Improving Laboratory Performance Through Scientific Subsampling Techniques

By Charles A. Ramsey and Jennifer Suggs

The rewards of proper application are worth the effort.

The entire analytical process has many steps including sample collection, sample preparation, actual analysis, quality control and data interpretation. If any of these steps are not properly performed, the resulting data is suspect. Much attention has been given to analytical chemistry, statistics, quality control and sample preparation, but sample collection in the laboratory is rarely mentioned. Some analysts do not think sampling is performed in the laboratory.

If the analytical process is to produce reliable data for decision-making purposes, the errors associated with laboratory subsampling must be understood and addressed. Only then can the data generated withstand the rigors of the scientific and legal community.

Described here are some of the basic scientific principles that should be part of any subsampling protocol using sampling of particulate matter as an example. It is by no means exhaustive, but rather the goal is to inform the reader that scientific principles do exist and can be applied to the problem of obtaining a representative subsample.

Concepts and Problems

The concepts of subsampling will be applied to the following fictional laboratory scenario: *LeadFreeCo has brought a soil sample into a testing lab, ExpertTestCo, requesting a lead content analysis on a sample with a mass of 250 grams.*

Generally, the analytical laboratory receives more sample (test material) than is typically used for a specific analytical method. A smaller amount of material, or a subsample, must be removed from the sample container. The techniques employed to remove this material have a great impact on the "representativeness" of the material that is analyzed. If the subsample does not represent the original sample, the data generated from the laboratory will be inaccurate regardless of the accuracy of the analytical technique.

There are two definitions that are imperative to understand:

- "Represent"–Achieving a like characteristic, ensuring that a subsample has the same chemical composition (relative to the analyte of interest) as the sample.
- "Representative Subsample"–The ideal representative subsample would be a miniature of the sample in all respects containing some of every type of particle in the same proportions as the original sample.

When a sample is sent to the laboratory for analysis, someone in the laboratory removes part of the material for analysis. It should be the goal of that person to remove a portion that is identical in all respects to the material in the entire container (i.e., representative subsample). *An ExpertTestCo chemist looks at the soil sample and sees a variety of particle sizes from very fine to about 2 mm in diameter.*

The nature of the sample determines the difficulty of this process. For example, if the sample is a single-phase liquid without suspended material, subsampling is easy. But if the sample consists of some mixture of particles, subsampling is more difficult. How does one equally represent the proportions of the mixture?

There are two problems with subsampling all materials. One is collecting enough sample mass to represent all the particles. The other is the segregation of finer, denser particles to the bottom of the container.

The Problem of Insufficient Subsample Mass. If only one particle is selected from the sample for analysis, it is impossible to achieve the correct concentration. Picture a homemade mixture of green M&Ms, blue M&Ms, Red Hots and mustard seeds. If a green M&M is selected, "analysis" will show a ND (not detected) for blue M&Ms, Red Hots and mustard seeds. If our goal is to determine the concentration of these "analytes," we will report an incorrect answer irrespective of the quality of the analysis. Obviously, the mass of only one particle is not enough to represent this sample. If 10 particles are selected as the subsample, the probability of a correct (or closer) answer increases.

Most chemists intuitively know that it takes less mass to represent a fine powder than a waste material with particles a half-inch in diameter. What is not always known is the scientific relationship between the mass required to adequately represent a sample and the characteristics of that sample.

Large particles are more difficult to represent than small ones. For a given mass there would be fewer large particles than small ones. Consequently, if there is enough mass to represent the large particles, the small particles will also be represented. Calculations for sample mass will thus only depend on the size of the largest particles.

The Problem of Sample Segregation. Assume the subsample mass needed for "analysis" of our homemade mixture is known. If the desired amount is scooped or poured off the top, the mustard seeds will not be represented and the analyst will report ND for the mustard seed concentration. "Subsampling error" is defined as the difference between the actual concentration in a sample and the

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actual concentration in the subsample.

The ExpertTestCo chemist needs to run a total lead analysis and toxicity characteristic leaching procedure (TCLP) on the sample. He scoops out a gram for the total metals digestion and 100 grams for the TCLP from the top of the container. It has been stated that all one needs to do is to stir the sample to decrease segregation. That is incorrect. No amount of mixing is going to allow you to adequately represent the mustard seeds. Segregation resulting from particles with different densities and sizes is often increased with stirring.

Sampling Theory as a Basis for Scientific Subsampling

There is a theory of sampling that describes these problems along with the solutions for proper subsampling.[1] There is always some subsampling error (as there is always some analytical error), but that error can be minimized so that it is tolerable for the desired data quality. A chemist might only consider error involved with the actual analysis: deviation from calibration standards, percent recovery of analytical or measurement spikes, and so on. But since final results can be tainted by improperly representing the original sample, subsampling error must be minimized at the start.

All subsampling error is a result of heterogeneity. There are two types of heterogeneity that must be addressed for any subsampling situation: compositional heterogeneity and distributional heterogeneity. If heterogeneity and the resulting errors are well understood, subsampling error can be controlled to any desired level.

Compositional Heterogeneity. This type of heterogeneity occurs when there is a difference in concentration between the particles in the sample. This type of heterogeneity always exists, even in the purest of materials. There is a direct relationship between the amount of compositional heterogeneity and the amount of subsampling error. Consider two cases:

- Case 1: The difference in lead concentration between the individual particles varies from 1.0 to 9.5 ppm. If the purpose of the analysis is to determine the average concentration of the sample, the wrong conclusion may be obtained by "accidentally" collecting material with only the 1.0 or only the 9.5 ppm concentration.
- Case 2: The difference in lead concentration between the individual particles varies from 1.00 to 99.5 ppm. Would this lead to more sampling error than Case 1?

Distributional Heterogeneity. This type of heterogeneity occurs when there is a nonrandom distribution of particles in the sample segregation. Due to the presence of gravity this error always exists. As expected, a larger distributional heterogeneity leads to a larger sampling error.

The easiest way to think of sampling error is as a relative standard deviation (RSD). RSD is used to measure many analytical errors. The standard deviation is nothing more than the variability of repeated measurements.

Two crucial factors in reducing variability and sampling error are the correct subsample mass (more particles) and the technique for removal of the subsample from the sample. The correct sample mass solves the problem of compositional heterogeneity and proper subsampling technique solves the problem of sample segregation or distributional heterogeneity.

Measuring Sampling Error

There are many types of sampling error, but this paper focuses on the two most important ones: "fundamental error" (FE), which results from compositional heterogeneity, and "segregation error," which results from distributional heterogeneity.

Fundamental Error. This calculation can be as simple or as complex as the application dictates. Very small errors (less than 1%) require more effort and sophisticated formulas. Fortunately, a rather simple formula provides adequate information for most environmental work. The equation below is only for non-calibrated materials (materials that are not all the same size; e.g., soil) in which the analyte of interest does not exist as a few discrete "nuggets." The equation also assumes that the material is generally rounded in shape. The amount of sample mass required to achieve a specified error (FE) is given by:

$$M_S = \frac{10 * \lambda * d^3}{FE_2}$$

Where:

λ = Density of material in g/cm^3
M_S = Mass of sample collected in grams
FE = Tolerable error (fundamental error)
d = Size of largest particles in centimeters

For example, maximum particle size is 4 mm, density is 2.5 g/cm^3, and the tolerable error from the subsampling process is 15% RSD. The minimum mass to represent all the particle sizes for this error is:

$$M_S = \frac{10 * 2.5 * 4^3}{0.15^2} = 71\ grams$$

The error for a particular mass can also be calculated using the formula:

$$FE^2 = \frac{10 * \lambda * d^3}{FE_2}$$

For *ExpertTestCo*, the sampling error caused from selecting one gram of material for total lead analysis with a particle size of 2 mm is:

$$FE^2 = \frac{10 * 2.5 * .2^3}{1} = 0.2$$

$$FE = 45\%$$

If the analysis requires 100 grams (TCLP) of material, the fundamental error is only 4%. In other words, it is reasonable to analyze 100 grams of this material, but analysis of only one gram of material will lead to large sampling errors resulting in decision errors and inde- fensible results. While it is commonplace in environmental testing laboratories to perform analysis on a one-gram subsample, the confidence that the analytical result is close to the truth is very small.

Table 1 gives sample masses for different maximum particle sizes (assume density = 1.0) and tolerable sampling error. This is the minimum subsample mass that must be removed and analyzed from a material with a specified maximum particle size in order to remain within a tolerable error. It is difficult to subsample and reduce error to low levels when particles are large. If the particle size is so large that the required mass is excessive, a grinder can reduce the particle size so that a manageable subsample mass is obtained.

For materials with densities other than one, Table 1 can be modified by multiplying the entries by the density of the material of interest. For example, when subsampling a material with a largest particle size of 2 mm, a tolerable subsampling RSD of 5% and a density of 2 would require 64 grams (32 x 2).

Segregation Error. The calculation for segregation error is very complex, but it can be simplified by following some initial assumptions to a logical conclusion

Tolerable RSD Component from Laboratory Subsampling*						
		15%	10%	5%	2%	1%
	.5 mm	0.06 g	0.13 g	0.5 g	3 g	12.5 g
Maximum	1 mm	.044 g	1 g	4 g	25 g	100 g
Particle	2 mm	4 g	8 g	32 g	200 g	400 g
Size	5 mm	56 g	125 g	500 g	3130 g	12,500 g
	10 mm	440 g	1,000 g	4,000 g	25,000 g	100,000 g
**This is an approximation. Table entries are sample mass in grams.*						

Table 1. Sample masses for different maximum particle sizes.

keeping in mind the goal of a representative subsample. Calculations regarding a recommended number of increments (or small, selective scoops of material) will not be discussed.

Gravity generally causes vertical segregation with lighter, larger particles on top of finer, denser particles. In many cases, the analyte of concern is concentrated in the dense fines (function of surface area). Clearly, it is better to select some material (an increment) from the top, middle and bottom of a sample than just from the top. In fact, a core from top to bottom, while not practical, would generally be a good subsample. Increasing the number of incremental slices or scoops from each level of the sample decreases the influence of segregation.

One of the requirements for representative subsampling is accessibility to the entire sample. In most cases it is necessary to remove the material from the original sample container and onto a flat, inert surface so that the entire sample is accessible. The increments are then collected at random, taking care to ensure that no particles are being discriminated against. A sampling tool should be selected that is thin enough to collect the fines, but not too small to collect the largest particles. The technique of reaching into the jar with a spatula to obtain material from the middle and bottom is not adequate.

The number of increments taken is a function of the distributional heterogeneity of the sample. As the distributional heterogeneity increases, the number of increments that should be taken should also increase. Generally, the number of increments should be 20 to 30. For very segregated materials this number can increase to 50 and for non-segregated materials this number should be about 10. More increments are always better than fewer increments (three is still better than one).

If the desired sample mass is 30 grams, then a good subsampling protocol would be to collect 30 one-gram increments at random to make the entire subsample. In the end, the more increments taken, the less sampling error due to distributional heterogeneity. The final subsample should look like an exact miniature of the sample. Some of all the particles in the same proportion should be present in the subsample.

Excuses to avoid proper subsampling such as lack of time, lack of workspace, contamination concerns, and so on, should be reconsidered to ensure data quality. If the analysis results are incorrect due to an unrepresentative subsample, then what are the penalties? If the data set shows more "outliers" than "inliers," if the % RSD is beyond acceptable laboratory limits, if the analyte concentration of the original sample is best described as "anybody's guess," how many excuses will be needed to explain those data quality problems?

Looking at the Big Picture

ExpertTestCo reviews the data and notices that the duplicate measurements for total lead have a RSD of 40%. Their contract requires a RSD of no more than 25%. Does ExpertTestCo reanalyze the duplicates until they fall within the 25% allowable error? Do they flag the data and state there was some matrix interference? Would any data that ExpertTestCo reports be adequate for decision-making purposes?

What if the samples were split with another lab that performed good subsampling? Who is the client likely to use next time? If the data is challenged in court, can ExpertTestCo defend their results and what are the consequences if they can't?

Some questions must be answered as part of a quality system: Where does laboratory subsampling fit in with the U.S. Environmental Protection Agency's (EPA) data quality objective (DQO) process? How will laboratory subsampling fit into performance-based measurement systems (PBMS)? Is laboratory subsampling part of a quality assurance project plan (QAPP)?

The rewards of proper laboratory subsampling will be worth the effort, if the following steps are taken every time the laboratory utilizes the approach described here:

- Determine the tolerable error for the laboratory subsampling step.
- Estimate the size of the largest sample particle; consider grinding to reduce particle size, if necessary.
- Calculate the mass necessary to achieve the desired fundamental error.
- Collect the subsample using 20 to 30 increments with a tool that allows equiprobable selection of all particles.

With just a little practice, the actual process of correct subsampling takes no more than a couple of minutes. Compared to the rest of the project–sample check-in, preparation, analysis, report generation and quality control–the few extra minutes are insignificant with the exception of the positive impact on the quality of the data.

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Reference

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