2013-001: DRAFT ANNEX TO ISPM 27 - *Candidatus* Liberibacter Solanacearum

Para	Text	Comment	SC responses to comments
G	(General Comment)	*Category : TECHNICAL* **(185) Venezuela (1 Oct 2016 2:54 AM)** El grupo de Venezuela no tiene comentarios por ahora	N/A
G	(General Comment)	*Category : EDITORIAL* **(184) Zambia (30 Sep 2016 11:19 PM)** Paragraph 426-427, 428-429, 440-441 and 442-444- rearranged to follow sequence in year of publication	**Considered, but not incorporated:** Did not change as the IPPC style lists references according by author rather than by year.
G	(General Comment)	*Category : SUBSTANTIVE* **(183) Canada (30 Sep 2016 9:24 PM)** Canada supports the Draft Annex to ISPM 27 – Candidatus Liberibacter solanacearum (2013-001)	N/A
G	(General Comment)	*Category : SUBSTANTIVE* **(182) Guyana (30 Sep 2016 6:35 PM)** We support the contents of the document.	N/A
G	(General Comment)	*Category : TECHNICAL* **(181) Mali (30 Sep 2016 11:29 AM)** o Les méthodes décrites dans ce protocole sont sophistiquées pour les niveaux d'équipement de nos laboratoires. Il serait intéressant que des études évoluent vers le développement de kits d'analyse rapide plus faciles à utiliser par les services d'inspection aux frontières. o Les niveaux de sensibilité de la PCR-temps réel doit être fournie pour permettre une comparaison entre cette technique et la PCR conventionnelle (paragraphes 60 et 77	Noted. This comment is beyond the TPDP capacity as it relates to potential implementation issues. It will be forwarded to the SC and apropriate bodies.
G	(General Comment)	*Category : TECHNICAL* **(172) Bolivia (29 Sep 2016 9:15 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification ares suggested as commented in others consultation periods according text previously agreed by the SC.	**Incorporated:** The standard footnote regarding brands as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(171) Bolivia (29 Sep 2016 9:12 PM)** Its is recommended to add a flow diagram	**Considered, but not incorporated:** A flow diagram will not add value to this

		for the identification of Candidatus Liberibacter solanacearum	diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : TECHNICAL* **(166) Peru (29 Sep 2016 8:20 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	**Incorporated:** The standard footnote regarding brands as in other diagnostic protocols, following the appropriate style and decisions.
G	(General Comment)	*Category : TECHNICAL* **(165) Peru (29 Sep 2016 8:20 PM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : TECHNICAL* **(164) Nigeria (29 Sep 2016 5:05 PM)** The diagnostic methods described are sophisticated and beyond the capacity of many national laboratories and inspectors. scientists should come up with simpler and quicker diagnostic kits ready to be deployed in at the national borders.	**Considered, but not incorporated:** The diagnostic protocol currently lists the methods available. The protocol will be reviewed when new diagnostic assays are developed.
G	(General Comment)	*Category : TECHNICAL* **(163) EPPO (29 Sep 2016 4:45 PM)** the terminology used should be checked. Eg. use PCR and not PCR assay.	**Modified:** in paragraph 119 of the draft DP, the wording was changed from "PCR assays" to "PCR tests". In other instances 'PCR' is used.
G	(General Comment)	*Category : TECHNICAL* **(116) Brazil (29 Sep 2016 4:27 PM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum. We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points. **Incorporated**: A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : SUBSTANTIVE* **(115) Barbados (29 Sep 2016 4:27 PM)** The draft annex has adequately covered the major areas with respect to the diagnosis of this pathogen.	Noted.
G	(General Comment)	*Category : TECHNICAL* **(57) Argentina (29 Sep 2016 4:17 AM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested	**Incorporated:** A standard footnote regarding brands will be added as in other diagnostic protocols.

		as commented in others consultation periods according text previously agreed by the SC.	
G	(General Comment)	*Category : SUBSTANTIVE* **(56) Argentina (29 Sep 2016 4:16 AM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : TECHNICAL* **(53) Chile (28 Sep 2016 6:13 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	**Incorporated:** A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(52) Chile (28 Sep 2016 6:12 PM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum.	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : TECHNICAL* **(51) Iraq (28 Sep 2016 11:03 AM)** No comments	N/A
G	(General Comment)	*Category : TECHNICAL* **(50) Burundi (28 Sep 2016 9:09 AM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. Studies should be continued to come out with quick diagnostic kits ready to be used by inspection services at the border points. Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods (Paragraphs 60 and 77).	**Considered, but not incorporated:** The diagnostic protocol currently lists the methods available. The protocol will be reviewed when new diagnostic assays are developed. The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.
G	(General Comment)	*Category : SUBSTANTIVE* **(49) New Zealand (27 Sep 2016 3:48 AM)** General Comment New Zealand assisted with the preparation of this protocol - so will not be commenting on it.	N/A
G	(General Comment)	*Category : TECHNICAL* **(46) Viet Nam (26 Sep 2016 12:04 PM)** Section 3.2 should include more specific rules on sampling and analysis criteria (form	**Considered, but not incorporated:** Paragraph 54 contains sufficient information for a diagnostician to perform

		of leaves, veins, root diseases ... etc need to collect in order to carry out PCR). Section 3.3.3 should be detailed and specific about the sequence of steps in the process of Real - Time PCR	the sampling. Section 3.3.3 is sufficiently detailed for a person experienced in real-time PCR to perform the test and is consistent with the information in other IPPC diagnostic protocols.
G	(General Comment)	*Category : EDITORIAL* **(45) PPPO (26 Sep 2016 12:23 AM)** PPPO has no comment on the draft ISPM	N/A
G	(General Comment)	*Category : SUBSTANTIVE* **(28) Thailand (21 Sep 2016 6:03 AM)** agree with this diagnostic protocol.	N/A
G	(General Comment)	*Category : TECHNICAL* **(25) Uruguay (15 Sep 2016 9:10 PM)** We request the TPDP to revise the use of footnotes associated to brand names for consistency and modifications are suggested as commented in other consultation periods according to the footnote text previously agreed by the SC.	**Incorporated:** A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(24) Uruguay (15 Sep 2016 9:09 PM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : SUBSTANTIVE* **(23) Zambia (8 Sep 2016 6:26 AM)** In agreement with the Draft Annex	N/A
G	(General Comment)	*Category : SUBSTANTIVE* **(22) Tajikistan (29 Aug 2016 1:48 PM)** I support the document as it is and I have no comments	N/A
G	(General Comment)	*Category : SUBSTANTIVE* **(19) Tajikistan (22 Aug 2016 12:15 PM)** I support the document as it is and I have no comments	N/A
G	(General Comment)	*Category : TECHNICAL* **(18) COSAVE (11 Aug 2016 11:18 PM)** It is recommended to add a flow diagram for the identification of Candidatus Liberibacter solanacearum	**Considered, but not incorporated:** A flow diagram will not add value to this diagnostic protocol as there is only one method and there are no decision points.
G	(General Comment)	*Category : TECHNICAL* **(15) COSAVE (11 Aug 2016 11:14 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	**Incorporated:** A standard footnote regarding brands will be added as in other diagnostic protocols.

G	(General Comment)	*Category : TECHNICAL* **(1) Sri Lanka (22 Jul 2016 10:54 AM)** The entire content is acceptable	N/A
G	(General Comment)	Category : TECHNICAL **Burkina Faso** The sensitivity levels of real-time PCR should be provided to allow a comparison between this technique and the conventional PCR (paragraphs 60 and 77).	**Considered, but not incorporated:** The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.
33	This diagnostic protocol was adopted by the Commission on Phytosanitary Measures in 20-.	*Category : TECHNICAL* **(176) Ghana (30 Sep 2016 12:31 AM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. We propose that more studies should be done to come out with better quick diagnostic kits to be used by inspection services at the each countries entry points.	**Considered, but not incorporated:** The diagnostic protocol currently lists the methods available. The protocol will be reviewed when new diagnostic assays are developed.
36	*Candidatus* ~~Liberibacter solanacearum -~~ *Candidatus Liberibacter solanacearum* is a phloem-limited, Gram-negative, unculturable bacterium that is associated with several emerging diseases. '*Ca*. L. solanacearum' was first identified in 2008 from the psyllid *Bactericera cockerelli* by Hansen *et al*. (2008) and from solanaceous plants by Liefting *et al*. (2008, 2009a, 2009b), and later from carrot and the carrot psyllid *Trioza apicalis* by Munyaneza *et al*. (2010). The bacterium has a rod-shaped morphology and is about 0.2 µm wide and 4 µm long (Liefting *et al*., 2009a; Secor *et al*., 2009).	*Category : TECHNICAL* **(177) Ghana (30 Sep 2016 12:34 AM)** Candidatus Liberibacter solanacearum (this should be italiced	**Considered, but not incorporated:** The accepted way to cite the name is '*Candidatus* Liberibacter solanacearum'.
36	~~*Candidatus*~~ *'Candidatus* Liberibacter ~~solanacearum~~ solanacearum' is a phloem-limited, Gram-negative, unculturable bacterium that is associated with several emerging diseases. '*Ca*. L. solanacearum' was first identified in 2008 from the psyllid *Bactericera cockerelli* by Hansen *et al*. (2008) and from solanaceous plants by Liefting *et al*. (2008, 2009a, 2009b), and later from carrot and the carrot psyllid *Trioza apicalis* by Munyaneza *et al*. (2010). The bacterium has a rod-shaped morphology and is about 0.2 µm wide and 4 µm long (Liefting *et al*., 2009a; Secor *et al*., 2009).	*Category : EDITORIAL* **(118) EPPO (29 Sep 2016 4:45 PM)**	**Incorporated:** The quotation marks have been included.
36	~~*Candidatus*~~ *'Candidatus* Liberibacter ~~solanacearum~~ solanacearum' is a phloem-limited, Gram-negative, unculturable bacterium that is associated with several emerging diseases. '*Ca*. L. solanacearum' was first identified in 2008 from the psyllid *Bactericera cockerelli* by Hansen *et al*. (2008) and from solanaceous plants by Liefting *et al*. (2008, 2009a, 2009b), and later from carrot and the carrot psyllid *Trioza apicalis* by Munyaneza *et al*. (2010). The bacterium has a rod-shaped	*Category : EDITORIAL* **(69) European Union (29 Sep 2016 2:41 PM)** Correction.	**Incorporated:** The quotation marks have been included.

	morphology and is about 0.2 µm wide and 4 µm long (Liefting *et al*., 2009a; Secor *et al*., 2009).		
36	*Candidatus* Liberibacter solanacearum is a phloem-limited, Gram-negative, unculturable bacterium that is associated with several emerging diseases. '*Ca*. L. solanacearum' was first identified in 2008 from the psyllid *Bactericera cockerelli* by Hansen *et al*. (2008) and from solanaceous plants by Liefting *et al*. (2008, 2009a, 2009b), potatoes by Abad et al.(2009), tomatoes by French et al. (2010), and later from carrot and the carrot psyllid *Trioza apicalis* by Munyaneza *et al*. (2010). The bacterium has a rod-shaped morphology and is about 0.2 µm wide and 4 µm long (Liefting *et al*., 2009a; Secor *et al*., 2009).	*Category : TECHNICAL* **(29) United States of America (21 Sep 2016 8:01 PM)** To add more information on the pest on potatoes and tomatoes.	**Modified**: The references of Liefting et al. (2008, 2009a) identified the liberibacter in potatoes and tomatoes. In order to keep the number of references to a minimum this sentence has been modified to reflect this.
37	Other '*Ca*. Liberibacter' species include those associated with Huanglongbing disease of citrus (also known as citrus greening disease): '*Ca*. L. africanus', '*Ca*. L. americanus' and '*Ca*. L. asiaticus' (Nelson *et al*., 2013a). Several new ~~liberibacter~~ '*Ca*. Liberibacter' species have recently been discovered such as '*Ca*. L. europaeus' (Raddadi *et al*., 2011), '*Ca*. L. caribbeanus' (Keremane *et al*., 2015) and the first ~~culturable species~~cultured species from this bacterial clade , *Liberibacter crescens* (Fagen *et al*., 2014). It is unclear if these new ~~liberibacter species~~ '*Ca*. Liberibacter'species are associated with plant disease. The discovery of additional '*Ca*. Liberibacter' species will continue with the application of new technologies such as next-generation sequencing to study the metagenomes of insects and plants.	*Category : TECHNICAL* **(119) EPPO (29 Sep 2016 4:45 PM)** It does not seem to be possible to enter both and editorial comment and a technical comment on the same paragraph.	N/A
37	Other '*Ca*. Liberibacter' species include those associated with Huanglongbing disease of citrus (also known as citrus greening disease): '*Ca*. L. africanus', '*Ca*. L. americanus' and '*Ca*. L. asiaticus' (Nelson *et al*., 2013a). Several new ~~liberibacter~~ '*Ca*. Liberibacter' species have recently been discovered such as '*Ca*. L. europaeus' (Raddadi *et al*., 2011), '*Ca*. L. caribbeanus' (Keremane *et al*., 2015) and the first ~~culturable species~~cultured species from this bacterial clade, *Liberibacter crescens* (Fagen *et al*., 2014). It is unclear if these new ~~liberibacter~~ '*Ca*. Liberibacter' species are associated with plant disease. The discovery of additional '*Ca*. Liberibacter' species ~~will~~ is likely to continue with the application of new technologies such as next-generation sequencing to study the metagenomes of insects and plants.	*Category : TECHNICAL* **(70) European Union (29 Sep 2016 2:46 PM)** Is liberibacter an accepted generic term? It has been used several times in the protocol. Should it be 'proposed' instead of 'discovered'? The rest is editorial.	**Modified:** Liberibacter is a common name to refer to all liberibacters but it is more appropriate to use '*Ca*. Liberibacter' in this paragraph describing different liberibacter species and so the suggested changes have been made. It should be 'discovered'.
37	Other '*Ca*. Liberibacter' species include those associated with ~~Huanglongbing disease of~~ citrus huanglongbing (also known as citrus greening disease): '*Ca*. L. africanus', '*Ca*. L. americanus' and '*Ca*. L. asiaticus' (Nelson *et al*., 2013a). Several new liberibacter species have recently been discovered such as '*Ca*. L. europaeus' (Raddadi *et al*., 2011), '*Ca*. L. caribbeanus' (Keremane *et al*., 2015) and the first culturable species, *Liberibacter crescens* (Fagen *et al*., 2014). It is unclear if these new liberibacter species are associated with plant disease. The	*Category : TECHNICAL* **(47) Japan (26 Sep 2016 4:52 PM)** "Huanglongbing" implies disease.	**Incorporated.**

	discovery of additional '*Ca*. Liberibacter' species will continue with the application of next-generation sequencing to study the metagenomes of insects and plants.		
37	Other '*Ca*. Liberibacter' species include those associated with Huanglongbing disease of citrus (also known as citrus greening disease): '*Ca*. L. africanus', '*Ca*. L. americanus' and '*Ca*. L. asiaticus' (Nelson *et al*., 2013a). Several new ~~liberibacter~~ Liberibacter species have recently been discovered such as '*Ca*. L. europaeus' (Raddadi *et al*., 2011), '*Ca*. L. caribbeanus' (Keremane *et al*., 2015) and the first culturable species, *Liberibacter crescens* (Fagen *et al*., 2014). It is unclear if these new ~~liberibacter~~ Liberibacter species are associated with plant disease. The discovery of additional '*Ca*. Liberibacter' species will continue with the application of next-generation sequencing to study the ~~metagenomes~~ metagenomics of insects and plants.	*Category : EDITORIAL* **(30) United States of America (21 Sep 2016 8:02 PM)** Liberibacter should be capitalized - global check Metagenomics is more appropriate wording	**Modified**: liberibacter has been changed to '*Ca*. Liberibacter' based on other comments. No longer relevant as this part of the sentence has been deleted.
38	In North and Central America and Oceania, '*Ca*. L. solanacearum' primarily infects solanaceous crops and weeds including *Solanum tuberosum* (potato), *Solanum lycopersicum* (tomato), *Capsicum annuum* (pepper), *Solanum betaceum* (tamarillo), *Nicotiana tabacum* (tobacco), *Solanum melongena* (eggplant), *Physalis peruviana* (cape gooseberry), *Solanum elaeagnifolium* (silverleaf nightshade), *Solanum ptycanthum* (eastern black nightshade) and *Lycium barbarum* (wolfberry) (EPPO 2013; Haapalainen, 2014). In Europe and North Africa, '*Ca*. L. solanacearum' has been associated with symptoms in species of the family Apiaceae, including ~~*Daucus carota*~~ (carrot), *Apium graveolens* (celery) and *Pastinaca sativa* (parsnip) (EPPO 2013; Teresani *et al*., 2014).	*Category : TECHNICAL* **(111) Algeria (29 Sep 2016 4:16 PM)**	**Modified**: It is unclear as to why the genus name for carrot is deleted but the latin name for carrot has been corrected to *Daucus carota* subsp. *sativus.*
39	In solanaceous plants, '*Ca*. L. solanacearum' is primarily spread from infected to healthy plants by the tomato and potato psyllid *B. cockerelli* (Munyaneza *et al*., 2007; Munyaneza, 2012; EPPO, 2013). '*Ca*. L. solanacearum' has not yet been shown to be transmitted through true seed from infected solanaceous plants (Munyaneza, 2012). It has been demonstrated that the bacterium can be disseminated with the transport of infected carrot seeds, although vertical transmission through seed has been shown only once (Bertolini *et al*., 2014). Horizontal transmission between plants from the family Apiaceae has been reported to occur by the psyllids *T. apicalis* (Nissinen *et al*., 2014) and *Bactericera trigonica* (Teresani *et al*., 2014, 2015). '*Ca*. L. solanacearum' can also be transmitted by grafting and via dodder (Crosslin and Munyaneza, 2009; Secor *et al*., 2009; Munyaneza, 2012; Haapalainen, 2014; Munyaneza, 2015). The bacterium is found in several organs and tissues of its psyllid host, including the alimentary canal, salivary glands, hemolymph and bacteriomes (Cooper *et al*., 2013), and is transmitted in a propagative, circulative and persistent manner (Sengoda *et al*., 2014). Vertical (transovarial) transmission of	*Category : EDITORIAL* **(120) EPPO (29 Sep 2016 4:45 PM)**	**Incorporated.**

	'*Ca*. L. solanacearum' has been reported in *B. cockerelli* (Hansen *et al*., 2008).		
39	In solanaceous plants, '*Ca*. L. solanacearum' is primarily spread from infected to healthy plants by the tomato and potato psyllid *B. cockerelli* (Munyaneza *et al*., 2007; Munyaneza, 2012; EPPO, 2013). '*Ca*. L. solanacearum' has not yet been shown to be transmitted through true seed from infected solanaceous plants (Munyaneza, 2012). It has been demonstrated that the bacterium can be disseminated with the transport of infected carrot seeds, although vertical transmission through seed has been shown only once (Bertolini *et al*., 2014). Horizontal transmission between plants from the family Apiaceae has been reported to occur by the psyllids *T. apicalis* (Nissinen *et al*., 2014) and *Bactericera trigonica* (Teresani *et al*., 2014, 2015). '*Ca*. L. solanacearum' can also be transmitted by grafting and dodder (Crosslin and Munyaneza, 2009; Secor *et al*., 2009; Munyaneza, 2012; Haapalainen, 2014; Munyaneza, 2015). The bacterium is found in several organs and tissues of its psyllid host, including the alimentary canal, salivary glands, hemolymph and ~~bacteriomes~~ bacterium (Cooper *et al*., 2013), and is transmitted in a propagative, circulative and persistent manner (Sengoda *et al*., 2014). Vertical (transovarial) transmission of '*Ca*. L. solanacearum' has been reported in *B. cockerelli* (Hansen *et al*., 2008).	*Category : TECHNICAL* **(112) Algeria (29 Sep 2016 4:19 PM)**	**Considered, but not incorporated:** This word should be bacteriome which is a specialized organ, found mainly in some insects, that hosts endosymbiotic bacteria.
39	In solanaceous plants, '*Ca*. L. solanacearum' is primarily spread from infected to healthy plants by the tomato and potato psyllid *B. cockerelli* (Munyaneza *et al*., 2007; Munyaneza, 2012; EPPO, 2013). '*Ca*. L. solanacearum' has not yet been shown to be transmitted through true seed from infected solanaceous plants (Munyaneza, 2012). It has been demonstrated that the bacterium can be disseminated with the transport of infected carrot seeds, although vertical transmission through seed has been shown only once (Bertolini *et al*., 2014). Horizontal transmission between plants from the family Apiaceae has been reported to occur by the psyllids *T. apicalis* (Nissinen *et al*., 2014) and *Bactericera trigonica* (Teresani *et al*., 2014, 2015). '*Ca*. L. solanacearum' can also be transmitted by grafting and via dodder (Crosslin and Munyaneza, 2009; Secor *et al*., 2009; Munyaneza, 2012; Haapalainen, 2014; Munyaneza, 2015). The bacterium is found in several organs and tissues of its psyllid host, including the alimentary canal, salivary glands, hemolymph and bacteriomes (Cooper *et al*., 2013), and is transmitted in a propagative, circulative and persistent manner (Sengoda *et al*., 2014). Vertical (transovarial) transmission of '*Ca*. L. solanacearum' has been reported in *B. cockerelli* (Hansen *et al*., 2008).	*Category : EDITORIAL* **(71) European Union (29 Sep 2016 2:47 PM)**	**Incorporated.**
39	In solanaceous plants, '*Ca*. L. solanacearum' is primarily spread from infected to healthy plants by the tomato and potato psyllid *B. cockerelli* (Munyaneza *et al*., 2007; Munyaneza, 2012; EPPO, 2013). '*Ca*. L. solanacearum' has not yet been shown to be transmitted through true seed from infected solanaceous plants	*Category : EDITORIAL* **(40) United States of America (21 Sep 2016 8:11 PM)** For clarity	**Incorporated.**

	(Munyaneza, 2012). It has been demonstrated that the bacterium can be disseminated with the transport of infected carrot seeds, although vertical transmission through seed has been ~~shown~~ reported only once (Bertolini *et al*., 2014). Horizontal transmission between plants from the family Apiaceae has been reported to occur by the psyllids *T. apicalis* (Nissinen *et al*., 2014) and *Bactericera trigonica* (Teresani *et al*., 2014, 2015). '*Ca*. L. solanacearum' can also be transmitted by grafting and dodder (Crosslin and Munyaneza, 2009; Secor *et al*., 2009; Munyaneza, 2012; Haapalainen, 2014; Munyaneza, 2015). The bacterium is found in several organs and tissues of its psyllid host, including the alimentary canal, salivary glands, hemolymph and bacteriomes (Cooper *et al*., 2013), and is transmitted in a propagative, circulative and persistent manner (Sengoda *et al*., 2014). Vertical (transovarial) transmission of '*Ca*. L. solanacearum' has been reported in *B. cockerelli* (Hansen *et al*., 2008).		
40	Five haplotypes of '*Ca*. L. solanacearum' have so far been described (Nelson *et al*., 2011, 2013b; Teresani *et al*., 2014). Two haplotypes (A and B) are associated with diseases in potato and other solanaceous ~~species~~species in America and Oceania, whereas the other three haplotypes (C, D and E) are associated with carrot and celery ~~crops~~crops in Europe and North Africa. The haplotypes were described by single nucleotide polymorphisms (SNPs) in the 16S ribosomal (r)RNA gene, 16S-23S rRNA intergenic spacer region (IGS), and 50S *rplJ* and *rplL* ribosomal protein genes.	*Category : TECHNICAL* **(121) EPPO (29 Sep 2016 4:45 PM)** In the current absence of studies about the behaviour of the five described haplotypes in different solanaceous and apiaceous hosts, it seems preferable to include text about their geographical distribution in the different botanical families. It has not been demonstrated that the haplotypes are host specific To our knowledge the haplotype of Pastinaca samples has not been identified yet. More details would also be needed of virulence features within the haplotype groups A+B and C-E; e.g. is haplotype C also pathogenic to cellary and Pastinaca sativa?, and which of the Solancaeous haplotypes is more agressive on potato (and tomato)?.	**1) Incorporated.** 2) Noted. 3) The authors are not aware of any accepted data to show that there are consistent differences in virulence between haplotypes and that the naming of the haplotypes is based on genetic differences.
40	Five haplotypes of '*Ca*. L. solanacearum' have so far been described (Nelson *et al*., 2011, 2013b; Teresani *et al*., 2014). Two haplotypes (A and B) are associated with diseases in potato and other solanaceous ~~species~~species in America and Oceania, whereas the other three haplotypes (C, D and E) are associated with carrot and celery ~~crops~~crops in Europe and North Africa. The haplotypes were described by single nucleotide polymorphisms (SNPs) in the 16S ribosomal (r)RNA gene, 16S-23S rRNA intergenic spacer region (IGS), and 50S *rplJ* and *rplL* ribosomal protein genes.	*Category : TECHNICAL* **(72) European Union (29 Sep 2016 2:49 PM)** In the current absence of studies about the behaviour of the five described haplotypes in different solanaceous and apiaceous hosts, it seems preferable to include text about their geographical distribution in the different botanical families. It has not been demonstrated that the haplotypes are host specific	**Incorporated.**

		To our knowledge the haplotype of Pastinaca samples has not been identified yet. More details would also be needed of virulence features within the haplotype groups A+B and C-E; e.g. is haplotype C also pathogenic to cellary and Pastinaca sativa?, and which of the Solancaeous haplotypes is more agressive on potato (and tomato)?	
40	Five haplotypes of '*Ca*. L. solanacearum' have so far been described (Nelson *et al*., 2011, 2013b; Teresani *et al*., 2014). Two haplotypes (A and B) are associated with diseases in potato and other solanaceous species, whereas the other three haplotypes (C, D and E) are associated with carrot and celery crops. The haplotypes were ~~described~~ differentiated by single nucleotide polymorphisms (SNPs) in the 16S ribosomal (r)RNA gene, 16S-23S rRNA intergenic spacer region (IGS), and 50S *rplJ* and *rplL* ribosomal protein genes.	*Category : EDITORIAL* **(41) United States of America (21 Sep 2016 8:11 PM)** Suggest this alternative wording	**Incorporated.**
41	Further information on '*Ca*. L. solanacearum', including its insect vectors, disease epidemiology, vector biology, and management, can be found in reviews by Secor et al. (2009), Munyaneza (2012, 2015), Nelson *et al*. (2013a) and Haapalainen (2014).	*Category : TECHNICAL* **(31) United States of America (21 Sep 2016 8:03 PM)** Secor et al. (2009) should be added as a reference here	**Incorporated.**
43	**Name:** '*Candidatus* ~~Liberibacter solanacearum'~~ -' *Candidatus Liberibacter solanacearum* (Liefting *et al*., 2009b)	*Category : TECHNICAL* **(178) Ghana (30 Sep 2016 12:37 AM)** Candidatus Liberibacter solanacearum (Should be italiced)	**Considered, but not incorporated:** The correct way to cite the name is '*Candidatus* Liberibacter solanacearum'.
44	**Synonym:** '*Candidatus* -' *Candidatus Liberibacter psyllaurous* ~~Liberibacter psyllaurous'~~ (Hansen *et al*., 2008)	*Category : TECHNICAL* **(180) Ghana (30 Sep 2016 12:47 AM)** Candidatus Liberibacter psyllaurous (this should be italiced)	**Considered, but not incorporated:** The correct way to cite the name is '*Candidatus* Liberibacter psyllaurous'.
45	**Taxonomic position:** Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, 'Candidatus ~~Liberibacter~~Liberibacter'	*Category : EDITORIAL* **(122) EPPO (29 Sep 2016 4:45 PM)**	**Incorporated.**
45	**Taxonomic position:** Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, ~~*Candidatus*~~'*Candidatus* ~~Liberibacter~~Liberibacter'	*Category : EDITORIAL* **(73) European Union (29 Sep 2016 2:50 PM)**	**Incorporated.**
48	Plants infected with '*Ca*. L. solanacearum' may be asymptomatic or the symptoms may be similar to those associated with other phloem-limited bacteria and physiological disorders. Therefore, specific tests are required for the detection and identification of '*Ca*. L. solanacearum'. Because of the inability to culture '*Ca*. L. solanacearum' and the overall low ~~titre~~ degree in which this bacterium occurs in its host plants, molecular tests are required for detection and	*Category : EDITORIAL* **(113) Algeria (29 Sep 2016 4:21 PM)**	**Considered, but not incorporated:** Titre and degree have the same meaning but generally titre is used to describe pathogen concentration.

	identification.		
48	Plants infected with '*Ca*. L. solanacearum' may be asymptomatic or the symptoms may be similar to those associated with other phloem-limited bacteria and physiological disorders. Therefore, specific tests are required for the detection and identification of '*Ca*. L. solanacearum'. Because of the inability to culture '*Ca*. L. solanacearum' and the overall low titre in which this bacterium occurs in its host plants, molecular tests are required for detection and identification. Add the symptoms pictures of other hosts	*Category : SUBSTANTIVE* **(68) China (29 Sep 2016 11:51 AM)** Add the symptoms pictures of other hosts.Symptoms pictures are visualized and useful for field investigation.	**Incorporated.** Photos of symptomatic tomato and celery have been included.
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous small, misshaped, poor quality fruits. In potato, the below-ground symptoms are characteristic of '*Ca*. L. solanacearum' (zebra chip) and include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).	*Category : TECHNICAL* **(123) EPPO (29 Sep 2016 4:45 PM)** It would be nice if a photo of symptoms in celery could be included so there is one for each host category. Photos on celeri can be retrieved from the EPPO Global database https://gd.eppo.int/taxon/LIBEPS/photos	**Incorporated.** Photos of symptomatic tomato and celery have been included.
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous small, misshaped, poor quality fruits. In potato, the below-ground symptoms are characteristic of '*Ca*. L. solanacearum' (zebra chip) and include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues	*Category : TECHNICAL* **(74) European Union (29 Sep 2016 2:51 PM)** It would be nice if a photo of symptoms in celery could be included so there is one for each host category. Photos on celery can be retrieved from the EPPO Global database https://gd.eppo.int/taxon/LIBEPS/photos .	**Incorporated.** Photos of symptomatic tomato and celery have been included.

	and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).		
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial ~~tubers~~tubers formation, leaf scorching, disruption of fruit set, and production of numerous small, misshaped, poor quality fruits. In potato, the below-ground symptoms are characteristic of '*Ca*. L. solanacearum' (zebra chip) and include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).	*Category : SUBSTANTIVE* **(60) Philippines (29 Sep 2016 9:18 AM)**	**Incorporated.**
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous small, misshaped, poor quality fruits. In potato, the below-ground symptoms are characteristic of '*Ca*. L. solanacearum' (zebra chip) and include collapsed stolons,	*Category : TECHNICAL* **(32) United States of America (21 Sep 2016 8:03 PM)** To add this concept. Add Lee et al 2006 as a reference	**Incorporated.**

	browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Freshly cut tubers, when infected, show in minutes necrotic browning in medullary ray tissue throughout the tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Lee et al., 2006; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).		
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous small, ~~misshaped~~misshapen, poor quality fruits. In potato, the below-ground symptoms ~~are~~ characteristic of '*Ca*. L. solanacearum' (zebra chip) ~~and~~ include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).	*Category : EDITORIAL* **(42) United States of America (21 Sep 2016 8:12 PM)** To clarify	**Incorporated.**
50	The above-ground plant symptoms associated with '*Ca*. L. solanacearum' infection in potato and other solanaceous species (Figures 1–2) resemble those associated with phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage, upward rolling of leaves, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial	*Category : EDITORIAL* **(20) Slovenia (24 Aug 2016 6:32 PM)** Common name of the disease should be 'zebra chips' or 'zebra chip complex'	**Considered, but not incorporated:** The common name 'zebra chip' is more commonly used. The authors are not familiar with 'zebra chips' terminology.

	tubers, leaf scorching, disruption of fruit set, and production of numerous small, misshaped, poor quality fruits. In potato, the below-ground symptoms are characteristic of '*Ca*. L. solanacearum' (zebra ~~chip)~~ chips) and include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from affected tubers show very dark blotches, stripes or streaks, rendering them commercially unacceptable (Figure 3). Symptoms in carrots associated with '*Ca*. L. solanacearum' infection include leaf curling, yellowish, bronze and purplish discolouration of leaves, stunting of the shoots and roots, and proliferation of secondary roots (Figure 4) (Munyaneza *et al*., 2010; Nissinen *et al*., 2014). These symptoms resemble those associated with phytoplasmas and *Spiroplasma citri* in carrots (Cebrián *et al*., 2010; Munyaneza *et al*., 2011). In celery, vegetative disorders associated with the pathogen include an abnormal number of shoots per plant and curled stems (Teresani *et al*., 2014).		
54	The within-plant distribution of '*Ca*. L. solanacearum' is highly variable, therefore careful sampling is required to improve the accuracy of diagnosis. Sampling protocols should consider that '*Ca*. L. solanacearum' may not be detectable by polymerase chain reaction (PCR) until three weeks after infective psyllids have fed on the plants (Levy *et al*., 2011). If typical foliar symptoms are present, three to five leaves and/or stems should be collected from symptomatic parts of the plant. In asymptomatic plants, leaves and/or stems from five to ten different parts of the plant should be sampled and should include newly developing leaves (Levy *et al*., 2011; Cooper *et al*., 2015). Below-ground plant parts such as potato tubers, carrot roots, silverleaf nightshade roots and stolons can also be used to detect '*Ca*. L. solanacearum'. Potato tubers showing obvious zebra chip symptoms will result in reliable detection. Detection from asymptomatic tubers will be less reliable, even if above-ground symptoms are present, as not all tubers from an infected plant will become infected by '*Ca*. L. solanacearum' (Buchman *et al*., 2011). The basal end (heel) of the tuber, the end which attaches to the stolon, is the recommended tissue to sample whether the potato tuber is symptomatic or not. Before extraction, ~~the~~ all plant material is subsampled so that the material used contains as much vascular tissue as possible (e.g. petioles, leaf midribs, cambium, and the heel end or vascular ring of potato tubers).	*Category : TECHNICAL* **(124) EPPO (29 Sep 2016 4:45 PM)** Heel end should be sampled Does silverleaf nightshade also show symptoms? Are asymptomatic tubers individually tested or can they be bulked? If bulking, what is the number of asymptomatic tubers that should be tested?	**Modified:** The sentence on sampling below-ground plant parts has been modified to make it more generic to avoid repeating the list of plant hosts from section 1. **Incorporated.** It is not recommended to test asymptomatic potato tubers individually or bulked and this has been made clear in the protocol. Other recommendations updated.
54	The within-plant distribution of '*Ca*. L. solanacearum' is highly variable, therefore careful sampling is required to improve the accuracy of diagnosis. Sampling protocols should consider that '*Ca*. L. solanacearum' may not be detectable by polymerase chain reaction (PCR) until three weeks after infective psyllids have fed on the plants (Levy *et al*., 2011). If typical foliar symptoms are present, three to	*Category : TECHNICAL* **(75) European Union (29 Sep 2016 2:54 PM)** Heel end should be sampled. Does silverleaf nightshade also show symptoms?	**Modified:** The sentence on sampling below-ground plant parts has been modified to make it more generic to avoid repeating the list of plant hosts from section 1.

	five leaves and/or stems should be collected from symptomatic parts of the plant. In asymptomatic plants, leaves and/or stems from five to ten different parts of the plant should be sampled and should include newly developing leaves (Levy *et al*., 2011; Cooper *et al*., 2015). Below-ground plant parts such as potato tubers, carrot roots, silverleaf nightshade roots and stolons can also be used to detect '*Ca*. L. solanacearum'. Potato tubers showing obvious zebra chip symptoms will result in reliable detection. Detection from asymptomatic tubers will be less reliable, even if above-ground symptoms are present, as not all tubers from an infected plant will become infected by '*Ca*. L. solanacearum' (Buchman *et al*., 2011). The basal end (heel) of the tuber, the end which attaches to the stolon, is the recommended tissue to sample whether the potato tuber is symptomatic or not. Before extraction, ~~the~~ all plant material is subsampled so that the material used contains as much vascular tissue as possible (e.g. petioles, leaf midribs, cambium, and the heel end or vascular ring of potato tubers).	Are asymptomatic tubers individually tested or can they be bulked? If bulking, what is the number of asymptomatic tubers that should be tested? And an editorial ('all plant' material).	**Incorporated.** It is not recommended to test asymptomatic potato tubers individually or bulked and this has been made clear in the protocol. Other recommendation updated.
54	The within-plant distribution of '*Ca*. L. solanacearum' is highly variable~~,~~; therefore careful sampling is required to improve the accuracy of diagnosis. Sampling protocols should consider that '*Ca*. L. solanacearum' may not be detectable by polymerase chain reaction (PCR) until three weeks after infective psyllids have fed on the plants (Levy *et al*., 2011). If typical foliar symptoms are present, three to five leaves and/or stems should be collected from symptomatic parts of the plant. In asymptomatic plants, leaves and/or stems from five to ten different parts of the plant should be sampled and should include newly developing leaves (Levy *et al*., 2011; Cooper *et al*., 2015). Below-ground plant parts such as potato tubers, carrot roots, silverleaf nightshade roots and stolons can also be used to detect '*Ca*. L. solanacearum'. Potato tubers showing obvious zebra chip symptoms will result in reliable detection. Detection from asymptomatic tubers will be less reliable, even if above-ground symptoms are present, as not all tubers from an infected plant will become infected by '*Ca*. L. solanacearum' (Buchman *et al*., 2011). The basal end (heel) of the tuber, the end which attaches to the stolon, is the recommended tissue to sample whether the potato tuber is symptomatic or not. Before extraction, the material is subsampled so that the material used contains as much vascular tissue as possible (e.g. petioles, leaf midribs, cambium, and the vascular ring of potato tubers).	*Category : EDITORIAL* **(43) United States of America (21 Sep 2016 8:14 PM)** Grammar fix	**Incorporated.**
54	The within-plant distribution of '*Ca*. L. solanacearum' is highly variable, therefore careful sampling is required to improve the accuracy of diagnosis. Sampling protocols should consider that '*Ca*. L. solanacearum' may not be detectable by polymerase chain reaction (PCR) until three weeks after infective psyllids have fed on the plants (Levy *et al*., 2011). If typical foliar symptoms are present, three to five leaves and/or stems should be collected from symptomatic parts of the plant.	*Category : TECHNICAL* **(33) United States of America (21 Sep 2016 8:04 PM)** Important technical addition	**Modified:** This sentence has been modified to make it more generic to avoid repeating the list of plant hosts from section 1.

	In asymptomatic plants, leaves and/or stems from five to ten different parts of the plant should be sampled and should include newly developing leaves (Levy *et al*., 2011; Cooper *et al*., 2015). Below-ground plant parts such as potato ~~tubers~~tubers and roots, carrot roots, silverleaf nightshade roots and stolons can also be used to detect '*Ca*. L. solanacearum'. Potato tubers showing obvious zebra chip symptoms will result in reliable detection. Detection from asymptomatic tubers will be less reliable, even if above-ground symptoms are present, as not all tubers from an infected plant will become infected by '*Ca*. L. solanacearum' (Buchman *et al*., 2011). The basal end (heel) of the tuber, the end which attaches to the stolon, is the recommended tissue to sample whether the potato tuber is symptomatic or not. Before extraction, the material is subsampled so that the material used contains as much vascular tissue as possible (e.g. petioles, leaf midribs, cambium, and the vascular ring of potato tubers).		
56	Insufficient data exist to recommend a sample size and bulking rate for seed testing. The single study of seed transmission in carrot by Bertolini *et al*. (2014) detected '*Ca*. L. solanacearum' in samples of 500 seeds. The International Seed Federation (ISF) recommends testing samples of 20 000 seeds composed of two subsamples of 10 000 seeds.	*Category : TECHNICAL* **(125) EPPO (29 Sep 2016 4:45 PM)** The ISF testing recommendation is 20 000 seeds and two subsamples of 10 000 seeds see: http://www.worldseed.org/wp-content/uploads/2016/07/Detection_Lso_carrot_seed_2016_1.pdf Is the sample size for all seeds or only carrot?	**Incorporated.**
56	Insufficient data exist to recommend a sample size and bulking rate for seed testing. The single study of seed transmission in carrot by Bertolini *et al*. (2014) detected '*Ca*. L. solanacearum' in samples of 500 seeds. The International Seed Federation (ISF) recommends testing subsamples of 10 000 seeds.	*Category : SUBSTANTIVE* **(114) Algeria (29 Sep 2016 4:25 PM)** préférable de quantifier le poids que d'utiliser le nombre c'est plus pratique pour l'analyse.	**Considered, but not incorporated.** The authors were reluctant to recommend a seed weight because of the differences that can occur between different varieties and seed lines. This is in keeping with other IPPC protocols e.g, PSTVd (DP 07) where numbers of seeds are specified rather than the weight.
56	Insufficient data exist to recommend a sample size and bulking rate for seed testing. The single study of seed transmission in carrot by Bertolini *et al*. (2014) detected '*Ca*. L. solanacearum' in samples of 500 seeds. The International Seed Federation (ISF) recommends testing samples of 20 000 seeds composed of two subsamples of 10 000 seeds.	*Category : TECHNICAL* **(76) European Union (29 Sep 2016 2:55 PM)** The ISF testing recommendation is 20 000 seeds and two subsamples of 10 000 seeds see: http://www.worldseed.org/wp-content/uploads/2016/07/Detection_Lso_carrot_seed_2016_1.pdf Is the sample size for all seeds or only carrot?	**Incorporated.** The sample size is only for carrot and this has now been made clear.

56	Insufficient data exist to recommend a sample size and bulking rate for seed testing. The single study of seed transmission in carrot by Bertolini *et al*. (2014) detected '*Ca*. L. solanacearum' in samples of 500 seeds. The International Seed Federation (ISF) recommends testing subsamples of 10 000 seeds.	*Category : TECHNICAL* **(21) Slovenia (24 Aug 2016 6:38 PM)** Before adoption of the protocol a reasonable sample size number should be recomended here, since discrepancy between 500 and 10000 is to big. ISTA should be asked for justification of their standard. Furthermore, it is not clear, if ISTA standard is specific for bacteria or general one.	**Considered, but not incorporated:** Due to a lack of experimental data a specific sample size has not been recommended.
58	Crosslin *et al*. (2011) determined that '*Ca*. L. solanacearum' can be reliably detected by conventional and real-time PCR in bulks of 30 laboratory reared adult *B. cockerelli*. However, it is best to limit bulking to ten psyllids if they are sampled from the field by either sticky traps or hand collection. If the insects are collected from sticky traps, it is not necessary to remove the glue before DNA extraction. But if desired, the glue may be removed before testing as described by Bertolini *et al*. (2014) and Teresani *et al*. (2014). '*Ca*. L. solanacearum' can be reliably detected in infected psyllids for up to ten months on sticky traps (Crosslin *et al*., 2011). For long-term storage, psyllids are preserved in 70% ethanol.	*Category : TECHNICAL* **(126) EPPO (29 Sep 2016 4:45 PM)** It is not clear what CLSo 'can be reliably detected in infected psyllids for up to ten months on sticky traps' means – can an insect still be positive after spending 10 months on a sticky trap in the environment? Also may climatic conditions affect this length of time? 'For long-term storage, psyllids are preserved in 70% ethanol.' Does this mean prior to testing?	**Incorporated.** Both of these statements have been clarified.
58	Crosslin *et al*. (2011) determined that '*Ca*. L. solanacearum' can be reliably detected by conventional and real-time PCR in bulks of 30 laboratory reared adult *B. cockerelli*. However, it is best to limit bulking to ten psyllids if they are sampled from the field by either sticky traps or hand collection. If the insects are collected from sticky traps, it is not necessary to remove the glue before DNA extraction. But if desired, the glue may be removed before testing as described by Bertolini *et al*. (2014) and Teresani *et al*. (2014). '*Ca*. L. solanacearum' can be reliably detected in infected psyllids for up to ten months on sticky traps (Crosslin *et al*., 2011). For long-term storage, psyllids are preserved in 70% ethanol.	*Category : TECHNICAL* **(77) European Union (29 Sep 2016 2:56 PM)** It is not clear what CLSo 'can be reliably detected in infected psyllids for up to ten months on sticky traps' means – can an insect still be positive after spending 10 months on a sticky trap in the environment? Also may climatic conditions affect this length of time? We believe that this is not valid for traps that have remained in the field. 'For long-term storage, psyllids are preserved in 70% ethanol.' Does this mean prior to testing?	**Incorporated.** Both of these statements have been clarified.
60	PCR is the method of choice for detection of '*Ca*. L. solanacearum'. Conventional PCR can be used, but real-time PCR is recommended because of its better sensitivity.	*Category : TECHNICAL* **(174) Ghana (30 Sep 2016 12:19 AM)** The levels of sensitivity and specificity of the proposed method should be indicated well in order to compare these methods with other methods.	**Considered, but not incorporated:** The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.
60	PCR is the method of choice for detection of '*Ca*. L. solanacearum'. Conventional PCR can be used, but real-time PCR is recommended because of its better sensitivity.	*Category : TECHNICAL* **(83) Kenya (29 Sep 2016 3:08 PM)** Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other	**Considered, but not incorporated:** The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.

		methods.	
62	Plant material may be homogenized using one of a variety of methods. The method chosen is dependent on the nature of the plant material. Soft plant tissue can be ground using homogenizers (e.g. Bioreba HOMEX 6[1], homogenizer hand model) or bead beater machines (e.g. Roche MagNA Lyser Instrument[1], BioSpec BeadBeater[1]). Alternatively, homogenization can be carried out by hammering plant material contained in a stomacher bag with a rubber or wooden hammer. This allows optimization of homogenization depending on the type of material. Hard plant tissue will need to be ground in a mortar with a pestle and if the tissue is very hard the grinding will need to be aided with the addition of liquid nitrogen. Whichever grinding method is used it is important that complete disruption of the plant vascular tissue is achieved in order to release any '*Ca*. L. solanacearum' present.	*Category : TECHNICAL* **(127) EPPO (29 Sep 2016 4:45 PM)** Alternative preparation	**Modified:** The first sentence has been included. It is unsure what is meant by the second sentence.
62	Plant material may be homogenized using one of a variety of methods. The method chosen is dependent on the nature of the plant material. Soft plant tissue can be ground using homogenizers (e.g. Bioreba HOMEX 6[1], homogenizer hand model) or bead beater machines (e.g. Roche MagNA Lyser Instrument[1], BioSpec BeadBeater[1]). Alternatively, homogenization can be carried out by hammering plant material contained in a stomacher bag with a rubber or wooden hammer. This allows optimization of homogenization depending on the type of material. Hard plant tissue will need to be ground in a mortar with a pestle and if the tissue is very hard the grinding will need to be aided with the addition of liquid nitrogen. Whichever grinding method is used it is important that complete disruption of the plant vascular tissue is achieved in order to release any '*Ca*. L. solanacearum' present.	*Category : TECHNICAL* **(78) European Union (29 Sep 2016 2:57 PM)** Alternative preparation.	**Modified**: The first sentence has been included. It is unsure what is meant by the second sentence.
63	~~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.~~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 in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results	*Category : TECHNICAL* **(169) Bolivia (29 Sep 2016 8:49 PM)** See general coments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
63	-The use of names of reagents, chemicals or equipment in these diagnostic protocols	*Category : TECHNICAL*	**Incorporated:** The standard

	implies no approval of them to the exclusion of others that may also be suitable. This information in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results~~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~~.	**(167) Peru (29 Sep 2016 8:22 PM)** See general coments	footnote regarding brands will be added as in other diagnostic protocols.
63	~~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.~~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 in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : SUBSTANTIVE* **(58) Argentina (29 Sep 2016 4:18 AM)** See general comment	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
63	~~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.~~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 in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : TECHNICAL* **(54) Chile (28 Sep 2016 6:14 PM)** See general coments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
63	~~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~~ This information is given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute an endorsement by the ~~standards~~ CPM of	*Category : TECHNICAL* **(26) Uruguay (15 Sep 2016 9:15 PM)** See general comment	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.

	~~individual laboratories~~the chemical, ~~provided that~~ reagent and/or equipment named. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead the same results.		
63	~~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.~~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 in given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and or equipment named. Equivalent products may be used if they can be shown to lead the same results.	*Category : TECHNICAL* **(17) COSAVE (11 Aug 2016 11:16 PM)** See general coments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
64	Seeds may be crushed with a pestle in a mortar, in a coffee grinder, or inside a plastic bag using a hammer. The ISF protocol for carrot seed recommends bag-mixing/stomaching rather than grinding. To remove fungicide treatments and to facilitate seed crushing, seeds are washed by shaking for 30' in 1:10 w:v 0.5% Triton X-100 and, after several rinses, let soften in water overnight.	*Category : TECHNICAL* **(128) EPPO (29 Sep 2016 4:45 PM)** The drafting team could consider the addition of a comment on how to deal with carrot seeds that have a seed treatment. Some information is provided in Bertolini et al. 2015. "Removal of fungicide treatment can be attempted by washing by shaking for 30' in 1:10 w:v 0.5% Triton X-100 (Bertolini et al. 2015)	**Incorporated.**
64	Seeds may be crushed with a pestle in a mortar, in a coffee grinder, or inside a plastic bag using a hammer. The ISF protocol for carrot seed recommends bag-mixing/stomaching rather than grinding.	*Category : TECHNICAL* **(79) European Union (29 Sep 2016 2:59 PM)** The drafting team could consider the addition of a comment on how to deal with carrot seeds that have a seed treatment. Some information is provided in Bertolini et al. 2015: "Removal of fungicide treatment can be attempted by washing by shaking for 30' in 1:10 w:v 0.5% Triton X-100." (Bertolini et al. 2015)	**Incorporated.**
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated	*Category : TECHNICAL* **(170) Bolivia (29 Sep 2016 9:08 PM)** See general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.

65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(168) Peru (29 Sep 2016 8:23 PM)** See general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(117) Brazil (29 Sep 2016 4:31 PM)** See general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(59) Argentina (29 Sep 2016 4:19 AM)** See general comment	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(55) Chile (28 Sep 2016 6:16 PM)** see general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(27) Uruguay (15 Sep 2016 9:18 PM)** See general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
65	Psyllids are easily homogenized in microfuge tubes with micropestles. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(16) COSAVE (11 Aug 2016 11:15 PM)** See general comments	**Incorporated:** The standard footnote regarding brands will be added as in other diagnostic protocols.
68	Samples may contain compounds that are inhibitory to PCR depending on the host	*Category : TECHNICAL* **(129) EPPO (29 Sep 2016 4:45 PM)**	**Modified:** 1. Details of the sephacryl spin

	species, plant tissue, age of the tissue and any treatments. Therefore it is important to check the PCR competency of the DNA extractions using internal control primers that amplify a gene from the host. Inhibitory effects of the host can be overcome by further purifying the DNA through a sephacryl spin column, by adding bovine serum albumin (BSA) to the PCR mixture at a final concentration of 0.5 mg/ml (Kreader, 1996) or by use of a commercial purification kit after DNA extraction (e.g. PVPP, polyvinylpyrrolidone spin column, Fluka P6755). Alternatively, inhibitors can be reduced by using a 1:10 (v/v) dilution of the template DNA.	Technical 1 More details are needed on the sephacryl spin column: item number, type or reference to a more detailed method. 2 Diluting template DNA will reduce the diagnostic sensitivity of detection although it is recognized that it can help when inibitors are present. A slight revision of the text is proposed. 3 Editorial Suggestion replace 'Therefore it is important to check the PCR competency of the DNA extractions by "check the efficiency of DNA extraction"	columns has been included. The information on PVP spin columns is not correct. 2. Agree with the point about diluting template DNA so this has been removed from the protocol. 3. It is better to use "competency" rather than "efficiency". The DNA extraction could have been efficient but could still contain inhibitors.
68	Samples may contain compounds that are inhibitory to PCR depending on the host species, plant tissue, age of the tissue and any treatments. Therefore it is important to check the PCR competency of the DNA extractions using internal control primers that amplify a gene from the host. Inhibitory effects of the host can be overcome by further purifying the DNA through a sephacryl spin column, by adding bovine serum albumin (BSA) to the PCR mixture at a final concentration of 0.5 mg/ml (Kreader, 1996) or by use of a commercial purification kit after DNA extraction (e.g. PVPP, polyvinylpyrrolidone spin column, Fluka P6755). Alternatively, inhibitors can be reduced by using a 1:10 (v/v) dilution of the template DNA.	*Category : TECHNICAL* **(80) European Union (29 Sep 2016 3:01 PM)** 1. More details are needed on the sephacryl spin column: item number, type or reference to a more detailed method. 2. Diluting template DNA will reduce the diagnostic sensitivity of detection although it is recognized that it can help when inibitors are present. A slight revision of the text is proposed. 3. Editorial suggestion: to replace "Therefore it is important to check the PCR competency of the DNA extractions" by "check the efficiency of DNA extraction".	**Modified:** 1. Details of the sephacryl spin columns has been included. The information on PVP spin columns is not correct. 2. Agree with the point about diluting template DNA so this has been removed from the protocol. 3. It is better to use "competency" rather than "efficiency". The DNA extraction could have been efficient but could still contain inhibitors.
69	***3.3.2.1 CTAB extraction***	*Category : TECHNICAL* **(130) EPPO (29 Sep 2016 4:45 PM)** A more simple CTAB procedure exists and works fine at least for Apiaceous plants. It is described in EPPO (2016) [PM7/0792 (2) Diagnostic protocol for Flavescence dorée).	**Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.
69	***3.3.2.1 CTAB extraction***	*Category : TECHNICAL* **(81) European Union (29 Sep 2016 3:06 PM)** A more simple CTAB procedure exists and works fine at least for Apiaceous plants. It is described in EPPO (2016) [PM7/0792 (2) Diagnostic protocol for Flavescence dorée).	**Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.

69	***3.3.2.1 CTAB extraction***	*Category : TECHNICAL* **(2) France (5 Aug 2016 4:14 PM)** A more simple CTAB procedure exists and works fine at least for Apiaceous plants. It is described in EPPO (2016) [PM7/0792 (2) Diagnostic protocol for Flavescence dorée).	**Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.
70	~~Plant~~ DNA extraction from plant tissue is ~~extracted~~ performed according to Munyaneza *et al*. (2010). In this method, 500 mg plant tissue is homogenized in 1 ml extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM ethylenediaminetetraacetic acid (EDTA), 500 mM NaCl and 10 mM 2-mercaptoethanol). The homogenate (300 µl) is mixed with 80 µl lysozyme (50 mg/ml in 10 mM Tris-HCl, pH 8.0) and incubated at 37 °C for 30 min. After incubation, 500 µl cetyl trimethylammonium bromide (CTAB) buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2% (w/v) CTAB, 1% (w/v) polyvinylpyrrolidone (PVP)-40 and 0.2% (v/v) 2-mercaptoethanol) is added to the homogenate and incubated at 65 °C for 30 min. The sample is allowed to cool at room temperature for 3 min before the addition of 500 µl ice-cold chloroform. Samples are mixed by vortexing and then centrifuged at 13 000 *g* for 10 min. The upper aqueous layer is transferred to a new microfuge tube, 0.6 volume of isopropanol is added and the tube is placed on ice for 20 min to precipitate the DNA. DNA is recovered by centrifugation as described above. The pellet is washed with ice-cold 75% ethanol and centrifuged at 13 000 *g* for 2 min. After removal of ethanol, the pellet is air-dried and resuspended in 100 µl sterile water.	*Category : EDITORIAL* **(132) EPPO (29 Sep 2016 4:45 PM)** to be consistent with the next proposal from GB on insects	**Incorporated.**
70	Plant tissue is extracted according to Munyaneza *et al*. (2010). In this method, 500 mg plant tissue is homogenized in 1 ml extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM ethylenediaminetetraacetic acid (EDTA), 500 mM NaCl and 10 mM 2-mercaptoethanol). The homogenate (300 µl) is mixed with 80 µl lysozyme (50 mg/ml in 10 mM Tris-HCl, pH 8.0) and incubated at 37 °C for 30 min. After incubation, 500 µl cetyl trimethylammonium bromide (CTAB) buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2% (w/v) CTAB, 1% (w/v) polyvinylpyrrolidone (PVP)-40 and 0.2% (v/v) 2-mercaptoethanol) is added to the homogenate and incubated at 65 °C for 30 min. The sample is allowed to cool at room temperature for 3 min before the addition of 500 µl ice-cold chloroform. Samples are mixed by vortexing and then centrifuged at 13 000 *g* for 10 min. The upper aqueous layer is transferred to a new microfuge tube, 0.6 volume of isopropanol is added and the tube is placed on ice for 20 min to precipitate the DNA. DNA is recovered by centrifugation as described above. The pellet is washed with ice-cold 75% ethanol and centrifuged at 13 000 *g* for 2 min. After removal of ethanol, the pellet is air-dried and resuspended in 100 µl sterile water.	*Category : TECHNICAL* **(131) EPPO (29 Sep 2016 4:45 PM)** How do the 500 mg relate to numbers of seeds or potato stolons?	**Considered, but not incorporated:** Not sure this information is relevant for the DNA extraction.

70	~~Plant~~ DNA extraction from plant tissue is ~~extracted~~ performed according to Munyaneza *et al*. (2010). In this method, 500 mg plant tissue is homogenized in 1 ml extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM ethylenediaminetetraacetic acid (EDTA), 500 mM NaCl and 10 mM 2-mercaptoethanol). The homogenate (300 µl) is mixed with 80 µl lysozyme (50 mg/ml in 10 mM Tris-HCl, pH 8.0) and incubated at 37 °C for 30 min. After incubation, 500 µl cetyl trimethylammonium bromide (CTAB) buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2% (w/v) CTAB, 1% (w/v) polyvinylpyrrolidone (PVP)-40 and 0.2% (v/v) 2-mercaptoethanol) is added to the homogenate and incubated at 65 °C for 30 min. The sample is allowed to cool at room temperature for 3 min before the addition of 500 µl ice-cold chloroform. Samples are mixed by vortexing and then centrifuged at 13 000 *g* for 10 min. The upper aqueous layer is transferred to a new microfuge tube, 0.6 volume of isopropanol is added and the tube is placed on ice for 20 min to precipitate the DNA. DNA is recovered by centrifugation as described above. The pellet is washed with ice-cold 75% ethanol and centrifuged at 13 000 *g* for 2 min. After removal of ethanol, the pellet is air-dried and resuspended in 100 µl sterile water.	*Category : TECHNICAL* **(82) European Union (29 Sep 2016 3:07 PM)** 1. How do the 500 mg relate to numbers of seeds or potato stolons? 2. (EDIT.) To be consistent with the next proposal on insects.	1. **Considered, but not incorporated:** Not sure this information is relevant for the DNA extraction. 2**. Incorporated**.
71	Insects are extracted as described by Goodwin *et al*. (1994) where the insects are homogenized in 125 µl CTAB extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% (w/v) CTAB and 1% (w/v) PVP-40). The homogenate is briefly vortexed and then incubated at 65 °C for 5 min. The suspension is extracted once with an equal volume of chloroform:isoamyl alcohol (24:1 (v/v)) and the DNA precipitated by adding 0.1 volume of 3 M sodium acetate, pH 5.2, and 2.5 volumes of ice-cold ethanol and incubating at –20 °C for at least 1 h. After centrifuging at 13 000 *g* for 15 min, the pellet is washed with 70% ice-cold ethanol, air-dried and resuspended in 15 µl sterile water.	*Category : TECHNICAL* **(134) EPPO (29 Sep 2016 4:45 PM)** The number of insects that can be processed with the amount of CTAB buffer should be given	**Incorporated**.
71	~~Insects are extracted as~~ DNA extraction from insects is described by Goodwin *et al*. (1994) where the insects are homogenized in 125 µl CTAB extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% (w/v) CTAB and 1% (w/v) PVP-40). The homogenate is briefly vortexed and then incubated at 65 °C for 5 min. The suspension is extracted once with an equal volume of chloroform:isoamyl alcohol (24:1 (v/v)) and the DNA precipitated by adding 0.1 volume of 3 M sodium acetate, pH 5.2, and 2.5 volumes of ice-cold ethanol and incubating at –20 °C for at least 1 h. After centrifuging at 13 000 *g* for 15 min, the pellet is washed with 70% ice-cold ethanol, air-dried and resuspended in 15 µl sterile water.	*Category : EDITORIAL* **(133) EPPO (29 Sep 2016 4:45 PM)**	**Incorporated**.
71	~~Insects are extracted as~~ DNA extraction from insects is described by Goodwin *et al*. (1994) where the insects are homogenized in 125 µl CTAB extraction buffer	*Category : TECHNICAL* **(84) European Union (29 Sep 2016 3:09 PM)**	**Incorporated**.

	(100 mM Tris, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% (w/v) CTAB and 1% (w/v) PVP-40). The homogenate is briefly vortexed and then incubated at 65 °C for 5 min. The suspension is extracted once with an equal volume of chloroform:isoamyl alcohol (24:1 (v/v)) and the DNA precipitated by adding 0.1 volume of 3 M sodium acetate, pH 5.2, and 2.5 volumes of ice-cold ethanol and incubating at –20 °C for at least 1 h. After centrifuging at 13 000 *g* for 15 min, the pellet is washed with 70% ice-cold ethanol, air-dried and resuspended in 15 µl sterile water.	1. The number of insects that can be processed with the amount of CTAB buffer should be given. 2. (EDIT.)	
73	Commercial kits based on silica spin columns (e.g. Qiagen DNeasy Plant Mini Kit[1] for plants, Qiagen DNeasy Blood and Tissue Kit[1] for insects) (Li *et al*., 2009) or magnetic beads (e.g. InviMag Plant DNA Mini Kit[1]) are used according to the manufacturer's instructions. The advantage of using magnetic beads is that the extractions can be performed on an automated workstation (e.g. Thermo Scientific KingFisher Magnetic Particle Processors[1]). For plant tissue that contains high levels of polyphenolic compounds a modified lysis step as described by Green *et al*. (1999) is recommended. The plant material is homogenized in CTAB extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2.5% (w/v) CTAB, 1% (w/v) PVP-40 and 0.2% (v/v) 2-mercaptoethanol added just before use). The homogenate (0.5 ml) is transferred to a microcentrifuge tube, mixed by inversion with 22 µl ribonuclease (RNase) A (20 mg/ml) and incubated at 65 °C with intermittent shaking for 25–35 min. The homogenate is then processed according to the manufacturer's instructions from the commercial kit being used.	*Category : TECHNICAL* **(135) EPPO (29 Sep 2016 4:45 PM)** Regarding the comment 'For plant tissue that contains high levels of polyphenolic compounds' it would be worth giving examples. Instead of homogenization of the plant material in CTAB buffer, it could be efficiently done in PBS buffer, which is even easier (see ISF protocol, 1.2 from July 2016).	**Incorporated**. For examples. **Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.
73	Commercial kits based on silica spin columns (e.g. Qiagen DNeasy Plant Mini Kit[1] for plants, Qiagen DNeasy Blood and Tissue Kit[1] for insects) (Li *et al*., 2009) or magnetic beads (e.g. InviMag Plant DNA Mini Kit[1]) are used according to the manufacturer's instructions. The advantage of using magnetic beads is that the extractions can be performed on an automated workstation (e.g. Thermo Scientific KingFisher Magnetic Particle Processors[1]). For plant tissue that contains high levels of polyphenolic compounds a modified lysis step as described by Green *et al*. (1999) is recommended. The plant material is homogenized in CTAB extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2.5% (w/v) CTAB, 1% (w/v) PVP-40 and 0.2% (v/v) 2-mercaptoethanol added just before use). The homogenate (0.5 ml) is transferred to a microcentrifuge tube, mixed by inversion with 22 µl ribonuclease (RNase) A (20 mg/ml) and incubated at 65 °C with intermittent shaking for 25–35 min. The homogenate is then processed according to the manufacturer's instructions from the commercial kit being used.	*Category : TECHNICAL* **(85) European Union (29 Sep 2016 3:11 PM)** 1. Regarding the comment 'For plant tissue that contains high levels of polyphenolic compounds' it would be worth giving examples. 2. Instead of homogenization of the plant material in CTAB buffer, it could be efficiently done in PBS buffer, which is even easier (see ISF protocol, version 1.2 from July 2016).	1. **Incorporated**. 2. **Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.

73	Commercial kits based on silica spin columns (e.g. Qiagen DNeasy Plant Mini Kit[1] for plants, Qiagen DNeasy Blood and Tissue Kit[1] for insects) (Li *et al*., 2009) or magnetic beads (e.g. InviMag Plant DNA Mini Kit[1]) are used according to the manufacturer's instructions. The advantage of using magnetic beads is that the extractions can be performed on an automated workstation (e.g. Thermo Scientific KingFisher Magnetic Particle Processors[1]). For plant tissue that contains high levels of polyphenolic compounds a modified lysis step as described by Green *et al*. (1999) is recommended. The plant material is homogenized in CTAB extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 50 mM EDTA, pH 8.0, 2.5% (w/v) CTAB, 1% (w/v) PVP-40 and 0.2% (v/v) 2-mercaptoethanol added just before use). The homogenate (0.5 ml) is transferred to a microcentrifuge tube, mixed by inversion with 22 µl ribonuclease (RNase) A (20 mg/ml) and incubated at 65 °C with intermittent shaking for 25–35 min. The homogenate is then processed according to the manufacturer's instructions from the commercial kit being used.	*Category : TECHNICAL* **(4) France (5 Aug 2016 4:20 PM)** Instead of homogenization of the plant material in CTAB buffer, it could be efficiently done in PBS buffer, which is even easier (see ISF protocol, version 1.1 from April 2016).	**Considered, but not incorporated:** There are many variations of the CTAB extraction method and they all cannot be included in the diagnostic protocol.
76	**3.3.3 Real-time PCR**	*Category : SUBSTANTIVE* **(136) EPPO (29 Sep 2016 4:45 PM)** There should be a reference to the real-time PCR of Teresani et al 2014 even if this is not described in full. This test is included in our draft protocol together with the test from Li et al 2009. THe co author of the protocl has been insisting to have it added with no success but the minimum would be to quote it.	**Incorporated**.
76	**3.3.3 Real-time PCR**	*Category : SUBSTANTIVE* **(86) European Union (29 Sep 2016 3:13 PM)** There should be a reference to the real-time PCR of Teresani et al 2014 even if this is not described in full in this protocol. This test is included in the draft EPPO protocol together with the test from Li et al 2009. The addition of this test was requested during the Expert Consultation. Although we understand that the protocol cannot include all tests it could be mentioned as not so many test exist for that pest.	**Incorporated**.
77	Real-time PCR is performed using the assay designed to target the 16S rRNA gene (Li *et al*., 2009). This assay is based on the real-time PCR designed to detect the three citrus-infecting liberibacter species (Huanglongbing) by Li *et al*. (2006). All liberibacter species use the same reverse primer and probe, whereas the forward primer is specific to each of the liberibacter species. The assay was specific as no	*Category : TECHNICAL* **(173) Ghana (30 Sep 2016 12:18 AM)** The levels of sensitivity and specificity of the proposed method should be indicated well in order to compare these methods with other methods.	**Considered, but not incorporated:** The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.

	cross-reactivity was observed with phytoplasmas, viruses, *Xylella fastidiosa*, the citrus-infecting liberibacters and 64 DNA extracts from healthy potato plants both when run as a simplex reaction and when multiplexed with the internal control primers that target the *cytochrome oxidase* (COX) gene (Li *et al*., 2009). The detection limit of the real-time PCR when multiplexed with the COX internal control primers was about 20 copies of the 16S rDNA templates of '*Ca*. L. solanacearum' for field-collected potato samples, and it was about tenfold more sensitive than conventional PCR with the LsoF/OI2c primer pair (Li *et al*., 2009).		
77	Real-time PCR is performed using the assay designed to target the 16S rRNA gene (Li *et al*., 2009). This assay is based on the real-time PCR designed to detect the three citrus-infecting ~~liberibacter~~ 'Ca. Liberibacter' species (Huanglongbing) by Li *et al*. (2006). All liberibacter species use the same reverse primer and probe, whereas the forward primer is specific to each of the liberibacter species. The assay was specific as no cross-reactivity was observed with phytoplasmas, viruses, *Xylella fastidiosa*, the citrus-infecting liberibacters and 64 DNA extracts from healthy potato plants both when run as a simplex reaction and when multiplexed with the internal control primers that target the *cytochrome oxidase* (COX) gene (Li *et al*., 2009). The detection limit of the real-time PCR when multiplexed with the COX internal control primers was about 20 copies of the 16S rDNA templates of '*Ca*. L. solanacearum' for field-collected potato samples, and it was about tenfold more sensitive than conventional PCR with the LsoF/OI2c primer pair (Li *et al*., 2009).	*Category : TECHNICAL* **(137) EPPO (29 Sep 2016 4:45 PM)** See previous comment	**Incorporated**.
77	Real-time PCR is performed using the assay designed to target the 16S rRNA gene (Li *et al*., 2009). This assay is based on the real-time PCR designed to detect the three citrus-infecting liberibacter species (Huanglongbing) by Li *et al*. (2006). All liberibacter species use the same reverse primer and probe, whereas the forward primer is specific to each of the liberibacter species. The assay was specific as no cross-reactivity was observed with phytoplasmas, viruses, *Xylella fastidiosa*, the citrus-infecting liberibacters and 64 DNA extracts from healthy potato plants both when run as a simplex reaction and when multiplexed with the internal control primers that target the *cytochrome oxidase* (COX) gene (Li *et al*., 2009). The detection limit of the real-time PCR when multiplexed with the COX internal control primers was about 20 copies of the 16S rDNA templates of '*Ca*. L. solanacearum' for field-collected potato samples, and it was about tenfold more sensitive than conventional PCR with the LsoF/OI2c primer pair (Li *et al*., 2009).	*Category : TECHNICAL* **(90) Kenya (29 Sep 2016 3:17 PM)** Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods.	**Considered, but not incorporated:** The comparison of sensitivity is described in the final sentence of Paragraph 77 and Paragraph 89.
77	Real-time PCR is performed using the assay designed to target the 16S rRNA gene (Li *et al*., 2009). This assay is based on the real-time PCR designed to detect the three citrus-infecting ~~liberibacter~~ 'Ca. Liberibacter' species (Huanglongbing) by Li	*Category : TECHNICAL* **(87) European Union (29 Sep 2016 3:14 PM)** See earlier comment.	**Incorporated**.

	et al. (2006). All liberibacter species use the same reverse primer and probe, whereas the forward primer is specific to each of the liberibacter species. The assay was specific as no cross-reactivity was observed with phytoplasmas, viruses, *Xylella fastidiosa*, the citrus-infecting liberibacters and 64 DNA extracts from healthy potato plants both when run as a simplex reaction and when multiplexed with the internal control primers that target the *cytochrome oxidase* (COX) gene (Li *et al*., 2009). The detection limit of the real-time PCR when multiplexed with the COX internal control primers was about 20 copies of the 16S rDNA templates of '*Ca*. L. solanacearum' for field-collected potato samples, and it was about tenfold more sensitive than conventional PCR with the LsoF/OI2c primer pair (Li *et al*., 2009).		
82	The 25 µl reaction mixture consists of a final concentration of 1× TaqMan real-time PCR master mix, 250 nM of each primer, 120 nM probe and 2 µl DNA template. Depending on the master mix used, additional $MgCl_2$ may need to be added to ensure that the final concentration is 6.0 mM. All samples are tested in duplicate. The amplification conditions are an initial denaturation step of 95 °C for 20 s followed by 40 cycles of 95 °C for 1 s and 58 °C for 40 s. Cycling conditions may vary depending on the type of master mix and machine used (e.g. some mixes require a polymerase activation step of 95 °C for 10 min, and mixes that contain uracil-DNA glycosylase (UDG) require an initial hold at 50 °C for 2 min). Real-time PCR results are analysed with the manufacturer's software.	*Category : TECHNICAL* **(138) EPPO (29 Sep 2016 4:45 PM)** Regarding the comment on cycling conditions, it may also be worth noting that denaturation times (within the cycling) are often longer than 1s, and this time may need optimizing to produce robust results with different PCR master mixes	**Incorporated**.
82	The 25 µl reaction mixture consists of a final concentration of 1× TaqMan real-time PCR master mix, 250 nM of each primer, 120 nM probe and 2 µl DNA template. Depending on the master mix used, additional $MgCl_2$ may need to be added to ensure that the final concentration is 6.0 mM. All samples are tested in duplicate. The amplification conditions are an initial denaturation step of 95 °C for 20 s followed by 40 cycles of 95 °C for 1 s and 58 °C for 40 s. Cycling conditions may vary depending on the type of master mix and machine used (e.g. some mixes require a polymerase activation step of 95 °C for 10 min, and mixes that contain uracil-DNA glycosylase (UDG) require an initial hold at 50 °C for 2 min). Real-time PCR results are analysed with the manufacturer's software.	*Category : TECHNICAL* **(88) European Union (29 Sep 2016 3:15 PM)** Regarding the comment on cycling conditions, it may also be worth noting that denaturation times (within the cycling) are often longer than 1s, and this time may need optimizing to produce robust results with different PCR master mixes.	**Incorporated**.
82	The 25 µl reaction mixture consists of a final concentration of 1× TaqMan real-time PCR master mix, 250 nM of each primer, 120 nM probe and 2 µl DNA template. Depending on the master mix used, additional $MgCl_2$ may need to be added to ensure that the final concentration is 6.0 mM. All samples are tested in duplicate. The amplification conditions are an initial denaturation step of 95 °C for 20 s followed by 40 cycles of 95 °C for 1 s and 58 °C for 40 s. Cycling conditions may vary depending on the type of master mix and machine used (e.g. some mixes	*Category : EDITORIAL* **(61) Philippines (29 Sep 2016 9:21 AM)** should be in a table format the same with other draft Annexes for ISPM 27	**Considered, but not incorporated:** It is not a requirement for the reaction mixture and cycling conditions to be in a table format in all diagnostic protocols.

	require a polymerase activation step of 95 °C for 10 min, and mixes that contain uracil-DNA glycosylase (UDG) require an initial hold at 50 °C for 2 min). Real-time PCR results are analysed with the manufacturer's software.		
83	The presence of amplifiable DNA in the plant extracts can be confirmed using the COX primers and probe of Weller *et al*. (2000):	*Category : TECHNICAL* **(139) EPPO (29 Sep 2016 4:45 PM)** The COX primers are for plant samples	**Incorporated**.
83	The presence of amplifiable DNA in the plant extracts can be confirmed using the COX primers and probe of Weller *et al*. (2000):	*Category : TECHNICAL* **(89) European Union (29 Sep 2016 3:17 PM)** The COX primers are for plant samples.	**Incorporated**.
87	The reaction mixture for the COX assay has the same components and is cycled under the same conditions as the '*Ca*. L. solanacearum' real-time PCR so the two assays can be run simultaneously in separate tubes. Alternatively, they can be multiplexed in the same tube if the probes are labelled with different reporter dyes and the primer and probe concentrations have been optimized to prevent low '*Ca*. L. solanacearum' levels being outcompeted by high levels of plant genes used as the internal control.	*Category : TECHNICAL* **(141) EPPO (29 Sep 2016 4:45 PM)** The cycling conditions stated for the 'Ca. L. solanacearum' test are NOT those in the original reference for the Weller et al COX assay. If it has been demonstrated that the alternate conditions work comparably then this should be stated.	**Incorporated**.The reaction mixture and cycling conditions for the COX assay has been updated as per the original reference.
87	The reaction mixture for the COX assay has the same components and is cycled under the same conditions as the '*Ca*. L. solanacearum' real-time PCR so the two assays can be run simultaneously in separate tubes. Alternatively, they can be multiplexed in the same tube if the probes are labelled with different reporter dyes and the primer and probe concentrations have been optimized to prevent low '*Ca*. L. solanacearum' levels being outcompeted by high levels of plant genes used as the internal control.	*Category : SUBSTANTIVE* **(140) EPPO (29 Sep 2016 4:45 PM)** In a multiplex format the analytical sensitivity decreases significantly and many false negative occur. It is suggested to remove this possibility.	**Incorporated**.The multiplex possibility has been removed.
87	The reaction mixture for the COX assay has the same components and is cycled under the same conditions as the '*Ca*. L. solanacearum' real-time PCR so the two assays can be run simultaneously in separate tubes. Alternatively, they can be multiplexed in the same tube if the probes are labelled with different reporter dyes and the primer and probe concentrations have been optimized to prevent low '*Ca*. L. solanacearum' levels being outcompeted by high levels of plant genes used as the internal control.	*Category : SUBSTANTIVE* **(91) European Union (29 Sep 2016 3:19 PM)** 1. In a multiplex format the analytical sensitivity decreases significantly and many false negative occur. It is suggested to remove this possibility. 2. (TECH.) The cycling conditions stated for the 'Ca. L. solanacearum' test are NOT those in the original reference for the Weller et al COX assay. If it has been demonstrated that the alternate conditions work comparably then this should be stated.	1. **Incorporated**. The multiplex possibility has been removed. 2. **Incorporated**. The reaction mixture and cycling conditions for the COX assay has been updated as per the original reference.
88	**3.3.4 Conventional PCR**	*Category : TECHNICAL* **(35) United States of America (21 Sep 2016 8:06 PM)** Suggest adding a section after 3.3.4 for Gel Electrophoresis.	**Considered, but not incorporated:** Gel electrophoresis is a standard laboratory technique so not necessary to include.

89	Conventional PCR is performed using the same forward primer as the real-time PCR assay designed by Li *et al*. (2009) to a region of the 16S rRNA gene that is unique to '*Ca*. L. solanacearum'. The forward primer is used in combination with the universal liberibacter reverse primer of Jagoueix *et al*. (1996). The conventional PCR primer pair LsoF/OI2c demonstrated the same specificity for '*Ca*. L. solanacearum' as the real-time PCR but was about tenfold less sensitive than it (Li *et al*., 2009).	*Category : SUBSTANTIVE* **(142) EPPO (29 Sep 2016 4:45 PM)** We do not think that this couple of primers should be recommended to perform conventional PCR in the framework of detection for the following reasons: i) The size of the amplicon is too long making the reaction less efficient and decreasing the sensitivity of the method; ii) As the DNA region amplified in real-time PCR with LsoF/HLBr primers is included in the region amplified in conventional PCR by LsoF/OI2c primers there is the risk of contamination from the conventional PCR to real-time PCR during the conventional PCR amplicon manipulation. To perform conventional PCR for detection it is suggested to use the primers (LsoTX16/23 F and R) of Ravindran et al (2011), that target the 16S/23S intergenic region. In addition, in our experience and as reported by Teresani et al., (2014) LsoTX16/23 F and R are most sensitive. However it is recognized that for haplotype determination amplification of the 16S rRNA region is needed. An option could be to transfer this test to the identification section and cross refer to it but recommended preferably the conventional PCR test of Ravindran et al (2011) to confirm positive results with the real-time PCR of Li et al (2009) (although sensitivity may be an issue) as noted in this paragraph	**Incorporated**.The conventional PCR has been changed to the one of Ravindran et al. (2011).
89	Conventional PCR is performed using the same forward primer as the real-time PCR assay designed by Li *et al*. (2009) to a region of the 16S rRNA gene that is unique to '*Ca*. L. solanacearum'. The forward primer is used in combination with the universal liberibacter reverse primer of Jagoueix *et al*. (1996). The conventional PCR primer pair LsoF/OI2c demonstrated the same specificity for '*Ca*. L. solanacearum' as the real-time PCR but was about tenfold less sensitive than it (Li *et al*., 2009).	*Category : SUBSTANTIVE* **(92) European Union (29 Sep 2016 3:20 PM)** We do not think that this couple of primers should be recommended to perform conventional PCR in the framework of detection for the following reasons: i) The size of the amplicon is too long making the reaction less efficient and decreasing the sensitivity of the method; ii) As the DNA region amplified in real-time PCR with LsoF/HLBr primers is included in the region amplified in conventional PCR by LsoF/OI2c primers there is the risk of contamination from the conventional PCR to real-time PCR during the conventional PCR amplicon manipulation. To perform	**Incorporated**.The conventional PCR has been changed to the one of Ravindran et al. (2011).

		conventional PCR for detection it is suggested to use the primers (LsoTX16/23 F and R) of Ravindran et al (2011), that target the 16S/23S intergenic region. In addition, in our experience and as reported by Teresani et al., (2014) LsoTX16/23 F and R are most sensitive. However it is recognized that for haplotype determination amplification of the 16S rRNA region is needed. An option could be to transfer this test to the identification section and cross refer to it but recommended preferably the conventional PCR test of Ravindran et al (2011) to confirm positive results with the real-time PCR of Li et al (2009) (although sensitivity may be an issue) as noted in this paragraph.	
89	Conventional PCR is performed using the same forward primer as the real-time PCR assay designed by Li *et al*. (2009) to a region of the 16S rRNA gene that is unique to '*Ca*. L. solanacearum'. The forward primer is used in combination with the universal liberibacter reverse primer of Jagoueix *et al*. (1996). The conventional PCR primer pair LsoF/OI2c demonstrated the same specificity for '*Ca*. L. solanacearum' as the real-time PCR but was about tenfold less sensitive than it (Li *et al*., 2009).	*Category : TECHNICAL* **(34) United States of America (21 Sep 2016 8:05 PM)** Regarding the reference for Li et al, 2009 in the last sentence, is this the correct Li 2009 article? Li et al published at least two articles in 2009, and the US thinks this information may be from one of the other 2009 articles, not the article referenced in this DP. Please check.	**Incorporated**. Yes, this is the correct reference.
89	Conventional PCR is performed using the same forward primer as the real-time PCR assay designed by Li *et al*. (2009) to a region of the 16S rRNA gene that is unique to '*Ca*. L. solanacearum'. The forward primer is used in combination with the universal liberibacter reverse primer of Jagoueix *et al*. (1996). The conventional PCR primer pair LsoF/OI2c demonstrated the same specificity for '*Ca*. L. solanacearum' as the real-time PCR but was about tenfold less sensitive ~~than it~~ (Li *et al*., 2009).	*Category : EDITORIAL* **(44) United States of America (21 Sep 2016 8:16 PM)** Grammar fix	**Incorporated**.
93	The 25 µl reaction mixture consists of a final concentration of 1× Taq DNA polymerase buffer containing 2.5 mM $MgCl_2$, 200 nM of each primer, 200 µM dNTPs, 1 U Taq DNA polymerase and 2 µl DNA template. The amplification conditions are an initial denaturation step of 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 1 min, and a final extension step of 72 °C for 10 min. Cycling conditions may vary depending on the type of master mix and machine used. The amplicon size is 1 163 base pairs (bp).	*Category : EDITORIAL* **(62) Philippines (29 Sep 2016 9:21 AM)** should be in a table format the same with other draft Annexes for ISPM 27	**Considered, but not incorporated:** It is not a requirement for the reaction mixture and cycling conditions to be in a table format in all diagnostic protocols.
97	The reaction mixture for the 28S rRNA assay has the same components and is cycled under the same conditions as the '*Ca*. L. solanacearum' conventional PCR	*Category : TECHNICAL* **(143) EPPO (29 Sep 2016 4:45 PM)** There is little experience with the primer in	**Modified:** The 28Sf/28Sr primers described here are universal to all eukaryotes. The

	so the two assays can be run simultaneously in separate tubes. The 28Sf/28Sr primer pair produces a 500–600 bp amplicon.	the region is 500-600 specific enough?	size of the amplicon varies depending on the presence of expansion domains. This has been clarified in the protocol.
97	The reaction mixture for the 28S rRNA assay has the same components and is cycled under the same conditions as the '*Ca*. L. solanacearum' conventional PCR so the two assays can be run simultaneously in separate tubes. The 28Sf/28Sr primer pair produces a 500–600 bp amplicon.	*Category : TECHNICAL* **(93) European Union (29 Sep 2016 3:21 PM)** There is little experience with the primer in the region is 500-600 specific enough?	**Modified:** The 28Sf/28Sr primers described here are universal to all eukaryotes. The size of the amplicon varies depending on the presence of expansion domains. This has been clarified in the protocol.
98	**3.3.5 Nested PCR** **3.3.6 Controls for molecular tests**	*Category : TECHNICAL* **(48) Japan (26 Sep 2016 4:55 PM)** "3.3.5 Nested PCR"should be added after "3.3.4 Conventional PCR" because there is a case that conventional PCR cannot amplify target DNA in a low concentration of bacteria.	**Considered, but not incorporated:** No validated nested PCR assays are available for this organism.
99	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used.	*Category : TECHNICAL* **(144) EPPO (29 Sep 2016 4:45 PM)** The internal control is described here as a minimum control to be included for molecular tests. Even if this control is appropriate to check the quality of the DNA extracted, this additional reaction may interfere with the pest specific reaction, by decreasing the sensitivity of the tests. It is not clear in this protocol if this internal control affects the sensitivity of the tests described. Additionnally this internal control may be conducted as a separate reaction, but then it would greatly increase the cost of the test. For these reasons, it is suggested to recommend the use of internal control, but not to make it obligatory.	**Incorporated:** Based on previous EPPO comments, the multiplex possibility with internal control primers has been removed. An internal control PCR is essential to check the competency of the nucleic acid extraction.
99	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used.	*Category : TECHNICAL* **(94) European Union (29 Sep 2016 3:22 PM)** The internal control is described here as a minimum control to be included for molecular tests. Even if this control is appropriate to check the quality of the DNA extracted, this additional reaction may interfere with the pest specific reaction, by decreasing the sensitivity of the tests. It is not clear in this protocol if this internal control affects the sensitivity of the tests described. Additionally this internal control may be conducted as a separate reaction, but then it would greatly increase the cost of	**Incorporated:** Based on previous EPPO comments, the multiplex possibility with internal control primers has been removed. An internal control PCR is essential to check the competency of the nucleic acid extraction.

		the test. For these reasons, it is suggested to recommend the use of internal control, but not to make it obligatory.	
99	For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used.	*Category : TECHNICAL* **(5) France (5 Aug 2016 4:28 PM)** The internal control is described here as a minimum control to be included for molecular tests. Even if this control is appropriate to check the quality of the DNA extracted, this additional reaction may interfere with the pest specific reaction, by decreasing the sensitivity of the tests. It is not clear in this protocol if this internal control affects the sensitivity of the tests described. Additionnally this internal control may be conducted as a separate reaction, but then it would greatly increase the cost of the test. For these reasons, it is suggested to recommend the use of internal control, but not to make it obligatory.	**Incorporated:** Based on previous EPPO comments, the multiplex possibility with internal control primers has been removed. An internal control PCR is essential to check the competency of the nucleic acid extraction.
100	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction), and specifically the amplification. Pre-prepared (stored) DNA extracted from an infected host or a synthetic control (e.g. cloned PCR product) may be used.	*Category : SUBSTANTIVE* **(63) Philippines (29 Sep 2016 9:23 AM)** where can we avail this?	It is the responsibility of the diagnostic laboratory to find a source of positive controls. It is difficult to specifically recommend where to source positive controls. For guidance you could contact the authors lisited in section 6.
100	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction), and specifically the amplification. Pre-prepared (stored) DNA extracted from an infected host or a synthetic control (e.g. cloned PCR product) may be used.	*Category : TECHNICAL* **(36) United States of America (21 Sep 2016 8:07 PM)** Do not recommend adding the example of cloned PCR products because those make the test prone to contaminations.	**Considered, but not incorporated:** In some instances a cloned PCR product is the only option available and when used at the correct concentration there is minimal risk of contamination.
103	Additional controls that could be considered for each series of nucleic acid extractions from the test samples are described below.	*Category : TECHNICAL* **(145) EPPO (29 Sep 2016 4:45 PM)** Should this also include a positive extraction control?	**Considered, but not incorporated:** No, this is not necessary when the internal control PCR is included.
103	Additional controls that could be considered for each series of nucleic acid extractions from the test samples are described below.	*Category : TECHNICAL* **(95) European Union (29 Sep 2016 3:22 PM)** Should this also include a positive extraction control?	**Considered, but not incorporated:** No, this is not necessary when the internal control PCR is included.
103	~~Additional controls that could be considered for each series of nucleic acid extractions from the test samples are described below.~~	*Category : EDITORIAL* **(64) Philippines (29 Sep 2016 9:25 AM)** No need to say additional control, we just simply add it to the above mentioned controls	**Considered, but not incorporated:** This is standard wording by the IPPC.

104	**Negative extraction control.** ~~This control~~ As an additional control, this is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positive samples are expected.	*Category : EDITORIAL* **(65) Philippines (29 Sep 2016 9:27 AM)**	**Considered, but not incorporated:** This is standard wording by the IPPC.
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010).	*Category : TECHNICAL* **(147) EPPO (29 Sep 2016 4:45 PM)** There is a mistake, the Ct value, as mentioned in this paragraph, has not to be checked in each laboratory, but the Ct cut off value has to be.	**Incorporated.**
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010).	*Category : TECHNICAL* **(146) EPPO (29 Sep 2016 4:45 PM)** The protocol recommends a procedure to check the Ct cut off value. But the method proposed by Chandelier et al. (2010) is not appropriate for the labs which will not have a large set of value for positive samples. This is obviously the case for the laboratories performing the test for the first time. Additionnally Chandelier et al. (2010) indicate to confirm real time PCR positive results by isolation, which is almost impossible for unculturable bacterium such as Candidatus Liberibacter solanacearum. Use of conventional PCR to confirm a real time PCR result is also questionable as it often leads to non confirmation due to a reduced sensitivity compared to real time PCR. This paragraph should be revised.	**Modified.** The authors of the published qPCR method in the diagnostic protocol did not provide any information on Ct cut-off values. The Chandelier et al. (2010) reference was provided as guidance to address a comment raised during expert consultation stage. After consideration of the comments raised during member consultation it was considered better to remove the sentence "The procedure for determining the Ct cut-off value is described in Chandelier et al. (2010)". This would then keep it consistent with the text used for this section with other IPPC DPs and leave it up to the individual laboratory to determine their own Ct cut-off values if needed.
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010).	*Category : TECHNICAL* **(96) European Union (29 Sep 2016 3:23 PM)** 1. There is a mistake, the Ct cut off value has to be checked in each laboratory not the Ct value, as mentioned in this paragraph. 2. The protocol recommends a procedure to check the Ct cut off value. But the method proposed by Chandelier et al. (2010) is not appropriate for the labs which will not have a large set of value for positive samples. This is obviously the case for the laboratories performing the test for the first time. Additionnally Chandelier et al. (2010) indicate to confirm real time PCR positive	**Incorporated**

		results by isolation, which is not possible for unculturable bacterium such as Candidatus Liberibacter solanacearum. Use of conventional PCR to confirm a real time PCR result is also questionable as it often leads to non confirmation due to a reduced sensitivity compared to real time PCR. This paragraph should be revised.	
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010). RT-PCR machines should be properly calibrated.	*Category : TECHNICAL* **(37) United States of America (21 Sep 2016 8:08 PM)** Suggested addition	**Considered, but not incorporated:** This statement is not considered necessary as it is standard laboratory practice.
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010).	*Category : TECHNICAL* **(7) France (5 Aug 2016 4:39 PM)** The protocol recommends a procedure to check the Ct cut off value. But the method proposed by Chandelier et al. (2010) is not appropriate for the labs which will not have a large set of value for positive samples. This is obviously the case for the laboratories performing the test for the first time. Additionnally Chandelier et al. (2010) indicate to confirm real time PCR positive results by isolation, which is almost impossible for unculturable bacterium such as Candidatus Liberibacter solanacearum. Use of conventional PCR to confirm a real time PCR result is also questionable as it often leads to non confirmation due to a reduced sensitivity compared to real time PCR. This paragraph should be revised.	**Incorporated.**
111	A sample will be considered positive if it produces an exponential amplification curve. The cycle threshold (Ct) value needs to be verified in each laboratory when implementing the test for the first time. The procedure for determining the Ct is described in Chandelier *et al*. (2010).	*Category : TECHNICAL* **(6) France (5 Aug 2016 4:31 PM)** There is a mistake, the Ct value, as mentioned in this paragraph, has not to be checked in each laboratory, but the Ct cut off value has to be.	**Incorporated.**
119	The minimum identification requirement for '*Ca*. L. solanacearum' is a positive result from one of the PCR assays described. The PCR assays described in this protocol are specific to '*Ca*. L. solanacearum'. However, if the outcome is critical (e.g. post-entry quarantine sample, new host record, new distribution), the conventional PCR should be performed (section 3.3.4) and the product should be sequenced. For the sequence to be considered as the same species as '*Ca*. L. solanacearum', it should be ≥99% identical to the sequence from the reference isolate (GenBank accession number EU834130).	*Category : SUBSTANTIVE* **(149) EPPO (29 Sep 2016 4:45 PM)** One of the conventional PCR recommended here is the one of Li et al. 2009 The cited accession number EU834130 corresponds to a sequence of the 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence; and tRNA-Ile and tRNA-Ala genes, complete sequence. With the conventional PCRs described here it is not possible to amplify	**Modified:** The conventional PCR has been changed to the one of Ravindran et al. (2011). It is not important that the reference sequence is longer than the amplicon it is being compared against. Blast analysis of the shorter amplicon will provide a nucleotide identity to the relevant region from the reference sequence. The level of

		the complete part of the genome. This means that the comparison between the amplified product and the reference sequence will be incomplete. Furthermore, for the 16S-23S ribosomal RNA intergenic spacer, the level of homology to be reached for the conclusion on identity is above 98%. For the 50S gene, the level of homology to be reached for the conclusion on identifity is above 97% rather than 99% as mentionned currently in the protocol It is suggested that the conventional PCR by Ravindran et al, (2011) as described here beneath (see 4.1.2) should also be used for confirmation (see previous comment in the section detection). Ravindran PCR is targeted to a different Target locus (the ITS region) instead of repeated detection of the 16S rRNA gene that was previously detected by the Real time PCR of Li et al 2009. The PCR product from the Ravindran PCR can thereafter be sequenced for higher confidence of the identity confirmation	identity has been updated to ≥98%.
119	The minimum identification requirement for '*Ca*. L. solanacearum' is a positive result from one of the PCR assays described. The PCR assays described in this protocol are specific to '*Ca*. L. solanacearum'. However, if the outcome is critical (e.g. post-entry quarantine sample, new host record, new distribution), the conventional PCR should be performed (section 3.3.4) and the product should be sequenced. For the sequence to be considered as the same species as '*Ca*. L. solanacearum', it should be ≥99% identical to the sequence from the reference isolate (GenBank accession number EU834130).	*Category : TECHNICAL* **(148) EPPO (29 Sep 2016 4:45 PM)** For consistency of the protocol, the reference sequence for 50S gene could be mentioned in this paragraph, additionnally to their mention in parapgraph 134 (EU834131).	**Modified:** The reference sequence for the 50S gene is not relevant in this section as the amplicon being sequenced is the 16S-23S rRNA IGS region. Has been added to para 134.
119	The minimum identification requirement for '*Ca*. L. solanacearum' is a positive result from one of the PCR assays described. The PCR assays described in this protocol are specific to '*Ca*. L. solanacearum'. However, if the outcome is critical (e.g. post-entry quarantine sample, new host record, new distribution), the conventional PCR should be performed (section 3.3.4) and the product should be sequenced. For the sequence to be considered as the same species as '*Ca*. L. solanacearum', it should be ≥99% identical to the sequence from the reference isolate (GenBank accession number EU834130).	*Category : SUBSTANTIVE* **(97) European Union (29 Sep 2016 3:25 PM)** 1. One of the conventional PCR recommended here is the one of Li et al. 2009 The cited accession number EU834130 corresponds to a sequence of the 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence; and tRNA-Ile and tRNA-Ala genes, complete sequence. With the conventional PCRs described here it is not possible to amplify the complete part of the genome. This means that the comparison between the	1. **Modified**: The conventional PCR has been changed to the one of Ravindran et al. (2011). It is not important that the reference sequence is longer than the amplicon it is being compared against. Blast analysis of the shorter amplicon will provide a nucleotide identity to the relevant region from the reference sequence. 2. **Modified:** The reference sequence for the 50S gene is not relevant in this section as this

		amplified product and the reference sequence will be incomplete. Furthermore, for the 16S-23S ribosomal RNA intergenic spacer, the level of homology to be reached for the conclusion on identity is above 98%. For the 50S gene, the level of homology to be reached for the conclusion on identity is above 97% rather than 99% as mentioned currently in the protocol. It is suggested that the conventional PCR by Ravindran et al, (2011) as described here beneath (see 4.1.2) should also be used for confirmation (see previous comment in the section detection). Ravindran PCR is targeted to a different Target locus (the ITS region) instead of repeated detection of the 16S rRNA gene that was previously detected by the Real time PCR of Li et al 2009. The PCR product from the Ravindran PCR can thereafter be sequenced for higher confidence of the identity confirmation. 2. (TECH.) For consistency of the protocol, the reference sequence for 50S gene could be mentioned in this paragraph, additionally to their mention in paragraph 134 (EU834131).	amplicon being sequenced is the 16S-23S rRNA IGS region. It has been added to paragraph 134.
119	The minimum identification requirement for '*Ca*. L. solanacearum' is a positive result from one of the PCR assays described. The PCR assays described in this protocol are specific to '*Ca*. L. solanacearum'. However, if the outcome is critical (e.g. post-entry quarantine sample, new host record, new distribution), the conventional PCR should be performed (section 3.3.4) and the product should be sequenced. For the sequence to be considered as the same species as '*Ca*. L. solanacearum', it should be ≥99% identical to the sequence from the reference isolate (GenBank accession number EU834130).	*Category : TECHNICAL* **(9) France (5 Aug 2016 4:49 PM)** For consistency of the protocol, the reference sequence for 50S gene could be mentioned in this paragraph, additionnally to their mention in parapgraph 134 (EU834131).	**Modified:** The reference sequence for the 50S gene is not relevant in this section as this amplicon being sequenced is the 16S-23S rRNA IGS region. It has been added to paragraph 134.
119	The minimum identification requirement for '*Ca*. L. solanacearum' is a positive result from one of the PCR assays described. The PCR assays described in this protocol are specific to '*Ca*. L. solanacearum'. However, if the outcome is critical (e.g. post-entry quarantine sample, new host record, new distribution), the conventional PCR should be performed (section 3.3.4) and the product should be sequenced. For the sequence to be considered as the same species as '*Ca*. L. solanacearum', it should be ≥99% identical to the sequence from the reference isolate (GenBank accession number EU834130).	*Category : SUBSTANTIVE* **(8) France (5 Aug 2016 4:48 PM)** The cited accession number EU834130 corresponds to a sequence of the 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence; and tRNA-Ile and tRNA-Ala genes, complete sequence. With the conventional PCRs described here it is not possible to amplify the complete part of the genome. This means that the comparison between the amplified product and the reference	**Modified:** The conventional PCR has been changed to the one of Ravindran et al. (2011). It is not important that the reference sequence is longer than the amplicon it is being compared against. Blast analysis of the shorter amplicon will provide a nucleotide identity to the relevant region from the reference sequence. The level of identity has been updated to

		sequence will be incomplete. Furthermore, for the 16S-23S ribosomal RNA intergenic spacer, the level of homology to be reached for the conclusion on identity is above 98%. For the 50S gene, the level of homology to be reached for the conclusion on identifity is above 97% rather than 99% as mentionned currently in the protocol.	≥98%.
125	The 16S-23S rRNA IGS region is amplified using the primers of Ravindran *et al*. (2011):	*Category : TECHNICAL* **(150) EPPO (29 Sep 2016 4:45 PM)** Even if these primers are more sensitive than Lp Frag4-(1611F)/Lp Frag4-480R (Hansen et al., 2008), they miss a part of the sequence containing additional discriminative SNPs (Nelson et al.,2011, 2013). If the aim of this additional reaction is to confirm a real-time PCR result, it is better to keep them and add a comment about their limit.	**Modified:** A note has been added about the primers missing some SNPs in the 16S-23S rRNA IGS region.
125	The 16S-23S rRNA IGS region is amplified using the primers of Ravindran *et al*. (2011):	*Category : TECHNICAL* **(98) European Union (29 Sep 2016 3:28 PM)** Even if these primers are more sensitive than Lp Frag4-(1611F)/Lp Frag4-480R (Hansen et al., 2008), they miss a part of the sequence containing additional discriminative SNPs (Nelson et al.,2011, 2013). If the aim of this additional reaction is to confirm a real-time PCR result, it is better add a comment about their limit.	**Modified:** A note has been added about the primers missing some SNPs in the 16S-23S rRNA IGS region.
125	The 16S-23S rRNA IGS region is amplified using the primers of Ravindran *et al*. (2011):	*Category : TECHNICAL* **(10) France (5 Aug 2016 4:51 PM)** Even if these primers are more sensitive than Lp Frag4-(1611F)/Lp Frag4-480R (Hansen et al., 2008), they miss a part of the sequence containing additional discriminative SNPs (Nelson et al.,2011, 2013). If the aim of this additional reaction is to confirm a real-time PCR result, it is better to keep them and add a comment about their limit.	**Modified:** A note has been added about the primers missing some SNPs in the 16S-23S rRNA IGS region.
135	The sequence from the unknown haplotype is aligned with the reference sequences for the 16S rRNA gene and the 16S-23S rRNA IGS region (GenBank accession number EU812559) and the 50S *rplJ* and *rplL* ribosomal protein genes (GenBank accession number EU834131). The haplotype is determined by comparing the sequence at each of the nucleotide positions listed in Table 1.	*Category : TECHNICAL* **(152) EPPO (29 Sep 2016 4:45 PM)** It would be helpful to add a comment regarding the number of snps required for a new haplotype to be assigned.	**Considered, but not incorporated:** The number of SNPs required to be considered a new haplotype has not yet been determined.
135	The sequence from the unknown haplotype is aligned with the reference sequences for the 16S rRNA gene and the 16S-23S rRNA IGS region (GenBank accession	*Category : TECHNICAL* **(151) EPPO (29 Sep 2016 4:45 PM)**	**Considered, but not incorporated:** The original

	number ~~EU812559)~~ EU812559.1) and the 50S *rplJ* and *rplL* ribosomal protein genes (GenBank accession number EU834131). The haplotype is determined by comparing the sequence at each of the nucleotide positions listed in Table 1.	Correction of accession number	number provided is correct. The ".1" suffix is not required.
135	The sequence from the unknown haplotype is aligned with the reference sequences for the 16S rRNA gene and the 16S-23S rRNA IGS region (GenBank accession number ~~EU812559)~~ EU812559.1) and the 50S *rplJ* and *rplL* ribosomal protein genes (GenBank accession number EU834131). The haplotype is determined by comparing the sequence at each of the nucleotide positions listed in Table 1.	*Category : TECHNICAL* **(100) European Union (29 Sep 2016 3:31 PM)** 1. Correction of accession number. 2. It would be helpful to add a comment regarding the number of snps required for a new haplotype to be assigned.	**Considered, but not incorporated:** 1. The original number provided is correct. The ".1" suffix is not required. 2. The number of SNPs required to be considered a new haplotype has not yet been determined.
160	~~C~~G	*Category : SUBSTANTIVE* **(153) EPPO (29 Sep 2016 4:45 PM)** The SNP for haplotype B is "G" and not "C" as described in table 2 in Nelson et al. (2011).	**Incorporated.**
160	~~C~~G	*Category : SUBSTANTIVE* **(101) European Union (29 Sep 2016 3:32 PM)** The SNP for haplotype B is "G" and not "C" as described in table 2 in Nelson et al. (2011).	**Incorporated.**
160	~~C~~G	*Category : SUBSTANTIVE* **(11) France (5 Aug 2016 4:53 PM)** The SNP for haplotype B is "G" and not "C" as described in table 2 in Nelson et al. (2011).	**Incorporated.**
176	16S rRNA / ~~1039~~1049	*Category : TECHNICAL* **(154) EPPO (29 Sep 2016 4:45 PM)** In Nelson et al 2013b the position is 1049	**Incorporated.**
176	16S rRNA / ~~1039~~1049	*Category : TECHNICAL* **(102) European Union (29 Sep 2016 3:33 PM)** In Nelson et al the 2013 position is 1049.	**Incorporated.**
242	16S-23S rRNA IGS / 1920	*Category : TECHNICAL* **(155) EPPO (29 Sep 2016 4:45 PM)** After position 1920, in Nelson et al 2013b there are also positions: 1943, 2055, 2081, 2218, 2260.	**Incorporated.**
242	16S-23S rRNA IGS / 1920	*Category : TECHNICAL* **(103) European Union (29 Sep 2016 3:34 PM)** After position 1920, in Nelson et al 2013b there are also positions: 1943, 2055, 2081, 2218, 2260.	**Incorporated.**
314	50S *rplJ* and *rplL* / ~~785~~786	*Category : TECHNICAL* **(156) EPPO (29 Sep 2016 4:45 PM)** In Nelson et al. 2013b this position is indicated as 786	**Incorporated.**

314	50S *rplJ* and *rplL* / ~~785~~786	*Category : TECHNICAL* **(104) European Union (29 Sep 2016 3:35 PM)** In Nelson et al. 2013b this position is indicated as 786.	**Incorporated.**
320	50S *rplJ* and *rplL* / ~~849~~850	*Category : TECHNICAL* **(157) EPPO (29 Sep 2016 4:45 PM)** In Nelson et al. 2013b this position is indicated as 850	**Incorporated.**
320	50S *rplJ* and *rplL* / ~~849~~850	*Category : TECHNICAL* **(105) European Union (29 Sep 2016 3:36 PM)** In Nelson et al. 2013b this position is indicated as 850.	**Incorporated.**
336	~~C~~T	*Category : SUBSTANTIVE* **(158) EPPO (29 Sep 2016 4:45 PM)** The SNP for haplotype D is "T" and not "C". This was observed by aligning the available 50S sequences in Genbank.	**Incorporated.**
336	~~C~~T	*Category : SUBSTANTIVE* **(106) European Union (29 Sep 2016 3:37 PM)** The SNP for haplotype D is "T" and not "C". This was observed by aligning the available 50S sequences in Genbank.	**Incorporated.**
336	~~C~~T	*Category : SUBSTANTIVE* **(12) France (5 Aug 2016 4:55 PM)** The SNP for haplotype D is "T" and not "C". This was observed by aligning the available 50S sequences in Genbank.	**Incorporated.**
343	–	*Category : TECHNICAL* **(159) EPPO (29 Sep 2016 4:45 PM)** A footnote to the table is required explaining what this means (presumably deletion?).	**Incorporated.**
343	–	*Category : TECHNICAL* **(107) European Union (29 Sep 2016 3:38 PM)** A footnote to the table is required explaining what this means (presumably deletion?).	**Incorporated.**
374	50S *rplJ* and *rplL* / ~~1068~~1072	*Category : TECHNICAL* **(160) EPPO (29 Sep 2016 4:45 PM)** In Nelson et al. 2013b this position is indicated as 1072	**Incorporated.**
374	50S *rplJ* and *rplL* / ~~1068~~1072	*Category : TECHNICAL* **(108) European Union (29 Sep 2016 3:38 PM)** In Nelson et al. 2013b this position is indicated as 1072.	**Incorporated.**
378	~~C~~T	*Category : SUBSTANTIVE* **(161) EPPO (29 Sep 2016 4:45 PM)**	**Incorporated.**

		The SNP for the haplotype D is "T" and not "C". This was observed after aligning the available 50S sequences from Genbank. Obviously if the value was C for all haplotypes, then, this is not a SNP that leads to identification of haplotypes.	
378	~~C~~T	*Category : SUBSTANTIVE* **(109) European Union (29 Sep 2016 3:39 PM)** The SNP for the haplotype D is "T" and not "C". This was observed after aligning the available 50S sequences from Genbank. Obviously if the value was C for all haplotypes, then, this is not a SNP that leads to identification of haplotypes.	**Incorporated.**
378	~~C~~T	*Category : SUBSTANTIVE* **(13) France (5 Aug 2016 4:57 PM)** The SNP for the haplotype D is "T" and not "C". This was observed after aligning the available 50S sequences from Genbank. Obviously if the value was C for all haplotypes, then, this is not a SNP that leads to identification of haplotypes.	**Incorporated.**
404	Source: Teresani *et al*. (2014).	*Category : SUBSTANTIVE* **(162) EPPO (29 Sep 2016 4:45 PM)** This reference (Teresani et al. (2014) includes several mistakes in SNP values, it would be good to mention that it is adapted from Teresani et al (2014) and Nelson et al. (2011).	**Incorporated.**
404	Source: Teresani *et al*. (2014).	*Category : SUBSTANTIVE* **(110) European Union (29 Sep 2016 3:40 PM)** This reference (Teresani et al. (2014) includes several mistakes in SNP values, it would be good to mention that it is adapted from Teresani et al (2014) and Nelson et al. (2011 & 2013b).	**Incorporated.**
404	Source: Teresani *et al*. (2014).	*Category : SUBSTANTIVE* **(14) France (5 Aug 2016 5:04 PM)** This reference (Teresani et al. (2014) includes several mistakes in SNP values, it would be good to mention that it is adapted from Teresani et al (2014) and Nelson et al. (2011).	**Incorporated.**
420	**8. References**	*Category : EDITORIAL* **(38) United States of America (21 Sep 2016 8:09 PM)** Recommend adding the following references per US comments:	**Modified:** The references of Liefting et al. (2008, 2009a) identified the liberibacter in potatoes and tomatoes. In order to keep the number of references

		Abad, J. A., Bandla, M., French-Monar, R. D., Liefting, L. W., and Clover, G. R. G. 2009. First report of the detection of 'Candidatus Liberibacter' species in Zebra Chip diseaseinfected potato plants in the United States. Plant Dis. 93:108. French-Monar, R.D., F. Patto, J.M. Douglas, J.A. Abad, G. Schuster, W. Wallace, and T.A. Wheeler. 2010. First report of "Candidatus Liberibacter solanacearum" on field tomatoes in the United States. Plant Disease 94: 481. Lee, I.-M., Bottner, K. D., Munyaneza, J. E., Davis, R. E., Crosslin, J. M., du Toit, L. J., & Crosby, T. 2006. Carrot purple leaf: A new spiroplasmal disease associated with carrots in Washington State. Plant Dis. 90:989-993.	to a minimum the appropriate sentence has been modified to reflect this. The Lee et al. (2006) reference has been included.
426	**Cooper, W.R., Alcala, P.E. & Barcenas, N.M.** 2015. Relationship between plant vascular architecture and within-plant distribution of '*Candidatus* Liberibacter solanacearum' in potato. *American Journal of Potato Research*, 92: 91–99.	*Category : EDITORIAL* **(175) Ghana (30 Sep 2016 12:28 AM)** In the references column, from Cooper, W.R., Alcala, P.E. & Barcenas, N.M. to Liefting, L.W., Weir, B.S., Pennycook, S.R. & Clover, G.R.G should be rearranged to follow sequence in the year of publications and also the number of authors.	**Considered, but not incorporated:** Did not change as the IPPC style lists references according by author rather than by year.
463	**9. Figures**	*Category : TECHNICAL* **(39) United States of America (21 Sep 2016 8:09 PM)** Suggest including pictures of RT-PCR and conventional PCR.	**Considered, but not incorporated:** PCR pictures are not generally included in IPPC diagnostic protocols.
470		*Category : SUBSTANTIVE* **(66) Philippines (29 Sep 2016 9:29 AM)** Add figure for control sample	**Considered, but not incorporated:** Figures have been added for diseased samples. A figure for a healthy potato tuber not considered necessary.
471	**Figure 3.** Slices of raw (left) and fried (right) tubers of *Solanum tuberosum* (potato) infected with '*Candidatus* Liberibacter solanacearum'~~.~~ Showing Zebra Chip symptom	*Category : SUBSTANTIVE* **(67) Philippines (29 Sep 2016 9:30 AM)** as shown in figure 3	**Considered, but not incorporated:** Zebra chip is mentioned in the body of the text that refers to this figure. This comment is not necessary.