

**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 ToCV by RT-PCR in tomato leaves
<b>Date, reference of the validation report</b>	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)
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Crinivirus tomatichlorosis (TOCV00)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular Conventional RT PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	For the RNA extraction, leaf samples was grinded in the RLT buffer (qiagen)
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Mini Kit
<b>Kit used following the manufacturer's instructions?</b>	
<b>Other information</b>	

<b>Other details on the test</b>	RNA was extracted with the Plant RNeasy minikit from Qiagen. R
<b>Method: Molecular Conventional RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	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
<b>Other information</b>	
<b>Other details on the test</b>	RT-PCR tests were carried out following the recommendation of the paper of Jacquemond et al (2009) et Louro et al (2000)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Crinivirus tomatichlorosis(TOCV00)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Not relevant
<b><u>Diagnostic sensitivity</u></b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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%
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	15 (see table as separate file or full validation report for detail)
<b>Specificity value</b>	
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	22 (see table as separate file or full validation report for detail)
<b>Specificity value</b>	Cross reaction observed with the triplex method
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Simplex RT-PCR (Louro, 2000): 100%; Duplex RT-PCR (Jacquemond, 2009): 100%; Triplex Rt-PCR (with Cox) (Jacquemond, 2009): 97.5% to 100%
<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="#">List of target strains and non-target organisms</a></li> <li>• <a href="#">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)</a></li> </ul>

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