Appendix 1 - Development and Validation Data Targets for Proposed NSHS Seed Health Testing Methods

Criterion	Sub-Criteria	Definition	Evidence	Preferred minimal data collection	Notes
Sensitivity-Method	Limit of Detection	The lowest level of contamination by the target organism that is consistently detected by the method	Dilution series performed with known contaminated seeds into clean (non-contaminated) seeds to show the limit of detection.	Minimally 5 samples at LOD	Also can be measured by spiking seed sample with cells, virions, DNA concentration, etc. Would be ideal to show this against a direct method (such as grow out) to show biological relevance of contamination rates vs. detection capability
	Diagnostic sensitivity	Rate of false negative results (percentage of true positives detected by the method)	Consistent detection of contaminated samples at appropriate levels of contamination	Minimally 5 samples at each level of contamination	Diagnostic sensitivity differs for different levels of contamination
Sensitivity-Assay	Limit of Detection	The lowest concentration of target pathogen that is consistently detected (>95%) by the [PCR, ELISA, etc.] assay	Dilution series performed with cells, virions, DNA concentration, etc. and replicated to show the limit of detection.	Minimally 20 replicates at LOD (with 19 detects) will achieve 95%	Not applicable to all assays (e.g., blotter tests)
Specificity	Inclusivity	Method detects all relevant variants of the target pathogen	Assay should be evaluated against an appropriate collection of strains/isolates/variants that represent different origins in geography, host, and time as are available; method should be evaluated using seed samples contaminated with variants of the target pathogen	Replicated samples of appropriate variants	Per availability of seed samples; method should detect all lots as positive that result in disease occurrence
	Exclusivity	Method excludes (minimally cross reacts with) non-target microbial strains including closely related species and look-alikes; method does not produce positive results for samples free of the target organism	Assay should be evaluated against an appropriate collection of microbial strains or isolates that reflect populations associated with routine testing samples; Method should be evaluated using seed samples from different geographic origins, production years, crop species that are free of target pathogen populations.	Minimally 5 negative control samples from different origins	
	Diagnostic specificity	Rate of false positive results (percentage of clean samples testing positive by the method)	See Exclusivity		
Selectivity		Ability of the method to detect the target pathogen(s) without being affected by seed matrix variations	Method should be evaluated using contaminated samples of seeds of different origins	Minimally 5 contaminated samples from different origins	Diverse samples can be spiked with a single variants of the pathogen; or naturally contaminated samples of different origins can be used
Repeatability		Agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the same lab and operating conditions over a short interval of time	Method should be repeated in a lab on replicate seed samples by the same technician, using the same reagents to show results (positive and negative) are replicable.	40 (10 positive and 10 negative replicates X 2 days)	
Reproducibility		Agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under operating conditions existing across different laboratories	Method should be performed across labs (minimally 3) on replicate seed samples with varying levels of pathogen contamination rates to show results (positive and negative) are reproducible. Lab conditions should include: different technicians, reagent sets, equipment, etc.	20 replicates per lab (10 positive and 10 negative replicates)	Temporary standard methods can be approved without reproducibility data
Robustness		A measure of the capacity to remain unaffected by small but deliberate variations in method parameters; provides an indication of reliability during normal usage	Can be demonstrated through reproducibility data and through systematic variation of method parameters (e.g., pipetting volumes, incubation times, etc.)	3 levels of each crucial parameter that is varied	Method parameter selection must be considered carefully but needn't be comprehensive