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More on Limits Based on Compendial Water Values

Last month we covered issues related to setting limits based on a 500 ppb TOC "requirement". This month we will expand that to cover other compendial water quality values that are sometimes used for cleaning validation limits. They include conductivity, bioburden and endotoxin.

The USP requirement for conductivity is a staged approach. Stage 1 is testing the sample without temperature compensation. Acceptable results depend on the measured temperature, with ≤1.3 µS/cm being acceptable if the temperature is 25°C and ≤2.4 µS/cm being acceptable if the temperature is 65°C (with different acceptable values being specified for different temperatures). If the Stage 1 requirement is not met, Stage 2 testing involves adjusting the temperature to 25°C, and then conductivities of ≤2.1 µS/cm are acceptable. If the Stage 2 requirement is not met, Stage 3 involves taking the Stage 2 sample, adding saturated KCl solution, and then measuring both pH and conductivity. The acceptable conductivity for this stage depends on the measured pH, with ≤2.4 µS/cm being acceptable at a pH of 6.0, and values closer to ≤4.6 µS/cm being acceptable at the pH extremes of 5.0 and 7.0.

A typical value sometimes proposed for rinse water samples in cleaning validation protocols is ≤2.4 µS/cm, since in many cases the rinse water will be near a temperature of 65°C. We must ask ourselves, however, what we are measuring with this conductivity value. Generally the approach is to measure the cleaning agent, which may be sodium hydroxide, phosphoric acid (especially if I am in the mode of following a caustic solution with an acidic solution), or a formulated cleaning agent (typically containing caustic or an acid, which is the major contributor to conductivity). If I am setting limits based on the toxicity of the cleaning agent, I can used whatever safety information is available, which for the most part is an LD50 study, an eye irritation study, and/ or a skin irritation study, in order to set an acceptable limit using a carryover calculation.

The next step is to prepare a conductivity versus concentration "curve" for the cleaning agent using values at and below the acceptance concentration limit in a rinse solution (at the appropriate temperature). The conductivity value corresponding to the calculated ppm limit of the cleaning agent represents a medically safe value. In many cases, the medically safe value may be 10 to 30 µS/cm, considerably above the USP limit. Because companies are typically able to achieve much lower values (lower than the 10 to 30 µS/cm value), they are tempted to place the limit at a value such as 2.4 µS/cm based on the USP water requirements. Is this okay?

It is certainly okay from a compliance perspective. If I set a limit more stringent than a medically safe level, there should be no objections from regulatory agencies. However, if I choose a level like 2.4 µS/cm, I might be setting myself up for a failure in my protocol, when really there is no effect on product safety or quality. The reason for this is not unlike the reasons given last month for the situation with TOC limits. The USP specification is for water in (or taken from) the recirculating loop. Once I take it out of that loop, and use it for rinsing equipment, I am likely to pick up at a minimum carbon dioxide from the air, which will give me higher conductivity values. It is for this reason that I generally recommend for companies that are able to consistently get low rinse water conductivity values to use either a conductivity value closer to 5 µS/cm, or else if the 2.4 µS/cm value is preferred, measure that as an increase over the baseline conductivity of the water used for final rinsing (that is, I subtract the baseline water conductivity from the test sample conductivity, and the resultant

value must be ≤2.4 µS/cm). Of course, this value must be less than the medically safe level determined by my carryover calculation.

Even where cleaning agent limits may not be set on a carryover calculation, I still prefer one of these two options for conductivity limits because otherwise I may be setting myself up for failure when there is no medical concern.

The second situation is limits for bioburden in a rinse solution. While there is no USP requirement for bioburden of rinse water, typical industry values are 100 CFU/mL for Purified Water (PW) and 10 CFU/100 mL for Water for Injection (WFI). A typical industry approach if PW is used as the final rinse is to set the limit at the PW "specification" of 100 CFU/mL. I generally support that approach. You might ask if I am inconsistent here in accepting the PW approach for bioburden, but not accepting it for TOC and conductivity. I guess that might be considered an inconsistency. However, the reason I accept that value is because carryover calculations for bioburden (based on what is acceptable in the next product) generally result in rinse water values considerably above the PW specifications. Therefore defaulting down to the PW specifications is acceptable. Realistically, if I am rinsing with hot PW as the final rinse, I am not expecting many vegetative organisms to survive. Unless there is something unusual about my cleaning process, I should readily meet that PW specification for a final rinse in a cleaning validation protocol.

The situation with WFI as the final rinse is slightly different. I generally see two extremes here. One side says "Hey, this is a sterile product, and I am doing a terminal sterilization by heat, I am doing a sterile filtration, and/or I am making the product aseptically; the equipment will be SIPed after the cleaning process. Why should I even have to measure bioburden in this situation?" Another side says, "Hey, I still am able to meet the WFI bioburden limit in this situation, so that will be my acceptance limit."

There is an element of truth in both statements. However, in response to those who want to skip bioburden testing, I would maintain that any sterilization process could be overwhelmed by the bioburden that might be present. Furthermore, if the bioburden is a Gram negative organism, it will release endotoxin in a heat/steam sterilization process (but not necessarily in the sterile filtration case). Finally, gross amounts of bioburden, even if they were handled by the sterilization process, are just not CGMP. To those who argue for the WFI limits, I would maintain that they may be putting in a value that is not required for product quality/safety. That strict bioburden limit is for bioburden in the recirculating water loop; once it is removed from that loop and passed through cleaned equipment, there is not necessarily an expectation that it still meet that WFI limit. For those reasons, I generally recommend a rinse limit in this situation of a value intermediate between the PW limit and WFI limits. Examples of such values are 10 CFU/mL and 1 CFU/mL.

The final case to examine in this Cleaning Memo is a limit for endotoxin in rinse water. Generally, there are endotoxin limits only if the subsequent product has endotoxin specifications. And, it is generally the case that the rinse water is WFI (or at least water with WFI quality). For endotoxin, the compendial water specification is ≤0.25 EU/mL. This is a case where I must confess I am inconsistent, because the industry almost always uses and I generally advocate that the compendial water specifications be adopted. The reason here is that it is difficult to have a rationale for something lower.

I have seen some companies, however, set a more conservative limit of around 0.125 EU/mL. The approach here is that there can be contributions of endotoxin in the next product from the water used for formulating that product, from the raw materials used for formulating that product, and from the cleaned equipment surfaces used to manufacture that product. It appears prudent to not allow 0.25 EU/mL from both the water and the cleaned equipment (although 0.25 EU/mL in the final rinse water for a CIP rinse ordinarily translates to a much smaller carryover to the next manufactured product).

I should also point out here that measuring endotoxin in the cleaning steps in biotech manufacture for E. coli fermentation and harvesting steps does not generally make sense, because the subsequent product made in those fermentation and harvesting vessels do not generally have strict endotoxin specifications. Any contribution of cleaned equipment to the endotoxin load in the next product in those steps is minor compared to what is already there from the bacterial fermentation itself. Furthermore, following the harvesting step, there generally are one or more endotoxin removal steps in the downstream manufacturing processes.

The purpose of this Cleaning Memo has been to help clarify issues in setting cleaning validation limits for conductivity, bioburden and endotoxin based on compendial water specifications.