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Limits for Rinse "Grab" Samples

There are generally two types of rinse samples taken for cleaning validation protocols. One is a separate sampling rinse (SSR) performed after the final process rinse (FPR). This SSR is only done in a protocol where one is attempting to measure residues to compare the results against acceptance limits. A second type of rinse sample is a grab sample of the FPR.

Setting limits for a SSR sample is relatively straightforward. The L3 limit (in µg/cm^2) is determined as conventionally done. Then the limit (L4 in µg/mL) in the SSR sample for a rinse sample volume (V in mL) used to sample a surface area (SA in cm^2) is as follows:

$$L4 = \frac{L3 \times SA}{V}$$

The issue that may not be as well understood is how limits are set for a *grab sample* of the FPR, such as in a CIP system. Whenever I talk about a grab sample for the rest of this Cleaning Memo, I will always be referring to a grab sample of the FPR. I still hear people express the view that limits in a grab sample cannot be scientifically determined. While this may be partly true for bulk biotech cleaning processes, for any situation where limits are based on a conventional *carryover calculation*, it is possible to appropriately set limits for the grab sample using scientific principles. This can be done by assuming the use of a SSR, even though the actual sampling will be a grab sample of the FPR.

What is the relationship between any residue measured in a grab sample (of the FPR) and that residue measured in a subsequent SSR? One way to evaluate this is to determine what would happen if the FPR were continued for an additional time, with that additional time being the time required for a SSR *if a SSR were to be used* (it in fact would not be used). Remembering that for a SSR we collect the entire rinse sample and measure the concentration of the residue, it is a reasonable expectation that the concentration of any measured residue in the SSR will be *the same or less than* the concentration of that same residue measured in a grab sample at the end of the FPR. Therefore if we set a limit based on the assumption of a SSR, but measure residues in the protocol using the grab sample of the FPR, then we are measuring residues in a worse case situation (the concentration of residue in the grab sample will be the same or higher than the concentration in a SSR if that SSR was performed).

Note here that it does not matter whether the grab sample at the end of the FPR is exactly the very last portion of that FPR. Any sample taken 30 seconds before the end of the FPR, or even 60 seconds before the end of the FPR, would constitute a worst case for measuring residues and comparing it to the acceptance criterion (it being a reasonable assumption that the concentration of residue in that grab sample *will not increase* with the additional time of the FPR).

A key decision in setting limits this way is the *volume* we select for the assumed SSR. Remember that we are not actually performing the SSR, but we have to calculate a limit *as if we were performing that SSR*. Using the

equation given previously for the limit in a SSR sample, the L3 limit and the sampled surface area are readily determinable. The *key* issue is what value we use for the rinse volume. It should be obvious that it may be possible to manipulate the rinse volume to produce a very high limit (which would be easier to achieve in our protocol). However, this needs to be recognized *and avoided*. For example, there is an FDA 483 where the company claimed to rinse a 1000 liter vessel with about 2 liters of rinse solution. Clearly this is not reasonable; it should take more than 2 liters to adequately contact all surfaces of a 1000 liter vessel.

Since we do not actually perform a SSR, what volume can we utilize for calculating limits? There are several options to consider. If a riboflavin coverage study was done on the vessel (which is assumed if it is a CIP spray device cleaning), it is possible to utilize whatever volume of water was used for the riboflavin procedure for that equipment. The riboflavin procedure demonstrates adequate contact with all surfaces of the equipment. Therefore, it can be a volume to use in the limits equation for the assumed SSR. If the rinse is done as a series of pulse rinses, another option is to use the volume of water in the final pulse (the volume can be readily determined by multiplying the flow rate by the time of that final pulse). A third option is to use some kind of "arbitrary" calculation based on the complexity of the equipment. Values that I usually advocate are 5% to 10% of the total equipment volume. A value of 5% is used for relatively simple equipment, such as a storage vessel. A value of 10% is used for relatively complex vessels, such as a fermentor. Whatever volume is chosen should pass the "smell test" – is this a reasonable volume to contact all surfaces in a CIP SSR procedure.

The volume selected for setting limits is also critical for any rinse recovery procedure. If I am using a rinse solution to measure residues to compare against acceptance criterion in protocols, it is important to perform rinse sampling recovery studies. A key feature in a rinse recovery study is the *ratio of rinse water used to surface area sampled*. In the rinse recovery procedure, it should be obvious that *more* water I use for rinsing a fixed surface area, the *higher* the recovery percentage will tend to be. Is there a way to determine an appropriate ratio of rinse water to surface area for my rinse recovery procedure? Yes - it is the *same ratio* I utilized in my limits calculation (both the sampled surface area and the rinse volume are critical for that limit calculation given above). So, the ratio of rinse volume to sampled surface area in the rinse recovery procedure should be the same or lower than the ratio used for the limits calculation. Note that this relationship between the ratio of volume to surface area in the recovery study and the limits calculation will hopefully prevent me from selecting an extremely low volume for my limits calculation, because if that ratio carries through to the recovery procedure, I will probably obtain inadequate recoveries.

The purpose of this Cleaning Memo is to not to proscribe nor prescribe grab sampling of the FPR. Rather the purpose is to elucidate one way to calculate limits for a grab sample of the FPR, and to provide some guidelines for determining the rinse volume of the assumed SSR in that situation.