2004-012: DRAFT ANNEX TO ISPM 27 – *Xanthomonas fragariae*

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
1.	*G*	Editorial		Although the importance of testing of asymptomatic material is recognized in the introduction it is not very clear if both type of samples are covered. Eg in the section detection it is mentioned 'Procedures for the detection of X. fragariae in plants with symptoms are presented below' so reading the text, the protocol seems to be restricted to symptomatic samples only. However later in sampling (3.2) and in some tests descriptions (3.9) asymptomatic samples are mentioned. The protocol would be clearer if it would make a clear distinction between symptomatic and asymptomatic material. It is also suggested to add pictures of X. fragariae (symptoms and exudates) and of X. arboricola pv. fragariae.	EPPO, European Union	**Incorporated:** the DP has been rewritten to make a clearer distinction between symptomatic and asymptomatic material. Figures have now been added to illustrate *X. fragariae* symptoms.
2.	*G*	Editorial		General comment: the technical panel must pay attention to detail when editing the document because in certain instances there appears to be no spacing between sentences in the document and also there are a few inconsistencies in terms of the referencing style.	South Africa	**Incorporated:** noted a further review for editorials/inconsistencies has been conducted.
3.	*G*	Editorial	Se hace una atenta solicitud para que los párrafos de estos protocolos se numeren para mayor claridad y mejor manejo de la información.	Para facilitar el proceso de consulta y de revisión de estos proyectos de NIMF (protocolos de diagnóstico).	Costa Rica, Mexico	?
4.	*G*	Substantive	I support the document as it is and I have no comments		Georgia, Indonesia, Lao People's Democratic Republic, United States of America, Nepal, Thailand, Mexico, Congo, Barbados, Bahrain,	**Noted**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
					Guyana, Belize, Ghana, Burundi	
5.	*G*	Substantive	Redraft section 4 'Identification' to recognise that the bacterium can be id entified without the need for pathogenicity tests or ELIS A tests.	The requirement for positive results from those three kinds of tests is asking too much and could be restrictive. A quick look at the literature shows a fair amount of work has been done to eliminate the need for pathogenicity tests. Scientists have moved to replace pathogenicity tests with PCRs and other DNA tests. Identification relies on the specificity of the test and there are several papers reporting specificity of DNA methods and reporting that certain DNA tests are specific enough to reliably distinguish Xanthomonas fragariae from related Xanthomonads. Mirmajlessi et al. (2015) have done a systematic review of the publications describing PCR based methods for detecting and identifying X. fragariae. They say that "Conventional PCR using species-specific primers is known to differentiate close species and used for detection of X. fragariae..." They also say that "A few loci suitable for the design of species specific primers for X. fragariae have been identified..." and then they provide references for the loci in RAPD-specific regions and within the hrp and gyrB genes. Albuquerque, P., Caridade, C. M., Marcal, A. R., Cruz, J., Cruz, L., Santos, C. L., ... & Tavares, F. (2011). Identification of Xanthomonas fragariae, Xanthomonas axonopodis pv. phaseoli, and Xanthomonas fuscans subsp. fuscans with Novel Markers and Using a Dot Blot Platform Coupled with Automatic Data Analysis. Applied and environmental microbiology, 77(16), 5619-5628. Mirmajlessi, S. M., Destefanis, M., Gottsberger, R. A., Mänd, M., & Loit, E. (2015). PCR-based specific techniques used for detecting the most important pathogens on strawberry: a systematic review. Systematic reviews, 4(1), 9. Vandroemme, J., Baeyen, S., Van Vaerenbergh, J., De Vos, P., & Maes, M. (2008). Sensitive real-time PCR detection of Xanthomonas	Australia	**Considered, but not incorporated:** For detections for regulatory purposes (e.g new country detections, area freedom issues) the requirement to fulfil Koch's postulates is an important one. The combined diagnostic approach will ensure that atypical isolates are not mis-identified and identification is confirmed by a biologically based test.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
				fragariae in strawberry plants. Plant Pathology, 57(3), 438-444.		
6.	*G*	Substantive	Insert some pictures of disease symptoms and flow chart for detection.	These information would be useful to identify Xanthomonas fragariae.	Japan	**Modified:** figures of disease symptoms will be included. We do not believe a flow chart is necessary to explain the requirements of detection.
7.	*G*	Technical	QBOL is a consortium of 20 partners (universities, research institutes and phytosanitary organizations) from all over the world working together and sharing their research expertise in the field of DNA barcoding of Arthropods, Bacteria, Fungi, Nematodes, Phytoplasmas and Viruses. Thereby, we would like to request the TPDP to evaluate the relevance to include this method in protocols. Regarding paragraph 49, we would like to request the TPDP to clarify the affirmation that "There is not always a good correlation between isolation, serological tests (i.e. immunofluorescence, ELISA) and/or PCR because isolation frequently fails."	See comment	Peru	**Modified:** While specific mention to QBOL was not mentioned the protocols in section 4.3.3 use a similar approach to that referenced by QBOL. Reference to QBOL website and protocol for x. fragariae has now been made in section 4.3.3 Paragraph 49 has been reworded to clarify the fastidious nature of X. fragariae.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
8.	*G*	Technical	Regarding paragraph 49, we would like to request the TPDP to clarify the affirmation that "There is not always a good correlation between isolation, serological tests (i.e. immunofluorescence, ELISA) and/or PCR because isolation frequently fails."	See comment	Argentina	**Incorporated:** see above
9.	*G*	Technical	Regarding paragraph 49, we would like to request the TPDP to clarify the afirmation that "There is not always a good correlation between isolation, serological tests (i.e. immunofluorescence, ELISA) and/or PCR because isolation frequently fails."	See comment	COSAVE, Uruguay, Chile, Paraguay	**Incorporated:** see above
10.	*G*	Technical	1. Regarding paragraph 49, we would like to request the TPDP to clarify the afirmation that "There is not always a good correlation between isolation, serological tests (i.e. immunofluorescence, ELISA) and/or PCR because isolation frequently fails." 2. QBOL is a consortium of 20 partners (universities, research institutes and phytosanitary organizations) from all over the world working together and sharing their research expertise in field of DNA barcoding of Arthropods, Bacteria, Fungi, Nematodes, Phytoplasma and Viruses. Thereby, we would like to request the TPDP to evaluate the relevance to include this method in this protocol.	See comment	Brazil	See comment 7.
11.	*G*	Technical	En el apartado 3.9.2, primer párrafo, se debe clarificar si el nivel de detección por el método Multiplex de PCR se refiere a un mínimo o un máximo nivel de detección. En el apartado 3.9.2, segundo párrafo, debe aclararse si la concentración de los componentes de la mezcla se refiere a concentración de la solución madre o a la concentración del mastermix. En el apartado 3.9.3, protocolo de Moltmann y Zimmerman primer párrafo, última oración, no queda claro si se está refiriendo al ADN total (incluye el de la planta) o solamente el ADN bacteriano. En el párrafo 3 primera oración de este protocolo debe indicarse	Aspecto relevantes que deben ser clarificados	Costa Rica	To FAO translation services

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			cuántas unidades de la Taq DNA polimeras ha de usarse, ya que no se indica En el protocolo de Roberts et al. tercer párrafo debe indicarse cuántas unidades de la Taq ADN polimerasa ha de usarse ya que no se indica			
12.	*1*	Substantive	Deletion of the name "Africa " and replacing it with the name "Ethiopia ".**DRAFT ANNEX to ISPM 27 – *Xanthomonas fragariae* (2004-012)**	According to CABI 2015, Ethiopia is the only country in Africa where Xanthomonas fragariae is known to occur rather than in the entire African continent	South Africa	**Modified:** The intention of the DP is to provide a generic description on geographic distribution of the disease rather than mention specific countries. Africa has now been deleted from text.
13.	*2*	Editorial	**Status box** This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption. **Date of this document** 2015-06-10 **Document category** Draft annex to ISPM 27 (*Diagnostic protocols for regulated pests)* **Current document stage** To member consultation **Origin** Work programme topic: Bacteria, CPM-1 (2006)	seperate the two words i.e to member and fragariae to	Kenya	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment		Explanation	Country	SC responses
				Original subject: *Xanthomonas fragariae* (2004-012)			
			Major stages	2004-11 SC added topic to work programme 2006-04CPM-1 added *Xanthomonas fragariae* to work programme (2004-012) 2008-06TPDP meeting 2014-01 Expert consultation 2014-07 TPDP meeting 2015-04 TPDP e-decision for submission to SC 2015-06 SC e-decision approval for submitting to MC (2015_eSC_Nov_03)			
			Discipline leads history	2006-07 SC Lum KENG-YEANG (MY) 2011-05 SC Robert TAYLOR (NZ)			

Comm. no.	Para. no.	Comment type	Comment		Explanation	Country	SC responses
			Consultation on technical level	The first draft of this protocol was written by: • Edwin L. CIVEROLO (USDA/ARS, United States) (retired) • Solke H. DE BOER (Centre for Animal and Plant Health, Canadian Food Inspection Agency) • John ELPHINSTONE (Plant and Environmental Bacteriology, Fera, United Kingdom) • María M. LÓPEZ (Centro de Protección Vegetal y			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Biotecnología, Instituto Valenciano de Investigaciones Agrarias, Spain). The following expert commented on the draft protocol on a voluntary basis during the expert consultation stage: • Stephan BIERIE (Canadian Food Inspection Agency, Canada) **Main discussion points during development of the diagnostic protocol** **Notes**: This is a draft document. 2015-03 Edited 2015-06 Status box last modified			
14.	*2*	Editorial	**Status box**	It is requested that the referencing method that is used in this text be consistent throuout the entire text	South Africa	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption. **Date of this document**: 2015-06-10 **Document category**: Draft annex to ISPM 27 (*Diagnostic protocols for regulated pests)* **Current document stage**: To member consultation **Origin**: Work programme topic: Bacteria, CPM-1 (2006) Original subject: *Xanthomonas fragariae* (2004-012) **Major stages**: 2004-11 SC added topic to work programme 2006-04CPM-1 added *Xanthomonas fragariae* to work programme (2004-012) 2008-06TPDP meeting 2014-01 Expert consultation			

Comm. no.	Para. no.	Comment type	Comment		Explanation	Country	SC responses
				2014-07 TPDP meeting 2015-04 TPDP e-decision for submission to SC 2015-06 SC e-decision approval for submitting to MC (2015_eSC_Nov_03)			
			Discipline leads history	2006-07 SC Lum KENG-YEANG (MY) 2011-05 SC Robert TAYLOR (NZ)			
			Consultation on technical level	The first draft of this protocol was written by: • Edwin L. CIVEROLO (USDA/ARS, United States) (retired) • Solke H. DE BOER (Centre for Animal and			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Plant Health, Canadian Food Inspection Agency) • John ELPHINSTONE (Plant and Environmental Bacteriology, Fera, United Kingdom) • María M. LÓPEZ (Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias, Spain). The following expert commented on the draft protocol on a voluntary basis during the expert consultation stage:			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			• Stephan BIERIE (Canadian Food Inspection Agency, Canada) **Main discussion points during development of the diagnostic protocol** **Notes**: This is a draft document. 2015-03 Edited 2015-06 Status box last modified			
15.	*2*	Substantive	**Status box** This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption. **Date of this document**: 2015-06-10 **Document category**: Draft annex to ISPM 27 (*Diagnostic protocols for regulated pests)*	Please correct Stephan Brière's name - BRIERE. It is spelled incorrectly in the table.	Canada	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Current document stage: To member consultation **Origin**: Work programme topic: Bacteria, CPM-1 (2006) Original subject: *Xanthomonas fragariae* (2004-012) **Major stages**: 2004-11 SC added topic to work programme 2006-04CPM-1 added *Xanthomonas fragariae* to work programme (2004-012) 2008-06TPDP meeting 2014-01 Expert consultation 2014-07 TPDP meeting 2015-04 TPDP e-decision for submission to SC 2015-06 SC e-decision approval for			

Comm. no.	Para. no.	Comment type	Comment		Explanation	Country	SC responses
				submitting to MC (2015_eSC_Nov_03)			
			Discipline leads history	2006-07 SC Lum KENG-YEANG (MY) 2011-05 SC Robert TAYLOR (NZ)			
			Consultation on technical level	The first draft of this protocol was written by: • Edwin L. CIVEROLO (USDA/ARS, United States) (retired) • Solke H. DE BOER (Centre for Animal and			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Plant Health, Canadian Food Inspection Agency) • John ELPHINSTONE (Plant and Environmental Bacteriology, Fera, United Kingdom) • María M. LÓPEZ (Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias, Spain). The following expert commented on the draft protocol on a voluntary basis during the expert consultation stage:			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			• Stephan BRIERLE (Canadian Food Inspection Agency, Canada) **Main discussion points during development of the diagnostic protocol** **Notes**: This is a draft document. 2015-03 Edited 2015-06 Status box last modified			
16.	*3*	Editorial	Contents Addition of a semi colon after the reference "Civerolo, 1980" for consistency with other listed references. Commission on Phytosanitary Measures (CPM) (NSPM, 2014; UH-CTAHR, 2006). Approximately	For consistency with other listed reference. Write out abbreviations in full the first time when they are used e.g. "CPM" in order to provide clarity for those who may not be familiar with the abbreviations in question. Addition of these references to validate the point that has been made. Replacement of the word "Approximatley" with "Approximately", it's grammatically correct. Replacement of the word "decribed" with "described", its grammatically correct Addition of this sentence which was in 3.5.2 below because it is more relevant in this paragraph than in 3.5.2. Deletion of this sentence and moving it to paragraph 3.5.1 above	South Africa	**Modified:** editorial comments fixed.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			described An alternative to isolation of X. fragariae from tissue is to streak aliquots of fresh exudates from lesions directly onto Wilbrink-N, YPGA, SPA or other commonly used media.	because the sentence is more relevant in paragraph 3.5.1.		
17.	*3*	Substantive	Immunofluorescence), Molecular (PCR) and Pathogenecity (Koch's Ppostulates)Contents	Deletion of Elisa, immunofluorescence and PCR reason being for consistency with point 4. In addition.; ELISA and immunofluorescence are both immunological tests and pathogenicity tests were not mentioned in this .paragraph.	South Africa	**Considered, but not incorporated:** The contents page will be added later. Comments do not relate to this section?
18.	*4*	Substantive	To be added later.or supernatant	Addition of "supernatant because it can also be used to confirm Koch's postulates .	South Africa	**Incorporated**
19.	*9*	Editorial	*Xanthomonas fragariae* Kennedy and King, 1962~~a~~ is the causal agent of bacterial angular leaf spot disease of strawberry. The disease is prevalent mainly in North America and was first reported in the United States in 1962 (Kennedy and King, 1962~~a~~; Hildebrand *et al*., 1967; Maas *et al*., 1995), but it has been subsequently reported in many strawberry growing areas around the world, including South America, Africa and Europe (CABI, 2015). *Fragaria* × *ananassa*, the predominant cultivated strawberry, is the primary host of *X. fragariae*. However, commercial cultivars vary in susceptibility, and other *Fragaria* species, including *F. chiloensis, F. virginiana* and *F. vesca*, as well as *Potentilla fruticosa* and *P. glandulosa* are also susceptible. Among *Fragaria* species only *F. moschata* is immune (Kennedy and King, 1962~~a~~; Kennedy, 1965; Maas, 1998).	The "Maas,1995" reference has multiple authors, as seen in the entry under the references section (Maas, J.L., Pooler, M. & Galletta, G.J. 1995. Bacterial angular leafspot disease of strawberry: Present status and prospects for control. Advances in Strawberry Research, 14: 18–24). It is proposed to delete the reference "Kennedy and King, 1962b" from the references section, as it was not cited in the draft annex. Hence, the reference "Kennedy and King, 1962a" should be changed to "Kennedy and King, 1962" instead.	Singapore	**Incorporated**
20.	*9*	Editorial	*Xanthomonas fragariae* Kennedy and King, 1962a is the causal agent of bacterial angular leaf spot disease of strawberry. The disease is prevalent mainly in North America and was first reported in the United States in 1962 (Kennedy and King, 1962a; Hildebrand *et al*., 1967; Maas, 1995), but it has been subsequently reported in many strawberry growing areas around the world,	Addition of these two references that were added in paragraph number: 2	South Africa	**Considered, but not incorporated:** references not relevant to that paragraph.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			including South America, Africa and Europe (CABI, 2015). *Fragaria* × *ananassa*, the predominant cultivated strawberry, is the primary host of *X. fragariae*. However, commercial cultivars vary in susceptibility, and other *Fragaria* species, including *F. chiloensis, F. virginiana* and *F. vesca*, as well as *Potentilla fruticosa* and *P. glandulosa* are also susceptible. Among *Fragaria* species only *F. moschata* is immune (Kennedy and King, 1962a; Kennedy, 1965; Maas, 1998). National Standards for Phytosanitary Measures (NSPM) approved by the NPPO and National Quarantine Committee of Nepal. 2014. Standard Technical protocols for collection and Handling of Disease samples.[Online] Available: http://www.moadwto.gov.np/downloadfile/NSPM-17pestreporting_1390904481.pdf-Nov 2015 pp5-6 University of Hawaii college of tropical agriculture and Human resources (UH-CTAHR), 2006. Collecting plant disease and Insects Pest Samples for problem diagnosis. Scot. C. Nelson and Brian C. Bushe. Cooperative Extension Service, Soil and Crop management. SCM-14. University of Hawaii. pp 2-3.			
21.	*10*	Technical	*X. fragariae* is readily transmitted via asymptomatic planting stock with latent infection. Inoculum sources for primary infection are infected but clinically asymptomatic daughter plants that develop on runners from infected nursery plants and that are used for planting in fruit production fields. Although *X. fragariae* is not free-living in the soil, it can overwinter in the soil in association with previously infected plant material and persist there for long periods of time (Maas, 1998). Residues of infected leaves and crown infections on runners used for planting are also sources of inoculum for primary infection.	AIt would be better to add a literature citation. One possible citation is the Maas (1998) Compendium of Strawberry Diseases (page 16 there is a reference to survival)	EPPO, European Union	**Incorporated**
22.	*13*	Editorial	**Name:***Xanthomonas fragariae* Kennedy and King, 1962~~a~~	It is proposed to delete the reference "Kennedy and King, 1962b" from the references section, as it was not cited in the draft annex. Hence, the reference "Kennedy	Singapore	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
				and King, 1962a" should be changed to "Kennedy and King, 1962" instead.		
23.	*19*	Editorial	Diagnosis of bacterial angular leaf spot disease of strawberry caused by *X. fragariae* is based on inspection for diagnostic symptoms, direct or indirect isolation of the pathogen, serological analyses (e.g. indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA)) and molecular methods, including polymerase chain reaction (PCR)-based techniques (López *et al*., 1985; Roberts *et al*., 1996; Civerolo *et al*., 1997a, 1997b; Hartung and Pooler, 1997;Zimmerman *et al.,* 2004; López *et al*., 2005). A detached leaf bioassay (Civerolo *et al.*, 1997a) is useful for ~~direct~~ presumptive diagnosis of *X. fragariae*. Analyses of field-collected or clinical samples are generally based on leaves with young water-soaked spots if available, or leaves with older lesions with or without dried bacterial exudates. If systemic infection is suspected, analysis of crown tissue is necessary (López *et al.*, 2005). The methods indicated, with the exception of the nested PCR, have been validated in a ring test project funded by the European Union (SMT-4-CT98-2252) (López *et al.,* 2005).	unnecessary word	EPPO, European Union	**Incorporated**
24.	*19*	Technical	Diagnosis of bacterial angular leaf spot disease of strawberry caused by *X. fragariae* is based on inspection for diagnostic symptoms, direct or indirect isolation of the pathogen, serological analyses (e.g. indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA)) and molecular methods, including polymerase chain reaction (PCR)-based techniques (López *et al*., 1985; Roberts *et al*., 1996; Civerolo *et al*., 1997a, 1997b; Hartung and Pooler, 1997;Zimmerman *et al.,* 2004; López *et al*., 2005). A detached leaf bioassay (Civerolo *et al.*, 1997a) is useful for direct presumptive diagnosis of *X. fragariae*. ~~Analyses of field-collected or clinical samples are generally based on leaves with young water-soaked spots if available, or leaves with older lesions with or without dried bacterial exudates. If systemic infection is suspected, analysis of crown tissue is necessary (López *et al.*, 2005).~~ The methods indicated,	it is suggested to replace the text on molecular tests by Several PCR detection tests each targeting different loci in the Xf genome have been developed (Roberts et al., 1996; Zimmerman et al., 2004; Weller et al., 2007; Vandroemme et al., 2007, 2008; Vermunt & van Beuningen, 2008; Turecheck et al., 2008). These tests can be used to confirm the presence of Xf in symptomatic plant material but several of them have also been used to for the detection of latent Xf-infections (Mahuku & Goodwin, 1997; Zimmerman et al., 2004; Moltman & Zimmerman, 2005; Vermunt & van Beuningen, 2008). More complete RIng test: We have been told by Quality assessors that the name "test performance study" was a better word than ring test and have decided to use it instead in EPPO documents. Proposed deletion: This text is repeated in section 3.2 and is better placed there In addition is the term clinical	EPPO	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			with the exception of the nested PCR, have been validated in a ring test project funded by the European Union (SMT-4-CT98-2252) (López *et al.,* 2005).	samples widely used? Not really in Europe does it mean laboratory samples?		
25.	*19*	Technical	Diagnosis of bacterial angular leaf spot disease of strawberry caused by *X. fragariae* is based on observation~~inspection~~ for diagnostic symptoms, direct or indirect isolation of the pathogen, serological analyses (e.g. indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA)) and molecular methods, including polymerase chain reaction (PCR)-based techniques (López *et al*., 1985; Roberts *et al*., 1996; Civerolo *et al*., 1997a, 1997b; Hartung and Pooler, 1997;Zimmerman *et al.,* 2004; López *et al*., 2005). A detached leaf bioassay (Civerolo *et al.*, 1997a) is useful for direct presumptive diagnosis of *X. fragariae*. Analyses of field-collected or [clinical samples] are generally based on leaves with young water-soaked spots if available, or leaves with older lesions with or without dried bacterial exudates. If systemic infection is suspected, analysis of crown tissue is necessary (López *et al.*, 2005). The methods indicated, with the exception of the nested PCR, have been validated in a ring test project funded by the European Union (SMT-4-CT98-2252) (López *et al.,* 2005).	Clarify Why clinical sample and yet we are dealing with plants.	Kenya	**Modified**: accept that clinical should be replaced with laboratory samples for better clarity. Inspection of plants is more correct.
26.	*19*	Technical	Diagnosis of bacterial angular leaf spot disease of strawberry caused by *X. fragariae* is based on inspection for diagnostic symptoms, direct or indirect isolation of the pathogen, serological analyses (e.g. indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA)) and molecular methods, including polymerase chain reaction (PCR)-based techniques (López *et al*., 1985; Roberts *et al*., 1996; Civerolo *et al*., 1997a, 1997b; Hartung and Pooler, 1997;Zimmerman *et al.,* 2004; López *et al*., 2005). A detached leaf bioassay (Civerolo *et al.*, 1997a) is useful for direct presumptive diagnosis of *X. fragariae* in the laboratory in cases where direct isolation is very slow or inhibited. ~~Analyses of field-collected or clinical samples are generally based on leaves with young water-soaked spots if available, or leaves with older lesions with or without dried bacterial exudates. If~~	it is suggested to replace the text on molecular tests by Several PCR detection tests each targeting different loci in the Xf genome have been developed (Roberts et al., 1996; Zimmerman et al., 2004; Weller et al., 2007; Vandroemme et al., 2007, 2008; Vermunt & van Beuningen, 2008; Turecheck et al., 2008). These tests can be used to confirm the presence of Xf in symptomatic plant material but several of them have also been used to for the detection of latent Xf-infections (Mahuku & Goodwin, 1997; Zimmerman et al., 2004; Moltman & Zimmerman, 2005; Vermunt & van Beuningen, 2008). More complete RIng test: We have been told by Quality assessors that the name "test performance study" was a better word than ring test and have decided to use it instead in EPPO documents. Proposed addition: Further clarity on the circumstances when te bioassay woulod be used. Proposed deletion:	European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			~~systemic infection is suspected, analysis of crown tissue is necessary (López *et al.*, 2005).~~ The methods indicated, with the exception of the nested PCR, have been validated in a ring test project funded by the European Union (SMT-4-CT98-2252) (López *et al.,* 2005).	This text is repeated in section 3.2 and is better placed there In addition is the term clinical samples widely used? Not really in Europe does it mean laboratory samples?		
27.	*21*	Technical	Procedures for the detection of *X. fragariae* in plants with symptoms are presented below.	See general comment (this sentence seemsto mean that only procedures for the detection of X. fragariae in plants with symptoms are included).	EPPO, European Union	**Incorporated**
28.	*22*	Editorial	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~(~~This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named.~~)~~. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	Remove the unnecessary brackets.	Canada	**Incorporated**
29.	*22*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. (This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named.)~~. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	Texted deleted and included in the footnote as previously agreed.	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Incorporated**
30.	*23*	Substantive	**3.1 Symptoms**	It is suggested that it would be most beneficial if pictures of the symptoms could be added.	New Zealand	**Incorporated**
31.	*24*	Editorial	Small (1–4 mm diameter) angular water-soaked spots (lesions) bounded by the smallest leaf veins appear	It is proposed to delete the reference "Kennedy and King, 1962b" from the references section, as it was not	Singapore	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			initially on the lower leaf surface. In the early stages of infection, these spots appear translucent yellow when viewed under transmitted light. The lesions enlarge and coalesce, eventually appearing on the upper leaf surface as angular water-soaked spots that become reddish brown. Viscous bacterial exudates that are white, milky, cream or yellow in colour develop from lesions under wet conditions or when the relative humidity is high. The exudates become dry scale-like masses that are opaque or brown. As the disease progresses, coalesced reddish-brown lesions become necrotic. Necrotic lesion tissue may tear or break off the leaf, and diseased leaves may appear blighted or ragged. Leaf infections often develop and form long lesions along major veins. In advanced stages of disease development, the foliar tissue around old coalesced reddish-brown lesions is generally chlorotic (Kennedy and King, 1962~~a~~; EPPO, 1992; Rat, 1993; Maas, 1998).	cited in the draft annex. Hence, the reference "Kennedy and King, 1962a" should be changed to "Kennedy and King, 1962" instead.		
32.	*24*	Technical	Small (1–4 mm diameter) angular water-soaked spots (lesions) bounded by the smallest leaf veins appear initially on the lower leaf surface. In the early stages of infection, these spots are bearly visible in the field and appear translucent yellow when viewed under transmitted light. The lesions enlarge and coalesce, eventually appearing on the upper leaf surface as angular water-soaked spots that become reddish brown. Viscous bacterial exudates that are white, milky, cream or yellow in colour develop from lesions under wet conditions or when the relative humidity is high. The exudates become dry scale-like masses that are opaque and whitish/silvery, becoming brown (Janse, 2005)~~or brown~~. As the disease progresses, coalesced reddish-brown lesions become necrotic. Necrotic lesion tissue may tear or break off the leaf, and diseased leaves may appear blighted or ragged. Leaf infections often develop and form long lesions along major veins. In advanced stages of disease development, the foliar tissue around old coalesced reddish-brown lesions is generally chlorotic	1 It is important to specify that the symptoms are not easy to see. 2 Bacterial slime on leaf spots dries as a silvery scale under dry conditions. The reference is Janse, J.D. 2005. Phytobacteriology: Principles and Practice. CABI Publishing, Wallingford, UK. PP. 224-226.	EPPO, European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			(Kennedy and King, 1962a; EPPO, 1992; Rat, 1993; Maas, 1998).			
33.	*25*	Technical	In contrast to angular leaf spot disease of strawberry, bacterial leaf blight of strawberry caused by *Xanthomonas arboricola* pv. *fragariae* is characterized by small reddish-brown lesions on the lower leaf surface that are neither water-soaked nor translucent; reddish spots on the upper leaf surface; lesions coalescing into large, dry brown spots surrounded by a chlorotic halo; and large brown V-shaped lesions along the leaf margin, midrib and major veins (Janse *et al*., 2001). Also, no bacterial exudation is associated with bacterial leaf blight lesions (Janse *et al*., 2001). In advanced stages, bacterial angular leaf spot is difficult to distinguish from fungal leaf-spotting diseases such as common leaf spot (*Mycosphaerella fragariae*) and leaf scorch (*Diplocarpon earliana*).provide reference	For Scientific justification and further cross reference	Kenya	**Incorporated**
34.	*26*	Substantive	Severe infections of *X. fragariae* may spread from the leaves to the crown where discrete water-soaked areas develop (Hildebrand *et al*., 1967). Severe crown infection can result in plants with decreased vigour that may collapse and eventually die. Leaves that develop from infected crowns are often systemically infected, with lesions that appear along the veins at the base of the leaves.join paragraph 26 and 27	For consistency since both refer to similar symptomps	Kenya	**Considered, but not incorporated:** the paras split the different types of symptoms.
35.	*26*	Technical	Severe infections of *X. fragariae* may spread from the leaves to the crown where discrete water-soaked areas develop (Hildebrand *et al*., 1967). Severe crown infection can result in plants with decreased vigour that may collapse and eventually die. Leaves that develop from infected crowns are often systemically infected, with lesions that appear along the veins at the base of the leaves. Slime may exude from vascular bundles when the crown is cut transversely.	Technical addition of a characteric symptom	EPPO, European Union	**Incorporated or modified if slime is not correct.**
36.	*27*	Substantive	In severe cases of disease, *X. fragariae* may attack flowers and cause blossom blight, but it does not directly infect fruits (Gubler *et al.,* 1999). Water-soaked lesions on infected calyx tissue are similar in appearance to foliar lesions (Gubler *et al.*, 1999). Fruit tissue near severely	For consistency	Kenya	See comment 34.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			infected calyx tissue may also become water-soaked. join with paragraph 26			
37.	*28*	Technical	*X. fragariae* can move systemically into the roots, crowns and runners without exhibiting obvious symptoms (Stefani *et al.*, 1989; Milholland *et al.,* 1996; Mahuku and Goodwin, 1997). This type of infection maycan result in the appearance of water-soaked areas at the base of newly emerged leaves followed shortly by sudden plant collapse and death although it is not always seen.	The sudden plant collapse and death is not frequently seen in Europe	EPPO, European Union	**Incorporated:**
38.	*30*	Editorial	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is necessary for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all tests as a positive control.	Spelling correction.	Singapore	**Incorporated:**
39.	*30*	Editorial	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot facilitatingand is neccessary for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. CWhen systemic infection is suspected it is necessary to analyse cCrown tissue should also be examinedfrom affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all tests as a positive control.	Simplification of the text	EPPO, European Union	**Incorporated:**
40.	*30*	Editorial	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is neccessary necessary for successful isolation of *X. fragariae*. Alternatively,	Incorret spelling	Jamaica	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all tests as a positive control.			
41.	*30*	Editorial	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is necessary ~~neccessary~~ for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all tests as a positive control.	spelling corrections	Kenya	**Incorporated:**
42.	*30*	Editorial	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is nec~~c~~essary for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all tests as a positive control.	Correct spelling of necessary	Canada	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
43.	*30*	Substantive	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is neccessary for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. A reference *X. fragariae* strain should be included in all [tests as a positive control].	Provide more information on sampling intensity	Kenya	**Considered but not incorporated:** Description on sampling methodology is outside the scope of the diagnostic protocol. Only methods of direct relevance for diagnostics are included in the protocol.
44.	*30*	Technical	For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot and is neccessary for successful isolation of *X. fragariae*. Alternatively, leaves with dry spots and with or without exudates can be used. When systemic infection is suspected it is necessary to analyse crown tissue from affected plants. For symptomless plants, it is recommended that several entire plants be selected and small amounts of tissue be excised from their leaves, petioles and crowns (EPPO, 2006). These can be used directly for PCR-based analyses as described in section 3.9. ~~A reference *X. fragariae* strain should be included in all tests as a positive control.~~	The last sentence should not belong to sampling but be included in the section related to the different tests.	EPPO, European Union	**Incorporated**
45.	*31*	Substantive	Samples should not be left in a wet condition after collection. Preferably samples should be partially dried, wrapped in paper, placed in polythene bags and kept cool. Samples should be transported in a well-insulated container and on arrival at their destination stored at 4 °C. join with paragraph 30	For consistency	Kenya	
46.	*31*	Technical	Samples should not be left in a wet condition after collection. Preferably samples should be partially dried, wrapped in paper, placed in polythene bags and kept cool. Samples should be transported in a well-insulated container and on arrival at their destination stored at 4 °C and processes as soon as possible.	technical important specification regarding the timing of processing of samples.	EPPO, European Union	**Modified:** "and processed as soon as possible."

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
47.	*32*	Technical	**3.3 Sample preparation**	Elements of sample preparation are scattered in 3.3 and 3.5 (where two methods for the preparation of samples are described. However isolation method 2 is also considered appropriate for PCR IF It is suggested to present the different sample preparation and indicate for each for which tests they are relevant and then describe the different tests.	EPPO, European Union	
48.	*33*	Editorial	The surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. Approximatelely 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as described in the following sections.	Spelling corrections.	Singapore	**Incorporated:**
49.	*33*	Editorial	The surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. Approximatelely 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as described in the following sections.	2 typing mistakes	EPPO, European Union	**Incorporated:**
50.	*33*	Editorial	The surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it	Incorrect spelling	Jamaica	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. ~~Approximatley~~ Approximately 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as ~~decribed~~ described in the following sections.			
51.	*33*	Editorial	The surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. Approximately ~~Approximatley~~ 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as ~~decribed~~described in the following sections.	spelling	Kenya	**Incorporated:**
52.	*33*	Editorial	The surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. Approximat~~l~~ely 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g	Two spelling issues (4th line - Approximately, 7th line - described)	Canada	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as described in the following sections.			
53.	*33*	Substantive	For symptomatic plants,tThe surfaces of plant tissue can be disinfested by wiping with 70% ethanol. If the plants show vascular symptoms, it is recommended that the roots and the leaves are removed, keeping the crown and petioles. The sample is rinsed in tap water to remove excess soil and then disinfested by immersing for 1 min in 70% ethanol followed by rinsing three times in sterile distilled water. Approximatley 0.1 g of leaf or crown and petiole tissue per sample is added to 9 ml phosphate-buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4{\cdot}12H_2O$, 0.2 g KH_2PO_4, distilled water to 1 litre; pH 7.2). The plant material is crushed and incubated at room temperature for 15 min. These sample tissue macerates are then used in ELISA, Immunofluorescence and PCR tests as decribed in the following sections. For asymptomatic plants, collect a 30 g sample at random from the 200 quarters. Place each sample in 150 mL of PBS. Shake for 30 min and use the washing liquid directly for detection, or after centrifugation at 10 000 g per10 min. Suspend the final pellet in sterile distilled water to obtain a final volume of 5 mL. After leaving to settle for 15 min collect the upper clarified part and prepare dilutions (1 : 10 and 1 : 100) in sterile distilled water. Keep 2 mL for analysis and put 1 mL at –20°C and 1 mL at –80°C with 30% glycerol.	The explanation on sample preparation should be divided into part of symptomatic plants and part of asymptomatic plants based on the reference (EPPO, 2006, pp.140)	Japan	**Modified:** Sample preparation section has been revised to clarify the differences. There is reference to testing of symptomless plants in para 30 and the EPPO reference is included.
54.	*35*	Editorial	Rapid screening tests facilitate detection of *X. fragariae*. As the bacterium is very difficult to isolate, tThree tests (ELISA, immunofluorescence and PCR) should be positive to confirm *X. fragariae* detection~~, as the bacterium is very difficult to isolate~~. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo *et al.*, 1997b). The detached leaf bioassay is a	Clearer wording.	Singapore	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			supplemental method for confirming the presence of viable *X. fragariae*.			
55.	*35*	Editorial	Rapid screening tests facilitate detection of *X. fragariae*. Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm *X. fragariae* detection, as the bacterium is very difficult to isolate. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo *et al.*, 1997b). The detached leaf bioassay is a supplemental test~~method~~ for confirming the presence of viable *X. fragariae*.	Consistency of terminology between IPPC protocols.	EPPO, European Union	**Incorporated**
56.	*35*	Substantive	Rapid screening tests facilitate [detection of *X. fragariae*]what rapid method are available for detection of X. fragariae?. Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm *X. fragariae* detection, as the bacterium is very difficult to isolate. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo *et al.*, 1997b). The detached leaf bioassay is a supplemental method for confirming the presence of viable *X. fragariae*.	Should provide more information on rapid screening.	Kenya	**Not incorporated:** Further information is provided on these tests in section 3.
57.	*35*	Technical	Rapid screening tests facilitate detection of *X. fragariae*. Three [tests (ELISA, immunofluorescence and PCR)] are the three tests rapid screening? should be positive to confirm *X. fragariae* detection, as the bacterium is very difficult to isolate. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo *et al.*, 1997b). The detached leaf bioassay is a supplemental method for confirming the presence of viable *X. fragariae*.	More technical information needed	Kenya	**Not incorporated:** Further information is provided on these tests in section 3.
58.	*35*	Technical	Rapid screening tests facilitate detection of *X. fragariae*. Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm *X. fragariae* detection, as the bacterium is very difficult to isolate. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo *et al.*, 1997b). The detached leaf bioassay is a supplemental assay~~method~~ for confirming the presence of viable *X. fragariae*.	Assay is a more precise term than method.	Canada	**Modified:** method has been changed to test. This term is used consistently across IPPC documents.
59.	*37*	Editorial	Direct isolation of *X. fragariae* is difficult, even in the presence of symptoms and exudates, because *X. fragariae* grows very slowly on artificial nutrient media and is rapidly overgrown by secondary organisms. Two media are recommended for isolation. Isolation is more	Correct writing of scientific name.	Singapore	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			successful on Wilbrink's medium with nitrate (Wilbrink-N) (10 g sucrose, 5 g proteose peptone (L85; Oxoid), 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_20$, 0.25 g $NaNO_3$, 15 g purified agar, distilled water to 1 litre; pH 7.0–7.2) (Koike, 1965). Isolation on YPGA medium (5 g yeast extract, 5 g Bacto peptone, 10 g glucose, 15 g purified agar, distilled water to 1 litre; adjust pH to 7.0–7.2; add 5 ml filter-sterilized cycloheximide (stock solution: 5 g cycloheximide per 100 ml absolute ethanol) after autoclaving) is less successful but still recommended. SPA medium (20 g sucrose, 5 g peptone, 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$, 15 g purified agar, distilled water to 1 litre; pH 7.2–7.4) may be useful for fastidious bacteria (Hayward, 1960); however, the reliability of this medium for isolating *X. fragariae* has not been validated (López *et al.*, 2005). The use of purified agar (Oxoid or Difco)[1] is recommended for all media as impurities in other commercial agars can inhibit the growth of *X. fragariae*.			
60.	*37*	Technical	Direct isolation of *X fragariae* is difficult, even in the presence of symptoms and exudates, because *X. fragariae* grows very slowly on artificial nutrient media and is rapidly overgrown by secondary organisms. Two media are recommended for isolation. Isolation is more successful on Wilbrink's medium with nitrate (Wilbrink-N) (10 g sucrose, 5 g proteose peptone (L85; Oxoid), 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_20$, 0.25 g $NaNO_3$, 15 g purified agar, distilled water to 1 litre; pH 7.0–7.2) (Koike, 1965). Isolation on YPGA medium (5 g yeast extract, 5 g Bacto peptone, 10 g glucose, 15 g purified agar, distilled water to 1 litre; adjust pH to 7.0–7.2; add 5 ml filter-sterilized cycloheximide (stock solution: 5 g cycloheximide per 100 ml absolute ethanol) after autoclaving) is less successful but still recommended. SPA medium (20 g sucrose, 5 g peptone, 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$, 15 g purified agar, distilled water to 1 litre; pH 7.2–7.4) may be useful for fastidious bacteria (Hayward, 1960); ~~however, the reliability of this medium for isolating *X. fragariae* has not been validated (López *et al.*, 2005)~~. The use of purified agar (Oxoid or Difco)[1] is	A comparison of six media was performed in a Portuguese laboratory. The SPA medium allowed the best recovery of X. fragarie cells from symptomatic material. This is confirmed by the experience of at least another laboratory in the EPPO region. If the editorial team wishes it more information can be asked to the laboratory.	EPPO, European Union	**Modified**: Paragraph may be reconsidered for revision once more information is made available on the results. Is this work published? It is still recommend that two media are used for isolation. Due to the fastidious nature of this bacterium caution is suggested on emphasizing reliance on one artificial nutrient medium for direct isolation.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			recommended for all media as impurities in other commercial agars can inhibit the growth of *X. fragariae*.			
61.	*38*	Substantive	***3.5.1 Isolation method 1*** ***Working area proceedure have been left out; for example disinfection of working surface with 70% ethanol***	The proceedure should form part of this sub heading	Kenya	**Not incorporated:** Not included this is standard laboratory practice.
62.	*39*	Editorial	For plants with symptoms, select leaves with initial lesions and disinfect the surface by wiping with 70% ethanol. Isolations should be made from initial water-soaked lesions or from the margins of older lesions by excising a small piece of tissue (0.5–1.0 cm^2) with a sharp sterile scalpel.	spelling	Kenya	**Incorporated**
63.	*40*	Editorial	Tissue is crushed in a few millilitres ~~mls~~ of sterile distilled water or PBS and incubated at room temperature for 10–15 min. Aliquots (50 – 100 µl) of lesion tissue macerates as well as dilutions (1:10, 1:100, 1:1 000 and 1:1 0000) are plated out onto the surface of Wilbrink-N and YPGA media. Similar aliquots of *X. fragariae* cell suspensions (10^4, 10^5 and 10^6 colony-forming units (cfu/ml) should also be plated out in order to verify the quality of the media and to compare the cultural characteristics of any bacterial colonies that develop. Incubate the plates at 25–27 °C for 7 days but mark the colonies appearing after 2–3 days as these will not be *X. fragariae.* Final readings should be performed after incubation at 25–27 °C for 7 days.	Suggest "millilitres" rather than "mls" as mls is not the correct short form for "millilitres"	Canada	**Modified:** replaced with "ml" as this term is used consistently with other protocols.
64.	*40*	Technical	Tissue is crushed in a few mls of sterile distilled water or PBS and incubated at room temperature for 10–15 min. Aliquots (50 – 100 µl) of lesion tissue macerates as well as dilutions (1:10, 1:100, 1:1 000 and 1:1 0000) are plated out onto the surface of Wilbrink-N, ~~and~~ YPGA and/or SPA media. Similar aliquots of *X. fragariae* cell suspensions (10^4, 10^5 and 10^6 colony-forming units (cfu/ml) should also be plated out in order to verify the quality of the media and to compare the cultural characteristics of any bacterial colonies that develop. Incubate the plates at 25–27 °C for 7 days but mark the colonies appearing after 2–3 days as these will not be	Aliquots (50 – 100 µl) of lesion tissue macerates : Could these aliquots also be used for other tests? This should be clarified. SPA is mentioned above	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			X. fragariae. Final readings should be performed after incubation at 25–27 °C for 7 days.			
65.	*40*	Technical	Tissue is crushed in a [few mls] should be specific of sterile distilled water or PBS and incubated at [room temperature] should provide a range for 10–15 min. Aliquots (50 – 100 µl) of lesion tissue macerates as well as dilutions (1:10, 1:100, 1:1 000 and 1:1 0000) are plated out onto the surface of Wilbrink-N and YPGA media. Similar aliquots of *X. fragariae* cell suspensions (10^4, 10^5 and 10^6 colony-forming units (cfu/ml) should also be plated out in order to verify the quality of the media and to compare the cultural characteristics of any bacterial colonies that develop. Incubate the plates at 25–27 °C for 7 days but mark the colonies appearing after 2–3 days as these will not be *X. fragariae.* Final readings should be performed after incubation at 25–27 °C for 7 days.	For clarity and Scientific justification	Kenya	**Modified**: the amount of sterile water or PBS can be left to the discretion of the diagnostician. A temperature range of 20 -25 has been included.
66.	*41*	Technical	*X. fragaria*e colonies on Wilbrink-N medium are initially off-white, becoming pale yellow, circular, slightly convex, smooth and mucoid after 4–6 days. On YPGA medium, the colonies are similar in morphology to those on Wilbrink-N, but they have a more intense yellow colour. Obtain pure cultures from individual suspect colonies of each sample (from each of the two media) by plating suspensions of the *Xanthomonasfragariae*-like colonies on Wilbrink-N medium.	A description of colonies on SPA is missing	EPPO, European Union	**Incorporated:**
67.	*43*	Technical	Excise pieces of leaf tissue with distinct water-soaked angular lesions and wash in 50 ml tap water and a few drops of Tween-20 and incubate at room temperature for 10 min. Rinse the leaf pieces in distilled water and blot dry. The surfaces of the leaf pieces can then be disinfected in 70% ethanol for 5 s and blot dried. Place the leaf pieces in 5 ml of 0.1 M PBS, mix and incubate at room temperature for 30 min to release any *X. fragariae* into the supernatant. Prepare a 1:100 dilution of supernatant in 0.1 M PBS and add 20 µl aliquots of the undiluted sample and 1:100 dilution to separate wells of a multi-well microscope slide. Fix the bacterial cells to the slide by flaming for later immunofluorescence analysis (section 3.8). Place 200 µl undiluted supernatant in a	regarding glycerol it hsould be mentioned at least 20% glycerol	EPPO, European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			microtube for later PCR analysis (section 3.9) and another 1 ml undiluted supernatant in a second microtube, adding a drop of glycerol, and store at –20 °C or –80 °C for reference purposes. The remaining supernatant can be used for isolation by dilution plating as described above and for inoculation of detached strawberry leaves (section 3.6).			
68.	*43*	Technical	Excise pieces of leaf tissue with distinct water-soaked angular lesions and wash in 50 ml tap water and a few drops of Tween-20 and incubate at room temperature for 10 min. Rinse the leaf pieces in distilled water and blot dry. The surfaces of the leaf pieces can then be disinfected in 70% ethanol for 5 s and blot dried. Cut leaf fragment into small pieces (1-4mm^2) and p~~P~~lace them~~the leaf pieces~~ in 5 ml of 0.1 M PBS, mix and incubate at room temperature for 30 min to release any *X. fragariae* into the supernatant. Prepare a 1:100 dilution of supernatant in 0.1 M PBS and add 20 µl aliquots of the undiluted sample and 1:100 dilution to separate wells of a multi-well microscope slide. Fix the bacterial cells to the slide by flaming for later immunofluorescence analysis (section 3.8). Place 200 µl undiluted supernatant in a microtube for later PCR analysis (section 3.9) and another 1 ml undiluted supernatant in a second microtube, adding a drop of glycerol, and store at –20 °C or –80 °C for reference purposes. The remaining supernatant can be used for isolation by dilution plating as described above and for inoculation of detached strawberry leaves (section 3.6).	For more clarity of the isolation procedure. This modification is consistent with (EPPO, 2006, pp.143)	Japan	**Incorporated:**
69.	*44*	Technical	~~An alternative to i~~Isolation of *X. fragariae* from tissue may also be peformed ~~if~~froms ~~to streak~~ aliquots of fresh exudates from lesions directly onto Wilbrink-N, YPGA, SPA or other commonly used media and may be performed in addition to the other isolation methods.	This isolation is not always successful and should not be proposed as an alternative methods but rather as an additional one.	EPPO, European Union	**Incorporated:**
70.	*46*	Editorial	The isolation is negative if no bacterial colonies with morphology similar to *X. fragariae* colonies are observed after 7 days on~~in~~ either of the two media (provided no growth inhibition due to competition or antagonism has	English correction	EPPO, European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			occurred) and typical *X. fragariae* colonies are found in the positive controls.			
71.	*46*	Technical	The isolation is negative if no bacterial colonies with morphology similar to *X. fragariae* colonies are observed after 7 days in either of the threetwo media (provided no growth inhibition due to competition or antagonism has occurred) and typical *X. fragariae* colonies are found in the positive controls.	Three media not two	EPPO, European Union	**Incorporated**
72.	*49*	Substantive	[There is not always a good] Need to be rephrased and combine with paragraph 48 correlation between isolation, serological tests (i.e. immunofluorescence, ELISA) and/or PCR because isolation frequently fails. The best isolation results are expected when using freshly prepared sample extracts from young lesions. Isolation onto media can also be achieved by *in planta* enrichment as described in section 3.6.	For consistency	Kenya	**Considered but not incorporated:** Stating this in a separate paragraph adds more emphasis.
73.	*52*	Editorial	Tissue sample preparations (section 3.3) can be used for inoculating detached strawberry leaves as soon as they are prepared in extraction buffer or distilled water (Civerolo *et al*., 1997a). Use young (7–14 days old) leaves of a cultivar susceptible to *X. fragariae* (e.g. Camarosa, Seascape, Selva, Korona) from greenhouse-grown, *X. fragariae*-free plants. The quality of the leaves and their age are essential considerations for a successful testassay.	Consistency opf terminology	EPPO, European Union	**Incorporated**
74.	*53*	Editorial	Aseptically remove three leaves (each one with three leaflets) from the greenhouse-grown plants and immediately place the petioles in glass tubes containing sterile water. Cut off the basal portion of the petioles then replace the petioles in their glass tubes containing sterile water.	Simplification	EPPO, European Union	**Incorporated**
75.	*53*	Technical	Aseptically remove three leaves (each one with three leaflets) from the greenhouse-grown plants and immediately place the petioles in glass tubes containing sterile water. Cut off the basal portion of the petioles then return replace the petioles in glass tubes containing sterile water.	scientific clarification	Kenya	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
76.	*55*	Editorial	Rinse off excess inoculum with sterile water 1 h after inoculation. Place leaves with their petioles in the~~ir~~ tubes in a humid chamber and incubate at 18–20 °C with a 12 h photoperiod for up to 21 days. The specified temperature and illumination during incubation is essential for avoiding false negative results. The inoculated leaves should not have visible injuries and water-soaking caused by the inoculum infiltration should disappear within 24 h.	simplification	EPPO, European Union	**Incorporated**
77.	*55*	Technical	Rinse off excess inoculum with sterile water 1 h after inoculation. Place leaves with their petioles in their tubes in a humid chamber (RH 95-100%) and incubate at 18–20 °C with a 12 h photoperiod for up to 21 days. The specified temperature and illumination during incubation is essential for avoiding false negative results. The inoculated leaves should not have visible injuries and water-soaking caused by the inoculum infiltration should disappear within 24 h.	It hsould be specified that HR is usually 100%	EPPO, European Union	**Incorporated**
78.	*57*	Technical	***3.6.2 Interpretation of detached leaf assay results***	A reference to the fact that the controls should give expected results should be added	EPPO, European Union	**Incorporated**
79.	*58*	Substantive	The detached leaf assay is negative if no typical *X. fragariae* angular leaf spots (i.e. dark, water-soaked when viewed with reflected light; translucent yellow when viewed with transmitted light) and/or chlorotic halos appear at some of the inoculated sites after 21 days. No water-soaked spots that appear translucent yellow when viewed with transmitted light should appear within inoculation sites infiltrated with negative controls.	It is suggested that it would be most useful to add a figure here with the expected symptoms, both for the leaf spots and the halos.	New Zealand	**Incorporated**
80.	*61*	Editorial	Select one leaf per sample from those inoculated in the detached leaf assay 48 h after inoculation for isolation onto media ~~after *in planta* enrichment~~. Excise 10–12 small discs 0.5 cm in diameter from each inoculated site per inoculated detached leaf and crush in 4.5 ml PBS. Prepare dilutions as for direct isolation (section 3.5) in PBS and streak 50 µl of each dilution onto the surface of Wilbrink-N medium in triplicate. Incubate plates at 25–27 ºC and record results for *X. fragariae*-like colonies after 5–7 days.	in planta enrichment is the detached leaf assay mentioned just in the line above.	EPPO, European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
81.	*65*	Editorial	The specificity of two ELISA tests~~protocols~~ with commercially available polyclonal anti-*X. fragariae* sera has been validated (López *et al*., 2005). Rowhani *et al.* (1994) showed that ELISA using polyclonal antibodies could specifically detect 34 strains of *X. fragariae* and the antibodies did not cross-react with other closely related pathovars or other bacteria isolated from strawberry plants. A test sensitivity of 10^5 cfu/ml has been reported for ELISA detection of *X. fragariae* (Rowhani *et al.,* 1994; Civerolo *et al.,* 1997b).	Consistency of terminology	EPPO, European Union	**Incorporated**
82.	*66*	Technical	Use cell suspensions prepared from pure cultures of *X. fragariae* and a non-*X. fragariae* strain as positive and negative controls in each microtiter plate. [Frequent cross-reactions among phytopathogenic or other bacteria can occur with polyclonal antibodies]clarify as it contradicts earlier statement. It is recommended that the appropriate working dilution of each polyclonal antiserum is determined.	further information needed for clarification.	Kenya	**Modified**: Deleted sentence with regards to cross-reaction of polyclonal antibodies as you are using validated antibodies.
83.	*68*	Editorial	Mix 210 µl of each test sample, the positive *X. fragariae* cell suspension (approximately 10^9 cfu/ml) and the negative non-*X. fragariae* cell suspension (approximately 10^9 cfu/ml) and the negative control (suspension of healthy strawberry material, see below) with 210 µl coating buffer (Na_2CO_3, $NaHCO_3$, distilled water to 1 litre) and add 200 µl of the sample and buffer mixture to each of two wells of a microtiter plate (PolySorp (Nunc)[2] or equivalent). For the negative plant material control, crush about 0.1 g healthy strawberry leaf, petiole or crown tissue in 0.9 ml PBS and add 0.9 ml coating buffer.	better wording	EPPO, European Union	**Incorporated**
84.	*68*	Technical	Mix 210 µl of each test sample, the positive *X. fragariae* cell suspension (approximately 10^9 cfu/ml) and the negative non-*X. fragariae* cell suspension (approximately 10^9 cfu/ml) and the negative control (healthy strawberry material) with 210 µl coating buffer (1.59g Na_2CO_3, 2.93g $NaHCO_3$, distilled water to 1 litre) and add 200 µl of the sample and buffer mixture to each of two wells of a microtiter plate (PolySorp (Nunc)[2] or equivalent). For the negative plant material control, crush about 0.1 g healthy	For more clarity of the ingredients in the buffer. This modification is consistent with (EPPO, 2006, pp.144)	Japan	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			strawberry leaf, petiole or crown tissue in 0.9 ml PBS and add 0.9 ml coating buffer.			
85.	*70*	Editorial	Prepare the appropriate working dilution separate according to the manufacturer's instructions, of the anti-*X. fragariae* serum in PBS and add 200 µl to each test well. Incubate at 37 °C for 2 h and then wash the plate three times in PBS-T. Add 200 µl of the antibody–enzyme conjugate at the appropriate dilution in PBS containing 0.2% BSA to each well. Incubate at 37 °C for 1 h and wash the plate four times in PBS-T. Add 200 µl freshly prepared substrate (1 mg p-nitrophenylphosphate/ml substrate buffer, pH 9.8) to each test well. Incubate in the dark at room temperature for 15, 30 and 60 min, and read the absorbance at 405 nm.	gramatical correction	Kenya	**Considered, but not incorporated**: the suggested revision is confusing.
86.	*72*	Editorial	For double antibody sandwich (DAS)-ELISA, add 200 µl of an appropriate dilution of anti-*X. fragariae* serum in thea coating buffer to each well of two microtiter plates (PolySorp (Nunc)[3] or equivalent). Incubate at 37 °C for 4 h and wash the wells three times with PBS-T. Add 200 µl of each tissue macerate sample, a positive and a negative control, as described for indirect ELISA, to each of two wells of each plate and incubate at 4 °C overnight. After washing the plates three times with PBS-T, add 200 µl of an appropriate dilution of the enzyme–antibody conjugate in PBS containing 0.2% BSA to each well. Incubate at 37 °C for 3 h. After washing the plates four times with PBS-T add 200 µl of freshly prepared substrate (1 mg ρ-nitrophenylphosphate/ml substrate buffer, pH 9.8) to each test well. Incubate in the dark at room temperature for 15, 30 and 60 min, and read the absorbance at 405 nm.	Better English	EPPO, European Union	**Incorporated**
87.	*77*	Substantive	Positive ELISA results for negative control wells indicate that cross-contamination or non-specific antibody binding has occurred. [The test should be repeated with fresh tissue or another test based on a different principle should be performed] join with paragraph 76.	for consistency	Kenya	**Considered, but not incorporated:** Stating this in a separate paragraph adds more emphasis.
88.	*79*	Editorial	Immunofluorescence procedures for identifying phytopathogenic bacteria are given in De Boer (1990). Two commercially available polyclonal anti-*X. fragariae*	COnssitency of terminology Simplification of English	EPPO, European Union	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			sera (Table 1) have been validated using fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulins (López *et al.,* 2005).This testmethod alolows thewill permit detection of approximately 10^3–10^4 cfu/ml *X. fragariae* in strawberry tissue (Calzolari and Mazzucchi, 1989).			
89.	*79*	Substantive	Immunofluorescence procedures for identifying phytopathogenic bacteria are given in [De Boer (1990)]is this the only method used and adopted by all diagnostic labs. Two commercially available polyclonal anti-*X. fragariae* sera (Table 1) have been validated using fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulins (López *et al.,* 2005).This method will permit detection of approximately 10^3–10^4 cfu/ml *X. fragariae* in strawberry tissue (Calzolari and Mazzucchi, 1989).	more information needed	Kenya	**Considered, but not incorporated:** This sentence refers to IF as a generic method I am not sure what the member country is referring to here? The additional of the reference in comment 90 may assist.
90.	*79*	Technical	Immunofluorescence procedures for identifying phytopathogenic bacteria are given in De Boer (1990) and in EPPO (2009). Two commercially available polyclonal anti-*X. fragariae* sera (Table 1) have been validated using fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulins (López *et al.,* 2005).This method will permit detection of approximately 10^3–10^4 cfu/ml *X. fragariae* in strawberry tissue (Calzolari and Mazzucchi, 1989).	Suggest making a reference to the EPPO Standard on IF A standard describing how to perform an indirect immunofluorescence test (IF) for plant pathogenic bacteria was approved in 2009	EPPO, European Union	**Incorporated**
91.	*81*	Editorial	Aliquots (20 µl) of test samples and positive and negative control suspensions are added to separate wells of a multi-well microscope slide. Preparations are air-dried and fixed by flaming or by soaking slides in acetone for 10 min followed by air-drying. Slides can be stored at –20 ºC until required. Primary *X. fragariae* antibody is diluted in PBS + 10% skim milk powder. Select the lowest antibody concentration that gives good staining when there is up to 100 positive cells per microscope field. It is advisableadvised that two dilutions of the antiserum areis	gramatical corrections	Kenya	**Incorporated**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			used to detect cross-reactions with other bacteria. Apply 20 µl of the primary antibody to each well and incubate the slides in a moist chamber at room temperature or at 37 °C for 30–60 min. The slides are then rinsed in PBS and washed by submerging in the same buffer for 10 min. The FITC-conjugated secondary antibody is diluted in PBS (optimum dilutions usually vary between 1:20 and 1:200). The wells of all slides are then covered with the secondary antibody and incubated in a moist chamber at room temperature or at 37 ºC for 30–60 min. The washing step is repeated and air-dried. The coverslips are mounted with mounting fluid (90 ml glycerol, 10 ml PBS) containing 1 mg ρ-phenylenediamine/ml) and slides viewed under oil immersion at 500–1 000× magnification. Count the cells that fluoresce and have a similar size to the reference *X. fragariae* strain (López *et al*., 2005).			
92.	*88*	Editorial	[1] Recommended for detection using immunofluorescence (validated in ring tests in a European Union-funded project (SMT-4-CT98-2252)) (López *et al.,* 2005).	consider replacing ring test by test performance study	EPPO, European Union	**Incorporated:**
93.	*89*	Editorial	[2] Recommended for detection using double antibody sandwich-enzyme-linked immunosorbent test~~assay~~ (validated in ring tests in a European Union-funded project (SMT-4-CT98-2252)) (López *et al.,* 2005).	consider replacing ring test by test performance study consistency of terminology	EPPO	See comment 92
94.	*90*	Substantive	**3.9 PCR** Real-time PCR assay wasn't included.	It is very important to develop a rapid, sensitive real-time PCR for detecting X. fragariae	China	**Modified:** agree that the inclusion of real-time PCR is important and this is now addressed in paragraph 93 and section 3.9.4.
95.	*91*	Editorial	The PCR methods described in this diagnostic protocol, with the exception of the nested PCR developed by Zimmerman *et al.* (2004), have been validated in a ring test funded by the European Union (SMT-4-CT98-2252)	Consider replacing ring test by test performance study	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			(López *et al.,* 2005). Nested PCR protocols were reported to increase sensitivity up to 100 times compared with conventional PCR protocols (Roberts *et al.,* 1996; Zimmerman *et al.,* 2004).			
96.	*91*	Substantive	The PCR methods described in this diagnostic protocol, with the exception of the nested PCR developed by Zimmerman *et al.* (2004), have been validated in a ring test funded by the European Union (SMT-4-CT98-2252) [(López *et al.,* 2005)]link not opening. Nested PCR protocols were reported to increase sensitivity up to 100 times compared with conventional PCR protocols (Roberts *et al.,* 1996; Zimmerman *et al.,* 2004).	provide more information	Kenya	**Incorporated:**
97.	*92*	Editorial	Protocols for DNA extraction from plant samples and PCR described in Pooler *et al.* (1996) and Hartung and Pooler (1997) have been validated (López *et al.*, 2005). A modified protocol using the REDExtract-N-Amp Plant PCR Kit (Sigma)[5] has also been reported to be appropriate for DNA extraction before amplification for testing large numbers of samples of asymptomatic leaves (Stöger and Ruppitsch, 2004). Other commercial kits for extracting DNA and for nested PCR and other primers (Roberts *et al.*, 1996) are available; however, these ~~may not be as reliable (e.g. reduced sensitivity) and~~ have not been validated yet ~~for clinical applications~~ (López *et al.*, 2005).	Simplification	EPPO, European Union	**Incorporated**
98.	*92*	Technical	Protocols for DNA extraction from plant samples and PCR described in Pooler *et al.* (1996) and Hartung and Pooler (1997) have been validated (López *et al.*, 2005). A modified protocol using the REDExtract-N-Amp Plant PCR Kit (Sigma)[5] has also been reported to be appropriate for DNA extraction before amplification for testing large numbers of samples of asymptomatic leaves (Stöger and Ruppitsch, 2004). Other commercial kits for extracting DNA and for nested PCR and other primers (Roberts *et al.*, 1996) are available; however, these may not be as reliable (e.g. reduced sensitivity) and have not been validated for [clinical applications] clarify (López *et al.*, 2005).	clarify why clinical and we are dealing with palnts	Kenya	**Modified:** this sentence has now been revised and "clinical" has been deleted.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
99.	*93*	Editorial	Two sensitive real-time PCR tests~~assays~~ have been described for detection of *X. fragariae* (Weller *et al.*, 2007; Vandroemme *et al.*, 2008) as well as differentiation of *X. fragariae* and *X. arboricola* pv. *fragariae* (Weller *et al.*, 2007) in strawberry tissue. The real-time PCR test~~assay~~ described by Weller *et al*. (2007) is based on primers designed within regions of the *gyr~~ase~~B* gene unique to *X. fragariae* and the *pep* gene unique to *X. arboricola* pv. *fragariae*. The real-time PCR test~~assay~~ developed by Vandroemme *et al*. (2008) is based on primers yielding a 41 base pair (bp) amplicon, designed from the 550 bp fragment amplicon described by Pooler *et al*. (1996). To date (March, 2015), neither of these tests~~methods~~ has been verified or validated (e.g. in a ring test). However, these tests~~methods~~ are potentially useful for detecting low levels of *X. fragariae* in asymptomatic or latent infections.	Consistency of terminology COnsider replacing ring-test by test performance study	EPPO, European Union	**Incorporated:**
100.	*93*	Editorial	Two sensitive real-time PCR assays have been described for detection of *X. fragariae* (Weller *et al.*, 2007; Vandroemme *et al.*, 2008) as well as differentiation of *X. fragariae* and *X. arboricola* pv. *fragariae* (Weller *et al.*, 2007) in strawberry tissue. The real-time PCR assay described by Weller *et al*. (2007) is based on primers designed within regions of the *gyraseB* gene unique to *X. fragariae* and the *pep* gene unique to *X. arboricola* pv. *fragariae*. The real-time PCR assay developed by Vandroemme *et al*. (2008) is based on primers yielding a 41 base pair (bp) amplicon, designed from the 550 bp fragment amplicon described by Pooler *et al*. (1996). To date (March, 2015), neither of these methods have~~has~~ been verified or validated (e.g. in a ring test). However, these methods are potentially useful for detecting low levels of *X. fragariae* in asymptomatic or latent infections.	gramatical correction	Kenya	**Incorporated:**
101.	*93*	Substantive	Two sensitive real-time PCR assays have been described for detection of *X. fragariae* (Weller *et al.*, 2007; Vandroemme *et al.*, 2008) as well as differentiation of *X. fragariae* and *X. arboricola* pv. *fragariae* (Weller *et al.*, 2007) in strawberry tissue. The real-time PCR assay described by Weller *et al*. (2007) is based on primers designed within regions of the *gyraseB* gene unique to *X. fragariae* and the *pep* gene unique to *X. arboricola* pv.	Sentence To date (March, 2015), neither of these tests methods has been verified or validated (e.g. in a ring test).). This is not correct, the real time PCR from Weller et al has now been validated in a Dutch laboratory and the validation has been uploaded on the EPPO database on diagnostic expertise http://dc.eppo.int/validationlist.php Search for	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			fragariae. The real-time PCR assay developed by Vandroemme *et al*. (2008) is based on primers yielding a 41 base pair (bp) amplicon, designed from the 550 bp fragment amplicon described by Pooler *et al*. (1996). To date (March, 2015), neither of these methods has been verified or validated (e.g. in a ring test). However, these methods are potentially useful for detecting low levels of *X. fragariae* in asymptomatic or latent infections.	Xanthomonas fragariae This test should be added to the protocol		
102.	*98*	Technical	***3.9.2 Multiplex PCR*** Conventional PCR by the primers 245A / 245B of three pairs of multiplex PCR primers are used instead of multiplex PCR.	The 300 bp band by the primers 245A / 245B is usually present when the extracts are from plants infected with X. fragariae but the other bands (550 and 615 bp) may appear occasionally (Pooler et al., 1996; Hartung and Pooler, 1997).	China	**Modified**: This has been revised to indicate that you can use primers 245A/B in conventional PCR as an alternative.
103.	*108*	Editorial	PCR cycling parameters are an initial activation step of 95 °C for 15 min; 35 cycles of 95 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min; and finally 72 °C for 7 min. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× Tris-acetate-EDTA (TAE) buffer (EPPO,2006, pp.142).	This condition is described in (EPPO, 2006, pp.142)	Japan	**Incorporated:**
104.	*111*	Editorial	The nested PCR protocol described by Moltmann and Zimmerman (2005) using primers developed by Pooler *et al*. (1996) and Zimmerman *et al.* (2004) is recommended for diagnosing *X. fragariae* in symptomatic strawberry plants as well as ~~. This protocol is also useful~~ for testing asymptomatic strawberry plants (frigo and green plants) ~~(Moltmann and Zimmerman, 2005)~~. The nested PCR test~~protocol~~ described by Roberts *et al.* (1996) also offers an alternative method for confirmation.	1 simplification 2 consistency of terminology	EPPO, European Union	**Incorporated:**
105.	*113*	Editorial	Specificity for this protocol was confirmed in a study with 14 isolates of *X. fragariae*, 30 isolates of *X. campestris* (representing 14 pathovars) and 17 isolates of unidentified bacteria associated with strawberry leaves. In addition, the specificity of the external primer set was verified by Pooler *et al.* (1996) (see previous section 3.9.2). NO cross reaction was observed with the isolated tested. ~~Only *X. fragariae* was detected (in all isolates)~~. This	1 clarification 2 consistency of terminology 3 simplification	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			test~~method~~ has been successfully applied to testing of samples collected during a survey of strawberry plants and imported plants (Moltmann and Zimmerman, 2005) and ~~. This nested PCR protocol~~ enabled detection to 200 fg DNA per reaction and was 100 times more sensitive than conventional PCR (Zimmerman *et al.,* 2004).			
106.	*114*	Technical	Incubate leaf, petiole and crown tissue (30–70 g) in 10–20 ml 0.01~~0.1~~ M sodium phosphate buffer~~butter~~ (pH 7.2) per gram of tissue at room temperature overnight. Extract DNA and analyse by single and nested PCR as described by Zimmerman *et al.* (2004). The primers are:	This modification is consistent with (Moltmann and Zimmermann, 2005, pp.53)	Japan	**Incorporated:**
107.	*119*	Editorial	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P-40, 2.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 94 °C for 4 min; 35 cycles of 94 °C for 1 min, 68 °C for 1 min and 72 °C for 1 min; and a final extension step of 72 °C for 7 min. For nested PCR, after amplification of DNA with the first round of primers (245A and 245B), 1 µl of the reaction mixture is used as template in a second PCR with the internal primers 245.5 and 245.267. The same PCR cycling conditions are used except the annealing temperature is 62 °C for the internal primers 245.5 and 245.267. PCR products are analysed by 1.2% agarose gel electrophoresis in 0.5× TAE buffer.	Re PCR in the first line - it is suggested that the same presentation be used throughout the file. The previous reaction description state the amount of buffer as well as the volume of added DNA template. It would be good if this system could be followed for all the tests.	New Zealand	**Incorporated:**
108.	*119*	Technical	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P-40, 2.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 94 °C for 4 min; 35 cycles of 94 °C for 1 min, 68 °C for 1 min and 72 °C for 1 min; and a final extension step of 72 °C for 7 min. For nested PCR, after amplification of DNA with the first round of primers (245A and 245B), 1 µl of the ~~reaction mixture~~ first PCR product is used as template in a second PCR with the internal primers 245.5 and 245.267. The same PCR cycling conditions are used except the	To clarify	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			annealing temperature is 62 °C for the internal primers 245.5 and 245.267. PCR products are analysed by 1.2% agarose gel electrophoresis in 0.5× TAE buffer.			
109.	*122*	Editorial	Specificity for this protocol was confirmed in a study with 30 isolates of *X. fragariae*, 17 isolates of *X. campestris* (representing 16 pathovars) and 9 isolates of non-pathogenic xanthomonads isolated from strawberry. No cross reaction was observed with the isolates tested ~~Only *X. fragariae* was detected (in all isolates)~~. This nested PCR technique enabled detection to approximately 18 *X. fragariae* cells in plant tissue (Roberts *et al.,* 1996).	Clarification	EPPO, European Union	**Incorporated:**
110.	*124*	Technical	• XF9: 5′-TGGGCCATGCCGGTGGAACTGTGTG~~TG~~G-3′	This modification is consistent with (Roberts et al.,1996, pp.1284)	Japan	**Incorporated:**
111.	*127*	Editorial	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 95 °C for 2 min; 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s; and a final extension step of 72 °C for 5 min. For the nested PCR, after amplification of DNA with the first round of primers (XF9 and XF11), 3 µl of the reaction mixture is used as template in a second PCR with the primers XF9 and XF12.The same PCR conditions as described for the first round are performed. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× TAE buffer.	typing mistake	EPPO, European Union	**Incorporated:**
112.	*127*	Editorial	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 95 °C for 2 min; 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s; and a final extension step of 72 °C for 5 min. For the nested PCR, after amplification of DNA with the first round of primers (XF9 and XF11), 3 µl of the reaction mixture is used as template in a second PCR with the primers XF9 and XF12.The same PCR conditions~~condtions~~ as described	spelling correction	Kenya	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			for the first round are performed. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× TAE buffer.			
113.	*127*	Technical	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 95 °C for 2 min; 3040 cycles of 95 °C for 30 s, 6560 °C for 30 s and 72 °C for 45 s; and a final extension step of 72 °C for 5 min. For the nested PCR, after amplification of DNA with the first round of primers (XF9 and XF11), 3 µl of the reaction mixture is used as template in a second PCR with the primers XF9 and XF12.The same PCR condtions as described for the first round are performed except that the aneealing temperature is 58 °C. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× TAE buffer.	This modification is consistent with (Roberts et al.,1996, pp.1284)	Japan	**Incorporated:**
114.	*127*	Technical	PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.2 µM each primer and 0.5 µl Taq DNA polymerase. The reaction conditions are an initial denaturation step of 95 °C for 2 min; 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s; and a final extension step of 72 °C for 5 min. For the nested PCR, after amplification of DNA with the first round of primers (XF9 and XF11), 3 µl of the reaction mixture first PCR product is used as template in a second PCR with the primers XF9 and XF12.The same PCR condtions as described for the first round are performed. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× TAE buffer.	To clarify	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Incorporated:**
115.	*128*	Substantive	Specific PCR amplicons for *X. fragariae* are 537 bp in the first round PCR using the XF9 and XF11 primers, and 458 bp in the semi-nested PCR using the primers XF9 and XF12. It is advised that the real-time PCR methods are introduced into PCR method section.	Two real-time PCR assays have been described for detection of X. fragariae in strawberry tissue(Weller et al., 2007; Vandroemme et al., 2008).	China	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
116.	*136*	Technical	***Positive nucleic acid control.*** This control is used to monitor the efficiency of PCR amplification. Pre-prepared (stored) nucleic acid, whole genome DNA or a synthetic control (e.g. a cloned PCR product) may be used. For this protocol, a suspension of pure culture *X fragariae* cells (10^4- 10^6cfu/ml) is recommended as a positive nucleic acid control.	It is better to recommend a range, for cfu/ml	EPPO, European Union	**Incorporated:**
117.	*139*	Technical	***Positive extraction control.*** This control is used to ensure that nucleic acid from the target is of sufficient quality for PCR amplification. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target near~~at~~ the concentration considered the detection limit of the protocol. The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the DNA extraction. For this protocol, *X. fragariae* tissue macerates spiked with ~~10^4 and~~ 10^6 cfu/ml of a reference *X. fragariae* strain are recommended as positive extraction controls. For PCR, care needs to be taken to avoid cross-contamination due to aerosols from the positive control or from positive samples (in particular for Nested PCR) . If required, the positive control used in the laboratory should be sequenced so that the sequence can be readily compared with sequences obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence that, again, can be compared with PCR amplicons of the correct size.	1 It is more correct to refer to near the limit of detection 2 It is better to have the higher concentration for DNA extraction from spiked samples 3 additionnal warning for nested PCR	EPPO, European Union	**Incorporated:**
118.	*145*	Editorial	*X. fragariae* has the common characteristics of all xanthomonads. Cells~~They~~ are Gram-negative, aerobic rods, with a single polar flagellum. ~~They do not reduce n~~Nitrates are not reduced, ~~they are~~ catalase test positive, ~~and~~ asparagine ~~is~~ not used ~~by them~~ as a sole source of carbon and nitrogen (Bradbury, 1977; Bradbury, 1984; Schaad *et al*., 2001). ~~They are w~~Weak production~~ers~~ of acids from carbohydrates. Colonies are mucoid, convex and shiny on YPGA and Wilbrink-N media (Dye, 1962; van den Mooter *et al.,* 1990; Swings *et al.,* 1993; Schaad *et al.,* 2001).*Xanthomonas* species are easily differentiated from the other genera of aerobic, Gram-	Simplification normal wording of biochemical characteristics	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			negative rod-shaped and other yellow-pigmented bacteria by the characteristics shown in Table 3 as~~and~~ described by~~in~~ Schaad *et al*. (2001).			
119.	*149*	Technical	**Table 3.** Phenotypic characteristics for differentiating *Xanthomonas* from *Pseudomonas* and other yellow-pigmented bacteria (*Flavobacterium* and *Pantoea)*	The following reference should be added Schaad NW, Jones JB and Chun W. 2001.Laboratory guide for the identification of plant pathogenic bacteria. APS press, St Paul, Minnesota. p. 177.	EPPO, European Union	**Incorporated:**
120.	*151*	Editorial	The most relevant or useful characteristics for distinguishing *X. fragariae* from other *Xanthomonas* (EPPO, 2006~~Schaad *et al.*, 2001; Janse *et al.*, 2001~~) are shown in Table 4.	Table 4. is described in (EPPO, 2006).	Japan	**Modified:** have included the EPPO reference but it is useful to keep the original references.
121.	*152*	Editorial	**Table 4.** Diagnostic tests to distinguish *Xanthomonas fragariae* from the *"X. campestris* group" and *X. arboricola* pv. *fragariae* (EPPO, 2006~~Janse *et al.*, 2001~~)	Table 4. is described in (EPPO, 2006).	Japan	**Modified:** have included the EPPO reference but it is useful to keep the original references.
122.	*157*	Substantive	**Table 5**. Reactions of *Xanthomonas fragariae* in API 20 NE tests	Need to add reaction for Phenyl-acetate - at bottom of table	New Zealand	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
123.	*158*	Technical	**Test** **Reaction (48 or 96 h)[1]**	In order to compare with other species of X. fragariae.	China	**Considered, but not incorporated:** Is this a strain issue? If so the described reactions are the same for 90% of strains tested see para 159.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Glucose fermentation – Arginine – Urease – Esculin + Gelatin + (weakly) Para-NitroPhenyl-ßDGalactopyranosidase (PNPG) + Assimilation of: + Glucose – Arabinose + Mannose – Mannitol + N-acetyl-glucosamine – Maltose – Gluconate – Caprate – Adipate +			

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Malate – Citrate – Phenyl-acetate It should be added the API 20 and API 50 results of other species of X. fragariae.			
124.	*160*	Editorial	For the API 50 CH strips[10], prepare bacterial cell suspensions of OD = 1.0 in PBS. Add 1 ml suspension to 20 ml modified medium C (0.5 g $NH_4H_2PO_4$, 0.5 g K_2HPO_4, 0.2 g $MgSO_4$,5 g NaCl, 1 g yeast extract, 70 ml b~~B~~romothymol blue (0.2%), distilled water to 1 litre; pH 6.8) (Dye, 1962). Follow the manufacturer's instructions for inoculation of the strips. Incubate at 25 °C under aerobic conditions and read after 2, 3 and 6 days. Utilization of the different carbohydrates is indicated by a	No capital needed	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			yellow colour in the wells after the incubation period (Table 6).			
125.	*162*	Technical	**Test[1]** / **Reaction (six days)** d-arabinose: Variable Galactose: + d-glucose: + d-fructose: + d-mannose: + N-acetyl-glucosamine: + Esculin: + Sucrose: + Trehalose: + d-lyxosa: + l-fucose: + It should be added the API 20 and API 50 results of other species of X. fragariae.	In order to compare with other species of X. fragariae.	China	See comment 123
126.	*165*	Editorial	Fatty acid methyl esters (FAMEs) associated with the cytoplasmic and outer membranes of Gram-negative bacteria are useful for bacterial identification (Sasser, 1990). Specific fatty acids that may be used to predict the	Conssitency of terminology	EPPO	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			genus of Gram-negative and Gram-positive bacteria are given by Dickstein *et al.* (2001). Identification is based on comparing the types and relative amounts of the fatty acids in a profile of an unknown strain with profiles from a wide variety of strains in a library database (e.g. TSBA40 library). It is critical that bacteria be grown under uniform conditions of time, temperature and nutrient media in order to obtain reproducible results. *X. fragariae* strains contain three major fatty acids (16:1ω-7 *cis*, 15:0 *anteiso* and 15:0 *iso*); while some strains give a good match to the library profile, other strains have differing fattytest~~acid~~ profiles that do not correspond well. Studies have shown that strains of *X. fragariae* show considerable diversity and fall into at least four distinct fatty acid groups (Roberts *et al.,* 1998). The method described by Roberts *et al*. (1998) is recommended for FAME profiling of *X. fragariae*. Test strains are grown on trypticase soy agar at 24 °C for 48 h, a fatty acid extraction procedure is applied and the extract is analysed using the Sherlock Microbial Identification System (MIDI).			
127.	*173*	Substantive	**4.3 Molecular tests** Real-time PCR assay wasn't included.	It is very important to develop a rapid, sensitive real-time PCR for detecting X. fragariae	China	**Incorporated**: See previous comments.
128.	*179*	Technical	Bacterial strains to be analysed are taken from streaks or individual colonies on Pierce's disease modified medium (5.0 g sucrose, 2.5 g Phytone (BBL Microbiology Systems, Baltimore, MD)[11], 10 g Phytagel (BBL Microbiology Systems)[12]; adjust pH to 7.5 with 2 N HCl before autoclaving; distilled water to 1 litre) (Opgenorth *et al.,* 1996).	REP PCR can be standardized in the lab using different growth medium This should be presented as an option	EPPO, European Union	**Incorporated**
129.	*185*	Technical	The reaction buffer contains 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 6.7 µM EDTA, 30 mM 2-mercaptoethanol, 0.17 mg BSA/ml, 10% (v/v) dimethyl sulfoxide, 1.2 mM of each dNTP, 62 pmol each primer and 2 U Taq DNA polymerase. Bacteria from a representative colony of the test strain are transferred, using a sterile 10 µl pipette tip (or other suitable implement), to a PCR reaction tube containing 25 µl of the reaction mixture.	Proposed deletion for consistency with other parts of the protocol where only agarose gels are mentioned.	EPPO, European Union	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			Cycling parameters are 95 °C for 6 min followed by 35 cycles at 94 °C for 1 min, 44 °C (REP primers) or 52 °C (ERIC primers) for 1 min and 65 °C for 8 min. The amplification cycles are followed by a final extension cycle of 68 °C for 16 min. The amplification products (5–10 µl) are electrophoresed in a 1.5% (w/v) agarose gel ~~at room temperature for 4 h at 5 V/cm in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA)~~. Amplified DNA fragments are visualized after staining with ethidium bromide by ultraviolet transillumination.			
130.	*187*	Substantive	Test bacterial strains are identified as *X. fragariae* if the same genomic fingerprints are obtained as those of the REP and ERIC genotypes of the reference strains (Pooler *et al*., 1996) amplified in the same test and run in the same gel. A small number of polymorphic bans may be obtained from different *X. fragariae* isolates due to low levels of genomic variability	Useful additionnal information	EPPO	**Incorporated:**
131.	*187*	Substantive	Test bacterial strains are identified as *X. fragariae* if the same genomic fingerprints are obtained as those of the REP and ERIC genotypes of the reference strains (Pooler *et al*., 1996) amplified in the same test and run in the same gel. A small number of polymorphic bans may be obtained from different *X. fragariae* isolates due to low levels of genomic variability	Useful additional information	European Union	Duplicate comment.
132.	*188*	Technical	***4.3.3 Multilocus sequence analysis*** Enumerate the housekeeping genes for identifying X. fragariae.	In order to standardize the process of identification.	China	**Modified**: examples of housekeeping genes have been provided.
133.	*189*	Editorial	A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (Almeida *et al.,* 2010; Hamza *et al.*, 2012; Parkinson *et al.,* 2007) and could be used for identification of *X. fragariae* especially now that a draft genome sequence is now available (Vandroemee *et al.,* 2013). However, it should be noted this methodology has not yet been validated for identification of *X. fragariae*. Housekeeping genes are amplified using primers and	Hamza et al., 2012 not included in references section.	Singapore	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			PCR conditions as described by Almeida *et al.* (2010) and Hamza *et al.,* (2012). MLSA consists of sequencing multiple loci (typically four to eight housekeeping genes) and comparing these sequences with reference sequences of *Xanthomonas* species deposited in nucleotide databases; for example, the Plant Associated Microbes Database (PAMDB) (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) (Almeida *et al.*, 2010) and the MLVAbank for microbe genotyping (https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/).			
134.	*189*	Editorial	A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (Almeida *et al.,* 2010; Hamza *et al.*, 2012; Parkinson *et al.,* 2007) and could be used for identification of *X. fragariae* especially now that a draft genome sequence is ~~now~~ available (Vandroemee *et al.,* 2013). However, it should be noted that this methodology has not yet been validated for identification of *X. fragariae*. Housekeeping genes are amplified using primers and PCR conditions as described by Almeida *et al.* (2010) and Hamza *et al.,* (2012). MLSA consists of sequencing multiple loci (typically four to eight housekeeping genes) and comparing these sequences with reference sequences of *Xanthomonas* species deposited in nucleotide databases; for example, the Plant Associated Microbes Database (PAMDB) (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) (Almeida *et al.*, 2010) and the MLVAbank for microbe genotyping (https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/).	1 unecessary word 2 word missing	EPPO	**Incorporated:**
135.	*189*	Editorial	A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (Almeida *et al.,* 2010; Hamza *et al.*, 2012; Parkinson *et al.,* 2007) and could be used for identification of *X. fragariae* especially now that a draft genome sequence is ~~now~~ available (Vandroemee *et al.,* 2013). However, it should be noted that this methodology has not	1. unnecessary word 2. word missing	European Union	Duplicate comment

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			yet been validated for identification of *X. fragariae*. Housekeeping genes are amplified using primers and PCR conditions as described by Almeida *et al.* (2010) and Hamza *et al.,* (2012). MLSA consists of sequencing multiple loci (typically four to eight housekeeping genes) and comparing these sequences with reference sequences of *Xanthomonas* species deposited in nucleotide databases; for example, the Plant Associated Microbes Database (PAMDB) (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) (Almeida *et al.*, 2010) and the MLVAbank for microbe genotyping (https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/).			
136.	*189*	Editorial	A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (Almeida *et al.,* 2010; Hamza *et al.*, 2012; Parkinson *et al.,* 2007) and could be used for identification of *X. fragariae* especially now that a draft genome sequence is now available (Vandroemee *et al.,* 2013). However, it should be noted this methodology has not yet been validated for identification of *X. fragariae*. Housekeeping genes are amplified using primers and PCR conditions as described by Almeida *et al.* (2010) ~~and Hamza *et al.*, (2012)~~. MLSA consists of sequencing multiple loci (typically four to eight housekeeping genes) and comparing these sequences with reference sequences of *Xanthomonas* species deposited in nucleotide databases; for example, the Plant Associated Microbes Database (PAMDB) (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) (Almeida *et al.*, 2010) and the MLVAbank for microbe genotyping (https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/).	This quotation is not included in the reference section. Fot this reason it should be deleted from the text or otherwise included in the reference section.	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Incorporated:**
137.	*194*	Technical	Prepare bacterial cell suspensions approximately(~~10^8~~ – 10^{6}~~^{9}~~ cfu/ml) in sterile distilled water or 10 mM PBS. Apply inoculum for each strain to the abaxial surfaces of three trifoliate leaves on each of two or three plants with a low pressure spray gun, airbrush or similar device (e.g. DeVilbiss) so as not to induce water-soaking. Infection may be facilitated by wounding leaves (e.g. puncturing the	The concentrations given is high for a pathogenicity test, It should be approximately 106 cfu/ml	EPPO	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			abaxial surface with a needle) before applying inoculum, although it is not necessary to do this. After inoculation, incubate plants in a chamber maintained at 20–25 °C with high humidity (>90%) and a 12–14 h photoperiod. Suspensions of cells of a reference *X. fragariae* strain (prepared in the same manner as the test strain) and sterile distilled water or 10 mM PBS serve as positive and negative controls, respectively, and should be inoculated in different trays. Evaluate lesion development weekly for three weeks (21 days) post-inoculation. Re-isolate the pathogen from such lesions, as described in section 3.5, and identify by ELISA, immunofluorescence or PCR.			
138.	*194*	Technical	Prepare bacterial cell suspensions approximately~~(10^8–~~ 10^{6}~~^{9}~~ cfu/ml) in sterile distilled water or 10 mM PBS. Apply inoculum for each strain to the abaxial surfaces of three trifoliate leaves on each of two or three plants with a low pressure spray gun, airbrush or similar device (e.g. DeVilbiss) so as not to induce water-soaking. Infection may be facilitated by wounding leaves (e.g. puncturing the abaxial surface with a needle) before applying inoculum, although it is not necessary to do this. After inoculation, incubate plants in a chamber maintained at 20–25 °C with high humidity (>90%) and a 12–14 h photoperiod. Suspensions of cells of a reference *X. fragariae* strain (prepared in the same manner as the test strain) and sterile distilled water or 10 mM PBS serve as positive and negative controls, respectively, and should be inoculated in different trays. Evaluate lesion development weekly for three weeks (21 days) post-inoculation. Re-isolate the pathogen from such lesions, as described in section 3.5, and identify by ELISA, immunofluorescence or PCR.	The concentration given is high for a pathogenicity test, It should be approximately 10 6 cfu/ml	European Union	**Duplicate comment**
139.	*196*	Technical	If the colonies are~~sample tissue extract inoculum contains~~ *X. fragariae*, initial symptoms will be dark, water-soaked (when viewed with reflected light) lesions on the lower leaf surfaces. These lesions appear translucent yellow when viewed with transmitted light. Later these lesions develop into necrotic spots surrounded by a yellow halo or marginal necrosis. The same symptoms should appear on	The pathogenicity test is based on pure cultures	EPPO, European Union	**Considered, but not incorporated:** This pathogenicity test can be performed with aqueous extracts of naturally or field

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			plants inoculated with a reference *X. fragariae* strain (positive control).			collected suspect angular leaf spots.
140.	*201*	Technical	Complete collapse and necrosis of the infiltrated tissue within 24–48 h post-inoculation is recorded as a positive test result. Most *X. fragariae* strains are HR positive. However, some may be HR negative, especially after being stored for some time. Similar reactions should not appear on leaves mock-inoculated with sterile distilled water or 10 mM PBS as a negative control.	According to Janse et al 2001 X. arboricola pv. fragariae has a positive HR reaction on tobacco and is differentiated from X. fragariae by this reaction. Therefore the reference on which information in this paragraph is based should be provided.	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Incorporated:**
141.	*225*	Technical	**EPPO** (European and Mediterranean Plant Protection Organization). 1992. Data sheet on *Xanthomonas fragariae*. *In* I.M. Smith, D.G. McNamara, P.R. Scott & K.M. Harris, eds. *Quarantine pests for Europe,* pp. 829–833. Data sheets on European Communities and for the European and Mediterranean Plant Protection Organization. Wallingford, UK, CABI. XX pp.	replace by EPPO. 1997. Data sheet on Xanthomonas fragariae. In EPPO/CABI (1997) Quarantine Pests for Europe. 2nd edition. Edited by Smith IM, McNamara DG, Scott PR, Holderness M. CABI, Wallingford, UK, 1425 pp pp 1124-1128. add EPPO (2009), PM 7/97 (1): Indirect immunofluorescence test for plant pathogenic bacteria. EPPO Bulletin, 39: 413–416.	EPPO, European Union	**Incorporated:**
142.	*233*	Technical	**Janse, J.D., Ross, M.P., Gorkink, R.F.J., Derks, J.H.J., Swings, J. Janssens, D. & Scortichini, M.** 2001. Bacterial leaf blight of strawberry (*Fragaria (×) ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. *fragariae* (pv. nov., comb. nov.). *Plant Pathology,* 50: 653–665.	add Janse, J.D. 2005. Phytobacteriology: Principles and Practice. CABI Publishing, Wallingford, UK. PP. 224-226.	EPPO, European Union	**Incorporated:**
143.	*235*	Editorial	**Kennedy, B.W. & King, T.H.** 1962~~a~~. Angular leaf spot of strawberry caused by *Xanthomonas fragariae* sp. nov. *Phytopathology,* 52: 873–875.	It is proposed to delete the reference "Kennedy and King, 1962b" from the references section, as it was not cited in the draft annex. Hence, "1962a" should be changed to "1962" instead.	Singapore	**Incorporated:**
144.	*236*	Editorial	~~**Kennedy, B.W. & King, T.H.** 1962b. Studies on epidemiology of bacterial angular leafspot of strawberry. *Plant Disease Reporter,* 46: 360–363.~~	It is proposed to delete this reference as it was not cited in the draft annex.	Singapore	**Incorporated:**
145.	*239*	Editorial	**López, M.M., Dominguez, F., Morente, C., Salcedo, C.I., Olmos, A. & Civerolo, E.** 2005. *Diagnostic protocols for*	For clarity.	Singapore	**Incorporated:**

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			organisms harmful to plants: Diagnosis Xanthomonas fragariae. SMT-4-CT98-2252. **Maas, J.L.,** ed. 1998. *Compendium of strawberry diseases*, 2nd edn. St Paul, MN, APS Press. XX pp.			
146.	*245*	Editorial	**Moltmann, E. & Zimmermann, C.** 2005. Detection of *Xanthomonas fragariae* in symptomless strawberry plants by nested PCR. *EPPO Bulletin,* 35: 53–54. National Standards for Phytosanitary Measures (NSPM) approved by the NPPO and National Quarantine Committee of Nepal. 2014. Standard Technical protocols for collection and Handling of Disease samples.[Online] Available: http://www.moadwto.gov.np/downloadfile/NSPM-17pestreporting_1390904481.pdf-Nov 2015 pp5-6	Addition of this reference that was added in paragraph: 3.2	South Africa	**Considered, but not incorporated:** see previous comments on these references.
147.	*263*	Editorial	University of Hawaii college of tropical agriculture and Human resources (UH-CTAHR), 2006. Collecting plant disease and Insects Pest Samples for problem diagnosis. Scot. C. Nelson and Brian C. Bushe. Cooperative Extension Service, Soil and Crop management. SCM-14. University of Hawaii. pp 2-3. **Van den Mooter, M. & Swings, J.** 1990. Numerical analyses of 295 phenotypic features of 266 *Xanthomonas* strains and related strains and an improved taxonomy of the genus. *International Journal of Systematic Bacteriology,* 40: 348–369.	Addition of this reference that was added in paragraph number: 3.2.	South Africa	**Considered, but not incorporated:** see previous comments on these references.
148.	*270*	Technical	**Footnote 1:** ~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved~~. The ~~U~~use of names of reagents chemicals or equipment in ~~these~~ this diagnostic protocol~~s~~ implies no approval of them to the exclusion of others that may also be suitable.	1. Text deleted because it is already included in the text of DP (paragraph 22). 2. Text added according to the previously agreed footnote.	COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay	**Modified**: This will be modified to incorporate the standard IPSM disclaimer that is present in all protocols.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC responses
			~~Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~ This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.			