

## CPM recommendation: High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes

### Status box

This is not an official part of the CPM Recommendation and it will be modified by the IPPC Secretariat after adoption.	
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## BACKGROUND

The Commission on Phytosanitary Measures (CPM) recognizes that accurate and timely pest diagnosis underpins export certification, import inspections and the application of appropriate phytosanitary measures<sup>1</sup>. It is widely accepted that the ability to detect and identify a plant pest varies with the accuracy, reproducibility and specificity of the detection tools.

High-throughput sequencing (HTS) technologies, also known as next generation sequencing (NGS) or deep sequencing technologies, are providing a powerful alternative to traditional diagnostic methods for the detection and identification of organisms (e.g. bacteria, fungi, phytoplasmas, viruses, and viroids). However, the HTS-based diagnostic outcomes may not be associated with evidence of living pests or damage to the plant or plant products by these organisms. As such, caution should be applied when interpreting the results of these highly sensitive technologies, such as HTS, for the detection and identification of pests. In particular, due consideration should be given to the risks and consequences of applying HTS-diagnostic results when implementing phytosanitary measures. Furthermore, HTS

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<sup>1</sup> See also CPM recommendation R-07: The importance of pest diagnosis (<https://www.ippc.int/en/publications/84234/>)

technologies may not suit all national plant protection organizations (NPPOs) due to the high cost in platform and other operational costs. It should also be noted that each NPPO may use a different HTS platform.

More information on HTS technologies is provided in Appendix 1.

## **ADDRESSED TO**

Contracting parties and regional plant protection organizations.

## **RECOMMENDATIONS**

The Commission notes that there are existing challenges and further work is needed on HTS technologies for pest detection and identification as the basis for applying phytosanitary regulations. Findings based on HTS technologies of an unknown microorganism need to be further investigated to demonstrate the potential of that microorganism to be a pest that would qualify as a regulated pest.

When a contracting party is proposing to use HTS technologies and their results as the basis for appropriate phytosanitary regulations, the Commission *encourages* contracting parties to:

- (a) *establish* guidelines on what actions should be taken after detection of an unknown organism (e.g. fungi, bacteria or virus) or detection of non-viable organisms in plant material
- (b) *ensure* that appropriate infrastructure and investments in Information Technology and bioinformatics, and education and training on bioinformatics, are in place for the appropriate data storage and interpretation of test results, and that there is effective implementation of these technologies
- (c) *standardise* and *apply* best-practice operational guidelines for HTS, including proper interpretation of results and quality control measures (e.g. procedure controls) that ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way
- (d) *validate* the reliability and accuracy of HTS by conducting trials comparing HTS against other existing diagnostic platforms
- (e) *communicate* information on the interpretation of HTS results, especially regarding conclusions about the phytosanitary risk of organisms detected, to the NPPO of the exporting country
- (f) *implement* HTS training programmes, including delivery of best laboratory practice courses online, and *coordinate* international proficiency testing to independently assess laboratory capability
- (g) *publish* HTS protocols (developed for corresponding HTS platforms), and *share* guidelines and training material for transparency
- (h) *publish* information on the unexpected biological associations of quarantine organisms in plants and plant products that are revealed by HTS.

## **RECOMMENDATION(S) SUPERSEDED BY THE ABOVE**

None.

This appendix is for reference purposes only and is not a prescriptive part of the CPM recommendation.

## **APPENDIX 1**

### **Background**

In December 2017, the Bureau of the Commission on Phytosanitary Measures (CPM) considered a paper prepared by the Standards Committee (SC) which reflected discussions by the IPPC Technical Panel on Diagnostic Protocols (TPDP) on opportunities and challenges in relation to the use of high-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes. The Bureau was asked to agree that the background paper be presented to CPM-13 with a request that the CPM note the challenges associated with the use of HTS technologies and that further work is needed on HTS technologies for pest detection and identification.

The SC prepared a paper on the use of HTS technologies as a diagnostic tool for phytosanitary purposes based on IPPC TPDP discussion on HTS opportunities and challenges.

In December 2017, the CPM Bureau was asked to agree that the background paper be presented to CPM-13 with a request to CPM to note the challenges associated with the use of HTS technologies and that further work is needed on HTS technologies for pest detection and identification.

The CPM Bureau agreed that since this was an emerging issue that would be of interest to contracting parties, a CPM Recommendation should be drafted to provide policy advice and guidance to contracting parties and regional plant protection organizations (RPPOs) on the use of HTS technologies as a diagnostic tool for phytosanitary purposes.

Australia, New Zealand and the European and Mediterranean Plant Protection Organization (EPPO) presented a draft CPM recommendation during CPM-13 and it was agreed to include the topic in the IPPC work programme for a CPM Recommendation on “High Throughput Sequencing technologies as a diagnostic tool for phytosanitary purposes”.

### **What is HTS and how is it different to other testing methods?**

High-throughput sequencing (HTS), also known as next generation sequencing (NGS) or deep sequencing technologies allow the sequencing of the whole genome and can be used for all types of organisms being of particular interest for non-culturable organisms (e.g. viruses and viroids, and some bacteria, oomycetes and fungi). HTS technologies can be used for targeted detection of regulated pests and also allow the detection of unknown organisms (i.e. without a priori knowledge). HTS technologies allow the sequencing of the genetic material, which can be used to identify the genome of microorganisms of phytosanitary interest that currently, with traditional technologies, have not been identified. Applying these technologies has recently resulted in the discovery of previously undetected microorganisms, such as fungi, bacteria, phytoplasmas and particularly viruses where the use of the technology is more advanced than for other pathogens (examples provided in this document are for viruses and viroids). Researchers and diagnosticians using HTS technologies will continue to identify and describe new taxa from among the large volume of as yet undiscovered organisms for which challenging and quick decisions will have to be taken by national plant protection organizations (NPPOs) on the basis of very limited information and imprecisely evaluated potential phytosanitary risks (Olmos *et al.*, 2018). These technologies, therefore, enable a new and comprehensive approach to the detection and characterization of potential pests in a biological sample.

Phytosanitary testing for viruses and viroids in plants and plant products currently relies on a combination of specific (molecular and serological) and generic (visual inspection, electron microscopy and biological indicators or bioassays) approaches. While these methods are currently the best available and widely used in plant pest diagnostic laboratories they have some inherent weaknesses. The specific

tests usually require a priori knowledge of the viral pathogens and each test needs to be developed and validated (including validation of the test for different pest-host combinations), making resource demands on NPPOs. Moreover, such specific tests can also detect nucleic acid or protein traces of disintegrated pathogen particles, resulting in an overestimation of actual pathogen presence. The host range of many pathogens is not well defined and exotic viruses and viroids may not be detected in new pest-host combinations. While bioassays have traditionally been used to detect unknown viruses, further molecular or serological testing is usually required to confirm the identity of the causal agent when disease symptoms are observed. Bioassays are heavily reliant on environmental conditions for symptom expression and often produce ambiguous results as false positives and false negatives.

The time taken for bioassays means that plants spend extended periods of time in post-entry quarantine stations, significantly adding to costs and delays for importers. A further drawback with bioassays is that strains may not be detected if they are asymptomatic on the indicator host. Studies conducted so far have demonstrated HTS to be equivalent to or better than biological indexing assays in detecting viruses and viroids of agronomic significance (Barrero *et al.*, 2017; Mackie *et al.*, 2017; Rott *et al.*, 2017; Rwahnih *et al.*, 2015). Most importantly, the studies demonstrated that HTS is able to produce results significantly quicker than bioassays. Nevertheless, HTS technologies are used alongside other existing tests and do not replace the need to confirm the biological significance of the detected organism.

Owing to the limitations of traditional diagnostic methods, new robust, reliable and cost-effective methods are required to rapidly and reliably screen plants and plant products for viruses and viroids but also for other non-culturable or fastidious pests, and HTS technologies open up such possibilities. Metabarcoding or HTS technologies applied to polymerase chain reaction (PCR) amplicons of a DNA barcode region also have diagnostic applications for phytosanitary purposes. Other than viruses, the aforementioned diagnostic applications for phytosanitary purposes could be the HTS approach that is most likely to have a diagnostic application.

In routine diagnostics, some opportunities and possibilities for the use of HTS technologies are for (1) understanding the pest status in a region through surveillance programmes, (2) certification of nuclear stock and plant propagation material, (3) (post-entry) quarantine testing, and (4) monitoring of imported commodities for new potential pest risks. HTS offers a wide range of benefits for all of these applications (Al Rwahnih *et al.*, 2015; Hadidi *et al.*, 2016; Rott *et al.*, 2017). Nevertheless, challenges are also associated with the implementation of these technologies, such as the requirements for laboratory infrastructure, bioinformatics, data sharing and validation of the data (Olmos *et al.*, 2018).

### **Regulatory and scientific challenges**

As new technologies become available, there are inherent challenges associated with them. HTS technologies have similar challenges to other molecular detection or sequence based detection technologies. However, research findings based on HTS technologies have significant implications within a phytosanitary framework. For example, there is a risk that the movement of plant material may be restricted due to the perceived presence of a (previously unknown) microorganism that does not have the potential to be pathogenic to this plant material. Not all organisms associated with plants are pests and instead are part of the plant microbiome; some may be mutualists providing benefit to the host plant or may be commensal agents. Ensuring that regulatory decisions are made on pests, and not on mutualists or commensal agents, is a key criterion to the adoption of whole genome sequencing as a diagnostic method. There is also the issue, as with other indirect methods, that HTS technologies may detect non-viable organisms.

Correctly identifying or predicting pests from whole genome sequences are two separate but important challenges using these technologies. The correct interpretation of results is another major challenge in using HTS technologies. Very large and well curated databases of the whole genomes or barcodes of known pests and microorganisms are required as the reference for comparison with HTS

generated sequence data. Because of the increased rate of new microorganism discovery, NPPOs will face the challenge of making decisions about the biological significance of a finding, for example the ability of a microorganism to infest plants or plant products, on the basis of nucleic acid data analysis without complete information (or even having no information). This decision-making process, of determining if the organism in question is a pest, distances the diagnostic outcome from any analysis of pathogenicity and poses questions in deciding whether the data are linked to the actual presence of a viable and pathogenic biological entity that is a quarantine pest. However, this same challenge is present with molecular and first generation sequencing methods and particularly for viruses that are “new to science”, so this is not a new problem. Other challenges in using HTS for regulatory purposes are noted by Martin *et al.* (2016), Massart *et al.* (2017) and Olmos *et al.* (2018).

To give NPPOs the confidence to adopt HTS technologies for pest diagnosis, internationally harmonized approaches are required, including the development of operational guidelines for reliably and repeatedly performing HTS including quality controls and validation data to interpret HTS outputs (Boonham *et al.*, 2014). Validation of the technology against existing methods, which also takes into account the limits of current procedures, is also needed. HTS technologies need to be thoroughly validated for each target pest and matrix to demonstrate that they are “fit-for-purpose”. Laboratory protocols would need to be available, along with a description of sample preparation, the process for data analysis and the databases to be used.

### **Global collaboration**

There are a number of initiatives underway in different regions of the world that are exploring the use of HTS technologies as a diagnostic tool for phytosanitary purposes (for example in Australasia, Europe and North America). These include discussions on associated policies that may be developed. Coordination of outcomes from these initiatives is required to progress the timely development of internationally harmonized standards for the use of HTS in a regulatory setting.

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