病毒颗粒计数的意义

我们知道噬斑法和 TCID50 法检测的是感染性病毒的浓度，并不体现病毒的总浓度（总颗粒数）。现在有越来越多的研究显示，很多病毒在包装过程中由于缺失基因组，形成空心病毒。或者在合成过程中，基因的突变或者缺陷导致蛋白的突变，造成病毒没有感染性。而这些非感染性病毒的数量在总病毒数量中所占的比例意义重大，能影响体内和体外的研究结果。所以快速的病毒总浓度定量方法是非常必要的。

流感疫苗的生产中，从鸡胚培养来源的流感疫苗在使用专门的试剂裂解后，收获免疫原蛋白 HA。非传染性的流感病毒（被证实含有衣壳蛋白和部分的基因组）在裂解后同样能贡献免疫原蛋白 HA。所以总病毒浓度的评估对流感疫苗的生产意义重大。

此外，总病毒浓度的定量也有助于减毒疫苗的生产。减毒疫苗是复制缺陷的非感染性的但是能引发机体免疫反应的一种疫苗。减毒疫苗既然不能复制，所以就不会引起细胞病变反应，而 CCID50 法则以细胞病变反应为判断基准。在某种意义上来说，所有的减毒疫苗是由非感染的病毒颗粒构成，因此，采用总病毒浓度定量的方法是减毒疫苗唯一可靠的方法。

在动物疾病的预防和治疗中，采用感染性方法测得病毒滴度来决定注射的剂量，往往忽视了非感染性病毒颗粒在动物免疫反应中的作用以及最终的药剂效果。比如噬斑法测得病毒滴度为 1E6 pfu,但是总病毒浓度是 1E8 vp/ml，在每一个感染颗粒中，有 100 个颗粒没有被计数，最终可能影响动物治疗结果。

考虑到非感染性病毒颗粒的生物学作用以及在疫苗生产的作用，感染性病毒颗粒和总病毒颗粒的定量对于病毒疫苗的生产和研究都至关重要，而病毒计数仪在 10min 内能快速获得病毒总浓度，所以能广泛应用于在病毒的研究和生产中。

WHY VIRAL PARTICLE QUANTIFICATION MATTERS

As was discussed in our previous White Paper “An Overview of Virus Quantification Techniques,” there are a variety of approaches for determining viral titer. Many rely on measuring infectivity or the amount of antigen, but do not enumerate viral particles. There is growing evidence, however, that the number of non-infectious viral particles is of significant biological importance and can impact both in vitro and in vivo studies. This mounting data suggests a need for rapid quantification of the total number of viral particles in a virus containing sample, which is now possible using the innovative ViroCyt® Virus Counter® 2100.

Background: The Biology and Biological Consequences of Non-Infective Viral Particles Although viral particles may be non-infective for a number of biological reasons, defective viral replication is often the cause.

For example, viral capsids which lack genomes, may be produced during the packaging phase, leading to empty particles.Mutations or defects in viral genomes also result in the production of viral particles which are incapable of supporting full replicative cycles. These include relatively minor mutations in key genes controlling the viral life cycle or much larger-scale defects.

In the case of so-called “defective interfering particles” (DIPs) discovered in influenza, very large portions of the viral genome are often missing1-4. A full replicative cycle is possible only if the DIP particles are in the presence of replication-competent, co-infecting viral particles. In such a case, DIPs may “piggyback” competent particle replication offsetting their defects, and as a result, DIPs compete for resources against replicative-competent particles and have even been shown to protect against lethal infections5. In addition to providing potential competition for critical resources, it has been more recently documented that DIPs affect the severity of infection through modulation of host immune response6. As a result of increasing amount of research into

non-infectious influenza particles, other classes of these particles have been discovered. Noninfectious cell-killing particles (niCKP) found in influenza cultures7, interferon-inducing particles (IFPs)8, and interferon induction-suppressing particles (ISPs)9 all play significant biological roles without causing viral infection. The observation that these non-infectious particle types actually make up the majority of particles in active influenza infections9, raises the question of whether these particles should be ignored. DIPS have also been documented in other virus types. In Dengue viral infections, they appear to play a role in natural biological attenuation10. In HIV, genome replication errors due to the reverse transcription process cause the formation of DIPs which actively contribute to infection through “priming” of CD4+ T lymphocytes11. In addition to their effect on biological systems, monitoring non-infectious particle numbers can be important for other applications as well. 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. Non-infectious particles that are known to have a protein capsid and a partial genome 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. Other types of vaccine production can also benefit from total particle quantification. 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 that most infectivity assays base their detection on. In a sense, all attenuated vaccines consist of non-infectious virus particles, and thus, the only methods to reliably quantify them are total particle quantification methods. Given the extensive biological role of these non-infective particles, as well as their impact on the development and manufacture of viral vaccines, infective titersand total particle numbers are both essential for accurate viral characterization.

The literature makes it clear that non-infectious 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-PFU” ratios for many types of virus can be quite large and may show considerable variability12, suggesting that parallel viral cultures with differing particle-to-PFU ratios may behave quite differently. Some viruses are known to have extremely high particle to PFU ratios. For example, varicella zoster virus has been shown to have a ratio of 40,000:113, while others – such as bacteriophages – have a particle to PFU ratio approaching one, meaning all viral particles are infective. As critical regulators of viral infection and of the immune system, non-infectious viral particles are a natural and necessary component of viral cultures, and complete characterization of viral cultures require that both infectious and non-infectious particles be quantified. Although, there are multiple methods which allow for infectious particle assessments to be made, until recently, options for non-infectious or total particle counting were limited primarily to visualization via transmission electron microscopy (TEM). However, 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 many.

To address the need for viral researchers to be able to accurately, reliably and easily quantify total viral particle count, the Virus Counter® 2100 was developed. The Virus Counter relies on fluorescent staining of surface proteins and nucleic acids followed by detection of fluorescent

signals using a specialized flow cytometer. Using laser excitation, intact viral particles are identified by coincidental protein and nucleic acid signals.

The Virus Counter® 2100, a Tool for "Universal Normalization" To truly normalize viral cultures, there is a clear need for both total and infectious particle numbers to be known. Due to the complex interactions and partially-understood relationships between infectious and non-infectious particles in active viral cultures, accurate normalization requires that both infectious and non-infectious viral particles (which may be deduced from total particle counts) be set at consistent levels. In the past, determination of particle-to-PFU ratios was often difficult and sometimes impractical,since the few methods that existed for quantifying total particle counts were both costly and time-consuming, requiring sophisticated, expensive and highly technical equipment. By contrast, implementation of the Virus Counter 2100 reduces the time required to roughly 30 minutes of sample staining and 5-10 minutes of instrument read time, limiting the cost, and lessening the technical expertise required to obtain results.

Use Scenario: Animal Studies – Accurate Determination of Viral Dosage

Viral challenge in the appropriate animal model is an important tool in the development of vaccines and therapies for the prevention and treatment of many diseases. However, the amount of virus used is often calculated solely based on infectivity-based assays and, as has been discussed, non-infective 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, but the total intact viral particle count is established to be 1E8 vp/ml, for every 1 infective particle, there are 100 particles that are not counted as infective, but may be influencing the experimental outcome, nonetheless. 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.

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 TCID50 assay or plaque titer, Virus Counter 2100 instrument and quantitative TEM. As shown, total particle counts determined by either TEM or the Virus Counter were statistically identical, while titer by TCID50 measured a fraction of the total particles, with counts ranging from 2-3.5 orders of magnitude lower than TEM or Virus Counter 2100 values. These results highlight the relative abundance of non-infective particles as a percentage of the total population across multiple virus types.

Use Scenario: Vaccine Production – Tracking and Optimizing Yield Throughout the Manufacturing Process

Although, there are many points during the process of developing, optimizing and producing vaccines that would benefit from rapid enumeration of viral particles, one of the most significant is tracking efficiency following harvest from egg- and cell-based systems. More often than not, 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 improvements to be made. Even small gains in efficiency at each step would lead to considerable financial benefits.

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