Method for the detection of *Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV) on Cucurbit seed

Crop:	Melon (*Cucumis melo*) and Cucumber (*Cucumis sativus*)
Pathogens:	Squash mosaic virus (SqMV), Cucumber green mottle mosaic virus (CGMMV) and Melon necrotic spot virus (MNSV)
Revision history:	Version 2, July 2017

Sample and sub sample size

For the DAS-ELISA method on seeds, the recommended minimum sample size is 2,000 seeds with a maximum sub-sample size of 100 seeds.

For the confirmatory method for SqMV in melon seed the recommended minimum sample size is 2,000 seeds.

Principle

Detection of viruses with a DAS-ELISA test followed by an optional confirmation based on a grow-out and DAS-ELISA method.

Restrictions on Use

DAS-ELISA method:

- The DAS-ELISA method is suitable for untreated seed.
- It may be suitable for seed treated with physical processes for disinfestation or seed that has been treated using chemicals (such as hydrochloric acid, peroxyacetic acid, etc.) for disinfestation provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- Although ELISA is compatible with some seed treatment chemicals (1), seed treatments may affect the performance of this test. It is the responsibility of the user to check for such antagonism and or inhibition by analysis, sample spiking, or experimental comparisons.

Method for confirmation of seed transmitted SqMV:

- The confirmation test is only suitable for SqMV. It has not been validated for MNSV and CGMMV.
- It has been validated on melon seed. In principle, all cucurbits can be evaluated with this method, however, the ELISA confirmation test on plant tissue samples should be evaluated and verified for the various cucurbits being evaluated in the grow-out test before accepting the results. This test method is suitable for untreated seed.

Note: The method was reviewed recently and found to be fit for purpose. The section **Validation** has been updated**.** A section on **Method Execution** has been added.

- This test method is suitable for seed that has been treated using chemicals or physical processes with the aim of disinfestation/disinfection, as well as seed treated with protective chemicals or biological substances.

Validation

Results of a comparative test using melon and cucumber seed on DAS-ELISA method were validated by ISTA (see www.seedtest.org >>Technical Committees >>Seed Health Committee >>Testing Methods >>Method Validation) and came into effect as an ISTA Rule (7-026) in January 2010.

The confirmation method for SqMV on melon seed was adopted by ISTA in June 2015 and came into effect in January 2016.

This method has also been approved by the US National Seed Health System (NSHS) as a Standard B (see http://seedhealth.org/seed-health-testing-methods/).

Used for many years by the industry for different cucurbit seed.

Method Execution

To ensure process standardization and valid results, it is strongly recommended to follow the best practices developed by ISHI-Veg for *ELISA Assays in Seed Health Tests* (see http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg/).

Method description

See www.seedtest.org (>>Technical Committees >>Seed Health Committee >>Testing Methods)

Reference

1. Pataky, J. K., Block, C. C., Michener, P. M., Shepherd, L. M., McGee, D. C. & White, D. G. (2004). Ability of an ELISA-based seed health test to detect *Erwinia stewartii* in maize seed treated with fungicides and insecticides. Plant Disease, 88, 633–640.