February 2006
Bioburden Proliferation in CEHT Protocols

A primary concern in clean equipment hold time (CEHT) protocols is that there should be a lack of bioburden or microbial proliferation. This emphasis has a good scientific rationale. In addition, it is specifically mentioned in the FDA cleaning validation guidance (1993): "There should be some evidence that routine cleaning and storage of equipment does not allow microbial proliferation. For example, equipment should be dried before storage, and under no circumstances should stagnant water be allowed to remain in equipment subsequent to cleaning operations."

The question then arises, "What constitutes microbial proliferation in such situations?" Well, one might say that proliferation is a "significant increase", but that begs the question; what then is a significant increase?

In answering that question, one has to defer to microbiologists and their views on microbial enumeration. Most microbiologists would generally not consider a 50% increase (or a 50% decrease) in bioburden in sampling the same surface using the same media batch and identical sampling and incubation conditions as a significant change. It merely reflects the impreciseness or variability that is inherent in enumerating living microorganisms. For example, in enumerating the same sample, results of 10 CFU and 15 CFU would not be considered significantly different.

When the comparison is made between a sample at time T=0 and a sample at time T= 14 days, which may involve different lots of media and different incubation days, a more typical view is that even a 0.5 log increase is not an indication of significant proliferation. It generally is the case that for CEHT validation protocols, a 1-log or greater increase would be considered "proliferation" such as to require action.

The first question to ask is, "Is this a reasonable requirement?" Is it too strict? Or, is it too lax? We can perhaps look at examples to evaluate reasonableness. Let's suppose we are sampling a surface by a contact plate of 25 cm2, and that after cleaning and drying the equipment, the results are 1 CFU/plate. A criterion of no more than 1-log increase would allow for as many as 10 CFU/plate after storage. Since a typical limit (see D. A. LeBlanc, "Equipment Cleaning Validation: Microbial Control Issues", Journal of Validation Technology 8:4, 40-46, August 2002) in such a situation would be 25 CFU/plate, obviously an increase to 10 CFU/plate should not be a concern.

But, you ask, what if the original bioburden is 10 CFU/plate? Isn't an increase to 100 CFU/plate unacceptable from a product point of view? Well, because cleaning validation limits typically set for bioburden are based more on process capability, and since those process capability limits are generally well below what could be considered a concern for product quality, a change from 10 CFU/plate to 100 CFU/plate would not constitute a significant concern for product quality.

If the criterion for "significant microbial proliferation" is to be a change of no more than 1-log, how do we deal with the situation where the initial bioburden from contact plates is "zero"; that is, the plates are "clean". How can we measure a 1-log change from zero, since there is no way to express "zero" on a log scale? What I generally recommend in such situations is to express the acceptance criterion for microbial proliferation as "A bioburden change of no more than 1-log or an absolute value for bioburden after storage no more than the

bioburden acceptance criteria in the cleaning validation protocol, whichever is less stringent." By the "cleaning validation protocol", I mean the original study to determine that cleaning is effective, with bioburden after cleaning being one acceptance criterion; this is in contrast to the "clean equipment hold time" protocol, which is the subject of this Cleaning Memo. I realize some companies choose to write one protocol including criteria for both the cleaning effectiveness and the storage effectiveness; however, I believe it is best to separate these as two different protocols (this will be the subject of next month's Cleaning Memo).

When I first present this to people, the first question I usually get is, "Don't you mean the more stringent?" And my answer is, "No, I mean the less stringent." Why is this the case? I will try to illustrate the wisdom of use of this criterion by giving some examples. Assuming the case of sampling by contact plate with a cleaning validation acceptance criterion of £25 CFU/contact plate (again assuming a contact plate of 25 cm2), suppose the initial bioburden (at the beginning of storage) is 5 CFU/plate. By my criterion of a change of no more than 1-log, this would allow 50 CFU/plate after storage. Since my cleaning validation limit is 25 CFU/plate, then the less stringent of these two would be 50 CFU/plate. That makes sense.

On the other hand, what if the initial bioburden were only 1 CFU/plate. In this case a 1-log change would be 10 CFU/plate. Would it be reasonable to fail a sample with 12 CFU/plate, when the initial criterion was twice as high (25 CFU/plate). I would argue that it is unreasonable. In this case, I would pick the less stringent of 10 CFU/plate (a 1-log change) and 25 CFU/plate (the original acceptance limit); therefore, my acceptance limit for the CEHT study would be 25 CFU/plate.

Let's take one more example and assume the initial bioburden results were all zeros. In this case, I can't express a 1-log change. Therefore my acceptance limit for the clean hold time study is the original cleaning validation acceptance limit of 25 CFU/plate. A possible argument against this is that if the initial results were all zero's, where would the bioburden come from to possibly produce 20 CFU/plate? This can be addressed by reminding the objector that a value of "zero" is really "<1 CFU". Regardless, it would make sense that having a bioburden after storage of £25 CFU/plate would not be a concern for product quality.

It should be remembered in the discussion that the issue of "objectionable" organisms (see the May 2005 Cleaning Memo on this subject, guest written by Dr. Tony Cundell) should also be considered in this evaluation of microbial or bioburden proliferation.

The purpose of this Cleaning Memo is to explore issues in setting acceptance criteria for bioburden proliferation in CEHT studies. Note that this concern about bioburden proliferation during storage is not limited to non-dedicated (multi-product) equipment; it applies equally to dedicated equipment. Further, it should be noted that there will be other acceptance criteria for a CEHT study (in addition to the bioburden proliferation criterion).