International Seed Federation
Seed is Life

Best Practices for Sweat Box Assays in Seed Health Tests
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This document describes best practices for the use of sweat box assays in routine seed health testing to ensure accurate and reliable results.

I Sweat Box Assays for Detection

Process controls and assay conditions in this document are defined for:

- Routine sweat box assays used for the detection of a specific bacterial pathogen on seed.
- Validation of new sweat box assays.

These process controls and assay conditions should be applied to all sweat box assays. Controls and conditions are designated as essential (must/shall be included) or recommended (can be included).

II Controls and their Purpose

The types of controls for routine sweat box assays are defined in Table 1.

The purpose of these controls is to verify both the quality of the materials used in the sweat box assay and proper test execution. Proper controls shall be included in every test to ensure reliable test results.

Table 1: Controls to be included in routine sweat box assays

Control type	**Negative process control (NPC)**	**Essential**
Definition	A known negative seed sample (with respect to the target pathogen) that is tested at the same time, using the same assay as the corresponding samples.	
Expected Result	No detection of the target pathogen.	
Description	The NPC serves as the negative control for the materials used in the sweat box assay and the sweat box assay process.	

Control type	**Positive process control (PPC)**	**Essential**
Definition	A known positive seed sample (naturally or artificially infected with the target pathogen) that is tested at the same time, using the same assay as the corresponding samples.	
Expected Result	Detection of the target pathogen.	
Description	The PPC serves as the positive control for the sweat box assay process, including the environmental conditions maintained during the test.	

III Sweat Box Assay Set-up

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

Table 2: Sweat box assay set-up

Description	Essential	Recommended
Quality Control (QC) information from the supplier: QC information on the substrate such as soil (e.g. if the soil is sterilized or has starter fertilizer) and vermiculite (e.g. its grade) must be requested from the supplier.	x	
Sanitization of the sweat box: Prior to re-use, sanitization of the sweat box container must be completed to prevent cross-contamination from the previous test.	x	
Efficacy of sanitation: Verification of the efficacy of sanitation can be done by running the test using known negative seeds in previously used sweat boxes.		x
Sanitization practices: To avoid cross-contamination between sweat boxes, proper aseptic practices must be used, including changing gloves between each seed lot and sanitation of all surfaces prior to beginning the test.	x	
Quantity of planting medium and water: The quantity of planting medium (e.g., soil or vermiculite) and water used in the sweat boxes must be optimized. Uniform conditions within each sweat box and between the sweat boxes are necessary to maintain uniform plant growth and disease development across all sweat boxes.	x	
Distribution of seed in the sweat box: Seeds must be distributed evenly over the surface of the planting medium. The cover medium needs to also be evenly distributed over the seed.	x	
Application of fungicide to substrate: A validated fungicide, as defined by protocol, may be used to control fungal infection/saprophytes by either directly treating the seeds or drenching the medium with a known quantity of fungicide in the sweatboxes. Validation of the fungicide must show that the recovery of target pathogen is not affected.		x

IV Essential Points

Certain components of a sweat box assay (e.g., environmental conditions) can greatly influence the outcome of the test. These components, described in Table 3, must be controlled for and monitored for the duration of each test.

Table 3: Essential components of a sweat box assay

Description	Essential	Recommended
Temperature: The Environmental Growth Chamber (EGC), as required by protocol, must be set to the defined temperature for the duration of the test. EGC temperatures are monitored by placing temperature recorders within each device for the duration of the test or by utilizing internal EGC data loggers.	x	

Description	Essential	Recommended
Temperature within the EGC must not deviate from the acceptable range (at most, target temperature ± 3°C) for the duration of the test.		
Photoperiod: Photoperiod, as defined by the protocol, must be maintained in the EGC for the duration of the test.	x	
Light conditions: Light of appropriate intensity and spectrum must be supplied to the seeds/ seedlings for adequate growth of the target pathogen, disease development and /or seedling germination. Bulbs or LEDs should be monitored for functionality and to ensure that excess heat is not being produced.	x	