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	GEVES 25 rue Georges Morel, 49070 Beaucouzé, France
Short description of the test	Detection of Xanthomonas phaseoli pv. phaseoli and Xanthomonas citri pv. fuscans by real time PCR in seed extract
Date, reference of the validation report	2023-07-03 - Rapport validation SE-qPCR Psp et Xap sur Haricot
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	Collaborative project with Vilmorin-Mikado and HM Clause
Description of the test	
Organism(s)	Xanthomonas phaseoli pv. phaseoli (XANTPH) Xanthomonas citri pv. fuscans (XANTFF)
Detection / identification	detection
Matrix(ces) tested	Pure culture, Seeds
Plant species tested	Phaseolus vulgaris
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Other information	
Other details on the test	See report
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	no

protocol	
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Duplex - Triplex
Other details on the test	Audy / Trb1 and Acat primers (some validation data were generated for simplex or duplex reactions)
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xanthomonas phaseoli pv. phaseoli(XANTPH)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Duplex real-time PCR (Trb1/Acat): Analytical sensitivity was evaluated by Vilmorin-Mikado (FR) by spiking the extract of 3 healthy seeds lots with different concentration of saprophytic flora (low, medium and highly concentrated) with a suspension of a target strain (CFBP 6546 identified as Xanthomonas axonopodis pv. phaseoli). Different dilutions of the target strain were tested. The results showed a detection threshold of 10 CFU/mL. The analytical sensitivity evaluated here corresponds to the detectable concentration present in the macerate (before enrichment).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	same as XANTFF
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	same as XANTFF
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	same as XANTFF
Specificity value	
Cross-reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	same as XANTFF
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	same as XANTFF
Repeatability	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	same as XANTFF
Organism 2.:	**Xanthomonas citri pv. fuscans(XANTFF)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Triplex real-time PCR (Trb1/Audy/Acat): Analytical sensitivity was evaluated by GEVES (FR) by spiking a target strain (CFBP 6473 identified as Xanthomonas fuscans subsp. fuscans) in a seed extract. Three different seed lots were tested, with low medium and high concentrations of saprophytic flora respectively. Different dilutions of the strain were tested in triplicate. The LOD was 6.67 CFU/mL for the Audy primers and for the Trb1 primers. The analytical sensitivity evaluated here corresponds to the detectable concentration present in the macerate (before enrichment).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Evaluated by Geves for the triplex PCR on 19 samples from healthy and naturally infected lots were analysed in each laboratory. For each lot, between 5 and 9 replicates were tested in each laboratory. 100% For the duplex (Trb1/Acat) evaluated in an ILC (with 3 laboratories) using the same samples : 100% Further data are available for the duplex PCR from intralaboratory study (see report VMK)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	The analytical specificity was evaluated by GEVES (FR). For Trb1 primers (evaluated in simplex reactions on isolated strains): Inclusivity: evaluated on 30 target strains in total of X. phaseoli pv. phaseoli and X. citri pv. fuscans (14 strains were non-fuscous, 12 strains were fuscous, and 4 other strains (fuscousity unknown)) For Audy primers (evaluated in duplex with IPC primers Wu (see Appendix 4)): Inclusivity: evaluated on 28 target strains of X. phaseoli pv. phaseoli and X. citri pv. fuscans
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	The analytical specificity was evaluated by GEVES (FR). For Trb1 primers (evaluated in simplex reactions on isolated strains): Exclusivity: evaluated on 40 non-target saprophytic isolates obtained from bean seeds: 100 %. The isolates were not identified but they do not correspond to X. phaseoli pv. phaseoli nor X. citri pv. fuscans based on the characteristics of the colonies on PTSCA media. For Audy primers (evaluated in duplex with IPC primers Wu (see Appendix 4)): Exclusivity: evaluated on 30 non-target strains (1 X. hortorum pv. carotae, 1 X. vesicatoria and 28 saprophytic isolates obtained from bean seeds)

Specificity value	For Trb1: 100% (considering X. phaseoli pv. phaseoli as a target) For Audy: 93.3% (considering X. phaseoli pv. phaseoli as a target). The 2 saprophytic isolates that cross reacted were not identified but they do not correspond to X. phaseoli pv. phaseoli nor X. citri pv. fuscans based on the characteristics of the colonies on PTSCA media.
Cross-reacts with	Xanthomonas phaseoli pv. phaseoli
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Evaluated by Geves for the triplex PCR on 19 samples from healthy and naturally infected lots were analysed in each laboratory. For each lot, between 5 and 9 replicates were tested in each laboratory. 100% For the duplex (Trb1/Acat) evaluated in an ILC (with 3 laboratories) using the same samples : 96% Further data are available for the duplex PCR from intralaboratory study (see report VMK)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Evaluated by Geves for the triplex PCR on 19 samples from healthy and naturally infected lots were analysed in each laboratory. For each lot, between 5 and 9 replicates were tested in each laboratory. 100% For the duplex (Trb1/Acat) evaluated in an ILC (with 3 laboratories) using the same samples : 93% for the healthy samples 100% for infected samples Further data are available for the duplex PCR from intralaboratory study (see report VMK)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Evaluated by Geves for the triplex PCR on 19 samples from healthy and naturally infected lots were analysed in each laboratory. For each lot, between 5 and 9 replicates were tested in each laboratory. 100% For the duplex (Trb1/Acat) evaluated in an ILC (with 3 laboratories) using the same samples : 93% for the healthy samples 100% for infected samples Further data are available for the duplex PCR from intralaboratory study (see report VMK)
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	For the duplex PCR Trb1/Acat: An interlaboratory comparison (ILC) was organized between three laboratories (GEVES, HM Clause and Vilmorin-Mikado (all FR)). 19 samples from healthy and naturally infected lots were analysed in each laboratory.
The following complementary files are available online:	• Dossier technique VMK L-qPCR Haricot • Rapport validation SE-qPCR Psp et Xap sur

	Haricot-incl annexes

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