Title: *In Vitro* Infectivity of Oncolytic Newcastle Disease Virus: Correlation between Plaque and Fluorescent Focus Assays

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In Vitro Infectivity of Oncolytic Newcastle Disease Virus: Correlation between Plaque and Fluorescent Focus Assays

Running Title: Correlation between Orthogonal Infectious Titer Assays for an Oncolytic Virus

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Highlights

- Optimized plaque and fluorescent focus assays (FFA) yield equivalent infectious titers for a recombinant, oncolytic Newcastle Disease Virus
- Manual and automated fluorescent foci analyses yield equivalent FFA titers
- FFA offers much higher throughput and faster turn-around than plaque assay

Abstract

Newcastle Disease Virus (NDV) is an avian paramyxovirus that has no significant pathogenicity in humans. Cancer cells with impaired immune defense mechanisms are susceptible to infection and lysis by NDV. A recombinant construct of a lentogenic form of NDV (rNDV) containing an insertion of granulocyte macrophage colony stimulating factor (GMCSF) transgene was earlier reported and shown to have acceptably low avian pathogenicity as well as oncolytic potential. Reliable measurement of infectious titer is key to determining the effectiveness of virus preparations to infect and lyse cells. We report here a comparative evaluation of two infectious titer assays as applied to rNDV: plaque assay and fluorescent focus assay (FFA). Optimization of assay conditions for both titer methods has produced concordant results spanning several orders of magnitude. While plaque formation is the gold standard measure of virus titer, FFA provides higher throughput and faster turn-around. FFA has been further evaluated on two different instrument platforms, for automated versus manual foci recognition and counting, with equivalent results. These results point to amenability of FFA to transfer between different laboratories and analysts, without introducing significant subjectivity in data analysis.

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[1]Abbreviations used: NDV, Newcastle Disease Virus; rNDV, recombinant NDV; FFA, Fluorescent Focus Assay; VCGM, Vero Cell Growth Medium; DMEM, Dulbeco's Modified Eagle Medium; FBS, Fetal Bovine