Spring 5-12-2016

A flow cytometric granularity assay for the quantification of infectious virus

Megan Logan
University of Waterloo, m3logan@uwaterloo.ca

Recommended Citation

A FLOW CYTOMETRIC GRANULARITY ASSAY FOR THE QUANTIFICATION OF INFECTIOUS VIRUS

Megan Logan, University of Waterloo
M3logan@uwaterloo.ca
Julia Manalil, University of Waterloo
Arno Zeister, Sanofi Pasteur Limited
Patrick Farrell, Sanofi Pasteur Limited
Marc Aucoin, University of Waterloo

Key Words: Granularity, infectivity assay, side scatter, herpes simplex virus, canarypox virus

A need exists for rapid, low-cost, and accurate infectious viral quantification method in the bio-pharmaceutical field for the production of vaccines and virus-based therapeutics. Two of the most common and traditionally employed methods to quantify infectious viruses are plaque assays and cell culture infectious dose 50 ($CCID_{50}$); both are relatively inexpensive, however they can be time consuming and demonstrate significant variability between operators. Here we present a method to quantify infectious viruses using the post-infection granular changes within the cell using flow cytometry to create a more rapid and high throughput quantification method. To validate this method, a recombinant Vero cell line was infected with a replication-deficient herpes simplex virus type 2 mutant and monitored over a 72 hour period for changes in granularity, using flow cytometry. Between 16-20 hours post infection (hpi), the percentage of the cell population displaying a high degree of granularity can be logarithmically correlated to the infectious titer of the viral sample. The granularity-based assay was used to estimate the infectious titer of 5 separate virus samples and the results were compared titers obtained through plaque assays. It was found that there was a maximum difference of 52.67% in the average titers between assays. To further demonstrate this as a universal method, Japanese quail muscle fibroblast cells (QT-35) were infected with a highly attenuated canarypox virus and their granularity was tracked. A similar increase in granularity post infection was detected, thereby giving credence to the utility of this viral enumeration technique across a potentially broad virus-host cell range. Given the high level of correlation between the proposed and traditional methods of viral quantification, the use of flow cytometry could aid in process development and optimization by decreasing the time required to assess the affect of production conditions on viral products.

Figure 1- Semilogarithmic plot correlating the percent of the population with high granularity to the concentration of infective virus (PFU) for 6 separate herpes virus samples. The trend line was calibrated using a standard sample with a well-defined titer

Table 1- A comparison of the infectious titers obtained from each assay with the variance associated with each measurement. The difference between the two assays was calculated by: (Mean titer of Granularity-based assay – Mean titer of Plaque assay) / (Mean titer of Plaque assay) x 100

Sample	Plaque Assay (10^6)	Coefficient of Variance	Granularity-based flow assay (10^6)	Coefficient of Variance	Difference between assays
A	6.00±1.81	30.11%	9.16±4.53	49.42%	-52.67%
B	6.33±4.13	65.35%	8.94±3.00	33.52%	-41.32%
C	6.34±2.20	34.62%	4.13±1.33	32.17%	34.85%
D	6.28±0.99	15.81%	4.96±1.50	30.23%	21.01%
E	61.7±7.67	12.43%	90.3±39.1	43.26%	-46.45%