International Seed Federation
Seed is Life

INTERNATIONAL SEED HEALTH INITIATIVE (ISHI)

Best Practices for ELISA Assays in Seed Health Tests

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I. ELISA FOR DETECTION

An ELISA will detect proteins associated with a target pathogen without discriminating whether the source of the protein is pathogenic, viable, or even intact. Such "indirect" tests, when used in seed health testing, do not yield any information about pathogen viability and are not sufficient to prove pathogenicity. While a negative ELISA result can be assumed to be conclusive evidence of a negative seed lot, a positive ELISA result must be followed by a direct test that confirms viability of the pathogen and its pathogenicity, see www.worldseed.org/wp-content/uploads/2015/10/Indirect_Seed_Health_Tests_2013.pdf. In such a situation, an ELISA could be considered a pre-screen for the subsequent direct test, such as a bio-assay or grow-out. An ELISA may also be used to detect a target pathogen in suspect plant tissue obtained from a bio-assay or grow-out.

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

- Routine ELISAs used for the detection of specific viral or bacterial pathogens on seed.
- Validation of new ELISAs.

Unless exceptions are stated, these process controls and assay conditions should be applied to all ELISAs. 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 ELISAs are defined in Table 1. Their purpose is to verify both the quality of the materials used in the sample extraction/ELISA process 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 ELISAs.

Control type	**Buffer (Blank) Control (BC) - Essential**
Definition	The buffers and reagents used in the ELISA, with no seed/tissue matrix ortarget pathogen.
Expected Result	No detection of the target pathogen.
Description	The BC is used to assess the background optical density (OD) value of the ELISA in the absence of seed/tissue matrix and target pathogen. The BC serves as a negative control for the ELISA process. The BC wells can also be used as blanking wells.

Control type	Negative Process Control (NPC) - Essential
Definition	Negative matrix (seed or tissue) that contains no target pathogen and istested at the same time, using the same assay as the corresponding samples.
Expected Result	No detection of the target pathogen.
Description	The NPC is used to establish a base-line OD value for negative samples of the same matrix (seed or tissue). This value is used to calculate the cut-offto distinguish between negative and positive samples. The NPC serves as a negative control for the sample extraction and ELISA processes.

Control type	Positive Process Control (PPC) - Essential
Definition	Positive matrix (seed or tissue) that contains the target pathogen and istested at the same time, using the same assay as the corresponding samples.
Expected Result	Detection of the target pathogen.
Description	The PPC serves as a positive control for the sample extraction and ELISA processes. It could be one of the following: - A seed sample that contains the target organism (known infected seed sample); - A seed sample spiked with target organism; - A seed extraction buffer spiked with target organism.

III. ELISA 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.	x	
Sample replicates: Run each sample in duplicate wells.	x	
Plate set-up: Border wells should not be used for samples or controls.		x
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.	x	
Cut-off determination: Use the vendor's recommended cut-off (e.g., an S:N ratio (TC:NPC) of 2). Alternatively, use the average NPC OD value plus 3 times the standard deviation of the NPC OD values. All samples with average OD values at or above the cut-off are considered positive.	x	

IV. 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
ELISA 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.	x	
Blending antibody batches: Do not mix different batches of antibodies from the same vendor or different vendors.	x	
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.	x	
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.		x
ELISA signal to noise 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 https://resources.rndsystems.com/pdfs/datasheets/edbapril02.pdf. To calculate the S: N ratio for an ELISA, divide the average PPC OD value by the average NPC OD value.		x
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 ISHI-Veg's Best Practices for PCRAssays in Seed Health Tests, see www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg-method-development.	x	