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Measuring Bioburden in Protocols

The question sometimes arises as to the value of establishing bioburden specifications and measuring bioburden following cleaning in validation protocols. Those involved in non-sterile manufacture sometimes object that bioburden is not significant. Those involved in aseptic processing sometimes object that bioburden is not relevant because the equipment undergoes a SIP (steam in place) cycle following cleaning. This Cleaning Memo addresses issues related to the measurement of bioburden after the cleaning procedure in cleaning validation protocols.

First, some general comments that apply to both non-sterile and sterile manufacturing. Performing bioburden testing after the cleaning process is not a burdensome requirement. If approved microbiological test procedures are used, the analytical method validation and the recovery studies (that are necessary with analytical procedures for actives, for example) are not required for bioburden testing. Collecting of (at a minimum) a final rinse water sample for bioburden sampling is not particularly burdensome. Furthermore, establishing limits for bioburden is not a complicated procedure (see my paper "Equipment Cleaning Validation: Microbial Control Issues", in Journal of Validation Technology 8:4, 40-46, August 2002). The only possible objection here might be that the number of swab sampling sites is limited, and it is not practical to sample certain sites for both chemical residues and for microbiological residues. If that is the case, it might still possible to test rinse samples

A further point is that if a cleaning equipment hold time (CEHT) study is to be done following the cleaning validation protocol, then a key feature of a CEHT study is to demonstrate no significant proliferation of bioburden. To demonstrate no significant proliferation, it is necessary to measure bioburden both at the start of storage (start of the hold time) and at the end of storage (end of the hold time). Since the "start of storage" is the same as the "end of cleaning", that same measurement of bioburden can be used both as the acceptance criterion for the cleaning validation protocol and the baseline data for the CEHT study. Admittedly, if the CEHT study is done separately from the cleaning validation protocol, this may not be an advantage.

For aseptic processing, the objection can be made that the equipment is subsequently sterilized, so measuring bioburden is not necessary. However, as in any sterilization process, there can come a point where the sterilization process is overcome by a high bioburden level. With effective cleaning, it is unlikely that one would achieve bioburden levels that could overcome the steam sterilization process. However, how does one know if the process is effective in producing low bioburden unless you measure it following cleaning? Furthermore, while the SIP process will provide assurance that any bioburden is killed, it does not deal with endotoxin issues. If the remaining bioburden is a gram negative bacteria at a sufficiently high level, the SIP process may effectively kill the bacteria, but it will release endotoxin (which usually is a concern in aseptic processing). Getting back to my original point, measuring bioburden is not a burdensome process, so it is prudent to include it in the validation protocol.

For non-sterile manufacturing, the situation is somewhat different. The argument can be made that a non-sterile drug product may have up to (for example) 100 CFU per gram of product. The equipment would have to have extremely high levels of bioburden to transfer that level of bioburden to the next product. Again, the counter to that objection is "how do you know that the bioburden being transferred is minimal unless you

measure it?" While conceivably it can be adequately documented during pre-validation studies, because bioburden testing is not burdensome, it is usually prudent to measure it in a cleaning validation protocol. With non-sterile manufacturing, there may be at least one exception. That is where an alcohol rinse or alcohol wipe is used following the last water rinse. This is sometimes used for small parts or small equipment. The alcohol serves one or more of several purposes: (1) it acts as a "polishing" rinse to further reduce residues, (2) it acts as a antimicrobial agent to assist in reducing bioburden on the surfaces, and (3) it serves to dry the equipment, thus reducing potential problems with bioburden proliferation during storage and/or rust (for mild steel equipment). I think a reasonable case can be made under these circumstances for not performing bioburden testing for cleaning validation. That said, because bioburden testing is so easy, I would probably do it anyway.

I should also mention that there may be circumstances specific to unusual situations where bioburden testing is not appropriate. If that is the case, a careful justification should be prepared as to why bioburden is not being evaluated following the cleaning process.