INTERNATIONAL SEED HEALTH INITIATIVE (ISHI)

Best Practices for
Sweat Box and Grow-Out Assays in Seed Health Tests

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This document describes best practices for the use of sweat box and grow-out 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. SWEAT BOX AND GROW-OUT ASSAYS FOR DETECTION OF PATHOGEN VIABILITY

Process controls and assay conditions in this document are defined for sweat box and grow-out assays used for the detection of a target pathogen on seeds.

II. CONTROLS AND THEIR PURPOSE

The types of controls for sweat box and grow-out assays are defined in Table 1. Their purpose is to verify both the quality of the material used 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 sweat box and grow-out assays.

Control type	**Negative process control (NPC) - Essential**
Definition	A known negative seed sample that is tested using the same assay at the same time as the test samples.
Expected Result	No development of specific disease symptoms
Description	A negative seed sample that is not infected by the target organism due to cross contamination during the assay.

Control type	Positive process control (PPC) - Essential
Definition	A known positive seed sample that is tested using the same assay at the same time as the test samples.
Expected Result	Development of specific disease symptoms due to transmission of the pathogen to the developing plants.
Description	Naturally or artificially infected seed used as a control of the assay, including the environmental conditions maintained throughout the test. In case of an artificially infected lot, the use of a marked target pathogen is recommended as it helps to verify that a positive result in the test samples is not due to cross contamination with the PPC. If no infected seeds are available, this control could be replaced by inoculating healthy plants with the target pathogen as a control for expected disease symptoms.

III. ASSAY SET-UP

The essential and recommended conditions for the set-up of a sweat box or grow-out assay are described in Table 2.

Table 2: Set-up for sweat box and grow-out assays.

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	
Efficacy of sanitation: Verifying the efficacy of sanitation by running the test using known negative seeds in previously used containers.		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. For grow-out assays a row or tray with a susceptible healthy plant between the different test samples to serve as an indicator 'catcher' may be included.		x
3. Placing positive controls in an area that is isolated from the assay plants.	x	
4. For assays that are dependent on high humidity and temperature, and if plants are not physically separated, placing positive controls	x	

Description	Essential	Recommended
at both ends of the compartment where assay plants are being raised.		
5. Sampling and physically handling positive controls for disease symptoms *after* all the assay plants have been evaluated.	x	
6. Placing foot baths with an effective sanitizing chemical at the entrance of each test location.		x
7. Restricting access to the test area to authorized and trained personnel only.		x
8. Using effective treatments to control insects, spiders, rodents and other known vectors of viruses and bacteria.		x
9. 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
10. Avoiding any physical contact with assay plants while watering.	x	
11. Avoiding physical contact or manual manipulation of seedlings until the final inspection.	x	
12. When using physical separations, these should be made with material that can be disinfected or that are disposable.	x	
Quantity of planting media: Optimising the quantity of planting media, such as soil or vermiculite for the species being raised, sowing seeds at the correct depth and ensuring uniform conditions necessary to maintain good and uniform plant growth and disease development.	x	
Quantity of water: Optimizing its use by species before sowing and during plant growth.	x	
Distribution of seed in the sweat box: Distributing seeds evenly on the surface of the planting medium and uniformly covering them with the cover medium.	x	
Application of fungicide to the substrate: Using a validated fungicide, as defined by the protocol, to control fungal infection/saprophytes by either directly treating seeds or drenching the medium with a known quantity. Validation of the fungicide must show that recovery of the target pathogen is not affected.		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.

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 relevant positionings, 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 used for optimum growth of the plants and disease development or symptom expression. The quality of bulbs or LEDs should be monitored for functionality and overheating.	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	