EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	This test can be used for the untargeted detection and identification of molecularly characterized ssRNA(+), ssRNA(-), dsRNA, cssRNA, dsDNA(-RT), ssDNA viruses and viroids in symptomatic plant samples.
Date, reference of the validation report	2020-07-13 - 2020.molbio.004 v1
Link to other validation data	- 2020.molbio.012 This validation data is for generic detection and identification of phytoplasmas. Phytoplasmas can be detected using real time PCR or conventional nested PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS.
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Viruses and viroids (1VIRUK)
Detection / identification	detection and identification
Method(s)	Molecular HTS
Method: Molecular HTS	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Roenhorst et al. (in preparation)
Is the test modified compared to the reference test	no

Other information	
Other details on the test	Included as VirDisc in EPPO PM7/151 - Appendix 1: Example of high throughput sequencing (HTS) tests for the detection and identification of viruses or viroids
Performance Criteria :	
Organism 1.:	**Viruses and viroids(1VIRUK)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	To determine the analytical sensitivity, a serial 10 times dilution (10^2 till 10^7) of infected S. lycopersicum homogenate in healthy S. lycopersicum homogenate was made in triplicate. HTS test results show that there is a correlation between the dilution and the sequence coverage, i.e. a 10 times dilution of the virus in the plant homogenate resulting in approx. 10 times less viral-sequence reads. As the threshold is set at 10 times read coverage to obtain consensus sequences, no coverage was obtained by de novo assembly at dilution 10^6 and 10^7. At a 10^4 dilution, (near) complete ToBRFV genomes were recovered and at 10^5 partial (fragmented) genomes were obtained. For subsequent virus species -host combinations, the LOD was calculated based on the hypothetical dilution at which (near) complete genomes could still be obtained .
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	The HTS test was successfully applied for the following virus/viroid host combinations, including but not limited to: 1. Citrus tristeza virus (Closterovirus) in Citrus confirmed with ELISA CTV + 2. Cotton leaf curl Gezira virus (Begomovirus) in Lavatera confirmed with RT-PCR-Sequencing generic Begomovirus + 3. Cucumber green mottle mosaic virus (Tobamovirus) in Cucumis sativus confirmed with ELISA CGMMV + 4. Cucumber mosaic virus (Cucumovirus) in Buddleja davidii confirmed with Bioassay P1++, bent-+, Wb+- 5. Cucumber mosaic virus (Cucumovirus) in Capsicum sp. confirmed with ELISA CMV + 6. Potato virus Y - O (Potyvirus) in Capsicum sp. confirmed with ELISA PVY + 7. Tomato chlorotic spot virus (Orthotospovirus) in Capsicum sp. confirmed with RT-PCR-Sequencing generic orthotospovirus TCSV+ 8. Strawberry latent ringspot virus (Stralarivirus) in Rubus idaeus confirmed with ELISA SLRSV + 9. Tobacco ringspot virus (Nepovirus) in Rosmarinus confirmed with ELISA TRSV + 10. Cherry leafroll virus (Nepovirus) in Sambucus nigra confirmed with ELISA CLRV + 11. Pepino mosaic virus (Potexvirus) in Solanum lycopersicum confirmed with ELISA PepMV + 12. Tomato brown rugose fruit virus (Tobamovirus) in Solanum lycopersicum confirmed with real-time RT-PCR specific ToBRFV + 13. Bean yellow mosaic virus (Potyvirus) in Vicia faba

	confirmed with RT-PCR-Sequencing generic potyvirus +
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	Not relevant for this test
Specificity value	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability and reproducibility of the test was investigated with biological material. From each dilution 10^2-10^5 three identical plant homogenate subsamples were made. RNA extraction of two of those subsamples was performed by one person at the same moment and the RNA was sequenced in the same batch (repeatability). The RNA of the third subsample was extracted by another person and sequenced at a different moment. The obtained sequence data was analysed by three qualified assessors independently. At low and medium dilutions (10^2-10^4) the ToBRFV genome was assembled in a single contiguous sequence representing the (near) complete genome with a sequence length between 6379-6353 nt and 100 % identical sequence.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	The repeatability and reproducibility of the test was investigated with biological material. From each dilution 10^2-10^5 three identical plant homogenate subsamples were made. RNA extraction of two of those subsamples was performed by one person at the same moment and the RNA was sequenced in the same batch (repeatability). The RNA of the third subsample was extracted by another person and sequenced at a different moment. The obtained sequence data was analysed by three qualified assessors independently. At low and medium dilutions (10^2-10^4) the ToBRFV genome was assembled in a single contiguous sequence representing the (near) complete genome with a sequence length between 6379-6353 nt and 100 % identical sequence.
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Validation report VirDisc • Validation report VirDisc - Appendices • Additional analyses Analytical Specificity • Additional analyses Analytical Specificity - Appendices • Additional analyses Analytical Sensitivity