Representative IncuCyte publications for pre-clinical Vaccine research

Vaccine *In Vitro* Model Development

1. Roberts GC, Zothner C, Remenyi R, Merits A, Stonehouse NJ, Harris M. Evaluation of a range of mammalian and mosquito cell lines for use in Chikungunya virus research. Sci Rep. 2017 Nov 7;7(1):14641. doi: 10.1038/s41598-017-15269-w.

   Author affiliations: University of Leeds, University of Tartu

   This study details the evaluation of a panel of cell lines in search of those that would be suitable to study the infectious cycle of Chikungunya virus (CHIKV), a mosquito born infection causing fever, rash, and joint pain from inflammatory tissue damage. The authors searched for an assay that would enable the *in vitro* study for analysis such as transfection, luciferase assays, immunofluorescence, Western blots, and virus infections. **Incucyte live-cell analysis** was used as part of an assessment of C2C12 (myoblast) and Huh7 (hepatocyte) cells morphology changes and cell differentiation during infection.

Antibody neutralization assessment for vaccine candidates

2. Rothan HA, Zhong Y, Sanborn MA, Teoh TC, Ruan J, Yusof R, Hang J, Henderson MJ, Fang S. Small molecule grp94 inhibitors block dengue and Zika virus replication. Antiviral Res. 2019 Nov;171:104590. doi: 10.1016/j.antiviral.2019.104590. Epub 2019 Aug 14.

   Author affiliations: University of Maryland, Walter Reed Army Institute of Research, University of Malaysia, NIH

   This study explored an alternative approach to target host cell components that are used by viruses for infection or replication. **Incucyte live-cell analysis** was used to capture and analyze the effect of a potential treatment, CDDO-me, and subsequent antibody neutralization on the proliferation of ZIKV-infected cells. **Incucyte** measurements of proliferation and confluence were used to determine treatment effects.

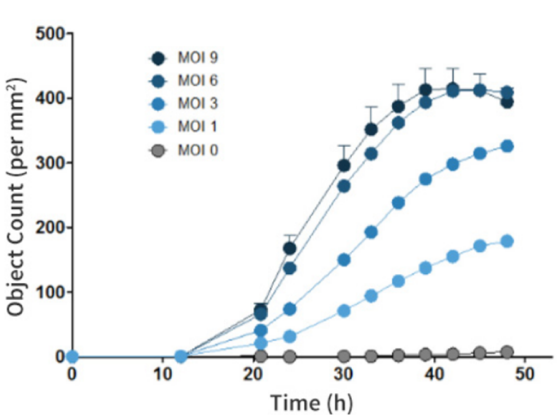
3. Brown AC, Reddy VRAP, Lee J, Nair V. Marek's disease virus oncoprotein Meq physically interacts with the chicken infectious anemia virus-encoded apoptotic protein apoptin Oncotarget. 2018 Jun 22;9(48):28910-28920. doi: 10.18632/oncotarget.25628. eCollection 2018 Jun 22.

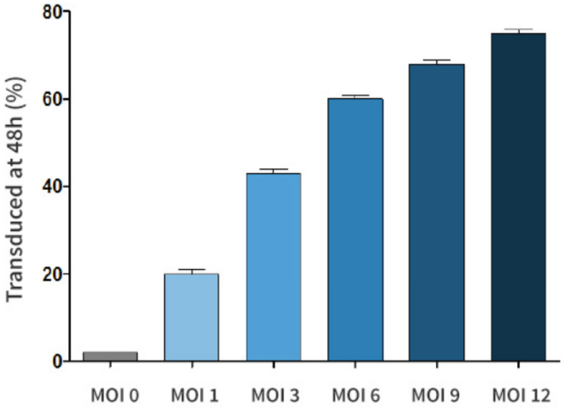
   Author affiliations: The Pirbright Institute, Wellcome Trust Genter for Human Genetics, Bristol Uiversity

   This study examined the interaction of critical proteins involved in viral infections of poultry : the Meq fromMarek's disease virus (MDV), and the apoptin protein in chicken anemia virus (CAV). Poultry commonly experience co-infections with these two viruses. **Incucyte live-cell analysis** and **IncuCyte Caspase-3/7 Apoptosis Reagent** were used to study the functional, kinetic effects of Meq-apoptin interactions on apoptosis on chicken fibroblast cells (DF-1) transfected with one or both proteins. Meq protein inhibited apoptin, which may have important implications for CAV vaccine produciton

Real time analysis of viral infection

- Fluorescent 'reporter' proteins enable quantification of virus infection
- Compare infection time-courses and efficiencies

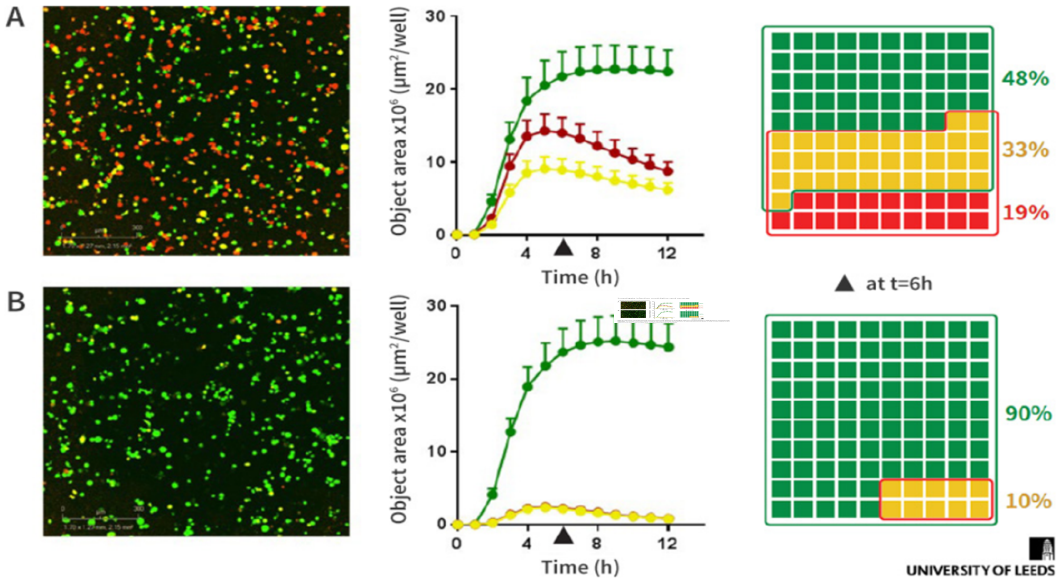




Time-course of nuclear GPF expression in A549 human lung carcinoma cells transduced with IncuCyte® NucLight Green lenti- virus. Transduction of cells with NucLight Green is titratable (HUVECs).

Investigate infection modulation and synergy

- Study factors that promote coinfection



BHK-21 cells were cotransfected with two foot-and-mouth disease virus (FMDV) replicons containing either a GFP (green) or mCherry (red) reporters. IncuCyte® coincident object analysis was used to quantify the proportion of cells expressing one (green or red) or both (yellow) of the replicons over time. Data courtesy of Dr. Morgan Herod (University of Leeds).

Vaccine Adjuvants to Improve Efficacy

6. Collinson-Pautz MR, Slawin KM, Levitt JM, Spencer DM. MyD88/CD40 Genetic Adjuvant Function in Cutaneous Atypical Antigen-Presenting Cells Contributes to DNA Vaccine Immunogenicity. PLoS One. 2016 Oct 14;11(10):e0164547. doi: 10.1371/journal.pone.0164547. eCollection 201.

   Author affiliations: Baylor College of Medicine, Bellicum Pharmaceuticals, Houston, TX

   The inclusion of immunological adjuvants in DNA vaccinations for cancer helps to activate tumor-specific T cells. The effect of a chimeric MyD88/CD40 (MC) adjuvant that can affect signaling in both innate and adaptive immune pathways was tested in the DNA vaccine against tumors. The MC adjuvant gave an enhanced CD8+ T cell response, higher levels of cytokine production, and greater anti-tumor response. **Incucyte live-cell analysis** was used to demonstrate increased rate of proliferation of NIH3T3 fibroblasts *in vitro* when activated by the MC adjuvant, as assessed by percent confluency.

7. Salyer, A.C.D. and David, SA. Transcriptomal signatures of vaccine adjuvants and accessory immunostimulation of sentinel cells by toll-like receptor 2/6 agonists Transcriptomal signatures of vaccine adjuvants and accessory immunostimulation of sentinel cells by toll-like receptor 2/6 agonists,
   Human Vaccines & Immunotherapeutics, 2018.14:7, 1686-1696, DOI: 10.1080/21645515.2018.1480284

   Author affiliations: University of Kansas, University of Minnesota

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https://www.accela.eu/essen-bioscience/incucyte-s3

This study attempted to use transcriptomal signatures of innate, immune stimulating molecules to identify new compounds that could be used as adjuvants to bolster vaccines. They identified several CC cytokines as candidates, with activation of non-hematopoietic cells such as fibroblasts and myocytes through TLR2. **Incuycte live-cell analysis** was used to assess PBMC chemotaxis with human foreskin (HFF) cells stimulated with TLR agonists.

Viral Vaccine Development

8. Tang, N, Zhang Y, Sadigh Y , Mo K., Shen, Z, Nair, V. and Yao, Y. Generation of a Triple Insert Live Avian Herpesvirus Vectored Vaccine Using CRISPR/Cas9-Based Gene Editing. Vaccines,2020 Feb 21;8(1). pii: E97. doi: 10.3390/vaccines8010097

   Author affiliations: The Pirbright Institute, Shandong Binzhou Animal Science and Veterinary Medicine Academy, Guangxi University, University of Oxford

   This group attempted to develop multivalent Herpesvirus of turkeys (HVT)-vectored vaccines that would be effective against multiple avian pathogens. They used a CRISPR/Cas9 gene editing protocol to create a triple insert HVT-VP2-fDgI-HA recombinant vaccine. The insertion into the viral genome was assessed, along with protein expression, and stability. This was the first reported successful creation of a triple gene insert HVT recombinant, **Incucyte live-cell imaging** was used to collect images to document the stability of triple insert recombinant HVT-VP2-gDgI-HA

Cancer Vaccines

10. Hassanzadeh G, Naing T, Graber T, Jafarnejad SM, Stojdl DF, Alain T, Holcik M. Characterizing Cellular Responses During Oncolytic Maraba Virus Infection.Int J Mol Sci. 2019 Jan 29;20(3). pii: E580. doi: 10.3390/ijms20030580.

    Author affiliations: Childrenos Hospital of Eastern Ontario Research Institute, Queenos University Belfast, Carleton University,

    Oncolytic viruses, such as Marba viruses, are used as a strategy for treating a variety of cancers as they kill malignant cells and have lower toxicity for normal cells. This study used an interferon-sensitive mutant Maraba virus (MG1), to better understand how oncolytic viruses interact with the host immune system and kill tumors. **Incucyte live-cell analysis** was used to monitor the rate of viral propagation through confluency of GFP signal in WT and S51 mouse embryonic fibroblasts (MEFs). **Incucyte® Cytotox Red Reagent** was used to monitor the infection of mock- or infected cells over the time course of infection. Incucyte was also used to observe the effect of knocking down the translation factor eIF5B on cell viability and rate of infection with MG1.

11. Sahin U, Derhovanessian E, Miller M, Kloke BP,, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017 Jul 13;547(7662):222-226. doi: 10.1038/nature23003. Epub 2017 Jul 5.

    Author affiliations: Biopharmaceutical New Technologies (BioNTech) Corporation, University Medical Center of Johannes, Gutenberg University, Langenbeckstraße,4EUFETS GmbH, Vollmersbachstraße 66, Medical University of Vienna, German Cancer Research Center (DKFZ),Im Neuenheimer, University Medical Center Mannheim, Cluster for Individualized Immunointervention e.V, Hölderlinstraße.

    This paper describes the development of individualized mutanome vaccines using RNA-based poly-epitopes for a first-in-human treatment for melanoma. A comprehensive identification of each individual

patient's mutations was performed along with computational prediction of the neo-epitopes. Customized vaccines were prepared based on individual mutational status. The **Incucyte system** and **Incucyte Caspase-3/7 Green Apoptosis Reagent** were used as part of their workflow to assess the apoptosis of melanoma cells co-cultured with effector T cells in vitro, assisting with vaccine characterization.

Bacterial Vaccines

12. Lalsiamthara J, Senevirathne A, So MY, Lee JH. Safety implication of Salmonella based Brucella vaccine candidate in mice and in vitro human cell culture. Vaccine. 2018 Mar 27;36(14):1837-1845. doi: 10.1016/j.vaccine.2018.02.069. Epub 2018 Feb 24.

    Author affiliations: Chonbuk National University

    The development of human anti-*Brucella* vaccine was not possible due to safety and formulation issues. This paper describes the assessment of an anti-*Brucella* vaccine using *Salmonella* as a vector to deliver *Brucella* proteins to increase vaccine safety. The vaccine was tested *in vivo* to determine lethal dose 50 (LD50) of vaccine strains in a mouse model as well as interaction with cultured human cells. An **Incucyte system** and **Incucyte® Cytotox Green Reagent** were used to assess cytotoxicity of the bacterial strains on macrophages and human Caco-2 cells (Caucasian colon adenocarcinoma). **Incucyte® Annexin V Reagent** was also used to quantify apoptosis in human cells infected with bacteria. Additionally, **Incucyte** was used as part of a "home-brew" assessment for nitric oxide production.