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Preparation of Virus Spikes Used for Virus Clearance Studies

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Preparation of Virus Spikes Used for Virus Clearance Studies Task Force

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1.0 Introduction

Assuring the viral safety of plasma derived biologicals and biopharmaceuticals is critical for safe use by healthcare consumers and successful marketing by industry of these vitally important healthcare products. Incidences of contamination of products derived from human plasma in the past have adversely impacted the health of hundreds of patients and tainted the image of certain segments of the healthcare industry. Today's recombinant biopharmaceuticals have never, as far as we know, presented a similar viral safety issue, and plasma derived products have a better safety record today. This is in large part due to stringent measures taken by the industry and regulators to mitigate viral safety risks.

The current strategy for ensuring viral safety involves multiple levels of control over the product and process, including cell bank screening, source material screening and/or inactivation, and incorporation of specific virus removal or inactivation steps into the production scheme. Validating the ability of the process to remove or inactivate viruses is key in understanding the ability of the manufacturing scheme to clear viruses, in the unlikely event that they do contaminate a process intermediate, and in providing a yard-stick to determine if the clearance capacity is large enough to assure viral safety.

Viral clearance studies start by designing scale-down models of the actual manufacturing unit operations. The objective of the scale-down model is to determine the performance and viral clearance that can be expected of a unit operation at full scale. First, key and critical process parameters, as defined in PDA Technical Report No. 42 ***(1)*** or ICH Q8(R2) ***(2)*** (e.g., resin contact time, filtration volume per membrane surface), are matched between the scale-down models and commercial large scale processing. Second, key and critical performance parameters, such as step yield and purity, must be representative of the large scale unit operation. Non-key/non-critical operating parameters, like column bed diameter and filter area, are lowered to allow reduction of the model unit operation to a scale practical for lab studies. Other key and critical parameters have to be considered if precipitation steps are investigated and virus is removed by distribution into the precipitate.

Viral clearance studies are conducted by spiking virus into the relevant intermediate and processing the spiked material in a scaled down unit operation. The reduction in the virus load by the unit operation demonstrates the effectiveness of the process step for virus removal or inactivation. The virus spike used in viral clearance studies should be representative of a potential contaminant to the extent achievable. Not only is the selection of appropriate relevant or model viruses important; the properties of the virus spike must also be considered. For example, the presence of serum in a virus spike may be problematic for a validation study of a serum-free manufacturing scheme. As another example, the presence of non-viral extraneous macromolecules, such as proteins and DNA, would be problematic for a validation of a downstream unit operation where the process fluid is presumably a highly purified, non-aggregated protein. It is important that contaminants in the virus spike itself do not impact key or critical performance parameters in a way that makes the scale-down model unrepresentative of the large scale process.

Achievement of these goals involves careful selection and design of virus spikes, both in terms of volume of spiking and purity of the preparations themselves. While it is relatively straightforward to modify the spiking volume to the point where it is non-interfering, achievement of spike purity is more complicated. Presently, some relatively crude spikes are produced directly from unprocessed clarified cell culture lysates or culture supernatants for direct use in validation studies. These spikes, like most biological systems, are relatively heterogeneous and difficult to control. Other virus preparations that are purified by ultracentrifugation/re-suspension, chromatography or other methods possess higher purity, but are still heterogeneous to some degree. The heterogeneities and

residual impurities associated with all virus preparations should be considered when designing and interpreting viral clearance validation studies.

The spike preparation procedures and quality attributes will inevitably vary between viruses; some are relatively easy to grow and prepare, while others are difficult. Viral clearance studies for multiple biopharmaceuticals and plasma derived biologicals are performed at multiple sites and multiple contractors and variations between laboratories are also inevitable. Minimizing this variation is desirable in order to maximize confidence of end-users and regulatory authorities in the current viral safety regime. The goal of the PDA's Virus Spike Preparation Task Force is to identify useful quality attributes for virus spikes and to identify the opportunities for spike quality optimization. This technical report will report on the generally-accepted criteria for spike selection for different biopharmaceuticals at different process steps, as well as provide some technical details of virus purification and characterization.

This technical report considers also the preparation of bacteriophages. Current regulatory expectations preclude replacement of mammalian viruses with phage for final process validation studies. Under specific conditions, bacteriophages can be useful tools to reduce costs and time. Examples are process optimization, development, and definition of acceptable operating range exercises, particularly for filtration steps.

Case studies presented in this technical report were drawn from members of the task force. In most instances, the case studies are summaries of oral presentations at three PDA Viral Safety meetings (Bethesda MD, 2001; Langen Germany, 2003; Bethesda MD, 2005), but the data was not published in written form. Others were provided by task force members directly for this technical report and are based on their firm's experience and data. Finally, surveys of interested parties were conducted by the task force in 2005 and 2007 to determine current practices and desired states for virus spike preparations; summarized results are reported here for the first time. Thus, this technical report is the first comprehensive written compilation of industry experience with the impact of virus production lot purity and other properties on clearance studies.

In summary, the scope of this technical report is the definition of quality attributes that may be applied to virus and bacteriophage spike preparations as well as to cell lines used for virus propagation and sample testing. It provides neither a standard for production of virus spike preparations nor standards for quality attributes for particular viruses, but rather guiding principles that can be used to select and define appropriate quality attributes for the virus in question, with an emphasis on minimizing the impact of the virus spike on the scale-down model of the unit operation under validation and the virus clearance observed.