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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	Detection of ToCV by RT-PCR in tomato leaves
Date, reference of the validation report	2011-07-01 - Loiseau M. et Cousseau P. 2011. Evaluation des méthodes de détection des jaunisses de la tomate – Tomato Infectious Chlorosis Virus (TICV) Tomato Chlorosis Virus (ToCV)
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
If yes, please specify	
Description of the test	
Organism(s)	Tomato chlorosis virus(TOCV00)
Detection / identification	detection
Matrix(ces) tested	Leaves leaves
Plant species tested	Solanum tuberosum
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Conventional RT PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Other information	

Other details on the test	For the RNA extraction, leaf samples was grinded in the RLT buffer (qiagen)
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	RNeasy Mini Kit
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	RNA was extracted with the Plant RNeasy minikit from Qiagen. R
Method: Molecular Conventional 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?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Jacquemond M., Verdin E., Dalmon A., Guilbaud L., Gognalons P., 2009. Serological and molecular detection of Tomato chlorosis virus and Tomato infectious chlorosis virus in tomato. Plant pathology 58:210-220. Louro D., Accotto G.P., Vaira A.M., 2000. Occurrence and diagnosis of Tomato chlorosis virus in Portugal. European Journal of Plant Pathology, 106: 589-592
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	RT-PCR tests were carried out following the recommendation of the paper of Jacquemond et al

	(2009) et Louro et al (2000)
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Tomato chlorosis virus(TOCV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Not relevant
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Simplex RT-PCR (Louro, 2000): 86.67% to 88.89%; Duplex RT-PCR (Jacquemond, 2009): 83.3%; Triplex Rt-PCR (with Cox) (Jacquemond, 2009): 49.02% to 54.9%
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	15 (see table as separate file or full validation report for detail)
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	22 (see table as separate file or full validation report for detai
Specificity value	Cross reaction observed with the triplex method
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Simplex RT-PCR (Louro, 2000): 100%; Duplex RT-PCR (Jacquemond, 2009): 100%; Triplex Rt-PCR (with Cox) (Jacquemond, 2009): 97.5% to 100%
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	

The following complementary files are available online:	• List of target strains and non-target organisms • Loiseau M. et Cousseau P. 2011. Evaluation des méthodes de détection des jaunisses de la tomate – Tomato Infectious Chlorosis Virus (TICV) Tomato Chlorosis Virus (ToCV)

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