International Seed Federation
Seed is Life

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

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DEVELOPED BY
International Seed Health Initiative (ISHI) of the International Seed Federation (ISF)

BEST PRACTICES AND THEIR PURPOSE

The following Best Practices have been developed to ensure accurate and reliable results when performing Sweat box and Grow-out assays and to provide general guidance to laboratories developing and using seed health tests.

These guidelines are not intended to serve as a legal reference. They are not binding on ISHI nor ISF members.

DISCLAIMER

This document has been developed based on the current technical state of the art. ISF cannot be held liable for any possible claims associated with the application of these guidelines.

INTRODUCTION

This document describes best practices for the use of sweat box and grow-out assays in seed health tests to ensure accurate and reliable results. Best practices include process controls and assay conditions that should be applied to all experiments.

Controls and conditions of the assays are designated either as essential (necessary to perform for proper test execution), recommended (advised to perform for proper test execution) or optional (not necessary to perform for proper test execution).

1. SWEAT BOX AND GROW-OUT ASSAYS: DIRECT TESTS FOR THE DETECTION OF PATHOGEN VIABILITY AND PATHOGENICITY

This document encompasses the following two types of assays:

- **Grow-out assay:** An assay in which seeds from a seed sample are sown under disease-conducive conditions, and plants are subsequently examined for the presence of disease symptoms caused by the pathogen to demonstrate seed to seedling transmission of a given pathogen.
- **Sweat box grow-out assay**: Type of grow-out assay in which seeds from a seed sample are sown, typically in vermiculite, under disease-conducive conditions in a closed plastic box that leads to high humidity. Plants are subsequently examined for disease symptoms to evaluate pathogen transmission from seed to seedling.

Grow-out and sweat box assays demonstrate presence, infectivity/viability and transmission of the pathogen from seed to seedling, and pathogenicity of a pathogen in the form of disease symptoms using seeds as source.

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

2. 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 in the assays and proper test execution. Appropriate negative and positive controls should be included in every assay to ensure reliability of the test results. When handling the positive controls, it is important to ensure no cross-contamination occurs. As such, it is recommended to handle the positive controls last after setting up the negative controls and sample (e.g., use filter tips when using a dissolved reference isolate).

Table 1. Controls defined for sweat box and grow-out assays.

	Negative Process Control (NPC)
Definition	Control that contains a well characterized healthy seed sample (with respect to the target pathogen) that is tested using the same assay at the same time as the samples.
Objective	To verify that growth conditions are suitable and that no cross-contamination occurred under the assay conditions.
Expected Result	Normal development of seedlings and no development of specific disease symptoms.
Description	Seed sample that contains no target pathogen.
Sweat Box	Essential
Grow-Out	Essential

	Positive Process Control (PPC)
Definition	Control that contains a well characterized positive seed sample, naturally infected or artificially contaminated with the target pathogen, that is tested and processed using the same assay at the same time as the samples.
Objective	To verify that growth conditions are suitable and that the target pathogen can be detected under the assay conditions in the presence of a seed matrix.
Expected Result	Development of seedlings with typical disease symptoms specific to the target pathogen.
Description	A naturally infected or artificially contaminated seed sample that contains the target pathogen.
Sweat Box	Essential
Grow-Out	Essential
Other Information	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.

3. 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.	×	
Sanitization of materials used: Sanitizing the greenhouse and growth chamber floors, wall benches, equipment and all planting trays, if being re-used.	×	
Efficacy of sanitation: Verifying the efficacy of sanitation by running the test using known negative seeds in previously used containers.		×

Description	Essential	Recommended
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.	×	
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.		×
3. Placing positive controls in an area that is isolated from the assay plants.	×	
4. For assays that are dependent on high humidity and temperature, and if plants are not physically separated, placing positive controls 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.	×	
6. Placing foot baths with an effective sanitizing chemical at the entrance of each test location.		×
7. Restricting access to the test area to authorized and trained personnel only.		×
8. Using effective treatments to control insects, spiders, rodents and other known vectors of viruses and bacteria.		×
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.		×
10. Avoiding any physical contact with assay plants while watering.	×	
11. Avoiding physical contact or manual manipulation of seedlings until the final inspection.	×	
12. When using physical separations, these should be made with material that can be disinfected or that are disposable.	×	
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.	×	
Quantity of water: Optimizing its use by species before sowing and during plant growth.	×	
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.	×	
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		×

Description	Essential	Recommended
quantity. Validation of the fungicide must show that recovery of the target pathogen is not affected.		

4. 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.	×	
Photoperiod: In the test location photoperiod must be maintained as per the requirements of the protocol for the duration of the test.	×	
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.	×	
Relative Humidity (RH): The RH must be maintained as per the requirements of the protocol and recorded using humidity probes for the duration of the test.	×	

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