technology has also been used for eukaryotic cell culture. To initiate culture, aliquots of the seed are first grown in a small volume, and the culture is incubated at increasingly larger volumes up to the volume of the final production-scale fermenter. Modern fermenters control and document environmental variables such as temperature, oxygen pressure, and pH enabling standardized production methods to be used.

Cell Culture
A master cell bank (MCB) consists of frozen vials of a cell line (generally kept in liquid nitrogen), manufactured at as low a passage number as reasonably possible. Documentation includes their origin and their passage histories, the number of passages since origination, storage, and cell culture conditions. Continuous cell lines are usually cloned from a single cell before an MCB is generated to assure purity of the cell line. The stock cultures in the MCB are used to generate the working cell bank (WCB), which is then extensively tested. Cell expansion can progress from flasks to 1 to 10 L spinner cultures and, for large-scale production, to 50 to 10,000 L bioreactor systems.

Harvest
The mode and method of harvest depends on the product being grown. For lytic viruses, the virus can be intracellular or lysed into the cell culture medium. For the latter, the cell culture supernatant is collected at different time points during production, sometimes over a few days. Perfusion systems where the spent culture supernatant is continuously harvested are popular.

Purification
After harvesting, the antigens are concentrated and purified using standard techniques such as centrifugation or ultrafiltration. Sometimes, no purification step is performed. For large-scale production systems, the virus can be concentrated using ultrafiltration, column chromatography, or gradient ultracentrifugation methods, followed by a sterile filtration step, which can be before or after inactivation.

Inactivation
Inactivation of purified antigens may be done chemically using reagents such as formaldehyde or peroxide, possibly in combination with heat. Inactivation parameters must be standardized and validated to ensure complete and consistent inactivation.

Formulation and Filling
The acceptable limits of variation for factors that impact consistency, such as the amount of antigen, stabilizers, adjuvants, pH, and volume, are ultimately related to the safety and efficacy demonstrated in clinical studies. The three steps of formulation, filling, and freeze-drying are the most critical steps of the entire production process regarding sterility. The reason is that most vaccines, especially those based on whole organisms or those incorporating adjuvants such as alum, cannot be terminally sterilized, in contrast to the majority of drugs and large-volume parenteral solutions. In addition, appropriate mixing of the vaccine must be ensured, in particular in the case of combination vaccines containing antigens against several different pathogens. The length of time of the filling process (e.g., over the course of several hours) must be validated. The definition of a lot, ''doses that are at the same risk of contamination,'' depends on the capacity of the filling process.

Packaging of a vaccine product may vary depending on the market. All packaging operations must also be under strict control, and the processes must be documented.

Testing
It is critical that biological materials used to generate products for human use are properly qualified. Qualification can include testing for identity, presence of any adventitious viruses, or microbial contamination. Safety assessments, such as lack of reversion for live attenuated vaccines or inactivation for killed vaccines, are also necessary and routine to demonstrate that the product itself remains safe. Furthermore, the amount of antigen is followed over the process to ensure efficacy. Testing is done at multiple steps in the production process. All tests must be standardized and validated.

Although the manufacturer has the responsibility for testing and quality assurance procedures on a lot-by-lot basis, all vaccine lots are subject to release by the relevant regulatory authority. In some cases, the release applies to bulk products, in others to the final container vaccine. In either case, time for lot release must be programmed into the manufacturer's planning process.

Storage and Shipping
Storage and shipping of vaccines are also subject to documentation and validation since heating or freezing can damage their integrity. The temperatures, storage, and shipping conditions must be such that the product is stable, and these conditions must be reproducible. Generally, vaccines are stored and shipped using a ''cold chain'' to maintain temperatures close to or below freezing, depending on the product. Manufacturers use temperature-monitoring devices to ensure that these temperatures are maintained. Real time data on the shipping process must be obtained to ensure product integrity.

GOOD MANUFACTURING PRACTICES
Basic Components of Good Manufacturing Practice
Compliance with the principles and guidelines of GMP is a statutory requirement applying to all pharmaceutical products, including vaccines. The GMP regulations govern those parts of quality assurance that ensure medicinal products are consistently produced and controlled to the quality standards appropriate to their intended use. Simply stated, production operations must follow clearly defined procedures. In addition, GMP requires evidence of prevention of cross-contamination in production; the performance of validation studies supporting the facilities, systems, processes, and equipment; control of starting materials; control of packaging materials; and handling of finished products. All aspects of these measures and methods of control are specifically defined by local regulations.

The term ''cGMP'' (current GMP) is part of a bigger system of quality assurance in the pharmaceutical industry. Quality assurance as a whole includes all subjects related to ensuring consistency of practice in all phases of a particular endeavor. Issues related to GMP need to be handled right from inception of the manufacturing process. It is the primary responsibility of the regulatory affairs/quality assurance department in a manufacturing facility to ensure that all manufacturing operations performed for production of human vaccines are in compliance with current GMP