Equipment Cleaning Validation: *Microbial Control Issues*

By Destin A. LeBlanc, M.A.
Cleaning Validation Technologies

The Parenteral Drug Association (PDA) spring conference was held in Las Vegas, Nevada in March 20, 2001. The conference showcased, cleaning validation, residue limits, bioburden, microbial limits, and sanitization.

The initial focus of regulatory documents relating to cleaning validation for process equipment in pharmaceutical manufacturing involved measuring residues of the drug active and the cleaning agent. For example, the introduction to the Food and Drug Administration (FDA) guidance document on cleaning validation[1] states: "This guide is intended to cover equipment cleaning for chemical residues only." While admitting that microbial residues are beyond the scope of the guideline, that guidance document further states, "microbiological aspects of equipment cleaning should be considered," particularly with reference to preventive measures so that microbial proliferation does not occur during storage. The European PIC/S document,[2] that was issued several years later, does explicitly mention microbial residues. In Section 6.2.1, contaminants to be removed include "the previous products, residues of cleaning agents as well as the control of potential microbial contaminants." However, Section 6.7 of this document that covers "Microbiological Aspects," focuses exclusively on the same issue discussed in the FDA guidance document, namely the issue of preventing microbial proliferation during storage.

"...it is becoming more common for regulatory authorities to cite manufacturers for deficiencies related to microbial control in cleaning validation programs."

As a practical matter, microbial residues on equipment surfaces are part of the contaminants that should be reduced to an acceptable level; that acceptable level being what is safe for the manufacture of the subsequently product. Unfortunately, very little has been written on what is a safe level for microorganisms following cleaning and/or sanitation.[3,4] Part of the reason for this is that microbial residues are significantly different from chemical residues. Chemical residues are "inert" in the sense that it is easy to calculate (especially using scenarios of uniform contamination in the subsequently product) the potential levels and effects of those chemical residues in the subsequently product should they be transferred to that subsequently product. With microbial residues left after the cleaning process, the situation is somewhat different. Because microorganisms are living organisms, those left as residues on equipment may change in number after the cleaning process, but before the manufacture of the subsequently product. Those microbes transferred to the

subsequently product may also change in number after they are incorporated into the subsequently product in the manufacturing step. This change may be a significant reduction in bioburden, either due to drying of the equipment or due to a preservative in the finished drug product, for example. This change may also involve rapid proliferation, either due to suitable growth conditions in wet equipment during storage, or due to suitable growth conditions in the finished drug product. Or, they may result in no significant change in microbial level, because the bioburden was due to bacterial spores (that will survive readily in dried equipment) or because the subsequently manufactured product was a dry product (with low water activity). Therefore, knowing the levels of microorganisms left on the equipment following cleaning does not necessarily give one the full story of the potential hazards of those microbial residues. Additional information is required to assess those potential hazards.

If that is the case, why has microbial evaluation during cleaning of process equipment been a little discussed topic? Part of the reason is that it is not a significant problem in process manufacturing. Yes, it could conceivably be a problem if cleaning and storage were inadequate. However, for the most part, cleaning and storage of process equipment, in so far as it applies to microbial residues, probably is done relatively well in most pharmaceutical manufacturing facilities. On the other hand, it is becoming more common for regulatory authorities to cite manufacturers for deficiencies related to microbial control in cleaning validation programs. One reason for this seeming anomaly is that while firms are adequately controlling microbial contamination of process equipment, there may be little documentation to support this. This lack of documentation includes any measurement of microbial residues during the cleaning validation and/or during routine monitoring. Some companies will measure the change in microbial levels on equipment surfaces during storage of the cleaned equipment. However, many times this does not include any assessment as to the effect of that unchanged bioburden level on the subsequently manufactured product.

“Some companies will measure the change in microbial levels on equipment surfaces during storage of the cleaned equipment. However, many times this does not include any assessment as to the effect of that unchanged bioburden level on the subsequently manufactured product.”

This paper will address issues, covering approaches to control of microorganisms in process equipment, the setting of acceptance limits, sampling techniques, and approaches to providing acceptable documentation.

Microbial Control Measures

Control measures to reduce the bioburden on cleaned process equipment include control of bioburden of raw materials, the cleaning process itself, a separate sanitizing step, and drying of the equipment following cleaning. Bioburden of raw materials includes the active, excipients, water, and any processing aids. In many cases, the manufacturer may have little control over the bioburden of raw materials other than to accept a specification by the raw material supplier. The most critical raw materials probably will be natural products, in which there may be considerable variation in the levels and types of microorganisms. A solid monitoring program to control incoming bioburden of raw material is necessary. If there could be significant variation in bioburden, then that should be addressed in the cleaning validation Performance Qualification (PQ) trials; at least one PQ trial should utilize the worst-case incoming bioburden of raw materials to demonstrate adequate cleaning and microbial control under those conditions.

A second means of microbial control is the cleaning process itself. The conditions of aqueous cleaning are often hostile to microbial survival. These

conditions include high temperature (commonly 60-80ºC), pH extremes (>11 and <4), and the presence of oxidizers (such as sodium hypochlorite in biotechnology manufacture). In addition, the presence of surfactants in the cleaning solution can assist in providing good physical removal of microbes (without necessarily killing them). Good cleaning is also beneficial to microbial control in that chemical residues left behind can provide a physical "microbial trap" to allow microorganisms to survive even in the presence of chemical sanitizers. Those chemical residues left behind might also serve as a nutrient source that allows microbes to proliferate during improper storage. Based on the author's experience, in most cases, effective control of microorganisms in pharmaceutical process equipment can be achieved with the use of an effective cleaning process, without the need for a separate chemical sanitizing step.

In some cases, a separate sanitizing step may be necessary. This may include sanitation by steam or by chemical sanitizers. Suitable chemical sanitizers for process equipment include sodium hypochlorite (chlorine bleach), quaternary ammonium compounds, alcohol (ethyl or isopropyl), hydrogen peroxide, and peracetic acid. It should be noted that, with the exception of alcohol and hydrogen peroxide, additional rinses would be necessary to remove any chemical residues of the sanitizer from the equipment. Those chemical residues may also have to be evaluated as residues to be measured in the cleaning validation protocol. For such chemical treatments, it is not an expectation that the equipment be sterile. Unless the final rinse is with sterile water, microorganisms will be reintroduced into the equipment from the use of Water-for-Injection (WFI) or purified water as the final rinse.

Some companies will use an alternative to sanitizing immediately after cleaning. This usually involves sanitizing after storage and immediately before use. This may be used in situations where it is difficult to control microbial recontamination or proliferation during storage. It should be noted that control of storage conditions, if possible, is preferable; the practice of relying solely on a separate sanitizing step immediately before manufacture should be discouraged. If this is practiced, then the sanitization step should be shown to be effective in reducing bioburden under the worst-case storage conditions ("initial" bioburden, time, temperature, and humidity). Needless to say, if the chemical sanitizing step is performed immediately prior to manufacture of the subsequently product, then removal of the sanitizer chemical residues to an acceptable level should also be demonstrated.

A fourth consideration for control of microorganisms is drying the process equipment surfaces following the final rinse. Drying the surfaces will further reduce the levels of vegetative organisms on the surface. In addition, drying will assist in preventing microbial proliferation during storage. Drying can be achieved by heated air, heated nitrogen, or by rinsing with alcohol. In all cases, the process can be assisted by application of a vacuum (to speed the evaporation of the water or, in the case of an alcohol rinse, of the alcohol itself).

Limits for Microbes

As mentioned earlier, it is possible to reasonably predict levels of chemical residues in subsequently manufactured products based on the levels present on equipment surfaces.[5,6] With microorganisms, it is possible to measure levels on equipment surfaces; however, the effect of those residues will depend on what happens to those microorganisms once they come in contact with the subsequently manufactured product. Things that may have to be evaluated include the species (including the so-called "objectionable" organisms), type of organism (vegetative bacteria versus bacterial spore, for example), the presence of preservatives in that subsequently product, the water activity of the subsequently product, as well as any subsequent sterilization process performed on that product. As a general rule, if the water activity is less than 0.6, then it can be expected that microorganisms will not proliferate (although they may continue to survive without reproducing).[7] Water activity is a physical-chemical measurement that expresses the water vapor pressure above the test sample as a fraction of the water vapor pressure of pure water at the same temperature as the test sample. For aqueous products with a neutral pH, microbial proliferation can generally be expected unless there is a preservative in the product. If there is a possibility of microbial proliferation because the product is unpreserved and neutral, then that should be addressed in setting limits.

Three methods to set microbial limits will be addressed. The first (Case I) involve limits where the

subsequent product does not allow microbial proliferation and is not subject to any further sterilization process. The second (Case II) involves subsequent products that are terminally sterilized. The third (Case III) involves subsequent products that are processed aseptically.

Case I Limits

If the subsequently manufactured product does not allow microbial proliferation, then the determination of acceptable microbial limits in the cleaned equipment can be calculated using the same principles used for chemical residues with one important exception. This process involves first determining the acceptance limit in the subsequently product. This limit is typically given in Colony Forming Units (CFU) per gram of product. Once this is determined, then the limit per surface area of equipment (assuming uniform contamination) can be calculated based on the batch size of the subsequently manufactured product and the equipment surface area.

How is the limit in the subsequently manufactured product determined? For chemical residues, it is based on dosing information for actives or toxicity information for cleaning agents. Such concepts cannot be directly applied to microbes. Fortunately, there are two good sources of information relating to levels of microorganisms in products. One is the manufacturer's own Quality Control (QC) specifications for the product, which may include a limit for bioburden in the product. A second source is information given in proposed United States Pharmacopeia (USP) <1111> relating to "Microbial Attributes of Nonsterile Pharmacopeial Articles."[8] Examples of those limits are given below:

Solid oral: ≤1000 CFU/g
Liquid oral; ≤100 CFU/g
Topicals: ≤100 CFU/g

Note: Although these limits were discussed and proposed in Pharmacopeial Forum, these specific recommendations were not adopted officially as part of the 24th edition of the USP.

Unfortunately, this is where the one exception to the conventional treatment arises. When one looks at the bioburden in a finished drug product, the equipment surfaces are not the only source of bioburden. One must also consider the raw materials themselves, as well as the primary packaging, as potential sources of microorganisms. The best way to deal with this issue is to develop information on the bioburden of the raw materials and the primary packaging, and factor these into the limits calculation. For example, if one were dealing with an oral liquid, one might calculate the contribution from the raw materials (assuming the upper limit bioburden for each raw material) as a maximum of 27 CFU/g. At the same time the contribution from the primary packaging is determined to be three (3) CFU/g. Therefore, the amount allowed from equipment surfaces would be 70 CFU/g (100 minus 27 minus three [3]). An additional safety factor should be used to account for the significant variability in microbiological enumeration. An appropriate factor may be on the order of five (5). Therefore, in this case, the limit (in CFU/g) that would be allowed solely due to the cleaned equipment surfaces would be 14 CFU/g (obtained by dividing 70 by five [5]). Higher safety factors also could be considered. These numbers are given for illustration purposes only; it should be realized that the contribution percentage allowed from cleaned equipment would vary depending on the contributions from the raw materials and the primary packaging.

Once the limit in the subsequently product allowed from the cleaned equipment surfaces is determined, the next step is to determine the limit per surface area (CFU/cm^2). This is calculated exactly as it would be for chemical residues:

$$\text{Limit per surface area} = \frac{(\text{limit in subsequently product})(\text{minimum batch size})}{(\text{product contact surface area})}$$

In the example above, if the batch size is 200 kg and the product contact surface area is 260,000 cm^2, then the microbial surface limit of the cleaned equipment is:

$$\text{Limit per surface area} = \frac{(70\ \text{CFU/g})(200{,}000\text{g})}{(260{,}000\ \text{cm}^2)} = 54\ \text{CFU/ cm}^2$$

If sampling were done with a typical contact plate of 25 cm^2, this would correspond to a limit of over 1300 CFU per contact plate. Since it is reasonable to count a maximum of only 250 CFU on a typical contact plate, this would clearly be in the TNTC (too

numerous to count) category. Needless to say, this will vary with the limit in the subsequently product, the portion allowed from cleaned surfaces, the safety factor used, the batch size, and the shared surfaces area. However, under most reasonable scenarios, the calculated limit due to microorganisms on the cleaned equipment surfaces will be significantly above what should be (and can be) achieved by proper cleaning. As a general rule, a good cleaning process should produce surfaces that contain no more than 25 CFU per contact plate (<1 CFU/cm^2). When failures occur, generally they will be gross failures, with counts generally above 100 CFU per-plate.

Case II Limits

This involves setting limits for cleaned equipment when the product subsequently manufactured in that equipment is to be sterilized. In this case, the microbial limit in the subsequently manufactured product can be established based on the assumed bioburden of that product at the time of sterilization. In other words, any validated sterilization process depends on an assumed bioburden of the item being sterilized. That assumed bioburden then becomes the limit in the subsequently product. Once that limit in the subsequently product is established, then the calculations are the same as for Case I – a certain portion of that total limit is allowed from cleaned equipment surfaces, a safety factor is applied, and then the limit per surface area is calculated using the minimum subsequent product batch size and the product contact surface area. It is significant that this issue is actually addressed in the FDA's cleaning validation guidance document states:

> *"…it is important to note that control of bioburden through adequate cleaning and storage of equipment is important to ensure that subsequent sterilization or sanitization procedures achieve the necessary assurance of sterility."*[9]

Case III Limits

This third case involves setting limits on equipment surfaces where the subsequently manufactured product is aseptically produced. This case is slightly different from Case II in that it is the equipment itself, and not the product, which is subsequently sterilized. This case is relatively straightforward, because the microbial limits on the surfaces of cleaned equipment are established based on assumed bioburden of the equipment surfaces for sterilization validation of that equipment. No information on batch sizes or surface areas is necessary. The assumed bioburden for the sterilization validation can be used directly for limit purposes. The only adjustment may be the incorporation of a safety factor (to accommodate normal variation in microbiological enumeration).

Measurement Techniques

Conventional tools used for microbial enumeration from surfaces can be used. These include rinse water sampling (usually with membrane filtration), swabbing (with desorption of the swab into a sterile solution and then a pour plate count), and use of a contact plate. The choice of recovery medium and incubation conditions is usually dictated by the expected organisms. As a general rule, the initial focus is on aerobic bacteria. However, if anaerobic bacteria or molds/yeasts are suspected problems, these should be also evaluated.

One issue that does not translate directly from chemical residue measurements is the idea of determining percent recovery using the sampling method. In the measurement of chemical residues, the target residue is spiked onto a model surface and the quantitative percent recovery is determined. The amount recovered as a percent of the amount spiked is considered the sampling method percent recovery. Percent recoveries in chemical sampling measurement are generally above 50 percent. This percent recovery is then used to convert an analyzed sample value; for example, if a chemical residue measured by a swabbing technique gives 0.6 µg of residue, then with a 50 percent recovery, this actually represents the possibility of 1.2 µg being on that surface. This concept cannot be applied directly to microbiological sampling. The reason for this is partly the inherent variability in microbiological testing. If one measured 10 CFU in one test and five (5) CFU in a duplicate test (a 50 percent difference), one would be hard pressed to say that those numbers are significantly different. In addition, how would one actually measure the percent recovery in a microbiological test? If a model surface is spiked with a specific number of a certain bacterium, and then that surface is allowed to dry and is sampled, just the process of drying might cause a low

recovery of bacteria (due to the dying of vegetative bacteria by drying). In addition, what species of bacteria would be used for the recovery study?

It is recognized that microbiological sampling methods may understate the number of microbes on a surface (indeed the concept of a CFU that may contain any number of bacteria, also clouds the issue). There are two ways to view such an issue. One is to make it clear that whatever variation exists in measuring microorganisms on surfaces is probably equally an issue when one sets limits based on product limits or sterilization bioburden limits. Therefore, the variability issue becomes a "wash." The other perspective is to account for such variation by choosing extremely high safety factors. In the calculation example for Case I, a factor of five (5) was used as a safety factor. Even if that safety factor were increased to 10 or 20, the calculated acceptance limits would have still been extremely high, and still beyond what one should achieve with a well-designed cleaning program.

"One issue that does not translate directly from chemical residue measurements is the idea of determining percent recovery using the sampling method."

Documentation Strategies

How these issues will be addressed will depend on the stage of the cleaning process development. For a new process being designed, the best strategy is to prepare a calculation of microbial limits, and then design the cleaning process to meet those acceptance criteria. Included in that evaluation should be any change in bioburden (in particular, any increase or proliferation) on storage of the equipment. The microbial acceptance limits should be included in the validation protocol, and measured as part of the three PQ trials. One should also include the absence of "objectionable" organisms as part of the acceptance criteria.

To deal with processes for which cleaning validation has already been completed, but for which no microbial evaluation has been done, there are two strategies available. The objective of each is to develop documentation that the cleaning process consistently provides equipment surfaces with acceptable bioburden. One option is to perform a cleaning validation PQ, measuring only bioburden on surfaces for comparison to calculated acceptance limits. The other option is to initiate a routine microbiological monitoring program as part of the monitoring of cleaning. This may involve something as simple as monitoring the bioburden in the final rinse water to demonstrate consistency. This data, combined with product QC data on bioburden, may satisfy the need for adequate documentation.

One should also consider one's motivation for wanting to obtain assurance that the bioburden is acceptably low after cleaning. If the impetus for action is due to lack of data, one should resist the impulse to immediately add a sanitizer into the cleaning program. The focus should be on developing data to demonstrate the sufficiency of the current cleaning process. Adding a separate sanitizing step only complicates matters by adding additional residue concerns. If the impetus for action is due to observed high microbial counts on equipment surfaces or (more likely) in manufactured product, then it is important to determine by careful investigation whether that unacceptable contamination is due to issues with the cleaning process, to issues with storage, or to both. In such a case, a separate sanitizing step should only be added if the data fully support it.

Conclusion

Bioburden on cleaned equipment is an important concern in the cleaning process. Fortunately, most aqueous cleaning processes, properly designed, should provide low and acceptable bioburden levels on equipment surfaces following the cleaning process. Proper drying and storage should provide assurance that microbial proliferation does not occur before the manufacture of the subsequently product in that equipment. Any scientifically justified determination of acceptable bioburden levels, particularly for non-sterile products, is generally far higher than what should be achieved in conventional practice.

This is becoming more of a regulatory and compliance issue, not because microbial contamination is a widespread problem, but rather because pharmaceutical manufacturers may lack appropriate documentation to support their practices. This can easily be remedied by a separate validation protocol to address microbial issues or by routine monitoring to demonstrate consistency. ❑

About the Author

Destin A. LeBlanc, M.A., is Vice President, Technical Support for the Scientific Division of STERIS Corporation. This includes responsibility for the STERIS contamination prevention products. He has 25 years experience in technologies of specialty chemicals. He is a graduate of the University of Michigan and of the University of Iowa.

References

1. FDA. "Guide to Inspections of Validation of Cleaning Processes." 1993.
2. Pharmaceutical Inspection Cooperation Scheme. Recommendations on Cleaning Validation. Document PR 1/99-2. Geneva, Switzerland. April 1, 2000.
3. A.M. Cundell. Microbial Monitoring. Presented at the 4th IIR Cleaning Validation Conference, October 20-22, 1997. (http://microbiol.org/files/PMFList/clean.ppt, accessed May 29, 2001).
4. S.E. Docherty. "Establishing Microbial Cleaning Limits for Non-sterile Manufacturing Equipment." *Pharmaceutical Engineering*. Vol. 19 No. 3. May/June 1999. Pp. 36-40.
5. G.L. Fourmen and M.V. Mullen. "Determining Cleaning Validation Acceptance Limits for Pharmaceutical Manufacturing Operations." *Pharmaceutical Technology.* Vol. 17 No. 4. 1993. Pp. 54-60.
6. D.A. LeBlanc. "Establishing Scientifically Justified Acceptance Criteria of Finished Drug Products." *Pharmaceutical Technology.* Vol. 19 No. 5. October 1998. Pp. 136-148.
7. R.R. Friedel. "The Application of Water Activity Measurements to Microbiological Attributes Testing of Raw Materials Used in the Manufacture of Nonsterile Pharmaceutical Products." *Pharmacopoeial Forum.* Vol. 25 No. 5. September-October 1999. pp. 8974-8981.
8. <1111> Microbial Attributes of Nonsterile Pharmacopoeial Articles (proposed). *Pharmacopoeial Forum*. Vol. 25 No. 2. March-April 1999. Pp. 77857791.
9. FDA. "Guide to Inspections of Validation of Cleaning Processes." 1993.

Article Acronym Listing

CFU:	Colony Forming Units
PDA:	Parenteral Drug Association
FDA:	Food and Drug Administration
PQ:	Performance Qualification
QC:	Quality Control
USP:	United States Pharmacopeia
WFI:	Water-For-Injection