 has therefore been moved to be the first paragraph in this section.	English	European Union
149.	103	Technical	Cubero and Graham (2002) developed PCR primers ~~for the *pth*Agene involved in virulence (all citrus canker strains) and~~ for the intergenic transcribed spacer (ITS) regions of 16S and 23S rDNAs specific to Xcc. Variation in the ITS sequences allowed the design of specific primers for Xcc and these primers detect the atypical strains A* and A^w(Cubero & Graham, 2002). The primers are:	Not necessary if paragraph 107 is moved above this paragraph	English	EPPO
150.	103	Technical	Cubero and Graham (2002) developed PCR primers ~~for the *pth*Agene involved in virulence (all citrus canker strains) and~~ for the intergenic transcribed spacer (ITS) regions of 16S and 23S rDNAs specific to Xcc. Variation in the ITS sequences allowed the design of specific primers for Xcc and these primers detect the atypical strains A* and A^w(Cubero & Graham, 2002). The primers are:	Not necessary if paragraph 107 is moved above this paragraph	English	European Union
151.	106	Editorial	PCR is carried out in 25 µl reaction mixtures containing 1×Taqbuffer, 1.5 mM $MgCl_2$, 0.04 µM primer J-RXg, 0.04 µM primer J-RXc2, 0.2 mM each dNTP and 1 UTaq DNA polymerase. The PCR amplification conditions are the same as those	pthA primers are described in 3.1.4.3	English	Uruguay

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			used with the *pthA* primers described in section 3.1.4.3~~1~~.			
152.	*106*	Editorial	PCR is carried out in 25 µl reaction mixtures containing 1×Taqbuffer, 1.5 mM $MgCl_2$, 0.04 µM primer J-RXg, 0.04 µM primer J-RXc2, 0.2 mM each dNTP and 1 UTaq DNA polymerase. The PCR amplification conditions are the same as those used with the *pthA* primers described in section 3.1.4.3~~1~~.	pthA primers are described in 3.1.4.3	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
153.	*106*	Technical	Primers based on the rpf region were designated (Coletta-Filho et al., 2006): Xac01 (5'-CGC CAT CCC CAC CAC CAC CAC GAC-3') Xac02 (5'-AAC CGC TCA ATG CCA TCC ACT TCA-3') PCR is carried out in 25 µl reaction mixtures containing 1×Taqbuffer, 1.5 mM $MgCl_2$, 0.04 µM primer J-RXg, 0.04 µM primer J-RXc2, 0.2 mM each dNTP and 1 UTaq DNA polymerase. The PCR amplification conditions are the same as those used with the *pthA* primers described in section 3.1.4.1.	Primers based on the rpf region provide improve detection of Xanthomonas axonopodis pv citri in naturally and artificially infected citrus plants	English	Uruguay
154.	*106*	Technical	Primers based on the rpf region were designated (Coletta-Filho et al., 2006): Xac01 (5'-CGC CAT CCC CAC CAC CAC CAC GAC-3') Xac02 (5'-AAC CGC TCA ATG CCA TCC ACT TCA-3') PCR is carried out in 25 µl reaction mixtures containing 1×Taqbuffer, 1.5 mM $MgCl_2$, 0.04 µM primer J-RXg, 0.04 µM primer J-RXc2, 0.2 mM each dNTP and 1 UTaq DNA polymerase. The PCR amplification conditions are the same as those used with the *pthA* primers described in section 3.1.4.1.	Primers based on the rpf region provide improve detection of Xanthomonas axonopodis pv citri in naturally and artificially infected citrus plants	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
155.	*107*	Editorial	It is recommended that in addition to the PCR protocol described in section 3.1.4.~~2~~3, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. and 3.1.4.3 Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.	Editorial to refer correctly to the corresponding sections	English	Uruguay
156.	*107*	Editorial	It is recommended that in addition to the PCR protocol described in section 3.1.4.~~2~~3, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham,	Editorial to refer correctly to the corresponding sections	English	COSAVE, Paraguay, Chile, Argentina, Peru,

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. and 3.1.4.3 Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.			Brazil
157.	107	Substantive	~~It is recommended that in addition to the PCR protocol described in section 3.1.4.2, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.~~	Moved to just below 102. Important point that should be emphasised.	English	EPPO
158.	107	Substantive	~~It is recommended that in addition to the PCR protocol described in section 3.1.4.2, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database.~~	Moved to just below 102. Important point that should be emphasised.	English	European Union
159.	107	Substantive	It is recommended that in addition to the PCR protocol described in section 3.1.4.2, the identification of pure cultures of suspect strains are confirmed by using two sets of primers, based on rDNA and the pthA gene (Cubero & Graham, 2002). The DNA extraction procedure, primer description and PCR method are as described in section 3.1.4.2. Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of Xcc strains deposited in the NCBI GenBank database. The size of amplified product by PCR primer J-Rxg/JRXc used in identification should be described.	The size of amplified products made by PCR is essential information in determining positive or negative for indentification.	English	Japan
160.	109	Editorial	For the DAS-ELISA, microtitre plates are coated with 200 µl/well carbonate coating buffer (Na_2CO_3, 1.59 g; $NaHCO_3$, 2.93 g; NaN_3, 0.2 g; distilled water, 1 litre; pH 9.6) containing appropriately diluted anti-Xcc immunoglobulins (IgG) and incubated overnight at 4 ºC. After washing the plates three times with PBS-Tween (NaCl, 8 g; KH_2PO_4, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KCl, 0.2 g; NaN_3, 0.2 g; Tween® 20, 0.25 ml; distilled water, 1 litre; pH 7.4), test sample, negative control (healthy plant material) or positive control (reference strain of Xcc) is added (200 µl/well). The plates are incubated for 2 h at 37 ºC. After washing, anti-Xcc IgG conjugated with alkaline phosphatase at the appropriate dilution in PBS-Tween is added (200 µl/well) and the plates are incubated for 2 h at 37 °C. After washing, p-	An abbreviation of colony forming unit is normally cfu.	English	Thailand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			nitrophenyl phosphate substrate buffer (1 mg/ml) is added (200 µl/well) and the plates are incubated for 30–60 min at room temperature. The absorbances are measured using a spectrophotometer equipped with a 405 nm filter. The criterion for determination of a sample as positive is two times the optical density (OD) value of the healthy plant material control. The detection limit of DAS-ELISA is 10^4–10^5 cfu ~~c.f.u.~~/ml (Civerolo & Fan, 1982). This method is not recommended for direct detection in plant tissue.			
161.	*109*	Technical	For the DAS-ELISA, microtitre plates are coated with ~~200~~100 µl/well carbonate coating buffer (Na_2CO_3, 1.59 g; $NaHCO_3$, 2.93 g; NaN_3, 0.2 g; distilled water, 1 litre; pH 9.6) containing appropriately diluted anti-Xcc immunoglobulins (IgG) and incubated overnight at 4 ºC. After washing the plates three times with PBS-Tween (NaCl, 8 g; KH_2PO_4, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KCl, 0.2 g; NaN_3, 0.2 g; Tween® 20, 0.25 ml; distilled water, 1 litre; pH 7.4), test sample, negative control (healthy plant material) or positive control (reference strain of Xcc) is added (200 µl/well). The plates are incubated for 2 h at 37 ºC. After washing, anti-Xcc IgG conjugated with alkaline phosphatase at the appropriate dilution in PBS-Tween is added (200 µl/well) and the plates are incubated for 2 h at 37 °C. After washing, p-nitrophenyl phosphate substrate buffer (1 mg/ml) is added (200 µl/well) and the plates are incubated for 30–60 min at room temperature. The absorbances are measured using a spectrophotometer equipped with a 405 nm filter. The criterion for determination of a sample as positive is two times the optical density (OD) value of the healthy plant material control. The detection limit of DAS-ELISA is 10^4–10^5 c.f.u./ml (Civerolo & Fan, 1982). This method is not recommended for direct detection in plant tissue.	Elisa protocols widely use 100 µl/well. The indication of higher volume may restrict the use of commercially available reagents validated using 100 µl/well volume. Therefore, the protocol should use the volume recommended by manufacturer	English	Uruguay
162.	*109*	Technical	For the DAS-ELISA, microtitre plates are coated with ~~200~~100 µl/well carbonate coating buffer (Na_2CO_3, 1.59 g; $NaHCO_3$, 2.93 g; NaN_3, 0.2 g; distilled water, 1 litre; pH 9.6) containing appropriately diluted anti-Xcc immunoglobulins (IgG) and incubated overnight at 4 ºC. After washing the plates three times with PBS-Tween (NaCl, 8 g; KH_2PO_4, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KCl, 0.2 g; NaN_3, 0.2 g; Tween® 20, 0.25 ml; distilled water, 1 litre; pH 7.4), test sample, negative control (healthy plant material) or positive control (reference strain of Xcc) is added (200 µl/well). The plates are incubated for 2 h at 37 ºC. After washing, anti-Xcc IgG conjugated with alkaline phosphatase at the appropriate dilution in PBS-Tween is added (200 µl/well) and the plates are incubated for 2 h at 37 °C. After washing, p-nitrophenyl phosphate substrate buffer (1 mg/ml) is added (200 µl/well) and the plates are incubated for 30–60 min at room temperature. The absorbances are measured using a spectrophotometer equipped with a 405 nm filter. The criterion for determination of a sample as positive is two times the optical density (OD)	Elisa protocols widely use 100 µl/well. The indication of higher volume may restrict the use of commercially available reagents validated using 100 µl/well volume. Therefore, the protocol should use the volume recommended by manufacturer	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			value of the healthy plant material control. The detection limit of DAS-ELISA is 10^4–10^5 c.f.u./ml (Civerolo & Fan, 1982). This method is not recommended for direct detection in plant tissue.			
163.	110	Technical	Monoclonal antibodies are available for ELISA, but are advised to be used only for identification of pure cultures because of their low sensitivity of detection in plant tissue. Commercial kits for detection of Xcc by ELISA are available (e.g. from Agdia, Inc.). For specificity data, refer to the technical information provided by the manufacturer. Some monoclonal antibodies have been reported to cross-react with *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas campestris* pv. *zinnea*, *Xanthomonas alfalfae* subsp. *citrumelonis*, which can be present on citrus, ~~*citromelo*~~ and *Xanthomonas hortorum* pv. ~~*P*~~*pelargonii*; however, these pathovars are unlikely to be present on citrus.	X citromelo is not an accepted name according to the references.	English	EPPO
164.	110	Technical	Monoclonal antibodies are available for ELISA, but are advised to be used only for identification of pure cultures because of their low sensitivity of detection in plant tissue. Commercial kits for detection of Xcc by ELISA are available (e.g. from Agdia, Inc.). For specificity data, refer to the technical information provided by the manufacturer. Some monoclonal antibodies have been reported to cross-react with *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas campestris* pv. *zinnea*, *Xanthomonas alfalfae* subsp. *citrumelonis*, which can be present on citrus, ~~*citromelo*~~ and *Xanthomonas hortorum* pv. ~~*P*~~*pelargonii*; however, these pathovars are unlikely to be present on citrus.	X citromelo is not an accepted name according to the references.	English	European Union
165.	111	Substantive	**4.3~~2.1~~ Indirect ELISA**	DAS-ELISA and Indirect ELISA is paratactic relationship.	English	China
166.	113	Editorial	Pure culture suspensions are centrifuged at approximately 10 000 *g* for 2 min and the supernatant is discarded. One millilitre of 1× PBS is added and the cells are resuspended by vortexing. The operation is repeated twice more. After the third wash, the cells are resuspended in coating buffer. The bacterial concentration is adjusted spectrophotometrically to OD_{600} 0.01 (approximately 2.5×10^7 cfu ~~c.f.u.~~/ml). Aliquots of the samples are loaded onto microtitre plates (two wells per sample, 100 µl/well). A positive control (a reference culture or sample provided by the manufacturer) and negative buffer control with another bacteria should be included. The plates are incubated overnight at 37 °C until they are dry. Blocking solution (5% non-fat dried milk powder in PBS) is added (200 µl/well). The plates are incubated for 30 min at room temperature and then washed twice with 1× PBS-Tween. Primary antibody at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-	An abbreviation of colony forming unit is normally cfu.	English	Thailand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			Tween. Enzyme conjugate at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Freshly prepared substrate solution containing 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) is added (100 µl/well). The plates are incubated for 30–60 min at room temperature. The OD is measured using a spectrophotometer with a 405 nm filter. Positive samples are determined as for DAS-ELISA.			
167.	*11 3*	Technical	Pure culture suspensions are centrifuged at approximately 10 000 *g* for 2 min and the supernatant is discarded. One ml~~millilitre of 1×~~ PBS is added and the cells are resuspended by vortexing. The operation is repeated twice more. After the third wash, the cells are resuspended in coating buffer. The bacterial concentration is adjusted spectrophotometrically to OD_{600} 0.01 (approximately 2.5×10^7 c.f.u./ml). Aliquots of the samples are loaded onto microtitre plates (two wells per sample, 100 µl/well). A positive control (a reference culture or sample provided by the manufacturer) and negative buffer control with another bacteria should be included. The plates are incubated overnight at 37 °C until they are dry. Blocking solution (5% non-fat dried milk powder in PBS) is added (200 µl/well). The plates are incubated for 30 min at room temperature and then washed twice with 1× PBS-Tween. Primary antibody at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Enzyme conjugate at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Freshly prepared substrate solution containing 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) is added (100 µl/well). The plates are incubated for 30–60 min at room temperature. The OD is measured using a spectrophotometer with a 405 nm filter. Positive samples are determined as for DAS-ELISA.	Simplification	English	EPPO
168.	*11 3*	Technical	Pure culture suspensions are centrifuged at approximately 10 000 *g* for 2 min and the supernatant is discarded. One ml~~millilitre of 1×~~ PBS is added and the cells are resuspended by vortexing. The operation is repeated twice more. After the third wash, the cells are resuspended in coating buffer. The bacterial concentration is adjusted spectrophotometrically to OD_{600} 0.01 (approximately 2.5×10^7 c.f.u./ml). Aliquots of the samples are loaded onto microtitre plates (two wells per sample, 100 µl/well). A positive control (a reference culture or sample provided by the manufacturer) and negative buffer control with another bacteria should be included. The plates are incubated overnight at 37 °C until they are dry. Blocking solution (5% non-fat dried milk powder in PBS) is added (200 µl/well). The plates	Simplification	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			are incubated for 30 min at room temperature and then washed twice with 1× PBS-Tween. Primary antibody at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Enzyme conjugate at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Freshly prepared substrate solution containing 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) is added (100 µl/well). The plates are incubated for 30–60 min at room temperature. The OD is measured using a spectrophotometer with a 405 nm filter. Positive samples are determined as for DAS-ELISA.			
169.	*116*	Editorial	Leaf assays by infiltration with a syringe with or without needle on susceptible cultivars of *Citrus* hosts allow demonstration of pathogenicity of bacterial colonies. Lesions develop 7–14 days after inoculation of intact leaves or detached leaves (Francis *et al.*, 2010; Koizumi, 1971) after incubation at 25 ºC in high humidity. With these assays,the eruptive callus-like reaction of Xcc can readily be distinguished. Bacteriagrown in liquid media or colonies from a freshly streaked agar plate are resuspended in sterile distilled water and the concentration is adjusted to 10^6–10^8 cfu ~~c.f.u.~~/ml for inoculation into hosts. A negative and a positive control should always be included. Plants inoculated with the positive control strain should be kept apart from test plants.	An abbreviation of colony forming unit is normally cfu.	English	Thailand
170.	*116*	Editorial	Leaf assays by infiltration with a syringe with or without needle on susceptible cultivars of *Citrus* hosts allow demonstration of pathogenicity of bacterial colonies. Lesions develop 7–14 days after inoculation of intact leaves or detached leaves (Francis *et al.*, 2010; Koizumi, 1971) after incubation at 25 ºC in high humidity. With these assays,the eruptive callus-like reaction of Xcc can readily be distinguished. Bacteriagrown in liquid media or colonies from a freshly streaked agar plate are resuspended in sterile distilled water and the concentration is adjusted to 10^6–10^8 c.f.u./ml for inoculation into hosts. A negative and a positive control should always be included. Plants inoculated with the positive control strain should be kept ~~apart~~ seperate from test plants.	For clarity	English	Ghana
171.	*116*	Technical	Leaf assays by infiltration with a syringe with or without needle on susceptible cultivars of *Citrus* hosts allow demonstration of pathogenicity of bacterial colonies. Immature leaves which are 50-70 % to fully expanded are preferred because of their high level of susceptibility. Lesions develop 7–14 days after inoculation of intact leaves or detached leaves (Francis *et al.*, 2010; Koizumi, 1971) after incubation at 25 ºC in high humidity. With these assays,the eruptive callus-like reaction of Xcc can readily be distinguished. Bacteriagrown in liquid media or	Further clarification	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			colonies from a freshly streaked agar plate are resuspended in sterile distilled water and the concentration is adjusted to 10^6–10^8 c.f.u./ml for inoculation into hosts. A negative and a positive control should always be included. Plants inoculated with the positive control strain should be kept apart from test plants.			
172.	*116*	Technical	Leaf assays by infiltration with a syringe with or without needle on susceptible cultivars of *Citrus* hosts allow demonstration of pathogenicity of bacterial colonies. Immature leaves which are 50-70 % to fully expanded are preferred because of their high level of susceptibility. Lesions develop 7–14 days after inoculation of intact leaves or detached leaves (Francis *et al.*, 2010; Koizumi, 1971) after incubation at 25 ºC in high humidity. With these assays,the eruptive callus-like reaction of Xcc can readily be distinguished. Bacteriagrown in liquid media or colonies from a freshly streaked agar plate are resuspended in sterile distilled water and the concentration is adjusted to 10^6–10^8 c.f.u./ml for inoculation into hosts. A negative and a positive control should always be included. Plants inoculated with the positive control strain should be kept apart from test plants.	Further clarification	English	European Union
173.	*117*	Substantive	**4.4 Description and biochemical characteristics**	This section should include information on the biochemical characteristics of other Xanthomonas pathovars pathogenic to citrus.	English	EPPO
174.	*117*	Substantive	**4.4 Description and biochemical characteristics**	This section should include information on the biochemical characteristics of other Xanthomonas pathovars pathogenic to citrus.	English	European Union
175.	*121*	Substantive	**4.5 Molecular identification**	Multilocus sequence analysis (MLSA) approach is being adopted for bacterial identification and specifically to X. citri (Young et al, 2008; Bui Thi Ngoc et al, IJSEM 2010; Almeida et al, Phytopathology 2010). A manuscript from a french team is ready to be submitted. Interlaboratory comparisons of fingerprints are possible and a dedicated online database has been started so that people can compare their strains to reference strains (http://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/) It is recommended that information on MLSA analysis is added when the above publication on X. citri is available.	English	EPPO
176	*12*	Subst	**4.5 Molecular identification**	Multilocus sequence analysis (MLSA) approach is being adopted for bacterial identification and	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
.	*1*	antive		specifically to X. citri (Young et al, 2008; Bui Thi Ngoc et al, IJSEM 2010; Almeida et al, Phytopathology 2010). A manuscript from a french team is ready to be submitted. Interlaboratory comparisons of fingerprints are possible and a dedicated online database has been started so that people can compare their strains to reference strains (http://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/) It is recommended that information on MLSA analysis is added when the above publication on X. citri is available.		
177.	*126*	Editorial	BOX PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 6 mM $MgCl_2$, 2.4 µM primer BOX1R (5′-CTACG-GCAAGGCGACGCTGCAG-3′)(Louws *et al*~~et al~~., 1994), 0.2 mM each dNTP, 2 U Taq polymerase and 5 µl DNA extracted from xanthomonad strains. The reaction conditions are an initial step of 94 °C for 5 min followed by 40 cycles of 94 °C (30 s), 48 °C (30 s) and 72 °C (1 min), and a final step of 72 °C for 10 min. PCR products are analysed in 3% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer (40 mmol/litre Tris-acetate; 1 mmol/litre EDTA; pH 8.0) for 2 h at 110 V and stained with ethidium bromide.	Typo	English	EPPO
178.	*126*	Editorial	BOX PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 6 mM $MgCl_2$, 2.4 µM primer BOX1R (5′-CTACG-GCAAGGCGACGCTGCAG-3′)(Louws *et al*~~et al~~., 1994), 0.2 mM each dNTP, 2 U Taq polymerase and 5 µl DNA extracted from xanthomonad strains. The reaction conditions are an initial step of 94 °C for 5 min followed by 40 cycles of 94 °C (30 s), 48 °C (30 s) and 72 °C (1 min), and a final step of 72 °C for 10 min. PCR products are analysed in 3% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer (40 mmol/litre Tris-acetate; 1 mmol/litre EDTA; pH 8.0) for 2 h at 110 V and stained with ethidium bromide.	Typo	English	European Union
179.	*126*	Editorial	BOX PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 6 mM $MgCl_2$, 2.4 µM primer BOX1R (5′-CTACG-GCAAGGCGACGCTGCAG-3′)(Louws *et al*~~et al~~., 1994), 0.2 mM each dNTP, 2 U Taq polymerase and 5 µl DNA extracted from xanthomonad strains. The reaction conditions are an initial step of 94 °C for 5 min followed by 40 cycles of 94 °C (30 s), 48 °C (30 s) and 72 °C (1 min), and a final step of 72 °C for 10 min. PCR products are analysed in 3% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer (40 mmol/litre Tris-acetate; 1 mmol/litre EDTA; pH 8.0) for 2 h at 110 V and stained with ethidium bromide.	An abbreviation et al. must be italicized.	English	Thailand
180	*12*	Subst	**4.5.2 Genomic DNA fingerprinting**	How reliable is this method for X. citri? In general this method is very old-fashioned and often not	English	EPPO

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
.	9	antive		reliable. Suggest to delete it.		
181.	129	Substantive	**4.5.2 Genomic DNA fingerprinting**	How reliable is this method for X. citri? In general this method is very old-fashioned and often not reliable. Suggest to delete it.	English	European Union
182.	131	Editorial	**Extraction of DNA** (Berman *et al*., 1981)	Berman et al., 1981 is missing from the references	English	EPPO
183.	131	Editorial	**Extraction of DNA** (Berman *et al*., 1981)	Berman et al., 1981 is missing from the references	English	European Union
184.	131	Substantive	**Extraction of DNA** (Berman *et al*., 1981)	A paper of Berman et al., 1981 is missing in section 8. References.	English	Thailand
185.	132	Technical	Two ten millilitre liquid Luria Bertani (LB) cultures of the test bacteria and of positive controls of Xcc in 50 ml flasks are grown with gentle rotary shaking at 27 °C for 18 h. Genomic DNA is prepared as follows. The pooled 20 mlculture is centrifuged at 10 000 *g* for 10 min and the pellet is resuspended in 10 ml PBS (20 mmol/litre KH_2PO_4buffer, pH 6.9, containing 150 mmol/litre NaCl). After a second centrifugation, the pellet is resuspended in 5 ml of 50 mmol/litre Tris, pH 8.0, containing 50 mmol/litre EDTA. Eggwhite lysozyme is added to a final concentration of 1 mg/ml and the tubes are incubated at 0 °C for 30 min. Then 1 ml of a freshly prepared lysing solution (0.5% SDS; 50 mmol/litre Tris-HCl, pH 7.5; 400 mmol/litre EDTA; 1 mg/ml pronase) is added to each tube, and the tubes are incubated at 50 °C until the suspension clears. The lysate is extracted with an equal volume of Tris buffer-saturated phenol (pH 7.8). After centrifugation (9 000 *g* for 10 min), the aqueous supernatant is transferred to a clean tube and sodium acetate is added to 0.3 mmol/litre. After addition of two volumes of ethanol and mixing by inversion, the nucleic acids are removed by spooling onto a glass pipette. They are dissolved in 3 ml Tris-EDTA (TE) buffer (10 mmol/litre Tris-HCl, pH 8.0; 1 mmol/litre EDTA) containing Ribonuclease (RNase) A (50 µg/ml). After 30 min at 37 °C, the solution is extracted with an equal volume of chloroform and the DNA is spooled out of the solution by a second ethanol precipitation. The DNA is dissolved in a minimal volume of TE buffer and stored at 4 °C until use. The concentration of DNA in the sample can be estimated spectrophotometrically.	Please clarify whether two ten ml cultures are used per bacterium	English	EPPO
186	13	Techn	Two ten millilitre liquid Luria Bertani (LB) cultures of the test bacteria and of positive controls of Xcc in 50 ml flasks are grown with gentle rotary shaking at	Please clarify whether two ten ml cultures are used	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
.	2	ical	27 °C for 18 h. Genomic DNA is prepared as follows. The pooled 20 mlculture is centrifuged at 10 000 *g* for 10 min and the pellet is resuspended in 10 ml PBS (20 mmol/litre KH_2PO_4buffer, pH 6.9, containing 150 mmol/litre NaCl). After a second centrifugation, the pellet is resuspended in 5 ml of 50 mmol/litre Tris, pH 8.0, containing 50 mmol/litre EDTA. Eggwhite lysozyme is added to a final concentration of 1 mg/ml and the tubes are incubated at 0 °C for 30 min. Then 1 ml of a freshly prepared lysing solution (0.5% SDS; 50 mmol/litre Tris-HCl, pH 7.5; 400 mmol/litre EDTA; 1 mg/ml pronase) is added to each tube, and the tubes are incubated at 50 °C until the suspension clears. The lysate is extracted with an equal volume of Tris buffer-saturated phenol (pH 7.8). After centrifugation (9 000 *g* for 10 min), the aqueous supernatant is transferred to a clean tube and sodium acetate is added to 0.3 mmol/litre. After addition of two volumes of ethanol and mixing by inversion, the nucleic acids are removed by spooling onto a glass pipette. They are dissolved in 3 ml Tris-EDTA (TE) buffer (10 mmol/litre Tris-HCl, pH 8.0; 1 mmol/litre EDTA) containing Ribonuclease (RNase) A (50 µg/ml). After 30 min at 37 °C, the solution is extracted with an equal volume of chloroform and the DNA is spooled out of the solution by a second ethanol precipitation. The DNA is dissolved in a minimal volume of TE buffer and stored at 4 °C until use. The concentration of DNA in the sample can be estimated spectrophotometrically.	per bacterium		
187.	13 4	Editorial	DNA extracts (3–5 µg) are digested with the restriction endonuclease *Eco*RI. Reaction volumes vary between 35 and 55 µl.~~,~~ ~~b~~Buffer conditions are those recommended by the supplier, and incubation is at 37 °C for 4 h. Samples are loaded on a 1.5 mm thick, 14 cm long vertical 5% polyacrylamide gel, and fragments are separated by electrophoresis at 14 mA constant current for 14 h in Tris-borate-EDTA (TBE) buffer (89 mmol/litre Tris; 89 mmol/litre boric acid; 2 mmol/litre EDTA). During electrophoresis, the voltage increases from 50 V to 90 V. Gels are stained with ethidium bromide (2 µg/ml) for 60 min, then photographed on a transilluminator using both an orange and a yellow filter. Genomic fingerprints of the test and reference extracts are compared using the photograph or using the negative and the aid of a photographic enlarger.	To clarify	English	EPPO
188.	13 4	Editorial	DNA extracts (3–5 µg) are digested with the restriction endonuclease *Eco*RI. Reaction volumes vary between 35 and 55 µl.~~,~~ ~~b~~Buffer conditions are those recommended by the supplier, and incubation is at 37 °C for 4 h. Samples are loaded on a 1.5 mm thick, 14 cm long vertical 5% polyacrylamide gel, and fragments are separated by electrophoresis at 14 mA constant current for 14 h in Tris-borate-EDTA (TBE) buffer (89 mmol/litre Tris; 89 mmol/litre boric acid; 2 mmol/litre EDTA). During electrophoresis, the voltage increases from 50 V to 90 V. Gels are stained with ethidium bromide (2 µg/ml) for 60 min, then	To clarify	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			photographed on a transilluminator using both an orange and a yellow filter. Genomic fingerprints of the test and reference extracts are compared using the photograph or using the negative and the aid of a photographic enlarger.			
189.	136	Editorial	Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.	ISPM 27 is not in the references	English	EPPO
190.	136	Editorial	Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.	ISPM 27 is not in the references	English	European Union
191.	137	Editorial	In instances where other contracting parties may be affected by the results of the diagnosis, retention of the original sample (labelled for traceability) culture(s) of the pest, preserved or mounted specimens, or test materials (e.g. photograph of gels, ELISA results printout, PCR amplicons) for at least for one year is recommended, especially in cases of non-compliance (ISPM 13:2001, *Guidelines for the notification of non-compliance and emergency action*) and where pests are found for the first time in a country or an area.	ISPM 13 is not in the references	English	EPPO
192.	137	Editorial	In instances where other contracting parties may be affected by the results of the diagnosis, retention of the original sample (labelled for traceability) culture(s) of the pest, preserved or mounted specimens, or test materials (e.g. photograph of gels, ELISA results printout, PCR amplicons) for at least for one year is recommended, especially in cases of non-compliance (ISPM 13:2001, *Guidelines for the notification of non-compliance and emergency action*) and where pests are found for the first time in a country or an area.	ISPM 13 is not in the references	English	European Union
193.	144	Editorial	**8. References**	Add references for Berman et al 1981, ISPM 13, ISPM 27 and Timmer et al 2000.	English	EPPO
194.	144	Editorial	**8. References**	Add references for Berman et al 1981, ISPM 13, ISPM 27 and Timmer et al 2000.	English	European Union
195.	146	Editorial	~~**Bradbury, J.F.** 1986. *Guide to plant pathogenic bacteria*. Wallingford, UK, CABI. 332 pp.~~	Not referred to in the text	English	EPPO
196.	146	Editorial	~~**Bradbury, J.F.** 1986. *Guide to plant pathogenic bacteria*. Wallingford, UK, CABI. 332 pp.~~	Not referred to in the text	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
197.	150	Editorial	~~**Civerolo, E.L. & Helkie, C.** 1981. Indirect enzyme-linked immunosorbent assay of *Xanthomonas campestris* pv. *citri*. *InProceedings of the Fifth International Conference on Plant Pathogenic Bacteria,* Cali, Colombia, August 16 – 23 pp. 105–112~~.	Not referred to in the text	English	EPPO
198.	150	Editorial	~~**Civerolo, E.L. & Helkie, C.** 1981. Indirect enzyme-linked immunosorbent assay of *Xanthomonas campestris* pv. *citri*. *InProceedings of the Fifth International Conference on Plant Pathogenic Bacteria,* Cali, Colombia, August 16 – 23 pp. 105–112~~.	Not referred to in the text	English	European Union
199.	151	Editorial	**Coletta-Filho HD, Takita MA, Souza AA, Rodrigues-Neto J, Destefano SAL, Hartung JS, Machado MA. 2006. Primers based on the rpf region provide improved detection of Xanthomonas axonopodis pv citri in naturally and artificially infected citrus plants. Journal of Applied Microbiology, 100: 279-285** **Cubero, J. & Graham, J.H.**2002. Genetic relationship among worldwide strains of *Xanthomonas* causing canker in citrus species and design of new primers for their identification by PCR. *Applied and Environmental Microbiology,* 68: 1257–1264.	Reference added because was cited in the text	English	Uruguay
200.	151	Editorial	**Coletta-Filho HD, Takita MA, Souza AA, Rodrigues-Neto J, Destefano SAL, Hartung JS, Machado MA. 2006. Primers based on the rpf region provide improved detection of Xanthomonas axonopodis pv citri in naturally and artificially infected citrus plants. Journal of Applied Microbiology, 100: 279-285** **Cubero, J. & Graham, J.H.**2002. Genetic relationship among worldwide strains of *Xanthomonas* causing canker in citrus species and design of new primers for their identification by PCR. *Applied and Environmental Microbiology,* 68: 1257–1264.	Reference added because was cited in the text	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
201.	158	Editorial	**Francis, M.I., Pena, A., Graham, J.H.** 2010. Detached leaf inoculation of germplasm for rapid screening of resistance to citrus canker and citrus bacterial spot. *European Journal of Plant Pathology*,127(4): 571–578.*1.（Gabriel,1989） Reinstatement of Xanthomonas citri (ex Hasse) and X . phaseoli (ex Smith) to Species and Reclassification of All X . campestris pv.citri Strains t. INTERNATIONALJ OURNAL OF SYSTEMATIBCA CTERIOLOGJaYn,. 1989, p. 14-22 2. Chao Jin, Xiao Qiming, Tan Zhoujin, Xie Xinwen. Study of Xanthomonas Campestris Separation Hunan Agricultural Sciences. 2005(5) : 53—55 Add two papers in the part of references: 1.（Gabriel,1989） Reinstatement of Xanthomonas citri (ex Hasse) and X . phaseoli (ex Smith) to Species and Reclassification of All X . campestris pv.citri Strains t. INTERNATIONALJ OURNAL*	This paper is important and should be added in the	English	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			OF SYSTEMATIBCA CTERIOLOGJaYn,. 1989, p. 14-22 2. Chao Jin, Xiao Qiming, Tan Zhoujin, Xie Xinwen. Study of Xanthomonas Campestris Separation Hunan Agricultural Sciences. 2005(5) : 53—55			
202.	*161*	Editorial	~~**Goto, M., Takahashi, T. & Messina, M.A.** 1980. A comparative study of the strains of *Xanthomonas campestris* pv. *citri* isolated from citrus canker in Japan and cancrosis B in Argentina. *Annals of the**Phytopathological Society of Japan*, 46: 329–338.~~	Not referred to in the text	English	EPPO
203.	*161*	Editorial	~~**Goto, M., Takahashi, T. & Messina, M.A.** 1980. A comparative study of the strains of *Xanthomonas campestris* pv. *citri* isolated from citrus canker in Japan and cancrosis B in Argentina. *Annals of the**Phytopathological Society of Japan*, 46: 329–338.~~	Not referred to in the text	English	European Union
204.	*164*	Editorial	**Hartung, J.S., Daniel, J.F., Pruvost, O.P. & Civerolo, E.L.** 1993. Detection of *Xanthomonas campestris* pv. *citri* by the polymerase chain reaction method. *Applied and Environmental Microbiology,* 59(4): 1143–1148. **ISPM 13.** 2001. Guidelines for the notification of non-compliance and emergency action. Rome, IPPC, FAO. **ISPM 27.** 2006. Diagnostic protocols for regulated pests. Rome, IPPC, FAO.	1) ISPM 13 is mentioned in [137]. 2) ISPM 27 is mentioned in [136].	English	EPPO
205.	*164*	Editorial	**Hartung, J.S., Daniel, J.F., Pruvost, O.P. & Civerolo, E.L.** 1993. Detection of *Xanthomonas campestris* pv. *citri* by the polymerase chain reaction method. *Applied and Environmental Microbiology,* 59(4): 1143–1148. **ISPM 13.** 2001. Guidelines for the notification of non-compliance and emergency action. Rome, IPPC, FAO.	1) ISPM 13 is mentioned in [137]. 2) ISPM 27 is mentioned in [136].	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			ISPM 27. 2006. Diagnostic protocols for regulated pests. Rome, IPPC, FAO.			
206.	*170*	Editorial	Mafra, V., Kubo, S.K, Alves-Ferreira, M., Ribeiro-Alves, M., Stuart, R. M., Boava, L. P., Rodrigues, C. M., Machado, M.A**. 2012. Reference genes for accurate t**ranscript normalization in citrus genotypes under different experimental conditions. PLoS One, e31263. **Mavrodieva, V., Levy, L. & Gabriel, D.W.**2004. Improved sampling methods for real-time polymerase chain reaction diagnosis of citrus canker from field samples.*Phytopathology,* 94: 61–68.	Reference added because it is cited in the text	English	Uruguay
207.	*170*	Editorial	Mafra, V., Kubo, S.K, Alves-Ferreira, M., Ribeiro-Alves, M., Stuart, R. M., Boava, L. P., Rodrigues, C. M., Machado, M.A**. 2012. Reference genes for accurate t**ranscript normalization in citrus genotypes under different experimental conditions. PLoS One, e31263. **Mavrodieva, V., Levy, L. & Gabriel, D.W.**2004. Improved sampling methods for real-time polymerase chain reaction diagnosis of citrus canker from field samples.*Phytopathology,* 94: 61–68.	Reference added because it is cited in the text	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
208.	*172*	Editorial	~~**Schaad, N.W., Postnikova, E., Lacy, G.H., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K. & Vidaver, A.K.** 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel et al., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv *malvacearum* (ex smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae*~~ subsp. *alfalfae* (ex Riker et al., 1935) sp. nov. nom. rev.; and "var. *fuscans*" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Systematic and Applied Microbiology,*28: 494–518.	This is not referred to in the text.	English	EPPO
209.	*172*	Editorial	~~**Schaad, N.W., Postnikova, E., Lacy, G.H., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K. & Vidaver, A.K.** 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel et al., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv *malvacearum* (ex smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and~~	This is not referred to in the text.	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			~~Jones, 1935) Dye 1978 as *X. alfalfae*~~ subsp. *alfalfae* (ex Riker et al., 1935) sp. nov. nom. rev.; and "var. *fuscans*" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Systematic and Applied Microbiology,*28: 494–518.			
210.	*172*	Editorial	**Schaad, N.W., Postnikova, E., Lacy, G.H., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K. & Vidaver, A.K.** 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel et al., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv *malvacearum* (ex Ssmith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker *et al*~~et al~~., 1935) sp. nov. nom. rev.; and "var. *fuscans*" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Systematic and Applied Microbiology,*28: 494–518.	- A name Smith should be used a capital letter "S". - An abbreviation et al. must be italicized.	English	Thailand
211.	*181*	Editorial	~~**Wu, W.C., Lee, S.T., Kuo, H.F. & Wang, L.Y.** 1993. Use of phages for identifying the citrus canker bacterium *Xanthomonas campestris* pv. *citri* in Taiwan. *Plant Pathology,*42: 389–395.~~	Not referred to in the text	English	EPPO
212.	*181*	Editorial	~~**Wu, W.C., Lee, S.T., Kuo, H.F. & Wang, L.Y.** 1993. Use of phages for identifying the citrus canker bacterium *Xanthomonas campestris* pv. *citri* in Taiwan. *Plant Pathology,*42: 389–395.~~	Not referred to in the text	English	European Union
213.	*182*	Editorial	~~**Wu, W.C., Chen, T.T. & Wang, Y.R.** 1996. Characterization of five filamentous phages from *Xanthomonas campestris* pv. *citri.**Plant Pathology Bulletin*, 5: 1–14.~~	Not referred to in the text	English	EPPO
214.	*182*	Editorial	~~**Wu, W.C., Chen, T.T. & Wang, Y.R.** 1996. Characterization of five filamentous phages from *Xanthomonas campestris* pv. *citri.**Plant Pathology Bulletin*, 5: 1–14.~~	Not referred to in the text	English	European Union
215.	*184*	Technical	**9. Figures**	1. One or two pictures of early symptoms on orange leaves and of a few pustules on orange fruit would be helpful. 2. Please indicate the source of photos in each case. 3. Please add latin names of host fruit to each figure title e.g. grapefruit (Citrus paradisi)	English	EPPO
216	*18*	Techn	**9. Figures**	1. One or two pictures of early symptoms on orange leaves and of a few pustules on orange	English	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
.	4	ical		fruit would be helpful. 2. Please indicate the source of photos in each case. 3. Please add latin names of host fruit to each figure title e.g. grapefruit (Citrus paradisi).		
217.	187	Editorial	**Figure 2.** Twig symptoms of citrus canker: early lesions on grapefruit (*Citrus paradisi*)	The latin name of grapefruit should be given.	English	EPPO
218.	187	Editorial	**Figure 2.** Twig symptoms of citrus canker: early lesions on grapefruit (*Citrus paradisi*)	The latin name of grapefruit should be given.	English	European Union
219.	189	Editorial	**Figure 3.** Fruit symptoms of citrus canker on sweet orange (*Citrus sinensis*) (left) and grapefruit (*Citrus paradisi*) (right)	The latin names of sweet orange and grapefruit should be given.	English	EPPO
220.	189	Editorial	**Figure 3.** Fruit symptoms of citrus canker on sweet orange (*Citrus sinensis*) (left) and grapefruit (*Citrus paradisi*) (right)	The latin names of sweet orange and grapefruit should be given.	English	European Union
221.	191	Editorial	**Figure 4.** Leaf symptoms of citrus canker on lemon (*Citrus limon*) exacerbated by citrus leaf miner wounding	The latin name of lemon should be given.	English	EPPO
222.	191	Editorial	**Figure 4.** Leaf symptoms of citrus canker on lemon (*Citrus limon*) exacerbated by citrus leaf miner wounding	The latin name of lemon should be given.	English	European Union