

**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>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	detection of Elsinoë species by Molecular real time PCR in Citrus Fruits
<b>Date, reference of the validation report</b>	2023-05-10 - 2020.molbio.003
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	Elsinoë(1ELSIG)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Real-Time PCR Detection of Elsinoë spp. on Citrus Ashleigh J. Elliott, Marcel M. J. P. van Raak, Ann V. Barnes, Christopher J. Field, Aron A. L. A. M. van Duijnhoven, Kathryn Webb, and Bart T. L. H. van de Vossenberg <i>PhytoFrontiers</i> <sup>™</sup> 2023 3:1, 164-172
<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>	Duplex
<b>Other details on the test</b>	18S-ITS1 Elsinoë generic real-time 18S generic internal control
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Elsinoë(1ELSIG)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The level at which both technical replicates produced positive results was averaged between three dilution series and resulted in a limit of detection 12.4 fg at a 99.7% confidence interval.
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	DNA extracted from pure cultures of 11 Elsinoë species were used to test the inclusivity of the test in vitro. All the tested Elsinoë spp. DNA extracts (including all three Elsinoë species known on Citrus and non-Citrus species) resulted in a positive Cq value, with a mean Cq value of 22.0 (SD = 2.4)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Nontarget fungal and bacterial species known to cause disease on citrus fruit were tested to determine the exclusivity of the test when used to test citrus fruit material for Elsinoë species. None of the nontarget fungi and bacteria produced false positive results. Analysis of the DNA extracted from mixed fungal cultures isolated from citrus fruit homogenates resulted in all negative results for the Elsinoë test, suggesting that no cross-reactions are expected with nonpathogenic fungi commonly found on Citrus fruits.
<b>Specificity value</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Interlaboratory comparison between FERA LTD. (United Kingdom) and NIVIP (the Netherlands)
<b>Other information</b>	
<b>Any other information considered useful</b>	The multiplex test was found to be specific to Elsinoë species. Elsinoë is highly host specific, with most pathogens exclusively found on one host species or genus; therefore, the combination of the multiplex real-time PCR, host species, sample origin, and typical symptoms can be used to draw conclusions on the Elsinoë spp. that are present. However, as the test is not specific at the species level, a confirmatory test is recommended for diagnosis in critical cases. Development and validation published at: <a href="https://doi.org/10.1094/PHYTOFR-03-22-0017-FI">https://doi.org/10.1094/PHYTOFR-03-22-0017-FI</a>

