Rapid Total Particle Quantification for Viral Families With High Potential for Pathogenic Emergence

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Rapid viral quantification during the emergence of a novel pathogenic virus is critical to quickly developing a vaccine. Current techniques for determining the quantity of virus particles in a sample, such as immunoassays, the plaque assay, TCID50 or measures of genomic content such as qPCR require a certain prior knowledge of the virus so that the correct cell lines can be used and the correct reagents manufactured. Building the suite of specific reagents for molecular or immunological studies can take weeks to months, with potentially devastating consequences during an emerging virus outbreak.

Here, we demonstrate the capabilities of a novel technology for generalised and rapid viral particle quantification: The Virus Counter® 3100 (VC 3100) instrument, coupled with the Virotag® DY ENV reagent is designed to stain and allow detection of viral protein and nucleic acid to directly quantify the total particle count of enveloped viral samples. This platform operates without the need for species- or strain-specific reagents. For this study, representative members from the Arenaviridae, Coronavirus, Flaviviridae, Orthomyxoviridae, and Togaviridae families were used. Tier results for each virus sample were obtained using the Virus Counter® platform and compared to the titer data from the suppliers’ Certificates of Analysis.

These results demonstrate the efficacy of the Virus Counter® 3100 platform for rapid and precise quantification of viruses from families with high potential for evolving or harboring new human pathogens. This technology can therefore improve time-to-result for critical experiments in vaccine manufacturing that require viral particle counts for a novel virus.

Virus Particle Detection

![Staining Protocol for Virus Samples](image1)

The two fluorogenic dye-components of Virotag® DY ENV are generalized stains for nucleic acid content and the hydrophobic regions of proteins. As these bound dyes flow through a laser beam, the emitted signals are detected on separate channels. The simultaneous signals are recorded as results (Figure 2).

Serial Dilutions of Viral Strains

![Serial Dilutions of Viral Strains](image2)

Shown are serial dilutions of representative species from families with high potential for emergence as human pathogens (Figure 3). The Virotag® DY ENV reagent enables rapid and specific detection of a wide variety of enveloped viral species. While the influenza strain shown here is a seasonal flu strain, this technology can be used to detect subtypes with pandemic potential.

Comparison of Total Particle vs. Infectious Titers

![Comparison of Total Particle vs. Infectious Titers](image3)

Tier comparisons of the VC 3100 results and infectious titer. VC 3100 results are shown with error bars representing the 95 percent confidence interval (Figure 4). The TCID50 results were provided by the sample vendors, and experimental error information was not available.

Conclusions

- The Virus Counter® 3100 Platform enables rapid quantification of a variety of enveloped viral species.
- Tier results for virus samples are available in less than one hour.
- Rapid method to quantify total virus particles, including the non-infectious population.
- No prior knowledge of genomic sequences or envelope epitopes is required.

This platform can improve time-to-result for critical experiments in vaccine manufacturing that require viral particle counts where other methods may not be readily available for a novel virus.