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MEMORANDUM

To: ALL TECHNOLOGY DEVELOPERS AND LICENSEES

From: GERALD GLENN F. PANGANIBAN, Ph.D.
Director
 Bureau of Plant Industry

Date: January 10, 2024

Subject: Guidelines for Sampling and Testing of Refuge Seeds of Corn for Insect Resistance Management (IRM) Pursuant to DA Memorandum Circular No. 2, Series of 2014 "Enhancing the IRM Strategy for Bt Corn Targeting Asian Corn Borer"

DEFINITION OF TERMS

a) Acceptable quality limit (AQL)	desired level of concentration of the tested parameter to accept the lot ¹
b) Composite sample	formed by combining and mixing all the primary samples taken from the seed lot ²
c) Confidence level	probability that the tested parameter falls within specific range of values
d) Primary sample	a portion taken from the seed lot during one single sampling action ²
e) Refuge seeds	non-Bt seeds intended to be planted with Bt crops to maintain susceptible insect populations that can mate with any resistant insects emerging from the Bt crop
f) Representative sample	a subset of the lot obtained to reflect or represent its characteristics
g) Retained sample	submitted sample stored in the laboratory for subsequent re-testing or validation of test results
h) Sampling	the process of obtaining a representative sample from a lot ³

i) Seed lot	specified quantity of seed that is physically and uniquely identifiable ²
j) Seed pool	collection of seeds obtained from the working sample (subsample) by which the analysis will be conducted
k) Submitted sample	sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a subsample thereof ²
l) Subsample	portion of the working sample obtained by sample reduction ²
m) Working sample	whole of the submitted sample or a subsample, thereof, on which the test/s is made ²

SAMPLING GUIDELINES

1. Technology developers/licensees shall notify in writing the BPI Biotechnology Office 1 week before the sampling schedule.
2. The technology developers shall provide the complete sample information (e.g. lot number, sample description, quantity represented) to BPI through the laboratory analysis request form (LAR form) before the sampling activity.
3. All sampling activities shall be conducted under BPI supervision. Safety precautions shall be observed by all personnel involved during sampling.
4. Random sampling shall be conducted for each lot to obtain representative samples. Sampling procedure shall be based on the International Rules for Seed Testing Chapter 2: Sampling (ISTA, 2023)^[4]. The following are the minimum recommendations based on ISTA:
 - a. Seeds may be sampled in containers. The containers holding the seeds must be fit for purpose (i.e. undamaged, clean to avoid cross contamination, and sealable).
 - b. The containers must be labelled or marked properly prior to sampling. The following information must be indicated legibly:
 - Lot Description
 - Lot Number
 - Quantity
 - c. The seed lot must be arranged so that each portion is conveniently accessible.
 - d. Minimum sampling intensity shall be followed (refer to Annex A).
 - e. When seed lot is in containers, containers to be sampled must be selected at random.
 - f. Primary samples must be drawn from top, middle, and bottom of the containers.

^[4] ² ISTA. (2023). International Rules for Seed Testing - Chapter 2: Sampling. *International Rules for Seed Testing, 2023*.
<https://www.seedtest.org/en/international-rules-for-seed-testing/chapter-2-sampling-product-1009.html>



- g. When seed lot is in containers, containers to be sampled must be selected at random.
 - h. Primary samples must be drawn from top, middle, and bottom of the containers.
 - i. The primary samples shall be combined to form the composite sample and shall have an approximate weight of no more than 2.0kgs for submission to the laboratory.
 - j. Sampling must be conducted using the appropriate equipment. All sampling apparatus must be cleaned before use to prevent cross contamination.
 - k. Triers must be long enough so that the opening at the tip reaches at least half of the diameter of the container.
5. Maximum seed lot size or weight shall not exceed 20,000kgs.
 6. The samples should be properly packed, labelled, handled, and submitted to the laboratory based on ISTA or BPI requirements:
 - a. Submitted samples must be labelled with the same identification as the seed lot.
 - b. Submitted samples must be packed securely and handled carefully to prevent damage, substitution, or manipulation during transit.
 7. Retained samples shall be stored in the laboratory for two (2) weeks after the release of results. The technology developers shall decide and inform BPI through a formal letter if they will subject it to retesting and/or return.
 8. The cost relative to sampling shall be borne by the technology developers/licensees.

TESTING GUIDELINES

1. Testing of refuge seeds shall be conducted or supervised by BPI.
2. Necessary forms and documents related to the sample (i.e., list of samples with lot number/code and quantity) shall be submitted by the technology developers/licensees to the BPI prior to laboratory testing.
3. Appropriate statistical tools for seed testing shall be used to determine the number of working samples to be obtained and tested from the submitted sample (e.g. USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) sample size calculator).
4. For the testing samples, an AQL of 4% should be accepted at 95% confidence level, as computed based on Annex C. Operating Characteristic Curves for Multiple Sample Plans with Qualitative Test.
5. Working sample shall be obtained from the submitted sample of refuge seeds.
6. To get the working sample, three hundred sixty (360) seeds will be randomly obtained from the primary sample. Subsamples will be obtained by dividing the working sample into twenty (20) seed pools, at 15 seeds per pool for testing.
7. Seeds or plumules from germinated seeds can be homogenized in an appropriate buffer. Any measures to prevent sample cross-contamination must be employed.
8. Serological-based methods such as lateral flow immunoassay can be used to detect the presence of Bt protein. Lateral flow strips specific for Bt protein shall be used to detect the presence of BT seeds in the test solution. Appearance of one line (control line) on the strip indicates a negative result while appearance of two lines on the strip indicates a positive result.
9. Test should meet the appropriate sensitivity (limit of detection) in the sample.

10. A maximum of 3 positive pools shall be allowed to qualify the lot as refuge materials. This limit corresponds to the Bt seed threshold level of $\leq 4\%$.
11. Travel costs relative to testing shall be borne by the technology developers/licensees such as transportation, food and lodging.
12. The technology developers/licensees shall provide necessary materials for testing and lateral flow strips for at least two insecticidal proteins present in their approved events. BPI personnel are entitled to charge overtime fees during sampling and testing based on existing policies and guidelines.

RE-TESTING

1. In the event of detecting a purity level of more than 4% (>3 seed pools) in the submitted samples, the technology developer/licensees may request re-testing upon receiving the initial test report.
2. Re-testing can be conducted using the retained or re-submitted samples by the technology developer/licensees.
3. A maximum of two re-tests for each lot shall be done if detected impurity is higher than 4%.

DECISION RULE

1. Result of laboratory test shall determine whether the lot shall be accepted or rejected as refuge seeds.
2. The following table shall be followed when re-testing will be conducted:

Initial Test	1 st Re-test	2 nd Re-test	Decision
≤ 3 positive seed pools	stop	N/A	Pass ¹
>3 positive seed pools (conduct re-test)	>3 positive seed pools	stop	Fail ²
>3 positive seed pools (conduct re-test)	≤ 3 positive seed pools (need to subject to 2 nd re-test)	≤ 3 positive seed pools	Pass ¹
>3 positive seed pools (conduct re-test)	≤ 3 positive seed pools (need to subject to 2 nd re-test)	>3 positive seed pools	Fail ²

¹Accept the lot. The lot can be used as refuge seed.
²Reject the lot. The lot cannot be used as refuge seed.

3. Rejected seed lots should not be used as refuge.
4. The technology developers/licensees may replace the seed lot and re-submit another sample/s for testing.



MONITORING OF COMPLIANCE

NON-COMPLIANCE

1. Non-compliance to this guideline shall be subject to sanctions deemed appropriate by the BPI.
2. The technology developers/licensees shall be notified for non-compliance.

EFFECTIVITY

This Memorandum shall take effect immediately and shall supersede all other policies inconsistent herewith.



ANNEX A

Minimum sampling intensity

For seed lots in containers holding up to and including 100kg, the minimum sampling intensity is based on the following table:

Number of containers	Minimum number of primary samples to be taken
1-4	3 primary samples from each container
5-8	2 primary samples from each container
9-15	1 primary sample from each container
16-30	15 primary samples, one each from 15 different containers
31-59	20 primary samples, one each from 20 different containers
60 or more	30 primary samples, one each from 30 different containers

For containers holding less than 15 kg of seed, containers must be combined into sampling units not exceeding 100 kg, e.g., 20 containers of 5kg, 3 containers of 3 kg or 100 containers of 1 kg. The sampling units must be regarded as containers based on table 1.

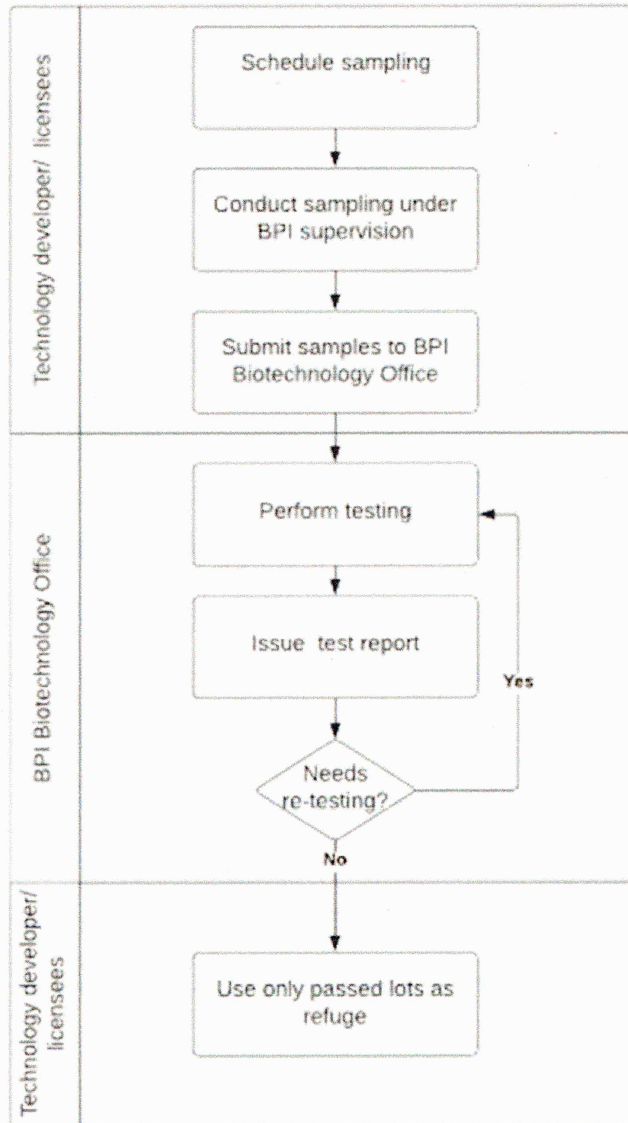
When sampling seed in containers holding more than 100 kg of seed, or from streams of seed entering containers, the sampling intensity according to the following must be regarded as the minimum requirement:

Seed lot size	Number of primary samples to be taken
Up to 500 kg	At least five primary samples
501 - 3,000 kg	One primary sample for each 300 kg, but not less than five
3,001 - 20,000 kg	One primary sample for each 500 kg, but not less than 10
20,001 kg and above	One primary sample for each 700 kg, but not less than 40



ANNEX B

Refuge Sampling and Testing Process Flow*



**After testing was done by the BPI Biotechnology Office, note the provisions on RETESTING and DECISION for reference.*

ANNEX C

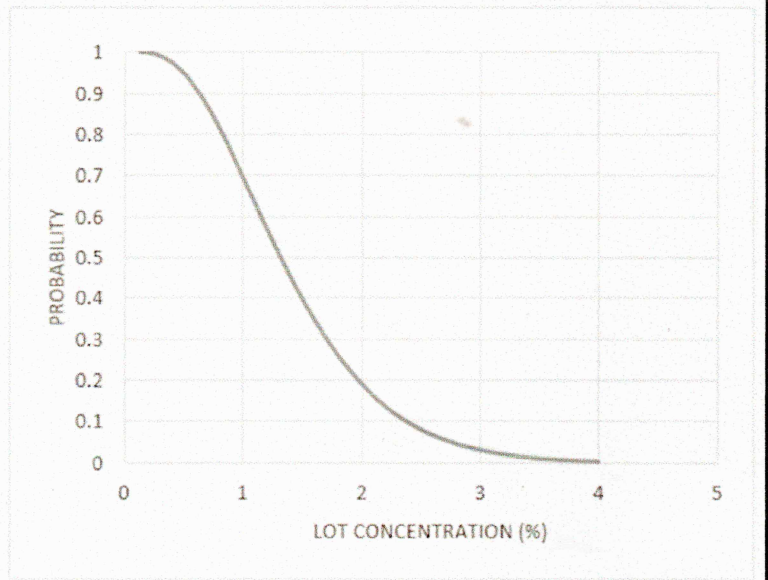
Operating Characteristic Curves for Multiple Sample Plans With Qualitative Test⁴

Three values are required to define a multiple sample plan.

1. The number of samples to be taken and tested.
2. The maximum number of positive tests allowed for the lot to be acceptable.
3. The number of kernels in each sample.

The plot gives the probability of accepting lots with the lot concentrations given on the x-axis. The probability curve reflects the multiple sample plan defined by the values entered in the following yellow boxes. The AQL stands for acceptable quality level and usually is a lot concentration that should be acceptable most of the time. For this worksheet, entering a lot concentration for AQL is a way to get the specific probability for the selected lot concentration.

# of Kernels per Sample	<input type="text" value="15"/>
# of Samples	<input type="text" value="20"/>
Maximum # of Positives	<input type="text" value="3"/>
AQL	<input type="text" value="4.00"/>
Probability of accepting AQL	0.004



⁴Remund, K., Dixon, D., Wright, D., & Holden, L. (2001). Statistical considerations in seed purity testing for transgenic traits. *Seed Science Research*, 11(2), 101-120. doi:10.1079/SSR200166