Cleaning Memo for December 2018
Clarifying Terms: Blanks vs. Controls

This month we'll cover the use of terms as used in the cleaning validation. It should be recognized that as long as a company clearly defines its terms in an appropriate document, and uses them consistently, then different uses may be acceptable.

As used for analytical measurements for cleaning validation studies, the two terms "blanks" and "controls" are sometimes used interchangeably. As I generally use the term, a *blank* is a sample for which the measured value is subtracted from a test sample to determine the analyte in the test sample due to the residue removed from the sampled surface. There are two ways a blank may be used. In one case (for example, in a spectrophotometric analysis) the blank is used to set a "zero" point for instrument output. Once the zero is set, I then place my test sample in the spectrophotometer and measure the absorbance, which I can then attribute to the residue I want to measure. This technique deals with impurities (for example) in the solvent I use for extraction of a swab. Now if I have a double beam spectrophotometer, the blank sample is measured at the same time as the test sample, and the instrument subtracts out the blank value.

A second way a blank is used can be illustrated by the use of TOC (Total Organic Carbon) as the analytical technique. In a test sample analyzed by TOC, there are multiple sources of carbon. What I am really interested in measuring is the carbon *removed from the surface*. But, other sources of carbon include the vial, the water, the swab itself (for swab sampling), and the air in the atmosphere where I prepare the sample. In addition to the test sample I prepare a blank with just the vial, water, and a swab in the same location where I take my test sample. The analytical lab runs both the test sample and the blank on the TOC instrument, and then subtracts the two to arrive at a TOC value representing the residue on the sampled surface.

Okay, what then is a *control*? A control is a sample that is analyzed where I am *expecting* to get a certain value or range of values. This control provides information as to the *validity* of my analysis. If the control is not in the expected range, then something is wrong. What is wrong may be a problem with the instrument, with the reagents used in the analysis, or with the sample preparation. An out-of-spec control calls into question the validity of the analytical results obtained for my test samples. Let's illustrate this again using TOC. One control might be running the analytical procedure on just water in a vial. I know based on past results that this control should be in the range of 30-60 ppb TOC. Another control might be running a KHP (potassium hydrogen phthalate) solution equivalent to 1.5 ppm TOC (the TOC limit for the protocol). If I don't measure within the expected values, this suggests something is wrong. Of course, if I were to run the 1.5 ppm KHP solution as a control, I may also have to run a blank (depending on whether my control range was based on the value in the solution itself or the value corrected for the blank). The advantage of running a blank in this situation is that if my KHP control is not correct, I don't know whether the source is the water I used or something else, making my investigation into the cause more complicated.

In terms of the use of controls, I might further define both *negative* controls and *positive* controls. The negative control is something that has no or very low response. In the TOC example given, the analysis of the water alone (well, alone in a vial) would be a negative control. The use of the 1.5 ppm KHP would represent a positive control, because it would represent a significant analytical response, in line with could be measured in my protocol (although if my limit were 1.5 ppm TOC, I would hope that my test samples were significantly below that concentration).

Now we get to the complicated part. A sample that is run (in addition to my test samples) may serve *both* as a blank and as a control. The TOC situation illustrates this possibility. As covered earlier, a TOC blank for swabbing may be a sample prepared containing carbon from the vial, the water, the swab, and the atmosphere it is prepared in. As a blank, the analytical TOC value is subtracted from any swab test sample to determine the TOC *sampled from the surface*. However, that same blank sample may also serve *functionally* as a control. How? Well, based on historical data the combination of the vial, water and swab has generally given values in a certain range (for example, 100-200 ppb TOC). If the blank value is outside that range, I should be careful about accepting any analytical data on my test samples. Here is an obvious example. Let's say my swab blank was 350 ppb and my test sample was 125 ppb. Do I subtract 350 ppb from 125 ppb and say that my test sample was below the detection limit? Or, do I look at those results and say something is wrong with that high blank? Perhaps the samples were labeled wrong, and the test sample was 350 ppb and the blank was 125 ppb. If that situation were actually the case, then the net TOC would be 225 ppb TOC, which may or may not be a passing result. So, in this situation, one sample functioned both as a blank and as a control. It is the same sample, but its function varied.

You might also be thinking of the 1.5 ppm KHP, which I indicated was a positive control, and saying that this should be called a *standard*. In the illustration given, that KHP is not strictly speaking a standard. Yes, I am preparing a known concentration. But the measurement of that solution is not functioning as a standard. A standard is a sample of known concentration that I use for calibration purposes. For example, if I were preparing a calibration curve, I would have known standards and measure the response of those standards. However, my assumption is that the analytical response is correct. Another example would be a pass/fail analytical procedure, where I run both a standard (at the protocol limit) and the test sample; if the response of the test sample were less than the response of the standard, then I have confirmed that the test sample has a concentration less than the concentration of the standard. Thus, the difference between a control and a standard is how the analytical result is used. In one case (the control), the analyst determines whether the result is acceptable or not. In the second (the standard), the result of the measurement is not questioned; it is accepted as correct.

Let me reiterate that your usages of these terms may be different. The important thing is that they are defined and used appropriately. But it is important to think about its function or use as a blank *and* as a control.