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Swab Sampling Recovery as a Function of Residue Level

I have generally taught that the percent swab sampling recovery decreases with increasing spiked level of residue, other things being equal. In other words, recovery at a level of X µg/cm^2 should be higher than recovery at a level of 2X µg/cm^2. I have not said how much higher the recovery percentage at the lower spiked level might be, but I believe that the difference based on spiked levels that differ by a factor of 2 or 3 would be minor, certainly compared to the variation of recovery percentages that are achieved by different operators or by the same operator on different days.

My rationale or explanation for such a belief is to present the analogy of using a snow shovel to pick up snow on a sidewalk. If I have one pass across the sidewalk to pick up as much snow as possible, it is likely that with a level of snow of only seven centimeters on the sidewalk, my one pass (one shovelful) might pick up a relatively large amount of snow in the "sampled" area. That value might be 60% to 80% of the snow present on the "sampled" area. On the other hand, if I were to use the same shovel and procedure on a sidewalk containing 70 centimeters of snow, in one shovelful I might get only 30% to 40% of the snow on the sidewalk. If snow and snow shovels are a foreign concept to you, you might translate the analogy into sand on a sidewalk and using a sand shovel.

It may be possible to take this analogy too far. If I were to propose only a layer of snow as thick as 0.1 millimeter, then the percentage of snow picked up by the shovel might be very low because the shovel would pass on top of the snow layer. However, I don't believe this situation applies to sampling in cleaning validation protocols.

A second way to explain a decreasing recovery percentage with increasing spiked residue is to appeal to a solubility analogy. If the only mechanism of removal of the residue in the swabbing procedure (don't get me wrong here; it probably is not the only removal mechanism), then it should take longer to dissolve a large amount of residue on the surface. That should result in recovery percentages decreasing as the spiked residue amount increased (again, other things being equal).

A third way to explain the situation is to appeal to saturation of the swab. Again, other things being equal, as the amount of residue spiked increases, it is more likely that the swab will be saturated with residue.

Now all that said, a recent publication, "A Risk Management Approach to Cleaning Validation" by B W Pack and J D Hofer (*Pharmaceutical Technology*, Vol. 34, No. 6, June 2010), came to the opposite conclusion based on their data. The authors specifically state that "the predominant trend was that the average recovery of a compound increased as the spiked amount increased on a given material of construction." Let me make it clear that the primary objective of this study was not to evaluate the effect of recovery as a function of spiked amount. This conclusion just appeared as an "offhand" comment in a discussion of "materials of construction". Here is a close approximation of a summary of their data this conclusion is based upon, which is for 316L stainless steel. They presented results for two compounds, Compound A (less soluble and more difficult to clean) and Compound B (more soluble and easier to clean). Note, however, when they made their conclusion about the "predominant trend", only the data for Compound B was discussed immediately to support that trend.

Spike level	% Recovery A	% Recovery B
0.5 µg/swab	~53	74
5 µg/swab	~80	90
50 µg/swab	~95	95

On initial look, this data seems to support the conclusion that recovery increases with increasing spiked level. On the other hand, could there be other factors accounting for these differences, such as performance on different days (the same analyst did the swabbing, so that probably is not a factor unless swabbing skill changed over time). In addition, there was significantly more variation at the lowest spiked level. For example, the data for Compound A at the 0.5 µg/swab level varied from a low of about 15% to a high of about 77%. The data for Compound A at the 5 µg/swab level varied from a low of about 73% to a high of about 87%. The data for Compound A at the 50 µg/swab level varied from a low of about 91% to a high of about 99%. Is this a reflection of variability in the analytical method at low levels, or is it a function of variability of removal of residue from the surface at lower levels?

One other data set reported in the publication caused me concern. A strategy was presented for introducing new materials of construction into the grouping program. That involved comparison of the data for the new material of construction with data run at the same time for a control. In the example given, the one control was 316L stainless with Compound A at 5.0 µg/swab (note that the publication lists the spiked amount as 5 $\mu g/in^2$; however, that must be a typo since that level was not part of the original study). In any case, the recovery at this level was consistently at 98% (with a range of 95% to 101%). My point is, that if this residue level is the same as reported in the original study, there is a significant difference between the 98% reported in the additional study as compared to the approximately 80% reported in the original study.

In any case, the Pack and Hofer publication caused me to go back through my files for previous publications where there might be recovery data as a function of spiked level.

One publication was by P. Yang et. al. ("Method Development of Swab Sampling for Cleaning Validation of a Residual Active Pharmaceutical Ingredient", *Pharmaceutical Technology*, January 2005, pp. 84-92). In this publication, recovery was done for an active on a nylon surface at two levels, 2.8 µg/swab and 4.0 µg/swab. The recovery was 80.4% at the lower level and 84.7 at the higher level. While this is consistent with the idea of increasing recovery with increasing spiked level, the difference between the two results is not practically significant to come to a conclusion.

Another publication is by S. Lombardo et. al. ("Development of Surface Swabbing Procedures for a Cleaning Validation Program in a Biopharmaceutical Manufacturing Facility", *Biotechnology and Bioengineering*, December 5, 1995, pp. 513-519). In Figures 4(a) and 5(a) in this paper, a linear relation was shown between the amount of residue spiked onto the coupon and the amount recovered. It should be noted that in most situations, the data was based on two spiked levels, but the curve was forced through "zero", which in essence gave three data points. The authors state that "a linear relationship prevails between the observed and anticipated contaminant values over the range investigated." The fact that the curves were linear suggests that the recoveries were essentially the same at different spiked levels (the slope of the straight line should give the recovery expressed as a decimal). In this study the range of spiked levels differed by no more than a factor of about three.

Another study was by K. Bader et. al. ("Online Total Organic (TOC) as a Process Analytical Technology for

Cleaning Validation Risk Management", *Pharmaceutical Engineering*, January/February 2009). In this publication, Figure 3 and Figure 4 are curves of recovered residue as compared to the positive control (the amount representing 100% recovery). Note that the two figures are the same data; however, Figure 3 presents individual swab technician results and Figure 4 presents the aggregate data. Although no conclusion is drawn from the data about the effect of spiked amount on percent recovery, the linear relationships suggests that percent recovery is the same over the evaluated range. For this study, the range from the low spiked level to the high spiked level was a factor of about five (5).

Another publication is by C. Glover ("Validation of the Total Organic Carbon (TOC) Swab Sampling and Test Method", *Journal of Pharmaceutical Science and Technology*, September-October 2006, pp. 284-290). In Table I, percent recovery is given as function of five spiked levels from 5 µg to 100 µg (a range representing a factor of 20). The reported data showed a general decrease in percent recovery as a function of increasing spiked amount. However, the data at the lower levels gave recoveries of greater than 150%, while the recoveries at the higher levels were close to 100%. That data suggests some issues with the TOC analysis.

A final publication is M. A. Strege et. al. ("Total Organic Carbon Analysis of Swab Samples for the Cleaning Validation of Bioprocess Fermentation Equipment", *BioPharm International*, April 1996). In this publication, Table 2 lists the percent recoveries from stainless steel for three dilutions of a fermentation cell paste. In this situation, the recovery increased with increasing spike level from a low of 75% to a high of 103%. This involved a range with a factor of four between the top and bottom spiked levels.

So, where does this leave us? The data from published studies sometimes show increasing percent recoveries with increasing spiked amounts, sometimes show no change in percent recoveries with increasing spiked amount, and sometimes show decreasing recoveries with increasing spiked amount. I should point out that in none of these studies cited was the stated objective to determine the relationship between the percent recovery and the amount of residue spiked.

If anyone has published any studies that can help elucidate this issue, I'd like to hear from them. If anyone would like to perform a study to specifically evaluate the relationship between percent recovery and spiked amount, I would be more than happy to assist in the design of it so that appropriate conclusions can be drawn. My only caution is that it would be best to avoid using TOC as the analytical method because of control of the sources of TOC. Furthermore, randomization of sampling order must be considered.

Until such time as a definitive study is published and confirmed, it would probably be best to stick with my original contention (based on a common sense understanding of what happens in a swabbing procedure) that other things being equal, percent recovery decreases with increasing spiked level, without stating how significant that decrease might be.