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<td>1.</td>
<td>G</td>
<td>Editorial</td>
<td></td>
<td>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.</td>
<td>EPPO, European Union</td>
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<tr>
<td>2.</td>
<td>G</td>
<td>Editorial</td>
<td></td>
<td>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.</td>
<td>South Africa</td>
</tr>
<tr>
<td>3.</td>
<td>G</td>
<td>Editorial</td>
<td>Se hace una atenta solicitud para que los párrafos de estos protocolos se numeren para mayor claridad y mejor manejo de la información.</td>
<td>Para facilitar el proceso de consulta y de revisión de estos proyectos de NIMF (protocolos de diagnóstico).</td>
<td>Costa Rica, Mexico</td>
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<tr>
<td>4.</td>
<td>G</td>
<td>Substantive</td>
<td>I support the document as it is and I have no comments</td>
<td></td>
<td>Georgia, Indonesia, Lao People's Democratic Republic, United States of America, Nepal, Thailand, Mexico, Congo, Barbados, Bahrain, Guyana, Belize, Ghana, Burundi</td>
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<tr>
<td>5.</td>
<td>G</td>
<td>Substantive</td>
<td>Redraft section 4 ‘Identification’ to recognise that the bacterium can be identified without the need for pathogenicity tests or ELISA tests.</td>
<td>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</td>
<td>Australia</td>
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<td>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., ... &amp; 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., &amp; 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., &amp; Maes, M. (2008). Sensitive real-time PCR detection of Xanthomonas fragariae in strawberry plants. Plant Pathology, 57(3), 438-444.</td>
<td>Japan</td>
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<td></td>
<td></td>
<td></td>
<td>Insert some pictures of disease symptoms and flow chart for detection. These information would be useful to identify Xanthomonas fragariae.</td>
<td>Japan</td>
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<tr>
<td>6</td>
<td>G</td>
<td>Substantive</td>
<td>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.</td>
<td>Peru</td>
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<td>Regarding paragraph 49, we would like to request the</td>
<td>Peru</td>
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<td>Technical</td>
<td>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.”</td>
<td>See comment</td>
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<td>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.”</td>
<td>See comment</td>
<td>COSAVE, Uruguay, Chile, Paraguay</td>
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<tr>
<td>10.</td>
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<td>Technical</td>
<td>1. 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.” 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.</td>
<td>See comment</td>
<td>Brazil</td>
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<tr>
<td>11.</td>
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<td>Technical</td>
<td>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 Aspecto relevantes que deben ser clarificados</td>
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<td>12.</td>
<td>1</td>
<td>Substantive</td>
<td>Deletion of the name “Africa” and replacing it with the name “Ethiopia”.</td>
<td>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</td>
<td>South Africa</td>
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<td>13.</td>
<td>2</td>
<td>Editorial</td>
<td>Status box This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption.</td>
<td>separate the two words i.e to member and fragariae to</td>
<td>Kenya</td>
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**Comment:** planta) o solamente el ADN bacteriano. En el párrafo 3 primera oración de este protocolo debe indicarse cuántas unidades de la Taq DNA polimerasa 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.

**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)
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<td>• Edwin L. CIVEROLO (USDA/ARS, United States) (retired)</td>
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<td></td>
<td>• Solke H. DE BOER (Centre for Animal and Plant Health, Canadian Food Inspection Agency)</td>
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<td></td>
<td>• John ELPHINSTONE (Plant and Environmental Bacteriology, Fera, United Kingdom)</td>
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The following expert commented on the draft protocol on a voluntary basis during the expert consultation stage:

- Stephan BIERIE (Canadian Food Inspection Agency, Canada)
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**Main discussion points during development of the diagnostic protocol**

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**Status box**

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**Date of this document**

2015-06-10

**Document category**

Draft annex to ISPM 27 (*Diagnostic protocols for regulated pests*)

**Current document stage**

To member

Please correct Stephan Briére's name - BRIERE. It is spelled incorrectly in the table.

Canada
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  - María M. | |
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2015-03 Edited

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<td>16.</td>
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<td>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 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. 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 because the sentence is more relevant in paragraph 3.5.1.</td>
<td>South Africa</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>3</td>
<td>Substantive</td>
<td>Immunofluorescence), Molecular (PCR) and Pathogenicity (Koch’s postulates)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.</td>
<td>South Africa</td>
<td></td>
</tr>
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<td>18.</td>
<td>4</td>
<td>Substantive</td>
<td>To be added later: supernatant Addition of “supernatant because it can also be used to confirm Koch’s postulates .</td>
<td>South Africa</td>
<td></td>
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<tr>
<td>19.</td>
<td>9</td>
<td>Editorial</td>
<td>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 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</td>
<td>Singapore</td>
<td></td>
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</tbody>
</table>

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.
<table>
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<td><em>Fragaria</em> species, including <em>F. chiloensis</em>, <em>F. virginiana</em> and <em>F. vesca</em>, as well as <em>Potentilla fruticosa</em> and <em>P. glandulosa</em> are also susceptible. Among <em>Fragaria</em> species only <em>F. moschata</em> is immune (Kennedy and King, 1962a; Kennedy, 1965; Maas, 1998).</td>
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<tr>
<td>20.</td>
<td>9</td>
<td>Editorial</td>
<td><em>Xanthomonas fragariae</em> 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 <em>et al</em>., 1967; Maas, 1995), but it has been subsequently reported in many strawberry growing areas around the world, including South America, Africa and Europe (CABI, 2015). <em>Fragaria × ananassa</em>, the predominant cultivated strawberry, is the primary host of <em>X. fragariae</em>. However, commercial cultivars vary in susceptibility, and other <em>Fragaria</em> species, including <em>F. chiloensis</em>, <em>F. virginiana</em> and <em>F. vesca</em>, as well as <em>Potentilla fruticosa</em> and <em>P. glandulosa</em> are also susceptible. Among <em>Fragaria</em> species only <em>F. moschata</em> is immune (Kennedy and King, 1962a; Kennedy, 1965; Maas, 1998).</td>
<td>Addition of these two references that were added in paragraph number: 2</td>
<td>South Africa</td>
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<tr>
<td>21.</td>
<td>10</td>
<td>Technical</td>
<td><em>X. fragariae</em> is readily transmitted via asymptomatic planting stock with latent infection. Inoculum sources for primary infection are infected but clinically asymptomatic</td>
<td>Alt 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)</td>
<td>EPPO, European Union</td>
</tr>
</tbody>
</table>
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.

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.

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;
<table>
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<tr>
<td>25.</td>
<td>19</td>
<td>Technical</td>
<td>Diagnosis of bacterial angular leaf spot disease of strawberry caused by <em>X. fragariae</em> 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 <em>X. fragariae</em>. 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).</td>
<td>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 &amp; 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 &amp; Goodwin, 1997;</td>
<td>Kenya</td>
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<tr>
<td>26.</td>
<td>19</td>
<td>Technical</td>
<td>Diagnosis of bacterial angular leaf spot disease of strawberry caused by <em>X. fragariae</em> 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 <em>X. fragariae</em>. 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).</td>
<td>Clarify Why clinical sample and yet we are dealing with plants.</td>
<td>European Union</td>
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<td>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 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).</td>
<td>Zimmerman et al., 2004; Moltman &amp; Zimmerman, 2005; Vermunt &amp; 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 would be used. Proposed deletion: 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?</td>
<td>EPPO, European Union</td>
<td></td>
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<td>27. 21</td>
<td>Technical</td>
<td>Procedures for the detection of X. fragariae in plants with symptoms are presented below.</td>
<td>See general comment (this sentence seems to mean that only procedures for the detection of X. fragariae in plants with symptoms are included).</td>
<td></td>
<td></td>
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<tr>
<td>28. 22</td>
<td>Editorial</td>
<td>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.</td>
<td>Remove the unnecessary brackets.</td>
<td>Canada</td>
<td></td>
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<tr>
<td>29. 22</td>
<td>Technical</td>
<td>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 Texted deleted and included in the footnote as previously agreed.</td>
<td>COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay</td>
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<td>30.</td>
<td>23</td>
<td>Substantive</td>
<td>3.1 Symptoms</td>
<td>procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.</td>
<td>New Zealand</td>
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<tr>
<td>31.</td>
<td>24</td>
<td>Editorial</td>
<td>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 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, 1962a; EPPO, 1992; Rat, 1993; Maas, 1998).</td>
<td>It is suggested that it would be most beneficial if pictures of the symptoms could be added.</td>
<td>Singapore</td>
</tr>
<tr>
<td>32.</td>
<td>24</td>
<td>Technical</td>
<td>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 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). 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, 1962a; EPPO, 1992; Rat, 1993; Maas, 1998).</td>
<td>It is proposed to delete the reference &quot;Kennedy and King, 1962b&quot; from the references section, as it was not cited in the draft annex. Hence, the reference &quot;Kennedy and King, 1962a&quot; should be changed to &quot;Kennedy and King, 1962&quot; instead.</td>
<td>EPPO, European Union</td>
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<tr>
<td>33.</td>
<td>25</td>
<td>Technical</td>
<td>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, 1962a; EPPO, 1992; Rat, 1993; Maas, 1998).</td>
<td>For Scientific justification and further cross reference</td>
<td>Kenya</td>
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<tr>
<td>34.</td>
<td>26</td>
<td>Substantive</td>
<td>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</td>
<td>For consistency since both refer to similar symptomps</td>
<td>Kenya</td>
</tr>
<tr>
<td>35.</td>
<td>26</td>
<td>Technical</td>
<td>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.</td>
<td>Technical addition of a characteric symptom</td>
<td>EPPO, European Union</td>
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<td>36.</td>
<td>27</td>
<td>Substantive</td>
<td>In severe cases of disease, <em>X. fragariae</em> 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 infected calyx tissue may also become water-soaked. <a href="#">Join with paragraph 26</a></td>
<td>For consistency</td>
<td>Kenya</td>
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<tr>
<td>37.</td>
<td>28</td>
<td>Technical</td>
<td><em>X. fragariae</em> 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 may 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.</td>
<td>The sudden plant collapse and death is not frequently seen in Europe</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>38.</td>
<td>30</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em>. 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>Spelling correction.</td>
<td>Singapore</td>
</tr>
<tr>
<td>39.</td>
<td>30</td>
<td>Editorial</td>
<td>For plants with symptoms, leaves with initial water-soaked spots are preferred as samples for the diagnosis of bacterial angular leaf spot facilitating and is necessary for successful isolation of <em>X. fragariae</em>. 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 should also be examined 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>Simplification of the text</td>
<td>EPPO, European Union</td>
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<td>Para. no.</td>
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<td>Explanation</td>
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<tr>
<td>40.</td>
<td>30</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em>. 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>Incorrect spelling</td>
<td>Jamaica</td>
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<tr>
<td>41.</td>
<td>30</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em>. 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>Spelling corrections</td>
<td>Kenya</td>
</tr>
<tr>
<td>42.</td>
<td>30</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em>. 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</td>
<td>Correct spelling of necessary</td>
<td>Canada</td>
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<td>43.</td>
<td>30</td>
<td>Substantive</td>
<td>described in section 3.9. A reference <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>Provide more information on sampling intensity</td>
<td>Kenya</td>
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<td>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 <em>X. fragariae</em>. 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
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<td>44.</td>
<td>30</td>
<td>Technical</td>
<td>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 <em>X. fragariae</em>. 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 <em>X. fragariae</em> strain should be included in all tests as a positive control.</td>
<td>The last sentence should not belong to sampling but be included in the section related to the different tests.</td>
<td>EPPO, European Union</td>
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<tr>
<td>45.</td>
<td>31</td>
<td>Substantive</td>
<td>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. <a href="#">Join with paragraph 30</a>.</td>
<td>For consistency</td>
<td>Kenya</td>
</tr>
<tr>
<td>46.</td>
<td>31</td>
<td>Technical</td>
<td>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 processed as soon as possible.</td>
<td>For consistency</td>
<td>EPPO, European Union</td>
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<td>Comm. no.</td>
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<td>47.</td>
<td>32</td>
<td>Technical</td>
<td>3.3 Sample preparation</td>
<td>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. 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.</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>48.</td>
<td>33</td>
<td>Editorial</td>
<td>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 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 Na2HPO4·12H2O, 0.2 g KH2PO4, 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.</td>
<td>Singapore</td>
<td></td>
</tr>
<tr>
<td>49.</td>
<td>33</td>
<td>Editorial</td>
<td>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 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 Na2HPO4·12H2O, 0.2 g KH2PO4, 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.</td>
<td>EPPO, European Union</td>
<td></td>
</tr>
<tr>
<td>50.</td>
<td>33</td>
<td>Editorial</td>
<td>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 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 Na2HPO4·12H2O, 0.2 g KH2PO4, 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.</td>
<td>Jamaica</td>
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<tr>
<td>51.</td>
<td>33</td>
<td>Editorial</td>
<td>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. <strong>Approximately</strong> 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 Na₂HPO₄·12H₂O, 0.2 g KH₂PO₄, 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 <strong>described</strong> in the following sections.</td>
<td>spelling</td>
<td>Kenya</td>
</tr>
<tr>
<td>52.</td>
<td>33</td>
<td>Editorial</td>
<td>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. <strong>Approximately</strong> 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 Na₂HPO₄·12H₂O, 0.2 g KH₂PO₄, 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 <strong>described</strong> in the following sections.</td>
<td>Two spelling issues (4th line - Approximately, 7th line - described)</td>
<td>Canada</td>
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<tr>
<td>Comm. no.</td>
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<tr>
<td>53.</td>
<td>33</td>
<td>Substantive</td>
<td>For symptomatic plants, 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 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 NaH_{2}PO_{4}.12H_{2}O, 0.2 g KH_{2}PO_{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. 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 per 10 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.</td>
<td>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)</td>
<td>Japan</td>
</tr>
<tr>
<td>54.</td>
<td>35</td>
<td>Editorial</td>
<td>Rapid screening tests facilitate detection of <em>X. fragariae</em>. As the bacterium is very difficult to isolate, three tests (ELISA, immunofluorescence and PCR) should be positive to confirm <em>X. fragariae</em> detection, as the bacterium is very difficult to isolate. The correlation among ELISA, PCR and detached leaf bioassay is usually high (Civerolo <em>et al.</em>, 1997b). The detached leaf bioassay is a supplemental method for confirming the presence of viable <em>X. fragariae</em>.</td>
<td>Clearer wording.</td>
<td>Singapore</td>
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<td>Comment no.</td>
<td>Para. no.</td>
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<td>Explanation</td>
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<tr>
<td>55.</td>
<td>35</td>
<td>Editorial</td>
<td>Rapid screening tests facilitate detection of <em>X. fragariae</em>. Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm <em>X. fragariae</em> 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 <em>X. fragariae</em>.</td>
<td>Consistency of terminology between IPPC protocols.</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>56.</td>
<td>35</td>
<td>Substantive</td>
<td>Rapid screening tests facilitate [detection of <em>X. fragariae</em>](what rapid method are available for detection of <em>X. fragariae</em>?). Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm <em>X. fragariae</em> 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 <em>X. fragariae</em>.</td>
<td>Should provide more information on rapid screening.</td>
<td>Kenya</td>
</tr>
<tr>
<td>57.</td>
<td>35</td>
<td>Technical</td>
<td>Rapid screening tests facilitate detection of <em>X. fragariae</em>. Three [tests (ELISA, immunofluorescence and PCR)](are the three tests rapid screening?) should be positive to confirm <em>X. fragariae</em> 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 <em>X. fragariae</em>.</td>
<td>More technical information needed</td>
<td>Kenya</td>
</tr>
<tr>
<td>58.</td>
<td>35</td>
<td>Technical</td>
<td>Rapid screening tests facilitate detection of <em>X. fragariae</em>. Three tests (ELISA, immunofluorescence and PCR) should be positive to confirm <em>X. fragariae</em> 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 <em>X. fragariae</em>.</td>
<td>Assay is a more precise term than method.</td>
<td>Canada</td>
</tr>
<tr>
<td>59.</td>
<td>37</td>
<td>Editorial</td>
<td>Direct isolation of <em>X. fragariae</em> is difficult, even in the presence of symptoms and exudates, because <em>X. fragariae</em> 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 (LB5; Oxoid), 0.5 g</td>
<td>Correct writing of scientific name.</td>
<td>Singapore</td>
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<td>Comm. no.</td>
<td>Para. no.</td>
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<td>Explanation</td>
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<td>K₂HPO₄, 0.25 g MgSO₄·7H₂O, 0.25 g NaNO₃, 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 K₂HPO₄, 0.25 g MgSO₄·7H₂O, 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.</td>
<td>A comparison of six media was performed in a Portuguese laboratory. The SPA medium allowed the best recovery of X. fragariae 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.</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>60.</td>
<td>37</td>
<td>Technical</td>
<td>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 K₂HPO₄, 0.25 g MgSO₄·7H₂O, 0.25 g NaNO₃, 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 K₂HPO₄, 0.25 g MgSO₄·7H₂O, 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.</td>
<td>EPPO, European Union</td>
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<tr>
<td>61.</td>
<td>38</td>
<td>Substantive</td>
<td><strong>3.5.1 Isolation method 1</strong>&lt;br&gt;&lt;i&gt;Working area procedure have been left out; for example disinfection of working surface with 70% ethanol&lt;/i&gt;</td>
<td>The procedure should form part of this sub heading</td>
<td>Kenya</td>
</tr>
<tr>
<td>62.</td>
<td>39</td>
<td>Editorial</td>
<td>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 cm²) with a sharp sterile scalpel.</td>
<td>spelling</td>
<td>Kenya</td>
</tr>
<tr>
<td>63.</td>
<td>40</td>
<td>Editorial</td>
<td>Tissue is crushed in a few <strong>millilitres</strong> 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 &lt;i&gt;X. fragariae&lt;/i&gt; cell suspensions (10⁴, 10⁵ and 10⁶ 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 &lt;i&gt;X. fragariae&lt;/i&gt;. Final readings should be performed after incubation at 25–27 °C for 7 days.</td>
<td>Suggest &quot;millilitres&quot; rather than &quot;mls&quot; as mls is not the correct short form for &quot;millilitres&quot;</td>
<td>Canada</td>
</tr>
<tr>
<td>64.</td>
<td>40</td>
<td>Technical</td>
<td>Tissue is crushed in a few <strong>millilitres</strong> 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 &lt;br&gt;and/or SPA media. Similar aliquots of &lt;i&gt;X. fragariae&lt;/i&gt; cell suspensions (10⁴, 10⁵ and 10⁶ 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 &lt;i&gt;X. fragariae&lt;/i&gt;. Final readings should be performed after incubation at 25–27 °C for 7 days.</td>
<td>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</td>
<td>EPPO, European Union</td>
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<td>Comm. no.</td>
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<td>Explanation</td>
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<tr>
<td>65.</td>
<td>40</td>
<td>Technical</td>
<td>Tissue is crushed in a [few mls] <em>should be specific</em> of sterile distilled water or PBS and incubated at [room temperature] <em>should provide a range</em> 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 <em>X. fragariae</em> cell suspensions (10⁴, 10⁵ and 10⁶ 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 <em>X. fragariae</em>. Final readings should be performed after incubation at 25–27 °C for 7 days.</td>
<td>For clarity and Scientific justification</td>
<td>Kenya</td>
</tr>
<tr>
<td>66.</td>
<td>41</td>
<td>Technical</td>
<td><em>X. fragariae</em> 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 <em>Xanthomonas fragariae</em>-like colonies on Wilbrink-N medium.</td>
<td>A description of colonies on SPA is missing</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>67.</td>
<td>43</td>
<td>Technical</td>
<td>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 <em>X. fragariae</em> 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 regarding glycerol it should be mentioned at least 20% glycerol</td>
<td>For clarity and Scientific justification</td>
<td>EPPO, European Union</td>
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<td>Comment no.</td>
<td>Para. no.</td>
<td>Comment type</td>
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<td>Explanation</td>
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<tr>
<td>68.</td>
<td>43</td>
<td>Technical</td>
<td>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 (1-4 mm²) and place them 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).</td>
<td>For more clarity of the isolation procedure. This modification is consistent with (EPPO, 2006, pp.143)</td>
<td>Japan</td>
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<tr>
<td>69.</td>
<td>44</td>
<td>Technical</td>
<td>An alternative to isolation of X. fragariae from tissue may also be performed if 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.</td>
<td>This isolation is not always successful and should not be proposed as an alternative methods but rather as an additional one.</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>70.</td>
<td>46</td>
<td>Editorial</td>
<td>The isolation is negative if no bacterial colonies with morphology similar to X. fragariae colonies are observed after 7 days on any of the two media (provided no growth inhibition due to competition or antagonism has occurred) and typical X. fragariae colonies are found in the positive controls.</td>
<td>English correction</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>71.</td>
<td>46</td>
<td>Technical</td>
<td>The isolation is negative if no bacterial colonies with morphology similar to X. fragariae colonies are observed</td>
<td>Three media not two</td>
<td>EPPO, European Union</td>
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<tr>
<td>Comm. no.</td>
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<tr>
<td>72.</td>
<td>49</td>
<td>Substantive</td>
<td>After 7 days in either of the three media (provided no growth inhibition due to competition or antagonism has occurred) and typical X. fragariae colonies are found in the positive controls.</td>
<td>For consistency</td>
<td>Kenya</td>
</tr>
<tr>
<td>73.</td>
<td>52</td>
<td>Editorial</td>
<td>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 test assay.</td>
<td>Consistency of terminology</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>74.</td>
<td>53</td>
<td>Editorial</td>
<td>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.</td>
<td>Simplification</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>75.</td>
<td>53</td>
<td>Technical</td>
<td>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.</td>
<td>Scientific clarification</td>
<td>Kenya</td>
</tr>
<tr>
<td>76.</td>
<td>55</td>
<td>Editorial</td>
<td>Rinse off excess inoculum with sterile water 1 h after inoculation. Place leaves with their petioles in the 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</td>
<td>Simplification</td>
<td>EPPO, European Union</td>
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<td>Comment no.</td>
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<td>Explanation</td>
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<tr>
<td>77.</td>
<td>55</td>
<td>Technical</td>
<td>Rinse off excess inoculum with sterile water 1 h after inoculation. Place leaves with their petioles in their 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.</td>
<td>It is should be specified that HR is usually 100%</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>78.</td>
<td>57</td>
<td>Technical</td>
<td>3.6.2 Interpretation of detached leaf assay results</td>
<td>A reference to the fact that the controls should give expected results should be added</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>79.</td>
<td>58</td>
<td>Substantive</td>
<td>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.</td>
<td>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.</td>
<td>New Zealand</td>
</tr>
<tr>
<td>80.</td>
<td>61</td>
<td>Editorial</td>
<td>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 °C and record results for X. fragariae-like colonies after 5–7 days.</td>
<td>in planta enrichment is the detached leaf assay mentioned just in the line above.</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>81.</td>
<td>65</td>
<td>Editorial</td>
<td>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).</td>
<td>Consistency of terminology</td>
<td>EPPO, European Union</td>
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<td></td>
<td>82</td>
<td>Technical</td>
<td>Use cell suspensions prepared from pure cultures of <em>X. fragariae</em> and a non-<em>X. fragariae</em> 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.</td>
<td>further information needed for clarification.</td>
<td>Kenya</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>Editorial</td>
<td>Mix 210 µl of each test sample, the positive <em>X. fragariae</em> cell suspension (approximately 10⁹ cfu/ml) and the negative non-<em>X. fragariae</em> cell suspension (approximately 10⁹ cfu/ml) and the negative control (suspension of healthy strawberry material, see below) with 210 µl coating buffer (Na₂CO₃, NaHCO₃, 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.</td>
<td>better wording</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>Technical</td>
<td>Mix 210 µl of each test sample, the positive <em>X. fragariae</em> cell suspension (approximately 10⁹ cfu/ml) and the negative non-<em>X. fragariae</em> cell suspension (approximately 10⁹ cfu/ml) and the negative control (healthy strawberry material) with 210 µl coating buffer (1.589g Na₂CO₃, 2.93g NaHCO₃, 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.</td>
<td>For more clarity of the ingredients in the buffer. This modification is consistent with (EPPO, 2006, pp.144)</td>
<td>Japan</td>
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<tr>
<td></td>
<td>85</td>
<td>Editorial</td>
<td>Prepare the appropriate working dilution separate according to the manufacturer's instructions, of the anti-<em>X. fragariae</em> 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</td>
<td>gramatical correction</td>
<td>Kenya</td>
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<td>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.</td>
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<tr>
<td>86.</td>
<td>72</td>
<td>Editorial</td>
<td>For double antibody sandwich (DAS)-ELISA, add 200 µl of an appropriate dilution of anti-X. fragariae serum in the coating buffer to each well of two microtiter plates (PolySorp (Nunc)² 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 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.</td>
<td>Better English</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>87.</td>
<td>77</td>
<td>Substantive</td>
<td>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.</td>
<td>for consistency</td>
<td>Kenya</td>
</tr>
<tr>
<td>88.</td>
<td>79</td>
<td>Editorial</td>
<td>Immunofluorescence procedures for identifying phytopathogenic bacteria are given in De Boer (1990). 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 test allows the detection of approximately (10^2)–(10^4) cfu/ml X. fragariae in strawberry tissue (Calzolari and Mazzucchi, 1989).</td>
<td>Consistency of terminology Simplification of English</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>89.</td>
<td>79</td>
<td>Substantive</td>
<td>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</td>
<td>more information needed</td>
<td>Kenya</td>
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<td>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).</td>
<td>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</td>
<td>EPPO, European Union</td>
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<tr>
<td></td>
<td>79</td>
<td>Technical</td>
<td>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</td>
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<tr>
<td>91</td>
<td>81</td>
<td>Editorial</td>
<td>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 °C 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 advisable that two dilutions of the antiserum are 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 °C 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 p-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).</td>
<td>gramatical corrections</td>
<td>Kenya</td>
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<td>92.</td>
<td>88</td>
<td>Editorial</td>
<td>1. Recommended for detection using immunofluorescence (validated in ring tests in a European Union-funded project (SMT-4-CT98-2252)) (López et al., 2005).</td>
<td>consider replacing ring test by test performance study</td>
<td>EPPO, European Union</td>
</tr>
<tr>
<td>93.</td>
<td>89</td>
<td>Editorial</td>
<td>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).</td>
<td>consider replacing ring test by test performance study consistency of terminology</td>
<td>EPPO</td>
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<tr>
<td>94.</td>
<td>90</td>
<td>Substantive</td>
<td>3.9 PCR</td>
<td>It is very important to develop a rapid, sensitive real-time PCR for detecting X. fragariae</td>
<td>China</td>
</tr>
<tr>
<td>95.</td>
<td>91</td>
<td>Editorial</td>
<td>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). 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).</td>
<td>Consider replacing ring test by test performance study</td>
<td>EPPO, European Union</td>
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<td>96.</td>
<td>91</td>
<td>Substantive</td>
<td>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). 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).</td>
<td>provide more information</td>
<td>Kenya</td>
</tr>
<tr>
<td>97.</td>
<td>92</td>
<td>Editorial</td>
<td>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 REXtract-N-Amp Plant PCR Kit (Sigma) 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).</td>
<td>Simplification</td>
<td>EPPO, European Union</td>
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<td>Comment no.</td>
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<tr>
<td>98.</td>
<td>92</td>
<td>Technical</td>
<td>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) 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).</td>
<td>clarify why clinical and we are dealing with plants</td>
<td>Kenya</td>
</tr>
<tr>
<td>99.</td>
<td>93</td>
<td>Editorial</td>
<td>Two sensitive real-time PCR assays have been described for detection of <em>X. fragariae</em> (Weller et al., 2007; Vandromme et al., 2008) as well as differentiation of <em>X. fragariae</em> and <em>X. arboricola pv. fragariae</em> (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 <em>gyraseB</em> gene unique to <em>X. fragariae</em> and the <em>pep</em> gene unique to <em>X. arboricola pv. fragariae</em>. The real-time PCR assay developed by Vandromme 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 tests are potentially useful for detecting low levels of <em>X. fragariae</em> in asymptomatic or latent infections.</td>
<td>Consistency of terminology CONsider replacing ring-test by test performance study</td>
<td>EPPO, European Union</td>
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<tr>
<td>100.</td>
<td>93</td>
<td>Editorial</td>
<td>Two sensitive real-time PCR assays have been described for detection of <em>X. fragariae</em> (Weller et al., 2007; Vandromme et al., 2008) as well as differentiation of <em>X. fragariae</em> and <em>X. arboricola pv. fragariae</em> (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 <em>gyraseB</em> gene unique to <em>X. fragariae</em> and the <em>pep</em> gene unique to <em>X. arboricola pv. fragariae</em>. The real-time PCR assay developed by Vandromme et al. (2008) is based on primers yielding a 41 base pair (bp) amplicon, designed from the 550 bp</td>
<td>gramatical correction</td>
<td>Kenya</td>
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<tr>
<td>101.</td>
<td>93</td>
<td>Substantive</td>
<td>Two sensitive real-time PCR assays have been described for detection of <em>X. fragariae</em> (Weller et al., 2007; Vandroemme et al., 2008) as well as differentiation of <em>X. fragariae</em> and <em>X. arboricola pv. fragariae</em> (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 <em>X. fragariae</em> and the pep gene unique to <em>X. arboricola pv. fragariae</em>. 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 <em>X. fragariae</em> in asymptomatic or latent infections.</td>
<td>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 <a href="http://dc.eppo.int/validationlist.php">http://dc.eppo.int/validationlist.php</a> Search for Xanthomonas fragariae This test should be added to the protocol EPPO, European Union</td>
<td></td>
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<tr>
<td>102.</td>
<td>98</td>
<td>Technical</td>
<td>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. 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). The 300 bp band by the primers 245A / 245B is usually present when the extracts are from plants infected with <em>X. fragariae</em> but the other bands (550 and 615 bp) may appear occasionally (Pooler et al., 1996; Hartung and Pooler, 1997).</td>
<td>China</td>
<td></td>
</tr>
<tr>
<td>103.</td>
<td>108</td>
<td>Editorial</td>
<td>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)</td>
<td>Japan</td>
<td></td>
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<tr>
<td>104.</td>
<td>111</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em> in symptomatic strawberry plants as well as for testing asymptomatic strawberry plants (frigo and green plants) (Moltmann and Zimmerman, 2005). The 1 simplification 2 consistency of terminology</td>
<td>EPPO, European Union</td>
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<td>105</td>
<td>113</td>
<td>Editorial</td>
<td>Specificity for this protocol was confirmed in a study with 14 isolates of <em>X. fragariae</em>, 30 isolates of <em>X. campestris</em> (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 <em>et al.</em> (1996) (see previous section 3.9.2). No cross reaction was observed with the isolated tested. Only <em>X. fragariae</em> was detected (in all isolates). This 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 <em>et al.</em>, 2004).</td>
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<td>Explanation</td>
<td>1 clarification 2 consistency of terminology 3 simplification</td>
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<td>Country</td>
<td>EPPO, European Union</td>
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<td>106</td>
<td>114</td>
<td>Technical</td>
<td>Incubate leaf, petiole and crown tissue (30–70 g) in 10–20 ml 0.010.1 M sodium phosphate buffer (pH 7.2) per gram of tissue at room temperature overnight. Extract DNA and analyse by single and nested PCR as described by Zimmerman <em>et al.</em> (2004). The primers are:</td>
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<td>This modification is consistent with (Moltmann and Zimmermann, 2005, pp.53)</td>
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<td>Country</td>
<td>Japan</td>
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<tr>
<td>107</td>
<td>119</td>
<td>Editorial</td>
<td>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 MgCl2), 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.</td>
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<td>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.</td>
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<td>New Zealand</td>
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<tr>
<td>108</td>
<td>119</td>
<td>Technical</td>
<td>PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet</td>
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<td>To clarify</td>
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<td>Country</td>
<td>COSAVE, Argentina, Peru,</td>
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<td>Comment no.</td>
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<tr>
<td>109.</td>
<td>122</td>
<td>Editorial</td>
<td>Specificity for this protocol was confirmed in a study with 30 isolates of <em>X. fragariae</em>, 17 isolates of <em>X. campestris</em> (representing 16 pathovars) and 9 isolates of non-pathogenic xanthomonads isolated from strawberry. No cross reaction was observed with the isolates tested. Only <em>X. fragariae</em> was detected (in all isolates). This nested PCR technique enabled detection to approximately 18 <em>X. fragariae</em> cells in plant tissue (Roberts et al., 1996).</td>
</tr>
<tr>
<td>110.</td>
<td>124</td>
<td>Technical</td>
<td>- XF9: 5′-TGGGCCATGCCGGTGGAACTGTGTGG-3′</td>
</tr>
<tr>
<td>111.</td>
<td>127</td>
<td>Editorial</td>
<td>PCR is carried out in 25 μl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂), 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.</td>
</tr>
<tr>
<td>112.</td>
<td>127</td>
<td>Editorial</td>
<td>PCR is carried out in 25 μl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂),</td>
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<td>Comm. no.</td>
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<tr>
<td>113.</td>
<td>127</td>
<td>Technical</td>
<td>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.</td>
</tr>
<tr>
<td>114.</td>
<td>127</td>
<td>Technical</td>
<td>PCR is carried out in 25 µl reaction mixtures containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂), 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 except that the annealing temperature is 58 °C. PCR products are analysed by 1.5% agarose gel electrophoresis in 0.5× TAE buffer.</td>
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<tr>
<td>115.</td>
<td>128</td>
<td>Substantive</td>
<td>Specific PCR amplicons for <em>X. fragariae</em> 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.</td>
</tr>
<tr>
<td>116.</td>
<td>136</td>
<td>Technical</td>
<td><strong>Positive nucleic acid control.</strong> 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 <em>X. fragariae</em> cells (10⁴ - 10⁶ cfu/ml) is recommended as a positive nucleic acid control.</td>
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<tr>
<td>117.</td>
<td>139</td>
<td>Technical</td>
<td><strong>Positive extraction control.</strong> 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, <em>X. fragariae</em> tissue macerates spiked with 10⁴ and 10⁶ cfu/ml of a reference <em>X. fragariae</em> 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.</td>
</tr>
<tr>
<td>118.</td>
<td>145</td>
<td>Editorial</td>
<td><em>X. fragariae</em> has the common characteristics of all xanthomonads. <strong>Cells.</strong> They are Gram-negative, aerobic rods, with a single polar flagellum. <strong>They do not reduce nitrates.</strong> Nitrates are not reduced, they are catalase test positive,</td>
</tr>
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</table>
\textbf{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 weak producers 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). \textit{Xanthomonas} species are easily differentiated from the other genera of aerobic, Gram-negative rod-shaped and other yellow-pigmented bacteria by the characteristics shown in Table 3 and described by Schaad et al. (2001).

119. \quad 149 \quad \textbf{Technical} \quad \textbf{Table 3. Phenotypic characteristics for differentiating \textit{Xanthomonas} from \textit{Pseudomonas} and other yellow-pigmented bacteria (\textit{Flavobacterium} and \textit{Pantoea})} \quad \text{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.} \quad \text{EPPO, European Union}

120. \quad 151 \quad \textbf{Editorial} \quad \textbf{The most relevant or useful characteristics for distinguishing \textit{X. fragariae} from other \textit{Xanthomonas} \textit{(EPPO, 2006Schaad et al., 2001; Janse et al., 2001)} are shown in Table 4.} \quad \text{Table 4. is described in (EPPO, 2006).} \quad \text{Japan}

121. \quad 152 \quad \textbf{Editorial} \quad \textbf{Table 4. Diagnostic tests to distinguish \textit{Xanthomonas fragariae} from the “\textit{X. campestris} group” and \textit{X. arboricola pv. fragariae} \textit{(EPPO, 2006Janse et al., 2001)}} \quad \text{Table 4. is described in (EPPO, 2006).} \quad \text{Japan}

122. \quad 157 \quad \textbf{Substantive} \quad \textbf{Table 5. Reactions of \textit{Xanthomonas fragariae} in API 20 NE tests} \quad \text{Need to add reaction for Phenyl-acetate - at bottom of table} \quad \text{New Zealand}

123. \quad 158 \quad \textbf{Technical} \quad \textbf{Test} \quad \textbf{Reaction (48 or 96 h)}

\text{In order to compare with other species of \textit{X. fragariae}.} \quad \text{China}
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<tr>
<th>Comment type</th>
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<th>Explanation</th>
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<tbody>
<tr>
<td>Glucose fermentation</td>
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<tr>
<td>Arginine</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Urease</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Esculin</td>
<td>+</td>
<td>-</td>
<td></td>
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<tr>
<td>Gelatin</td>
<td>-</td>
<td>+ (weakly)</td>
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<tr>
<td>Para-NitroPhenyl-ß-D-Galactopyranosidase (PNPG)</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Assimilation of:</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>Glucose</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Mannose</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Mannitol</td>
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<tr>
<td>N-acetyl-glucosamine</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Gluconate</td>
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<td>Caprate</td>
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<td>+</td>
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<td>Adipate</td>
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<td>Malate</td>
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<td>Citrate</td>
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<td></td>
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<td>Phenyl-acetate</td>
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</table>

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, prepare bacterial cell suspensions of OD = 1.0 in PBS. Add 1 ml suspension to 20 ml modified medium C (0.5 g NH₄H₂PO₄, 0.5 g K₂HPO₄, 0.2 g MgSO₄, 5 g NaCl, 1 g yeast extract, 70 ml Bromothymol 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 yellow colour in the wells after the incubation period.

No capital needed

EPPO, European Union
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<tr>
<th>Comm. no.</th>
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<tr>
<td>125.</td>
<td>162</td>
<td>Technical</td>
<td>Test 1</td>
<td>Variable</td>
<td>China</td>
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<td></td>
<td></td>
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<td>d-arabinose</td>
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<td>Galactose</td>
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<td></td>
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<td>d-glucose</td>
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<td></td>
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<td></td>
<td>d-fructose</td>
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<td></td>
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<td>d-mannose</td>
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<td></td>
<td></td>
<td></td>
<td>N-acetyl-glucosamine</td>
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<td></td>
<td></td>
<td></td>
<td>Esculin</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td>Sucrose</td>
<td>+</td>
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<td></td>
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<td></td>
<td>Trehalose</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td>d-lyxosa</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td>L-fucose</td>
<td>+</td>
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</table>

It should be added the API 20 and API 50 results of other species of *X. fragariae*.

<p>| 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 genus of Gram-negative and Gram-positive bacteria are consis... | Consistency of terminology | EPPO    |</p>
<table>
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<td>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. <em>X. fragariae</em> 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 fatty acid profiles that do not correspond well. Studies have shown that strains of <em>X. fragariae</em> 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 <em>X. fragariae</em>. 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).</td>
<td>It is very important to develop a rapid, sensitive real-time PCR for detecting <em>X. fragariae</em></td>
<td>China</td>
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<tr>
<td>127.</td>
<td>173</td>
<td>Substantive</td>
<td><strong>4.3 Molecular tests</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
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<td><strong>Real-time PCR assay wasn’t included.</strong></td>
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<tr>
<td>128.</td>
<td>179</td>
<td>Technical</td>
<td>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)&lt;sup&gt;12&lt;/sup&gt;, 10 g Phytage (BBL Microbiology Systems)&lt;sup&gt;12&lt;/sup&gt;; adjust pH to 7.5 with 2 N HCl before autoclaving; distilled water to 1 litre) (Opgenorth et al., 1996).</td>
<td>REP PCR can be standardized in the lab using different growth medium This should be presented as an option</td>
<td>EPPO, European Union</td>
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<tr>
<td>129.</td>
<td>185</td>
<td>Technical</td>
<td>The reaction buffer contains 16.6 mM (NH₄)₂SO₄, 67 mM Tris-Cl (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. 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</td>
<td>Proposed deletion for consistency with other parts of the protocol where only agarose gels are mentioned.</td>
<td>EPPO, European Union</td>
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<td>130</td>
<td>187</td>
<td>Substantive</td>
<td>(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.</td>
<td>Useful additionnal information</td>
<td>EPPO</td>
</tr>
<tr>
<td>131</td>
<td>187</td>
<td>Substantive</td>
<td>Test bacterial strains are identified as <em>X. fragariae</em> if the same genomic fingerprints are obtained as those of the REP and ERIC genotypes of the reference strains (Pooler <em>et al.</em>, 1996) amplified in the same test and run in the same gel. A small number of polymorphic bands may be obtained from different <em>X. fragariae</em> isolates due to low levels of genomic variability.</td>
<td>Useful additional information</td>
<td>European Union</td>
</tr>
</tbody>
</table>
| 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 |
<p>| 133       | 189       | Editorial   | A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (Almeida <em>et al.</em>, 2010; Hamza <em>et al.</em>, 2012; Parkinson <em>et al.</em>, 2007) and could be used for identification of <em>X. fragariae</em> especially now that a draft genome sequence is now available (Vandroemee <em>et al.</em>, 2013). However, it should be noted this methodology has not yet been validated for identification of <em>X. fragariae</em>. Housekeeping genes are amplified using primers and PCR conditions as described by Almeida <em>et al.</em> (2010) and Hamza <em>et al.</em>, (2012). MLSA consists of sequencing. | Hamza <em>et al.</em>, 2012 not included in references section. | Singapore |</p>
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<td>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) (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl</a>) (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al., 2010</a>) and the MLVAbank for microbe genotyping (<a href="https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/">https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/</a>).</td>
<td>editorial</td>
<td>EPPO</td>
</tr>
<tr>
<td>134. 189</td>
<td>Editorial</td>
<td>A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al., 2010</a>; <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Hamza et al., 2012</a>; <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Parkinson et al., 2007</a>) and could be used for identification of X. fragariae especially now that a draft genome sequence is now available (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Vandroemee et al., 2013</a>). 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 <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al. (2010)</a> and <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Hamza et al. (2012)</a>. 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) (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl</a>) (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al., 2010</a>) and the MLVAbank for microbe genotyping (<a href="https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/">https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/</a>).</td>
<td>1 unnecessary word 2 word missing</td>
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<tr>
<td>135. 189</td>
<td>Editorial</td>
<td>A multilocus sequence analysis (MLSA) approach has been widely used for the specific identification of xanthomonads (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al., 2010</a>; <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Hamza et al., 2012</a>; <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Parkinson et al., 2007</a>) and could be used for identification of X. fragariae especially now that a draft genome sequence is now available (<a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Vandroemee et al., 2013</a>). 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 <a href="http://genome.pwws.vt.edu/cgi-bin/MLST/home.pl">Almeida et al. (2010)</a> and</td>
<td>1 unnecessary word 2 word missing</td>
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<td>136.</td>
<td>189</td>
<td>Editorial</td>
<td>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 <em>X. fragariae</em> 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 <em>X. fragariae</em>. 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) (<a href="http://genome.ppw.vt.edu/cgi-bin/MLST/home.pl">http://genome.ppw.vt.edu/cgi-bin/MLST/home.pl</a>) (Almeida et al., 2010) and the MLVAbank for microbe genotyping (<a href="https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/">https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/</a>).</td>
<td>This quotation is not included in the reference section. For this reason it should be deleted from the text or otherwise included in the reference section.</td>
<td>COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay</td>
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<td>137.</td>
<td>194</td>
<td>Technical</td>
<td>Prepare bacterial cell suspensions approximately ((10^8 - 10^9)) 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</td>
<td>The concentrations given is high for a pathogenicity test, It should be approximately 106 cfu/ml</td>
<td>EPPO</td>
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<td>Comm. no.</td>
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<td>138. 194</td>
<td>Technical</td>
<td>Prepare bacterial cell suspensions approximately (10^8 - 10^9) 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 (&gt;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.</td>
<td>The concentration given is high for a pathogenicity test, it should be approximately 10^6 cfu/ml</td>
<td>European Union</td>
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<td>139. 196</td>
<td>Technical</td>
<td>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 plants inoculated with a reference X. fragariae strain (positive control).</td>
<td>The pathogenicity test is based on pure cultures</td>
<td>EPPO, European Union</td>
<td></td>
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<td>140. 201</td>
<td>Technical</td>
<td>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.</td>
<td>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</td>
<td>COSAVE, Argentina, Brazil, Uruguay, Chile, Paraguay</td>
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<td>143.</td>
<td>235</td>
<td>Editorial</td>
<td>Kennedy, B.W. &amp; King, T.H. 1962a. Angular leaf spot of strawberry caused by <em>Xanthomonas fragariae</em> sp. nov. <em>Phytopathology</em>, 52: 873–875.</td>
<td>It is proposed to delete the reference &quot;Kennedy and King, 1962b&quot; from the references section, as it was not cited in the draft annex. Hence, &quot;1962a&quot; should be changed to &quot;1962&quot; instead.</td>
<td>Singapore</td>
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<td>144.</td>
<td>236</td>
<td>Editorial</td>
<td>Kennedy, B.W. &amp; King, T.H. 1962b. <em>Studies on epidemiology of bacterial angular leafspot of strawberry</em>. <em>Plant Disease Reporter</em>, 46: 360–363.</td>
<td>It is proposed to delete this reference as it was not cited in the draft annex.</td>
<td>Singapore</td>
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<td>146.</td>
<td>245</td>
<td>Editorial</td>
<td>Moltmann, E. &amp; Zimmermann, C. 2005. Detection of <em>Xanthomonas fragariae</em> in symptomless strawberry plants</td>
<td>Addition of this reference that was added in paragraph: 3.2</td>
<td>South Africa</td>
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<td>Comm. no.</td>
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<td>148.</td>
<td>270</td>
<td>Technical</td>
<td><strong>Footnote 1:</strong> In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. 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.</td>
<td>1. Text deleted because it is already included in the text of DP (paragraph 22). 2. Text added according to the previously agreed footnote.</td>
<td>COSAVE, Argentina, Peru, Brazil, Uruguay, Chile, Paraguay</td>
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