

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	Walloon Agricultural Research Centre (CRA-W) Département Sciences du Vivant Unité Santé des plantes et forêts Bâtiment Marchal Rue de Liroux, 4, 5030 Gembloux, Belgium
Short description of the test	Detection of BWYV by RTqPCR
Date, reference of the validation report	2025-11-13 - Detection of BWYV by RTqPCR
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	VIROBETT project (PRW 204/3, grant -D65-1429)
Description of the test	
Organism(s)	Polerovirus BWYV (BWYV00)
Detection / identification	detection and identification
Matrix(ces) tested	Leaves
Plant species tested	Beta vulgaris, Capsella bursa-pastoris, Capsicum annuum, Cucumis sativus, Nicotiana tabacum, Solanum lycopersicum, Spinacia oleracea
Method(s)	Molecular real time RT PCR
Method: Molecular real time RT PCR	
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?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Hellin et al. (under review)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no

Other information	
Reaction type	Duplex
Other details on the test	Reactions were performed in 25µL of final volume using One-Step Takyon Ultra Probe 4X MasterMix (Eurogentec, Belgium) and primers and probe at 250 nM and 80 nM, respectively. The primers and probe set (CyOXID-F, R and -TAQ) designed by Papayiannis et al. (2011) to quantify the level of the mitochondrion cytochrome oxidase subunit I (mtCOXI) from plants were also added in multiplex (at 300 and 100 nM, respectively) to serve as an internal control. Samples were run in duplicates with 3 µL of sample RNA per reaction. The cycling program consisted of a 15 min step at 50°C to enable reverse transcription followed by an activation step at 95°C for 3 min, then by 40 cycles of denaturation at 95°C for 3 s and annealing/extension at 60°C for 1 min, with the intensity of fluorescence captured at the end of every cycle.
Performance Criteria :	
Organism 1.:	Polerovirus BWYV(BWYV00)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Up to 10 ⁵ fold dilution of starting material infected with BWYV isolate P6-6D-W11_BWYV
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% for BWYV isolates
Standard test(s)	BWYV TAS-ELISA (RT-0049) kit from DSMZ
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Target isolates P6-6D-W11_BWYV, BWYV-USA, BWYV-USDA and BLYV-NN as well as synthetic oligonucleotide controls for BWYV (Genbank MW349137.1, HM804471.1, KU521324.1, MW674791.1, OL449448.1) and BLYV (GenBank LC428352.1)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	BChV, BMYV, TuYV, PLRV, CABYV, MABYV (only synthetic controls), CYDV-RPV, UPoIV, PeVYV, BtMV, BYV, BNYVV, CMV, AMV, BSBMV, BSBV and BVQ
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	BWYV TAS-ELISA (RT-0049) kit from DSMZ

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	no

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