Method for the Detection of \textit{Xanthomonas axonopodis} pv. \textit{phaseoli} on Bean Seed

**Crop:** Bean (\textit{Phaseolus vulgaris} L.)

**Pathogen:** \textit{Xanthomonas axonopodis} pv. \textit{phaseoli} (\textit{X. a. pv. phaseoli}) (syn. \textit{Xanthomonas campestris} pv. \textit{phaseoli} (Smith) Dye)

**Date:** Version 2, July 2017

**Sample and sub-sample size**
The test is done on a minimum sample size of 5,000 seeds and a maximum sub-sample size of 1,000 seeds.

Note: The method was validated using a minimum of 5,000 seeds. However, sample size depends on the risk management strategy of each user, and thus the choice of sample size is at the user’s discretion.

**Principle**
- Detection of viable bacteria (both fuscans and non-fuscans types) based on soaking seeds and plating the liquid obtained on semi selective media.
- Confirmation of suspect bacterial colonies is completed by a pathogenicity assay or a PCR method.

**Restrictions on Use**
- This test method is suitable for untreated seed.
- The ability to recover \textit{Xanthomonas axonopodis} pv. \textit{phaseoli} on plates can be influenced by the presence of other microorganisms and/or inhibitory chemicals used for seed disinfestation/ disinfection. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- This test method has not been validated for seed treated with protective chemicals or biological substances. If a user chooses to test treated seed using this method, it is the responsibility of the user to determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on the method results.

**Validation**
The method for detecting viable fuscans and non-fuscans types of bacteria was first adopted as an ISTA Rule (7-021) in January 2007 (see www.seedtest.org >>Technical Committees >>Seed Health Committee >>Testing Methods >>Method Validation for the results of the comparative test validated by ISTA).

A revised method that includes a new pathogenicity assay and a PCR method for the confirmation of suspect bacterial colonies was validated by ISTA in June 2013 based on the results of a comparative test, see www.seedtest.org (>>Technical Committees

Note: The method was reviewed recently and found to be fit for purpose. The sections Sample and sub-sample size and Validation have been updated. A new section Method Execution has been added.
The revised ISTA Rule (7-021) came into force in January 2014. The method has also been approved by the US National Seed Health System (NSHS) as a Standard A (see http://seedhealth.org/seed-health-testing-methods/).

**Method Execution**

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

**Method description**

See [www.seedtest.org](http://www.seedtest.org) (>>Technical Committees >>Seed Health Committee >>Testing Methods)