INTERNATIONAL SEED HEALTH INITIATIVE (ISHI)

Best Practices for Biological Assays in Seed Health Tests

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This document describes best practices for the use of biological assays in seed health testing to ensure accurate and reliable results. Best practices include process controls and assay conditions that should be applied to all trials.

Controls and conditions are designated as essential (must/shall be included) or recommended (can be included).

I. BIOLOGICAL ASSAYS FOR DETECTION OF PATHOGEN VIABILITY

This document encompases the two following types of biological assays:

- Bioassay: An assay in which plant tissue (e.g., seed or its extract) suspected of being infected with a pathogen is applied to a test organism (typically an indicator plant) to assess infectivity relative to a positive control.
- Pathogenicity assay: An assay in which a pure culture of a putative pathogen isolated from a seed lot is inoculated onto a suitable host plant under disease-conducive conditions to assess the development of typical disease symptoms.

The bioassay demonstrates infectivity / viability of the pathogen in the form of a hyper senstitive reaction using seed extracts as matrix. The pathogenicity assay demonstrates pathogenicity, in the form of disease symptoms, using pure isolates as matrix.

The best practices described below are for both types of assays, unless otherwise stated.

II. CONTROLS AND THEIR PURPOSE

The types of controls for biological assays are defined in Table 1. Their purpose is to verify both the quality of the material used in the test and proper test execution. Proper negative and positive controls should be included in every assay to ensure reliable test results.

Table 1: Controls to be included in biological assays.

Control type	Negative control (NC) - Recommended
Definition	The extraction or dilution buffer used to prepare the sample that is tested using the same assay at the same time as the test samples.
Expected Result	No response on the indicator plants (pathogenicity assay) or symptom in the host plant (bioassay).
Description	The extraction buffer is used for the bioassay while the dilution buffer for the pathogenicity assay. In cases when no extraction or dilution buffer is used, the indicator/host plants are simply rubbed or stabbed with the inoculation instrument alone.

Control type	Negative Process Control (NPC) - Essential
Definition	A known negative seed or plant part sample tested using the same assay at the same time as the test samples.
Expected Result	No response on the indicator plants (pathogenicity assay) or symptom in the host plant (bioassay).
Description	It could be either seed or a plant part, or a non-pathogenic strain depending on whether it is a bioassay or a pathogenicity assay.

Control type	Positive process control (PPC) for a bioassay - Essential
Definition	A known positive seed or plant part sample (naturally or artificially infected with the target pathogen) tested using the same assay at the same time as the test samples.
Expected Result	Specific response on the indicator plants caused by the target pathogen.
Description	A seed sample that contains the target organism (known infected seed sample) **OR** Known infected plant tissue **OR** A seed sample spiked with target organism **OR** Seed extract sample spiked with target organism.

Note: If more than one pathogen is targeted in a sample and if those pathogens give different responses on the indicator plants, it is essential to use as many PPC as the number of targeted pathogens.

Control type	Positive process control (PPC) for a pathogenicity assay - Essential
Definition	A known isolated strain of the target pathogen tested using the same buffer and assay, at the same time as the test samples.
Expected Result	Specific symptoms on the host plants caused by the target pathogen.
Description	Dilution buffer spiked with target organism **OR** Pure culture of target organism.

Note: If more than one pathogen is targeted in a sample and if those pathogens give different symptoms on the host plants, it is essential to use as many PPC as the number of targeted pathogens.

III. ASSAY SET-UP

The essential and recommended conditions for the set-up of a biological assay are described in Table 2.

Table 2: Set-up for a biological assay.

Description	Essential	Recommended
Quality Control (QC): Requesting a certificate from the supplier on that appropriate hygiene measures were applied to the substrate (soil or vermiculite) to be used.	x	
Sanitization of materials used: Sanitizing the greenhouse and growth chamber floors, wall benches, equipment, and all planting trays, if being re-used.	x	
Sanitization practices to avoid cross contamination: 1. Using proper aseptic practices, including changing gloves between each sample and sanitising all surfaces prior to beginning the test.	x	
2. Maintaining adequate distance or using physical barriers between plants where appropriate (e.g. separate inoculated plants from healthy plants and separate plants inoculated with different samples). Eventually, add a row or tray with a susceptible healthy plant between the different test samples to serve as an indicator 'catcher'.		x
3. Placing positive controls in an area that is isolated from the assay plants.	x	
4. Sampling and physically handling positive controls for symptoms *after* all the assay plants have been evaluated.	x	
5. Placing foot baths with an effective sanitizing chemical at the entrance of each test location.		x
6. Restricting access to the test area to authorized and trained personnel only.		x
7. Using effective treatments to control insects, spiders, rodents and other known vectors of viruses and bacteria.		x
8. Protective clothing, designated by name and exclusively for the area where the assays are being run, for technicians performing various tasks specific to the assays.		x
9. Avoiding any physical contact with assay plants while watering.	x	
10. Strictly following the protocol for the inoculation stage of the indicator/host plants.	x	

IV. ENVIRONMENTAL CONDITIONS

Environmental conditions can greatly influence the outcome of the test and must be controlled and monitored for the duration of each test as described in Table 3.

Table 3: Environmental conditions for a biological assay.

Description	Essential	Recommended
Temperature: For the duration of the test, the temperature of the test location must be set according to the requirements specified in the protocol. It must also be monitored using temperature probes placed in a relevant position, and recorded for the entire period and must not deviate from the acceptable range by more than ± 2°C.	x	
Photoperiod: In the test location photoperiod must be maintained as per the requirements of the protocol for the duration of the test.	x	
Light conditions: Light of the appropriate intensity and spectrum must be supplied for optimum growth of the indicator/host plants and disease development or symptom expression. The quality of bulbs or LEDs should be monitored for functionality and overheating. Care must be taken to ensure that light intensity and spectrum are not decreased below the acceptable limit for the assay.	x	
Relative Humidity: The relative humidity must be maintained as per the requirements of the protocol and recorded using humidity probes for the duration of the test.	x	