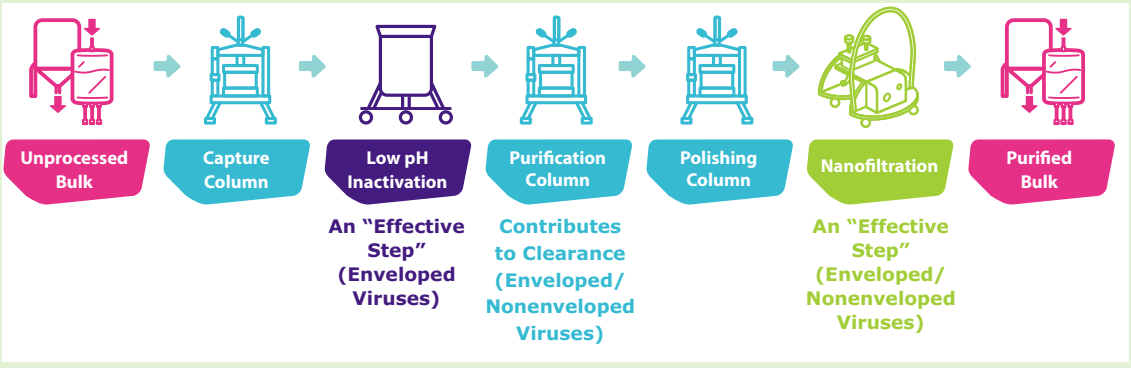
Figure 3: Typical monoclonal antibody purification process



Box 3: Regulatory Guidance Documents

ICH Q5A: *Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cells Lines of Human or Animal Origin,* CPMP/ICH/295/95

Guideline on Virus Safety *Evaluation of Biotechnological Investigational Medicinal Products,* EMEA/CHMP/BWP/398498

Note for Guidance on Viral Validation Studies: *The Design, Contribution, and Interpretation of Studies Validating the Inactivation and Removal of Viruses.* CPMP/BWP/268/95

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. FDA/CBER, 1997

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, FDA/CBER, 1993

that can be analyzed in that assay as a proportion of the total amount of material. For *in vitro* tests, particular cell lines can be more or less susceptible to infection by known or novel viruses. Some materials may interfere with the ability of an assay to detect viruses or cytotoxic to detector cells used in an assay. A negative test result does not indicate that an adventitious agent is absent, but rather it indicates that an adventitious agent is below the assay LOD. Another limitation of detection assays is that they generally are designed to detect specific virus pathogens, which means you often find only what you're looking for.

Testing for virus is performed on an MCB, cells at maximum use, and bulk harvest. Typically, a minimum of three lots of bulk harvest are tested for bioburden, mycoplasma, and viruses using an *in vitro* virus assay before clinical trials. Those assays are performed on each batch of bulk harvest as part of routine process quality control. For rodent cells, the amount of expressed retrovirus particles is determined by TEM or quantitative PCR assays.

The most recent European Pharmacopoeia chapter (EP 5.2.3) notes the availability of new sensitive molecular technologies with broad detection capabilities such as massively parallel sequencing (MPS), degenerate PCR for whole virus families, oligonucleotide arrays, and mass spectrometry (**8**). As mentioned above, these methods can be used either as an alternative to *in vivo* or specific NAT or as a supplement/ alternative to *in vitro* culture with approval from national regulators.

NGS sometimes is called high-throughput sequencing, deep sequencing, or MPS. NGS generates high millions or billions of sequencing reads from a nucleic acid library template. Those reads are then analyzed using powerful bioinformatics computing processing and compared with databases of known sequences. A critical component of NGS is computational analysis of data. Initially, poor-quality reads are eliminated from the analysis, and then a multistep basic local alignment search tool (BLAST) analysis is performed — where reads from different mammalian cell housekeeping genes act as controls to determine breadth and depth of sequence coverage. Sequences of expected components then are subtracted, enabling analysis of cell line samples to focus on any adventitious virus sequences.

An NGS interest group with members from industry and regulatory agencies is developing some practical guidance and standards for NGS and bioinformatics analysis. In addition, an ongoing study is evaluating the relative sensitivity of NGS testing for viral detection. The power of NGS may not be increased sensitivity but rather