Cleaning Memo for June 2002

Recovery Studies for Microbial Sampling?

Measurement of potential microbial contamination has become a more of an expectation in cleaning validation protocols for process manufacturing equipment. Sampling for bioburden on surfaces has usually involved contact plates, swabs and rinse water (with membrane filtration) analyses. A common question that arises for this microbial sampling is whether or not recovery studies (analogous to recovery studies for chemical residue sampling in cleaning validation) need to be done, and if they are done, are the recovery percentages used to transform the bioburden numbers?

The answers to these questions partly depend on what one means by recovery studies. If by "recovery" one means procedures such as USP <1227>, "Validation of Microbial Recovery from Pharmacopoeial Articles", then the concepts presented there are appropriate to make sure there is nothing in the procedure that would inhibit growth of microbes if microbes were present. This "microbial recovery" thus deals with such things as adequate neutralization, appropriate recovery medium, and growth conditions.

However, if by "recovery" one means calculating a recovery *percentage* and using that percentage to *transform* actual CFU values obtained in a given test, then the answer (at least right now) is that such recovery studies are probably not appropriate and/or practical. One reason for this concern is that it is unclear how any such "percentage recovery" test should be run. If one spikes a test surface with a fixed CFU of microorganisms, allows the surface to dry (reflecting actual surface conditions), and then samples the surface to measure the actual recovered CFU, then the act of drying the surface will kill a significant number of organisms if vegetative bacteria are used. This can be resolved partly by using only spore-forming bacteria in the spore form; however, the question is still open as whether that data also applies to the same organism in the vegetative state (or to different species of microbes). Another way to avoid the "drying" issue is to spike the surface and then sample it wet (without drying). While this may work with swab sampling, it is not practical with contact plates. Further, one still ask the relevance of sampling surfaces wet in a recovery study if the surfaces will be sampled dry in actual protocols.

A further issue is just the inherent variability of microbial enumeration. If one spikes a surface with 50 CFU and then samples the surfaces and recovers 40 CFU, should this be considered a recovery of 80%? And should that percentage be applied to transform values measured in cleaning validation protocols. The general consensus is "no", because measuring the same 50 CFU innoculum twice might yield results of 50 CFU one time and 40 CFU the next time. That is, the data is not significantly different.

The value of doing recovery studies to determine specific percentages and then applying those percentages to transform experimental results is questionable. One alternative some companies have considered is to do percentage recovery studies as part of the test method

qualification only. In other words, a minimum percentage recovery is established, and this minimum percentage must be met or exceeded to consider the procedure valid; however, that percentage is *not* used to transform experimental data in a cleaning validation protocol. In such cases the minimum recovery is in the range 0f 20%.

In short, the use of percentage recoveries in microbial sampling to transform experimental data generally should be avoided. Yes, this is a source of variability in microbial testing, but without better methods or better procedures for utilizing recovery percentages, for most companies there are probably better and more effective ways to allocate resources to improve drug manufacturing consistency and quality.

Next month's Cleaning Memo: "Worst-case" Process Conditions