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	detection of Elsinoë species by Molecular real time PCR in Citrus Fruits
Date, reference of the validation report	2023-05-10 - 2020.molbio.003
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?	no
Description of the test	
Organism(s)	Elsinoë (1ELSIG)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time 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?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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 PhytoFrontiers™ 2023 3:1, 164-172
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	18S-ITS1 Elsinoe generic real-time 18S generic internal control
Performance Criteria :	
Organism 1.:	**Elsinoë(1ELSIG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	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)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Nontarget fungal and bacterial species known to cause disease on citrus fruit were tested to determine the exclusively 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.
Specificity value	100%
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	Interlaboratory comparison between FERA LTD. (United Kingdom) and NIVIP (the Netherlands)
Other information	
Any other information considered useful	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: https://doi.org/10.1094/PHYTOFR-03-22-0017-FI

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