

Rapid and highly sensitive determination of virus titers using the ConSense™ Analyzer

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Abstract

We describe here a straightforward method to detect influenza virus in very low concentrations in a confocal reader system with single molecule sensitivity. The individual virus particles are labeled with Nile red following a simple and fast protocol. This enables the “counting” of individual virus particles diffusing through the focus of the ConSense and therefore directly relates to the number of virus particles in the sample. This method is applicable to all types of viruses that incorporate dyes like Nile red.

Introduction

The determination of low virus titers can be a rather time consuming process that slows down development and process control. Most of these methods rely on cell culture systems which can easily take several days. Other direct methods use separation technologies like size-exclusion chromatography and yield additional information about the size of virus aggregates. This is important for setting up robust processes for the production of virus vaccine preparations.

In contrast to these approaches the method described herein allows to measure very low virus titers below 1 µg/ml within minutes and with excellent precision. In addition, information on the aggregation behavior of the virus particles can also be derived from the measurement data.

Materials and method

Beta propiolactone inactivated Influenza A virus from vaccine production (in TRIS buffer) was used as a test system for titer determinations. The stock solution contained 1.7 mg/ml virus.

Nile red is a hydrophobic molecule with low solubility in water. It is established for the detection of hydrophobic areas on the surface of proteins. The fluorescence maxima are at 550 nm (excitation) and at 662 nm (emission), respectively.

Instrumentation

Measurements were conducted with the ConSense Analyzer. Excitation of Nile red was done with the 532 nm single mode, single frequency laser at a power of 160 µW. Three µl of each virus mixture were transferred into a detection chamber on a ConSense Type A microchip and measured for 60 seconds.

Results were obtained with Virtual Lab software applying accurate stochastic fluorescence spectroscopy (ASFS) technology.

Assay setup

Nile red was dissolved in ethanol, sonicated and added to the PBS buffer solution (final ethanol concentration 10%). Diluted virus solution was added and the samples were incubated for one hour. The reaction conditions (e.g. dye concentration, incubation temperature and buffer) were optimized in order to increase the solubility of Nile red, to facilitate the integration of dye into the virus particles and thereby improve the stability and robustness of the assay.

Two dilution series were prepared to cover different concentration ranges and each concentration measured in three replicates in order to determine the precision of the assay.

Tab 1: Virus titer preparations

No.	Series I Virus $\mu\text{g/ml}$	Series II Virus in $\mu\text{g/ml}$
1	0	0
2	25	0.17
3	100	1.7
4	200	17
5	400	170

Results

The calibration curves generated for both concentration ranges (Fig. 1 and 2) show linear behavior. The standard deviations for all data points are given in Tabs 2 and 3. They are in the range of 10% for virus titers below approx. 20 $\mu\text{g/ml}$ and significantly lower (< 5%) for higher concentrations. The detection limit for influenza virus in this experimental setup is therefore lower than 1 $\mu\text{g/ml}$.

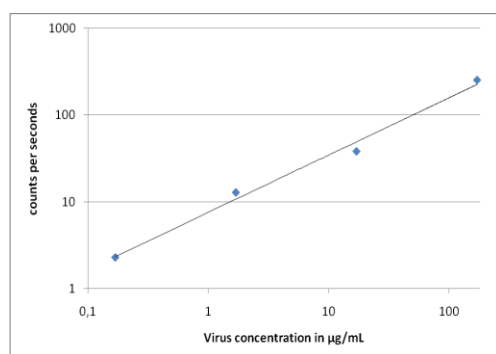


Fig 1: Calibration curve for virus titers between 0.17 and 170 $\mu\text{g/ml}$.

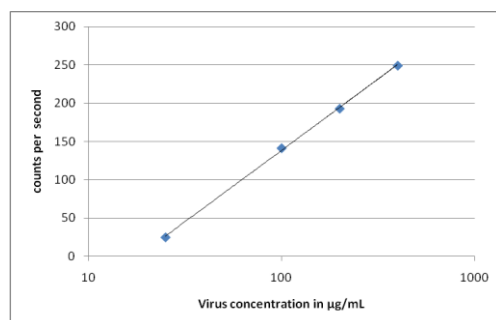


Fig 2: Calibration curve for virus titers between 25 and 400 $\mu\text{g/ml}$.

In this report we have focused on virus titer determination with the ConSense and the protocol has been optimized to reduce aggregate formation and detect single virus particles.

Under different assay conditions the size distribution of virus aggregates can be directly detected without using additional separation techniques. This, in addition to the overall sensitivity of the system shall lead to a powerful combination for the characterization of virus preparations and help to improve manufacturing and formulation processes.

Tab 2: Individual measurement results and CV values for low concentration series. Count rates were dye background corrected.

Virus titer [$\mu\text{g/ml}$]	Run1 [kcps]	Run 2 [kcps]	Run 3 [kcps]	Mean [kcps]	CV
0.17	2.20	2.56	2.13	2.30	10.05
1.7	12.93	10.32	15.34	12.86	19.52
17	34.61	37.45	42.55	38.20	10.53
170	256.3	244.9	257.6	252.9	2.57

Tab 3: Individual measurement results and CV values for high concentration series.

Virus titer [$\mu\text{g/ml}$]	Run1 [kcps]	Run 2 [kcps]	Run 3 [kcps]	Mean [kcps]	CV
0	4.57	4.52	4.48	4.52	1,00
25	27.24	28.82	31.66	29.24	7.66
100	144.5	147.3	149.4	147.1	1.66
200	194.5	196.0	202.2	197.6	2.08
400	254.6	251.7	255.5	253.9	0.77