

February 2004 Endotoxin Issues in Cleaning Validation

The questions that will be addressed in the Cleaning Memo include:

- “When should I measure endotoxin in a cleaning validation protocol?”
- “How should I sample for endotoxin?”
- “What acceptance criterion should I establish for endotoxin?”

As a general principle for *finished drug* manufacture, endotoxin should be included as part of the protocol testing for products that have endotoxin specifications. This generally means injectable products. One could argue that since the final product is tested for endotoxin, then it is not necessary to test for endotoxin. After all, the reason we test for cleaning agent or previous drug active in cleaning validation protocols is that we do not test for those potential residues in the finished drug product. This approach of depending on the testing of endotoxin in the finished drug product could be acceptable if testing of the finished drug product represents “100% verification”. A general principle in validation (which is expressed more clearly in medical device validation documents) is that if the results of a process can be “fully verified” by inspection and testing, then validation is *not* required. The question then becomes, “Is testing of part of the bulk finished product or testing of a certain number of vials ‘full verification’?” Whatever the answer to that question, as discussed below, adding an endotoxin test to a cleaning validation is relatively uncomplicated such that it is generally recommend for injectable products.

What about manufacture of bulk APIs? In this case, if there is an endotoxin specification for the final bulk API, it is generally advisable to include endotoxin testing as part of the cleaning validation protocol. One exception to this is for the early stages of manufacture of a bulk active (particularly biotech actives) for which there is a subsequent endotoxin reduction step. Any endotoxin that is left following cleaning would carry over to the next batch and be removed by the endotoxin reduction steps.

How should endotoxin be sampled? The most common way is by taking a sample of the final rinse water. It is readily acknowledged that endotoxins can adhere to and be difficult to remove from surfaces. While sampling surfaces by swabbing has been explored for cleaning validation, there is no clear evidence that it is any better than rinse sampling for recovery of endotoxin from surfaces.

The acceptance criterion for a rinse sample should be at least the WFI criterion for endotoxin, namely 0.25 E.U./mL. To be somewhat conservative, some companies may choose to be at half that level, or 0.125 E.U./mL. Why is this acceptable? If one accepts the “worst case” principles for establishing limits for rinse samples (see my article “Rinse Sampling for Cleaning Validation Studies”, in *Pharmaceutical Technology* 22:5, 66-74, May 1998), then what is measured in the rinse sample is the same as the potential contamination of the next product. Therefore, measuring 0.125 E.U./mL in the rinse sample means a potential carryover of 0.125 E.U./mL into the next product. If the assumptions of CIP rinsing (a topic which I’ve covered in my seminars many times, and which I plan to publish later this year) are applied, measuring 0.125 E.U./mL in the rinse sample means a potential carryover of *much less* than 0.125 E.U./mL into the next product. Unless the next manufactured product has a “natural” endotoxin level of greater than 0.1 E.U./mL, neither of these measured levels in rinse samples should pose a problem. I should note that when endotoxin is measured in finished drug manufacture, the results in the *rinse sample* are generally well below 0.05 E.U./mL.