International Seed Federation
Seed is Life

Best Practices for Biological Assays in Seed Health Tests

MAY 2025, VERSION 2

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 Biological 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 biological 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 (voluntary to perform for proper test execution).

1. BIOLOGICAL ASSAYS: DIRECT TESTS FOR THE DETECTION OF PATHOGEN VIABILITY AND PATHOGENICITY

This document encompasses the two following types of biological assays:

- **Bioassay**: An assay in which a plant tissue (e.g., seed or leaves) that is suspected of being infected with a pathogen is applied to an indicator plant to assess viability/infectivity.
- **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 and viability of a pathogen in the form of either a hypersensitive reaction on the indicator plant or, in the case of a systemic infection, a specific response on the indicator plants using seed extracts as matrix. The pathogenicity assay demonstrates pathogenicity, in the form of disease symptoms, using pure isolates as matrix. Typically, seed extracts are only subjected to a biological assay for confirmation of prior assay results.

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

2. 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 used 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 bioassays and pathogenicity assays.

	Negative Buffer Control (NBC)
Definition	Control that contains all buffers and reagents used to prepare the sample that is tested and processed using the same assay at the same time as the samples. This control contains no target pathogen and no spike.
Objective	To verify the sterility of the buffer used in the absence of samples.
Expected Result	No response on the indicator plants (bioassay) or no symptom on the host plants (pathogenicity assay).
Description	Extraction buffer for the bioassay OR Dilution and/or inoculation buffer for the pathogenicity assay OR If no buffer is used, rub, or stab the indicator/host plants with a clean inoculation instrument alone.
Bioassay	Essential
Pathogenicity Assay	Essential

	Negative Process Control (NPC)
Definition	Control that contains a well characterized healthy seed sample (with respect to the target pathogen) for a bioassay, or a non-target isolate strain for a pathogenicity assay, that is tested and processed using the same assay at the same time as the samples.
Objective	To verify that no cross-contamination occurred under the assay conditions.
Expected Result	No response on the indicator plants (bioassay) or symptom in the host plants (pathogenicity assay).
Description	Seed sample that contains no target pathogen for the bioassay OR Freshly prepared suspension from a colony of a non-target pathogen
Bioassay	Recommended
Pathogenicity Assay	Recommended

	Positive Control (PC)
Definition	Control that contains a known (isolated) target pathogen in the absence of seed that is tested and processed using the same assay at the same time as the samples.
Objective	To verify that the target pathogen can be detected under the assay conditions in the absence of a seed matrix.
Expected Result	For pathogenicity assay, development of disease symptoms on the host plants, which are typical to the target pathogen. For bioassay, in case of local lesion assay, the number of necroses/necrotic spots observed should be within the expected range. In case of systemic infection, a specific response on the indicator plants caused by the target pathogen should be observed.
Description	Dilution buffer spiked with a known pathogenic reference strain/isolate of the target pathogen (pathogenicity assay/bioassay) or with leaves contaminated with the target pathogen (bioassay).
Bioassay	Optional
Pathogenicity Assay	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 the target pathogen can be detected under the assay conditions in the presence of a seed matrix.
Expected Result	For pathogenicity assay, development of disease symptoms on the host plants, which are typical to the target pathogen. For bioassay, in case of local lesion assay, the number of necroses/necrotic spots observed should be within the expected range. In case of systemic infection, a specific response on the indicator plants caused by the target pathogen should be observed.
Description	A naturally infected or artificially contaminated seed sample that contains the target pathogen OR A seed sample or seed extract spiked with living target pathogen or infected plant tissue extract.
Bioassay	Essential
Pathogenicity Assay	Recommended

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.

3. 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.	×	
Sanitization of materials used: Sanitizing the greenhouse and growth chamber floors, wall benches, equipment, and all planting trays, if being re-used.	×	
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). Eventually, add a row or tray with a susceptible healthy plant between the different test samples to serve as an indicator 'catcher'.		×
3. Placing positive controls in an area that is isolated from the assay plants.	×	
4. Sampling and physically handling positive controls for symptoms after all the assay plants have been evaluated.	×	

Description	Essential	Recommended
5. Placing foot baths with an effective sanitizing chemical at the entrance of each test location.		×
6. Restricting access to the test area to authorized and trained personnel only.		×
7. Using effective treatments to control insects, spiders, rodents, and other known vectors of viruses and bacteria.		×
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.		×
9. Avoiding any physical contact with assay plants while watering.	×	
10. Strictly following the protocol for the inoculation stage of the indicator/host plants.	×	

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 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.		×
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 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.	×	
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.	×	

Seed is Life