Simplifying Progress

Rapid Quantification of Adeno-Associated Virus (AAV) and Lentiviral Vectors with the Virus Counter® Platform: Near-Real-Time Monitoring of Viral Vector Production

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Abstract

Adeno-Associated Virus (AAV) and Lentivirus are commonly used vectors in modern gene and cell therapies. History has shown that in-depth sample characterization and precise enumeration of total viral vectors in final formulations are critical for the safety of these promising new therapies. Real-time monitoring of complex viral vector manufacturing processes is needed to address the rapidly-growing demand for viral vectors. The Virus Counter® platform offers an emerging method to quantify total intact particles for AAV and Lentiviral vectors with high speed and precision. The Virus Counter® platform achieves this by utilizing antibody-based reagents (Virotag AB), Virotag® AAV2-3 and Virotag® VSVG, to measure virus titers with high specificity for their respective targets. Using a patented no-wash assay, biologically relevant total particle counts can be obtained in three minutes following a 30 minute incubation. Virotag® AAV 2-3 demonstrated rapid and precise enumeration of AAV2 and AAV3 viruses. Virotag® VSVG is capable of detecting the VSV-G -pseudotype in Lentivirus and
BacMam expression vectors. The results of the Virus Counter® method for total particle quantification was compared to other enumeration methods. Many quantification methods rely on the enumeration of a single virus building block to calculate virus titer. This can lead to overestimated virus concentrations. Virus Counter® assay results tend to be less variable and exhibit little to no statistically significant cross-reactivity against related viruses. The Virus Counter® Platform, a rapid and dependable method for virus quantification, can accelerate process development and increase the efficiency of manufacturing potent viral vectors.

Introduction

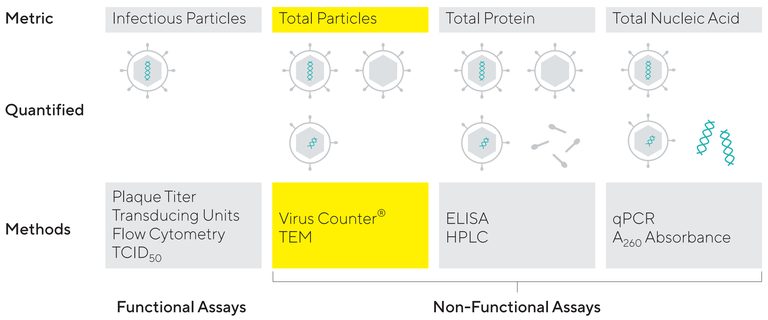

Figure 1: Virus quantification methods measure different subpopulations or viral building blocks to determine viral titers. The icons shown in the quantified population box refer to intact viral particles, empty particles, defective particles, free protein and unassociated nucleic acid. The quantified subpopulation box summarizes which fractions different methods measure in their titer determination. This explains the divergence in titers measured for a single virus sample with different methology.

The Virus Counter® instrument detects and measures viral particles in a fluid stream.

Integrated software directs instrument function and enables data analysis.

Virotag® AB and Virotag® DY assay kits label virus particles using direct, no-wash assays.

Figure 2: The Virus Counter® platform offers an integrated solution to measure virus titers specifically and in near-real-time.

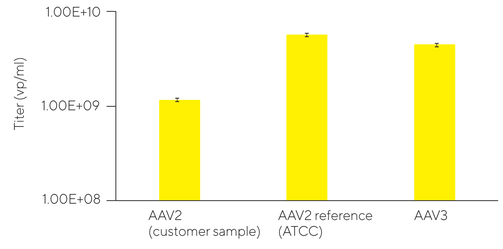

Figure 3: Virotag® AAV2-3 reagent quantifies AAV2 and AAV3 virus samples with high speed and precision. Final titers (triplicate measurements) were 1.16 × 10⁹ vp/ml (1.33 % c.v.) for AAV2 (customer sample), 5.61 x 10⁹ vp/ml (2.79 % c.v.) for AAV2 reference material (ATCC) and 4.39 × 10⁹ vp/ml (1.90 % c.v.) for AAV3 sample.

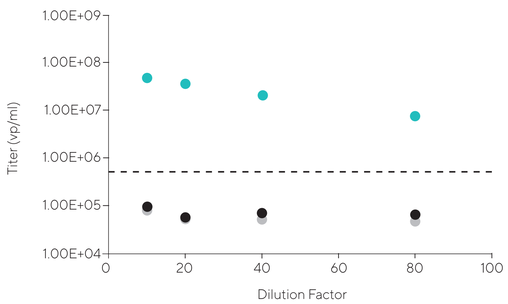

Figure 4: Virotag® AAV2-3 reagent is highly specific for AAV serotypes 2 and 3. The Virotag® reagent binds specifically to an epitope on these viruses, enabling quantification, while excluding other viruses (Baculovirus and Adenovirus data shown).

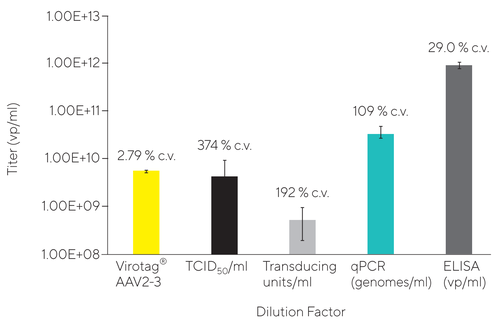

Figure 5: Comparison of AAV2 reference strain (ATCC) quantification with different methods. These results emphasize the importance of understanding which subpopulations or viral building blocks within a sample are enumerated.
Virotag® reagents quantify total viral particles with high precision while other methods measure infectious units ($TCID_{50}$ and TU) or virus building blocks (qPCR, ELISA) to calculate the total virus concentration of a sample. Data from Lock M et. al.

Results

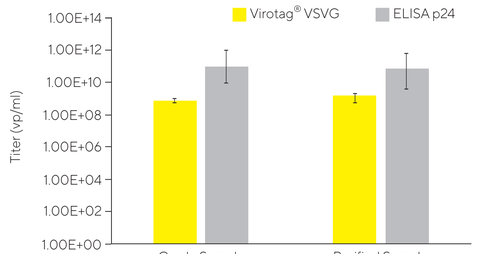

Figure 6: Virotag® VSVG reagent and ELISA assay were used to quantify crude and purified Lentivirus samples. Virotag® reagent shows high precision in enumerating samples. Both lentivirus samples were measured with Virotag® VSVG in triplicate, resulting in average titers of 1.00 × 10⁸ vp/ml (9 % c.v.) for the crude sample and 1.49 × 10⁹ vp/ml (14 % c.v.) for the purified sample. ELISA results for both Lentivirus samples had >50 % c.v.

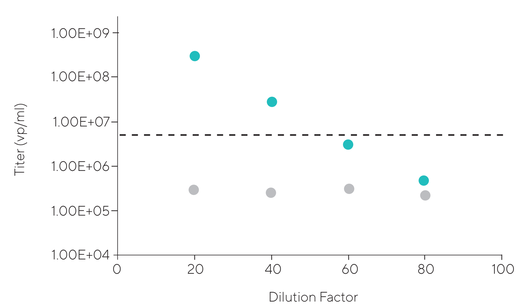

Figure 7: Virotag® VSVG reagent is highly specific VSV-G epitope. Only BacMam particles are recognized and quantified while wildtype Baculovirus, that does not express the VSV-G epitope, is excluded. BacMam is specifically recognized and measured, titers are linearly correlated with dilution factor.

Discussion & Conclusion

Traditional quantification methods such as $TCID_{50}$ and ELISA can be time-consuming and variable, while also yielding indirect data in regard to total particle counts. As an emerging state-of-the art the Virus Counter® Platform provides unique insight into the levels of non-infective particles in virus preparations.

The use of Virotag® reagents AAV2-3 and VSVG with the Virus Counter® instrument provides more precise uantification of total particles. Both reagents exhibit high specificity for their respective targets. Virotag® VSVG reagent demonstrates that VSV-G-expressing BacMam particles can be detected, while excluding native Baculovirus and other negative controls. Virotag® reagents demonstrate lower counts in comparison with ELISA and qPCR methods, suggesting overestimation of total particle counts by these latter approaches . This is likely due to recognition of unassociated proteins, virus fragments and free nucleic acid in the assay.

Antibody-based quantification delivers biologically relevant and highly specific read-out of total particles concentration in a viral sample.

Citations: Lock, M. et al, Rapid, Simple, and Versatile Manufacturing of Recombinant Adeno-Associated Viral Vectors at Scale, Human Gene Therapy (2010)