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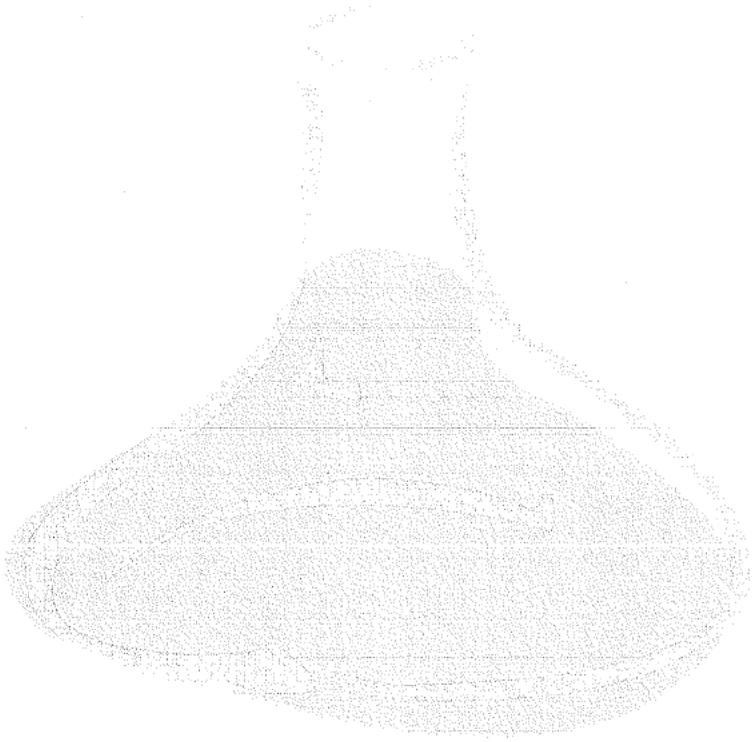
10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

Validation of bio-assay to determine the infectivity of pepino mosaic virus



Document: Validation of bio-assay to determine the infectivity of pepino mosaic virus.
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Introduction

This document describes the validation of a method to determine the infectivity of pepino mosaic virus. The method is a bio-assay, since samples are tested on living plants. Samples containing PepMV can be tested whether they are infectious by inoculation of tomato seedlings. After two weeks, infection of the plantlets can be checked by ELISA. The percentage of infected plantlets at a certain dilution is a measure for the infectivity of PepMV in the tested sample. The bioassay is performed according to SPV A519 and ELISA is performed according to SPV A517, both are standard operating procedures (SOPs) of the laboratory of [redacted]
This document describes the accuracy and reliability of the bio-assay. As external requirements the directives of the Working Document SANCO/3030/00 rev.4 11/07/00 are applied.

Summary of Method

Ten tomato seedlings are inoculated by a diluted PepMV sample. Inoculation is carried out by rubbing the leaves with a virus-carborundum suspension at a certain dilution. After two weeks the percentage of infected plantlets is tested by ELISA. The percentage PepMV-positive plantlets is a measure for the infectivity. The criterion of good infectivity was set at a percentage equal or higher than 70% with a particular diluted sample.

Identification and qualitative result

High concentrations of infectious PepMV will lead to infected plants after inoculation. Low concentrations will not lead to infections. For the detection of PepMV in the inoculated plants a polyclonal antiserum against PepMV is used (PrimeDiagnostics, Wageningen) for the method ELISA (SPV A517). The method validated here is a qualitative method. The result of the bio-assay is whether or not the sample is infectious at a 50-fold dilution.

Validation design

The goal of this validation is to confirm a reliable and accurate method to test the infectivity of PepMV in a sample. The following parameters were checked:

- Repeatability: one sample was tested 5 times with the standard dilution 50x (Appendix 1)
- Robustness: five dilutions (10x, 50x, 100x, 500x, 1000x) were tested for two samples (Appendix 2).

Type of matrix

- Plant Extract

[redacted]

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

Results and Discussion

Repeatability

Repeatability is defined as the variation that occurs among measurements made by the same operator under the same conditions. Five samples containing PepMV were inoculated on tomato seedlings with the standard 50-fold dilution. All 10 plantlets of all five samples were positive with PepMV ELISA (Appendix 1).

Table 1. Repeatability of bio-assay PepMV (n=5)	
Average % positive plants	100
Standard deviation	0
Relative standard deviation (%)	0

Robustness:

Robustness is defined here as a degree of insensitivity of the measuring result to deviations in dilutions. The robustness was assessed by testing 5 serial dilutions of two typical PepMV batches (VX1 and VC1). Results are shown in Appendix 2. Diluting more than 1000-times was not carried out, because batches of PepMV are tested at a standard dilution of 50 and for application in practice of the product, the batches are also diluted 50-times. Therefore dilutions were chosen around the 50x dilution. A VX1 batch at a 100x dilution was able to infect more than 70% of the plants, VC1 showed at 500-times dilution 70% PepMV-positive plants. At 50-times dilutions (which is the standard dilution in the bio-assay), both batches showed 100% PepMV-positive plants. To calculate the robustness, the following dilutions were considered: 10x, 50x and 100x for both VC1 and VX1. The robustness for these 6 dilutions is 100%, because all of these dilutions resulted in more than 70% PepMV-positive plants.

Conclusions

According to the quality of the results the conclusion can be drawn that this bio-assay allows for reliable measurements of the infectivity of PepMV in samples. It is justified to use a standard dilution of 50x and to have set a criterion of $\geq$ 70% PepMV-positive plants for PepMV-infectivity of samples.

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Appendix 1. Repeatability

Preparation of samples:

- 0,5 ml Batch VCN281112 (MEX121217625)
- 0,5 ml Batch VX271112 (MEX121217624)
- 49 ml PBS

This mixture (50x diluted sample) was five times inoculated in 10 tomato seedlings (cultivar Capricia) according to SPV A519.

Date of inoculation: 18-12-2012
Date of sampling: 31-12-2012
Date of ELISA: 02-01-2013

Results:

Treatment	PepMV positive/ total plants	Percentage positive plants (%)
1	10/10	100
2	10/10	100
3	10/10	100
4	10/10	100
5	10/10	100

Repeatability of bio-assay PepMV at a dilution of 50x.
Average (n=5): 100%
SD: 0
RSD: 0%

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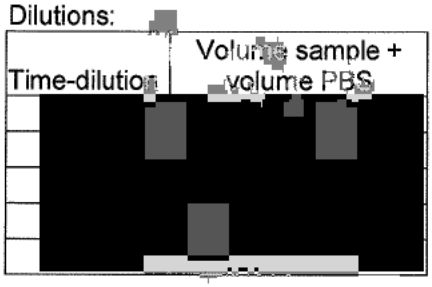
Appendix 2. Robustness

Dilutions:



Each dilution was inoculated [redacted]0-times in tomato seedlings.
PBS was used for the dilutions.

Two PepMV batches were tested:

- VX061112; date inoculation: 13-11-2012; date sampling: 26-11-2012; date ELISA: 27-11-2012.
- VCN281112; date inoculation: 18-12-2012; date sampling: 31-12-2012; date ELISA: 02-01-2013.

Results VX1 batch:

Dilution	PepMV positive/ total plants	Percentage PepMV-positive plants (%)
[redacted]	10/10	100
	10/10	100
	10/10	90
	5/10	50
	2/10	20

100-times is the highest dilution, which shows a percentage of 70% or more of PepMV-positive plants.

Results VC1 batch:

Dilution	PepMV positive/ total plants	Percentage PepMV-positive plants (%)
[redacted]	10/10	100
	10/10	100
	10/10	100
	7/10	70
	6/10	60

500-times is the highest dilution, which shows a percentage of 70% or more of PepMV-positive plants.

Robustness of dilutions around 50-times dilution for VX1 and VC1 (10x, 50x and 100x) with results of ≥ 70% PepMV-infected plants.
Average (n=6): 100%
SD: 0
RSD: 0%