A Q&A

Quantifying Viruses? Here's What Matters Most

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With viruses playing a greater role in everything from vaccine development to gene therapy, ensuring their rapid, accurate, and biologically relevant enumeration isn't just an analytical "plus"; it's the first step in the development of biological tools that support human health.

The catch? Traditional virus-quantification methods haven't always cleared the high bar that contemporary needs set. That began to change a few years back when a startup in Boulder, CO, designed a new platform for rapidly enumerating total virus particles using reagent technologies that are both biologically relevant and biologically specific.

Seeing the potential in this emerging technology—called the Virus Counter platform—Sartorius Stedim Biotech acquired it in 2016 and made it even better, engineering it into a robust commercial platform. We sat down with Antje Schickert, product manager for virus analytics at Sartorius Stedim Biotech, to learn how the Virus Counter makes even the trickiest virus enumerations…well, *count*.

BioPharm International: Which industries have a need for virus quantification?

Schickert: Virus enumeration plays a critical role in vaccine development and production, viral vector manufacturing, and many other processes where the viruses are often the final product. The increasing demand for viral vectors, requires more efficient manufacturing processes to keep pace with the needed quantities of viruses.

Optimization of those manufacturing processes commands a very detailed understanding of how the concentration, and also the quality, of the product—which, in this case, is a virus—is influenced by different parameters such as different growth conditions or purification steps.

In applications such as gene therapy, viruses are often directly administered to patients as a therapeutic, and in this case, it's really vital to characterize these viral therapies in detail to ensure the safety of the patient. Enumeration and a very good understanding of the composition of these products are part of that in-depth characterization.

In the past, the availability of quick and reliable quantification methods often failed to effectively provide these important insights.

BioPharm International: How did these industries traditionally enumerate viruses?

Schickert: Traditional methods of virus enumeration include plaque titer assays or $TCID_{50}$. These are often referred to as functional assays, but they present a drawback in the delay they impose because of the labor-intensive nature of the techniques. Results often aren't available for days or weeks because we have to wait until the viruses replicate in the cell to actually quantify them. That can slow or pause manufacturing processes, or provide results that might not be as relevant as when the sample was taken.

Many of these traditional methods are also inherently variable, and that can decrease confidence in the results that they deliver. So, these methods really cannot keep pace with the modern requirements of cell and gene therapies.

BioPharm International: What are some of the more modern approaches that are used to prevent the delays caused by traditional assays?

Schickert: To address the delays that functional assays cause, methods such as PCR and ELISA were more recently introduced into the virus quantification field. These methods are more rapid than more traditional functional assays, but they often rely upon measurements of viral components only such as viral proteins or nucleic acids; then, they calculate the titer from these measurements. That means that results can potentially be biased, as they're derived values rather than values based upon direct measurements of intact viruses.

BioPharm International: How does the Virus Counter address these gaps in the field of virus quantification?

Schickert: The Virus Counter platform was designed to measure virus titers directly and with high speed and precision. The readout is biologically relevant because we look at total viral particles that are directly measured using specific binding reagents. That can have a significant impact on, for example, patient safety.

Sartorius offers different reagents to address diverse customer needs. We have a more universal quantification reagent that has very broad utility and can be used with a large variety of different viruses, but we also offer an antibody-based reagent line that allows virus detection with high affinity and specificity.

We also emphasize the ease of use of our instrument. From system operation to data analysis, everything is software assisted, and that means we limit the risk of any kind of user error or variability.

BioPharm International: Can you describe how the Virus Counter platform works?

Schickert: The Virus Counter platform was purpose-built to enumerate viruses. As mentioned, the platform includes an instrument, software, and the reagents we're using to detect viruses. Viruses are directly labeled with either fluorescent dyes or antibody-based reagents. Inside the instrument, the virus sample is guided in a fluidic stream and focused to a small core of moving fluid. A laser is then used to excite the fluorophores that are directly associated with the viruses as they pass the laser. The Virus Counter instrument then detects the emitted light and uses it, together with the sample flow rate, to calculate total particle concentration in the virus sample.

BioPharm International: What is the significance of counting total virus particles in a direct manner?

Schickert: Virus samples can be incredibly heterogeneous mixes of infectious viruses, nonfunctional particles, and unassociated nucleic acids and proteins that weren't assembled into virus particles.

Diverse virus quantification methods actually quantify different sub-fractions of the virus sample. For example, plaque assays only enumerate functional particles. Depending on the virus type and the manufacturing process, however, functional particles can actually be a very small fraction of total particles within the sample. ELISA assays usually quantify viral proteins in the sample, and then use that data to calculate a virus titer. PCR quantifies virus-specific nucleic acid sequences in the sample and derives a titer value from that measurement.

So, the heterogeneity of the sample and the nature of these quantification assays are responsible for the diverse results that we obtain with different quantification methods when we measure the same virus sample.

The Virus Counter directly quantifies all intact virus particles by measuring a fluorescent signal—so the readout here is total particles, and it's really critical for the in-depth understanding of the virus sample composition and for the safety of the patient.

BioPharm International: Where do you see the advantages of the Virus Counter technology over existing enumeration assays?

Schickert: The Virus Counter platform enables users to measure virus samples in near real time and with very high precision, and that makes Virus Counter results actionable. The technology empowers our users to increase viral vector yields by comparing, for example, different growth conditions and recovery during process development. Users can also track virus titers throughout their process and determine ideal conditions and even detect possible challenges early on in their process. Additionally, the Virus Counter platform increases safety and efficiency by facilitating the in-depth characterization of virus samples and increases the understanding of the total particle concentration in the final product.