Why Viral Particle Quantitation Matters

There are a variety of approaches for determining viral titer; many rely on measuring infectivity, genome copy number, or the amount of antigen present in a sample. However, these approaches do not directly quantify viral particles and may not recognize defective or noninfectious particles. Growing evidence demonstrates that the number of noninfectious viral particles in a sample is of significant biological importance and can impact both in vitro and in vivo studies. Now, rapid quantitation of the total number of viral particles in a sample is possible using the innovative ViroCyt® Virus Counter® platform.

Background: Biology and Biological Consequences of Noninfective Viral Particles

Although viral particles may be noninfective for numerous biological reasons, defects in the viral replication cycle are often the cause. For example, viral capsids that lack genomes may be produced during the packaging phase, leading to empty particles that remain immunogenic but are noninfective. Defective or mutated genomes may also be packaged into viral particles, resulting in a particle that can infect cells and drive immune responses without producing infectious virions.

In the case of defective interfering particles (DIPs) first identified in influenza preparations, very large portions of the viral genome were often missing or duplicated, leading to replication-deficient populations.⁵⁻⁷ DIPS have also been documented with other virus types, including Dengue viral infections, where they appear to play a role in natural biological attenuation.⁵ Or in HIV, where genome replication errors due to the reverse transcription process cause the formation of DIPs which actively contribute to infection through priming of CD4+ T lymphocytes.⁶ These defective particles still play a role in the host response to infection and the eventual outcome of infection, in that populations with high levels of defective particles often result in attenuated infections due to reduced population fitness in the host.⁷ These observations have consequences for all applications that utilize viruses in an industrial process, including vaccine manufacture, bioprocessing, and gene therapy.⁸
Painting a Complete Picture of Viral Cultures

The literature makes it clear that noninfectious viral particles are of far more biological interest than the inert errors they were once thought to be. It has been known for decades that the so-called particle to plaque-forming unit (PFU) ratios for many types of virus can be quite large and may show considerable variability, suggesting that parallel viral cultures with differing particle-to-PFU ratios may behave quite differently. For example, varicella zoster virus has been shown to have a ratio of 40,000:1, while other species such as Sindbis virus have a particle-to-PFU ratio approaching 1:1 where nearly all viral particles are infective. In addition to their effect on biological systems, monitoring noninfectious particle numbers can be important for numerous other applications. During production of the seasonal flu vaccine, influenza is grown, then purified from chicken eggs. Following purification, the virus particles are split apart using a specialized reagent, and the immunogenic HA proteins are harvested from this solution. Noninfectious particles will also contribute the immunogenic HA protein after being split. It is therefore essential during this process to have a rapid method for accurately measuring total particle concentrations in conjunction with traditional methods of assaying for infectivity.

Other types of vaccine production can also benefit from total particle quantitation. Attenuated vaccines use a replication-deficient version of a virus to cause an immune response, but with little to no viral infection. Since these attenuated viruses do not replicate, they will not cause the cytopathic effect on which most infectivity assays base their detection. In addition, newer approaches, such as virus-like particles (VLPs), produce immunogenic particles which are nonlytic and often do not contain nucleic acids, making them incompatible with assays such as 50% tissue culture infective dose (TCID<sub>50</sub>) and qPCR. Given the extensive biological role of these noninfective particles and their impact on the development and manufacture of viral vaccines, infective titers and total particle numbers are both essential for accurate characterization of a viral sample.

ViroCyt Virus Counter Case Study: Vaccine Production—Tracking and Optimizing Yield Throughout; the Manufacturing Process

There are many points during the process of developing, optimizing, and producing vaccines that would benefit from rapid enumeration of total viral particles. One of the most significant is tracking efficiency following harvest from egg- and cell-based systems. The long and complex steps of taking crude material and transforming it into a product ready for patients results in substantial loss of material. The ability to track essentially in real time the quantity of virus at beginning and end of each distinct stage will identify where losses are occurring, and allow for process improvements to be made. Even small gains in efficiency at each step would lead to considerable financial benefits.

There are multiple methods which allow for infectious particle assessments to be made. Until recently, options for noninfectious or total particle counting were limited primarily to visualization via transmission electron microscopy (TEM). Due to the high level of technical expertise required to conduct these measurements as well as the need for sophisticated and costly equipment, this technique has proven impractical for industrial scale applications. To address the need for viral researchers to be able to accurately, reliably, and easily quantify total viral particle count, the ViroCyt Virus Counter was developed.
The ViroCyt Virus Counter

The ViroCyt Virus Counter is a detection system which has been optimized to quantify nanoparticles, such as viruses. It detects viral particles which have been labeled with fluorescent stains. Two fluorescent reagents allow for flexibility in sample detection depending on the virus type and buffer composition.

The ComboDye® Reagent Kit universally labels enveloped viruses using a proprietary two-component dye that marks viral protein and nucleic acids, thereby identifying intact viral particles that contain genomic content. The ViroTag® Antibody-Based Detection System is specific for a single viral species and is effective at labeling both enveloped and nonenveloped viral particles. The ViroTag Antibody-Based Detection System labels the particles and gives a total particle count, including particles lacking nucleic acid.

Depending upon the virus of interest and sample composition, one or both approaches may be suitable for sample quantitation.

Comparing Infectious and Total Particle Counts

To compare infectious titers with total particle count, samples of influenza H1N1, cytomegalovirus (CMV), respiratory syncytial virus (RSV), and rubella were measured by TCID$_{50}$ assay or plaque titer, ViroCyt Virus Counter instrument, and quantitative TEM. As shown, total particle counts determined by either TEM or the ViroCyt Virus Counter were statistically identical, while titer by TCID$_{50}$ measured a fraction of the total particles, with counts ranging from 2–3.5 orders of magnitude lower than TEM or ViroCyt Virus Counter values. These results highlight the relative abundance of noninfective particles as a percentage of the total population across multiple virus types.

ViroCyt Virus Counter Case Study: Gene Therapy Studies—Accurate Determination of Vector Dosage

Many new human gene therapies are delivered to target cells by way of a viral vector. However, the amount of virus used is often calculated solely on infectivity- or transducing-units-based assays; and as has been discussed, noninfective particles can often have either a positive or negative impact in the immune response and the ultimate effectiveness of the agent. For example, if the infective titer is determined by plaque assay to be 1E6 pfu/ml but the total intact viral particle count is established to be 1E8 vp/ml, for every one infective particle there are 100 particles that are not counted as infective, yet may be influencing the experimental outcome.

This is a critical component of ensuring safety of gene therapy products, as particle number is a key determinant of immunogenicity. Indeed, after the failed gene therapy trials utilizing adenovirus in the 1990’s, the FDA now states that “given the potential toxicity of the adenoviral particles themselves, CBER recommends that patient dosing be based on particle number.” By tracking each of these properties for different lots of virus, dates, and other variables, a clear and accurate picture of the relative contribution of each variant is possible. Therefore, the ViroCyt Virus Counter allows for the rapid quantitation of total particle count to facilitate the development of viral vectors for gene therapy applications.
Conclusion

Using laser excitation, intact viral particles are identified by their fluorescent signals (single signal for the ViroTag Antibody-Based Detection System, simultaneous signals of both nucleic and protein stains for the ComboDye Reagent Kit). Implementation of the ViroCyt Virus Counter significantly reduces the time required to titer a viral sample when compared to traditional methods such as qPCR and TCID_{50}. Instead of hours or even days to titer a viral sample, the ViroCyt Virus Counter makes it possible to titer a viral sample within approximately 30 minutes of sample staining and 3 minutes of instrument read time per test. This no-wash staining protocol limits the cost and lessens the technical expertise and time required to obtain results as compared to classical methods.

References


