sartorius stedim biotech

Virus Counter® Reagents

Rapid, Direct, Biologically Relevant Virus Quantification

turning science into solutions

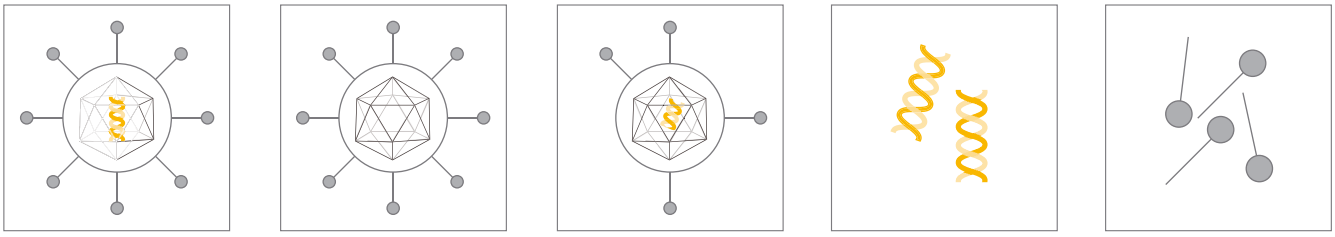
Historical Challenges with Virus Quantification

Traditional methods of virus enumeration, including $TCID_{50}$ or plaque titer assays, quantify infectious particles or units. These methods require considerable time and are burdened with high variability. Other methods for virus quantification, like qPCR and ELISA, quantify a particular component of the virus of interest (nucleic acid or viral protein, respectively) and result in derived titers versus direct quantification.

Quantification of a single virus sample with multiple methods often demontsrates the high degree of variability among methods. This is a result of the inherent heterogeneity of virus samples, which can contain a mixture of infectious particles, empty particles, defective particles, or unassosciated nucleic acid and proteins that derive from unassembled virus and cell components. Different quantification methods may enumerate a subpopulation or component within the sample, explaining the disparity among the results.

ELISA assays enumerate the total protein content in the sample and qPCR delivers a readout of total nucleic acid. These techniques can result in a falsely elevated result by the amplification of viral components, rather than intact virus, within the sample. While these methods are generally less time consuming and more reproducible than traditional functional assays, they may fail in effectively relating their readout to total virus counts. In contrast, functional assays quantify the subpopulation of viruses that can successfully infect cells but they cannot be used to investigate non-infectious particles in the sample. These non-infectious particles may be large portion of the total virus popluation, with a potential impact on the safety of virus-derived products and treatments.

Heterogeneity within Virus Samples



Infective Particle | Empty Particle | Defective Particle | Unassociated Nucleic Acid | Unassociated Protein

The Virus Counter® 3100 Platform

The Virus Counter® 3100 platform is a powerful analytical tool that provides real-time virus counts and can be used throughout virus growth and purification processes. This approach allows differentiation among unassociated proteins and nucleic acid in the sample, as well as discrete particle types, providing a clear advantage of this technology over quantification approaches that only measure one subcomponent of the virus.

The platform enables users to identify optimal harvesting times, validate processing steps, and define final formulations with speed and confidence. The Virus Counter® platform features multiple detection methods, offering the right solution for individual quantification needs.

Speed – Real-time quantification for process monitoring and production optimization

Biologically-relevant readout – Total viral particle quantification

Versatility – High-specificity and universal quantification reagents

Ease of use – software-assisted system operation and data analysis (including 21 CFR Part 11 capabilities)

Reliability – Rugged design for industrial settings, easy system maintenance

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

Integrated software directs instrument function and enables data analysis.

ViroTag® Assay and Combo Dye® Assay Kits label virus particles using direct, no-wash assays.

The Virus Counter® Platform offers Real-Time Insights into Virus Titers

- Assists in optimization of each processing step.
- Allows increased viral vector yield by comparing growth conditions and recovery during process development.
- Enhances protein expression yields and shortened timelines by harvesting with pinpoint precision.
- Provides early identification of problems during manufacturing, minimizing time and cost impacts.
- Increases safety and efficiency by facilitating in-depth characterization of total particle concentration in a final product.

Total Particle Quantification Matters

Non-infectious particles can represent a large portion of a virus sample (Figure 1). For this reason, enumerating infectious particle counts alone is not sufficient for in-depth virus sample characterization.

Severe setbacks in early gene therapy clinical trials are illustrative of the importance of total particle quantification. These events induced the FDA to issue new guidelines to minimize the risk of adverse outcomes when using viral vectors: "Given the potential toxicity of the adenoviral particles themselves, CBER recommends that patient dosing be based on particle number" (Guidance for Human Somatic Cell Therapy & Gene Therapy, FDA Centers for Biologics Evaluation and Research, 1998).

In-depth sample characterization of viral vectors is essential to ensure therapy is safe and efficient. This depends not only on the number of infectious particles, but, also on an understanding of potentially adverse side-effects that may be caused by the total viral particles in a dose. Non-infectious particles can represent a large portion of a virus sample, underlining the fact that infectious particle counts alone are not sufficient for thorough virus sample characterization. In the rapidly growing area of viral vector therapy, where the industry struggles to meet demand, optimization of virus manufacturing is crucial. Rapid and reliable virus quantification allows timely identification of optimal growth conditions and harvesting times, and can increase the productivity of viral vector manufacturing.

Comparison of Infectious Titers with Total Particle Count

Samples of Influenza H1N1 (FLU) cytomegalovirus (CMV), respiratory syncytial virus (RSV), and rubella virus (rubella) were measured by 50% tissue culture infective dose assay ($TCID_{50}$), Virus Counter® platform and quantitative transmission electron microscopy (TEM). Total particle counts determined by either TEM or the Virus Counter® platform were statistically undistinguishable. Titer measured by $TCID_{50}$ was a fraction of total particles, with counts ranging from 2 – 4 orders of magnitude lower than TEM or Virus Counter® platform values.

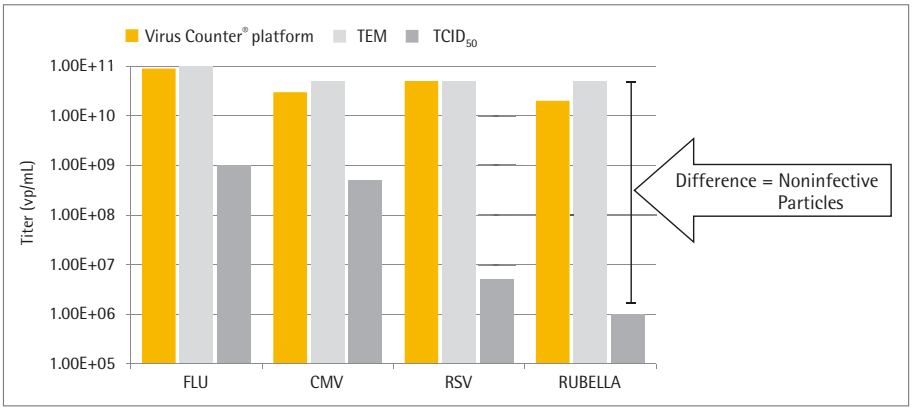


Figure 1: This comparison of infectious titer with total particle counts shows that noninfective particles can be a large portion of the total particle concentration of a virus sample.

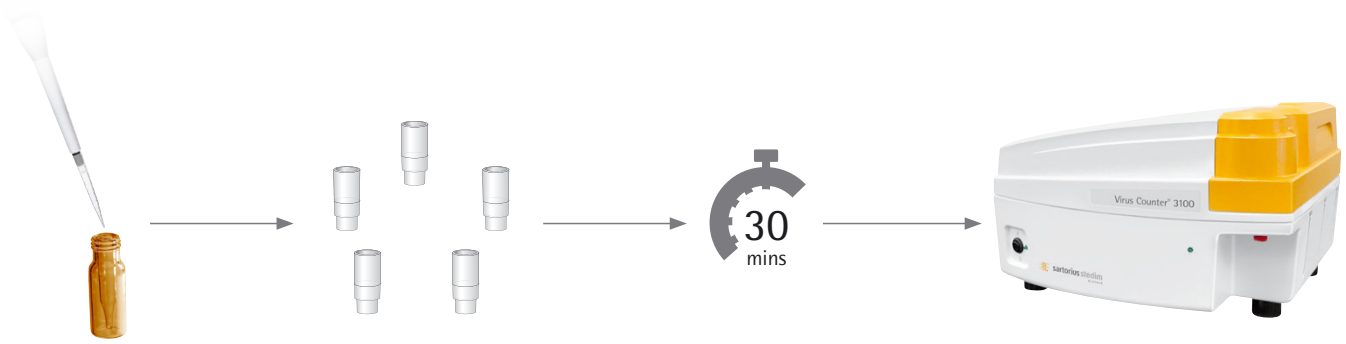
Simple and Quick No-Wash Assay

Utilizing a no-wash assay, virus samples are mixed with Combo Dye® or ViroTag® reagents and incubated for 30 minutes*, protected from light.

*A few ViroTag® reagents might require longer incubation times. Please refer to the Product Information Sheet

No Wash Assay

Both Combo Dye® and ViroTag® labeling systems use a rapid, no-wash workflow.

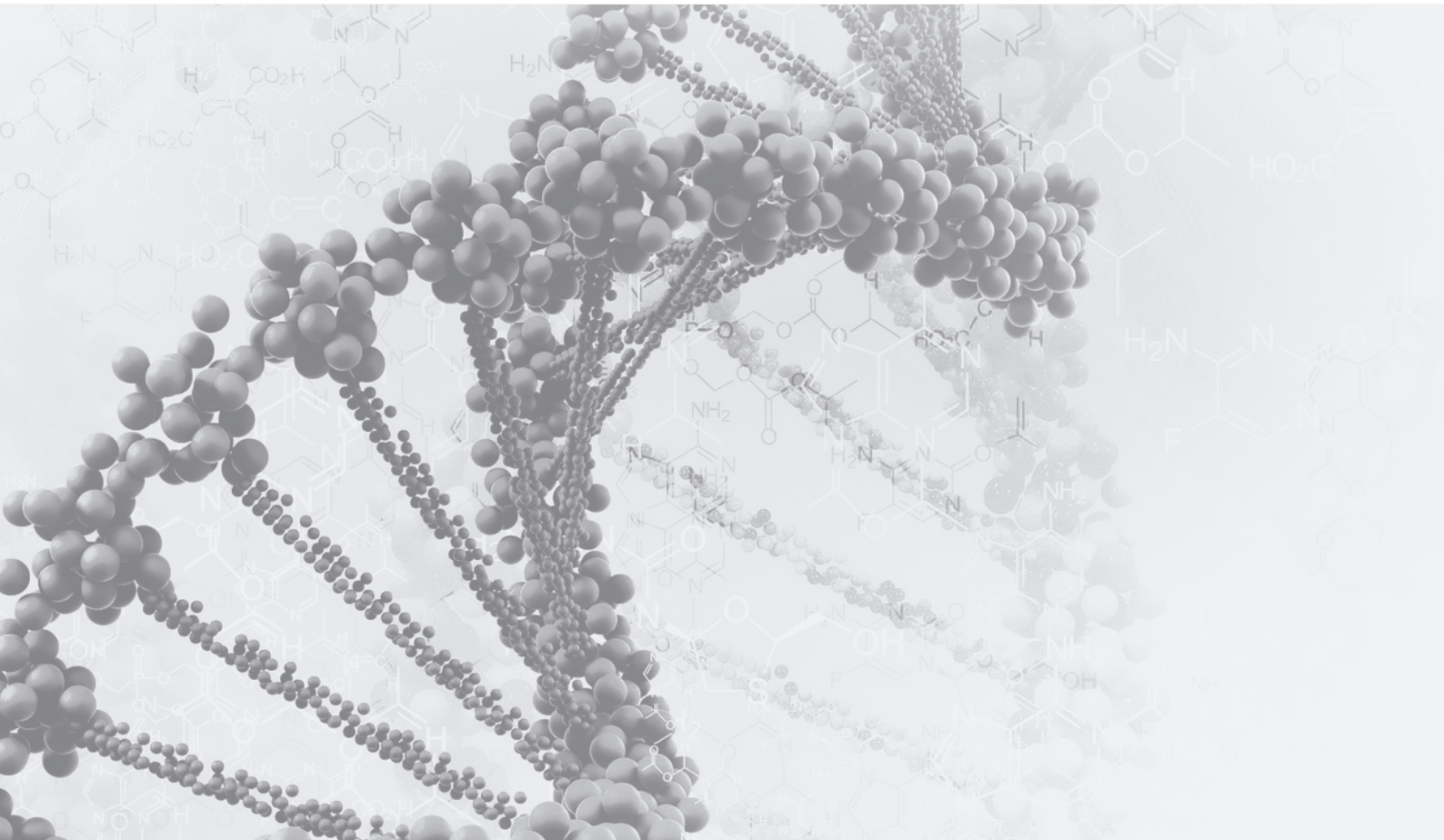


Pipet reagent…

…into sample vials containing sample with virus within the dynamic range of the instrument.

Mix and incubate protected from light for 30 minutes.

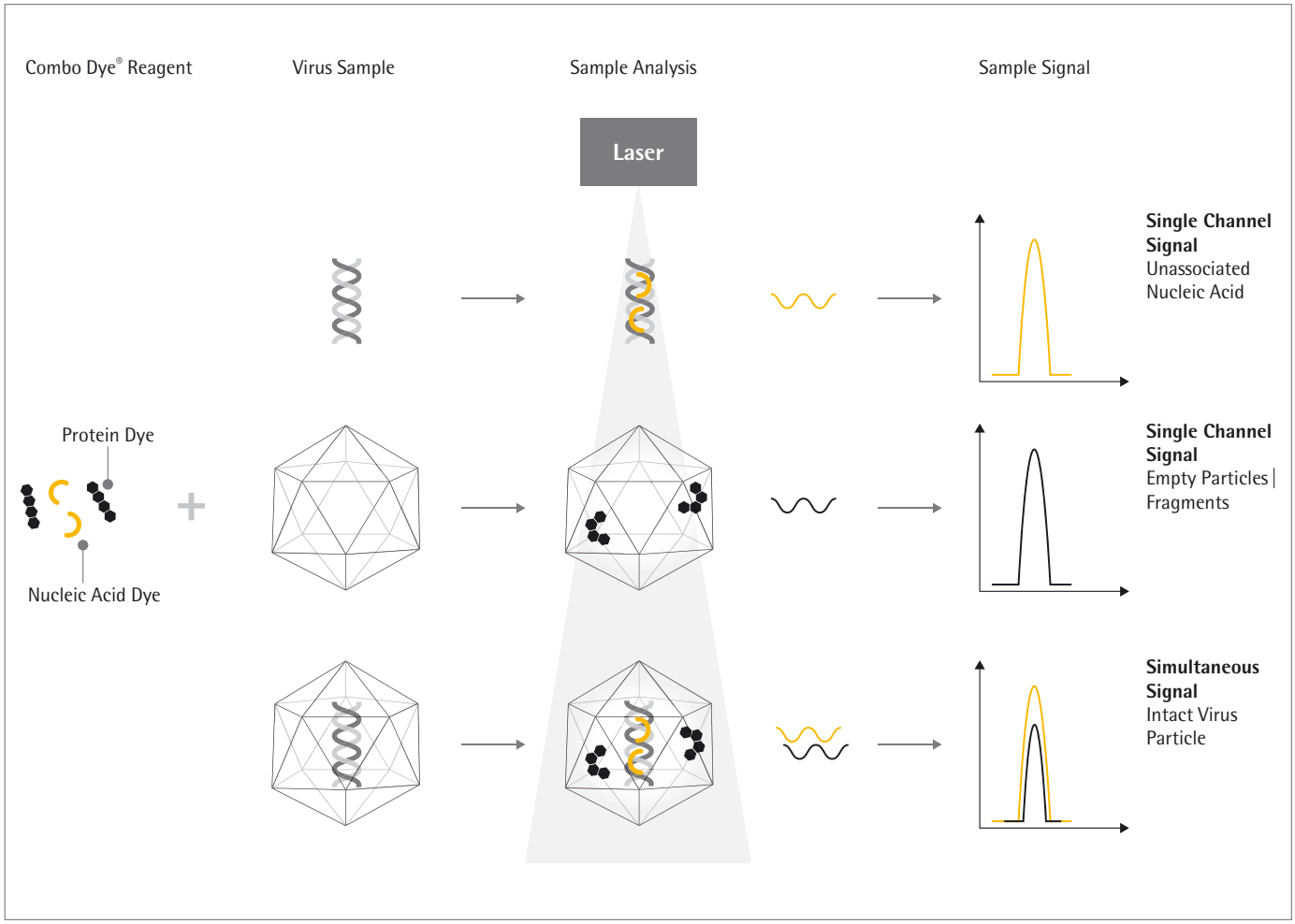
Analyze.



Combo Dye® Reagent: Virus Quantification using a Dual Stain Approach

Combo Dye® reagent is a versatile staining system used in the Virus Counter® platform to rapidly quantify total virus particles. The reagent is based on a dual fluorogenic stain approach: a protein binding dye and a nucleic acid binding dye added simultaneously to virus samples. Upon binding to their respective targets, the quantum yield of the dyes increases significantly. Washing steps to remove unbound dye are unnecessary due to their low, unbound background signal.

Stained samples are guided through the fluidic system of the Virus Counter® instrument where virus bound fluorochromes emit light in response to laser excitation. The emitted light from protein and nucleic acide dyes is measured in two separate channels. Detection of simultaneous signals is interpreted as an intact virus particle. Unassociated nucleic acid or empty capsid are detected as signals in a single channel and are not counted as viruses. The number of events counted during the analysis time is used in combination with sample flow rate to calculate the concentration of virus particles per milliliter of sample.



Versatility of Combo Dye® Reagent

Combo Dye® is suited for enveloped viruses with single- or double-stranded DNA or RNA genomes. Examples of viruses compatible with the Virus Counter® Platform and Combo Dye® reagent:

Arenaviridae
Pichinde virus

Filoviridae
Ebola virus
Marburg virus

Poxviridae
Pox virus
Vaccinia virus

Baculoviridae
Baculovirus

Herpesviridae
Herpes Simplex Virus I
Cytomegalovirus

Retroviridae
Lentivirus
γ-Retrovirus

Coronaviridae
Coronavirus
Infecious bronchitis virus

Orthomyxoviridae
Influenza virus

Rhabdoviridae
Rabies virus
Vesicular Stomatitis virus

Flaviviridae
Dengue virus
Zika virus

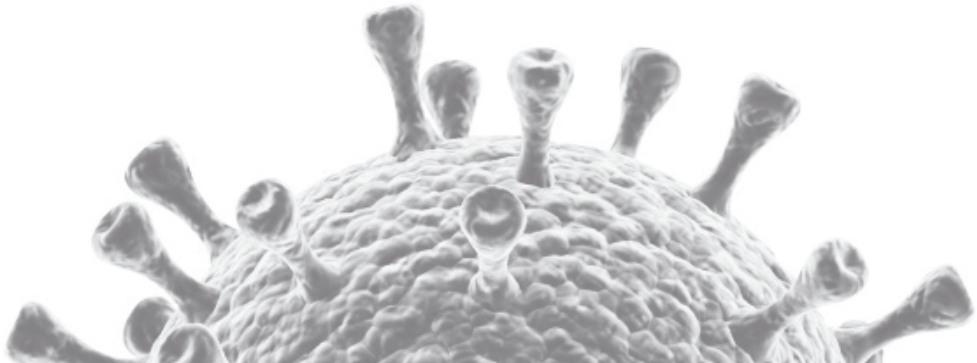
Paramyxoviridae
Measles virus
Newcastle disease virus

Togaviridae
Chikungunya

For a complete list please visit: **www.sartorius.com/virus-analytics**

"We have basically stopped running plaque assays on our P0 and P1 virus stocks because the accuracy of the titers obtained with the Virus Counter® leads to better virus amplifications than those obtained using plaque assay titers. The instrument saves one to two weeks on our virus production time-line and it is very helpful to know within a day or two that a transfection or co-transfection has yielded virus particles."

Kempbio



Titer Determination with High Precision

When analyzing a virus sample for the first time, it is recommended to determine the linear range of the assay (Figure 2) before measuring the titer at an optimal dilution (Figure 3). This protocol ensures measurements in the optimal range of the sample and instrument, and provides high quality data with little variability. Sample titers are determined with high precision and low sample-to-sample (Figure 3) and day-to-day variability (Figure 4).

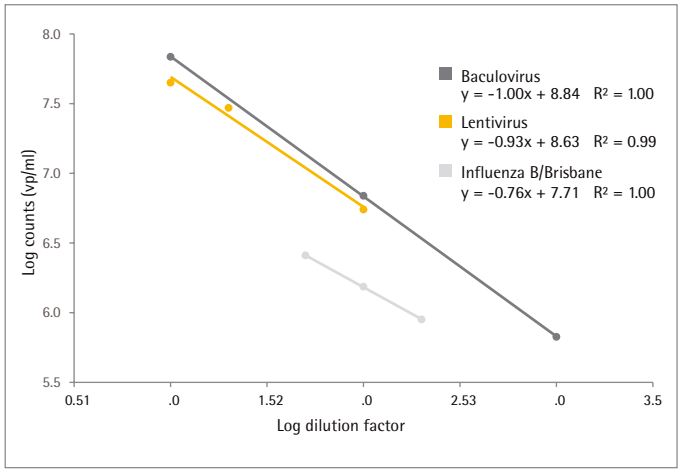


Figure 2: Determination of linear range of Baculovirus, Influenza virus B/Brisbane and Lentivirus samples using the Virus Counter® Platform.

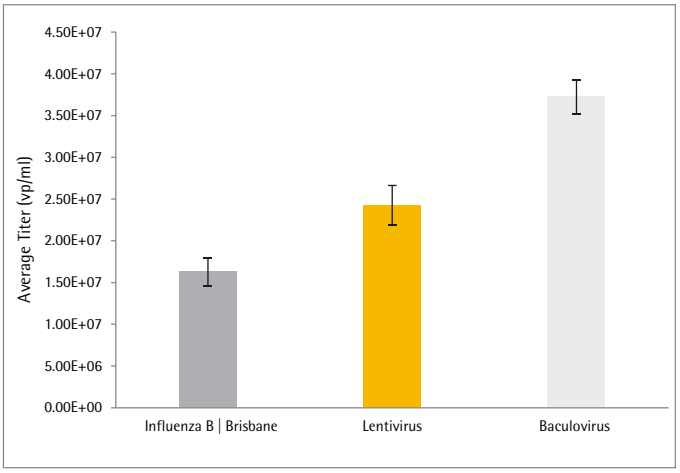


Figure 3: Optimal dilution within the linear range of the instrument was chosen to determine sample counts in replicates of five. Measured coefficients of variance (CVs) are 10.3% (Influenza B), 10.1% (Lentivirus) and 5.7% (Baculovirus).

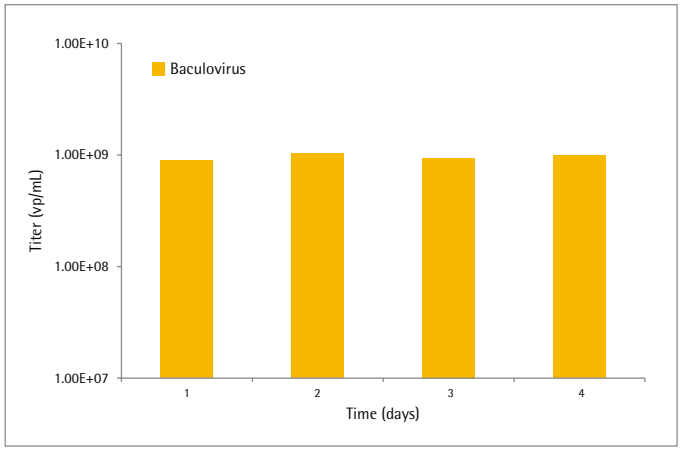


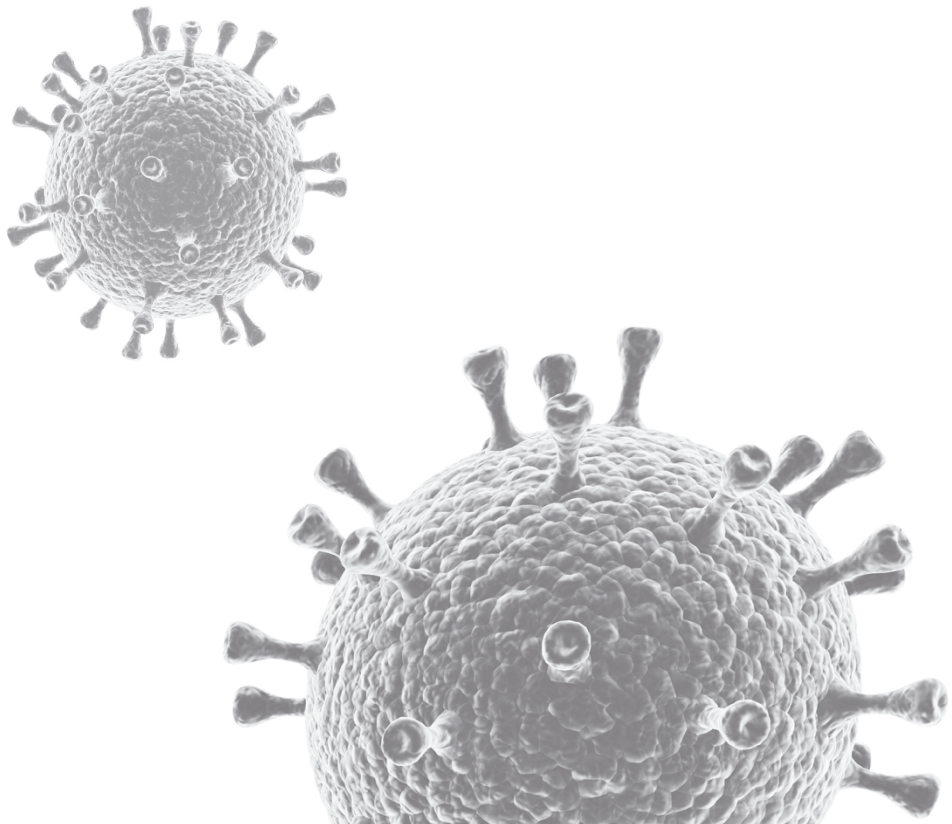
Figure 4: Baculovirus titer was determined over a four day period. The measured CV of day-to-day variablity was 6.6%

ViroTag® Reagents: Virus Quantification with Antibody-Based Reagents

Antibody-based ViroTag® reagents are conjugated to fluorophores and bind to their respective targets with high specificity. Viruses that are labeled with ViroTag® reagents are guided through the Virus Counter® instrument and interrogated by a laser, where the conjugated dyes emit light upon excitation. The Virus Counter® instrument detects the emitted light and uses it together with the sample flow rate to calculate total particle concentration. ViroTag® reagents allow quantification of viruses in crude and purified samples making them the optimal tool to follow virus titer during the whole manufacturing process. ViroTag® reagents are also compatible with VLPs and other particles that do not contain nucleic acids.

Comparison of Quantification Methods

Virus titers obtained with ViroTag® reagents using Virus Counter® Platform show a very tight standard deviation compared to many other quantification methods (Figures 5 – 8). Discrepancies among quantification methods are due to the heterogeneity of virus samples. While ELISA and qPCR enumerate total protein or nucleic acid content of a sample, these methods can lead to an overestimation of viral titer due to inclusion of unassociated particles in the quantification process (Figure 5 and 6). Methods such as plaque titer assays and $TCID_{50}$ quantify only infectious units, while the Virus Counter® platform generates total particle counts (Figure 7 and 8).



Titer Determination with High Specificity

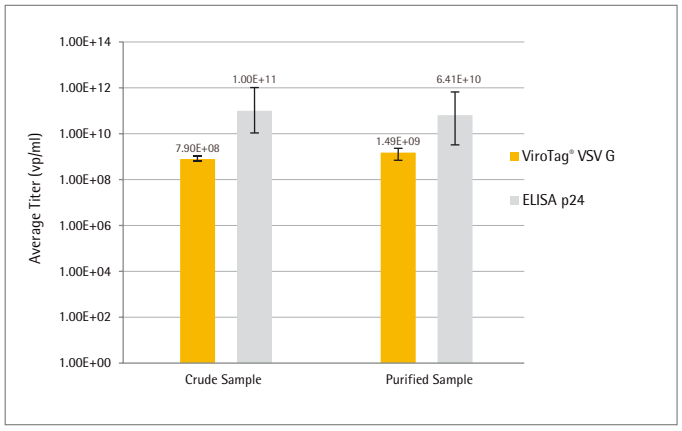


Figure 5: ViroTag® VSVG reagent was used to quantify to VSV-G pseudotyped Lentivirus samples (crude and purified). CV for Virus Counter® platform measurements were 9 % and 14 %, for p24 ELISA CVs were >50 % for crude and purified sample.

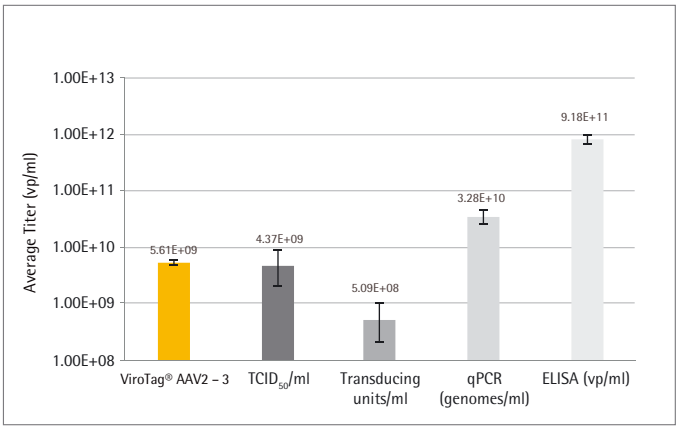


Figure 6: ViroTag® AAV 2/3 was used with other quantification methods to quantify AAV 2 virus standard (ATCC). CV for Virus Counter® platform measurement 2.79 %. CVs measured for other methods from left to right 374 % c.v., 192 % c.v., 109 % c.v., 29.0 % c.v. Data from Lock *et al.*

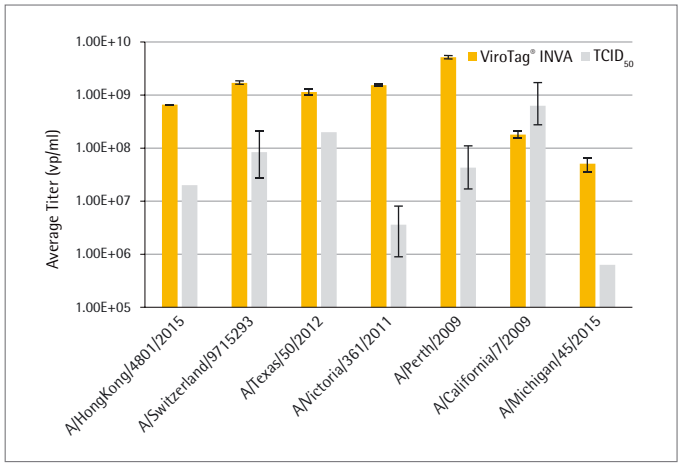


Figure 7: Quantification of influenza A strains with ViroTag® INVA reagent and $TCID_{50}$. ViroTag® result show high precision. ViroTag® reagents quantify total virus particles in the sample compared to $TCID_{50}$ which enumerates only the infectious subpopulation.

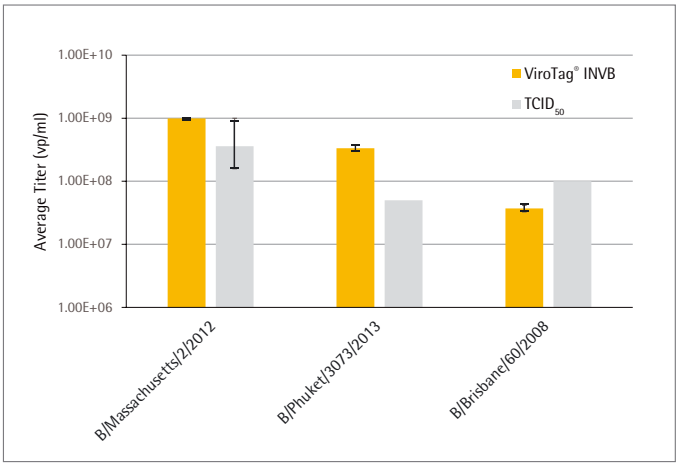
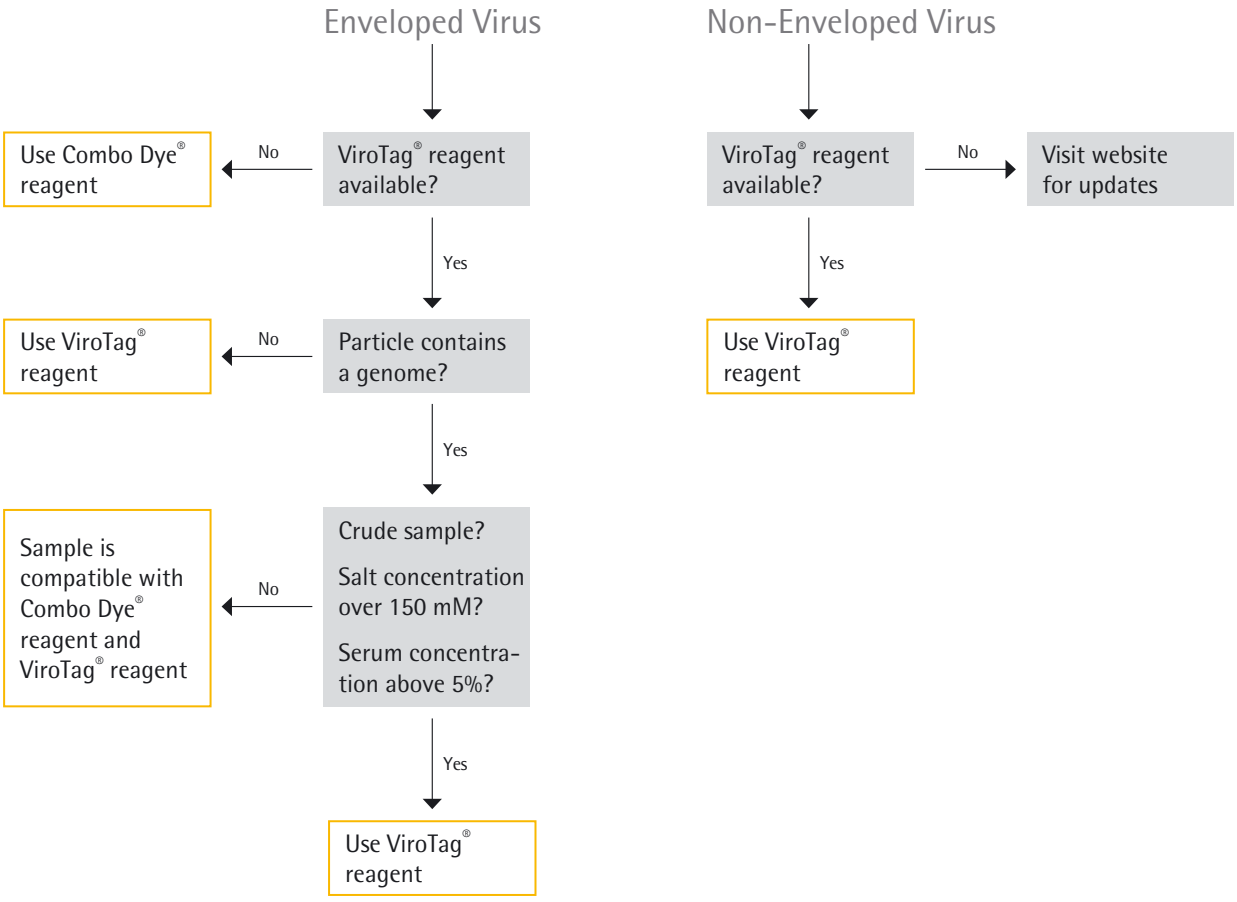


Figure 8: Quantification of influenza B strains with ViroTag® INVB reagent and $TCID_{50}$. ViroTag® result show high precision and higher quantification results than $TCID_{50}$. This is due to the quantification of total virus particles with ViroTag® compared to infectious units enumerated with $TCID_{50}$.

Which Reagent is Optimal for my Sample?



Ordering Information

Reagent	Target	Order Number	Quantity*
AAV2-3	Intact AAV2 and AAV3 capsids	VIR-92117	200 samples per kit
BCVB	Budded Baculovirus	VIR-92108	200 samples per kit
INVA	Influenza A Viruses (H1 and H3 subtype)	VIR-91151	200 samples per kit
INVB	Influenza B Viruses	VIR-91152	200 samples per kit
VSVG	VSV-G Protein	VIR-92332	200 samples per kit
Combo Dye®	Universal stain for enveloped viruses	VIR-92333	200 samples per kit

* Smaller kits available, 100 samples per kit, 50 samples per kit. Please request article code with your local sales representative.

Replacement Consumables		
Sheath Fluid (Virus Counter® 3100)	VIR-92302	1 L bottle
Inter-Sample Wash (ISW)	VIR-92303	6 vials
Cleanliness Verification Fluid (CVF)	VIR-92295	6 vials
Startup | Shutdown Rinse (SSR)	VIR-92338	6 vials

Instrument	
Virus Counter® 3100	VIR-92341

Selected References

- Evaluation of the Virus Counter® for Rapid Baculovirus Quantitation. Journal of Virological Methods, 2011, 171(1); Ferris *et al.*
- Evaluation of ViroCyt® Virus Counter® for Rapid Filovirus Quantitation. Viruses, 2015, 7; pp 857-872; Rossi *et al.*
- Non-plaque-forming virions of Modified Vaccinia virus Ankara express viral genes. Virology, 2016, 499, pp 322-330; Lülf *et al.*
- Rapid and Effective Monitoring of Baculovirus Concentrations in Bioprocess Fluid Using the ViroCyt® Virus Counter®. BioProcessing Journal, 2014, 13(2), pp32-39; Birch *et al.*
- Characterization of Human Influenza Viruses Propagated by Madin-Darby Canine Kidney Cells or Embryonated Eggs. International Journal of Virology and AIDS 2014; Plant *et al.*
- Virotherapy Process Optimization. Bioprocessing Journal, 2015, 14(1); Artinger *et al.*
- Rapid, Simple, and Versatile Manufacturing of Recombinant Adeno-Associated Viral Vectors at Scale. Human Gene Therapy (2010); Lock, M. *et al.*

Learn why leaders in vaccine development, protein expression, and viral therapeutics choose the Virus Counter® 3100 Platform as their tool of choice for virus quantitation. Please visit: **www.sartorius.com/virus-analytics**

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