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Feature

Fundamentals Of Viral Clearance Studies
Part 3: – Study Design Factors For Optimal Log Reduction Factors

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When evaluating viral reduction for a given process step, the reduction is the difference between the total virus in the input sample and the total virus in the post-purification, product-containing fraction. The results of viral assays are typically provided as a titer; that is viral units (e.g., TCID50, PFU, genome equivalents, etc.) per unit volume. For calculation of total virus in each fraction, the volume of each fraction be must be considered, and this is done by multiplying the volume of the input and output fractions by the virus titers. Virus reduction, then, can be calculated as (ICH Topic Q5A (R1) Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, CPMP/ICH/295/95):

$$10\text{^reduction} = \frac{10\text{^log titer}_{input} \times \text{volume}_{input}}{10\text{^log titer}_{output} \times \text{volume}_{output}}$$

For example, if a TCID50 assay was used to determine viral clearance across a bind and elute chromatography step, and the virus titers and volumes of the input and output fractions were:

Column Fraction	Volume	Virus Titer
Column Load Volume (Input)	200 mL	6.5 $\log_{10}$ $TCID_{50}$/mL
Eluate Volume (Output)	50 mL	3.2 $\log_{10}$ $TCID_{50}$/mL

then reduction would be calculated to be:

$$10\text{^3.9} = \frac{10\text{^6.5} \times 200}{10\text{^3.2} \times 50}$$

If the volume of each fraction has been considered, then virus reduction can be calculated simply as the difference between the log total virus of the input fraction and the log total virus of the output sample. In the example above, 3.9 log reduction is the difference between 8.8 log total virus in the input sample and 4.9 log total virus in the output sample. The reduction for each purification step in the manufacturing process that is evaluated for viral clearance can be summed to determine the overall process reduction. Due to the inherent variability in the biological assays used for virus detection, however, reductions of one log or less cannot be included in the overall process reduction.

Virus reduction is expressed on a logarithmic scale, and while virus can be reduced to very low levels, it can never be reduced to zero. If no virus is detected in a sample, a theoretical endpoint is calculated. Using the Poisson distribution, the concentration of virus that would be present in the sample in order for the small volume that is tested in the virus assay to not contain a virus particle 95% of the time (Figure 1).

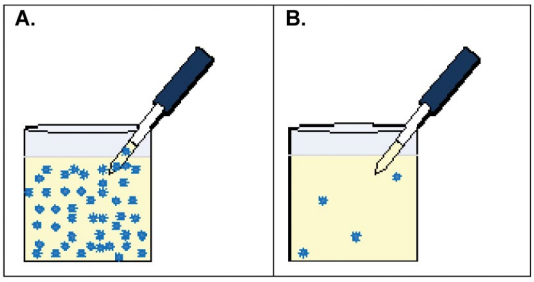

Figure 1. At high virus concentrations (A.), the volume sampled for assay is likely to contain a representative concentration of virus. At very low virus concentrations (B.), the volume sampled may or may not contain infectious virus. In these cases a theoretical endpoint is calculated as the concentration of virus that would result in the volume sampled not containing an infectious virus 95% of the time.

This can be calculated using the Poisson distribution and the following equation:

$c = \ln p / -v$,

where:

c = concentration of infectious virus particles in the process intermediate,

p = probability (typically at 95%)

v = the volume of the sample that is actually tested in the assay.

From this it is readily apparent that as the volume of sample tested decreases, the concentration of virus (or what we colloquially refer to as our assay limit) increases (Table 1). Consequently, when the presence of cytotoxicity or viral

interference necessitates dilution of the process intermediate, the volume of the intermediate in the sample decreases, and the assay limit will increase (Figure 2, A. and B.). Testing less volume increases the assay limit, but testing more volume will decrease the assay limit. This is the basis of a large volume assay, in which much more sample is assayed (Table 1). For every ten-fold increase in the volume assayed, the assay limit decreases by approximately one log. There are practical limitations, however, to the volume that can be assayed; while a 10-fold or 100-fold increase in sample volume may be manageable for the laboratory, larger increases in volume may prove too unwieldy to be used. Large volume testing is only useful when no virus is detected in the sample. It must be remembered, too, that the more volume that is assayed, the greater the probability that an infectious virus will be detected.

Dilution of Process Intermediate to be Assayed	Assay Limit (Log_{10} $TCID_{50}$/mL)	
	Standard Titration	Large Volume Testing
Undilute	≤ 0.87	≤ - 1.13
1:3	≤ 1.35	≤ - 0.65
1:10	≤ 1.87	≤ - 0.13
1:30	≤ 2.36	≤ 0.36
1:100	≤ 2.87	≤ 0.87

Table 1. The assay limit increases as the volume of sample assayed decreases. The use of a large volume assay results in a lower assay limit. It must be noted that this is only valid when no virus is detected in the assay.

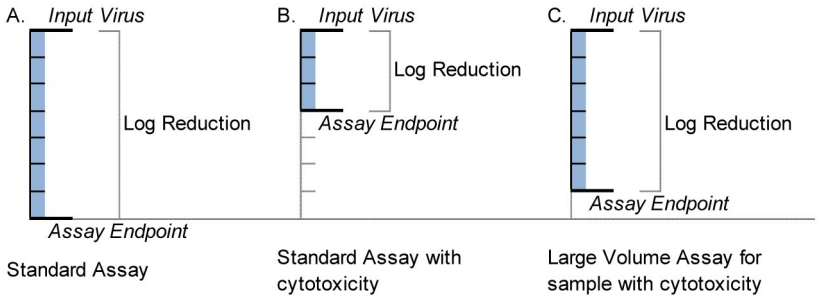

Figure 2. Schematic representation of log virus reduction (blue bar) in (A.) a standard assay, (B.) in a standard assay with a highly cytotoxic process intermediate and (C.) using a large volume assay for the same highly cytotoxic process intermediate.

Large volume testing can be used to increase the log reduction factor for a process step. In the situation where a process intermediate must be diluted due to cytotoxicity or viral interference, the log reduction is reduced because the assay limit is increased (Figure 2B). Large volume testing can potentially increase the level of clearance that can be claimed by decreasing the assay limit (Figure 2C). Knowing the extent that a process intermediate is cytotoxic or interferes with the virus detection assay prior to initiation of the spiking study will allow large volume assays to be utilized to maximize potential reduction.