International Seed Federation
Seed is Life

Best Practices for Blotter Assays in Seed Health Tests
(August 2018)

This document describes best practices for the use of blotter assays in routine seed health testing to ensure accurate and reliable results.

I Blotter Assays for Detection

There are two types of blotter assays that are in the scope of this document:

- Blotter assays in which seeds are incubated and seeds and/or seedlings are then evaluated for symptoms or signs of the target disease or pathogen.
- Blotter assays in which seeds are treated (with cold temperature or chemical application) to prevent germination and un-germinated seeds are then evaluated for growth of the target pathogen.

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

- Routine blotter assays used for the detection of a specific fungal pathogen on seed.
- Validation of new blotter assays.

Unless exceptions are stated, these process controls and assay conditions should be applied to all blotter assays. Controls and conditions are designated as essential (must/shall be included) or recommended (can be included).

II Controls and their Purpose

The purpose of these controls is to verify both the quality of the materials used in the blotter 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 blotter 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 blotter assay and the blotter 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. The only exception is in the case of obligate parasites when naturally infected seed is not available. In these cases, an alternative host/pathogen control could be used to confirm that conducive conditions were maintained during the test.	
Expected Result	Detection of the target pathogen.	
Description	The PPC serves as the positive control for the blotter assay process, including the environmental conditions maintained during the test.	

III Blotter Assay Set-up

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

Table 2: Blotter assay set-up

Description	Essential	Recommended
Substrate: Prior to use, blotter/filter paper (substrate) may be sterilized.		x
Incubation container: Prior to re-use, blotter boxes/petri plates (incubation containers) must be sanitized using a validated sanitization method (e.g., bleach treatment) to prevent cross-contamination from the previous test.	x	
Sanitization practices: To avoid cross-contamination between seed samples during distribution of seed and seed/seedling evaluation, appropriate sanitization practices must be used (e.g., changing gloves and sanitizing the seed spreader with ethanol between seed samples).	x	
Saturation of substrate: The quantity of liquid (water or fungicide) used to saturate the substrate in each incubation container must be optimized for the assay as performed in each laboratory. Uniform conditions between incubation containers are necessary to maintain uniformity in growth of the target pathogen, disease development, or seedling germination.	x	
Application of fungicide to substrate: A validated fungicide solution, as defined by the protocol (e.g., dichloran solution), may be used to saturate the substrate to minimize the growth and spread of saprophytic fungi. Validation of the fungicide must show that recovery of the target pathogen is not affected when applied to substrate.		x
Seed sanitization: A validated seed sanitization step, as defined by the protocol (e.g., dilute sodium hypochlorite treatment), may be used to minimize the growth of saprophytic fungi. Validation of the seed sanitization technique must show that recovery of the target pathogen is not affected.		x

Description	Essential	Recommended
Distribution of seed on substrate: Seed must be distributed evenly over the substrate surface within each incubation container with seed spacing (number of seed per cm^2 blotter paper) as defined by the protocol.	x	

IV Essential Points

Certain components of a blotter 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 blotter assay

Description	Essential	Recommended
Temperature: The Environmental Growth Chamber (EGC) or freezer, as required by the protocol, must be set to the defined temperature for the duration of the test. EGC and freezer temperatures are monitored by placing temperature recorders within each device for the duration of the test or by utilizing internal ECG or freezer data loggers. Temperature within the EGC or freezer must not deviate from the acceptable range (at most, target temperature ± 3°C) for the duration of the test.	x	
Relative humidity: Incubation containers must provide the relative humidity required by the protocol or be maintained in EGCs that provide the relative humidity required by the protocol.	x	
Photoperiod: Photoperiod, as defined by the protocol, must be maintained in the EGC or freezer 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, or seedling germination. Care must be taken when stacking incubation containers to ensure that light intensity and spectrum are not decreased below the acceptable limit for the assay.	x	