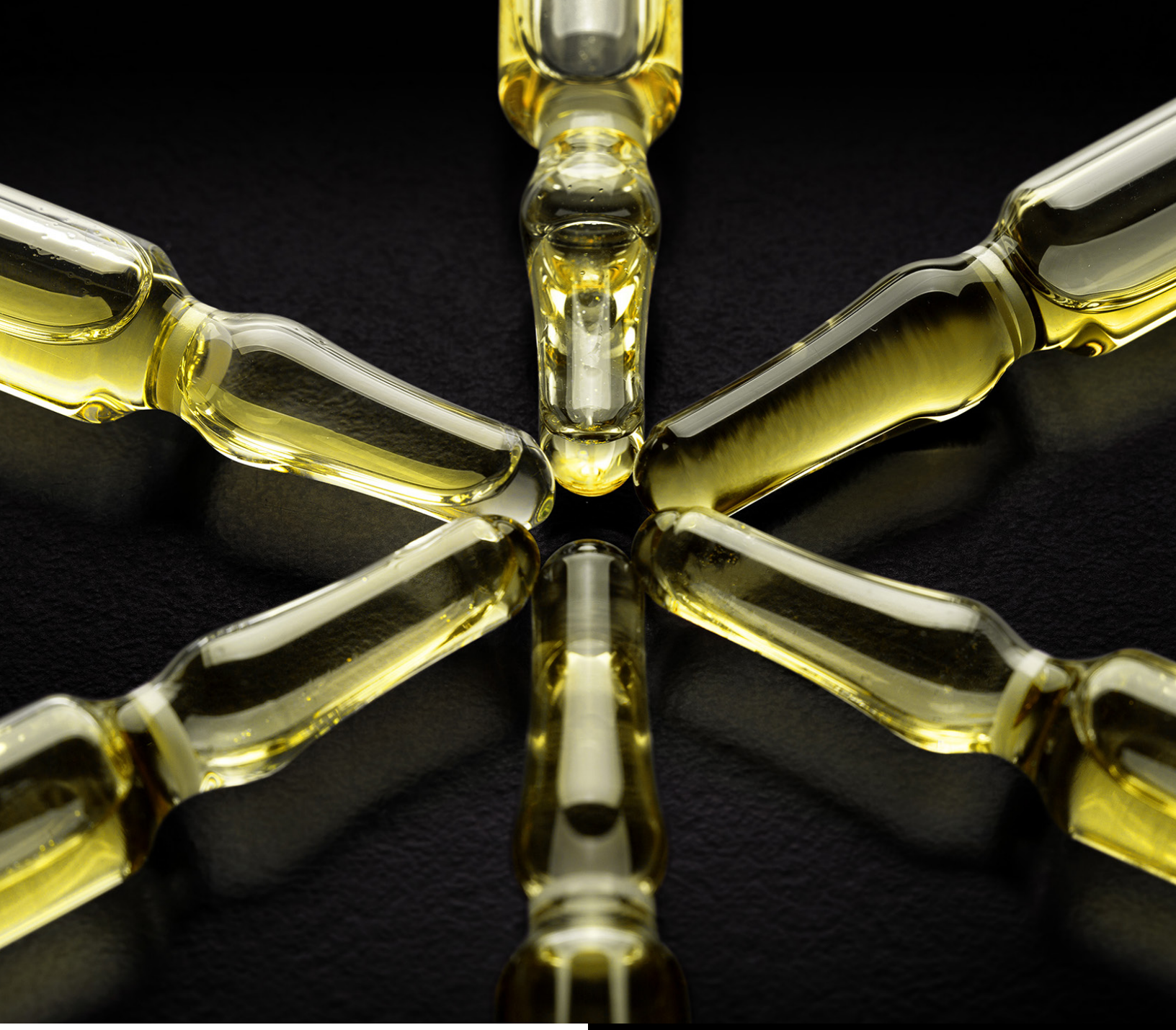

Simplifying Progress

Overcoming the Challenges of Infectious Diseases with Label-Free Approaches

GEN Genetic Engineering & Biotechnology News
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Simplifying Progress

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Overcoming the Challenges of Infectious Diseases with Label-Free Approaches

Introduction

In response to the global COVID-19 pandemic, vaccine research and manufacturing processes have seen unprecedented advancements over a short timeframe. While the worldwide attention to public health has put the spotlight on the multi-step process of vaccine development, adapting our platforms to meet the growing global demand for life-saving vaccines has always been top-of-mind for infectious disease researchers.

Sartorius supports scientists throughout every step of the vaccine development process—from analysis of virus biology, infection mechanisms, host responses, and determining therapeutic mechanisms, to candidate selection, pre-clinical and clinical assessment, and regulatory approval for use in humans. High-throughput label-free technologies, like the Octet® Bio-Layer Interferometry (BLI) systems are accelerating the selection of antigenic targets by enabling kinetic interaction analysis in real-time.

Traditional methods for analyzing biomolecular interactions such as ELISA provide only end-point data without taking into consideration the association and dissociation kinetics. Furthermore, they are often cumbersome and may require time-consuming sample preparation. When the world's attention is on rapid development of viable vaccine and therapeutic candidates, fast and flexible solutions become indispensable. The fluidic-free design of the Octet® BLI platform combined with a wide variety of off-the-shelf dip-and-read biosensors play a critical role in determining vaccine potency, stability, and titer measurements in upstream and downstream vaccine and biotherapeutic development and manufacturing.

This eBook explores the latest methodologies and suggests alternative strategies in the development of future vaccines to fight the huge variety of infectious diseases. The first article reports a new type of cholera vaccine, consisting of polysaccharides displayed on virus-like particles. Tested in mice, the new vaccine was found to generate long-lasting antibody responses against the causative bacterium, Vibrio cholera. The second selection highlights the latest in immunotherapy approaches, such as mRNA vaccines, monoclonal antibodies (mAbs), and designer DNA vaccines, targeting infectious diseases. The third article reviews the findings of a study showing broad-spectrum activity of two human antibodies against Ebolaviruses. The fourth article summarizes structural data on the attachment glycoprotein of a Henipavirus, Nipah virus, in complex with an antibody fragment, informing new therapeutic strategies for tackling these deadly infections. The final article looks at how primary infection with a pathogen affects our immune response to a secondary infection with SARS-CoV-2.

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Virus-Like Particles Used to Generate Potentially Longer-Lasting Cholera Vaccine

This image was captured in 2016, and depicted Global RRT, Tier-1 Epidemiologist, Ashley L. Greiner, MD, working long hours, while conducting cholera case investigations in Haiti after Hurricane Matthew.
[Content provider, CDC/Keisha Pressley; Photo credit, Coralie Giese]

For their new vaccine construct the researchers developed a method to efficiently link multiple copies of OSP to Qβ.

An international research team including scientists at Michigan State University and Massachusetts General Hospital has developed a new type of cholera vaccine, consisting of polysaccharides displayed on virus-like particles. Tested in mice, the new vaccine was found to generate long-lasting antibody responses against the causative bacterium, Vibrio cholera. First author Zahra Rashidijahanabad, PhD, at Michigan State University, and colleagues reported on their development of the new vaccine in ACS Infectious Diseases, in a paper titled, "Virus-like Particle Display of Vibrio cholerae O-Specific Polysaccharide as a Potential Vaccine against Cholera."

Cholera is an acute, secretory diarrheal disease caused by V. cholerae, a highly transmissible, highly motile Gram-negative bacterium, the authors wrote. There are 2–3 million cases of cholera each year, resulting in tens of thousands of deaths annually. And although there are more than 200 serogroups of V. cholerae based on the

O-antigen of surface lipopolysaccharide (LPS) structures, only serogroups O1 and O139 are capable of causing epidemic cholera.

Global cholera control strategies have been "transformative," the team continued, but existing oral vaccines have the lowest level and duration of protection in young children, who bear " … a large share of global cholera burden," especially in cholera-endemic countries. "As such, there is a need to develop new cholera vaccines that can provide high-level and long-term immunity."

Existing cholera vaccines contain killed or weakened V. cholerae bacteria and are administered orally. "Current cholera vaccines include oral killed whole cell vaccine with or without cholera toxin B subunit (CtxB), and attenuated oral cholera vaccine," the investigators continued. These vaccines trigger the immune system to produce antibodies against the O-specific polysaccharide (OSP) on the surface of V. cholerae, but this polysaccharide in isolation does not generate a strong, long-lasting immune response. "… as O-antigens are T cell-independent B cells antigens, direct administration of the O-antigens often only leads to low titers of low-affinity IgM antibodies with limited duration of antibody responses and a lack of induction of immunological memory, rendering O-antigen-based vaccination suboptimal," the investigators noted.

Rashidijahanabad and colleagues wondered if attaching OSP to virus-like particles (VSP) could induce stronger, longer-lasting immunity. The team had previously demonstrated that self-assembled virus-like particles, such as the bacteriophage Qβ—a virus-like particle that infects bacteria—could be used to conjugate with carbohydrate antigens as potential vaccines. "The resulting glycoconjugates were able to induce strong glycan specific IgG antibody responses," they wrote. But to date, only low molecular weight glycans had been tested in Qβ based anticarbohydrate vaccine studies. "It is not known whether bacterial polysaccharide antigens could be conjugated with Qβ and whether such conjugates could induce strong IgG antibody responses to polysaccharides," the team acknowledged.

For their new vaccine construct the researchers developed a method to efficiently link multiple copies of OSP to Qβ. They first demonstrated that the modified virus-like particles were recognized by antibodies in blood taken from recovering cholera patients, but not from patients with typhoid, another bacterial disease.

When the scientists then immunized mice using Qβ-OSP, they found that three doses caused a strong antibody response that persisted at least 265 days after the first dose. The immunized mice had antibodies that recognized the OSP from the natural lipopolysaccharide of V. cholerae. When the researchers mixed serum antibodies from the mice with other immune system proteins that kill bacteria and with live V. cholerae, antibodies from two of the five mice triggered more bacterial death than those from mice immunized with Qβ alone. The researchers said the virus-like particle could mimic natural bacteria by presenting multiple copies of OSP on its surface. "High levels of antipolysaccharide IgG antibodies were induced by the conjugate

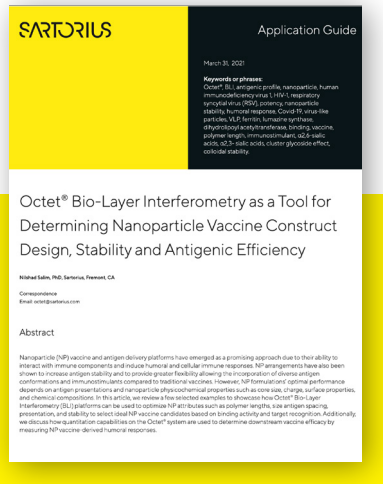
in mice, and the antibodies were effective in killing the bacteria," they wrote.

Encouragingly, anti-OSP IgG antibody levels were hugely increased in mice that were then given a booster vaccination 265 days after their initial vaccination. "These results suggest that Qβ-OSP vaccination induced memory B cell responses and the anti-OSP humoral immunity could be boosted," the scientists noted.

"This was the first time that Qβ was conjugated with a bacterial polysaccharide for vaccine development, broadening the scope of this powerful carrier," they commented, suggesting that the VLP-based display of bacterial OSP may warrant additional evaluation as a next-generation anti-cholera vaccine. "Bacteriophage Qβ VLP is a promising platform for organized display to induce antibody responses against a target antigen."

Read the Application Guide...

Nanoparticle (NP) vaccine and antigen delivery platforms have emerged as a promising approach allowing diverse antigen conformations.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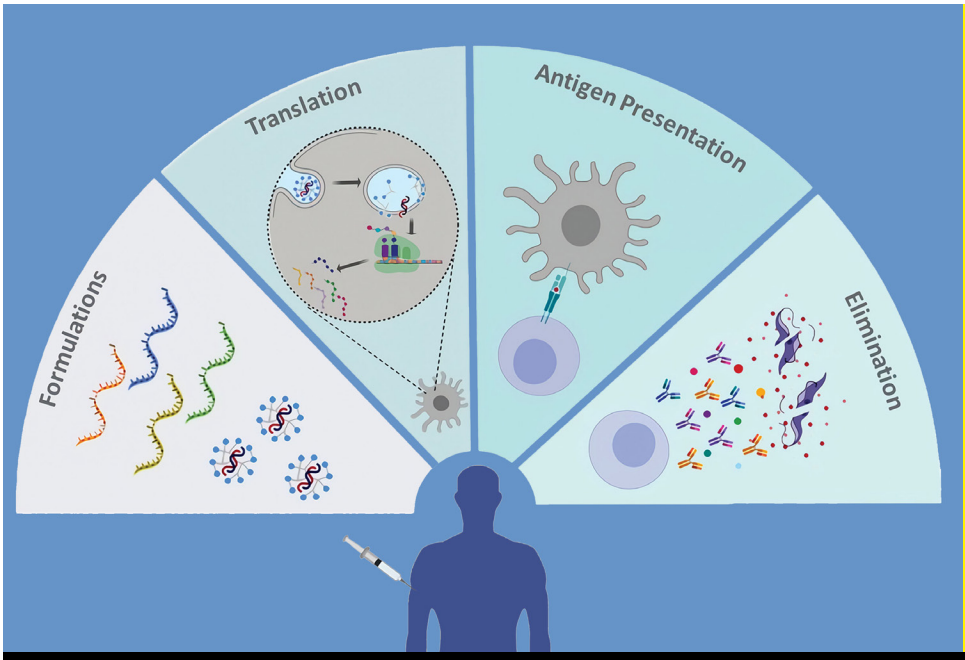
Application Guide

March 31, 2021

Octet® Bio-Layer Interferometry as a Tool for Determining Nanoparticle Vaccine Construct Design, Stability and Antigenic Efficiency

Abstract

Immunotherapy Targets Emerging Infectious Diseases



Biologics and vaccines against Ebola, Zika, and Chikungunya are at the forefront of development.

At the Baylor College of Medicine, Jeroen Pollett, PhD, and colleagues are developing mRNA-encoded antibody therapies. The scientists have leveraged improvements in mRNA vaccine formulations to enhance the transfection of antigen-presenting cells and subsequent translation to therapeutic proteins. For example, they have developed a multivalent antiparasitic mRNA vaccine against Chagas disease.

Fear of deadly contagion drives the action in The Hot Zone, a television drama inspired by the true story of Ebola's first arrival in the United States in 1989. Although the Ebola strain that breached our borders back then was eventually found to be incapable of causing illness in humans, The Hot Zone recreates flashback scenes depicting the terrifying 1976 Ebola outbreak in Africa before cutting back to the spectacle of hazmat-suited U.S. Army personnel euthanizing infected animals and decontaminating lab facilities.

More recent African outbreaks attest to the deadly nature of this hemorrhagic fever and other emerging infectious diseases and highlight the diligent efforts to create new therapies. As in The Hot Zone, the challenges faced by everyday scientists sometimes prompt them to rise to the occasion and become real-life microbe-fighting heroes. They are already making headway developing novel immunotherapeutic strategies to attack and neutralize menacing and highly infectious viruses. (Immunotherapy is also targeting other areas from cancer to antibiotic resistance.)

The inaugural "Immunotherapy for Infectious Diseases" conference was recently held "to provide a forum for exchange of results and ideas and to establish a community of interested parties in the interphase between academia and industry,"

commented meeting organizer Magnus Hook, PhD, professor, director, Center for Infectious and Inflammatory Diseases, Texas A&M Health Center. A second conference is planned for 2020 in Italy.

Conference topics included coaxing lung cells to combat infections, leveraging new technological innovations to create mRNA vaccines, assessing unique therapeutic monoclonal antibodies (mAbs) against Ebola, Zika, and other infectious diseases, and utilizing electroporation-assisted delivery of naked DNA to create "designer DNA vaccines."

Luring lungs to attack pathogens

The epithelium of the lung, akin to a military perimeter, defends against infection both as a passive barrier and as a battery of active killing components, noted Burton F. Dickey, MD, professor and chair of the department of pulmonary medicine at the University of Texas MD Anderson Cancer Center. "Rather than outsourcing all active antimicrobial defenses to leukocytes, barrier epithelial cells, which face the onslaught of pathogen attack, have developed their own potent innate defenses," Dickey explained.

Dickey along with Scott E. Evans, MD, associate professor in the same department, and colleagues, developed a strategy to induce lung cells to ramp up their defenses. The team identified a synergistic combination of two Toll-like receptor (TLR) agonists that, when inhaled, could induce rapid lung resistance to infection from more than 15 bacteria, viruses, and fungi.

According to Dickey, the discovery was exciting, but also puzzling. "There was," Dickey pointed out, "no obvious link between the two agonists that consisted of a diacylated lipopeptide ligand for TLR2/6 (that is, Pam2CSK4) and a class C unmethylated 2'-deoxyribocytidine-phosphate-guanosine (CpG) ligand for TLR9 (that is, ODN M362).

The investigators next pursued the biology of the effect. "The combo (Pam2-ODN) induced production of reactive oxygen species without reliance on type I interferon signaling. Essentially, the lung epithelial cells were producing 'Clorox' to kill pathogens," quipped Dickey.

The scientists also recently discovered the mechanism of action, which presented another surprise. Dickey reported, "The ODN in the mix binds a cytoplasmic DNA sensor that is required for the rather magical effect in which all three players are engaged to induce pathogen killing."

The PAM2-ODN therapeutic is currently in a Phase IIa trial. "We are testing its ability to block bronchitis caused by rhinovirus in a challenge study," Dickey said. However, other uses include treating cancer patients undergoing myeloablative chemotherapy, who are very susceptible to pneumonia, asv well as organ transplantation patients and others on immunosuppressants. Dickey concluded that "there are many strategies that could be developed, now that we know combinations of innate ligands delivered by aerosol to the lungs are capable of inducing a high level of broad host resistance against a variety of pathogens."

mRNA vaccines: The body as bioreactor

Billions of people worldwide are at risk from endemic and newly emerging tropical infectious

diseases. Although traditional vaccines have had an enormous impact on preventing disease and saving lives, hurdles remain to more rapid vaccine development and deployment.

Some believe that the introduction of mRNA vaccines could usher in a new era in vaccinology. Although early reports of successful use of in vitro transcribed mRNA in animals appeared more than 30 years ago, only recently have major technological innovations allowed mRNA to begin taking its place as a viable therapeutic.

"Typical vaccines often utilize recombinant proteins, but the need to produce and purify them are major hurdles," explained Jeroen Pollet, PhD, assistant professor of pediatrics at the National School of Tropical Medicine at Baylor College of Medicine in Houston. "Once a platform is developed, the process can be streamlined. It is even conceivable to combine mRNAs against different antigens to increase potency."

According to Pollet, mRNA vaccines offer some significant advantages: "There is no risk of genomic integration. The cellular immune response can be regulated both by nucleoside modifications and delivery methods, and mRNA vaccines can be produced by rapid, inexpensive, and scalable means."

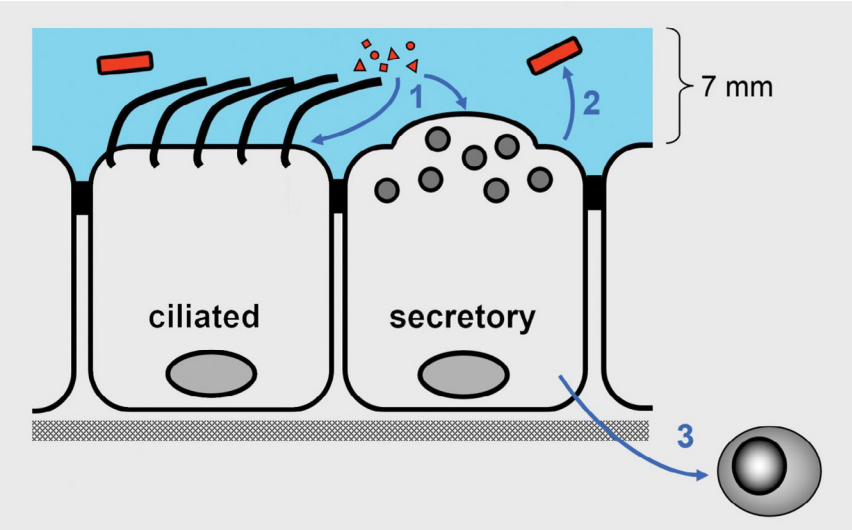
Pollet and colleagues at Texas Children's Hospital Center for Vaccine Development are studying Chagas disease, which is caused by the protozoan parasite Trypanosoma cruzi. An estimated eight million people in Latin America are afflicted with the parasite, and the incidence is increasing. Pollet explained his approach: "We created a vaccine that combined six unique mRNAs encoding different parasite proteins and administered that to mice. We've had exciting results thus far; however, our in vivo experiments are complex because we aim to affect the lengthy chronic phase of Chagas disease."

Pollet reported that this same strategy is being adapted to develop therapeutics for other infectious diseases such as Zika and rabies. In addition, Pollet pointed out that there are other lucrative uses of the technology: "Any therapeutic mAb could be developed into an mRNA-encoded antibody therapy. This would allow patients to make their own Abs in any of their transfected cells. Certainly, progress in overcoming challenges related to mRNA stability, immunogenicity, and delivery can now begin to drive a large and expanding commercial application of mRNA therapeutics."

Consortium for standardizing mAbs

Although a number of investigators have produced and evaluated mAbs against Ebola virus (EBOV), making comparisons is challenging without standardization of assays and interpretations among the various groups. To help solve the problem, the Viral Hemorrhagic Fever Immunotherapeutic Consortium (VIC) assembled with the goal of gathering a broad pool of antibodies to EBOV and other viruses and analyzing them using a systematic strategy employing identical assay conditions. Gary P. Kobinger, PhD, professor and director of the Infectious Disease Research Centre at the Université Laval is a member of VIC. He gave the keynote address at the conference titled, "The Ascent of mAb Therapies against Infectious Diseases."

Issues confronting mAb therapeutics include identifying which in vitro tests best predict the in vivo



Researchers at MD Anderson Cancer Center led by Burton F. Dickey, MD, have shown that airway epithelial cells sense the presence of pathogens through innate immune receptors such as Toll-like receptors (TLRs). Pathogen sensing and response steps unfold as follows: (1) TLRs bind molecules shed by microbes. (2) Cells are prompted to kill pathogens by releasing reactive oxygen species and antimicrobial peptides. (3) Cells also recruit leukocytes to assist in pathogen defense. The response steps can be induced by aerosolized drugs that mimic pathogen-associated molecules.

efficacy of mAbs. VIC recently published a report describing development of a comprehensive dataset examining more than 170 mAbs evaluated in each of 30 assays. The various mAbs included chimeric Abs, human survivor mAbs, and those raised by immunization. They concluded that no single neutralization assay alone can always predict protection, and that the mAb epitope is not the sole determinant of neutralization behavior. Despite these findings, the group compiled other sets of key information to serve as a framework for future studies of EBOV and other human pathogens.

Single human mAb quells Ebola

At the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center (VRC), Nancy J. Sullivan, PhD, chief of the Biodefense Research Section, and colleagues have developed a therapeutic mAb (mAb114) derived from blood drawn from an Ebola survivor 11 years after infection.

Sullivan and team first verified the presence of circulating antibodies against the EBOV's surface glycoprotein (GP). Then, they sorted the patient's memory B cells, immortalized individual clones, and chose one with specific properties they had determined from previous research to increase mAb potency. After cloning its gene, purifying mAb protein, and testing in a rhesus macaque model, they found mAb114 could protect against infection even when given five days after challenge.

Seeking to identify the structural and molecular basis for mAb114's striking activity, Sullivan and colleagues at Dartmouth College obtained crystals and solved the ternary structure of the mAb/GP complex. Sullivan explained that the GP, found in abundance on the virus surface, consists of a trimer of monomers with two subunits, GP1 and GP2. They found the mAb binds to a novel site of vulnerability on GP1, attesting to the value of identifying natural defenses targeted by the host immune system.

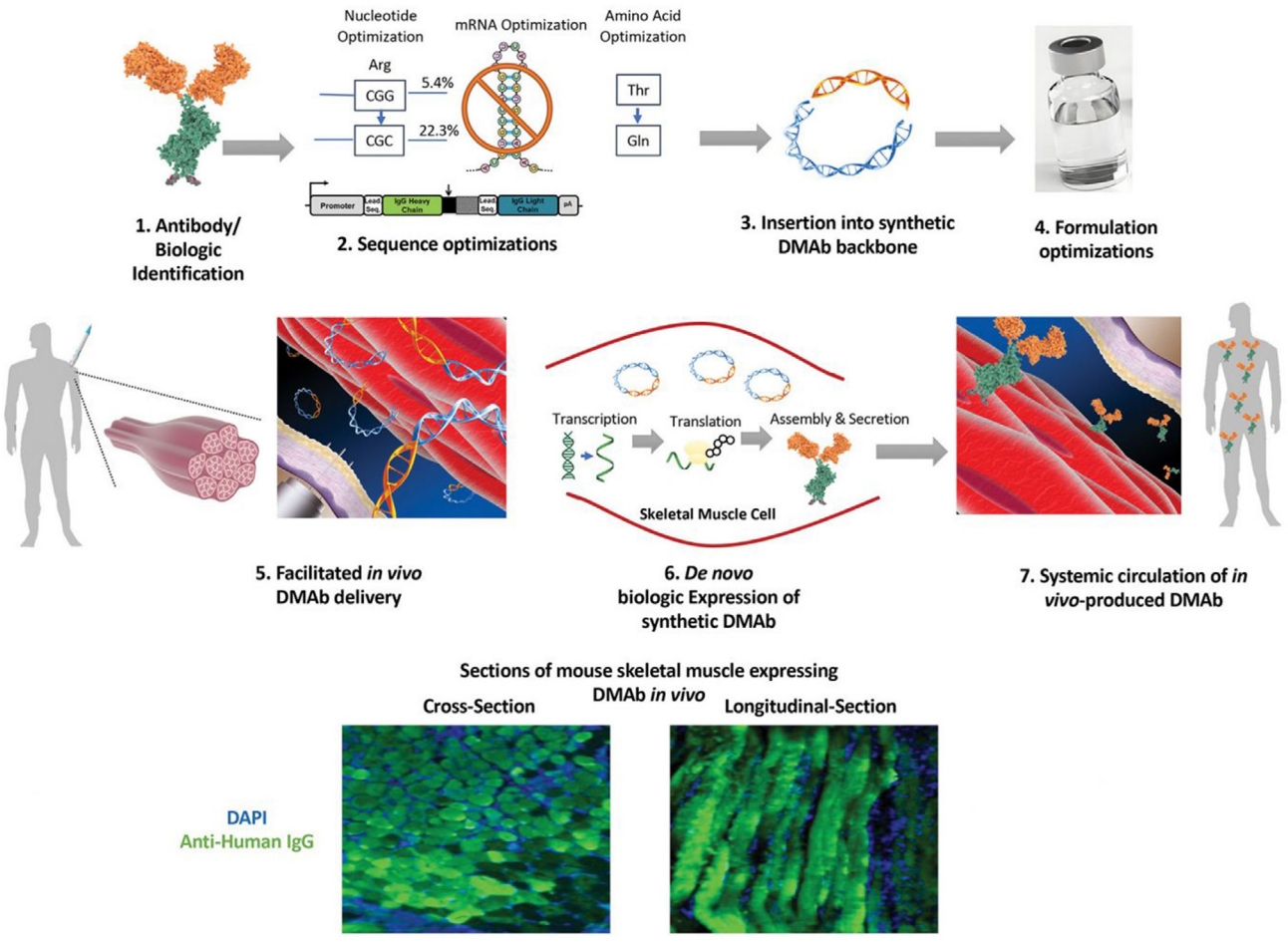
Subsequently, Julie E. Ledgerwood, DO, chief, clinical trials program at the VRC, and her team, led by Martin Gaudinski, MD, medical director at the VRC, completed the first-in-human open label-Phase I trial of mAb114. Ledgerwood reported (Lancet

2019) that even after a single 30-minute intravenous infusion (and patient monitoring for 24 weeks), the mAb was well tolerated, showed linear pharmacokinetics, and was rapidly infused, making it suitable for rapid deployment as a treatment for outbreaks. Thus, one of the striking features of mAb114 is that a single infusion, rather than several over multiple days, protects the subject.

Ridgeback Biotherapeutics is leading further clinical development of mAb114. Sullivan also reported that due to a recent African outbreak of EBOV, Jean Jacques Muyembe, director general of the Institut National de Recherche Biomédicale in Kinshasa, DRC, is treating patients under a WHO compassionate use protocol.

Designer DNA immunotherapies

Another form of immunotherapy, DNA vaccines, sparked interest in the scientific community in the 1990s with the technology's theoretical ability



The Wistar Institute laboratory of David B. Weiner, PhD, has developed a workflow for generating DNA-encoding monoclonal antibodies (DMAbs) against infectious disease targets. (1) An antibody of interest is selected. (2) Sequence optimizations are performed. (3) The synthetic nucleotide is cloned into a plasmid backbone. (4) An optimized buffer is formulated to improve shelf-life. (5) The plasmid is injected intramuscularly using facilitated in vivo delivery at the same site to enhance plasmid uptake. (6) The plasmid is transcribed in vivo; the mRNA transcript is translated; and the encoded product is assembled and targeted to the cellular secretory pathway. (7) Sustained, robust, and systematic expression of the antibody is achieved. [Ziyang Xu, Megan Wise, Ami Patel, James Hayden]

to generate broad immune responses without the need for live attenuated virus. However, early clinical trials were disappointing, despite a good safety record. Recently, many of those hurdles have been overcome, propelling "designer DNA vaccines" once again into the limelight.

"Some of the earlier challenges were low response rates in humans, poor reproducibility, and lack of an immunogenic response," reported David B. Weiner, PhD, executive vice president, director of the Vaccine & Immunotherapy Center, and W.W. Smith Charitable Trust Professor in Cancer Research at the Wistar Institute. Weiner, a pioneer in the field, said several key innovations are driving the new resurgence of interest: "First, we can now engineer inserts into DNA plasmids that will result in 25–50 times more protein expression per cell. Second, we've learned to increase DNA formulations that also have reduced volume. Third, we've dramatically increased our delivery with advanced electroporation-assisted devices that provide for cellular update of plasmid more than 1000-fold as compared to plasmid delivery alone."

A search on clinicaltrials.gov revealed 610 current clinical trials using DNA vaccines for the treatment of cancers, influenza, and infectious diseases. Weiner feels that DNA vaccines provide a valuable immunotherapy especially for rapidly emerging infectious diseases. He gave the example of Zika virus. "During the 2015 outbreak, there were no drugs and no vaccine available to treat Zika," he recalled. "Our vaccine, a synthetic DNA cassette featuring a Zika-specific antigen, was the first into the clinic and took only about 6.5 months to develop from bench to bedside."

However, Weiner and colleagues have also taken another approach to fighting Zika. They have engineered designer synthetic plasmids with a DNA-encoding mAb, ZK190, that can produce a full-length functional antibody known to potently neutralize Zika in animal studies. The team found that when delivered in vivo, the DMAb-ZK190 was produced in the living animal and proved protective to Zika challenge in both mice and rhesus macaques. Weiner explained, "Unlike viral vector platforms, this platform is nonlive, nonintegrating, and noninfectious while promoting rapid and transient highly targeted DMAb generation."

Weiner sees a broad horizon for DNA platforms, from fighting emerging infectious diseases to attacking cancer. "As improvements continue to broaden our scope and accelerate the pace of success," he says, "it is possible we are entering the 'Designer DNA Vaccine and Immunotherapy Era.'"

Ebola Virus Antibodies Offer Protection from Infection in Nonhuman Primates

While there are antibody therapies against Ebola virus, some antibodies in these therapies don't actually neutralize the virus.

Out of the five Ebolavirus species—Ebola virus (Zaire ebolavirus); Sudan virus (Sudan ebolavirus); Taï Forest virus (Taï Forest ebolavirus, formerly Côte d'Ivoire ebolavirus); Bundibugyo virus (Bundibugyo ebolavirus); Reston virus (Reston ebolavirus)—vaccines and monoclonal antibody cocktails are available to treat only Ebola virus infections. However, broad-spectrum therapies are needed that neutralize multiple Ebolavirus species. To that end, researchers are trying to develop human antibodies that target vulnerable sites across Ebolavirus species.

Current cocktails contain antibodies that cross-react with the virus's secreted soluble glycoprotein (sGP) that absorbs virus-neutralizing antibodies. Researchers sorted memory B cells from Ebola virus infection survivors to isolate two broadly reactive anti-GP monoclonal antibodies—1C3 and 1C11. These antibodies potently neutralize, protect rodents from disease, and lack sGP cross-reactivity. A cocktail of both antibodies, the researchers noted, completely protected nonhuman primates from Ebola virus and Sudan virus infections, indicating their potential clinical value.

The work is published in Cell in the paper, "Asymmetric and non-stoichiometric glycoprotein recognition by two distinct antibodies results in broad protection against ebolaviruses."

The new study shows that two human antibodies can target both Ebolavirus species Ebola virus and Sudan virus. These two species are responsible for the biggest, deadliest outbreaks. The new report suggests researchers could combine these two potent antibodies to make a powerful antiviral therapy.

"Finding antibodies with this breadth is important because we don't know which virus in the genus of ebolaviruses is going to break out next," said Erica Ollmann Saphire, PhD, president & CEO of the Center for Infectious Disease and Vaccine Research.

The team used cryo-electron microscopy (cryo-EM) to learn how these antibodies neutralize ebolaviruses. This imaging gave them a clear view of how the two antibodies, 1C3 and 1C11, bind to vulnerable sites on an ebolavirus glycoprotein. Both antibodies recognize quaternary epitopes in trimeric ebolavirus GP.

They were surprised to see that 1C3 attacked the glycoprotein in an unexpected way. "This antibody might punch above its weight," said Saphire. "The antibody is able to block three sites on the virus at the same time using different loops and structures to anchor into each one. That is remarkable."

Meanwhile, the paired antibody 1C11 binds to the fusion machinery the virus would normally use to enter and infect host cells. As Saphire explained, because the fusion machinery has such a critical job, it looks very similar between Sudan virus and Ebola virus. "This is a site of very broad recognition and resistance to any antibody escape," she added. "That's how this antibody gets its breadth."

More specifically, 1C11 bridges adjacent protomers via the fusion loop while 1C3 has a tripartite epitope in the center of the trimer apex, the authors noted. "One 1C3 antigen-binding fragment anchors simultaneously to the three receptor-binding sites in the GP trimer, and separate 1C3 paratope regions interact differently with identical residues on the three protomers," they wrote.

While there are antibody therapies against Ebola virus, some antibodies in these therapies don't actually neutralize the virus. Instead, the antibodies home in on a decoy protein, called soluble glycoprotein, that the virus makes. Fortunately, 1C3 and 1C11 ignore the decoy and go straight for the virus's actual surface glycoprotein structure. This means the researchers could use fewer antibodies to effectively target Ebola virus and Sudan virus. "If 80–90% of what's there is some kind of smokescreen, having antibodies that can target the vulnerable spot is valuable," Saphire said.

The two antibodies performed very well outside the lab. Study collaborators found that combining 1C3 and 1C11 in an antibody therapy could protect against Ebola virus and Sudan virus disease in nonhuman primates, reversing severe symptoms. The broad-spectrum effects of the two antibodies make them a promising therapy

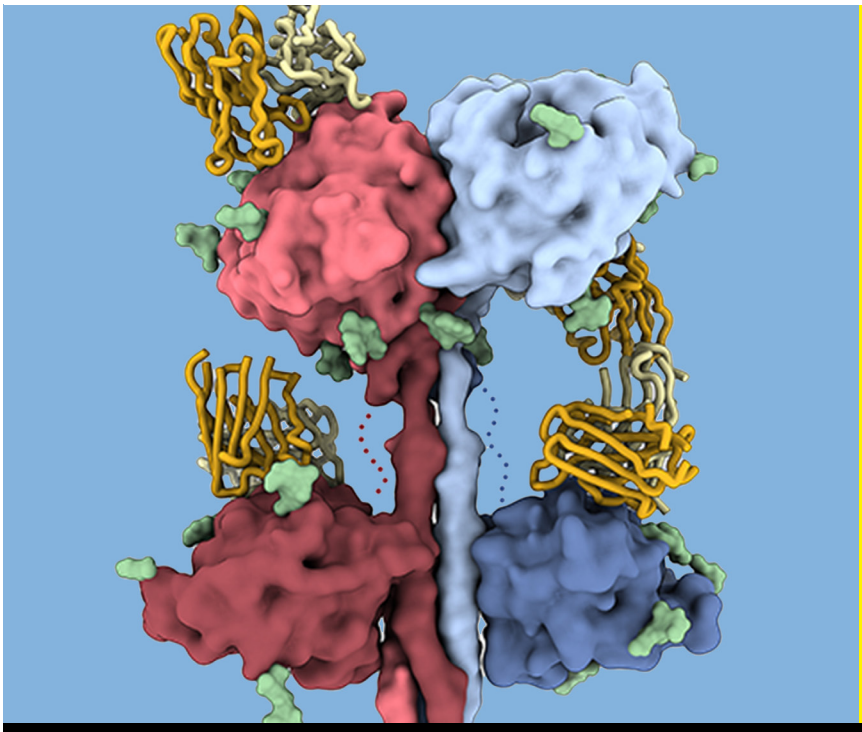
for situations when doctors don't have time to figure out which Ebolavirus species is responsible.

Even better, these antibodies may be effective even when given late in the course of the disease. This late treatment would be extremely valuable because many patients with either Ebola virus or Sudan virus have already progressed far into infection when they are diagnosed.

"The first symptoms of Ebola virus tend to be a fever and a headache, which can look like a lot of different diseases," Saphire said. "An antibody that can be used later in the course of disease is a lot more useful."

Going forward, the team is trying to figure out how much lower the dosage could be. In the nonhuman primate trial, even the lowest dose provided 100% protection. This is an important question to answer because lower doses would make the therapy much cheaper to produce.

Nipah Virus Attachment Protein Structure Determined Through Cryo-EM



It's estimated that two billion people live in the parts of the world where henipavirus spillovers from bats, or intermediary animal vectors, could be a threat.

A structural image of the Nipah virus attachment protein in a complex with an nAH1.3 antibody Fab fragment
[Zhaoqian Wang/Veesler Lab]

Nipah virus and Hendra virus are bat-borne zoonotic pathogens responsible for outbreaks of encephalitis and respiratory illness. Notably, these henipaviruses have fatality rates between 50% and 100%. Over the past two decades, Nipah virus has spilled over into humans almost annually in Bangladesh with other outbreaks occurring in India and the Philippines. There are no approved vaccines or therapeutics for use in people against these infections.

The entry of Henipaviruses into host cells requires the attachment (G) and fusion (F) glycoproteins which are the main targets of antibody responses.

To further the understanding of viral infection and host immunity, researchers have determined a cryo-electron microscopy structure of Nipah virus's G homotetrameric ectodomain in complex with a broadly neutralizing antibody Fab fragment.

These findings offer new details on how Nipah and Hendra viruses attack cells, and the immune responses that try to counter them. The results point toward multi-pronged tactics to prevent and treat these deadly illnesses. This research is reported in *Science* in the paper, "Architecture and antigenicity of the Nipah virus attachment glycoprotein."

Using cryo-electron microscopy, a research team led by David Veesler, PhD, associate professor of biochemistry at the University of Washington School of Medicine and a Howard Hughes Medical investigator, was able to determine the structure of the Nipah virus G homotetrameric ectodomain in complex with the nAH1.3 broadly neutralizing antibody Fab fragment. The scientists went on to show that a cocktail of two non-overlapping G-specific antibodies neutralizes Nipah virus and Hendra virus synergistically. The combination of forces also helped keep escape mutants from emerging to sidestep the antibody response.

Examining the antibody response in laboratory animals provided vital information. More specifically, the analysis of polyclonal serum antibody responses elicited by vaccination of macaques with the Nipah virus G protein indicates that the receptor-binding head domain is immunodominant.

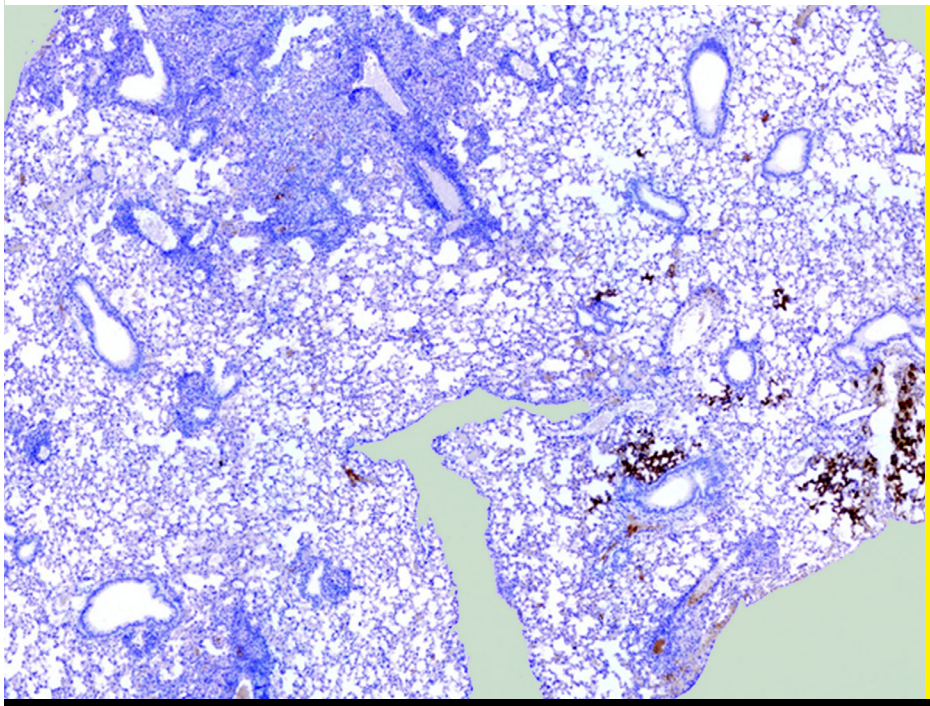
Before this study, the researchers said, no information was available on the structure of the G protein. This lack of information was an obstacle to understanding immunity and to improving the design of vaccine candidates.

The scientists noted that the architecture "adopts a unique two heads up and two heads down conformation that is different from any other paramyxovirus attachment glycoprotein." The paramyxovirus is a large family of single-strand RNA viruses including measles, mumps, distemper, parainfluenza, and henipavirus.

Now that the researchers have uncovered the 3D organization, and some of the conformational dynamics of the G protein, they may be closer to creating a template for building new and improved vaccines. These findings, the researchers noted, "provide a blueprint for engineering next-generation vaccine candidates with improved stability and immunogenicity," with a focus on the vulnerability of the head domain. They anticipate a design approach like that employed for newer computer-engineered SARS-CoV-2 and respiratory syncytial virus candidates. A mosaic of head antigens would be presented to the body in an ordered array on a multivalent display. Using only the head domain rather than the full G protein could also make manufacturing large supplies of vaccines simpler.

New attempts to design life-saving preventatives and treatments became even more urgent after a new strain of Hendra was discovered a few months ago. Henipavirus antibodies have been detected in people and Pteropus bats in Africa. It's estimated that two billion people live in the parts of the world where henipavirus spillovers from bats, or intermediary animal vectors, could be a threat.

COVID-19 Potentially Thwarted by TB Infection... at Least in Mice



Currently, M. tuberculosis, and SARS-CoV-2, are the leading causes of death from infectious disease worldwide.

Magnified image of a lung that is infected with the respiratory pathogens that cause tuberculosis (TB) and COVID. The dense blue area in the upper left is infected with Mycobacterium tuberculosis (the cause of TB), while the brown area in the lower right is infected with SARS-CoV-2 (the cause of COVID).
[Erin S. Gloag]

COVID-19 and tuberculosis (TB) top the list of infectious disease-related causes of death around the globe. But what is the effect of one of these respiratory infections on the other? A team of researchers asked that question, with a focus on the role of a tuberculosis infection, and it's immune response, on a secondary infection with SARS-CoV-2.

The team used two mouse models of COVID-19, using mice that were chronically infected with Mycobacterium tuberculosis. In both model systems, the mice infected with tuberculosis were, they said, "resistant to the pathological consequences of secondary CoV2 infection, and CoV2 infection did not affect M. tuberculosis burdens." The findings support the hypothesis that, in mice, the immune response mounted against tuberculosis prevents them from developing COVID-19.

The work is published in PLOS Pathogens in the article, "Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2."

"TB and COVID are pandemics that affect every part of the world," Richard Robinson, PhD, asso-

ciate professor of microbial infection and immunity at the Ohio State University (OSU) noted. "Our study reflects the work of a diverse and talented group of OSU scientists to better understand how these two diseases influence one another, a surprising observation being that mice with TB are resistant to COVID in a lab setting."

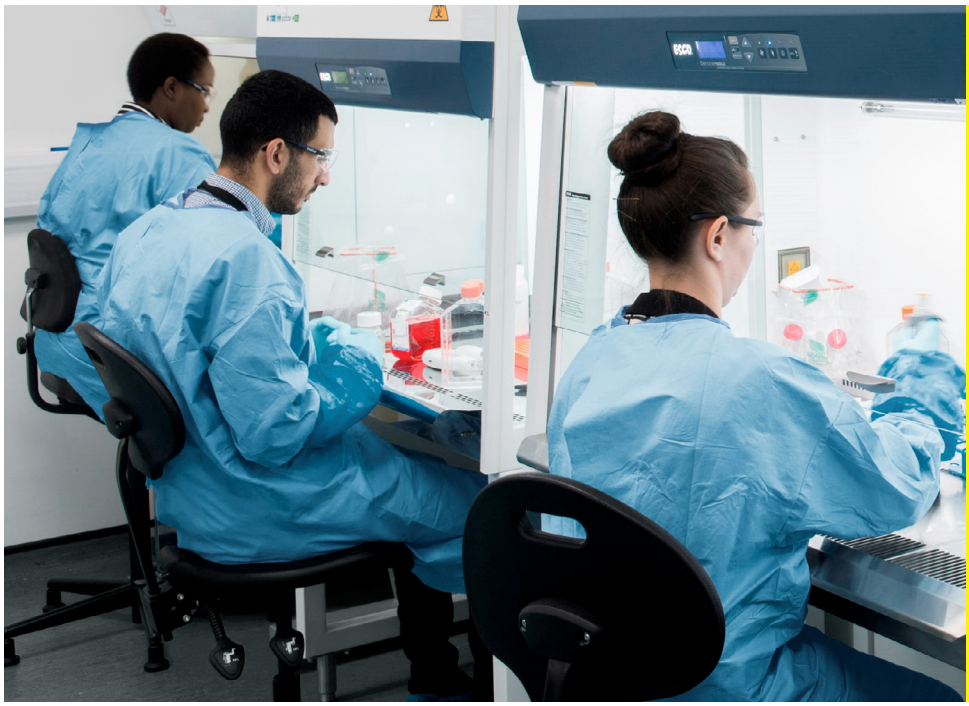
Currently, the bacterium that causes tuberculosis, M. tuberculosis, and the virus that causes COVID-19, SARS-CoV-2, are the leading causes of death from infectious disease worldwide.

Tuberculosis is widespread, and scientists have questioned whether the immune response triggered by this serious respiratory infection might protect people from developing COVID-19. To find out more, researchers worked with two different strains of mice and infected them with M. tuberculosis. Then they exposed the mice to SARS-CoV-2 and monitored them for signs of infection. They discovered that mice with tuberculosis showed no signs of COVID-19, likely because the pre-existing immune response to tuberculosis prevented the virus from proliferating in the lungs.

More specifically, single-cell RNA sequencing of coinfected and monoinfected lungs demonstrated the resistance of M. tuberculosis infected mice is associated with expansion of T- and B-cell subsets upon viral challenge.

The findings demonstrate that tuberculosis infection makes the lungs inhospitable to SARS-CoV-2 in mice. If the same is true for humans, this discovery may be one reason why there have been few reports of individuals with both TB and COVID-19 in the absence of other complications. The findings may also explain why countries tend to have high rates of infection of COVID-19 or TB, but not both. The researchers propose that future research should focus on the interaction between COVID-19 and TB infections in humans.

Addressing the Challenges of Quality Control Labs in Vaccine Manufacturing



To ensure the safety, efficacy and quality of new vaccines a meaningful combination of measures need to be in place...

Different vaccines types against COVID-19 are currently being manufactured and offered to the global population.

Besides the mRNA vaccines which are completely chemically synthesized, the viral vector, inactivated and recombinant protein (sub-unit) vaccines are produced using cell culture-based manufacturing processes.

To ensure the safety, efficacy and quality of these vaccines a meaningful combination of measures need to be in place that comply with the principles of good manufacturing practices, including proving the consistency of the manufacturing process and testing for quality.

As is the case with other biopharmaceutical products, the quality control (QC) of vaccines relies on three key components: control of the starting materials, control of the production process and control of the final product. This poses significant challenges to quality control labs in terms of time and sample testing requirements; it is estimated that about 70% of the production time of a vaccine is dedicated to quality control.

Testing for microbial contaminations throughout the manufacturing process is key to ensure the safety of the patients.

Human, animal and insect cells are commonly used in culture-based manufacturing processes

and are typically susceptible to mycoplasma infections. Mycoplasma belong to the smallest known bacteria. They lack a cell wall and as a result can take on a very dynamic shape or form. If they enter the process, they can negatively impact the cultured cells and subsequently the product. This is manifested in alterations in the cell growth rates and morphologies, product yields as well as in DNA, RNA and protein synthesis. Testing for Mycoplasma is therefore necessary for the biologically derived raw materials and cell banks and each batch of the unprocessed bulk material (prior to harvest of the production bioreactor).

The traditional culture-based method challenges quality control in two ways. Firstly, viable but non-culturable (VBNC) Mycoplasma can go undetected when using the traditional method of mycoplasma cultivation, risking false-negative results. Secondly, a minimum of four weeks incubation time is required to be able to detect the presence of mycoplasma with certainty. It is especially challenging in continuous manufacturing processes where the bioreactor harvest can often not be held back too long; as a result, the process typically proceeds without Mycoplasma test results.

Sartorius' Microsart® qPCR kit offers a rapid solution for the early detection of Mycoplasma contamination. These kits are fast, highly specific, sensitive, and compliant with international guidelines. Alternative testing methods such as real-time PCR are gaining increasing acceptance by regulators with a need for a faster time-to-result when compared to traditional or compendial testing.

Microbial enumeration (bioburden) testing is another in-process quality control measure to demonstrate the safety and performance of the manufacturing process. In cell culture-based manufacturing processes it is required at the following stages:

Sartorius' Microsart® qPCR kit offers a rapid solution for the early detection of Mycoplasma contamination. These kits are fast, highly specific, sensitive, and compliant with international guidelines. Alternative testing methods such as real-time PCR are gaining increasing acceptance by regulators with a need for a faster time-to-result when compared to traditional or compendial testing.

- Raw materials (biologically derived)
- Cell banks (Master Cell Bank, Working Cell Bank)
- Unprocessed Bulk Material (prior to bioreactor harvest)
- Appropriate stages in the downstream process
- Drug substance

Membrane filtration is the regulatory-preferred method for microbial enumeration testing of liquids. However, handling a large amount of test sample materials including transferring the membrane filter to the microbiological growth medium is one of the most common sources of secondary contamination and false-positive results. Simple but effective – as often smart solutions can make a difference to daily routine tasks and simplify workflow steps. The Microsart® @filter combined with the Microsart® @media is a unique system which effortlessly positions the membrane on an agar plate completely touch-free. With only a turn of the lid, it's locked and ready to incubate, reducing the risk of contamination.

Alternatively, a rapid PCR-based total bacteria and fungi test can provide a quick result within 3 hours.

At the end of the process and as an essential product release parameter, sterility testing ensures that viable microorganisms are not present as contaminants in the final parenteral products, as most vaccines are administered as injectables.

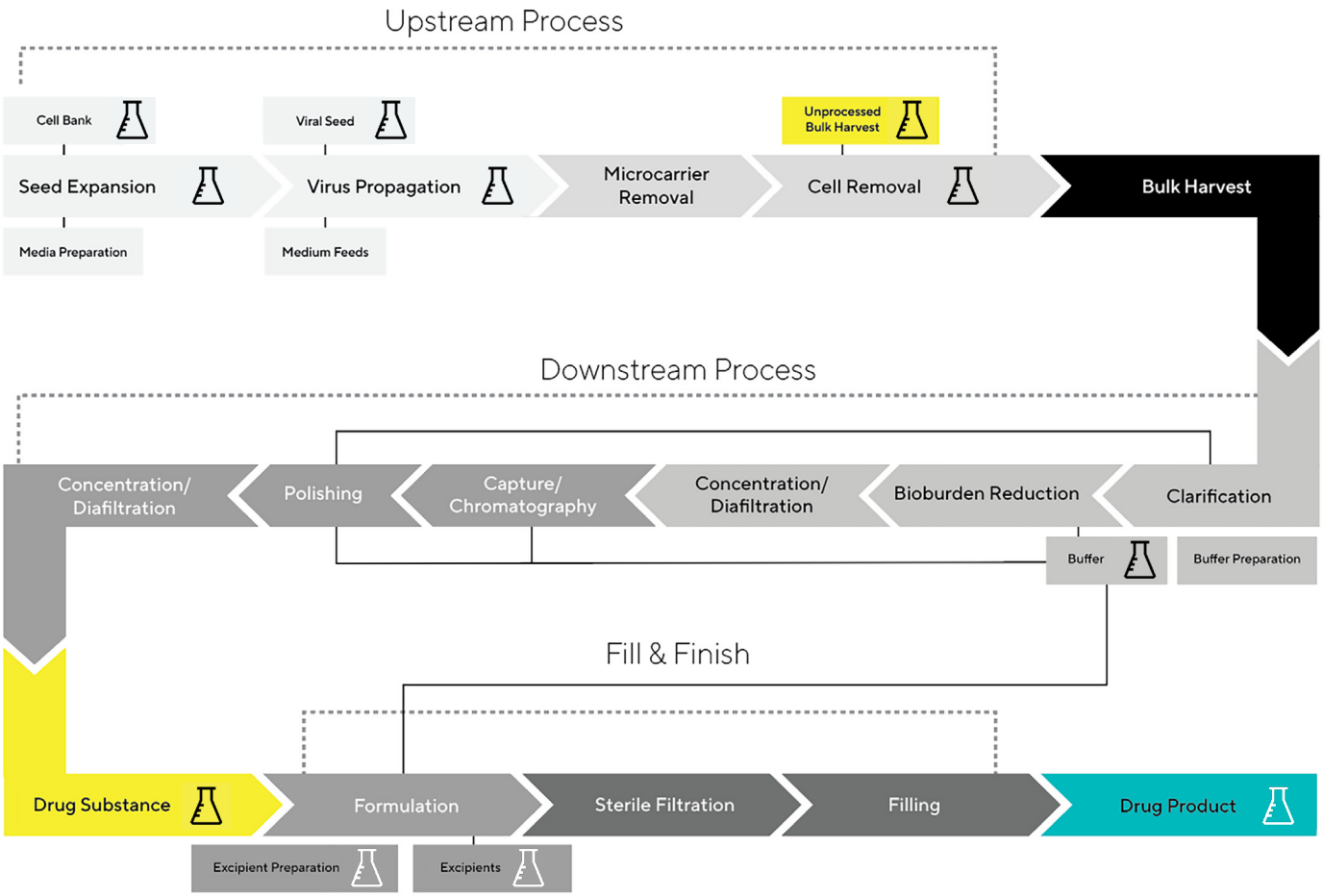
Membrane filtration is the prescribed pharmacopeial method for sterility testing, the chapters USP<71> and the Ph. Eur. 2.6.1 specify that the 'technique of membrane filtration is used whenever the nature of the product permits'. Its advantage is that by filtering large volumes of a sterile product, even a single CFU (colony forming unit) in large volumes, perhaps litres, can effectively be retained on the membrane filter and subsequently cultured. The method also permits for the elimina-

tion of compounds with bacteriostatic or fungistatic properties through filtration and rinsing.

Rapid methods, as previously described, are gaining acceptance for the testing of short-shelf life products, such as cell and gene therapies as well as ATMPs and radiotracers used in PETs, and may also be used for traditional sterile pharmaceuticals. The Sterisart® device enables aseptic sampling during incubation by means of a septum port in the canister, ensuring compliance with global regulations. Product samples are filtered, the canisters filled with growth media and incubated for a period of 5-6 days. A sample is then drawn aseptically via the septum port and transferred to the rapid testing platform Celsis® (Charles River) for testing. This way quality control labs can maintain a growth-based strategy and at the same time achieve an earlier result. Further, the filtration method ensures that only viable microorganisms are reliably detected, avoiding any undue concern on background or noise, leading to false positives.

There are multiple sources of potential microbial contamination in any manufacturing process and

Quality control testing is applied at several testing points (⚗) along the manufacturing process. Take a look below at a typical cell-based vaccine manufacturing process and see where Sartorius products and services can help you overcome your quality control challenge.



cell culture-based processes are no exception. In sterile pharmaceutical production environments, the monitoring of the air is a key requirement and part of biocontamination control strategies as described in related standards and guidelines, such as the EN 17141 and the recently revised EU GMP Annex 1. According to the EU GMP Annex 1 Revision, continuous viable air monitoring has to be ensured during the full duration of critical processing, including equipment (aseptic set-up) assembly and filling operations and any risk caused by the intervention of the monitoring process should be avoided.

To comply with the need for long-term sampling, gelatin membrane filters are the optimal solution, and can be used non-stop for the whole production run, e.g. for 8h using only one single filter.

The MD8 Airscan® command unit utilizes a single, sterile gelatin membrane filter to capture and retain even the smallest airborne microorganisms, even viruses, over an 8 hour period. This USP-approved membrane filter can then be placed on any standard agar plate for routine incubation as per environmental monitoring protocols. This method of continuous active air monitoring keeps quality control labs compliant and avoids the risk of false positive results due to sampling errors.

In addition to microbial contaminations, other process-related impurities such us residual host cell proteins (HCP), produced during cell culturing and residual protein A (RPA) can also); a potential product purification contaminant can negatively impact the safety and efficacy of the vaccines produced in living cells. Therefore, these tests need to be included before in the final release testing package. Quality control labs typically use the Octet® Bio-Layer Interferometry (BLI) platform for such impurity lot-release assays. The Octet® system is also heavily used for other QC assessments including vaccine potency assessment through ligand binding assays, as well as to develop stability indicating methods that assess changes in activity through stressed and forced degradation assays. This easy to use platform helps the daily routine work of quality control technicians, and in combination with the high-throughput capability it enhances productivity.

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Application Note

Cell Line Development: Accelerating Process Optimization by Combining Ambr® 15 Cell Culture with Octet® Titer Measurements

Jonathan Kirby[2], Alison Rees-Manley[2], David Apiyo[3], Lukas Klein[1], Eva Diem[1], Tim Dale[2]

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Introduction

Cell line development involves the screening of thousands of clones to find those that are stable, produce high yields of the bioproduct and exhibit desired critical quality attributes (CQAs). Typically screening and process optimization activities will be carried out at the small scale in bioreactor cultures to ensure that results translate up to larger bioreactor scales. Performance data is primarily based on cell growth, cell viability, metabolite analysis and product titer, and assessed over the entire culture process duration. Often product titer data will have a longer turn-around time where samples are submitted to analytical groups for analysis. Introducing analytical technology into the cell culture process development workflow can greatly speed up the time to results. The Octet® platform is an analytical instrument that can easily be implemented in cell line development labs to facilitate rapid determination of product yield. This capability allows informed decisions directly at the

end of the process, for example which samples need to be sent off for quality analysis, reducing overall sample numbers. It also enables the next experiments to be planned based on all of the performance data, rather than waiting days or weeks for product yield results to be available. Here we show the power of using the Ambr® 15 Cell Culture system with an integrated Vi-CELL XR and Ambr® Analysis Module together with an Octet® to identify optimum yield conditions in media screening and process optimization experiments.

Ambr® 15 Cell Culture

The Ambr® 15 Cell Culture, is an automated, high throughput single-use bioreactor system that provides parallel operation of up to 48 single-use microbioreactors at a time and can be managed by a single operator.

Comparing cultures in identically-sized, multi-parallel bioreactors, allows scientists to screen more clones in a representative bioreactor format early on in the cell line development workflow and obtain meaningful results in a shorter time frame. Multiple experiments can be set-up to evaluate different cell lines or clones, and to investigate the effect of process parameters, such as temperature, feeding, media composition, gassing rates and inoculation densities.Together these features enable cell line selection, media selection, and process optimization to be executed in a quick and efficient way.

Ambr® 15 Cell Culture is the industry standard microbioreactor system, implemented in laboratories world wide. Studies in Ambr® 15 have shown greatly improved results when compared to shake flask or shaking plate cultures due to the high level of automation combined with reliable and independent process control for pH and dissolved oxygen. Flexible operation allows cultures to be run in batch, fed-batch and even perfusion mimic mode. With low working volumes, from 10 - 15 mL, the small-scale stirred bioreactor vessels reduce the cost per experiment by saving substantial amounts on media and feeds.

For ease of product quantification and CQA assessment the Ambr® 15 and Octet Bio-Layer Interferometry can be implemented as platform technologies to manage the cell line development workflow (Figure 1, on the following page).

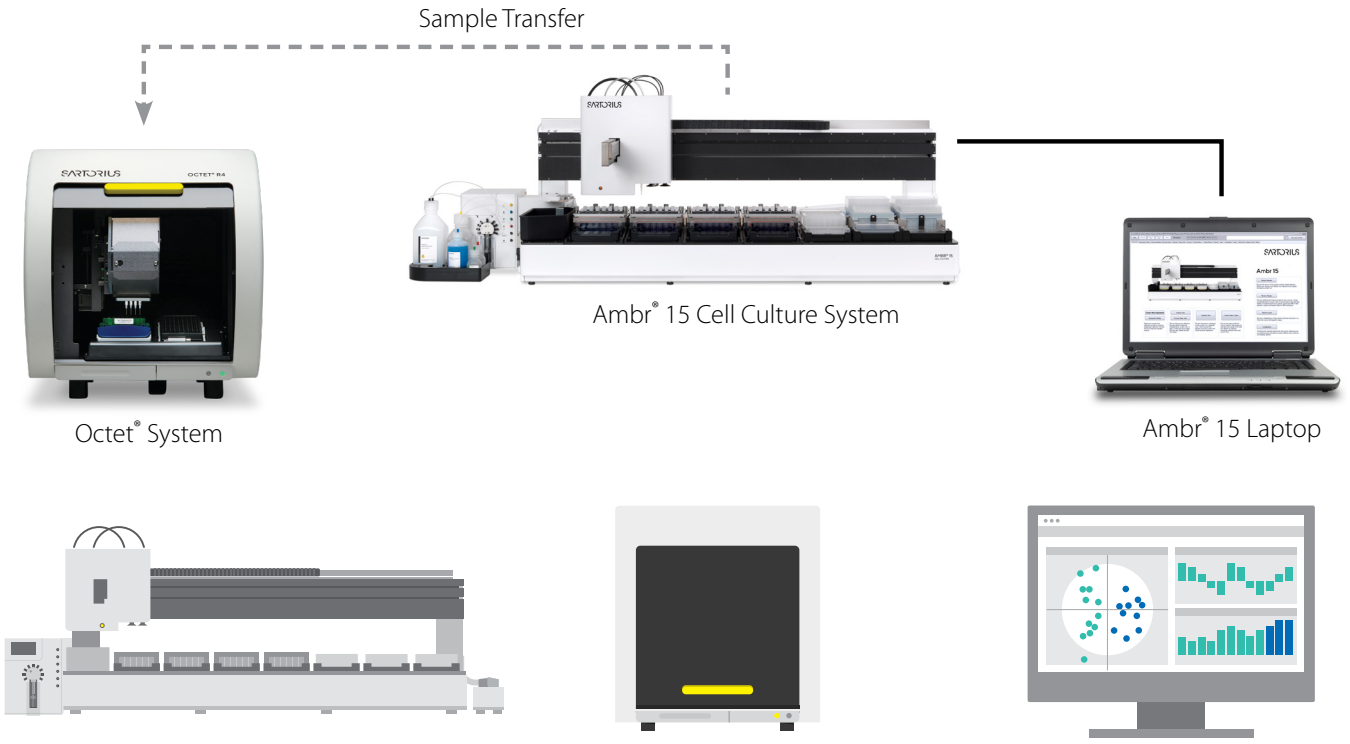
Octet® Bio-layer Interferometry (BLI) Platform

Octet® systems operate on the label free, non-fluidic based BLI technology with Dip and Read assay format, that utilizes micro-titer plates and propriety biosensors coated with protein ligands to enable specific binding between the ligand and the relevant binding partner. These systems are commonly used for kinetics and affinity constants analysis for receptors binding to antibodies, viral particles, recombinant proteins and many other biological molecules. For IgG titer determination Protein A or Protein G coated Octet® biosensors can be dipped into IgG samples with the binding expected to occur between the biosensor and the IgG through the Fc region. BLI measures changes in light interference patterns originating from the tip of the biosensor surface where light wavelengths are made to reflect from

two layers; a biocompatible layer at the end of the biosensor surface and an internal reference layer. The spectral pattern of the reflected light changes as a function of the optical thickness of the molecular layer. This spectral shift is monitored at the detector and reported on a sensorgram as a change in wavelength (nm shift).

The key feature for Octet® analysis is that the refractive index changes in the sample do not affect shifts in the interference pattern. This leads to reduced matrix effects enabling the Octet® to quantify IgG concentration from both purified samples and heterogeneous crude lysates. The advantage allows for significant reduction in the analysis time as samples can be simply transferred into micro-titer plates and Protein A or G biosensors dipped in one step for rapid analysis. In addition, the newly launched Sartorius GlyS and GlyM

Figure 1: *The Octet® and Ambr® 15 Cell Culture Setup for Clone and Media Selection*



Ambr® 15 Cell Culture
- Multiparallel microbioreactor cultures
- Clone screening and ranking
- Media screening
- Process optimization

Octet® Label-free Biomolecular Interaction Analysis
- Titer determination
- Evaluate critical quality attributes (CQAs)
- Quality and stability monitoring

Data Collection and Analysis Using MODDE® Software
- Assimilate data from platforms providing enhanced decision making
- Helps teams to select better clones, screen more media and optimize processes faster

kits for sialic acid and mannose content screening respectively can be used in tandem with the titer measurement biosensors to better understand product quality changes as a function of different media or processing conditions.

Key benefits of the Octet® platform include:

- Significantly reduce analysis time by using crude samples and ready to use Protein A or Protein G biosensors; product titer can be obtained in less than 5 minutes from as many as 96 samples on the Octet® RH96
- Save costs and complete more projects on the Octet® with minimal analyst time required (Table 1 compares the Octet® to other platforms commonly used for titer determination)

A comparison between the Octet® platform, HPLC and manual ELISA for mAb titer. A project in theis example is defined as the titer determination for a total of 10,000 mAbs in a high throughput screening process. The data in the table assumes an analysis labor time of 0.2 hours, 0.5 hours and 3 hours for the Octet® ELISA and HPLC respectively.

Table 1

	Octet®	ELISA	HPLC
FTE labor costs	X	15X	3X
Time to results (hrs)	52	625	1040
# projects/year	40	3	2

Materials and Methods

Media Screening Experiment in Ambr® 15

Ambr® 15 Cell Culture standard microbioreactors with sparge tube (Figure 2) were inoculated with a density of 3E5 cells/mL in a starting volume of 13 mL. Each microbioreactor has individual gassing via sparge tube into the culture. The pH and DO control loops maintained target set-points by applying CO_2 and O_2 as required, along with a fixed ballast gas (air) set at 0.15 mL/min throughout the process. Set-points were as follows; pH 7.0 (upper limit of 7.1), DO 40%, temperature 36.8 °C and stirring speed 1300 rpm.

Figure 2:
Ambr® 15 Cell Culture Microbioreactor (With Sparge Tube)

Five different media were investigated as part of this experiment with two different clones.

Process Optimization Experiment in Ambr® 15

Ambr® 15 Cell Culture standard microbioreactors with sparge tube were inoculated with a density of 3E5 cells/mL at three different starting volumes ranging from 12 to 14 mL. Each microbioreactor has individual gassing via sparge tube into the culture. The pH and DO control loops maintained target set-points by applying CO_2 and O_2 as required, along with a fixed ballast gas (air). In

this experiment the ballast gas (air) flow rates were between 0.05 to 0.25 mL/min. The process set-points were as follows; pH 7.0 (upper limit of 7.1), DO 40% and temperature 36.8 °C.

Four different stirring speeds were investigated as part of the DOE experiment, these ranged from 1050 to 1650 rpm.

Media and Reagent Additions

Proprietary basal and feed media were used for the experiments. Daily feed and antifoam solutions were added to each vessel automatically for both experiments.

Sampling and Analysis

Viable cell concentration (VCC) counts were obtained via a coupled Vi-CELL XR (Beckman Coulter, USA). pH measurements were obtained via an integrated Ambr® Analysis Module. Glucose and lactate measurements were performed off-line with an EKF BIOSEN S-Line device (EKF-Diagnostic GmbH, Germany) according to manufacturer's protocol. Cell viability and cell count were determined daily with a cut-off for titer measurement set at 70% viability i.e. when the viability fell below 70%, titer was not measured. For titer measurement daily samples were taken automatically by the Ambr® 15 Cell Culture. These samples

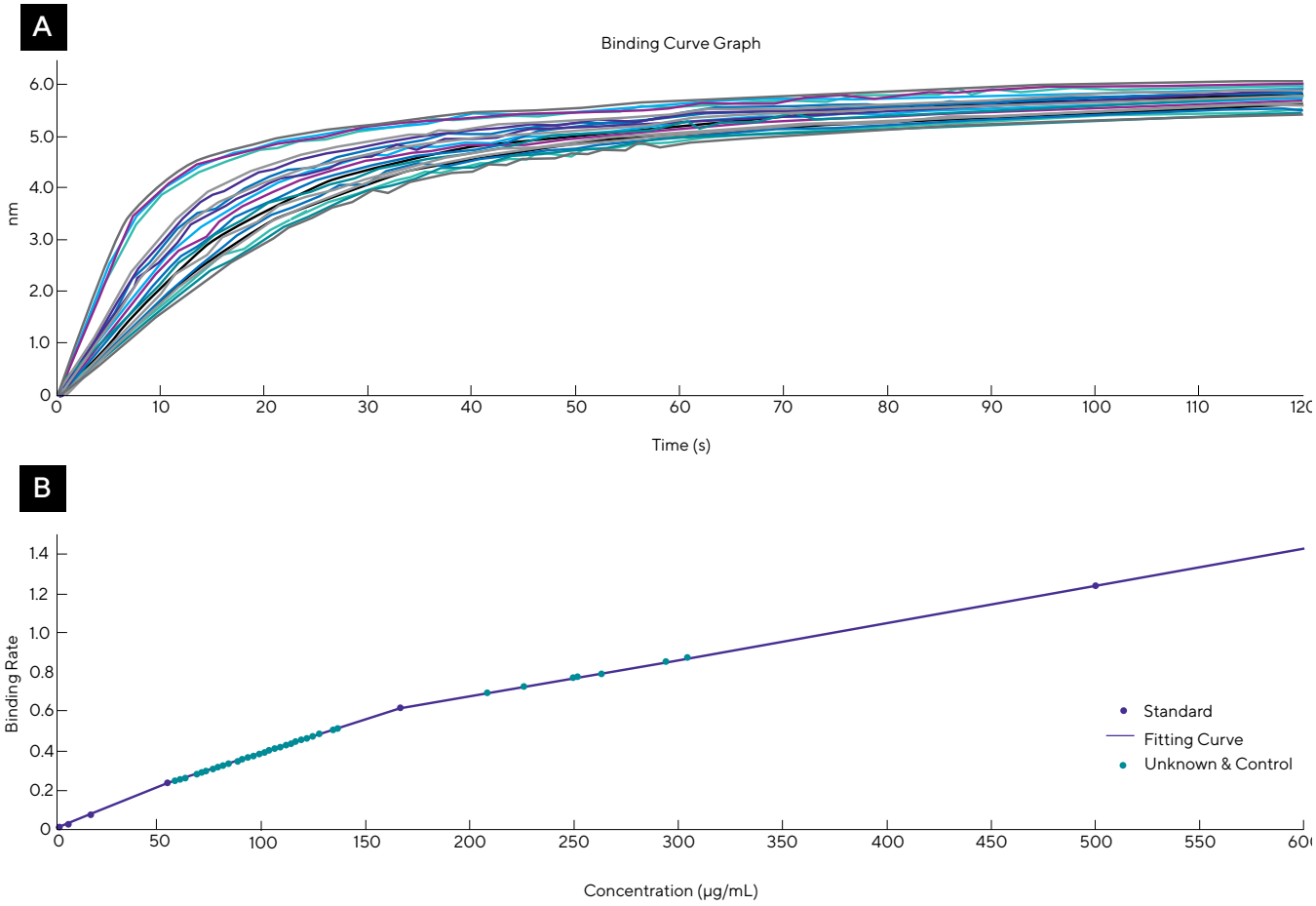


Figure 3. *IgG Titers: (A) Protein a Biosensor Binding Curves of IgG Molecules Generated From Two Replicate in Each of the Five Media Growth Conditions. (B) All 96 Sample IgG Concentrations (Orange) Plotted on the Calibrator Curve (Blue, 0-500 (µg/mL)*

were centrifuged for 5 min at 6600 xg, the cell-free supernatant was removed and retained for titer measurement.

Titer Measurement in Octet®

The cell-free supernatant samples were diluted in the required 96-well plate. The plate was then vortexed for at least 1 minute at a moderate level. An Octet® QKe was used with Protein-A biosensors. The system is suitable for measurement of protein concentration between 1 and 500 µg/mL, for higher protein titers the samples were diluted in media. Calibrator samples with known concentration were used for the generation of the standard curve (Figure 3). Biosensors were regenerated for re-use by dipping into regeneration buffer followed by neutralization buffer for 5 seconds each. Three cycles of regeneration were used. Quantitation assay time was set to 120 seconds with the sample plate temperature set to 30 °C.

Results and Discussion

Media Screening Experiment

Implementation of the Octet® platform alongside the Ambr® 15 Cell Culture allows cell line developers to identify the best path forward for the choice of the top clone and the best

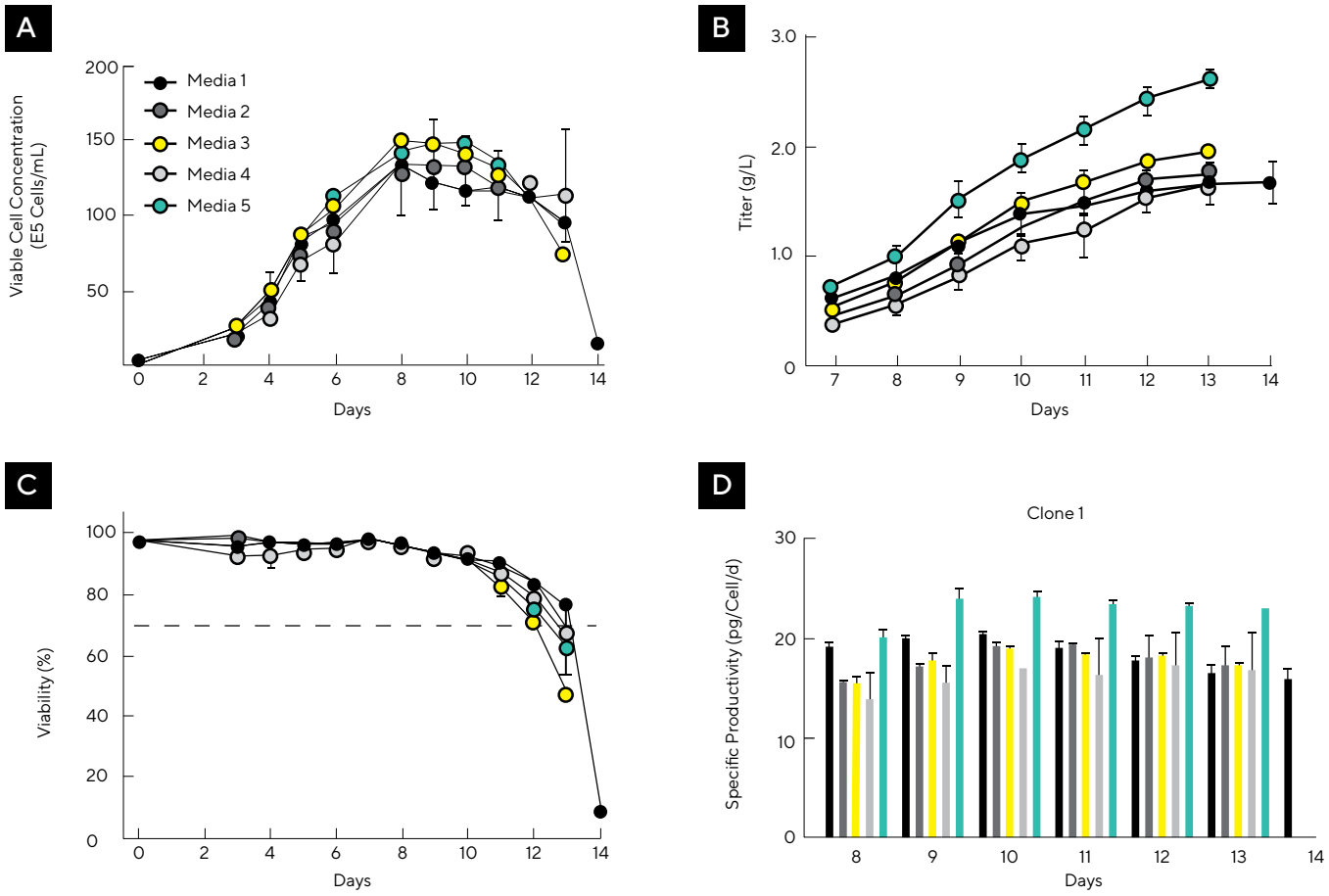


Figure 4. *Time-Course Cell Count and Titer Analysis for Clone 1 in Different Media Types: Cell Count Was Performed Using Vi-CELL XR While the Octet® Was Used for Titer Determination*

media combination during the early stages of development. This study demonstrated the ease, simplicity and speed of performing multiple conditions in one experiment using Ambr® 15 Cell Culture by assessing five different media compositions on two different clones.

One clone (Figure 4 - Clone 1, shown on the previous page) shows all five media types resulted in similar viable cell count profiles and cell viabilities, with slightly higher peak cell densities for Media 3 and Media 5. The product titer results (Figure 4B) clearly highlight Media 5 as the best performing media for mAb production, and this is shown in the cell specific productivity (Qp) graph (Figure 4D).

Results from a second clone (Figure 5 - Clone 2) shows comparable cell growth in all five media types up until day 6, after which both Media 1 and Media 2 outperform the other media in terms of peak viable cell densities and also cell viabilities. For this clone the cell viabilities in Media 3, 4 and 5 start to decline fairly early on, from day 8 onwards. In the titer graph (Figure 5B) we can see higher product titers in both Media

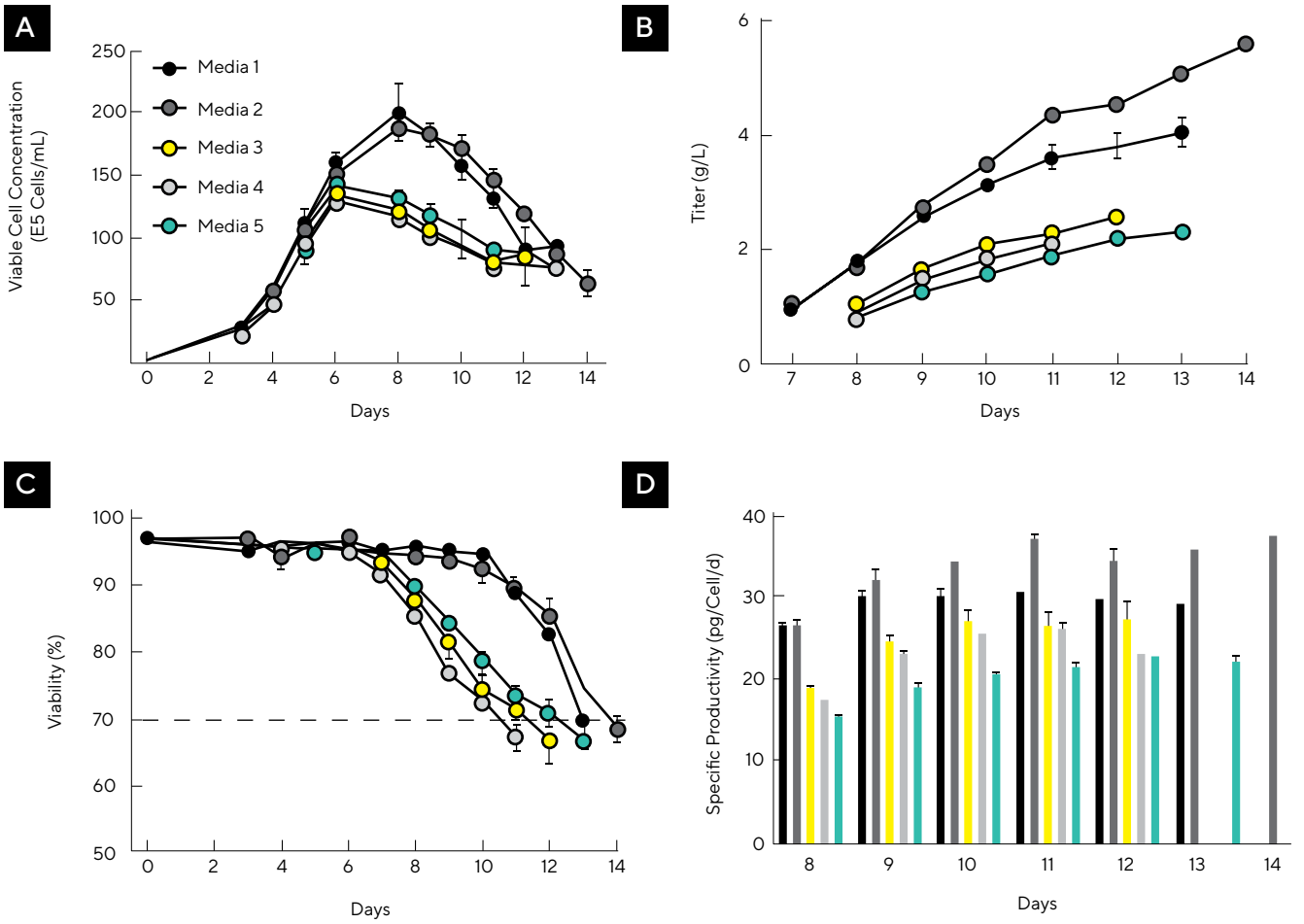


Figure 5. *Time-Course Cell Count and Titer Analysis for Clone 2 in Different Media Types. Cell Count Was Performed Using Vi-CELL XR While the Octet® Was Used for Titer Determination*

1 and Media 2, but overall Media 2 has higher productivity after day 8 and for the remainder of the process resulting in higher Qp (cell specific productivity) values (Figure 5D).

Furthermore comparison of both clones, shows that overall Clone 2 achieved a maximum product titer of more than double the best results for Clone 1. However had Media 5 been chosen as the best media then the results would have indicated Clone 2 to have a lower performance, since Media 5 gave lower productivity in combination with this clone. Therefore it should not be underestimated the power of screening multiple clones and different media types together since performance can vary significantly.

Process Optimization Experiment

Design of Experiments (DOE) is a rational and cost-effective approach to practical experimentation that can provide a great deal of information about the effect on a response variable due to one or more factors. DOE can also identify important interactions that may be missed when experimenting with one factor at a time.

The Ambr® 15 software includes a license for MODDE® DOE which has been integrated to allow planning of DOE experiments and already factors in the set up of the Ambr® 15 system. Process parameters that are included in the DOE study can be tagged in the Ambr® 15; this facilitates running of the experiment and allows easy transfer and analysis of the results in MODDE® on completion. The DOE MODDE® software integrated in the Ambr® 15 software enables scientists to quickly establish a Design Space where relevant bioprocessing conditions are varied simultaneously. Analysis of product titer using the Octet® through the process allows data analysis and further

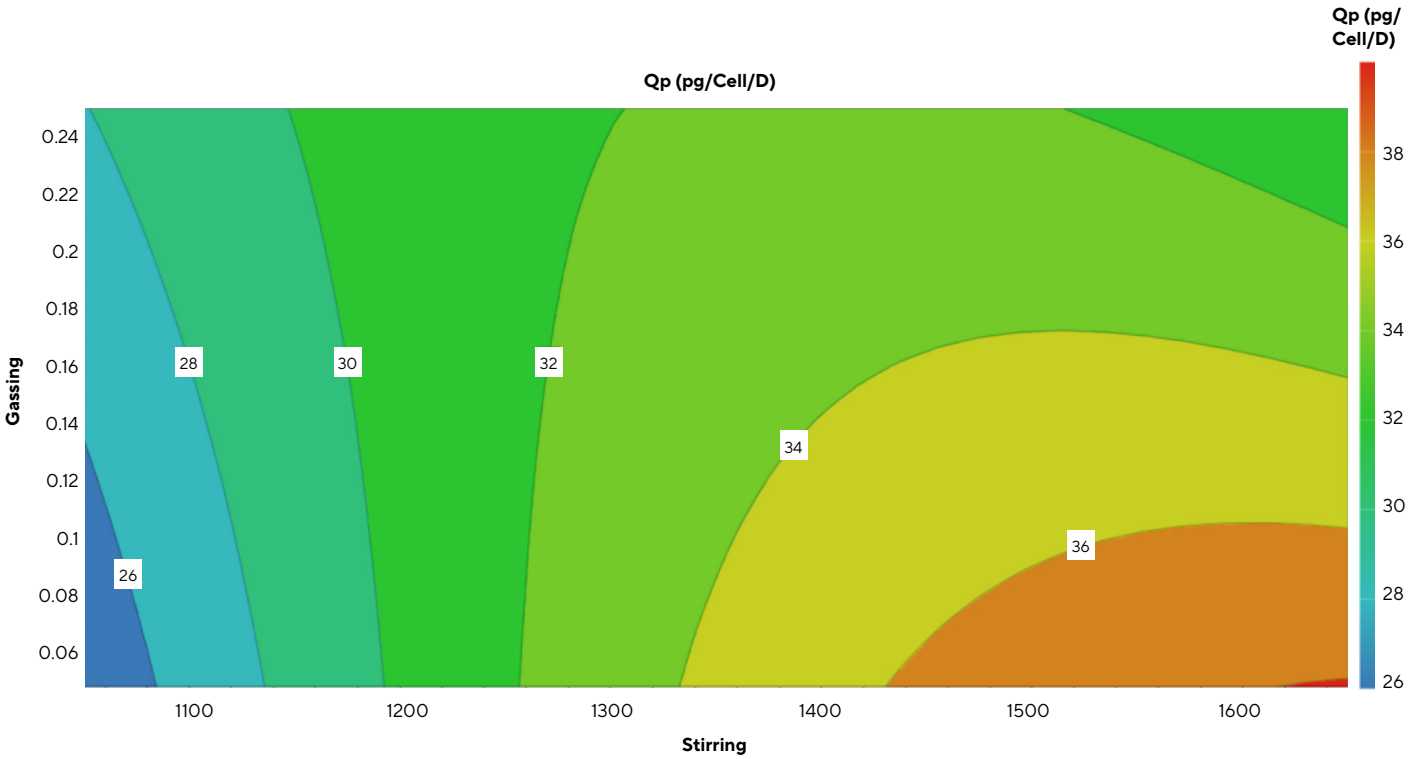


Figure 6. *Response Contour Plot of Effect of Stirring vs Ballast Gassing on Qp at Endpoint; analyzed in MODDE® Software*

experiment planning to occur without delay and can have a positive impact on project scheduling.

In this experiment, stirring speed, ballast gas flow rates and starting volume were all factors that were varied in one experiment (Table 2). A reduced combinatorial design was used to strike a good balance between the factors investigated with limited number of experiments and replicates. Cell counts and product titer results were used to calculate Qp as a response.

Table 2: Overview of Process Parameters and Responses of the DOE Study

Process Parameters	Range
Stirring speed	1050 - 1650 rpm
Ballast gassing (air)	0.05 - 0.25 mL/ min
Starting volume	12 - 14 mL
Responses	Qp (cell specific productivity)

The DOE software MODDE® provides an easy-to-use, user-friendly interface for experimental design as well as statistical data analysis and visualization. Together with the design Wizard and analysis Wizard, MODDE® provides the user with guidance through their process investigations.

The response contour plot (Figure 6, on the previous page) shows how Qp is impacted by the combination of stirring speed and ballast gassing. At low stirring speeds increasing the ballast (air) gas flow rate has a small but positive effect, as the stirring speed increases to 1200 rpm there appears to be no effect from the ballast gas flow rate. At the highest stirring speeds however, it actually has a detrimental effect to Qp and lower ballast gas flow rates are desirable. Overall, the highest specific productivity values are seen at the higher stirring speeds combined with lower ballast gas flow rates.

In general, as stirring speeds increase so does the kLa - the mass transfer coefficient; a measurement of the capacity of the bioreactor to transfer oxygen into the culture. This is due to the energy input of the stirrer providing better distribution and reduction in size of the gas bubbles. Smaller bubbles improve oxygen transfer since they have a larger gas-liquid interface per unit of liquid volume and they have longer residence time in the medium, when compared with larger bubbles. Together with an increase in the amount of gas sparged into the cultures, this will further increase kLa, however higher shear stress exerted on cells may impact cell viability, which in turn will decrease cell productivity.

The main effect plot is a plot of the mean response values at each level of a single process variable. The stirring speed main effect plot (Figure 7A) shows only the relationship between stirring speed and cell specific productivity Qp. The productivity of the cells improves with stirring speeds up to approximately 1500 rpm, after which the cell productivity plateaus and at the highest stirring speeds tested there is a very slight decrease in Qp. Cell viability profiles (data not shown) displayed a slightly faster decline over time under the higher gassing and higher stirring conditions. Whilst the maximum Qp was obtained at the higher stirring speeds tested, for this particular clone it appears that some shear sensitivity was observed.

The starting volume main effect plot in this study (Figure 7B) suggests a lower fill volume provides a higher Qp (higher yield per cell). Changing the volume inside a bioreactor changes the total

surface area for gas exchange. In Ambr® 15 Cell Culture the interfacial area between headspace volume and liquid volume is a higher contributor to the overall vessel kLa than might be the case in a larger bioreactor. Additionally at lower working volumes, for the same stirring speed the power input per unit volume rises, which means that the energetic input going directly into disrupting and encouraging suspension of gas bubbles in the liquid is greater, which will also make an important contribution to the kLa. Thus, the relationship of fill volume with other parameters must be considered carefully during process optimization.

The DOE experiment provides great insights into how product yield is affected by the different process parameters, and can be viewed individually (main effects plot), in combination (contour plot), or in other formats which may to help to identify which variables are critical to a particular

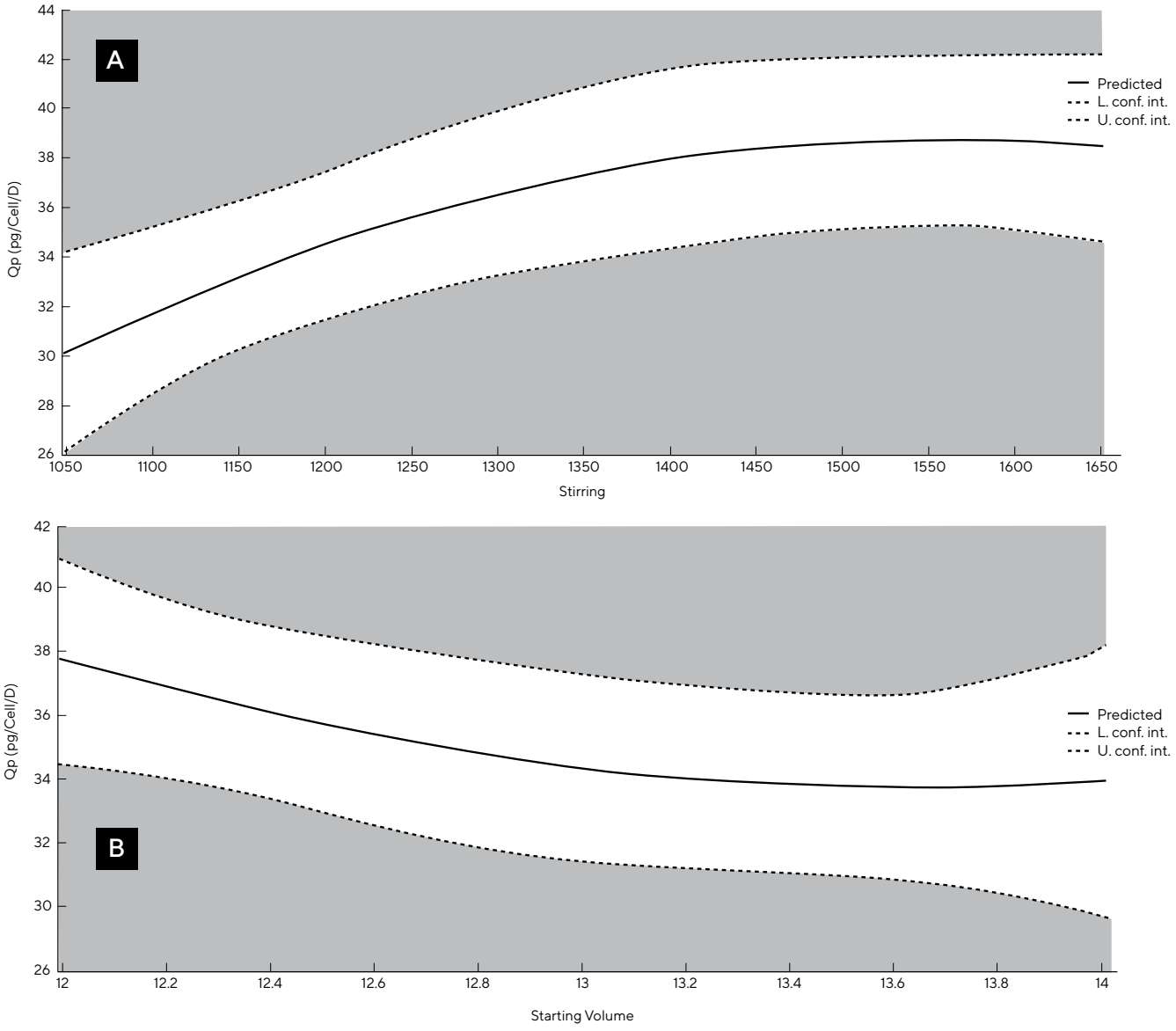


Figure 7. *Main Effect Plots for Specific Productivity (Qp) for (A) Stirring (B) Starting Volume; analyzed in MODDE® Software*

cell line and | or process, and this knowledge can be applied to support further optimization work. The results from this study showed that for the tested clone, higher stirring speed, lower ballast gassing and lower starting volume resulted in the highest specific productivity, however care must be taken when defining the optimum setpoints, to ensure that other important factors, such as cell viability, are also taken into account.

The powerful features in MODDE® embedded within Ambr® 15 allows high throughput DOE studies to be performed together with product titer analysis using the Octet®, the results of which assist with understanding the relationships between the process parameters and the cell specific productivity of a particular clone(s).

Summary

Implementation of the Octet® platform alongside the Ambr® 15 Cell Culture system within the cell line development workflow allows easy and rapid product quantification, and could also enable assessment of critical quality attributes when combined with the newly launched Sartorius Octet® GlyS and GlyM kits.

The Ambr® 15 Cell Culture automated micro-bioreactor system for mammalian cell culture, is able to run up to 48 × 15 mL cultures per experiment, thus offering considerable advantages for screening of multiple cell lines or clones in parallel, and reducing the experimental costs associated with media and feeds, especially for fed-batch or intensified bioreactor processes. The Octet® on the other hand can perform process analytics on crude samples with minimal sample processing. Compared to traditional titer determination techniques such as ELISA or HPLC, the Octet® enables a faster turnaround of yield analysis with minimal analyst involvement.

In these studies, we've demonstrated the power of combining an Ambr® 15 Cell Culture with the Octet® platform by comparing cultures in identically-sized, multi-parallel bioreactors for the rapid identification of the best combinations of media and clones based on the product titers and cell specific productivity. A DOE experiment was then used to examine the role of different physical process parameters; starting volume, stirring and ballast gassing rates on product yield.

Reference

1. Biolayer Interferometry as an Alternative to HPLC for Measuring Product Concentration in Fermentation Broth, Anurag S. et al., LCGC, Volume 35, Issue 12, 870–877.

Guide to Abbreviations

BLI: Bio-Layer Interferometry
CQA: Critical Quality Attribute
DO: Dissolved Oxygen
DOE: Design of Experiment
ELISA: Enzyme-Linked Immunosorbent Assay
HPLC: High-Performance Liquid Chromatography
IgG: Immunoglobulin G
Qp: Cell Specific Productivity (pg Protein/Cell/Day)

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