



UPDATE ON ACTIVITIES OF THE TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS (TPDP) FROM MAY 2017 TO APRIL 2018

(Prepared by the IPPC Secretariat with input from the TPDP Steward)

1. Background

[1] The Stewards for the Technical Panel on Diagnostic Protocols (TPDP) are:

- Ms Jane CHARD (Steward)
- Ms Janyani Nimanthika WATHUKARAGE (Assistant Steward)

[2] The IPPC Secretariat support for the TPDP are:

- Ms Adriana G. MOREIRA (lead)
- Ms Sandra GORITSCHNIG (support)

[3] The TPDP membership and contact information can be found on [IPP](#)¹. Table 1 shows a simplified version of the TPDP membership as of April 2018.

Table 1. TPDP membership (as of April 2018) and expertise of its members.

| Participant role | Name (country) | Expertise | Term expires |
|-------------------|---|---|--------------------------------------|
| Steward | Ms Jane CHARD (United Kingdom) | | |
| Assistant steward | Ms Janyani Nimanthika WATHUKARAGE (Sri Lanka) | | |
| Member | Mr Robert TAYLOR (New Zealand) | Bacteriology (and backup for mycology) | May 2021 (2 nd term) |
| Member | Ms Liping YIN (China) | Botany | April 2018 (2 nd term) |
| Member | Mr Norman B. BARR (United States) | Entomology | July 2022 (2 nd term) |
| Member | Ms Juliet GOLDSMITH (Jamaica) | Entomology | November 2019 (1 st term) |
| Member | Ms Géraldine ANTHOINE (France) | Nematology | April 2019 (2 nd term) |
| Member | Mr Delano JAMES (Canada) | Virology | November 2020 (2 nd term) |
| Member | Mr Brendan RODONI (Australia) | Virology (and back up for bacteriology) | July 2022 (2 nd term) |

[4] It is noted that the term of Ms Liping YIN (China) is ending in 2018. Ms YIN has confirmed her employer's support and her willingness to be considered for an additional 5 years term.

[5] In April 2018, the term of Mr Hans DE GRUYTER (Mycology) ended. Depending on the outcome of the Call for Topics: Standards and Implementation and the potential addition of Mycology topics to the List of Topics for IPPC Standards, the SC may consider asking the Secretariat to issue a call for experts in Mycology.

2. TPDP volume of work

[6] The TPDP work programme currently comprises 11 diagnostic protocols (DPs) under six disciplines in various stages of development (Figure 1). All DPs are drafted, except for the DP "Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-

¹ TPDP main page on IPP: <https://www.ippc.int/en/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols/>

028)", which due to a lack of validated and verified data on molecular methods for identification of fruit fly larvae of all genera has "pending status"².

[7] A total of eight draft DPs progressed in the standard setting process in 2017. In 2018, nine of the 11 draft DPs that are currently on the TPDP work programme are projected to advance through the standard setting process (Figure 2). The timeline of adopted DPs shows the high number of DPs adopted in the recent years, representing the management of over 100 DP authors (Figure 3). To date, there are 24 adopted DPs, published as annexes to ISPM 27 (*Diagnostic protocols for regulated pests*).

[8] The TPDP work programme is delivered through several activities. Since May 2017, the activities were as follows:

- one consultation³ (01 July – 30 September 2017): six draft DPs
- one DP notification period⁴ (01 July – 15 August 2017): two draft DPs
- four TPDP e-decisions: two draft DPs and two topics of interest for IPPC DPs.

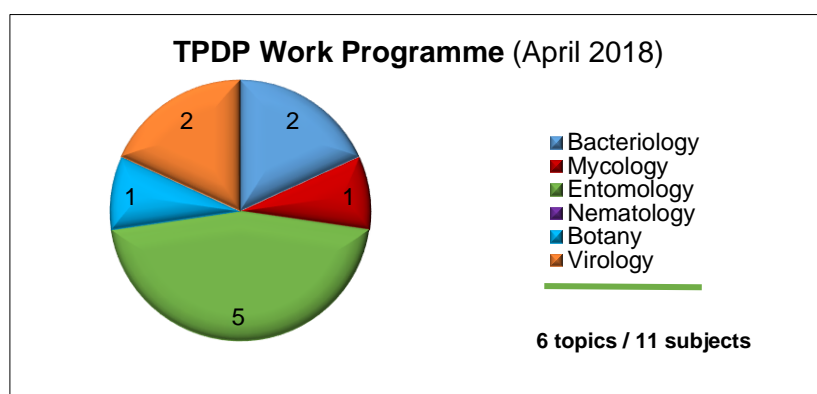


Figure 1. Number of diagnostic protocols per discipline currently in the TPDP work programme.

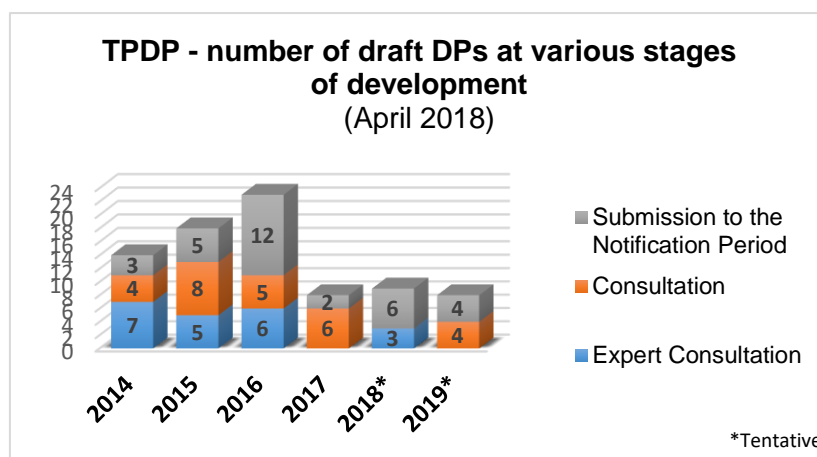


Figure 2. Stages of development of draft diagnostic protocols (annexes to ISPM 27). As for 2018 and 2019, these are tentative numbers.

² List of topics for IPPC standards: <https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/>

³ Consultation on draft ISPMs: <https://www.ippc.int/en/core-activities/standards-setting/member-consultation-draft-ispms/>

⁴ DP notification period: <https://www.ippc.int/en/core-activities/standards-setting/draft-ispms/notification-period-dps/>

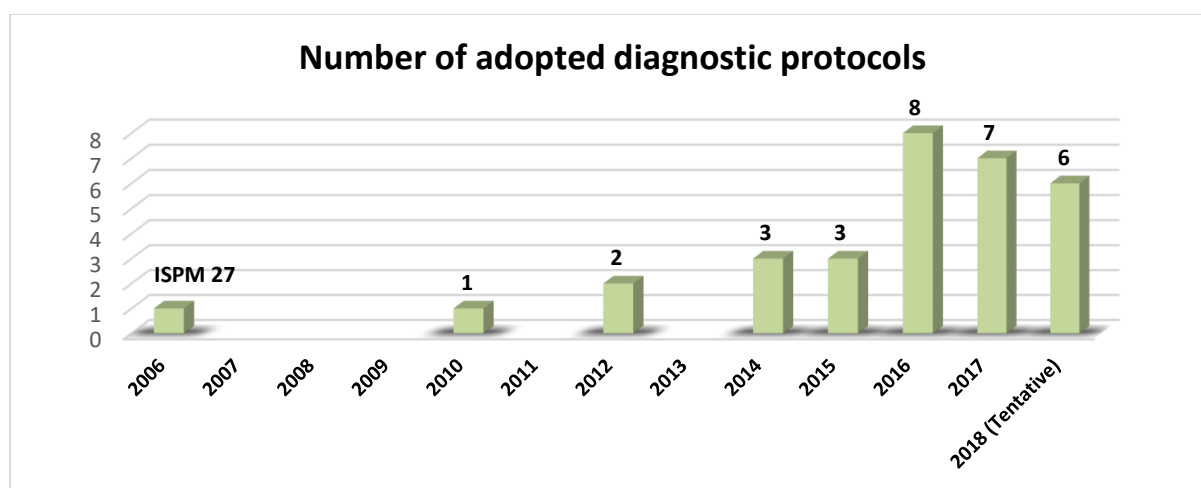


Figure 3. Number of adopted diagnostic protocols (annexes to ISPM 27) per year. As for 2018, this is a tentative number.

3. Highlights of the work

- [9] The TPDP continued to deliver its work programme during the May 2017 to April 2018 period, managing more than 40 DP authors from various countries⁵. In 2017, a total of seven DPs were adopted as annexes to ISPM 27, and six draft DPs were moved through the consultation stage. Detailed information on the draft DPs submitted to the various steps of the standard setting process can be found in the Commission on Phytosanitary Measures (CPM-13) document CPM 2018/12 (*Report on the activities of the Standards Committee*)⁶.
- [10] In addition to drafting of DPs, the panel engaged in several discussions on horizontal issues that may affect diagnostics, such as quality assurance, best practices for DNA sequencing, controls for molecular methods, next generation sequencing (NGS) technologies and interpretation of the results of serological tests. During the recent consultation period in 2017 several issues concerning implementation of the DPs were raised by contracting parties, highlighting the need for further integration of the standard setting and implementation activities of the IPPC.
- [11] The panel also engaged in discussions on the ongoing need to develop new DPs and to update the adopted ones, including discussions on their usefulness, stressing that the DPs are an essential part of surveillance programmes and the foundations for pest reporting. Diagnostic protocols support pest eradication programmes, export certification, import inspections and the application of appropriate phytosanitary treatments. In light of rapid advances in molecular methodologies and the spread of emerging threats, the panel foresees the need for the development of additional DPs and for revisions of existing DPs. The panel also discussed “emerging pests” and agreed that, if an emerging pest is identified, a DP should be developed if there is no appropriate diagnostic protocol available. They also expressed their interest in the results of the 2016 Implementation Review and Support System (IRSS) general survey, which indicated good rates of implementation and highlighted the usefulness of adopted DPs by the NPPOs.
- [12] The TPDP reviewed its working procedures and updated information for the DP drafting groups by revising the Instructions to Authors⁷. The panel highlighted the need for focussed face-to-face meetings for their work, noting the advances achieved during these meetings. In order to efficiently and effectively proceed with the TPDP workplan, noting that there would be at least four draft DPs to have in depth

⁵ IPPC Diagnostic Protocols (DPs) drafting groups: <https://www.ippc.int/en/publications/2582/>

⁶ CPM 2018/12: Report of the activities of the Standards Committee: <https://www.ippc.int/en/publications/85461/>

⁷ Instructions to Authors of Diagnostic Protocols: <https://www.ippc.int/en/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols/>

discussions, the panel proposed having a meeting in January 2019 and the TPDP member from Australia offered to host the meeting in Melbourne.

4. TPDP Meetings

[13] The TPDP held one meeting since May 2017:

- 2018 TPDP February 2018 (face-to-face) meeting: 5 - 9 February 2018, (EPPO, Paris, France)⁸

[14] A summary of the discussions and outcomes of the meeting is detailed below.

2018 TPDP February meeting (Paris, France)

[15] The main objective of the meeting was to revise draft diagnostic protocols (DPs) in various stages of development, discuss several horizontal issues related to DPs and review the TPDP work programme with strategic discussions on the future of the IPPC DPs. The meeting was chaired by Ms Géraldine Anthoine (France) and attended by all seven TPDP members (from Australia, Canada, China, France, New Zealand, the United States and the Caribbean Agricultural Health and Food Safety Agency (CAHFSA)/Jamaica). Additionally, the meeting had the participation of the TPDP Steward Ms Jane Chard, (United Kingdom), the Assistant Steward and co-author of the draft DP for *Striga* spp. Ms Jayani Wathukarage (Sri Lanka), the host and expert Ms Françoise Petter (EPPO), and representatives from the IPPC Secretariat.

[16] The following draft DPs along with comments received during the first consultation⁹ (July – September 2017) were revised in detail:

1. Revision of DP 2: Plum *pox virus* (2016-007)
2. *Xylella fastidiosa* (2004-024)
3. *Austropuccinia psidii* (2006-018)
4. *Bactrocera dorsalis* complex (2006-026)
5. *Conotrachelus nenuphar* (2013-002)
6. *Ips* spp. (2006-020)

[17] The revised versions of these DPs were recommended to the SC for adoption by e-decisions.

[18] With reference to the revision of DP 2: Plum *pox virus* (2016-007) the panel noted the requirement for a major revision of this draft DP, extending beyond the initially intended minor revisions. With this in mind and based on the speed of scientific advances in diagnostic methodology (especially molecular methods), the panel foresees that many of the adopted DPs will be requiring updates and revisions in the coming years.

[19] During the consultation for the draft DP *Bactrocera dorsalis* complex (2006-026) one contracting party requested a future revision of this DP to include larvae identification, once methods are available (see Table 1 in Appendix 1).

[20] Several implementation issues were raised in the consultation comments (see Tables 2 and 3 in Appendix 1). For example, the need for developing capacity was highlighted in view of the increasing use of molecular methods in diagnostic protocols, especially with respect to laboratory infrastructure and staff expertise. Another issue concerned the access to protocols referenced in DPs, and the TPDP recommended that the contact points in the DPs should be contacted for assistance, if necessary. Finally, the panel highlighted the difficulty to obtain access to quarantine pests as reference material as an important implementation issue, since positive controls are essential components in phytosanitary

⁸ TPDP February 2018 meeting report available at: <https://www.ippc.int/en/publications/85736/>.

⁹ Consultation period page: <https://www.ippc.int/en/core-activities/standards-setting/member-consultation-draft-ispms/>

diagnostics. Although the panel acknowledged that this is a country specific implementation issue and that DPs should not provide direct guidance on how to obtain positive controls, the panel felt that it is important to further discuss this issue in the relevant IPPC bodies.

[21] Two draft DPs were also revised and discussed by the panel. These draft DPs are planned to be submitted for expert consultation in 2018 and therefore, to be submitted for first consultation period in 2019. These draft DPs were:

1. *Striga* spp. (2006-020)
2. Begomoviruses transmitted by *Bemisia tabaci* (2006-023)

[22] It was noted that the drafting of the DP for *Candidatus Liberibacter* spp. on *Citrus* (2004-010) had had faced some delays, as the lead author of the DP had to discontinue her role due to other work commitments. A new lead author was agreed by the TPDP, in order to move this draft DP forward as it is considered of high importance and relevance for contracting parties.

[23] As consequential adjustments from the revision of the draft DPs, the TPDP updated the IPPC Instruction to Authors¹⁰. The TPDP has previously said they would develop guidance for DP authors on criteria for inclusion of next generation sequencing (NGS) or high throughput sequencing (HTS) technologies in IPPC DPs. However, this was not yet done because additional information on NGS and the interpretation of NGS results still need to be addressed, noting that CPM-13 will be providing guidance and directions on the use of NGS technologies for diagnostic purposes. In response to several comments from contracting parties on the standard disclaimer for laboratory methods and the use of brand names, and on the duplication of text in the DPs, the TPDP agreed to adjust the disclaimer text to avoid unnecessary duplication. The TPDP further agreed to include the general disclaimers in all draft DPs because it is considered important. The revised standard disclaimers agreed were:

- **Paragraph (in DP body text):** In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define 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.
- **Footnote (only necessary if brand names are mentioned in the methods):** 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.

[24] The Secretariat updated the panel on relevant discussions from the CPM Bureau and SC meetings. Considering the issue of detection of non-viable pests with molecular methods brought up by the TPDP in their last meeting, the panel was informed on the decision of the Bureau to not address pest viability at this time, as there are currently no solutions to the problem of distinguishing live and non-viable pests using molecular methods.

[25] The Secretariat informed the panel about the draft strategic framework (2020-2030) for the IPPC and invited them to review it and provide comments to the discussion at CPM-13 via their CPM representative. The TPDP was very pleased that the formation of a network of diagnostic laboratories was included in the strategic framework, as it highlights the importance of phytosanitary diagnostics and the need to build and share capacity among contracting parties.

[26] The panel members were content with the rate of implementation of DPs by the contracting parties, as evidenced by the 2016 IRSS survey¹¹. The survey revealed a good level of implementation of the IPPC

¹⁰ TP Diagnostic Protocols - Instructions to Authors of diagnostic protocols: <https://www.ippc.int/en/publications/83612/>

¹¹ IRSS 2016 general survey: <http://www.fao.org/3/I7637EN/i7637en.pdf>

DPs that varied between 25 to 40% implementation. The TPDP also briefly discussed the findings from the 2016 IPPC regional workshops questionnaire on global emerging issues¹².

- [27] In continuation of their discussion on the use of NGS technologies in phytosanitary diagnostics, the TPDP noted and supported the CPM-13 side session on gene sequencing and molecular technologies. The panel further agreed to provide any comments on the possible CPM recommendation on the use of NGS technologies for phytosanitary purposes, which was discussed during CPM-13 and will be presented to member consultation in 2018, to their CPM representatives or NPPOs contact points, and to continue their expert support for questions on the use of NGS technologies for routine phytosanitary activities.
- [28] The panel continued discussions on the following horizontal issues relating to phytosanitary diagnostics:
- Guidance on the controls for immunocapture RT-PCR
 - Controls and interpretation of results of ELISA tests
 - Control options for molecular tests for pest group categories
 - Best practices for DNA sequencing
 - Quality assurance for DPs
- [29] The TPDP identified additional areas where they could contribute their expertise in future work. The TPDP noted that, depending on the outcome of the discussion during the CPM-13 on the joint call for standards and implementations, that six pests, which had previously been identified by the panel and forwarded to the SC, be forwarded to be included as gaps in the Framework for Standards and Implementation. A panel member proposed two additional potential topics for international DPs. The TPDP filled the criteria forms for these topics, discussed them in TPDP e-decisions and agreed on forwarding them to the SC for consideration.
- [30] As per SC request on an analysis of the consequences of updating or not updating a DP, the TPDP also noted that there will likely be revisions of existing adopted DPs in the future, following advances in methodologies. It was stressed that if DPs are not updated properly, they have the risk to become outdated and therefore unable to be used. One member questioned whether papers on horizontal topics should be considered in future work plans (e.g. draft CPM recommendation on NGS) and what value those could have for the IPPC contracting parties.
- [31] The TPDP had a strategic discussion on the future of the panel and the suggested arrangements for delivery of the work programme. It had been suggested that, due to financial constraints in the IPPC Secretariat, the work of the panel would slow down, the Secretariat support for the panel be reduced and face-to-face meetings of the panel be replaced by virtual meetings for the time being. However, the panel noted that in view of the efficiency of their work during this meeting, where they processed eight draft DPs in various stages of development, this would be impractical. The Secretariat noted the workload for organizing and processing virtual meetings and suggested that, since several virtual meetings are required for the discussion of a single DP, the workload for the Secretariat would not be reduced by this move. The panel therefore deemed it appropriate to continue having face-to-face meetings for broader and in depth discussions on draft DPs, highlighting that there is still the need to finalize the DPs in their work programme. They considered the possibility of small group virtual meetings to discuss specific topics.
- [32] The TPDP was updated by the invited expert on the development of regional diagnostic protocols by EPPO. The panel noted work done by EPPO on the NGS technologies, on flexible scope in the accreditation of phytosanitary laboratories, on access to reference material and on the importance of proper communication between diagnosticians and pest risk managers.

¹² Global emerging issues – A report of findings from the 2016 IPPC regional workshops questionnaire. URL: <http://www.fao.org/3/a-i8016e.pdf>

- [33] One TPDP member informed the panel that the ISO standard ISO/TC 34/SC 16/13484: *Molecular Biomarker Analysis: General requirements for molecular biology analysis for detection and identification of plant pests* was still in the adoption process, having been rejected twice in the vote. At the moment it is unclear in which form this standard may be adopted, but the TPDP was reminded to be aware of the developments, as outlined in the TPDP specification (Specification TP 1)¹³.
- [34] The panel, through its members, also liaises with the Convention on Biological Diversity (CBD) Secretariat on diagnostic issues related to the Global Taxonomy Initiative (GTI). The TPDP noted that in 2017 the GTI issued a call for proposals for training courses in DNA based methods, and that eleven of these have been selected and will be held throughout 2018. The panel noted the importance of such initiatives to build capacity in novel methods in phytosanitary diagnostics.

5. Tentative work plan for the period May 2018 – April 2019

- [35] The next face to face meeting is tentatively planned to be convened on 28 January – 01 February 2019. The tentative agenda covers an in-depth discussion of four draft DPs and discussions on the future activities of the TPDP and any other points identified by the SC, especially if the CPM-13 agrees on having a call for topics and implementation issues.
- [36] The TPDP will continue to work on the 11 draft DPs remaining on its work programme. It is expected that most of them will be adopted by 2019, with the remainder progressing through the standard setting process as planned. Two expert consultations are tentatively planned to take place in the 3rd and 4th quarter of 2018 for three draft diagnostic protocols.
- [37] In addition, from the new call of topics: standards and implementation, potentially additional draft DPs may be recommended for inclusion in the work programme.
- [38] The TPDP tentative work plan for May 2017 – April 2018 is summarized in Figure 4.

| | 2018 | | | | 2019 | | | | 2020 | | | |
|--|------|----|----|----|------|----|----|----|------|----|----|----|
| Draft DP | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| <i>Xylella fastidiosa</i> (2004-024) | | | | | | | | | | | | |
| <i>Austropuccinia psidii</i> (2006-018) | | | | | | | | | | | | |
| Revision of DP 2: <i>Plum pox virus</i> (2016-007) | | | | | | | | | | | | |
| <i>Bactrocera dorsalis</i> complex (2006-026) | | | | | | | | | | | | |
| <i>Conotrachelus nenuphar</i> (2013-002) | | | | | | | | | | | | |
| <i>Ips</i> spp. (2006-020) | | | | | | | | | | | | |
| Begomoviruses transmitted by <i>Bemisia tabaci</i> (2006-023) | | | | | | | | | | | | |
| <i>Striga</i> spp. (2008-009) | | | | | | | | | | | | |
| <i>Candidatus Liberibacter</i> spp. on <i>Citrus</i> spp. (2004-010) | | | | | | | | | | | | |
| Genus <i>Ceratit</i> (2016-001) | | | | | | | | | | | | |

Legend:

| | |
|--|------------------------|
| | DP with drafting group |
| | expert consultation |
| | first consultation |
| | notification period |

Figure 4: Tentative work plan of the TPDP for the years 2018-2020 with important stages in the drafting process of the current draft DPs in the work programme highlighted. This work plan is based on the current list of topics and may be adjusted as necessary.

¹³ Specification TP 1 - Technical Panel on Diagnostic Protocols: <https://www.ippc.int/en/publications/1297/>

6. Recommendations to the SC

[39] The SC is invited to:

- (1) *agree* that Ms Liping YIN (China) be renewed as TPDP member for Botany for another five-year term, starting in May 2018.
- (2) *acknowledge* the contribution of Mr Hans DE GRUTER who left the TPDP in 2017
- (3) *consider* asking the Secretariat to open a call for experts in Mycology depending on the outcome of the Call for Topics: Standards and Implementation
- (4) *note* the 2018 TPDP February meeting report;
- (5) *note* the TPDP tentative work plan for May 2018– April 2019 (summarized in Figure 4);
- (6) *note* the revised TPDP Instructions to authors of diagnostic protocols (posted on IPP¹⁴ on the TPDP webpage), especially for the standard texts on the use of brand names;
- (7) *note* the request from a contracting party of future revision of the DP on “*Bactrocera dorsalis* Complex (2006-026)” to include larvae identification, once methods are available (see comment 52 of the compiled comments) and *archive* this request for the future;
- (8) *note* the comments and their responses (comments 1, 9, 77 and 123) from the consultation on Revision of the DP 02: *Plum pox virus* (2016-007) on possible implementation issues with regards to appropriate laboratory infrastructure, staff expertise and access to protocols referenced in the DP and *forward* the comments to the Implementation and Capacity Development Committee (IC);
- (9) *note* the comments (comments 176 and 206) from the consultation on *Xylella fastidiosa* (2004-024) on possible implementation issues on the acquisition of positive controls to perform diagnosis and *forward* the comments to the Implementation and Capacity Development Committee (IC);
- (10) *include* in the Framework for Standards and Implementation, as gaps, the following:
 - *Citrus leprosis virus*
 - *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) on *Triticum* spp.
 - *Microcyclus ulei*
 - *Mononychellus tanajoa*
 - *Puccinia graminis* f.sp. *tritici* UG 99
 - *Moniliophthora roreri*
 - *Amaranthus palmeri*
 - *Solanum rostratum*;
- (11) *consider* that Ms Françoise PETTER (EPPO) be invited to the next TPDP face-to-face meeting, as invited expert.

¹⁴ TPDP Instructions to Authors: <https://www.ippc.int/en/publications/1180/>

Appendix 1: Contracting party comments and TPDP's responses from the 2017 consultation to the attention of the SC.

Table 1: Contracting party comment requesting future revision of the DP and TPDP's response from the 2017 consultation of the draft Annex to ISPM27: *Bactrocera dorsalis* complex (2006-026).

| # | Para | Text | Comment | TPDP's response and approved by the SC (2018_eSC_May_06) |
|----|------|---|--|--|
| 52 | 117 | Identification at the level of the species or the <i>Bactrocera dorsalis</i> complex requires morphological examination of adult flies. It is generally difficult and not reliable to morphologically identify eggs, larvae or pupae to the species level. It is not possible to identify a fly to the <i>Bactrocera dorsalis</i> complex using immature life stages. | Russian Federation We consider it necessary either to develop identification of larvae stage as it is the stage that mostly spreads on plant products, e.g. tropical fruits, or to adopt this draft, adding information on larvae identification during further revision of the standard. <i>Category : SUBSTANTIVE</i> | Considered but not Incorporated. Inclusion of new methods for identification in future versions would add value to the protocol. The protocol only includes methods that are currently available. Noted the suggestion for future revision of this DP to include larvae identification. The IPPC Secretariat will archive this proposal for the future. |

Table 2: Contracting party comments highlighting potential implementation issues and TPDP's responses from the 2017 consultation of the draft revision of Annex to ISPM27: DP 02 Plum pox virus (2016-007).

| # | Para | Text | Comment | TPDP's response and approved by the SC (2018_eSC_May_09) |
|---|------|-------------------|--|--|
| 1 | G | (General Comment) | Cameroon Les préoccupations que nous avons sont celles relatives à l'infrastructure et le niveau technique requis pour conduire de tels tests. Les formations et le développement de kits de diagnostic rapide pourraient aider à combler ces lacunes pour les pays de notre région en général. <i>Category : TECHNICAL</i> | Noted. NPPOs are encouraged to seek suitable training from labs/experts identified in Section 6. Contact Points. However, the comment is more an implementation issue and it is outside of the TPDP's remit. It will be forwarded to the relevant IPPC bodies. |
| 9 | G | (General Comment) | Saint Vincent and The Grenadines No additional comments. This standard is highly technical and would be difficult | NOTED NPPOs are encouraged to seek suitable training from |

| # | Para | Text | Comment | TPDP's response and approved by the SC (2018_eSC_May_09) |
|-----|------|---|---|---|
| | | | to be implemented by St. Vincent and the Grenadines <i>Category : SUBSTANTIVE</i> | labs/experts identified in Section 6. Contact Points. However, the comment is more an implementation issue and it is outside of the TPDP's remit. It will be forwarded to the relevant IPPC bodies. |
| 77 | 77 | The only monoclonal antibody currently demonstrated to detect all strains of PPV with high reliability, specificity and sensitivity is 5B-IVIA (Cambra <i>et al.</i> , 2006a). Optimal detection of isolates of strain CR requires adjustment of the extraction buffer to pH 6.0 (Chirkov <i>et al.</i> , 2013; Glasa <i>et al.</i> , 2013). In a DIAGPRO ¹ ring-test conducted by 17 laboratories using a panel of 10 samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using the 5B-IVIA monoclonal antibody was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR) which was 94% accurate (Olmos <i>et al.</i> , 2007; Cambra <i>et al.</i> , 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using the 5B-IVIA monoclonal antibody was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote <i>et al.</i> (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using the 5B-IVIA monoclonal antibody was a true positive. | Philippines Would this protocol/procedure be available online for free once this Annex is approved <i>Category : SUBSTANTIVE</i> | Considered, but not incorporated (All adopted ISPMs and their annexes are publically available on the IPPC website. NPPOs and RPPOs need to be informed of availability online, and the existence of contact points to provide any assistance required. Procedures are provided with the commercial kits) |
| 123 | 145 | DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra <i>et al.</i> (1994), using D- and M-specific monoclonal antibodies (Cambra <i>et al.</i> , 1994; Boscia <i>et al.</i> , 1997), according to the manufacturer's instructions. | Philippines provide protocol as attachment to this Annex <i>Category : SUBSTANTIVE</i> | Considered but not incorporated All adopted ISPMs and their annexes are publically available on the IPPC website. NPPOs and RPPOs need to be informed of availability online, and the existence of contact points to provide any assistance required. Procedures are provided with the commercial kits |

Table 3: Contracting party comments highlighting potential implementation issues and TPDP's responses from the 2017 consultation of the draft Annex to ISPM27: *Xylella fastidiosa* (2002-024).

| # | Para | Text | Comment | TPDP's response and approved by the SC (2018_eSC_May_07) |
|-----|------|--|--|--|
| 176 | 417 | <i>Positive nucleic acid control.</i> This control is used to monitor the efficiency of PCR amplification. Pre-prepared (stored) nucleic acid, whole genomic DNA or a synthetic control (e.g. cloned PCR product) may be used. For this protocol, genomic DNA (50 ng/μl) extracted from either a culture of <i>X. fastidiosa</i> or naturally infected tissue is recommended as a positive nucleic acid control. | Philippines Very difficult to obtain positive controls (PC). If detection will be carried out by countries with no reported occurrence of the pathogen, where to source the PC is a problem. Are there NPPOs willing to share whole genomic DNA for this purpose? <i>Category : SUBSTANTIVE</i> | Considered but not incorporated. The access to reference material is possible from public collections. Table 10 provides guidance on where to source type/pathotype strains. (see response to comment 206) |
| 206 | 541 | The reference <i>X. fastidiosa</i> strains available from different collections are listed in Table 10. These strains are suggested for use as positive controls in biochemical and molecular tests. | Philippines This addresses the concern on difficulty of obtaining positive controls. Section for requesting positive may also be included. NPPO needs guidance on how requests will be conducted. <i>Category : SUBSTANTIVE</i> | Considered, but not incorporated Section on reference material is already included (Section 4.1.2) and it can be obtained from public collections. However, the comment is more an implementation issue and will be forwarded to the relevant IPPC bodies. |