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REPORT

Technical Panel on Diagnostic Protocols (TPDP)

**Virtual Meeting
23 November 2021**

IPPC Secretariat

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1. Opening of the Meeting

1.1 Welcome

- [1] The Standard Setting Specialist Ms Aoife CASSIN, from the International Plant Protection Convention (IPPC) Secretariat (hereafter referred to as “the Secretariat”), welcomed the participants to this focused virtual meeting of the Technical Panel on Diagnostic Protocols (TPDP) to revise and recommend the draft DP *Candidatus Liberibacter on Citrus* spp. (2004-010) to recommend to the Standards Committee (SC) for adoption.

2. Meeting arrangements

2.1 Selection of the Chairperson

- [2] The IPPC Secretariat acted as Chairperson for this meeting.

2.2 Selection of the Rapporteur

- [3] Mr Robert TAYLOR (Plant Health & Environment Laboratory, New Zealand) was selected as Rapporteur.

2.3 Adoption of the agenda

- [4] The TPDP adopted the agenda (Appendix 1).

3. Administrative matters

- [5] The IPPC Secretariat noted that Mr Álvaro SEPÚLVEDA LUQUE, Ms Liping YIN, Ms Colette C. JACONO, Mr Norman B. BARR, Ms Juliet GOLDSMITH, Ms Gèraldine ANTHOINE and Mr Brendan RODONI could not attend the meeting. The participants list is presented in Appendix 2.

4. Recommendations to the SC for adoption

4.1 Revision and approval of *Candidatus Liberibacter on Citrus* spp. (2004-010)

- [6] The draft diagnostic protocol (DP) for *Candidatus Liberibacter* spp. on *Citrus* spp. (2004-010) is in the TPDP work programme with priority 2¹. It was submitted to Expert Consultation in 2016² and discussed by the TPDP at their July 2016 meeting³.
- [7] Despite some challenges with the drafting group to get their commitment to revise the draft DP, it was revised to address the main substantial points and to update with recent information. The draft DP was presented to the TPDP in March 2021 via eForum, the same document that was discussed by the TPDP in April 2021⁴, for approval for recommending it to the Standards Committee (SC) for consultation period.
- [8] The draft DP went to the 2021 consultation period from 01 July 2021 to 30 September 2021⁵. Three hundred and seven comments were received. Mr Robert TAYLOR, Referee and author of this draft DP, presented the background document draft DP for *Candidatus Liberibacter on Citrus* spp. (2004-010),

¹ List of topics for IPPC standards: <https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/list>

² Expert Consultation on Draft Diagnostic Protocols (ECDPs): <https://www.ippc.int/en/core-activities/expert-consultation-draft-diagnostic-protocols/2016/04/candidatus-liberibacter-spp-on-citrus-spp-2004-010/>

³ 2016-07 TPDP meeting report:

https://assets.ippc.int/static/media/files/publication/en/2016/10/Report_TPDP_2016_July_2016-10-20_UOMfwV7.pdf

⁴ 2021-04 TPDP Virtual Meeting Report: <https://www.ippc.int/en/publications/89834/>

⁵ Consultation of draft ISPMs: <https://www.ippc.int/en/core-activities/standards-setting/member-consultation-draft-ispm/>

which is the same document that was presented to the TPDP via eForum⁶ in November 2021, ahead of the virtual meeting. It was noted that some country comments were duplicated or editorial comments. Therefore, there were some substantive comments to be considered by TPDP.

[9] Although the quorum was not achieved, the TPDP proceeded with the revision of the draft DP, as some TPDP members provided their comments prior to the meeting via eForum⁶. The substantive comments were introduced for the consideration of the TPDP, as follows:

- The DP has no PCR protocols published later than 2014. Additional PCR protocols (Fujikawa and Iwanami, 2012; Zheng *et al.* 2016) have been recommended by two countries.
- EPPO on behalf of the EU provided many technical comments and are about to publish a revised version of their *Liberibacter* diagnostic protocol.
- Several member countries wanted additional information added to the pest information section on sampling and the distribution and concentration of *Liberibacter*s within the plant. The DP author commented that EPPO protocol has new information that could be added.
- Several comments were on geographic distribution for specific countries which has since been addressed by referring to broad geographic regions instead of individual countries. The DP author explained that this protocol is specifically listing countries and to address this issue the specific countries were deleted and kept the broad regions, as stated in the instruction to authors;
- A request by one country for the addition of a flowchart;
- EPPO has suggested a revision to the sampling number and selection and the author informed the TPDP that the draft was revised following the EPPO protocol;
- One country did not consider biological indexing a reliable sole testing measure. The wording was revised by the author to show that this was not the intent of the DP.
- EPPO has suggested a new rapid DNA extraction method which has been validated. This has been included in the revised protocol. Several member countries pointed out the missing nucleotide G from primer sequences OI1 (Jagoueix *et al.* 1996) and BLas (Li *et al.* 2006). Bao *et al.*, (2020) found that the HLBas (forward primer) described in Li *et al.*, 2006 was missing a nucleotide G based on analysis of CLas genome sequences and that this affected test sensitivity. The author noted that these primer sequences have been amended accordingly;
- A recommendation to delete Bertolini *et al.* 2014 qPCR method due to a high rate of false positives. The author mentioned that EPPO are no longer using this method, and that he is not aware this qPCR been widely used. Then, the author mentioned that this protocol has now been removed.
- To consider the amplification and sequence analysis of genes other than 16S for the identification of species of *Ca. Liberibacter* spp. The author mentioned that a sentence to suggest this has been included and he is still trying to track down references to support this statement. Sequence analysis appears to be mainly conducted on rDNA genes so this sentence will be re-written.

[10] The TPDP considered these issues and reviewed the text of the draft DP.

[11] **Pest information.** One TPDP member commented on the acronyms HLB x CLas or CLaf, saying that HLB is widely used, sometimes instead of CLas, and asked the TPDP to make a decision on it. The

⁶ 2021_eTPDP_Nov_01: Review of draft DP *Candidatus Liberibacter* spp. on *Citrus* spp. (2004-010): https://www.ippc.int/en/forum/2021_etpdp_nov_01-review-of-draft-dp-candidatus-liberibacter-spp-on-citrus-spp-2004-010/

author reminded the group that HLB is used as the abbreviation of the disease and it should be used when the text refers to it. Otherwise, CLas or CLaf should be used, when referring to the pathogen. The Secretariat will ask the editor to cross-check the two acronyms to check their consistency.

- [12] One TPDP member provided a comment suggesting a change of the order of two sentences to the end of the paragraph, which was incorporated. The same TPDP member suggested to remove the term “upwards” from the sentence, once it was contradicting with the earlier sentence that says that *Ca. Liberibacter* species are “restricted” to the sieve tubes within the phloem. The comment was discussed by the TPDP and the author noted to check the reference and update the draft, if necessary.
- [13] ‘*Candidatus Liberibacter americanus*’. One TPDP member sent a comment⁷ to define the studied temperatures. The author noted to check the reference to address the comment and update the draft, if necessary.
- [14] Some country comments requested to include the references for the papers cited in the text in the References section. The Secretariat noted that the editor will review all of this and update the References section as needed.
- [15] One TPDP commented that the term Huanglongbing was already mentioned previously, so the acronym HLB could be used. The comment was addressed, the text adjusted and a reminder to check the acronyms in the whole text was done.
- [16] **Taxonomic information.** The TPDP discussed a country comment regarding the synonyms ‘Liberobacter’ and ‘Liberibacter’. The author replied that he had difficulties finding the original paper, but he believes that this is not a reason for an objection to the adoption of the protocol.
- [17] One TPDP member asked for clarification on the sentence regarding the new species discovered in Brazil (CLam). The author proposed a rephrasing of the sentence during the meeting and the TPDP members agreed.
- [18] The TPDP also discussed a comment on the “target DNA” and the author noted that he will update his response to the compiled comments document that will be made available to IPPC Contracting Parties (CPs), since part of the text was removed during the meeting to address other comment.
- [19] **Detection.** One TPDP member suggested three editorial changes to the first paragraph of this section. The TPDP agreed and the changes were incorporated. Another TPDP member commented on repetition of information in the same paragraph regarding CLas not being culturable, which the author noted and will revise accordingly.
- [20] Regarding the second paragraph of this section, one TPDP member commented that the information on the high titre should not be understood as being absolute, since there are plenty of psyllids in HLB positive orchards that tested negative for HLB. The text was adjusted by the author to address the comment and other editorial changes.
- [21] **Symptoms.** There was a general comment from a TPDP member regarding the description of symptoms and how these can be confused with nutritional disorders and other diseases, which can be used as an initial diagnostic tool, not excluding additional steps and analysis. The suggestion was to re-write the sentence to complete the information. The Secretariat reminded the TPDP members that symptoms are an important part of the diagnostic process. The TPDP discussed and the author will check the information to make sure that the comment will be properly addressed and consistent with adopted DPs, as necessary.
- [22] One TPDP member suggested some editorial changes to the last sentence of the first paragraph of this section. The TPDP agreed and the suggested changes were incorporated to the text. The author will check the compiled comments to make sure they were properly addressed.

- [23] **Sampling and sample preparation.** One TPDP member suggested an editorial change in the first paragraph of this section, which the author accepted.
- [24] **Biological detection (graft transmission).** One TPDP member commented that graft transmission is not reliable for diagnosis as it depends on many factors to be considered a reliable tool, and also considering the low rate of grafting transmission mentioned in the text. The author agreed that 'biological indexing is not the best method and agreed to rephrase the sentence and update the country comment to "modified" instead of "incorporated".
- [25] **CTAB extraction.** One TPDP member suggested to add text regarding the options for incubation at 65°C using a thermometer or a water bath with tube inversion, which was accepted and the text was rephrased to accommodate both options. The same TPDP member suggested a change in the volume of the tube because the way it was originally written meant that there is no room for mixing and could also cause aerosols when the tubes are opened, which can potentially cause contamination. The author agreed to update the text on the size of the tube and provide a reference for the method.
- [26] **Conventional PCR.** An editorial change regarding the removal of the reference to Levy (2007) from the sentence was addressed during the meeting.
- [27] **Conventional PCR using the primers of Jagoueix et al. (1996).** One TPDP member asked if the organisms mentioned in the sixth paragraph of this section were tested using these primers and suggested to re-write the sentence for clarification. The DP author reminded the TPDP that in the Instructions to DP authors it is recommended to provide information about the specificity of the test. The sentence was rephrased.
- [28] **Table 1.** This table presents the master mix composition, cycling conditions and amplicons for conventional PCR using the primers of Jagoueix *et al.* (1996). One TPDP member queried if the primers final concentrations of 1.0 µM are correct, since it seem to be very high. The author noted the concern and agreed to review the original paper to check the primer concentrations, mainly regarding the DNA volume, as well as the others present in this protocol.
- [29] **HLBas (forward primer).** One TPDP member commented that the CLas-4G forward primer sequence is not correct and the sequence that was deleted would be the corrected one. She reminded that also the name of the primer is different, not only the sequence. The author explained that this forward primer sequence (CLas-4G) was suggested by some countries during the consultation based on a reference from 2020. One of the country comments mentioned that a nucleotide was missing in the previous sequence (HLBas). One TPDP member reinforced that a nucleotide was missing, but that the new sequence is completely different from the previous one. The author informed the TPDP that the reasons for the recommendation of the CLas-4G sequence by the countries was to provide more sensitivity to the assays and to prevent potential false negative results. One TPDP member raised the idea to include the nucleotide missing in the HLBas sequence and provide the two options of sequences, but the TPDP noted that in the reference the nucleotide is missing. The author and TPDP agreed to include more text in the new paragraph for clarification and the two sequences of primers with the respective references.
- [30] **Real-time PCR using the primers and probes of Bertolini et al. (2014).** One TPDP member asked if the last paragraph of this subsection regarding the diagnostic kit was meant to be deleted. The author confirmed and explained that the diagnostic kit was based on the reference from 2014 cited in the title of the subsection, which was deleted.
- [31] **Table 7.** This table presents the master mix composition and cycling conditions for combined nested PCR and real-time PCR. One TPDP member posed a question regarding the nested PCR and the possibility of using the terms internal" and "external" replacing "inner" and "outer", respectively. One TPDP member reminded that a country also commented on the nested PCR. Another TPDP member suggested another way to refer to the primers as "first round primers" and "second round primers". The

author will review the reference on these primers descriptions and update them if they differ from the reference.

[32] **Positive extraction control.** There was a country comment on the first sentence of the second paragraph of this subsection, requiring a reference that corroborates with the statement: “The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the DNA extraction”. The TPDP discussed and agreed that this initial sentence should be deleted.

[33] **Negative extraction control.** There was a country comment regarding the absence of a negative control and the use of a clean extraction buffer, which was followed by a TPDP discussion. The author explained that this comment will not be incorporated since it is a cross-issue and can affect the whole control section, but he highlighted that the control section needs to be revised in the future.

[34] **Identification** One TPDP member commented via eForum⁶ on this topic noting that the section does not indicate a minimum size of a 16S sequence needed to complete a confirmed identification and asking a question about an authentic sequence for species identification. The author agreed to re-write this section providing the information required and references.

[35] **Records.** Some country comments recommended to include more PCR protocols. The author responded that there is a lack of validation to include one of the suggestions and the other PCR protocol suggested has validation data and could be included. The author asked the TPDP’s opinion. One TPDP member provided some explanation on validation data. The author will incorporate the PCR protocol that has the validation data and not incorporate the other one, with the rationale for this decision.

[36] Another country comment suggested to include a flowchart into this section. One TPDP member commented that based on earlier discussions and attempts, it was concluded that a diagram did not provide clarity because simplified flowcharts oversimplify the process (leading to incorrect information) or become too complicated that they do not explain process better than text and would require significant revision if additional methods are included. The TPDP agreed to not include the flowchart based on this rationale.

[37] The TPDP:

- (1) *thanked* Mr Robert TAYLOR, the Referee and one of the drafting group authors for the updates done in the draft;
- (2) *agreed* for the author to revise the draft DP based on TPDP comments at this meeting, and provide a final version to the IPPC Secretariat by 29 November 2021;
- (3) *agreed to review* the revised draft DP *Candidatus* Liberibacter on *Citrus* spp. via eForum to be presented to the SC to be adopted on behalf of the CPM.

7. Any Other Business

[38] There was no other business.

8. Close of the Meeting

[39] Prior to closing the meeting, the Secretariat informed that the current TPDP eForum⁶ on this draft DP will remain open and once the revised draft is ready, the Secretariat will upload it there to have the agreement of the TPDP members to recommend to the SC for approval.

[40] The Secretariat closed the meeting and thanked the participants for their participation.

[41] A list of action items are presented in Appendix 3 of this report

Appendix 1: Agenda

**2021 VIRTUAL MEETING OF THE
TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS (TPDP)**

23 November 2021

AGENDA

Agenda Item		Document No.	Presenter
1.	Opening of the Meeting		
1.1	Welcome by the IPPC Secretariat	--	CASSIN / MOREIRA
2.	Meeting Arrangements		
2.1	Selection of Chairperson	--	CASSIN / MOREIRA
2.2	Selection of the Rapporteur	--	Chairperson
2.3	Adoption of the Agenda	01_TPDP_Tel_2021_Nov	Chairperson
3.	Administrative Matters		
3.1	Participants / membership	TPDP membership list	MANGILI
3.2	Connections to Zoom and virtual meetings	Short guideline for participants	
4.	Recommendation to the SC for approval for the DP notification period		
4.1	Revision and approval of <i>Candidatus Liberibacter</i> on <i>Citrus</i> spp. (2004-010) <ul style="list-style-type: none"> - Background paper - Compiled comments for Draft DP with Lead's response 	02_TPDP_Tel_2021_Nov (presented in the TPDP eForum) 03_TPDP_Tel_2021_Nov	TAYLOR
5.	Any other business	-	Chairperson
6.	Closing of the meeting <ul style="list-style-type: none"> - Recommendations to SC or IPPC Secretariat 	-	CASSIN/ Chairperson

Appendix 2: Participants list

2021 VIRTUAL MEETING OF THE TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS (TPDP)

23 November 2021

PARTICIPANTS LIST

A check (✓) in column 1 indicates confirmed attendance at the meeting by the time this paper was posted.

	Participant role	Name, mailing, address, telephone	Email address	Term begins	Term ends
	Steward	Mr Álvaro SEPÚLVEDA LUQUE Servicio Agrícola y Ganadero División de Protección Agrícola y Forestal Av. Presidente Bulnes 140, 4 th floor, Santiago, CHILE Tel: + 56-2 234 5120	alvaro.sepulveda@sag.gob.cl		
✓	Bacteriology	Mr Robert TAYLOR Plant Health & Environment Laboratory New Zealand Ministry for Primary Industries 231 Morrin Road St Johns PO Box 2095 Auckland 1140 New Zealand Tel: (+64) 9 909 3548 Fax: (+64) 9 909 5739	Robert.Taylor@mpi.govt.nz	May 2011	2021 (2 nd term)
	Botany	Ms Liping YIN Plant Quarantine Laboratory Animal and Plant Inspection and Quarantine Technology Center Shanghai Entry-Exit Inspection and Quarantine Bureau 1208 Minsheng Road Shanghai, 200135 China Tel: (+86) 21 6854 0577 Fax: (+86) 21 6854 6481	yinlp@shciq.gov.cn ; yinlp2013@hotmail.com	April 2008	2023 (3 rd term)
	Botany	Ms Colette C. JACONO USA / USDA-APHIS-PPQ National Identification Services National Taxonomist - Botany Address: 10300 Baltimore Ave., BARC-W Bldg 12, Rm 10., Beltsville MD 20705-2350 USA Tel. (+1) 240 428 9658	Colette.Jacono@usda.gov	October 2020	2025 (1 st term)

	Participant role	Name, mailing, address, telephone	Email address	Term begins	Term ends
	Entomology	Mr Norman B. BARR Assistant Director Mission Laboratory 22675 N. Moorefield Rd. Moore Air Base Bldg. S-6414 Edinburg, TX 78541 USA Tel. (+1) 956 205 7658 Fax: (+1) 956 205 7680	Norman.B.Barr@aphis.usda.gov	July 2012	2022 (2 nd term)
	Entomology	Ms Juliet GOLDSMITH Plant Health Specialist Caribbean Agricultural Health and Food Safety Agency (CAHFSA) Letitia Vriesdelaan 10 Paramaribo Suriname Tel: (+597) 422 546 Mobile: (+597) 725 2922	julietgoldsmith@gmail.com	November 2014	2024 (2 nd term)
	Nematology	Ms G�r�aldine ANTHOINE Directrice adjointe / Deputy head Chef d'unit� coordination de la r�f�rence / Head of unit "coordination of reference activities" 7 rue Jean Dixm�ras 49044 ANGERS cedex 01 France Tel: (33) 241207431 Fax: (33) 240207430	geraldine.anthoine@anses.fr	April 2009	2024 (3 rd term)
	Virology, and backup for bacteriology	Mr Brendan RODONI Biosciences Research Division AgriBio Centre Ring Road La Trobe University Bundoora 3083 Australia Tel: (+61) 3 9032 7319 Fax: (+61) 3 9800 3521	brendan.rodoni@ecodev.vic.gov.au	July 2012	2022 (2 nd term)
✓	Virology	Ms Vessela Assenova MAVRODIEVA Assistant Laboratory Director, USDA APHIS, PPQ, Beltsville, MD, USA Tel: (+1) 301-313-9208	vessela.a.mavrodieva@usda.gov ; ;	March 2020	2025 (1 st term)
✓	Mycology	Ms Julie PATTEMORE Assistant Director: Plant Pathology, Department of Agriculture, Water and the Environment, Melbourne, Australia Tel: (+61) 3 83186957	julie.pattemore@awe.gov.au	March 2020	2025 (1 st term)
✓	Mycology	Ms Yazmin RIVERA Molecular Biologist, USDA APHIS, PPQ, Beltsville, MD, USA Tel: (+1) 301-313-9273	Yazmin.Rivera@usda.gov ;	March 2020	2025 (1 st term)

	Participant role	Name, mailing, address, telephone	Email address	Term begins	Term ends
✓	IPPC Secretariat Lead	Ms Adriana MOREIRA Standards Officer / Deputy Assistant to Unit Leader IPPC Secretariat / FAO Viale delle Terme di Caracalla 00153 Rome, Italy	Adriana.Moreira@fao.org ;		
✓	IPPC Secretariat Support	Ms Aoife CASSIN Standard Setting Specialist IPPC Secretariat / FAO Viale delle Terme di Caracalla 00153 Rome, Italy	Aoife.Cassin@fao.org		
✓	IPPC Secretariat Support	Ms Erika MANGILI ANDRE Standard Setting Specialist IPPC Secretariat / FAO Viale delle Terme di Caracalla 00153 Rome, Italy	Erika.MangiliAndre@fao.org		

Appendix 3: Action points arising from the November 2021 TPDP meeting**ACTION POINTS ARISING FROM THIS MEETING**

(by agenda item)

Action		Agenda Item	Responsible	Deadline
1.	The TPDP agreed that the discipline lead will revise the draft DP based on TPDP comments at this meeting, and provide a final version to the IPPC Secretariat	4.1	Robert Taylor	29 November 2021
2.	Keep eForum open for the TPDP to approve the draft for <i>Candidatus Liberibacter</i> on <i>Citrus</i> spp. to be presented to the SC for adoption	4.1	IPPC Secretariat	December 2021