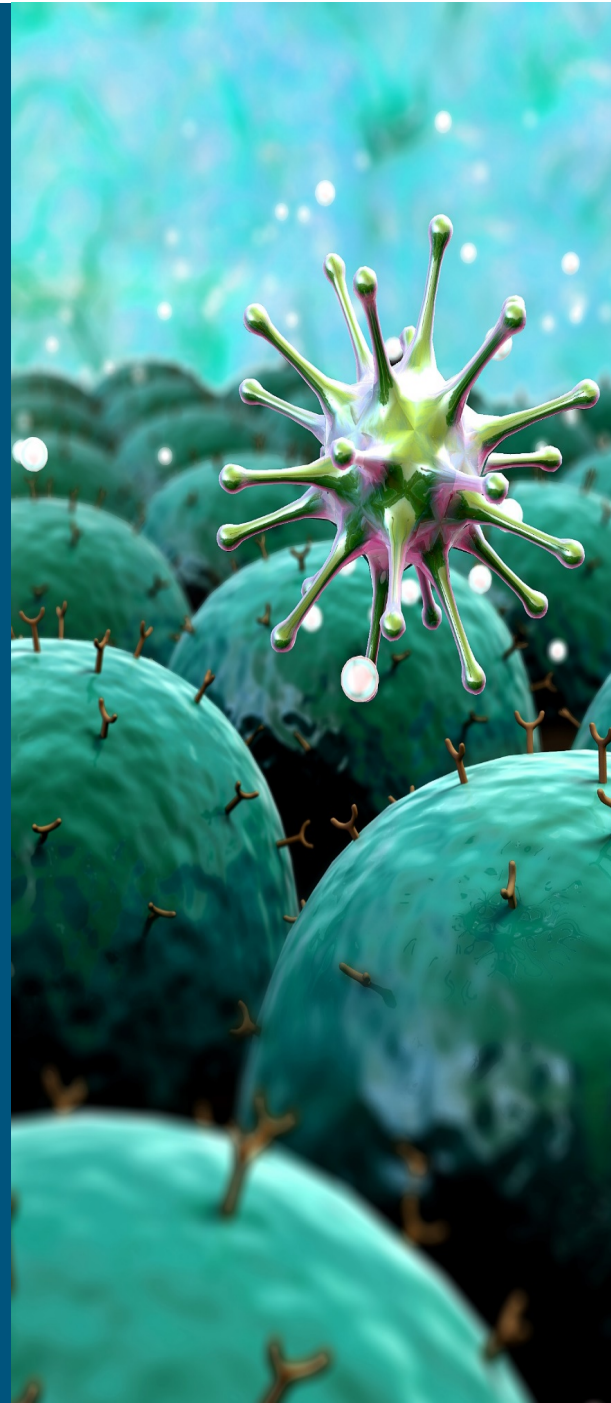


xCELLigence® Real-Time Cell Analysis

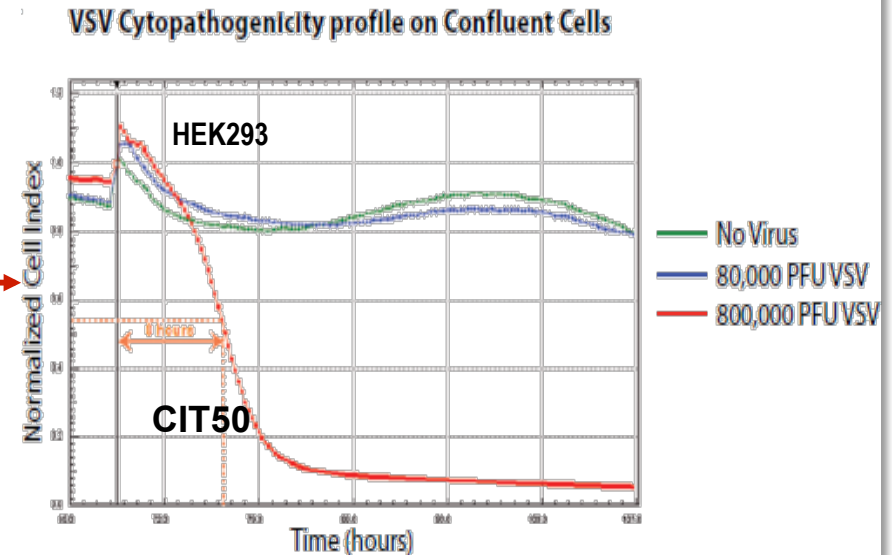
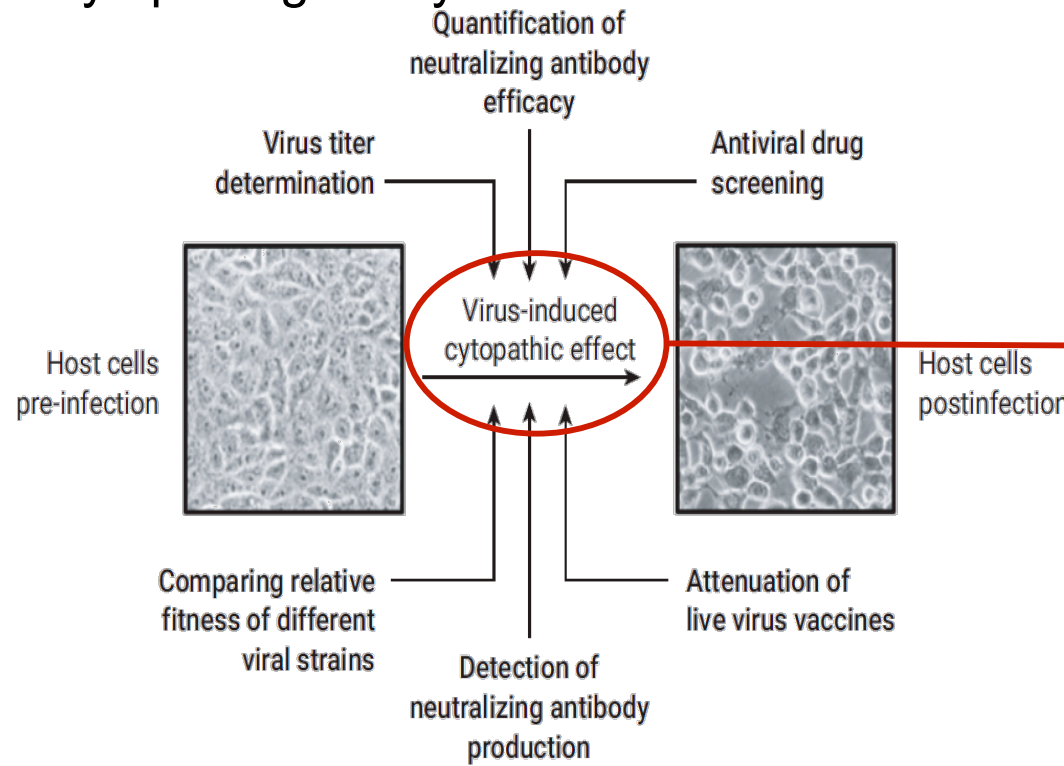
Vaccine and Virology Applications

Live Cell Analysis Division
ACEA Biosciences – a Part of Agilent



A Great Need for Vaccine and Virology Research

xCELLigence RTCA systems are a reliable tool for studying virus-mediated cytopathogenicity



*xCELLigence RTCA Application Note No. 9/
A New Way to Monitor Virus-Mediated
Cytopathogenicity*

Agilent xCELLigence RTCA handbook/Vaccine and Virology Applications

According to the WHO, approximately 30% of deaths globally are due to infectious diseases every year. At least 6 out of the top 10 infectious diseases can be caused by viruses.

Traditional Endpoint Methods to Measure Viral Cytopathic Effect (CPE) versus xCELLigence Real-Time Cell Analysis (RTCA)



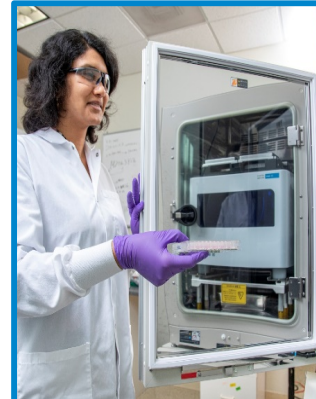
Endpoint

Plaque assays

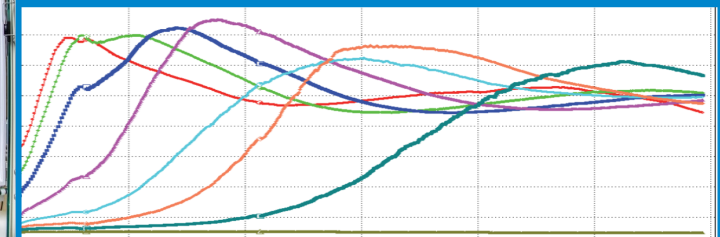
Immunofluorescence assays (IFA)

Indirect measurement

- Labor intensive
- Time consuming
- Difficult to reproduce
- Wrong timepoint can result in inaccurate calculation of viral titer and lytic activity
- Exposure to biohazard



RTCA Method:

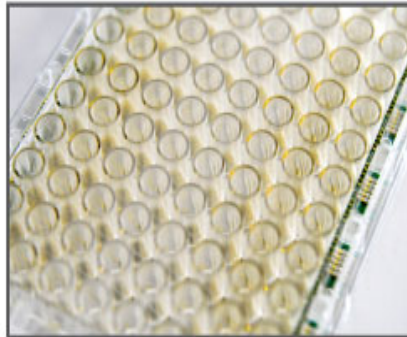


- Label free and imaging methods combined.
- Fast and high throughput
- Real-Time: Quantitative monitoring of CPE
- Easy Work Flow: Requires only the addition of virus to host cells
- Accurate, Precise and Highly Reproducible
- Automatic data plotting and objective analysis, precluding the subjective data vetting that is common to plaque assays.

Three timepoints 3 wells vs Hundreds of timepoints 1 well

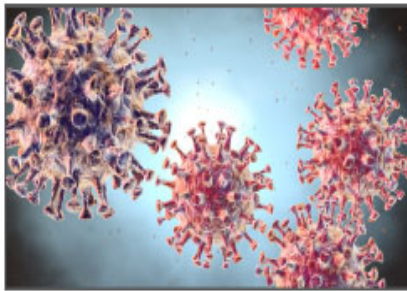
xCELLigence Real Time Cell Analysis – Simple Workflow

Easy walk-away and fully automated operation



Step 1: Grow Cells in E-Plate

Adherent cells are first seeded in E-Plate wells. Microelectronic biosensors enable the RTCA assay to offer dynamic, real-time, label-free, and noninvasive analysis of virus-mediated cytolysis.



Step 2: Infect with Virus

Cells are infected with virus in the presence or absence of neutralizing antibody or anti-viral drugs.



Step 3: Monitor Viral CPE in real time

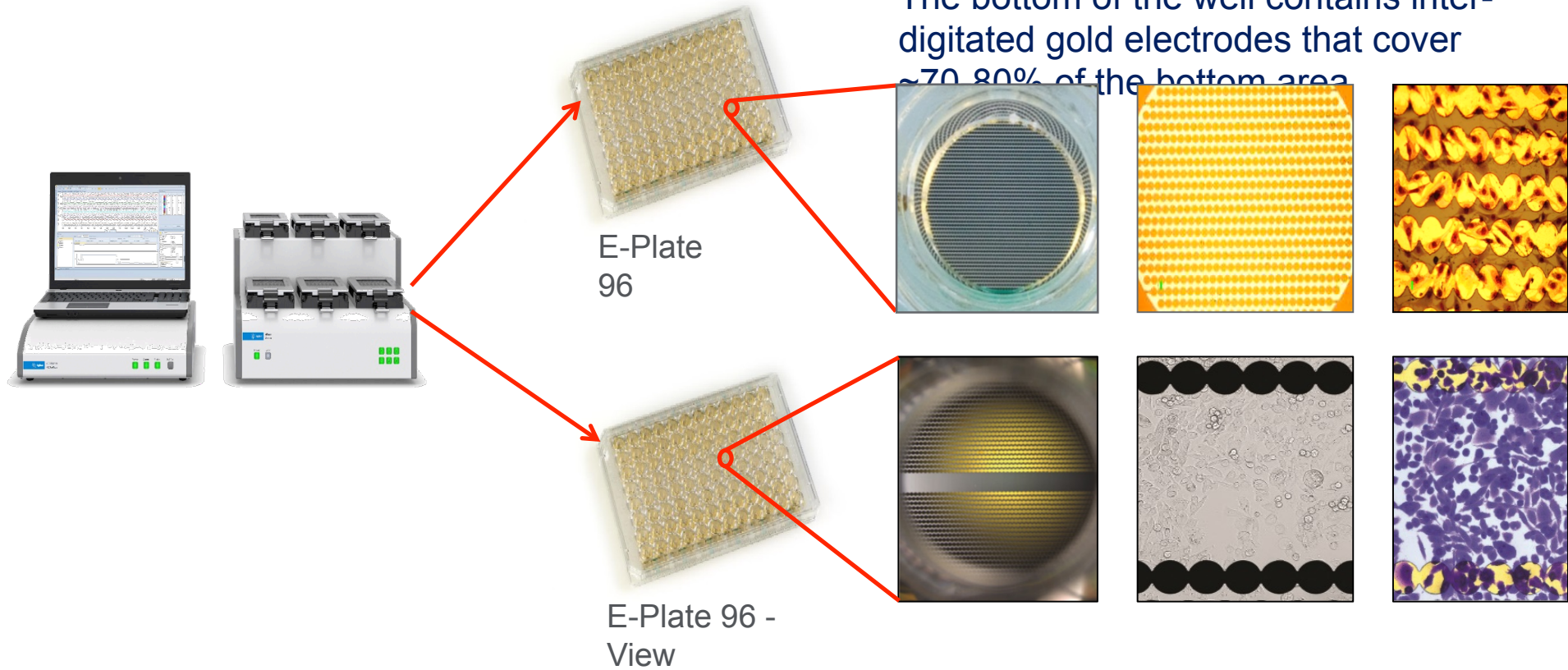
The xCELLigence system is housed inside a CO₂ incubator and automatically acquires data in real-time, minimizing manual sample handling and risk of exposure.

xCELLigence Real Time Cell Analysis – E-Plate Technology

Impedance biosensors

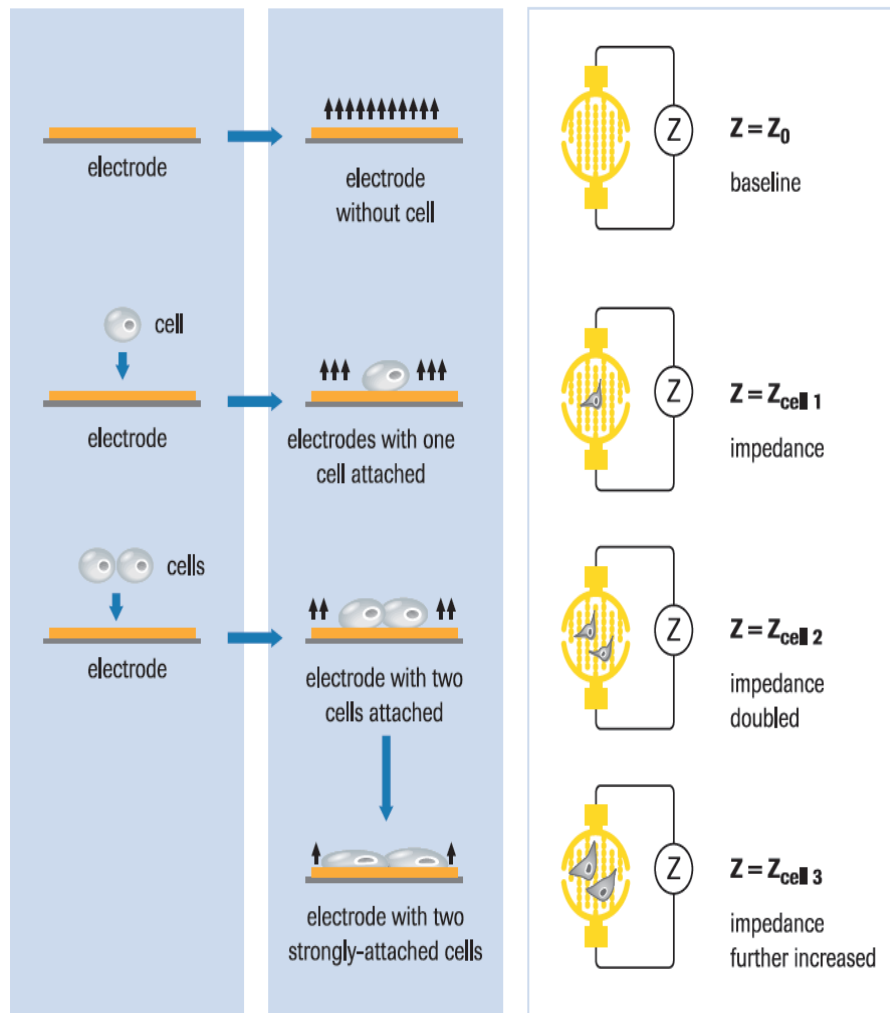
Heart of the technology: *E-Plate*[®]

The bottom of the well contains interdigitated gold electrodes that cover ~70-80% of the bottom area.

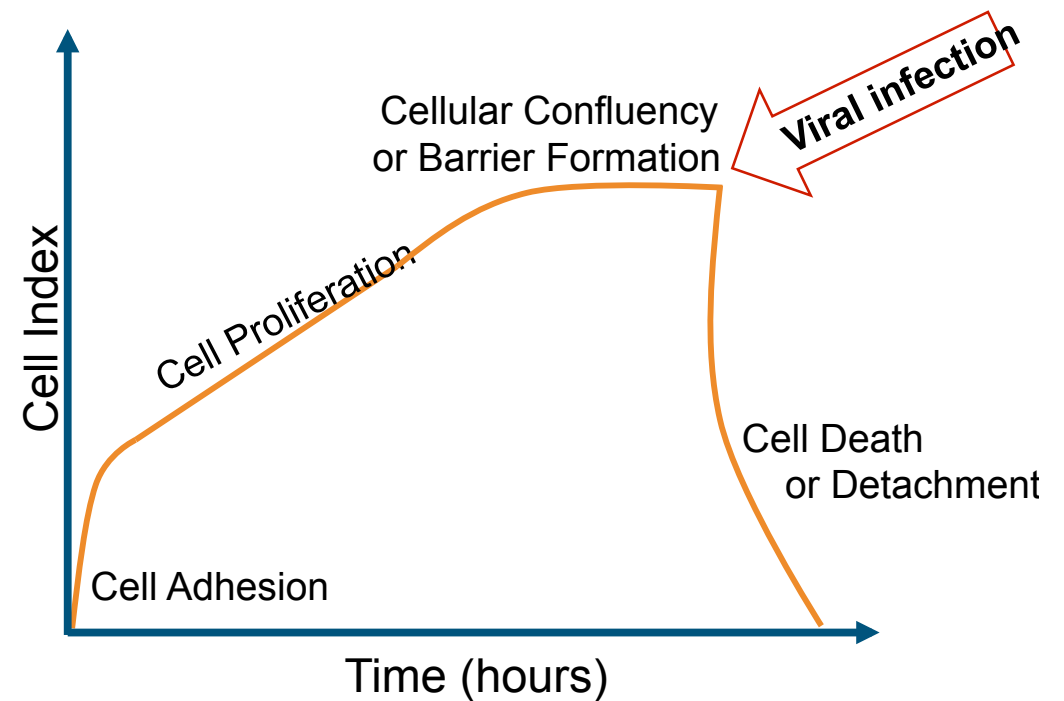


xCELLigence Real Time Cell Analysis Technology

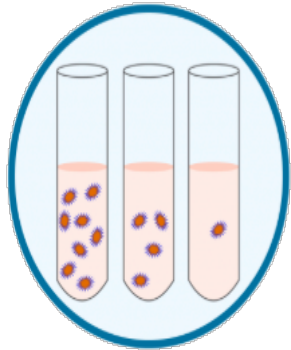
Principle of Detection & typical Cell Index Profile of viral CPE



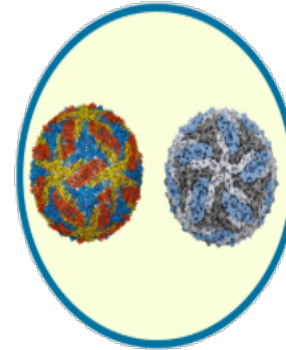
$\frac{\text{Measured Impedance (t)} - \text{Medium impedance}}{Z_0}$
Nominal impedance constant



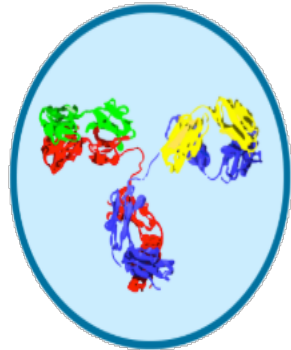
Key xCELLigence Virology/Vaccine applications:



1. Virus Titer Determination



4. Viral Fitness Comparison



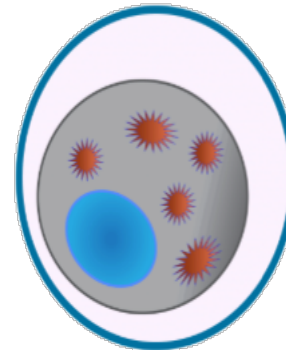
2. Neutralizing Antibody Detection & Quantification



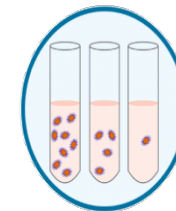
5. Virucide Efficacy



3. Antiviral Drug Studies

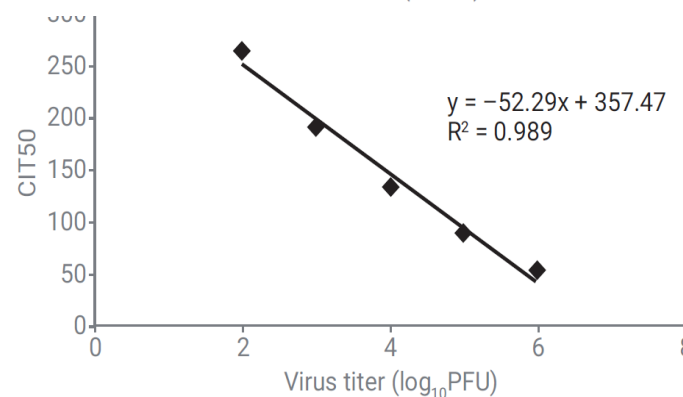
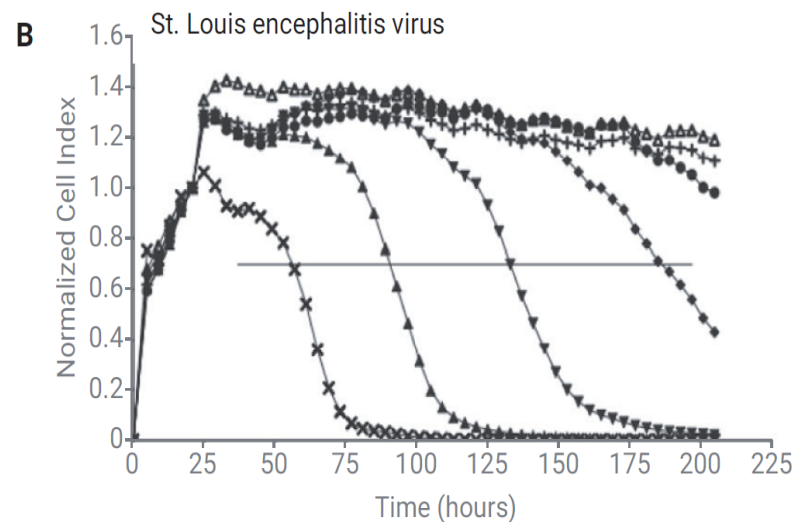
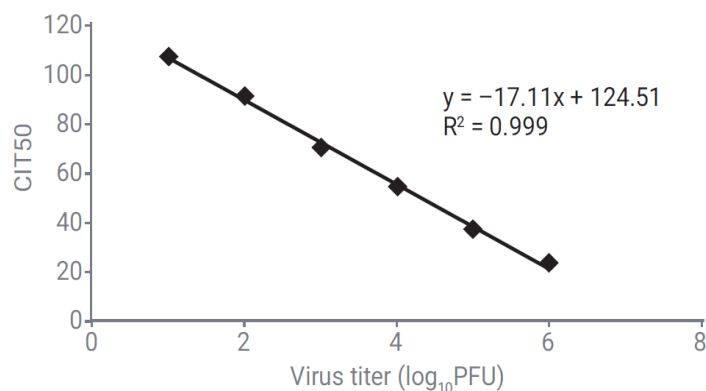
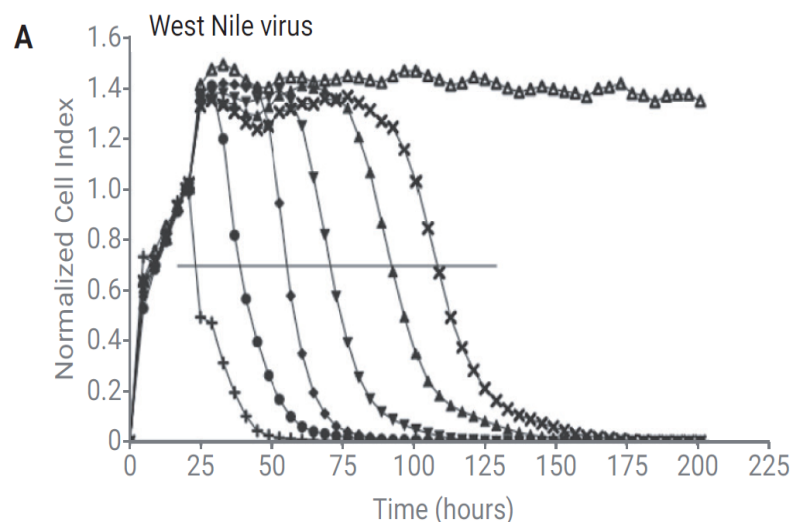


6. Oncolytic Viruses

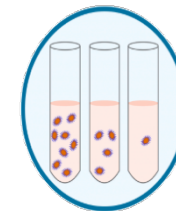


1. Virus Titer Determination

Impedance analysis shows distinctive cytopathic effect (CPE) patterns for certain viruses



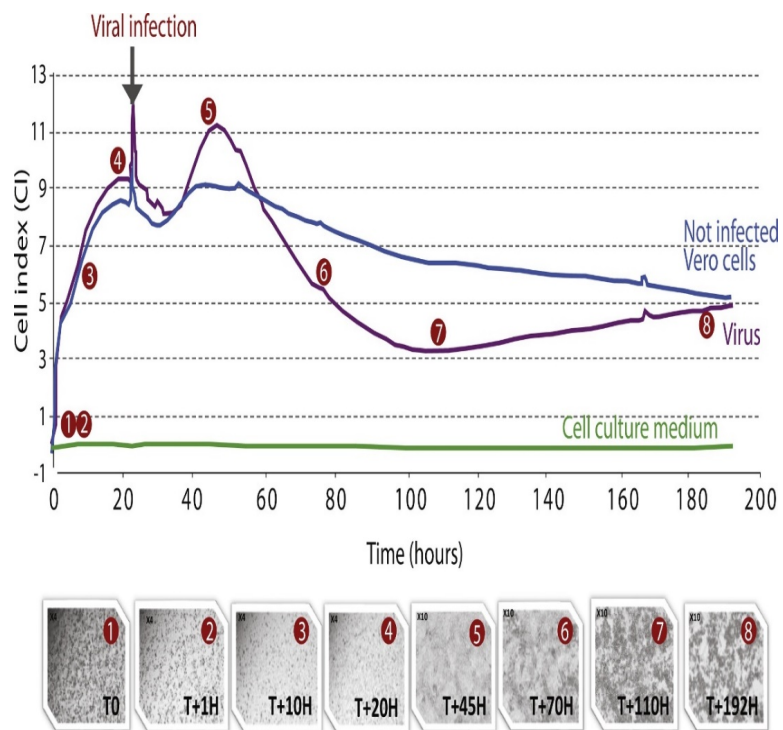
Journal of Virological Methods, volume 173(2), Fang, Y. et al., "Real-Time Monitoring of Flavivirus Induced Cytopathogenesis Using Cell Electric Impedance Technology," pages 251–8. Copyright 2011




1. Virus Titer Determination

Quantitative detection of virus infection and virus titer

Viral Infection – CPE (Cytopathic Effect)

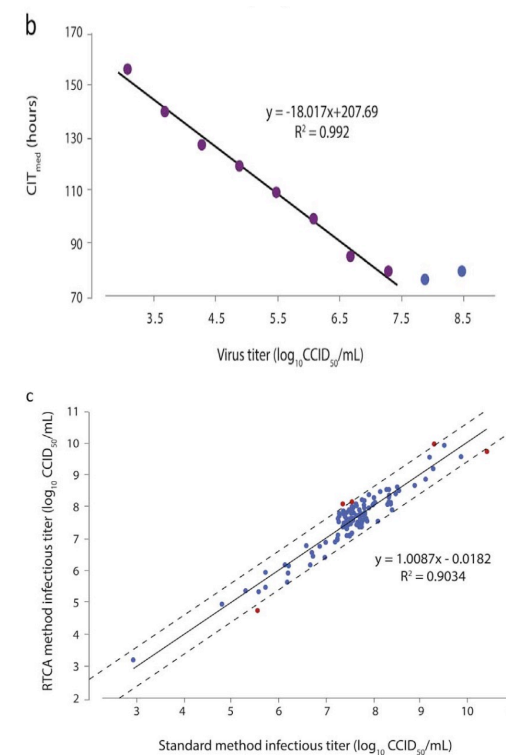
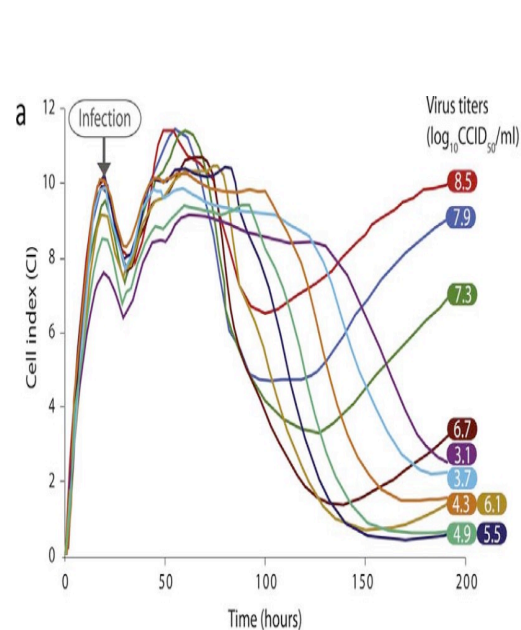




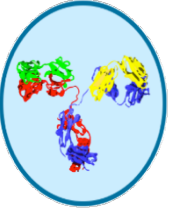
New Techniques in Viral CPE Assessment using Real Time Cell Analysis

Watch the webinar to learn how xCELLigence Real Time Cell Analysis technology can be used for vaccine development.

[Watch webinar on demand](#)

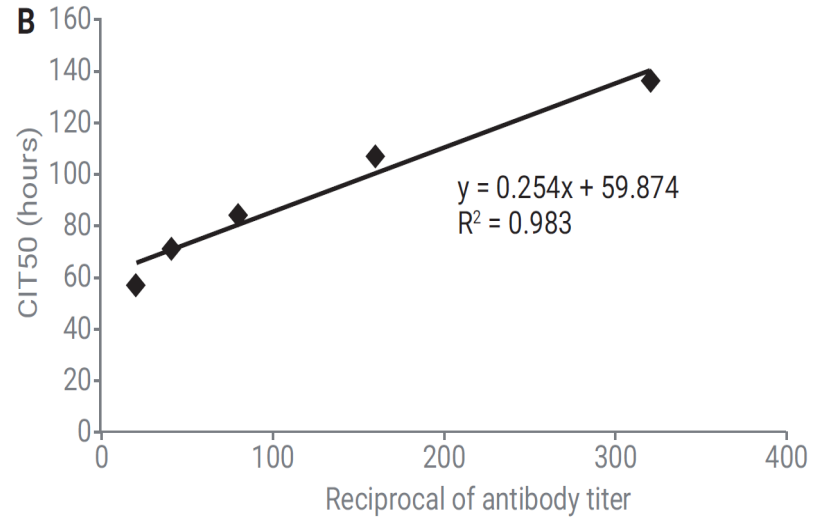
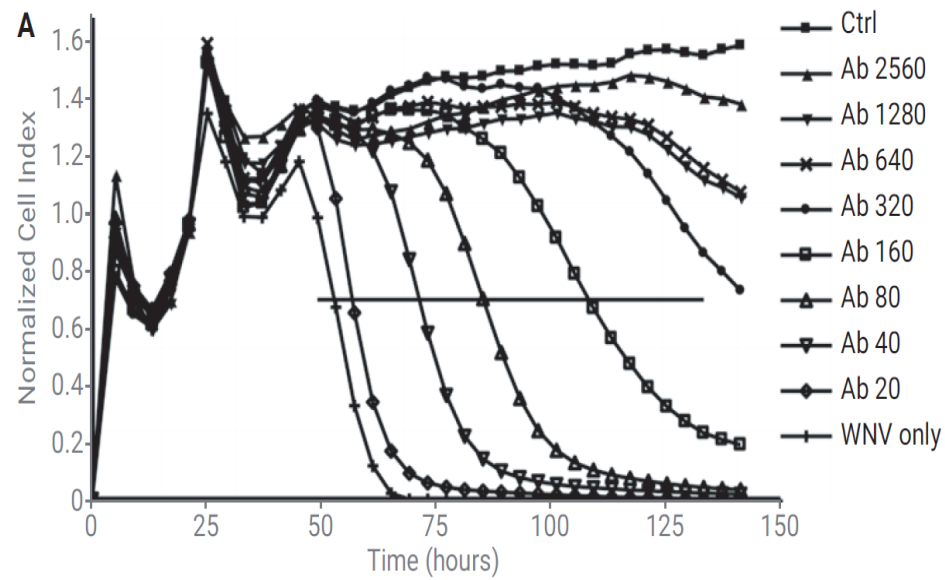


Journal of Virological Methods, volume 252 Charretier C., et al. "Robust real-time cell analysis method for determining viral infectious titers during development of a viral vaccine production process" pages:57-64.

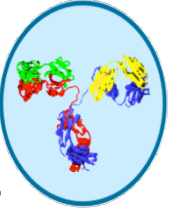


2. Neutralizing Antibody Detection & Quantification

The concentration of an antibody correlates with the onset of virus-induced CPE

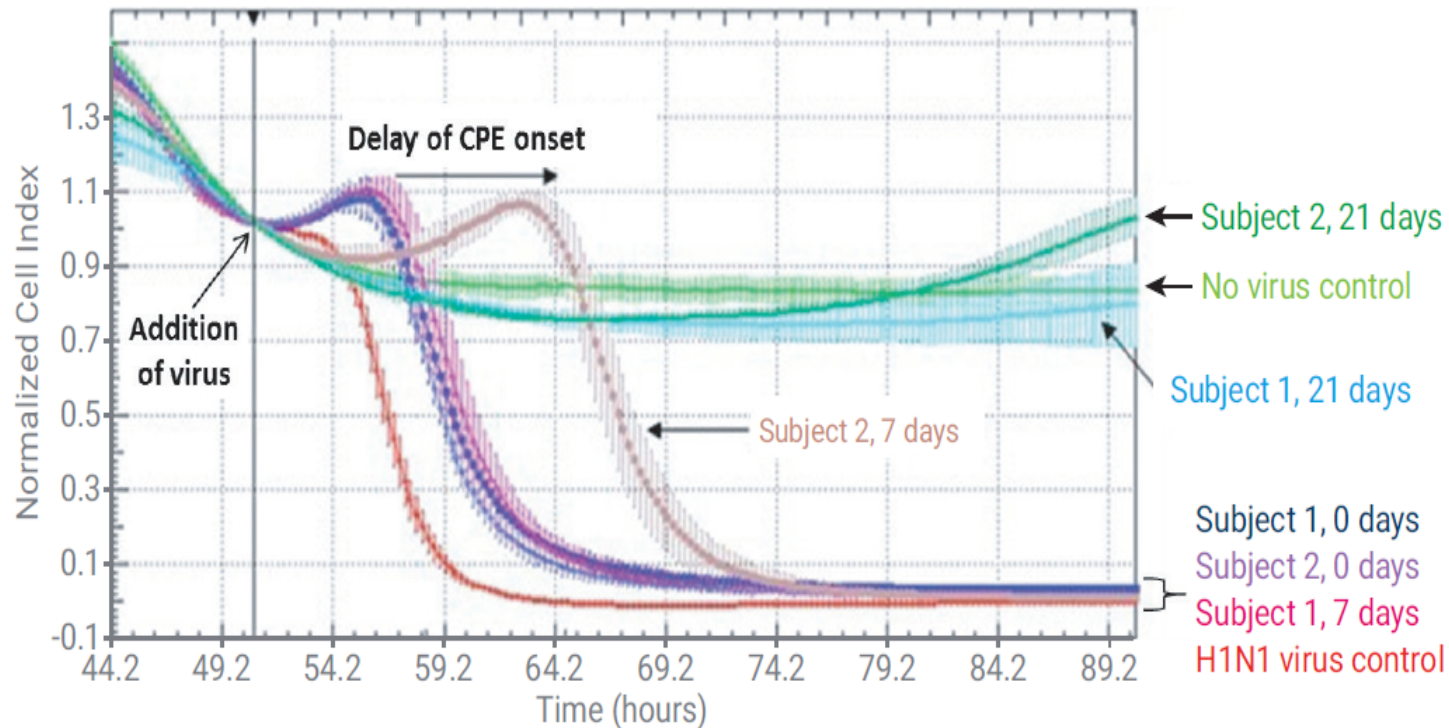


Journal of Virological Methods, volume 173(2), Fang, Y. et al., "Real-Time Monitoring of Flavivirus Induced Cytopathogenesis Using Cell Electric Impedance Technology," pages 251–8. Copyright 2011



2. Neutralizing Antibody Detection & Quantification

Quantitative assessment of vaccine efficacy and kinetics of virus resistance emergence

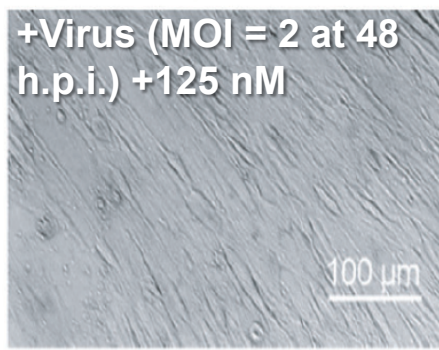
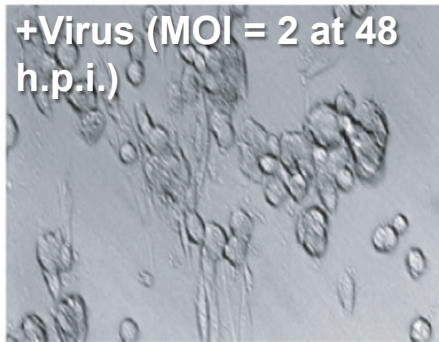
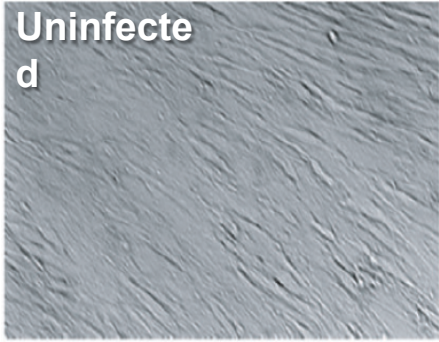
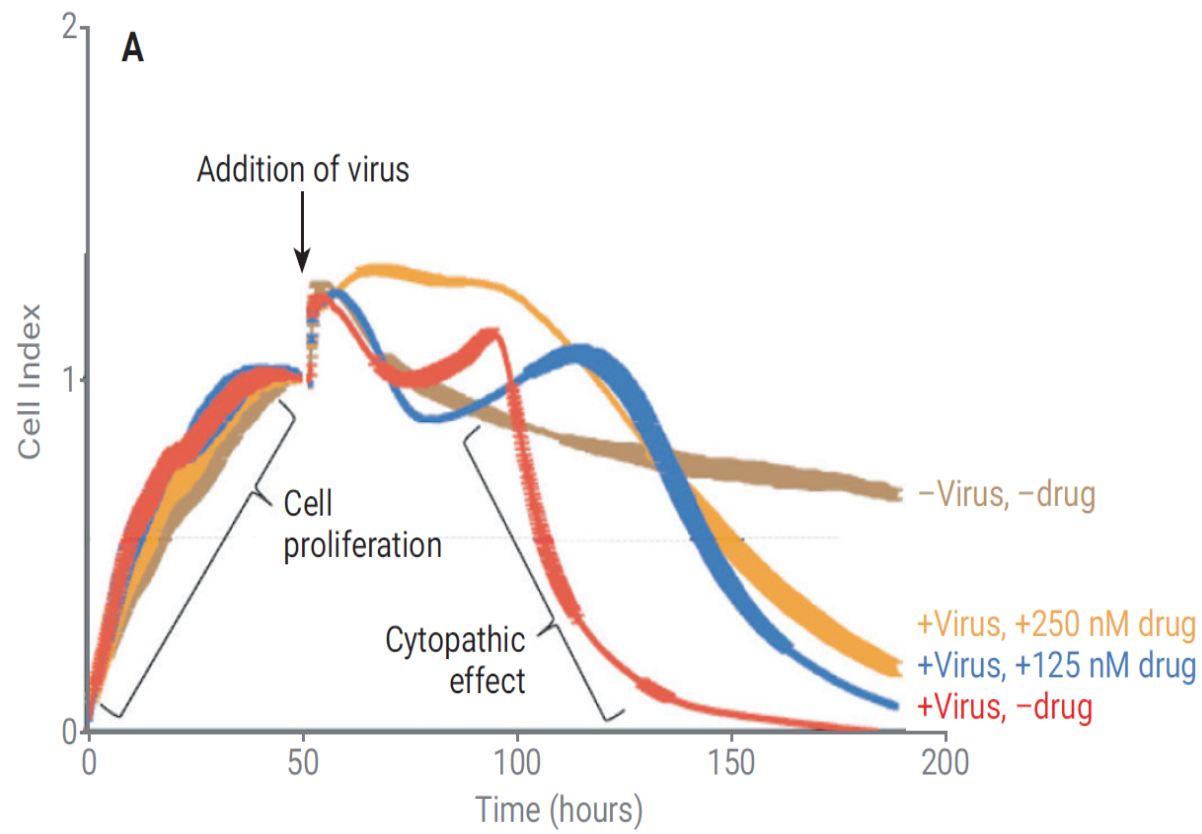


Asia Pacific Biotech News, volume 14(10), Lu, H. et al. "Label-free Real-time Cell Based Assay System for Evaluating H1N1 Vaccination Success," pages 31–32. Copyright 2010



3. Antiviral Drug Studies

High-throughput screening of antiviral compounds

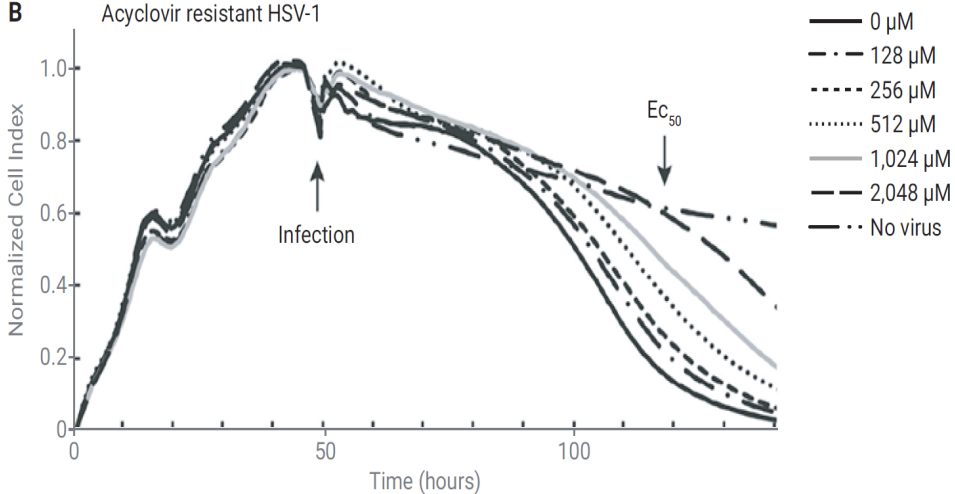
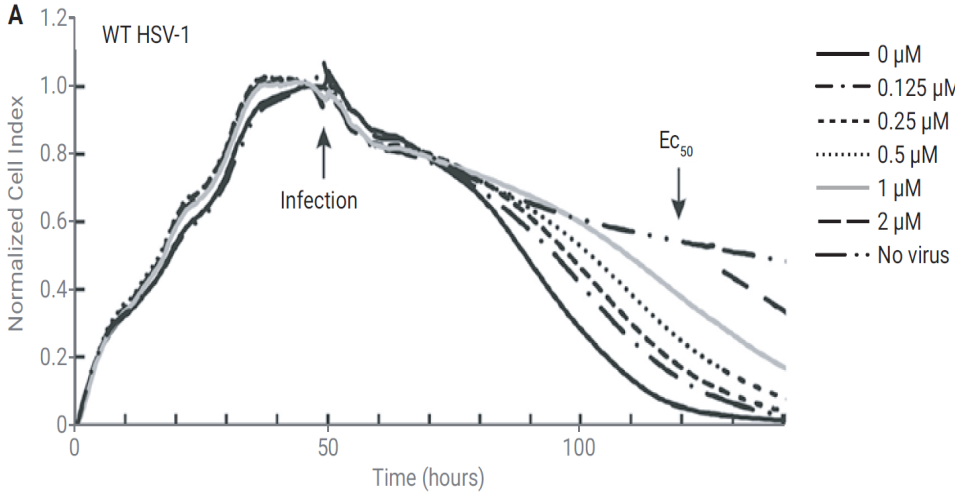


American Chemical Society Infectious Diseases, volume 3(6), Prasad, V. et al. "Cell Cycle-Dependent Kinase Cdk9 Is a Postexposure Drug Target Against Human Adenoviruses," pages 398–405. Copyright 2017

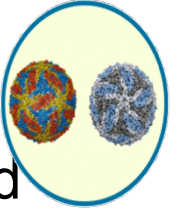


3. Antiviral Drug Studies

Screening for treatment sensitivity and resistance

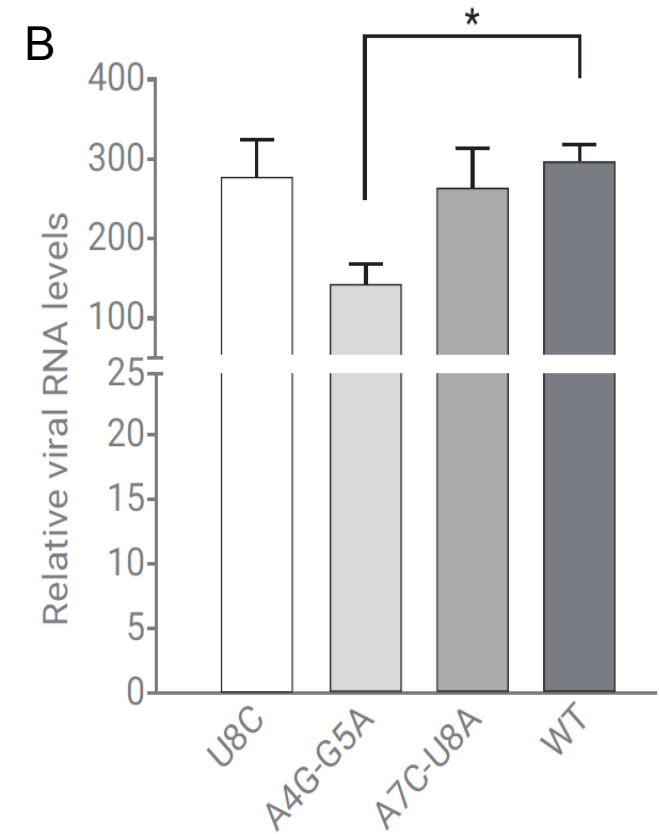
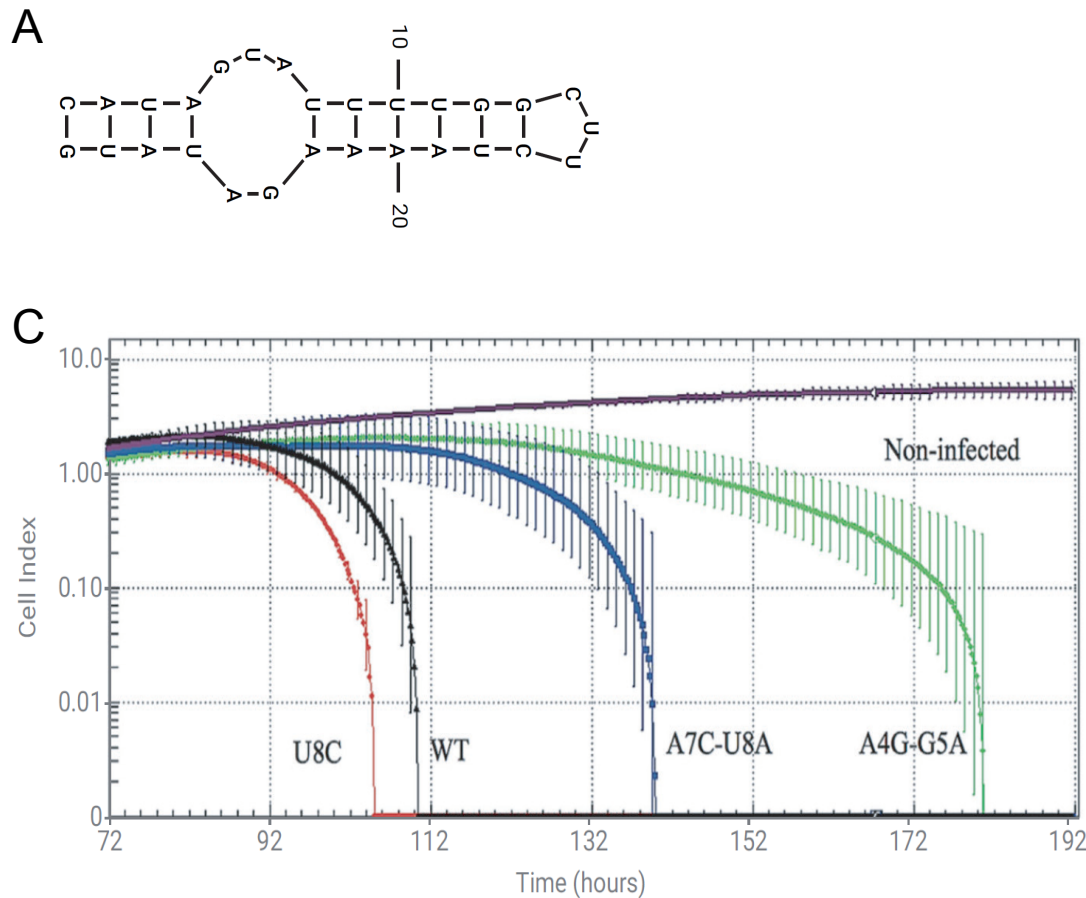


Journal of Clinical Microbiology, volume 54(8), Piret, J. et al. "Novel Method Based on Real-Time Cell Analysis for Drug Susceptibility Testing of Herpes Simplex Virus and Human Cytomegalovirus." Copyright 2016

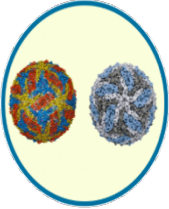


4. Viral Fitness Comparison

Assessing the degree to which a live viral vaccine has been attenuated

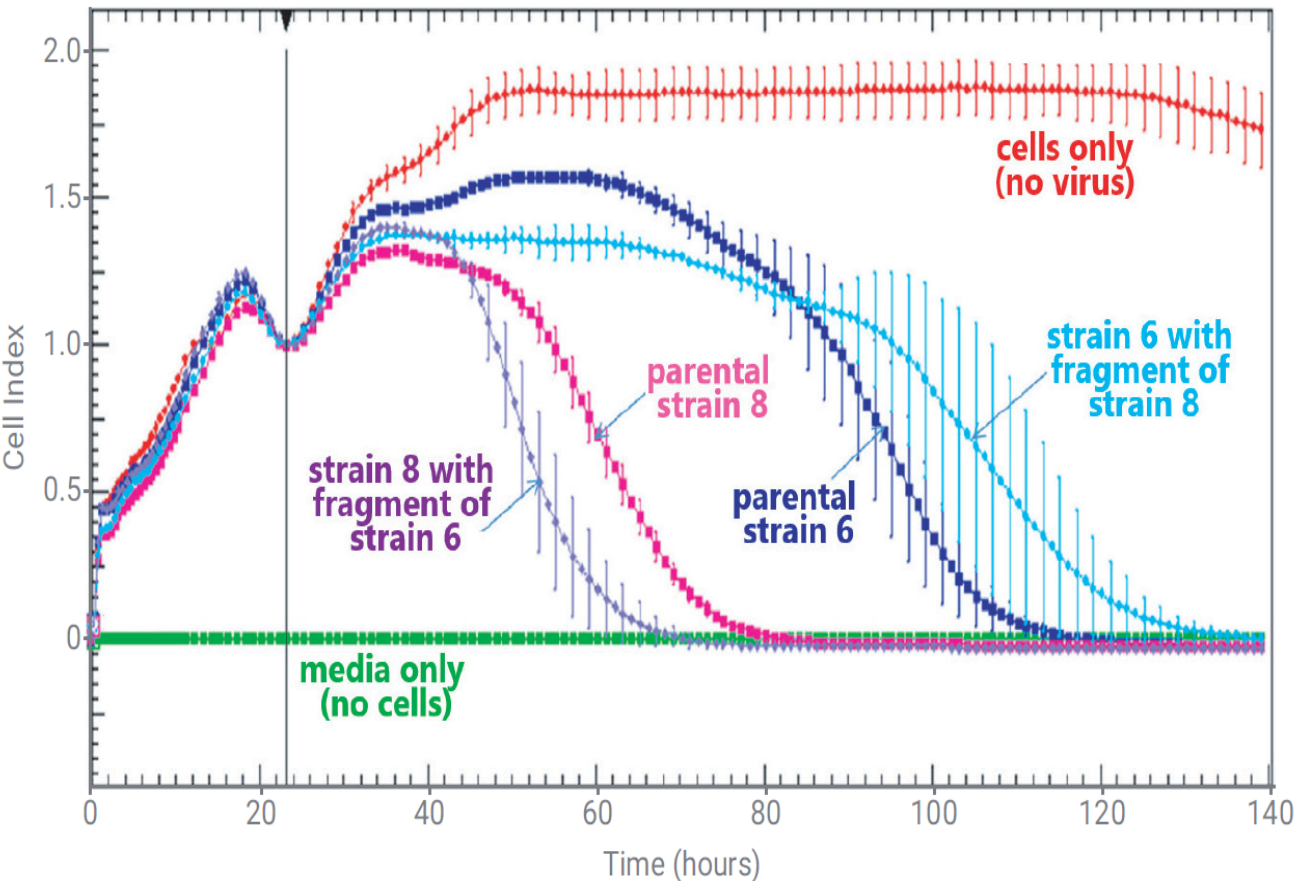


Virology, volume 476, Kim, S. H. *et al.* "Specific Nucleotides at the 3'-Terminal Promoter of Viral Hemorrhagic Septicemia Virus are Important for Virulence In Vitro and In Vivo," pages 226–32. Copyright **2015**,



4. Viral Fitness Comparison

Precise identification of viral phenotypes



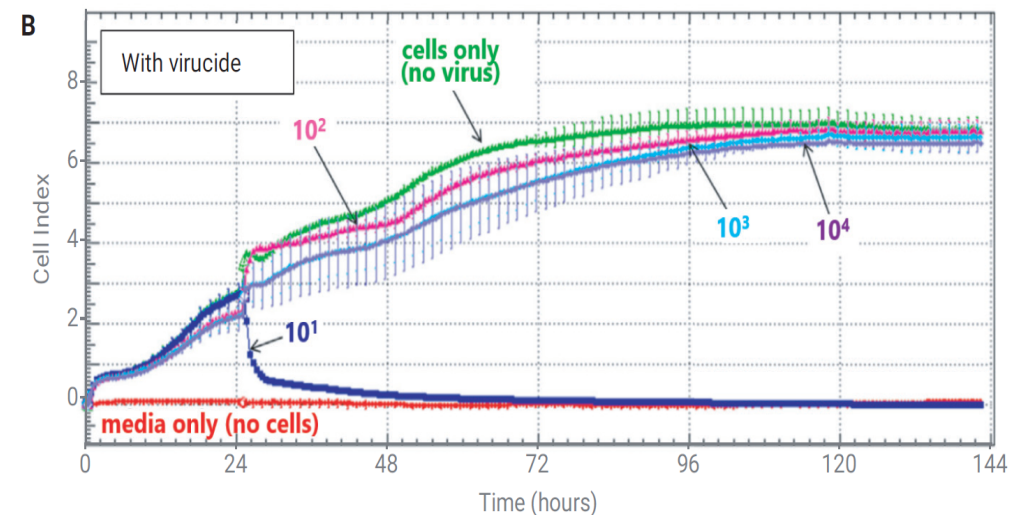
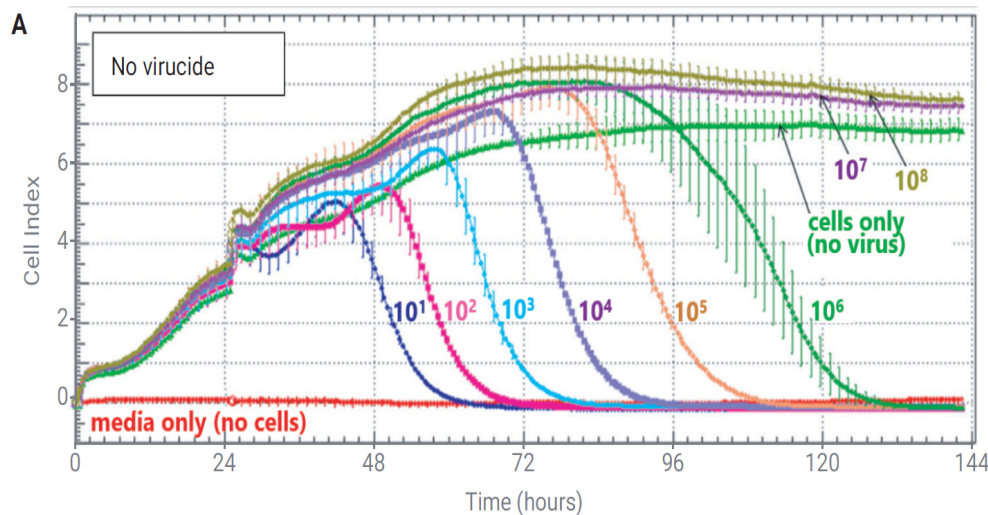
Veterinary Microbiology, volume 171 (1-2), Coetzee, P. et al. "Viral Replication Kinetics and In Vitro Cytopathogenicity of Parental and Reassortant Strains of Bluetongue Virus Serotype 1, 6 and 8," pages 53–

65. Copyright 2014

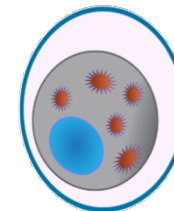


5. Virucide Efficacy

Precise and reproducible assessment of disinfectant efficacy and cytotoxicity

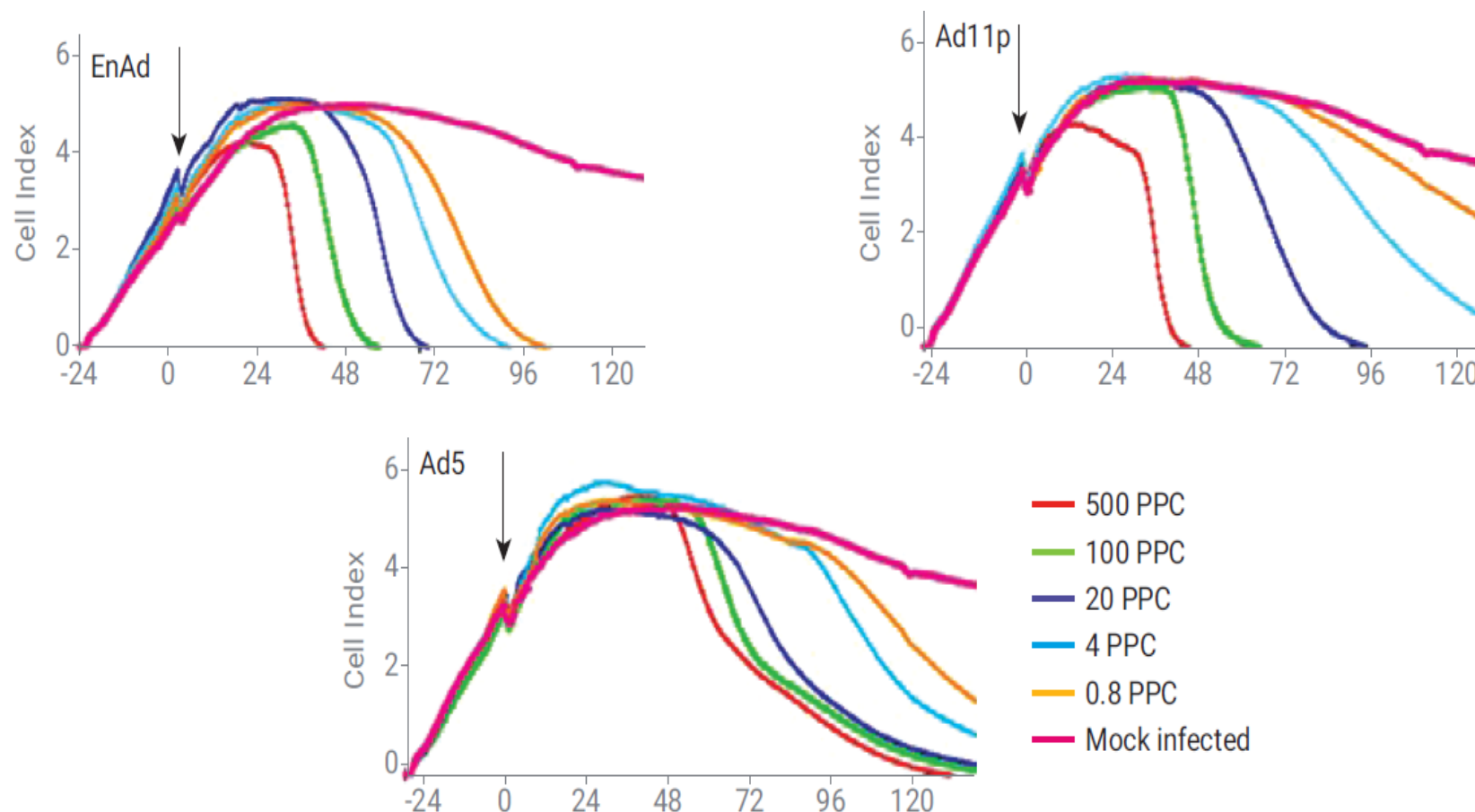


Journal of Virological Methods, volume 199, Ebersohn, K. *et al.* "An Improved Method for Determining Virucidal Efficacy of a Chemical Disinfectant Using an Electrical Impedance Assay," pages 25–28. Copyright



6. Oncolytic Viruses

Killing of A549 Lung Cancer Cells by Different Adenoviruses

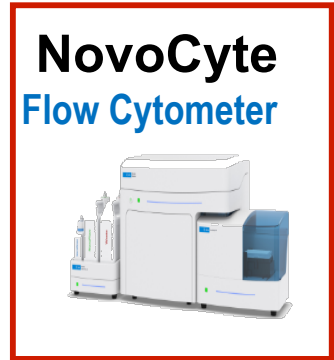


Molecular Therapy Oncolytics, volume 10(4), Dyer, A. et al. "Oncolytic Group B Adenovirus Enadenotucirev Mediates Non-Apoptotic Cell Death with Membrane Disruption and Release of Inflammatory Mediators," pages

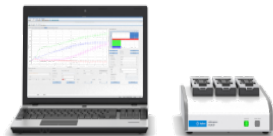
18-30. Copyright 2016

The xCELLigence Real Time Cell Analysis (RTCA)

Advancing discovery through innovation – all throughput needs are covered!



Jun 2008
RTCA SP



Mar 2009
RTCA DP



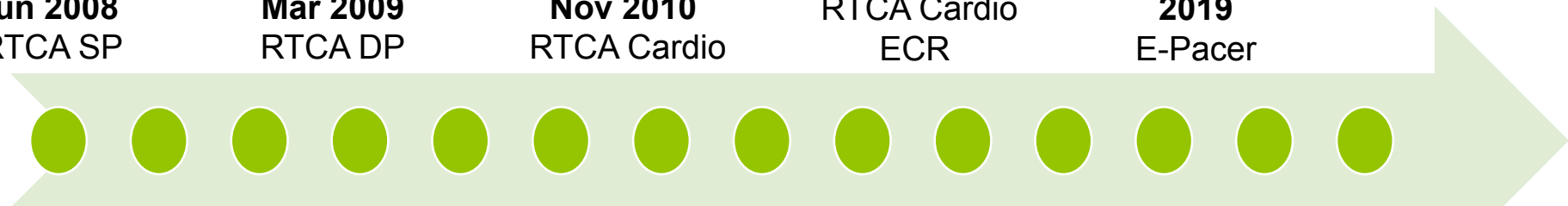
Nov 2010
RTCA Cardio



Oct 2014
RTCA Cardio
ECR



March 2019
E-Pacer



Sept 2008
RTCA MP



Nov 2010
RTCA HT



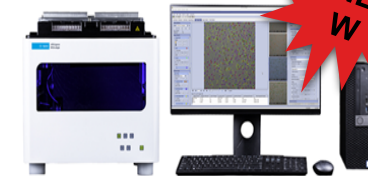
Jul 2012
RTCA iCELLigence



Apr 2018
RTCA S16



August 2019
eSight



Thank you!

