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Antibody-Based Influenza Virus Particle Detection With the Virus Counter® Platform

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Introduction

The Sartorius Virus Counter® 3100 platform is an emerging technology that enables rapid, direct and precise quantification of seasonal Influenza A and B viral particles in solution. The system's fluidics and flow cell are specifically engineered for the detection of particles <1 µm. Virus particles are labeled with patented antibody-based detection reagents that allow for Influenza virus particles to be quantified in minutes rather than hours or days. Virotag® reagents are fluorescent, antibody-based reagents that specifically bind viral particles, facilitating quantification on the Virus Counter® 3100 platform. Virotag® INVA and Virotag® INVB reagents allow for the rapid and precise quantification of both seasonal Influenza A and B virus particles, respectively. Here we demonstrate the ability of this platform to rapidly and precisely quantify seasonal Influenza virus A and B strains that have been used in the Southern and Northern Hemisphere seasonal Influenza vaccines. These studies demonstrate that the Virus Counter® 3100 platform can rapidly and reliably enumerate viral titers based on total particle counts while excluding viral debris that might be erroneously counted with techniques such a qPCR and ELISA. This characterization allows a more complete assessment of Influenza virus samples than traditional methods currently in use.

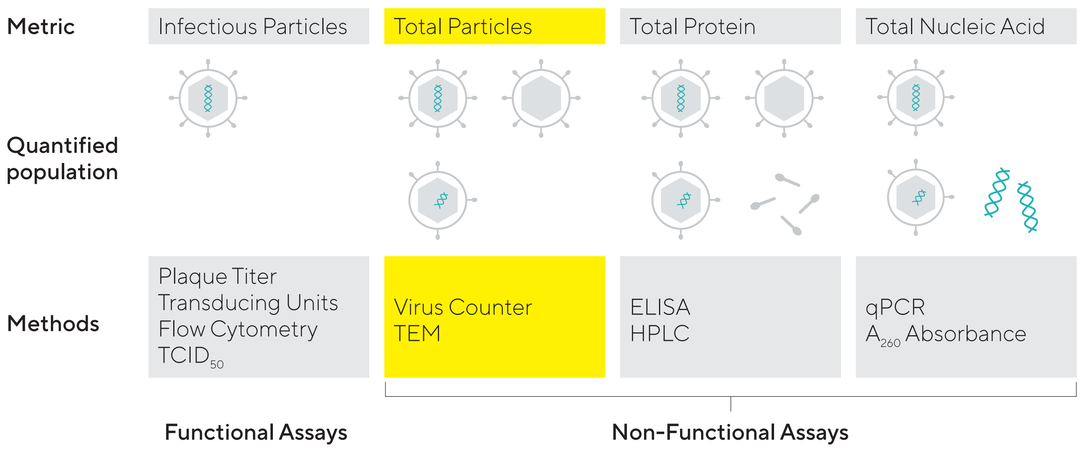

Figure 1: Virus quantification methods measure different subpopulations or viral building blocks to determine viral titers. The icons shown in the quantified population box refer to intact viral particles, empty particles, defective particles, free protein and unassociated nucleic acid. The quantified 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® Assay and Virotag® DY ENV 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.

Results

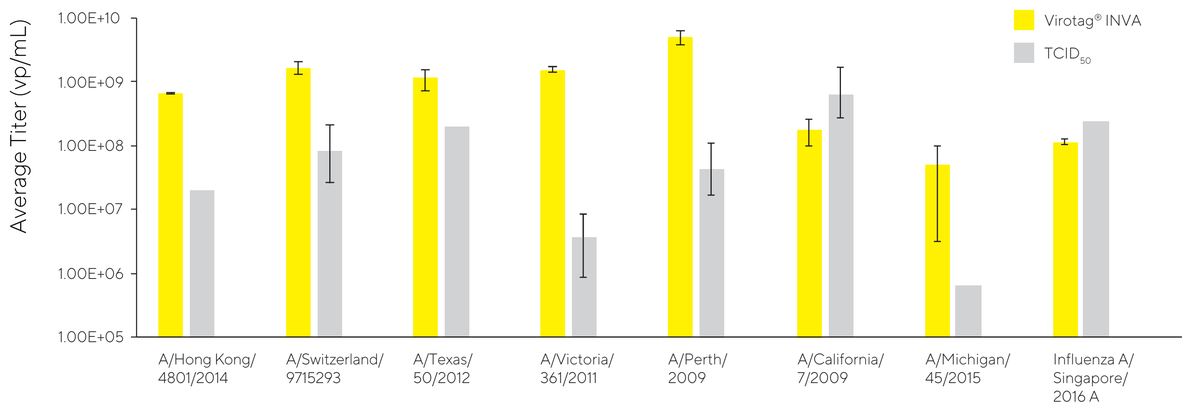

Figure 3: Quantification of Influenza A vaccine strains with Virotag® INVA reagent and TCID50. Using the optimal dilutions determined from dilution series of Influenza A virus, dilutions of Influenza A and allantoic fluid controls were prepared (n=3) and used to calculate the viral titer in particles/mL as well as the standard deviation. Influenza A titers determined through use of the Virus Counter® and Virotag® INVA reagent were then compared to the TCID50 values* on the CoA provided by the manufacturer. Allantoic fluid result was below detection limit.

* TCID50 values not available for all commercial samples.

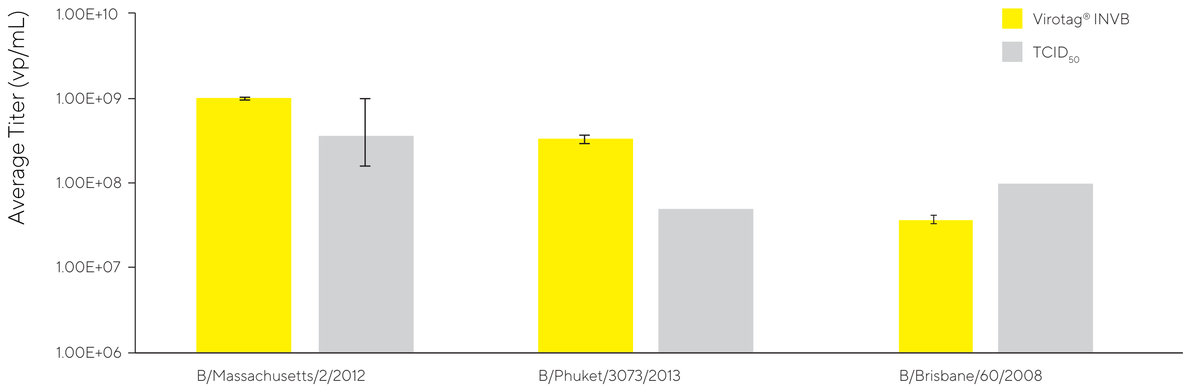

Figure 4: Quantification of Influenza B vaccine strains with Virotag® INVB reagent and TCID50. Using the optimal dilutions determined from dilution series of Influenza B virus, dilutions of Influenza B and allantoic fluid controls were prepared (n=3) and used to calculate the viral titer in particles/mL as well as the standard deviation. Influenza B titers determined through use of the Virus Counter® and Virotag® INVB reagent were then compared to the TCID50 values* on the CoA provided by the manufacturer. Allantoic fluid result was below detection limit.

* TCID50 values not available for all commercial samples.

Table 1: Multiple reagents support Influenza virus particle quantification with the Virus Counter® 3100 platform. The optimal reagent for a sample will depend on the sample type (cell culture-grown or egg-grown), the strain of virus (Influenza A or B types), and whether detection is based upon epitope recognition or two-channel quantification of protein and nucleic acid (Virotag® vs. Virotag® DY ENV reagent, respectively).

	Epitope recognition	2 Channel recognition	Influenza A strains	Influenza B strains	Egg-grown virus	Cell-culture grown virus
Virotag® INVA	+		+		+	+
Virotag® INVB	+			+	+	+
Virotag® DY ENV		+	+	+		+

Discussion

Here we have shown that the Virus Counter® 3100 platform and the Virotag® INVA and INVB reagents allow for the rapid and precise quantification of Influenza A and B strains, respectively. Results demonstrate the enhanced precision of Influenza virus particle quantification using the Virus Counter® 3100 instrument and Virotag® reagents compared to the TCID50 method: and, also highlights the differences inherent in measuring infective particles vs. total particles in a sample. By utilizing the Virus Counter® 3100 and antibody-based reagents for the quantification of Influenza virus samples, users can shorten their time to getting critical results and improve workflows, resulting in high confidence in the precision of their results.

Conclusion

- The Virus Counter® 3100 platform allows for rapid, precise virus particle quantification.
- Direct and rapid quantification utilizing the Virus Counter® platform has distinct advantages when compared to alternate methods of viral quantification like qPCR, TCID50, and ELISA.
- Antibody-based reagents Virotag® INVA and INVB allow for high-precision detection of Influenza viruses.
- Virotag® INVA and INVB reagents optimized for detection of both egg- and cell culture-grown samples.