



## The Challenge of Finding the Unknown

Advanced analytical methods are speeding up the targeted evaluation of potential viral contaminants.

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Unlike with blood and plasma for which possible viral contaminants are generally known, because the raw materials used in the manufacture of biologic drugs can come from animal, plant, and manmade sources, it is not possible to predict all potential viral contaminants. Analytical methods, therefore, must be able to detect both known and unknown viruses. Traditional cell-based assays are effective but have limitations, including an extensive assay procedure time. Newer nucleic acid-based methods are much more rapid, but those implemented to date are generally designed for specific viral targets. Both biopharmaceutical compa-

nies and FDA, however, believe that new sequencing techniques capable of identifying multiple viruses can be adopted by the industry in the future.

### NO SAFETY CONCERNS

First, it must be stressed that the interest in new viral detection methods does not stem from any issues or problem cases related to the safety of biotherapeutics. "Biotechnology drugs are very safe from a viral contamination standpoint. The international standard ICH Q5A, which was promulgated in 1998, assures

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viral safety by requiring both testing of cell banks, raw materials, and bioreactor harvests and viral clearance using downstream purification processes,” says Kurt Brorson, a staff scientist for monoclonal antibodies with the Center for Drug Evaluation and Research (CDER) at FDA.

Regardless of the analytical method, adds Ivar Kljavin, director of adventitious agent management with Genentech, because testing is trying to not only detect unknown viruses, but also do the impossible and prove a negative result, it is not sufficient by itself. “It is very important to realize that testing is crucial but only one part of an effective solution for viral contamination prevention. It is absolutely imperative that steps be taken to minimize the risk of viral infection, such as heat treatment, viral filtration, and the tracking and tracing of all raw materials to their original sources in order to be able to identify potential risks,” he asserts.

### **CURRENT METHODS EFFECTIVE BUT WITH LIMITATIONS**

The traditional test method is the *in-vitro* adventitious virus assay, which uses various indicator cell lines for detection of viruses for in-process or lot-release testing of the bioreactor at the end of the run, according to Dominick Vacante, scientific director for virology with Janssen Pharmaceutical R&D. “The assay is very sensitive and can potentially detect one infectious virus particle. The limitation is that the virus must replicate in at least one of the indicator cell types and produce some type of effect that is detected by the readouts of the assays, which are visual for cytopathic effects and/or hemadsorption or hemagglutination of specific red blood cells,” he

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explains. Problems do arise when a virus replicates but causes no signs of cytopathic effects, or is infectious but does not replicate in the cell types selected for the assay, which in both cases yields a false-negative result.

In some cases, the test article may interfere with the ability of a virus to infect the cells or display signs of infection, according to Kljavin. Cell-based methods can also be variable; a virus that is detected in one assay may not be found when the test is repeated. “Equally importantly, it takes time for the viruses to replicate and grow, which leads to a very long test time of 14–28 days. A significant amount of product can be produced and sent downstream during that time, and if found to be infected must be disposed. The entire production facility, not just cell-culture areas, must be decontaminated, which can lead to a disruption in the supply of the drug to patients, a situation that is unacceptable,” Kljavin states.

He again stresses, though, that cell-based assays are effective when performed in conjunction with other risk-mitigating steps like viral clearance validation. The current issues revolve around development of new assays to test for viruses at the various sampling points that are more rapid, more sensitive, and broader. Gaining a greater understanding of how

purification unit operations clear viruses, how robust they are, and how to best validate the clearance of different viruses are other measures that mitigate gaps in testing, according to Brorson.

### **EVOLVING TECHNOLOGY**

While ICH Q5A serves the industry and regulators well, Brorson notes that technology has evolved since 1998, leading to the development of different approaches, such as modular validation for robust unit operations and the introduction of new assays for cell-line characterization, like genome sequencing-based methods. “ICH Q5A is silent on these new technologies since it was written before their introduction, and could stand to be updated with developments of the past 15 years,” he says. FDA has been following new pan virus detection methods/deep-sequencing methods for cell-line characterization, which are at the current time, according to Brorson, great for investigations and broad surveys of cell lines or raw materials. “What isn’t clear yet, though, is whether they have reached a state where they can be used in a routine QC setting.” FDA expects, however, that the situation could change over the next dozen years or so.

### **NUCLEIC ACID-BASED METHODS**

More simple and specific polymerase chain reaction (PCR) methods are currently used by many biopharmaceutical manufacturers. Each PCR test accurately detects a DNA sequence from a specific virus and is completed rapidly compared to cell-based assays (e.g., within one day vs. three to four weeks). Presently, however, PCR is not applicable for the detection of multiple viruses at once. Massive parallel sequencing, or deep

sequencing, on the other hand, is attracting a lot of interest because it can be used to detect multiple DNA sequences from different viruses.

In addition to deep sequencing, PCR combined with mass spectrometry (MS) and microarrays are considered advanced technologies for virus detection. PCR with degenerate probes may also be included as an advancement of PCR, according to Vacante. "These technologies may improve virus detection by enabling the detection of a broad range of viruses, either in an unbiased manner as with deep sequencing or through the detection of consensus sequences of many or all viruses in a virus family. The assays also by their nature provide information on the virus detected and taxonomy, including the virus family, subfamily, and genus, which may be helpful for quickly determining where a contamination may have originated," Vacante observes.

It is important to remember, however, that with these methods, only viral DNA sequences are detected, and not actual live particles, according to Kljavin. "These tests do not indicate if any live virus is present, only that a part of the DNA of the virus is present. The challenge then becomes how to respond if a positive result is obtained," he explains. It is necessary to work backward and run other tests to determine if there is an actual infection. "If no infection is found, then a decision must be made regarding how to proceed. Both industry and FDA will have to figure out how to go forward in such a situation," Kljavin adds.

### REAL ADVANTAGES

Both Genentech and Janssen have employed these nucleic-acid techniques when dealing with possible viral contamination. In 1993 and 1994, Genentech experienced contamination by the rodent parvovi-

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rus, or minute virus of mice (MVM). In 1993, the infection was detected using cell-based assays, but only after production continued for three to four weeks, thus the facility was contaminated and significant clean-up and production delays resulted. By 1994, a PCR method was developed, and the virus was detected before harvest from the bioreactor. "In this latter case, production was halted before further downstream processing progressed, and decontamination efforts were limited to the impacted bioreactor, and other normal operations in the facility continued," notes Kljavin.

A few years ago at Janssen, in response to a suspected viral contamination, deep sequencing, and DNA amplification followed by MS, previously referred to as TIGER and now termed PLEX-ID, was used to show that there was no contamination, according to Vacante. "Analysis of the effect observed in cell culture and extensive screening using the advanced tests provided convincing evidence for the lack of a viral contamination or other biological agent," Vacante adds. Using established standard operating procedures, the impacted lots were then retested and found to be actually negative. The initial test result was then assigned "false-positive status," and the product was ultimately released to the market (1).

### INDUSTRY AND AGENCY ACTIVITIES

To help evaluate the current status of analytical technology with this goal in mind, a task force of members of the Parenteral Drug Association (PDA) that includes industry, government, and academia are preparing a white paper on emerging methods for virus detection. "This white paper describes both conventional methods and new technologies, including the benefits and limitations of each and their application to biologic drugs," Vacante says. He also notes that a PDA interest group was formed to exchange ideas and practices, evaluate these technologies, and possibly develop best practices and standards or standardized approaches for comparison of methods across different laboratories. FDA has been involved with both the white paper and the interest group, and Arifa Khan at the Center for Biologics Evaluation and Research has provided leadership regarding the evaluation of these new technologies for virus detection, according to Vacante.

CDER has also been involved with industry and other players in directed research on standardization and improved methods for validating viral clearance unit operations. "For example, CDER performed crucial lab work that contributed to the development of the first ever standard nomenclature for virus retentive filters promulgated in PDA's TR41, Virus Retentive Filters. We also laid part of the groundwork for ASTM E2888 Standard Practice for Process for Inactivation of Rodent Retrovirus by pH, and participated in the committee writing the standard. A challenge for industry is to implement these new standards, assays, and validation approaches," observes Brorson.

### REFERENCE

1. L.C. Hendricks et al., *PDA J. Pharm. Sci. and Tech.*, 64(5), 471-480 (2010). **BP**