# HIGH CONTENT SCREENING IN ACTION

## a powerful tool to identify novel antivirals and vaccines

High content screening (HCS) has played a significant role in infectious disease research and drug discovery to date and can be a strong tool in the age of COVID-19 and beyond. With a focus on viral diseases, this article will describe how HCS has been used by laboratories around the world to help with three key interventional strategies: drug repurposing to fast track a previously-approved drug to treat new diseases; development of new therapeutics; and vaccine development.

By Dr Karin Böttcher

As being experienced with the COVID-19 pandemic and formerly seen by outbreaks from Ebola, MERS, SARS and Zika, infectious diseases caused by bacterial, as well as viral and parasitic pathogens, are a major burden to global health and without effective vaccines or anti-microbial treatments can be devastating. The increased globalisation of modern society, with travel and trade that facilitates the spread of emerging and re-emerging infectious diseases, only underscores the critical need for new preventative and therapeutic approaches.

As pharmaceutical companies and medical researchers around the globe push to fast-track drug discovery, development and clinical trials in response to COVID-19, they are focusing on three key interventional strategies:

- Repurposing of existing drugs
- Development of new therapeutics
- Development of vaccines

To accelerate this process, they can leverage existing technologies, including high content screening (HCS), which is already well-established as playing a significant role in viral disease research and drug discovery.

Through its wide range of applications, from basic research of viruses through to drug efficacy screening, virus neutralisation and early toxicity testing, HCS helps with all of the aforementioned strategies being adopted to address COVID-19.

### The value of HCS in virology

High content screening, also known as high content analysis, combines high-throughput automated cellular imaging with sophisticated image analysis to extract quantitative multi-parametric data at the single-cell level.

Originally developed as a complementary technology to traditional biochemical high-throughput screening (HTS) in drug discovery, today HCS is established in a far broader area of the life science space as an unbiased and quantitative imaging method to assess cellular behaviour and function.

In the context of virology HCS brings:

- More meaningful results

Screening can be performed within the context of a host cell, allowing drugs that target host cell or

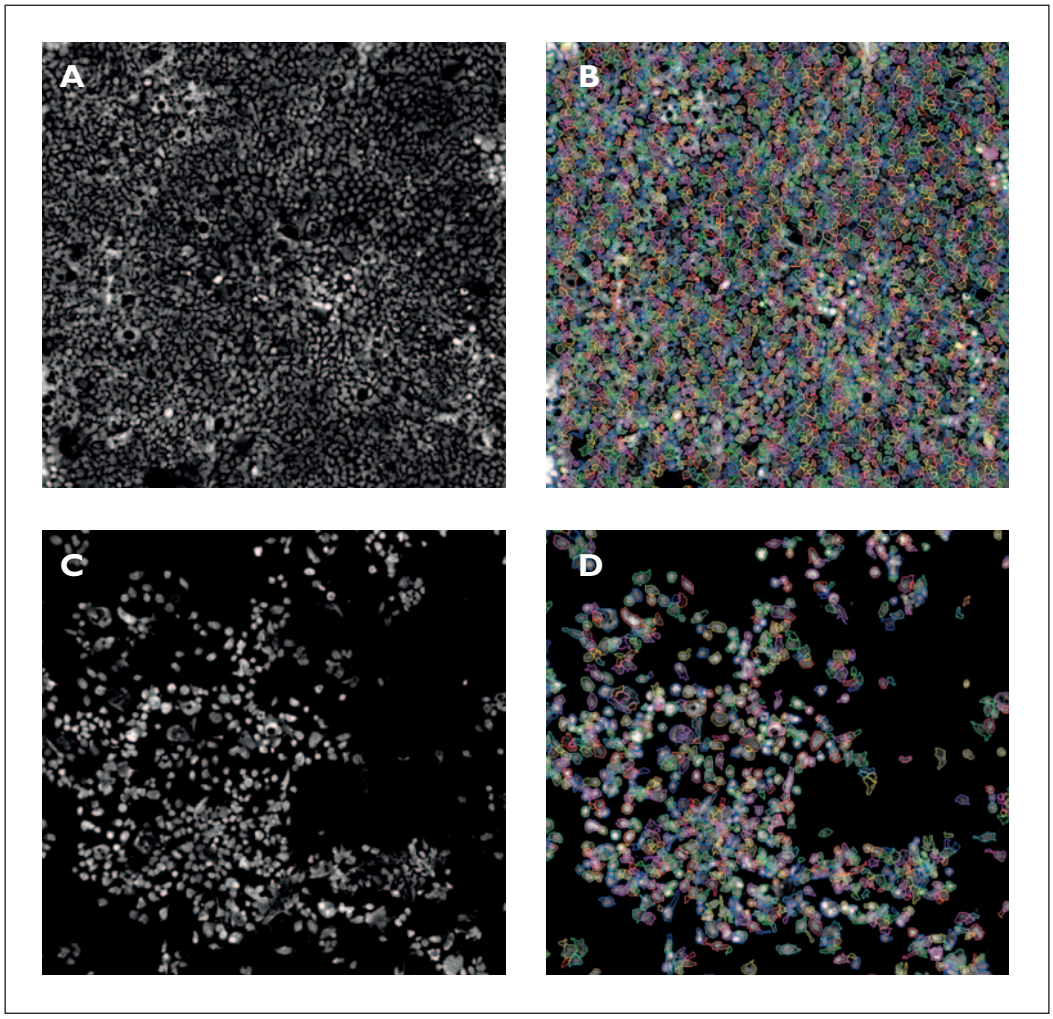


**Figure 1**
Digital phase contrast images from the drug-repurposing screen against SARS-CoV-2 performed on the PerkinElmer Operetta CLS System. Caco-2 cells were grown to confluence and left uninfected (A + B) or were incubated with SARS-CoV-2 virus for 48 hours prior to fixation (C + D). Quantification of viral growth was performed in Harmony software by quantifying Caco-2 cell counts/viability (B + D). *Image Courtesy of Dr Bernhard Ellinger, Fraunhofer IME, Hamburg*

virus proteins to be discovered in the same screen. HCS allows for the analysis of complex processes such as host cell entry, intracellular trafficking or cell-to-cell spread using fixed samples or live-cell imaging. HCS also supports the use of more complex cellular models such as lung organoids, providing a more physiologically-relevant model system for screening for compounds against respiratory viruses such as SARS-CoV-2.

● More information from cellular samples
Fully-automated imaging and unbiased quantitative image analysis exploits the full potential of microscopy, allowing characterisation of pathogens as well as host cell phenotypes. Infection rates can be determined with high sensitivity, since individual infected cells can be identified. The process of infection can be described with up to hundreds of readouts per cell, allowing the linkage of infection rates to other readouts such as host cell morphology or host cell signalling.

● More questions answered simultaneously
Data sets are more information-rich compared to non-imaging read-outs, allowing a number of conclusions to be drawn from one experiment, such as drug efficacy against a pathogen as well as drug toxicity on host cells.

● More samples analysed
The automation of imaging tasks allows the analysis of significantly more samples compared to conventional microscopy, enabling genome-wide or kinome-wide siRNA screens, screening of large compound libraries, or targeted libraries such as FDA-approved drug libraries.

## HCS and drug repurposing in action

### SARS-CoV-2

One approach to fast-track medicines is to build on previous knowledge and repurpose existing drugs. Approved drugs that have been developed against other diseases have established safety profiles and,

therefore, can give a head start for COVID-19 drug development. However, the efficacy against SARS-CoV-2 needs to be proven first. This is usually done by a drug repurposing screen; a screen of FDA-approved or shelved drugs for efficacy against a new virus or disease in a relevant cell model. As FDA-approved drug libraries contain drugs directed against a variety of different drug targets, HCS is a particularly useful technology as it is a phenotypic method that works independently of the drug target. It can also identify drugs that either work directly against the virus or target the virus inside its host cells.

Researchers from the Fraunhofer Institute for Molecular Biology and Applied Ecology, Screening Port Hamburg, performed a large-scale SARS-CoV-2 drug repurposing screen on an HCS system to identify potential candidates for progression towards clinical studies[1]. The screening collection included 5,632 compounds, of which more than 3,000 have undergone clinical investigations previously, across 600 different indications or diseases. The high content assay screened exclusively for virus-induced cytotoxicity, using both digital phase-contrast imaging and fluorescent imaging of the host cell nucleus via Hoechst staining. Virus inhibition calculated from digital phase images correlated well with nuclear staining readouts, thereby showing how a simple label-free assay provides reliable results and an average Z' value of 0.75. The screening strategy consisted of a screen using a single dose, followed by testing responses to a range of concentrations. Of the 5,632 compounds, 19 inhibited SARS-CoV-2 with high potency. From these 19 compounds, 90% had not been previously reported to be active against SARS-CoV-2. Some were also targeting potential drug targets in the host cell.

However, the screen also confirmed *in vitro* activities against SARS-CoV-2, which had been described earlier.

Results were further validated by comparing the antiviral activities identified with results from previous screens against related coronaviruses such as SARS-CoV or MERS, which may have the benefit of identifying a treatment that is applicable to a wider range of coronavirus-related illnesses (**Figure 1**).

**MERS-CoV**

Previously, researchers from the Institute Pasteur, Korea (IPK) have used HCS for a drug repurposing screen against the Middle East Respiratory Syndrome Coronavirus (MERS-CoV)2. The MERS virus emerged in 2012 in Saudi Arabia and to date (May 2020) has caused 2,494 laboratory-confirmed cases and 858 deaths (WHO, 2020 https://www.who.int/emergencies/mers-cov/en/). Compared to the screen run at the Fraunhofer Institute, the IPK's MERS-CoV screen was performed on a similar number of compounds; however, it used a different cell line (Vero cells) and an assay based on measuring the expression levels of the virus spike protein with immunofluorescence. Cytotoxicity of the compounds on host cells was also measured by counting the number of host cell nuclei based on Hoechst staining. From the initial 5,406 compounds, 12 FDA-approved drugs were selected and tested in time-of-addition studies to investigate whether they act on early or late stages of the viral life cycle (pre- or post-entry). The time-of-addition study is also based on the virus spike protein immunofluorescence assay measured; however, compounds were added one hour prior to infection or at zero-, one-, two-, three- and four-hour post-infection. In this way, researchers are able to understand, for example, if drugs block the endocytosis of the virus or act on the later stages (**Figure 2**).

**ZIKV**

In fairly recent history, another virus gaining attention in the news headlines was the Zika virus (ZIKV). Zika is a flavivirus that is primarily transmitted via Aedes mosquitoes and sexual contact. Maternal Zika virus infection during pregnancy has the potential to result in significant birth defects, including microcephaly, and the virus is also strongly associated with neurologic complications such as encephalitis, meningoencephalitis, and development of Guillain-Barre syndrome. There are currently no targeted therapeutics for its treatment, nor is there a vaccine. HCS was used in a study that aimed to identify novel ZIKV inhibitors from a library of 774 existing, FDA-approved drugs that could potentially be used for the treatment of Zika infection[3]. Using an immunofluorescence assay measuring Zika viral envelope protein expression, 20 compounds with antiviral activity were selected. Given the evidence for sexual transmission of the virus and the dramatic effect of Zika on the fetal neuronal development, selected compounds were further validated in cell lines derived from genital, placental or neural tissues. Compounds that were found to have inhibitory properties included those with established anti-flaviviral activity, as well as some that had not been previously associated with antiviral properties. In addition, some of the compounds identified had already been approved for use in pregnancy.

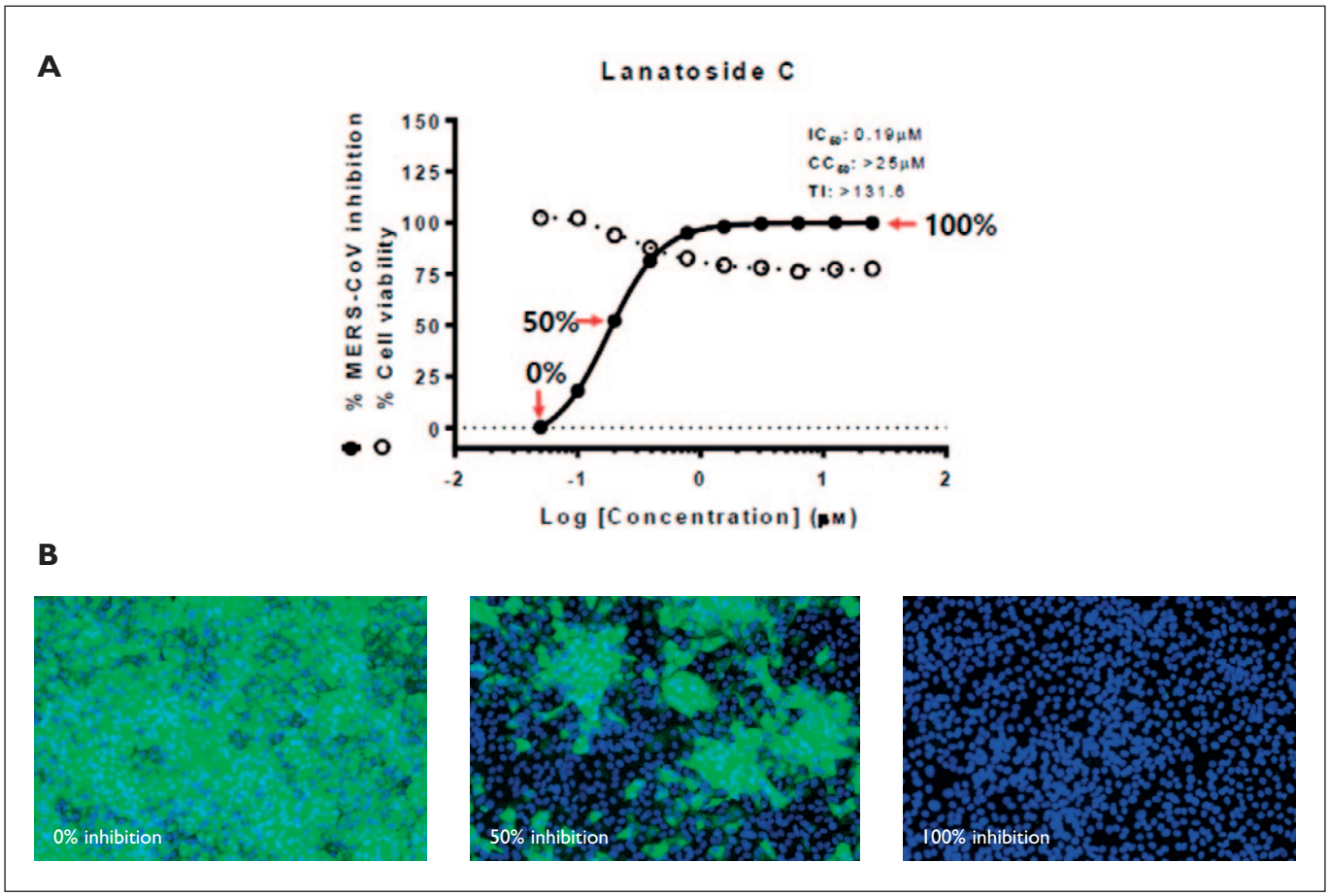


**Figure 2**
A MERS-CoV drug repurposing screen performed on the PerkinElmer Operetta system. A) Dose-response curve for Lanatoside S, illustrating the % inhibition of MERS-CoV in Vero cells as well as the % cell viability at the respective concentration. B) Representative immunofluorescence images showing MERS-CoV inhibition based on measuring the expression of MERS-CoV spike protein. *Image Courtesy of Dr Marc Windisch, Institute Pasteur Korea*

**EBOV and MARV**

Ebola and Marburg viruses cause severe hemorrhagic fevers, with mortality rates of more than 90% in instances of disseminated disease. A 2014 outbreak in West Africa resulted in around 30,000 reported cases and more than 11,000 deaths[4]. The high mortality associated with these diseases, combined with the current lack of FDA-approved vaccines and therapeutics, would seem to make them ripe for study. However, both pathogens are considered biosafety level 4, meaning they must be studied under particularly strict conditions. Their stability in aerosolised form, high case-fatality rate and lack of approved vaccine or treatment has limited their study somewhat, until recently.

Using an HIV-1-based surrogate assay derived from authentic EBOV and MARV isolates, a team of researchers was able to study EBOV at a biosafety level 2 containment. Infectivity of EBOV and MARV components was confirmed via immunostaining and high content imaging. One long-standing strategy regarding the development of therapeutics was to target filovirus entry, which is mediated entirely by glycoprotein (G-protein) heterodimers of two subunits, GP1 and GP2. A potential antihistamine lead encouraged the team to begin investigating this further. Through the use of docking studies, the researchers discerned that H1 receptor blockers bind the same EBOV glycoprotein as toremifene, an aromatase inhibitor previously known to destabilise the viral glycoproteins. Based on this, the research team decided to formally study the potential of first-generation histamine receptor blockers as anti-filovirus agents, due to their antagonism of G-protein coupled receptors.

## HCS and new therapeutic development in action

The development of novel therapeutics depends upon a detailed understanding of the virus and its interaction with the host cell. Viruses cannot replicate themselves; instead, they rely on a host cell to sustain their lifecycle. To enter the host cell and employ the cell's machinery for replication, viruses interact with many different host-cell proteins. These protein-protein interactions are potential drug targets as their inhibition provides a means of inhibiting viral growth. In principle, antiviral drug

treatments can be directed against a virus protein, against a host-cell protein or a protein-protein interaction, the latter two having the advantage of being less prone to the development of drug resistance by the virus.

### CHIKV or VEEV

siRNA screening using a high content approach can serve as a tool to identify cellular proteins required for virus replication, thus advancing basic virus research as well as drug target identification. The principle is to knockdown cellular proteins using an siRNA and analyse how that knockdown affects virus replication. Researchers at USAMRIID have recently performed this type of screen on an HCS system to identify host-cell factors required for Alphavirus infection[5]. Alphaviruses such as Chikungunya (CHIKV) or Venezuelan equine encephalitis virus (VEEV) are significant human pathogens causing arthritis or fatal encephalitis in humans. Targeting proteins required for host-cell trafficking, 140 different siRNAs were transfected into Hela cells that were subsequently infected with VEEV and then analysed for the presence of the virus surface protein E2 on the host cell surface. The screen revealed a novel finding: alphaviruses heavily rely on regulators of the actin cytoskeleton. Further assays helped to characterise the actin remodelling pathway and provided the hypothesis that actin foci are induced and employed by the virus to mediate virus E2 protein transport to the cell surface.

## HCS and vaccine development

While COVID-19 therapeutics will be life-saving for patients that have the disease, it is vaccines that will make a difference for the rest of the population. Vaccines help a person to generate an immune response against an infection without first being exposed to the pathogen.

The gold standard functional assay to confirm if a person has developed a protective immune response is a cell-based neutralisation assay. This assay tests if a patient's serum can prevent virus infection of new host cells. However, this assay requires live virus, which can, in the case of SARS-CoV-2, only be handled in biosafety level 3 laboratories.

Researchers from the National Institute of Diagnostics and Vaccine Development in Infectious Diseases in Xiamen, China, have therefore developed a robust neutralisation assay based on a pseudovirus bearing the SARS-CoV-2 S-protein that can be handled under reduced safety measures[6]. Two different virus pseudotypes expressing the GFP reporter gene were created based on vesicular stomatitis virus, one expressing full-length S-protein and one expressing a truncated version (Sdel18). Using HCS combined with fluorescence quantification and image data storage and analysis systems, the group first optimised VSV packaging.

They identified the VSV expressing SARS-CoV-2-Sdel18 as being packaged more efficiently compared to the full-length S-protein bearing VSV. By quantifying the number of GFP positive cells, they also determined that BHK21 cells expressing the human ACE2 protein were most susceptible to infection with the developed pseudovirus. A live-cell time course assay on the HCS system then identified the optimal timepoint for the pseudovirus neutralisation assay – 12-24-hour post-infection – which is much faster than assays run with live SARS-CoV-2 that peak after 48 hours. The pseudotype infection model was finally tested with neutralising monoclonal antibodies from mice as well as sera from convalescent patients that recovered from COVID-19. In summary, the authors developed a pseudotype infection model that can greatly benefit both vaccine development as well as therapeutic antibody development against SARS-CoV-2.

## Conclusion

Given the current urgent need for effective treatments for COVID-19 and the likelihood of other viruses detrimental to human health emerging in the coming years, research teams will need to leverage technologies that provide both detailed insights into the complexities of viral infections and the versatility to address a variety of interventional strategies.

As described here, researchers are already reaping the rewards of high content screening technology – identifying existing drugs which may impact newly-emerging viruses for rapid translation into therapies, developing a deep understanding of virus – host-cell interactions as a precursor to discovering new therapeutics and finding novel ways of addressing the challenges of testing vaccines against highly-infectious agents. DDW

*As Strategy Leader, Biotech Research at PerkinElmer,* ***Dr Karin Böttcher*** *works in close collaboration with the high content imaging laboratory at PerkinElmer to identify new application areas for high content imaging and manage scientific collaborations. She holds a doctorate in microbiology from the University of Goettingen where she studied human parasites using live cell microscopy. Following a postdoc in the field of identification of new drug targets at the University medical centre of Goettingen, she joined PerkinElmer where she worked first as an application scientist and then team leader of the high content imaging laboratory.*

## References

**1** Ellinger, B et al (2010). Identification of inhibitors of SARS-CoV-2 in-vitro cellular toxicity in human (Caco-2) cells using a large-scale drug repurposing collection. Preprint available at: https://www.researchsquare.com/article/rs-23951/v1.

**2** Ko, M et al (2020). Screening of FDA-approved drugs using a MERS-CoV clinical isolate from South Korea identifies potential therapeutic options for COVID-19. bioRxiv. Cold Spring Harbor Laboratory, p. 2020.02.25.965582. doi: 10.1101/2020.02.25.965582.

**3** Barrows, NJ et al (2016). A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection. Cell Host and Microbe, 20(2), pp. 259–270. doi: 10.1016/j.chom.2016.07.004.

**4** CDC https://www.cdc.gov/vhf/ebola/outbreaks/2014-west- africa/case-counts.html.

**5** Radoshitzky, SR et al (2016). siRNA Screen Identifies Trafficking Host Factors that Modulate Alphavirus Infection. PLoS pathogens. Public Library of Science, 12(3), p. e1005466. doi: 10.1371/journal.ppat.1005466.

**6** Xiong, H-L et al (2020). Robust neutralization assay based on SARS-CoV-2 S-bearing vesicular stomatitis virus (VSV) pseudovirus and ACE2-overexpressed BHK21 cells. Preprint available at: https://www.biorxiv.org/content/10.1101/2020.04.08.026948v2.