



Diagnostic Protocol Notification Period : 01 July – 15 August 2016 Formal Objections

Formal objection submitted by: The European Union and its 28 Member States and supported by Turkey

Date: 29 July 2016 (Turkey supported on 14 August 2016)

Draft DP: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019)

General comment: The draft diagnostic protocol is not ready for adoption, to which we therefore express hereby our formal objection. The main reason is that it would already be outdated before publication. As mentioned in the comments sent during member consultation, it was noted that the tests were quite old and that specificity data was lacking. We believe that at least a warning should have been added for users of the protocol as in some parts the tests are claimed to be specific for different species.

Specific comments:

Some examples for raising our formal objection are given below (more can be provided if needed)

Paragraph 36 Comment

With regard to serological tests with specific antibodies it should be noted that in the case of tospoviruses specific (polyclonal) antisera may cross react with other species. So a serological test might not be sufficient for identification of the species.

Also for the molecular tests described, no data are available on the specificity of the tests. Moreover, it is not known which species will be detected by the generic RT-PCR. Since the RT-PCR amplifies part of the N gene, which sequence is an important criterion for species demarcation, this might allow identification in cases an amplicon is obtained.

SC response

The primers of Mumford et al. 1994 and 1996a are described as specific for TSWV and INSV, respectively. The primers by Chu et al. 2001 are described as specific for WSMoV. This is supported by the DIAGPRO test performance validation study. Paragraph 109 indicates that sequence of amplicons may be carried out if an NPPO requires additional confidence.

Since primers for TSWV have only been tested with INSV and CMV and primers for INSV only with TSWV and GRSV, the conclusion that the test is specific for TSWV and INSV is not substantiated.

Paragraph 52 Comment

Regarding the last sentence, the question is 'is this still the case?'. Since the test performance study additional tospovirus species have been described. Consider adding more data.

SC response

If a citable reference states otherwise this reference should be provided

At least it should clarify which virus species had been tested.

Paragraph 53

Regarding the interpretation of results, (paragraph 53) a similar comment was made by the EU in the Protocol for *Citrus Tristeza virus* and changes were implemented in the later protocol.

We do not understand why a similar change was not made in the protocol for Tospoviruses.

Other information

Finally, please find below data from the paper Hassani-Mehraban *et al* (2016) on cross reactions of antisera.

Supplemental data S1. Cross reactions of antisera raised against tospoviruses

Species ¹	Serogroup/type	Target (protein)	Type of antiserum ²	Test method	Cross reacting species ³	Reference
BeNMV	-	N	Pab	Dot-blot	GRSV, TSWV	De Oliveira et al., 2012
CSNV	-	N	Pab	DAS-ELISA	TCSV, TSWV	Ciuffo et al., 2008
GRSV	II / II	N	Pab	DAS-ELISA	TCSV, TSWV	Boben et al., 2007; Hassani-Mehraban et al., 2005; Williams et al., 2001
INSV	III / -	NSs	Pab	Indirect ELISA, Western blot	GRSV, INSV, TCSV, TSWV	Heinze et al., 2000
MeSMV	-	N	Pab	Western blot	TSWV	Ciuffo et al., 2009
MYSV	IV / -	N	Pab	Indirect ELISA, Western blot	CaCV, CCSV, GBNV, WBNV	Chen et al., 2010
PCFV	PYSV ⁴ / -	N	Pab	Indirect ELISA	PYSV ⁴	Kang et al., 2014
PYSV ⁴	PYSV ⁴ / -	N	Pab	Western blot	PCFV	Kang et al., 2014
PolRSV	IYSV / -	N	Pab	DAS-ELISA, Western blot	IYSV, TYRV	Ciuffo et al., 2008
TCSV	II / I	N	Pab	DAS-ELISA	CSNV, TSWV	Boben et al., 2007; Ciuffo et al., 2009; Hassani-Mehraban et al., 2010; Williams et al., 2001
TSWV	I / -	N	Pab	DAS-ELISA, Western blot	ANSV, GRSV, TCSV	Hassani-Mehraban et al., 2005; Hassani-Mehraban et al., 2010; Williams et al., 2001
WSMoV	IV / -	N	Mab	DAS-ELISA	CSNV	Matsuura et al., 2007
		N	Pab	Indirect ELISA, Western blot	CaCV, CCSV, GBNV, MYSV, WBNV	Chen et al., 2010
		N	Mab	Indirect ELISA, Western blot	CaCV, GBNV, WBNV	Chen et al., 2010
		NSs	Mab	Indirect ELISA, Western blot	CaCV, CCSV, IYSV, MYSV, TYRV, WSMoV	Chen et al., 2011

¹Species to which antibody was raised, indicated by acronyms as in Figure 1; ²Pab: polyclonal antibody, Mab: monoclonal antibody; ³Species cross reacting with the antiserum, acronyms representing positive test results; test results for other tospovirus species are not reported or unknown; ⁴Peanut yellow spot virus is synonym for Groundnut yellow spot virus.