International Seed Federation
Seed is Life

Best Practices for ELISA 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 ELISA 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 Enzyme-linked immunosorbent assay (ELISA) 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. ELISA ASSAY: INDIRECT TEST FOR THE DETECTION OF PATHOGENS

An ELISA detects antigens from a specific organism by the process of immobilisation on a coated surface using antibodies directed against the antigen to be measured. An ELISA assay is an indirect test that does not discriminate whether the source of the antigen is pathogenic or viable and therefore, it is not sufficient to prove pathogenicity. While a negative ELISA assay result can be assumed to be a conclusive evidence of a healthy seed lot, a positive ELISA assay result must be followed by a direct test to inform about the viability of the pathogen and its pathogenicity (see ISF viewpoint on indirect seed health tests). An ELISA assay is considered as a pre-screen performed prior to a direct test, such as a bioassay or a grow-out assay. An ELISA may also be used to confirm the detection of a target pathogen and/or its identification in suspect plant tissues obtained from a bioassay or grow-out assay.

2. CONTROLS AND THEIR PURPOSE

The types of controls for ELISA 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 ELISA assays.

	Non Target Control (NTC)
Definition	Control that contains 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 extraction buffer, no target pathogen and no spike.
Objective	To verify the sterility of the reagents used in the absence of extraction buffer and samples.
Expected Result	No detection of any pathogen and saprophyte, including the target.
Description	ELISA reagents free from any buffer, pathogen, or seed.
ELISA	Optional

	Negative Buffer (Blank) 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 putative contamination of the extraction buffer used in the absence of samples and to assess the background optical density (OD) value (i.e., blanking wells).
Expected Result	No detection of the target pathogen.
Description	Extraction buffer
ELISA	Essential

	Negative Process Control (NPC)
Definition	Control that contains a well characterized healthy seed sample (with respect to the target pathogen) 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, and to establish a baseline OD value.
Expected Result	No detection of the target pathogen.
Description	Seed or tissue sample that contains no target pathogen
ELISA	Essential

	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 seed matrix.
Expected Result	Detection of the target pathogen.
Description	Extraction buffer spiked with a known reference isolate of the target pathogen.
ELISA	Recommended

Note: The reference isolate can be obtained from an external supplier.
Note: Users should at least perform either the PC or PPC.

	Positive Process Control (PPC)
Definition	Control that contains a well characterized positive seed sample, naturally infected or artificially contaminated with the target pathogen and is tested and processed using the same assay and 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	Detection of the target pathogen.
Description	A seed sample that contains the target pathogen (known infected seed sample) **OR** A seed sample spiked with target pathogen or infected plant extract.
ELISA	Recommended

Note: Users should at least perform either the PC or PPC.

	Inhibition Control (IC)
Definition	Control that contains an internal spiking of a predetermined quantity of an isolated target or related pathogen, added directly to the seed samples that is tested using the same assay and at the same time as the samples.
Objective	To verify that no inhibition due to the sample seed matrix (e.g., chemical residues if treated and saprophytic load if not treated) occurs under the assay conditions used.
Expected Result	Detection of the spiked pathogen
Description	Spiking of a predetermined quantity target pathogen added to a split subsample of the sample being tested ("early spike") **OR** Spiking of a predetermined quantity target pathogen into the seed sample being tested after extraction ("late spike").
ELISA	Recommended

Note: Users are recommended to use this control in case of testing disinfected and/or treated seeds.

3. ASSAY SET-UP

Control and sample replicates must be included in ELISAs for routine seed health testing. The minimum number of replicates and recommendations for ELISA plate set-up and the determination of sample results are described in Table 2.

Table 2. ELISA plate set-up and results determination.

Description	Essential	Recommended
Control replicates: Run each control in duplicate wells on each plate.	×	
Sample replicates: Run each sample in duplicate wells.	×	
Plate set-up: Border wells should not be used for samples or controls.		×
Substrate incubation: Follow the vendor's suggested substrate incubation temperature and times prior to measuring OD values. Measure OD values using the wavelength that is suggested by the vendor. If no times are provided or if the ELISA is generated in-house, use the substrate incubation time that generates the optimum signal: noise (S:N) ratio.	×	
Cut-off determination: Use the vendor's recommended cut-off (e.g., a signal over noise (S:N) ratio (TC:NPC) of 2). Alternatively, use the average NPC OD value plus three times the standard deviation of the NPC OD values. All samples with average OD values at or above the cut-off are considered positive.	×	

4. OPTIMISATION OF ELISA TESTS

Certain components of an ELISA influence the performance and outcome of the test. They are described in Table 3 and should be controlled for quality and validated prior to use in routine seed health testing.

Table 3. Components of ELISA testing.

Description	Essential	Recommended
Quality Control (QC): A request should be made to the vendor to provide any QC information on the antibody/ ELISA kit components as well as performance expectations. Verify that all performance specifications are met when the ELISA is performed with the materials and conditions used for routine seed health testing. This is necessary to avoid the risk of false negative sample results.	×	
Blending antibody batches: Do not mix different batches of antibodies from the same vendor or different vendors.	×	
Comparison of antibody batches: Compare the performance of each new batch of antibody to those that were previously in use to ensure performance is equivalent or better. The optimal dilution of the new batch of coating and conjugate antibodies may be determined using a checkerboard dilution series.	×	
Coating antibody and conjugate batches: It is recommended to use the batch of coating antibody and the batch of conjugate that were obtained at the same time. If coating antibody and conjugate batches are received at different times, it is recommended that the laboratory ensure that performance is equivalent or better.		×
S/N ratio: It is recommended that an ELISA have an S:N ratio of at least 10:1 to ensure sufficient separation between positive and negative results, see ELISA Development Guide. To calculate the S:N ratio for an ELISA, divide the average PPC OD value by the average NPC OD value.		×
Use of molecular techniques in conjunction with ELISA: If molecular techniques (e.g., PCR) are used in conjunction with ELISA, all appropriate controls must be included, as defined in the ISHI Best Practices for PCR Assays in Seed Health Tests.	×	

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