May 2010
Final Notes on "Stratified Sampling"

In the past two months I have covered the use of "stratified sampling", first applying stratified sampling to segments within a given piece of equipment and then applying it to a series of equipment items in a manufacturing equipment train. This month I will address some additional questions in the use of stratified sampling.

The first question should be an obvious one. If stratified sampling can be applied to segments within a given piece of equipment and it can also be applied to a series of equipment items in a manufacturing equipment train, is it possible to apply stratified sampling where I stratify both segments within one piece of equipment and a series of equipment items in a manufacturing train? And the answer is "Yes", although it significantly complicates the pre-protocol work that must be done, as well as the calculations necessary in the protocol execution itself. However, there is nothing logically or scientifically invalid about applying stratified sampling principles in these cases (provided of course, the restrictions or limitations discussed in the two previous Cleaning Memos are considered).

The second question involves the use of rinse sampling and swab sampling for a stratified approach (the examples given in the previous months mostly involved swab sampling). I will consider three cases.

> Case I: Is it possible to use this approach where only rinse sampling is performed (on separate equipment items in a train)?
> Case II: Is it possible to use this approach in an equipment train where some of the equipment is sampled by swabbing and some equipment is sampled by rinsing?
> Case III: Is it possible to use this approach in an equipment train where a given equipment item is sampled by both rinsing and swabbing?

The answer to all questions is "Yes". The key is just to use the stratified sampling principles appropriately.

In Case I (everything is only rinsed), it is important that the rinse limit be established on carryover calculation principles. Then, the total actual carryover for a given equipment item can be determined by multiplying the concentration of the residue in the rinse solution by the volume of the rinse solution. The total actual carryover for the equipment train can be determined by adding up the actual carryovers for each equipment item. That total is then compared to the total carryover limit determined by the MAC calculation.

In Case II (some items in a train are only swabbed and some are only rinsed), it is important that both the rinse limit and the swab limit be established on carryover calculation principles. For equipment items rinsed, the total actual carryover for a given equipment item can be determined by multiplying the concentration of the residue in the rinse solution by the volume of the rinse solution. For equipment items swabbed, the total actual carryover for a given equipment item can be determined by using the calculations shown in the March 2010 Cleaning Memo. The total actual carryover for the equipment train can be determined by adding up the actual carryovers for each equipment item (whether it was sampled by swabbing or rinsing). That total is then compared to the total carryover limit determined by the MAC calculation.

In Case III (some items in a train are sampled by both swabbing and rinsing), it is again important that both the rinse limit and the swab limit be established on carryover calculation principles. For each equipment item both swabbed and rinsed, the total actual carryover for that equipment item can be first determined by using the swabbing data to determine the maximum actual carryover for that item based on swabbing. Then for the same equipment item, the maximum actual carryover is calculated based on the rinse data (using the principles in Case I above). Assuming both the swab data and the rinse data each give results representing the total actual carryover, the larger of the two actual carryover results is then used for the total carryover for that equipment item. [Note that other things being equal, the results based on swabbing will ordinarily give a higher result than the data based on rinsing.] This is done for each equipment item, and the total actual carryover for the equipment train can be determined by adding up the actual carryovers for each equipment item (however that item is sampled). That total is then compared to the total carryover limit determined by the MAC calculation.

This sounds like a lot of work, and it does represent extra calculations. However, there is another alternative in the use of stratified sampling. That alternative is to use a *staged* approach to determine whether the acceptance criterion in a protocol is met. This staged approach involves an initial evaluation of every swab and rinse sample result, and comparing it to the acceptance limits for swabbing (perhaps based on a limit expressed as $\mu g/cm^2$) and for rinse samples (perhaps based on a limit expressed as ppm or µg/mL). This is how it is ordinarily done in a cleaning validation protocol. If all those results are at or below the acceptance limit, then there is no point in going further in stratified sampling; the residue limit criterion is met. However, if one data point (or more) for a swab or rinse sample is above the limit, then the next step is to proceed with a stratified sampling approach to see whether the total carryover is acceptable. In this case, it is preferable not to say the initial evaluation "failed" the acceptance criterion. It is better to say something like "Stage 1 criteria were not met, and we will proceed to a Stage 2 evaluation to determine acceptability". If the stratified sample approach demonstrates that the total carryover was acceptable, then the residue limit criterion was met.

In this staged approach, one cannot have "unacceptable" results from a typical (Stage 1) evaluation, and then decide that it might pass by a stratified sampling evaluation. This staged approach should be written into the protocol. Furthermore, if segments within an equipment item are to be stratified, those segments should be identified in advance (in part to prevent the temptation to "adjust" the segments or segment surface areas based on the data obtained, so that the end result is more likely to meet the limit based on stratified principles).

This staged approach should not be foreign to those in pharmaceutical manufacturing. It is something we use on a regular basis in the USP testing for conductivity in Purified Water and WFI systems.

The purpose of the Cleaning Memo is to address additional issues in the use of a stratified sampling approach for determining compliance with residue acceptance criteria in a protocol. This approach, while not commonly used, is based on good science and good logic. I should reiterate some conditions for utilizing stratified sampling. This method can only be used if the residues from equipment surfaces are uniformly dispersed throughout the next manufactured product. Furthermore, it is preferably only used proactively. That is, define the segments in advance, select the worst case location(s) in each segment, and write your protocol with this approach. It is also preferable that this approach be permitted in your cleaning validation master plan or high level policy. Furthermore, this Cleaning Memo should be read in conjunction with the March and April 2010 Cleaning Memos.