

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
<b>Short description of the test</b>	Detection of <i>Xylella fastidiosa</i> by real-time PCR Harper et al., 2010 on composite sample of <i>Helichrysum italicum</i>
<b>Date, reference of the validation report</b>	2020-12-24 - Detection of <i>Xylella fastidiosa</i> by real-time PCR Harper et al., 2010 on composite sample of <i>Helichrysum italicum</i> version 1
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xylella fastidiosa</i> (XYLEFA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 <i>Xylella fastidiosa</i> (version 4)
<b>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 25 DP 25: <i>Xylella fastidiosa</i> (version 2018)
<b>Name of the test</b>	QuickPick SML Plant DNA kit (Bio-Nobile)
<b>Is the test modified compared to the reference test</b>	yes Crushing into sterile demineralized water rather than PBS Centrifugation speeds and durations

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Other details on the test</b>	Pool of 50 plants 2 pieces of stem of 2-3 cm per plant 2 steps of bacterial concentration centrifugation A sonication step is applied
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/024 Xylella fastidiosa (version 4)
<b>Name of the test</b>	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
<b>As or adapted from an IPPC diagnostic protocol</b>	yes
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
<b>Name of the test</b>	Real-time PCR -simplex (Harper et al., 2010;errtum 2013)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Other details on the test</b>	A cut-off of 38 is applied
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xylella fastidiosa(XYLEFA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	10 <sup>4</sup> cells/ with a detetection rate of 100%
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100%
<b>Standard test(s)</b>	QuickPick SML plant DNA kit + Harper et al., 2010 applied on a single naturally infected sample in

	comparison with the evaluated test on the same sample pooled with 49 healthy plants
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	55 target strains Cf. Validation data MA039 v5
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	18 non target strains Cf. Validation data MA039 v5
<b>Specificity value</b>	100%
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100%
<b>Specify the test(s)</b>	QuickPick SML plant DNA kit + Harper et al., 2010 applied on a single naturally infected sample
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Pool of 50 plants Bacterial concentration (Cells/mL) Reproducibility 10 <sup>3</sup> 92,6% 10 <sup>4</sup> 100% 10 <sup>5</sup> 100% Evaluated with 3 replicates per concentration by operators on 3 different days
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Pool of 50 plants Bacterial concentration (Cells/mL) Repeatability 10 <sup>3</sup> 93,4% 10 <sup>4</sup> 100% 10 <sup>5</sup> 100% Evaluated with 3 replicates per concentration by 1 operator on 3 different days
<b>Test performance study</b>	
<b>Test performance study?</b>	no
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Rapport de validation Pool Helichrysum v1</a></li> </ul>

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