Sampling of Grain and Seed to Estimate the Adventitious Presence of Biotechnology-Derived Seeds in a Lot

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In this article the basics of seed and grain sampling and the uncertainties that are an integral part of examining samples from a lot rather than the entire lot are described for analysts who do not have significant training in statistics. Sampling, sampling statistics, and test methods as applied to measuring low concentrations of biotechnology-derived materials (also known as genetically engineered [GE] materials or genetically modified organisms [GMOs]) in seed and grain lots are discussed. The term “adventitious presence” (AP) is used to describe a low concentration of seeds or grains containing biotechnology-derived materials in a lot. How sampling plans may be developed and applied also are discussed. For details of sampling procedures, the reader is referred to the references listed at the end of the article.

The Role of Sampling

Typically, buyers of bulk commodity lots have a set of specific characteristics they want a lot to possess. The price of a lot is negotiated based on its characteristics. The size of a bulk commodity lot can vary widely—ranging from smaller lots, such as the contents of a farmer’s truck, to very large lots, such as the contents of a large oceangoing vessel. Both the buyer and seller need to know that a lot has the characteristics specified in the contract. Examining an entire lot generally is prohibitively time-consuming and expensive. If the test is destructive, such as testing for the presence of biotechnology-derived seed or grain, testing the entire lot would have some obvious disadvantages, with the foremost being destruction of the lot.

An alternative to testing the entire lot is to test a small fraction of the lot. This small fraction is called a sample. Analysis of samples is an economical and practical means of estimating the characteristics of an entire lot; however, it does have some disadvantages. The main disadvantage of sampling is that the characteristics of a sample are rarely exactly the same as the characteristics of the lot from which it is taken. For example, the percentage of AP seeds in a sample is unlikely to be exactly the same percentage as is present in the entire lot, due to the inherent limitations of sampling plans.

If samples are taken from the lot using proper sampling methods, statistical theory describes how much the samples are likely to deviate from the lot content as a whole. This variation among samples is related to sample size and can be managed by choosing sample sizes that produce acceptable variation at an acceptable cost for testing.

Sample deviations cause uncertainty for both sellers and buyers. The seller may take a sample to estimate the characteristic of the lot before shipment, while the buyer may take a sample to estimate the characteristic of the lot upon arrival at its destination. Because the seller and buyer have different samples, each will likely have different estimates. Knowledge of variation can help the seller and buyer choose appropriate sample sizes and acceptance criteria. The size of the difference between their two samples can be anticipated, and understanding this difference will result in fewer disputes.

Although sampling from a lot is a significant source of variation, it is not the only source of variation. Once a sample is taken from a lot, it usually requires some processing before a measurement is made using an analytical method. The increase in variation due to sample preparation and measurement depends heavily on the technology used for processing and analysis. Some technologies may add very little to the variation, while others may increase variation significantly.

The sampling and testing process consists of a number of steps. Each must be performed with sufficient care and understanding...
of the process to obtain a good estimate of the concentration of the analyte in the lot. The following steps comprise the process:

1) Determine the type of lot (static or flowing) and, thus, the type of sampling strategy that is appropriate. The strategy may include representative or random approaches and the use of an appropriate probe or sampler.
2) Take multiple increment samples and combine the increments into one bulk sample.
3) Mix the bulk sample thoroughly and withdraw at least one sample to send for analysis (the laboratory sample). One or more extra samples can be taken at this point (file samples) for future reference. It should be noted that file sample(s) may or may not contain the same concentration of analyte as the laboratory sample due to sampling considerations.
4) Test the laboratory sample using the appropriate testing strategy (quantitative, qualitative, or subsampling).

The types of samples used in testing are described in the side bar.

Relation of Sample Size to Lot Size

Sampling from Large Lots. Sample size, once the lot exceeds a certain size, is not dictated by the size of the lot, but by the sample needed to perform the required testing. For example, the sample sizes sent to a laboratory will not differ based on whether the testing is for the presence of biotechnology-derived material in the lot. The types of samples used in testing are described in the side bar.

Sampling from Small Lots. For small lots, some published acceptance sampling plans (e.g., ISO 24333:2009 [3] and the ISTA Handbook on Seed Sampling [4]) call for smaller sample sizes than may be taken for larger lots for purely economic reasons: the value of the seed that is destroyed by testing may be significant (e.g., in the case of high-value vegetable seeds). A smaller sample size means that the probability of accepting a higher concentration of analyte in a lot is greater. In some situations, accepting a higher concentration in a small lot may be acceptable, but the results of such testing should be understood before accepting the lot. Taking a smaller sample size for smaller lots, thus, is a compromise between the loss of seed due to destructive testing and the cost of accepting a higher analyte concentration. Sampling of small seed lots and small amounts of grain, where the laboratory sample exceeds 10% of the total lot, is a special case and not covered in detail in this article.

Sampling Procedures

Random samples are desired for estimating lot characteristics. A simple random sample is one that is selected through a process that gives every possible sample an equal chance of being selected. Random samples can be taken from some lots, such as lots that have computerized records of the elements of the lot. However, obtaining random samples of this type from large bulk commodity lots is not practical.

In theory, almost any sample taken from a lot that has a perfectly uniform (homogeneous) distribution of seeds also will result in a random sample. However, in practice, bulk lots are rarely homogenous unless measures have been taken to make them so. For example, a lot with a low concentration of target (AP) seed that is not homogenous (i.e., heterogeneous) will have areas of the lot with higher concentrations and other areas of the lot with lower concentrations. Variability caused by heterogeneity can be reduced by using proper sampling procedures such as sampling a sufficient number of increments.

Practical sampling procedures that take into account the possibility that lots can be heterogeneous have been developed for bulk commodity lots. Descriptions of practical sampling procedures can be found in documents such as the USDA GIPSA Grain Inspection Handbook—Book I, Grain Sampling (7) or ISO 24333:2009 (3). Samples taken using practical sampling procedures are called representative samples.
Representative Samples. The concept behind practical sampling procedures is to take multiple small samples from throughout a lot. These small samples are sometimes called increment samples. If the lot happens to be heterogeneous, taking increment samples throughout the lot improves the chances of sampling high and low concentrations (e.g., AP seeds) in different areas of the lot. Although heterogeneity in the lot can contribute to the variability of estimates of lot averages, if most or all areas of a heterogeneous lot can be sampled, then the contribution to variability from heterogeneity is reduced or eliminated.

Practical sampling procedures have been developed for sampling all areas of flowing and static grain lots. The equipment and techniques differ for flowing and static lots, but the concept is the same—take many small samples from throughout the lot.

Large commercial facilities typically use an automatic sampler to sample the flowing grain stream. These samplers, sometimes called diverter samplers, are installed in a grain spout. A device inside the diverter sampler periodically passes through the grain stream and takes a small slice as it passes through the stream. The composite of the increment samples is the bulk sample for the lot. A lot in a large commercial facility may be the contents of a bin or silo that may contain 500 tons or more of grain. As many as 100 equally spaced increment samples may be taken from the flowing lot.

Flowing grain streams of smaller lots can also be sampled at the farm level, e.g., at the tailgate of a truck or the spout of a harvester. Containers have been developed to manually pass through a grain stream, but other containers, such as a small bucket, can be used. The concept is the same as in a commercial facility: multiple increment samples are taken from the lot. The grain stream must be accessible, such that the sampling container can be passed safely through the grain stream at approximately equally spaced intervals. For small farm lots, a minimum of four increment samples is reasonable.

A hand probe, a long tube that is pointed on one end, is the only effective device for sampling static lots. Probes are made in different lengths for sampling containers with different depths, such as trucks, railcars, and barges. A hand probe is inserted into a lot the full depth of the lot. It is inserted slightly off vertical (=10 degrees off vertical). A handle on the end of the probe is turned to open ports on the side of the probe to let grain fill the probe. After filling, the handle in the end of the probe is turned to close the ports, and the probe is removed from the lot. The content of the probe constitutes one increment sample from the lot. The composite of the increment samples is the bulk sample for the lot. Multiple increment samples are taken from the lot following an appropriate pattern for the container being sampled. An example of a probe pattern is given in Figure 1. The probe pattern followed is taken from the USDA GIPSA Grain Inspection Handbook—Book I, Grain Sampling (7) and is recommended for hopper-bottom trucks and trailers. Probe samplers extended on booms and operated remotely are typically used at grain facilities to simplify sampling of large numbers of trucks and railcars.

Random Samples. A simple random sample is a sample taken through a process in which every possible sample from the lot has an equal chance of being selected. For example, if a sample of 10 items is to be selected from a lot, the first 10 items in the lot have the same chance of being selected as the last 10 items. A random sample can be taken from a lot when the items in the lot can be uniquely identified. For large lots, the number of combinations of items in the sample can be extremely large. Choosing random samples is easiest if the elements are in a computerized database or some other electronic form.

As an example, suppose a random sample of 3 items is to be selected from a lot of 10 items. A simple random sample can be obtained from the lot by assigning each item in the lot a random number using a uniform random number generator, which are included in many software packages. The three items in the lot with the three smallest random numbers are chosen for the random sample. Sorting by the random number is helpful in identifying the random sample. Note, the random sample could also be the items with the three largest random numbers. The decision on how to choose the three samples must be made before assigning the random numbers (Table 1). As with any sampling process, these samples are then combined to form the bulk sample.

Reduction of Bulk Sample to Test Sample

Representative and random sampling result in a number of increment samples that are combined to form a bulk sample. The bulk sample must be as large as, and is usually much larger than, the sample needed to perform the analysis. Thus, the bulk sample typically must be reduced to provide one or more samples to be sent for analysis (the laboratory sample). In addition, it is common practice to retain one or more extra samples as file samples for use as a reference or in case of possible disputes.

Reduction of the bulk sample to obtain the laboratory and file sample(s) must be done with proper consideration so the laboratory sample is the best possible representation of the bulk sample. It must be well mixed to make it as homogeneous as possible. The sample can be reduced in size manually using accepted approaches, including using a suitable divider (such as Boerner divider). When the bulk sample has been reduced to the size required for the next step, the samples should be clearly identified, and chain of custody of the sample should be maintained.

Testing the Laboratory Sample—Qualitative or Quantitative?

Once the bulk sample has been reduced to the laboratory sample, a decision must be made as to which type of test to ap-

![Example of a probe pattern for hopper-bottom trucks and trailers (6).](Image)

Fig. 1. Example of a probe pattern for hopper-bottom trucks and trailers.

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Table 1. Choosing 3 items from 10 using randomly assigned numbers

<table>
<thead>
<tr>
<th>Lot Item</th>
<th>Step 1: Assign Random Numbers</th>
<th>Step 2: Choose Three with Smallest Random Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Item</td>
<td>Random Number</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
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<td>6</td>
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<td>7</td>
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<td>0.998077</td>
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<td>8</td>
<td>8</td>
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<td>9</td>
<td>9</td>
<td>0.998077</td>
</tr>
</tbody>
</table>
A lot with 2% AP seed (seeds containing biotechnology-derived materials) is nearly as likely. If a qualitative test is used (a test that gives a negative if no AP seeds are present and a positive otherwise), the factor of interest in the chart is the probability of having zero AP seeds. The probability of having no AP seeds in the sample is ≈0.13. Alternatively, the probability of having one or more AP seeds in the sample in this example is ≈0.87.

The probabilities of the most likely outcomes if the sample size is doubled to 200 seeds are shown in Figure 3. If the lot contains 2% AP seed, the most likely outcome is that the sample will contain 2% AP seed (4 AP seeds in 200 seeds). If a qualitative test is being used, the probability of having no AP seeds in the sample is ≈0.02. Increasing the sample size from 100 to 200 seeds increases the probability of having at least 1 AP seed in the sample from ≈0.87 to ≈0.98. The probabilities shown in Figure 3 are dependent only on the sample size and the concentration in the lot. The lot size does not enter the calculation as long as the sample is <10% of the lot.

Acceptance Sampling Using a Single Sample. A qualitative test produces a negative result if there are no AP seeds in the sample and a positive result if there are one or more AP seeds in the sample. In acceptance sampling using qualitative testing, a lot is accepted when a negative result is obtained for the sample. The probability of accepting a lot depends on the concentration of AP seeds in the lot and the sample size.

At any particular sample size, the chance of finding a sample containing no AP seed will depend on the level of AP seed in the lot. If a lot with 0.1% AP seed is sampled and the sample contains 100 seeds, the probability of accepting the lot (finding no AP seeds in the sample) is 0.90. That is, 90% of the samples tested will not contain an AP seed. A 100 seed sample from a lot with 1.0% AP seed has a 0.37 probability of being accepted (37% of the samples will not contain an AP seed). Probabilities of accepting lots using 100 seed samples can be computed for many lot concentrations. These probabilities are plotted against various lot concentrations in Figure 4. The plot shows that a lot containing 3% AP seed has an ≈0.05 probability of acceptance. If a buyer does not want to accept a lot containing more than 3% AP seed, a qualitative test using a 100 seed sample would be an appropriate test because it provides a 95% certainty of identifying a lot that has this concentration of AP seed (5% of the samples will be negative).

Sample sizes can be compared by putting probability curves for different sample sizes in the same graph. The plots for samples containing 100, 150, and 300 seeds are shown in Figure 5. These sample sizes would have a 0.95 (95%) probability of rejecting lots with 3, 2, and 1% AP seed concentrations, respectively.

Sample size, thus, is an important consideration when managing the risks of accepting lots containing AP seeds. As an example, if a buyer wants to detect lots containing 0.1% AP seed with a 0.95 probability, a sample of 2,995 seeds is needed. However, qualitative test methods usually specify a maximum sample size, which depends on the limit of detection (LOD) of the test method. For example, some qualitative test methods may specify a maximum sample size of 800 seeds. In effect, the method has a limit of detection of 1/800, or ≈0.125%. If the recommended sample size for the method is exceeded, one AP seed in the sample would be less than the LOD, and the test might not reliably detect its presence. False negatives would occur, and the probability of detection would be less than desired. When the
desired sample size exceeds the recommended sample size for a test method, the sample must be divided into smaller samples. These smaller samples should be no greater than the maximum sample size allowed for the method. If a 2,995 seed sample is to be tested using a method that has a maximum sample size of 800, the 2,995 seed sample should be divided into four samples of ≈750 seeds each. Each of the four samples would be tested, and a positive on any test would mean the entire sample tested positive. This subsampling approach can also be used to yield a quantitative result (described below).

**Acceptance Sampling Using Multiple Subsamples.** Qualitative testing can be used to control the risk of accepting a high concentration of AP seed in a lot. For example, as illustrated in Figure 5, testing a 300 seed sample results in only a 0.05 probability of accepting a lot containing 1% AP seed. This type of testing provides reasonable protection for the buyer or importer that a lot does not contain significantly more than 1% AP seed. However, if the curve for 300 seeds in Figure 5 is examined, the AP concentration has to be near zero (well below 1%) for the lot to have a very high probability of being accepted. If 1% AP seed in the lot is acceptable, the seller or exporter may wish to have concentrations somewhat greater than zero to have a reasonable chance of being accepted.

Quantitative testing is necessary for AP concentrations greater than zero to have a reasonable chance of being accepted. Quantitative results can be obtained using a quantitative method such as quantitative real-time PCR (5) or qualitative tests when using multiple sampling plans. Multiple sampling plans test more than one sample, and quantitative results can be obtained for use in those situations where some positive results are acceptable. An example of a multiple sample plan is to test 39 samples of 100 seeds each; the lot would be acceptable if 19 or fewer samples test positive for the presence of AP seeds. The probability curves for this multiple sample plan and a single qualitative test using a 300 seed sample are shown in Figure 6. Both sample plans will detect lots containing 1% AP seed with at least a 0.95 probability. The difference between the plans can be seen at lower concentrations. For lots containing 0.5% AP seed, the 300 seed sample will accept the lot with a 0.22 probability; lots containing 0.5% AP seed will be rejected with a probability of 0.78. Using the multiple sample plan, lots containing 0.5% AP seed will be accepted with a 0.91 probability. Multiple sample plans will reject fewer lots that are acceptable. Tools to derive single and multiple sampling plans and calculations of probabilities and acceptance curves are provided by the ISTA tool SeedCalc (6).

![Fig. 4. Probability of accepting various lot concentrations of AP seed (seeds containing biotechnology-derived materials) using a 100 seed sample.](image1)

![Fig. 5. Probabilities of accepting various lot concentrations of AP seed (seeds containing biotechnology-derived materials) using different test sample sizes.](image2)

![Fig. 6. Probabilities of accepting various lot concentrations of AP seed (seeds containing biotechnology-derived materials) using a single sample plan and a multiple sample plan.](image3)
Comparison of Single Sample and Multiple Subsampling Approaches. Acceptance plans for subsample testing have three components: number of subsamples to test, size of subsamples, and maximum number of positive tests. Changing any one of the three components will change the shape of the probability curve. By changing all three, many different acceptance sampling plans can be found that have an ≈0.05 probability of accepting a lot containing, for example, 1% AP seed. The purpose of subsample testing is to provide more protection for the seller or exporter by accepting more lots with AP levels lower than 1%. To increase the chances of accepting lots with AP seed levels <1%, the number of subsamples tested would have to increase. Increasing protection for the seller or exporter comes with the increased costs of testing more subsamples.

Another way in which the possibility of rejecting good lots, or accepting lots that are around the threshold, is multiple step sampling. In this approach, the results of the first test are used to decide whether to test a second sample (which may or may not be the same size). Such plans can reduce the total cost of testing while still allowing good lots to be accepted without increased risk of accepting bad lots. Seedcalc (6) allows the generation of two-step sampling plans.

Statistics of Quantitative Testing

As discussed in the section on testing multiple subsamples using qualitative testing, qualitative testing can be employed to obtain quantitative information. In particular, the risks of rejecting good lots can be reduced by this approach when some level of AP seed is acceptable in a lot. Of course, the same risk reductions can be obtained with a single quantitative test if the acceptance sampling plan is appropriately specified. An acceptance sampling plan with quantitative testing typically specifies the sample size and acceptance limit (AL). A representative sample is taken from the lot, and a test sample of the specified size is obtained. A specified test is performed on the sample, and the resulting measurement is compared to the AL. In this approach the statistical uncertainty of the quantitative test method must be taken into account. A decision to accept or reject the lot is based on the comparison to the AL. Typically, a lot would be accepted if the measurement for the sample is below or equal to the AL. The lot would be rejected if the measurement for the sample is above the AL.

With quantitative testing, the AL can be any non-negative value. Once a sampling plan has been specified, a curve of probabilities for acceptance of various lot concentrations can be constructed. This curve of probabilities is called an operating characteristic (OC) curve. The shape of a typical OC curve is illustrated in Figure 7. The probabilities in this curve must take into account the uncertainty of the measurement method and sampling variation. Although computation of these probabilities is beyond the scope of this article, some aspects of selecting a sampling plan can be discussed.

As shown in Figure 7, a lot that contains a concentration at the AL has an ≈50% chance of being accepted. Intuitively, a sample from a lot that has a concentration at the AL will have about the same probability of being below or above the AL. This is true regardless of the sample size. The sample size will influence the steepness of the curve, but the curve will cross the acceptance limit at ≈50% probability.

Sometimes it is desired that a specific lot concentration be accepted most of the time (e.g., 95% of the time). This concentration is called the acceptable quality limit (AQL). If there is an AQL, the AL cannot be set at the AQL; the AL must be set somewhat higher than the AQL. The relationship between the AQL and AL is shown in Figure 8.

Likewise, sometimes it is desired that a specific lot concentration be accepted rarely (e.g., 10% of the time). This concentration is called the lower quality level (LQL). Again, the AL cannot be set at the LQL; the AL must be set lower than the LQL. The relationship between the AL and LQL is shown in Figure 9.

To reduce the number of lots that are erroneously accepted or rejected, it is usually desired that the AL be as close as possible to the AQL or LQL. To accomplish this, the variability must be reduced. Increasing the test sample size will reduce the sampling variability component of the overall variation. Of course, practical considerations may restrict how large the test sample can be. Uncertainty due to the measurement method can be reduced by making multiple measurements and averaging the
results. Unfortunately, this will increase the cost of testing. In practice, a compromise between cost and desired risk must often be made.

Conclusions

Sampling and testing lots is typically the only practical way to obtain information about lot characteristics. Unfortunately, uncertainty concerning the data provided by measurements is unavoidable when sampling and testing lots. The level of uncertainty can be managed by selecting an appropriate sampling plan. A sampling plan consists of a sample size and AL. When the AL is zero, qualitative testing is the appropriate testing methodology to use with the sampling plan. When the AL is greater than zero, quantitative testing is an appropriate testing methodology. Qualitative testing can be used with sampling plans that utilize multiple subsamples to obtain quantitative results.

Managing uncertainty is not the only consideration when choosing a sampling plan. Practical considerations such as cost, availability of testing methods and laboratories, and timeliness of obtaining results must also be considered.

Selecting an appropriate sampling plan is complicated. Consulting an expert on the subject is advised.

References


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