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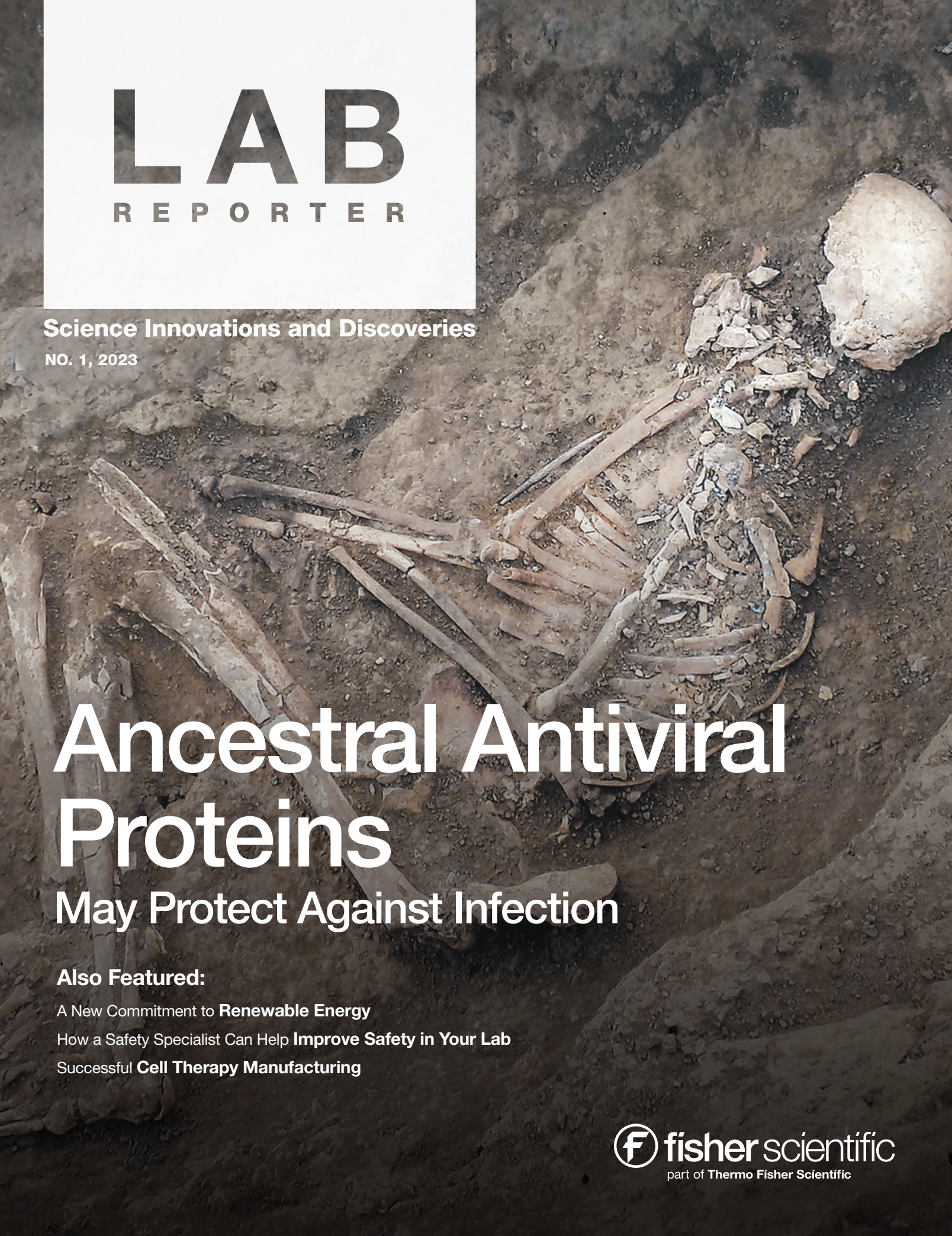
NO. 1, 2023



Ancestral Antiviral Proteins

May Protect Against Infection

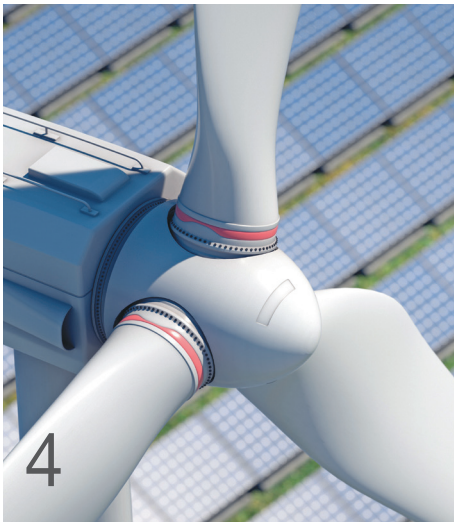
Also Featured:

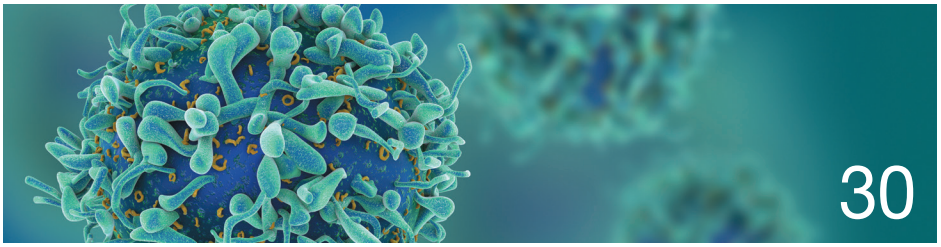
A New Commitment to **Renewable Energy**

How a Safety Specialist Can Help **Improve Safety in Your Lab**

Successful **Cell Therapy Manufacturing**

CONTENTS





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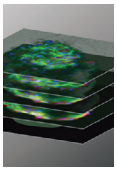

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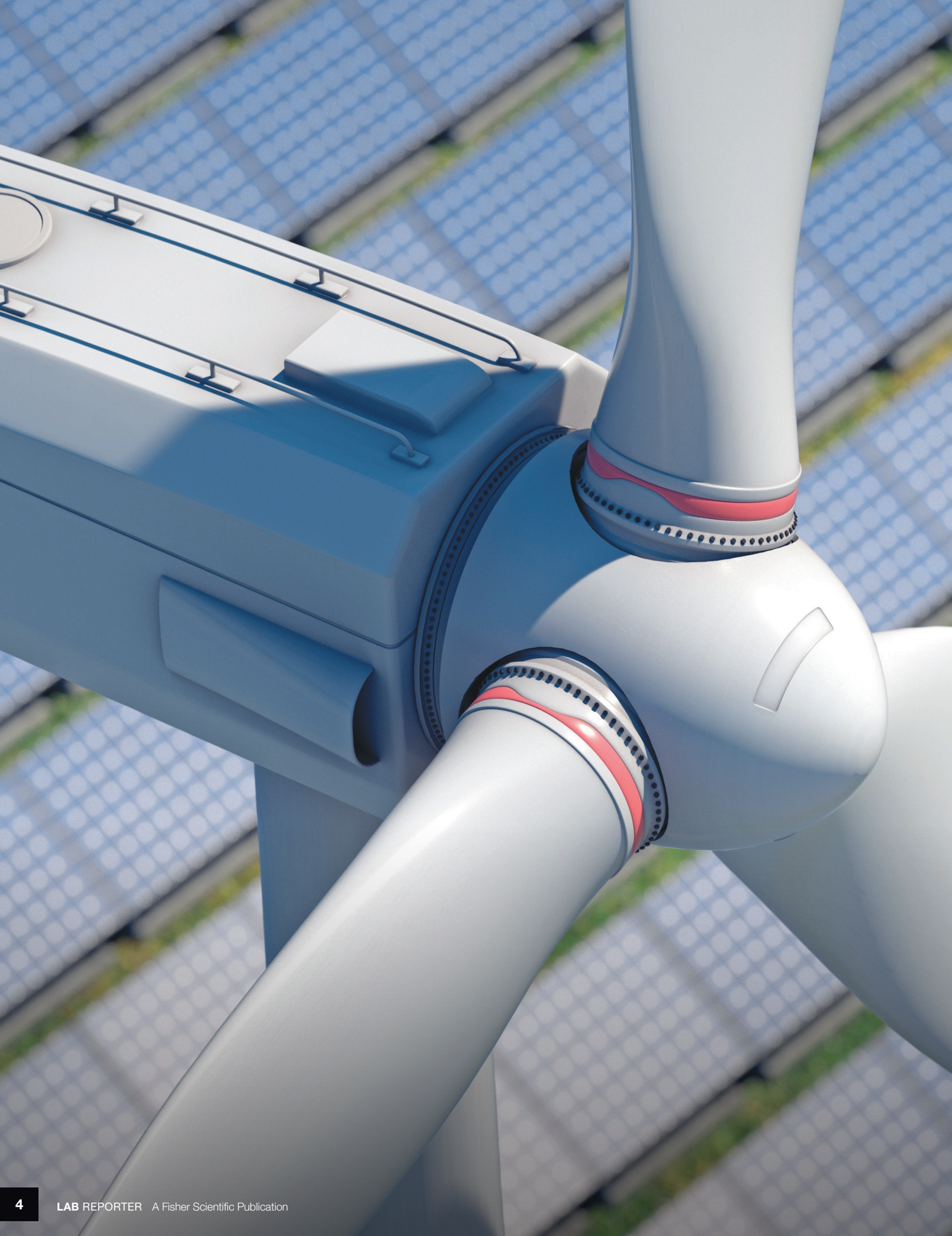

A New Commitment to Renewable Energy

By Kylie Wolfe

States across the Midwest are leading the quest for renewable energy in America. After Texas and Iowa, Oklahoma is the third largest producer of wind energy in the United States. Its open landscapes are home to over a dozen wind farms, one of which is helping Thermo Fisher Scientific reach critical sustainability milestones.

In September 2022, Thermo Fisher furthered its commitment to renewable energy, purchasing a 90-megawatt portion of the Seven Cowboy wind project in western Oklahoma. This step supports the company's long-term plan to reduce emissions.

Harnessing Wind in Western Oklahoma

Managed by Enel Green Power, the Seven Cowboy wind project is comprised of 107 wind turbines that are expected to generate roughly 1.3 terawatt hours of energy each year. This amount of power could keep over 120,000 U.S. households running and at least 758,000 tons of CO_2 emissions out of the atmosphere.

Through an eight-year virtual power purchasing agreement (VPPA), Thermo Fisher secured 90 megawatts of the overall system. This equates to 400,000 megawatt hours of renewable electricity each year, half of the company's electricity usage in the U.S.

"If we were to take every site and cover every roof, parking lot, and adjacent property with solar panels or wind turbines, we still wouldn't be able to match the total combined electricity used across the organization. These VPPAs, or off-site projects, give us that flexibility and the ability to fundamentally address our Scope 2 emissions at the scale that we're actually producing them," said Scott Self, director of procurement at Thermo Fisher Scientific.

An Important Step Forward

The U.S. Environmental Protection Agency (EPA) has different classifications for emissions. Scope 1 emissions are defined as "direct greenhouse (GHG) emissions that occur from sources that are controlled or owned by an organization." Scope 2 emissions are those that result from the purchase of utilities like electricity. These are operational emissions associated with buildings and on-site manufacturing efforts. Scope 3 emissions are indirect and associated with an organization's supply chain, often the largest sources of emissions.

In 2021, Thermo Fisher joined the Business Ambition for 1.5°C campaign—led by the Science Based Targets initiative—and committed to net-zero emissions by 2050. This included reducing Scope 1 and 2 emissions by 30 percent compared to 2018 values. This goal was recently increased to 50 percent.

The Seven Cowboy wind project helps address Scope 2 emissions by generating renewable energy credits. It's those credits that are used to match emissions from sites across the country. By improving Scope 2 emissions, Thermo Fisher is not only supporting clean energy but also helping customers meet their Scope 3 reduction targets. That's because a company's Scope 1 and 2 emissions contribute to their customer's Scope 3 emissions.

> "These VPPAs, or off-site projects, give us that flexibility and the ability fundamentally to address our Scope 2 emissions at the scale that we're actually producing them."
>
> **Scott Self**
> *Director of Procurement*
> *Thermo Fisher Scientific*

"When you think about the relationships between us and our customers and even us and our suppliers, it's like links in a chain that all support each other," said Self.

The project is expected to be operational in the second half of 2023.

Plans for a Sustainable Future

Today, Thermo Fisher has more than 60 sites around the world that exclusively use renewable electricity. All sites in Germany and Italy fall into this category, relying on sources like the Seven Cowboy project. Some sites also have their own solar panels and wind turbines, generating electricity physically on site. In Cork, Ireland, a wind turbine helps match a lot of the emissions at the facility.

Self says that the company is looking to expand on-site solar initiatives wherever possible. Over 20 projects are currently in development, including two physical installations at Massachusetts-based sites. His team is also looking to remove equipment that uses fossil fuels. By taking steps to add renewable systems on site and leverage long-term power purchasing agreements, Thermo Fisher is making strides toward a more sustainable future.

Kylie Wolfe is a Thermo Fisher Scientific staff writer.

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How a Safety Specialist Can Help Improve Safety in Your Lab

By Christina P. Hooton

Underlying hazards exist in every facility, and the lab is no different. The key to maintaining a safe work environment for your employees, faculty, and students is identifying hazards and putting in place the personal protective equipment (PPE), training, and resources required to mitigate those risks.

Whether you're just starting your safety program or need to reinvigorate your existing safety processes, a safety specialist can serve as your trusted resource and offer the support you need to prioritize safety in your lab.

Identifying Hazards

When designing a safety program, the lab manager or the environmental, health, and safety (EH&S) professional supporting the facility will perform a hazard assessment. During the assessment, they will identify all the hazards within a lab, prioritize each, and define the proper engineering controls, work practices, PPE, or other safety supplies to use in those situations.

"Safety specialists with a distributor or a PPE manufacturer can help support a hazard assessment, providing core information and then personalizing it to their particular organization and protocol," said Don Doyle, Fisher Scientific senior safety specialist, Thermo Fisher Scientific.

For example, hazardous waste may be present in the lab. In this situation, Charlie Fink, Fisher Scientific senior safety specialist, Thermo Fisher Scientific, recommends a site assessment. "It involves a walkthrough of your lab by a safety specialist who compiles a confidential report indicating where you may or may not be complying with applicable standards. I provide assessments to my customers who want to make sure they are properly storing hazardous materials and wastes and have the right products and supplies on hand to safely transport and dispose of wastes," said Fink.

Selecting PPE

After PPE requirements are identified through the hazard assessment, a safety specialist can make product suggestions for your lab based on your specific needs. For example, hand protection is needed in every lab, but selecting the right option depends on the work being done, regulatory requirements, and your business performance goals.

"Most customers are dealing with sharps, which could be blades, broken glass, etc., so they need a good solution for cut protection. In these situations, I usually recommend an 18-gauge cut liner that can be worn underneath their exam gloves," said Doyle. "Cryo gloves are another common solution. Although they are generally large, puffy gloves, there are options that offer high dexterity, are suitable for −80°C cryo work, and are great for handling frozen cryo tubes."

Optimizing Education and Training

Your lab personnel need to be aware of and comply with the standards and regulations of various government agencies, including the Occupational Safety and Health Administration (OSHA), the U.S. Department of Transportation (DOT), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA), depending on their work. It's also important for them to be aware of your organization's internal standard operating procedures (SOP) and the requirements of state and local governments.

The initial assessment will help reveal which standards and regulations lab personnel need to adhere to and identify training and education opportunities. Safety specialists can make additional suggestions and connect labs with safety training and services as well as training in specific areas, such as contamination control.

Focusing on Sustainability

In addition to maintaining safety standards, many labs need to meet sustainability goals that are intertwined with safety practices and products. Recycling and disposal can be challenging because of the many different waste streams—from hazardous and regulated medical waste to more traditional streams such as glass and plastic.

"From a lab and safety perspective, implementing sustainability measures could mean following green chemistry practices; recycling gloves, apparel, masks, and pipette tips; using biodegradable gloves and other products; and just properly disposing of and reducing waste of everyday safety supplies," said Josh Boyle, Fisher Scientific senior safety specialist, Thermo Fisher Scientific.

Relying on a safety specialist can make this process less daunting. They can provide greener product recommendations as well as suggest recycling programs that simplify the complexities.

No matter the hazard or area of focus, safety specialists can provide the extra support you need to improve safety in your lab every step of the way.

Visit **fishersci.com/contact-safety-specialist** or **fishersci.ca/contact-safety-specialist** to find out who your Fisher Scientific safety specialist is or if you need assistance with any of your safety or PPE needs.

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Christina P. Hooton is a Thermo Fisher Scientific staff writer.

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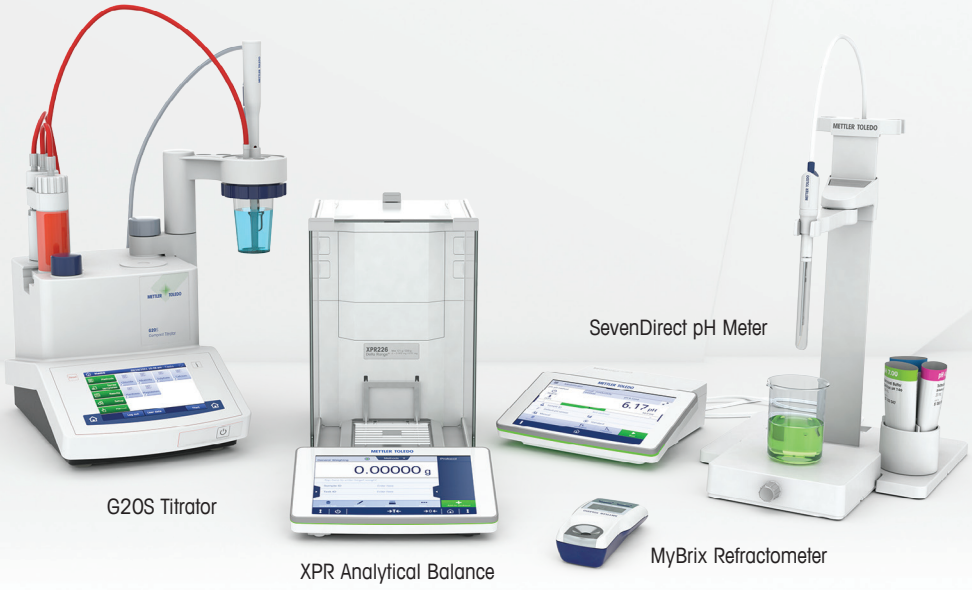

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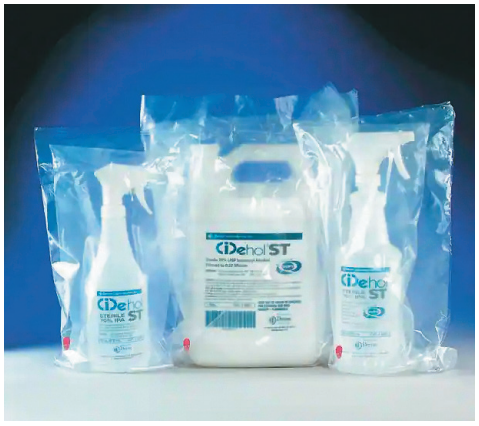

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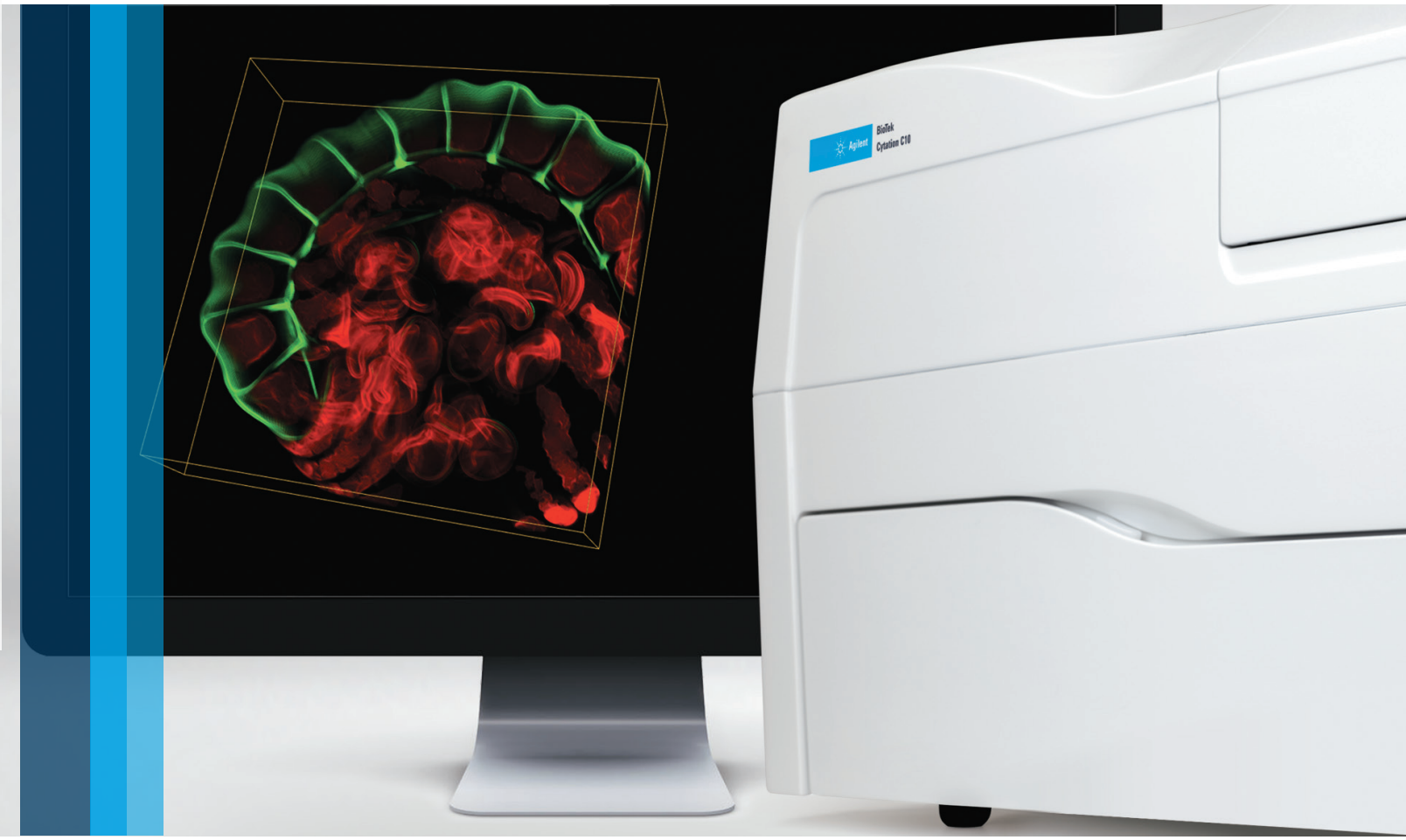

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Cleanroom-Compatible CO_2 Incubators for Cell Therapy

Cell therapy process development and manufacturing requires improved technologies for CO_2 incubators as well as other cleanroom equipment. These products must use materials optimized for stringent cleaning protocols and reduce the potential for microorganism and non-viable particulate contamination. CO_2 incubators must also provide optimal growth conditions for patient cell samples and adjustable shelving configurations to maintain high throughput per footprint.

Important Considerations

Cleaning and Disinfection Compatibility

CO_2 incubators with a brushed 304 stainless-steel exterior are recommended for cell therapy development and production. More resistant than a traditional painted steel exterior, this finish can tolerate chemical disinfectants and vaporized hydrogen peroxide (VHP) procedures.

Likewise, electropolished stainless steel components in the chambers have fewer microscopic structures that can become havens for microbial growth. Moisture-resistant housings and casings (rated IP54) and silicone-sealed touchscreen displays protect the electronics and can tolerate common cleanroom protocols.

Low Particulate Emission and Microbial Contamination Prevention

Particulate management remains a primary concern in cell therapy manufacturing since cell-based products for human injection cannot be easily purified. Although not the only contaminant of concern, microbial contamination remains the number one reason for biological pharmaceutical recalls.

Choose a CO_2 incubator that offers active particle control for ISO Class 5 and Grade A/B cleanroom environments. Your CO_2 incubator should also offer contamination control as well as control parameters to help cells express their optimal characteristics and potency.

- In-chamber circulating fans provide fast recovery, improve uniformity following door openings, and offer in-chamber HEPA filtration
- Carefully designed air circulation systems help parameters recover within 10 minutes after a thirty-second door opening
- Combined air circulation and HEPA filtration will help you capture particulates of all sizes and reach ISO Class 5 conditions in five minutes or less
- On-demand dry heat sterilization cycle tested by an independent, third-party laboratory will help to verify that it reaches a 12-log sterilization assurance level (SAL)
- Multiple-point temperature mapping demonstrates consistency throughout the chamber

Certificates and Documentation

Certified cleanroom-compatible CO_2 incubators are tested against ISO 14644-1 requirements and adhere to air quality limits within grade A/B cleanroom standards. In addition, each unit should undergo complete end-of-line testing. The manufacturer should also provide certificates and documentation to facilitate and support the audit process for on-site qualification, including:

- Product certificates, specifications, and materials of composition
- Factory acceptance test documentation
- Clear guidance for cleaning, disinfecting, routine maintenance, accessory and part replacement, and expected performance

Optimized Space to Help Achieve High Throughput

CO_2 incubators should accommodate appropriately sized culture vessels and produce an environment that promotes proper growth and the expression of desired receptors and optimum critical quality attributes (CQAs). Accessory incubator shelving systems can also help maximize limited incubator space, providing up to one and a half times more chamber capacity for higher output per footprint.

Visit **fishersci.com/co2-incubators** or **fishersci.ca/co2-incubators** to learn more.

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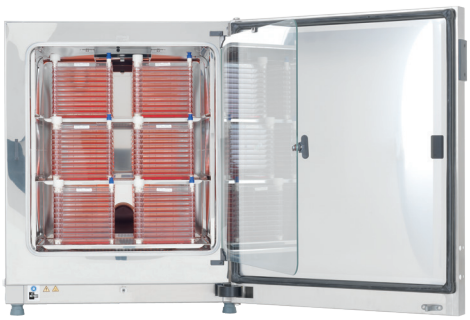

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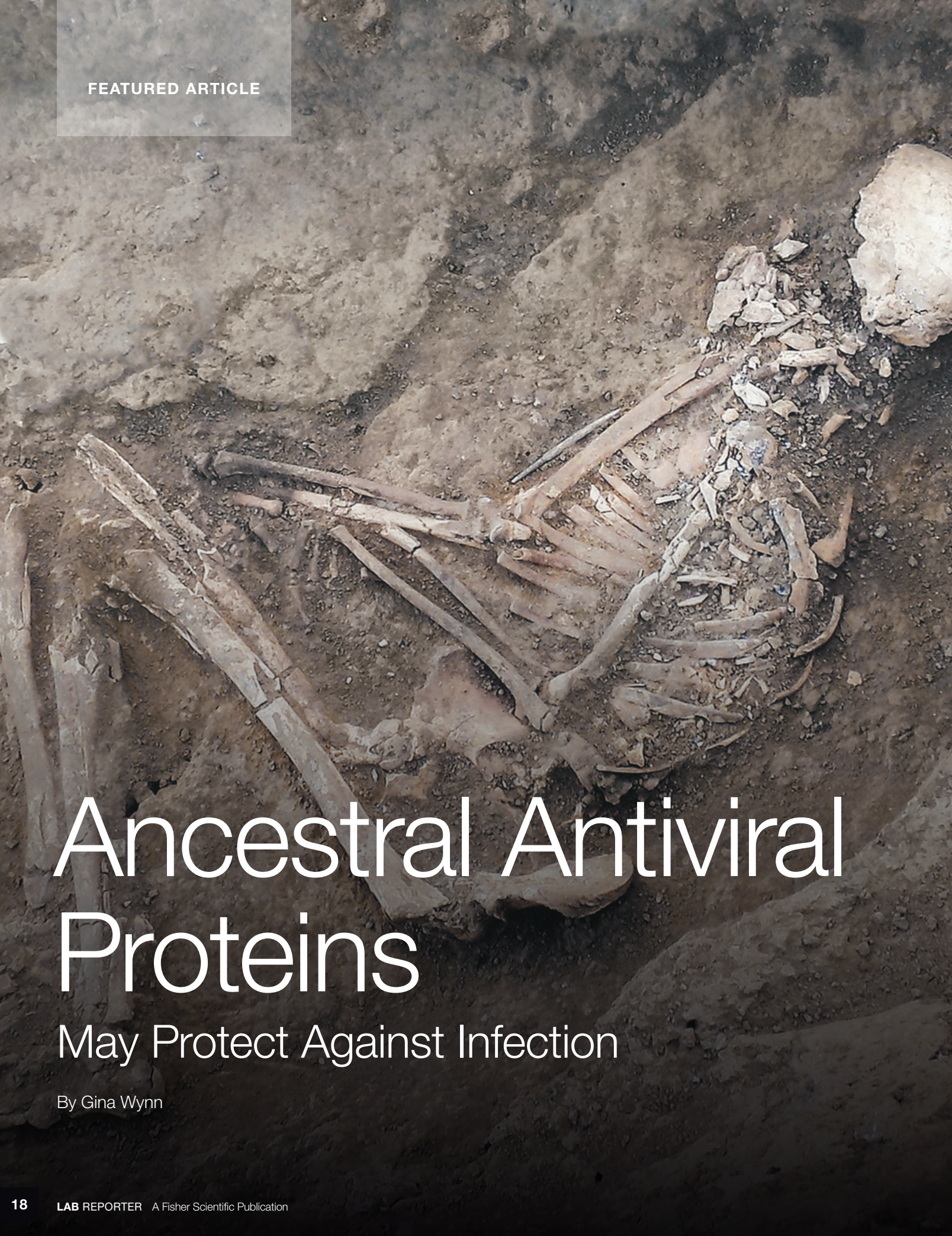


Ancestral Antiviral Proteins

May Protect Against Infection

By Gina Wynn

We can thank our ancestors for many of our inherited physical traits, including our eye color, height, and complexion. Now we may be able to add immunity to viral infections to that list, according to a recent Cornell University study.

By Gina Wynn

When examining cultured human cells in the laboratory, researchers found that proteins from ancient viral DNA were passed down from our ancestors. These human endogenous retroviruses (HERVs) may provide immunity by preventing modern viruses from entering cells that could become hosts. Previous studies have already documented this occurrence in mice, chickens, cats, sheep, and other primates.

"The results show that in the human genome, we have a reservoir of proteins that have the potential to block a broad range of viruses," said research team member Cédric Feschotte, PhD, Cornell professor of molecular biology and genetics, as reported in the *Cornell Chronicle* article "Ancient viral DNA in human genome guards against infections."

Feschotte's former graduate student, John Frank, PhD, who is now a Yale University postdoctoral researcher, was the first author of the study. The group's findings were published in the *Science* article "Evolution and Antiviral Activity of a Human Protein of Retroviral Origin."

A Pool of Protective Proteins

Further investigation could lead to the discovery of antiviral proteins that could help develop treatments that don't cause autoimmune side effects. Because the proteins are already incorporated into human DNA, they aren't seen as foreign to the body and don't prompt an immune response.

Approximately eight percent of DNA in the human genome is made up of HERVs, which is at least quadruple the amount of DNA in the genes that code for proteins. Our genome could be harboring a vast defense system with significant implications for healthcare.

Gaining Genomic Access

Viruses, in the form of proviruses, can introduce their RNA into a host cell, where it is converted to DNA and integrated into the host's genome. The hijacked cell then follows the genetic instructions from the virus to replicate it. If this occurs in germ or reproductive cells, the viral DNA becomes a permanent part of the genetic code and is passed down through generations.

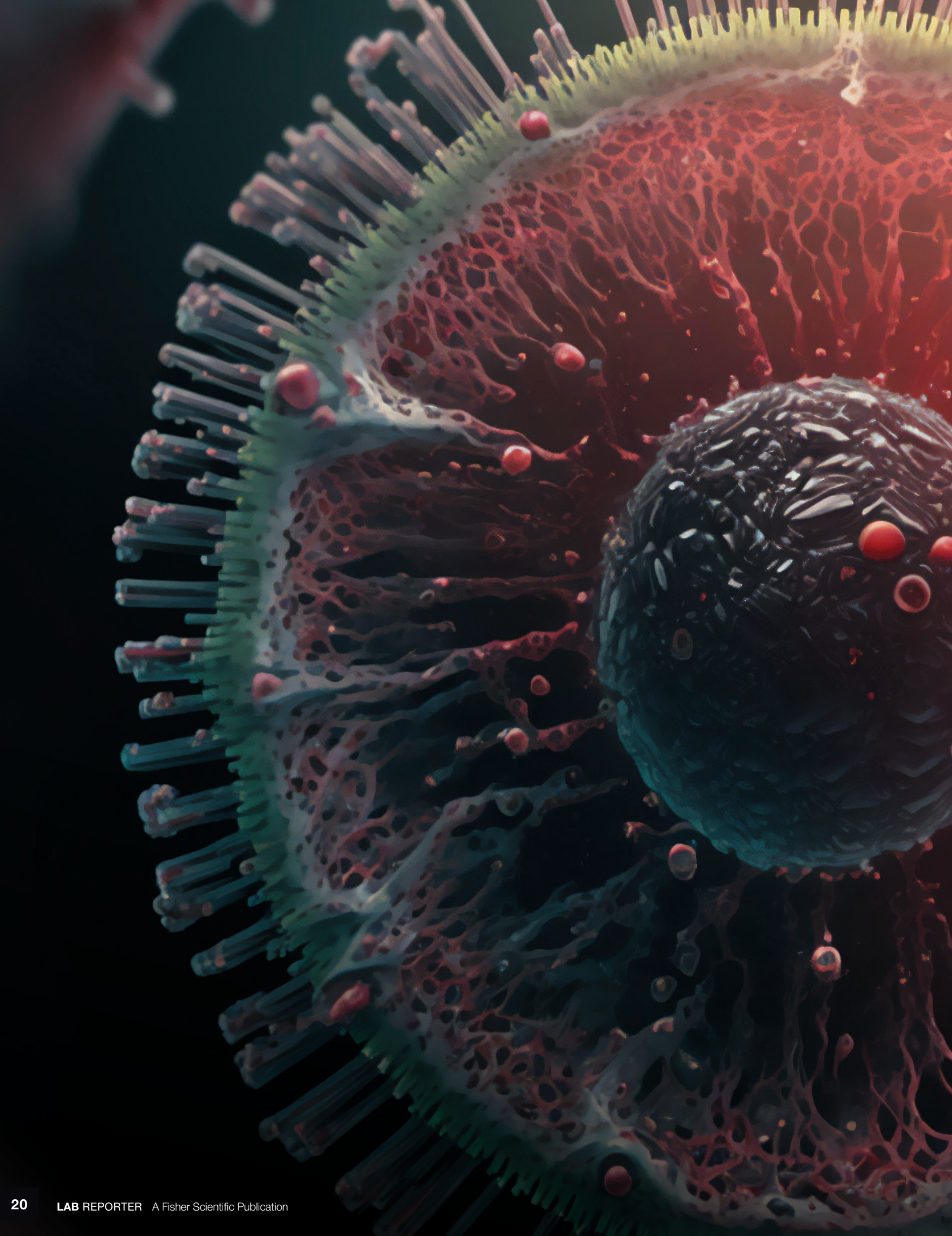
> "The results show that in the human genome, we have a reservoir of proteins that have the potential to block a broad range of viruses."
>
> **Cédric Feschotte**
> *Professor of Molecular Biology and Genetics*
> *Cornell College of Agriculture and Life Sciences*

HERVs are evidence of viruses that became fixed in the human genome millions of years ago. They are remnants of the countless pandemics to which humans have been exposed throughout history. Scientists believe these viruses were widespread in ancient human populations since they have also been found in chimpanzee, gorilla, and other primate genomes, according to the Aidan Burn article in *Scientific American* "How the Ancient Viral DNA in Our Genome Affects Disease and Development."

The Key to Entry

To enter a cell, a virus first needs to be admitted by a viral envelope or spike protein. The envelope protein binds to a receptor on the cell surface, like a key in a lock, and opens the cell to admit the virus. For their study, Frank and team used computational genomics to scan the human genome to find viral envelope proteins that they could investigate.

After cataloging the potential retroviral envelope protein-coding sequences that potentially retained receptor binding activity, they identified which genes were actively expressing retroviral envelope gene products. The team found clear evidence of expression in the early embryo and germ cells. They also noted a subset of antiviral proteins that are expressed in immune cells upon infection.



continued from page 19

Ancestral Antiviral Proteins May Protect Against Infection

Testing with Suppressyn

To test the susceptibility of different cell types to viral infection, the group focused on suppressyn (SUPYN), an antiviral envelope protein that is known to bind the alanine-serine-cysteine transporter 2 (ASCT2) receptor. SUPYN is prevalent in very early human embryonic development and placental cells, and ASCT2 is the cellular gateway for type D retroviruses.

Because placental cells are commonly targeted by viruses, Frank and his colleagues experimented with exposing human placental-like cells to RD114, a type D retrovirus that typically infects domestic cats and other felines. Although other human cell types not expressing SUPYN were easily infected, the placental and embryonic stem cells that did express SUPYN were not affected by the virus. When the team removed SUPYN from the cells, they became infected with RD114; when SUPYN was reintroduced, viral resistance returned.

Frank and his colleagues took their experimentation a step further and introduced SUPYN to embryonic kidney cells that don't typically express it. Normally the cells are susceptible to RD114, but with the added SUPYN, they were resistant to the virus.

Infection Insight

These results demonstrate how a human retroviral protein can deny viruses entry into cells by using antiviral envelope proteins to block cell receptors. They provide insight into how ancient retroviruses in the human genome may protect developing embryos from infection by related viruses. Feschotte hopes to study other envelope-derived proteins in the human genome to see if they have the same antiviral effect on cells, according to the *Cornell Chronicle* article.

Pushing the Envelope

In a similar study conducted in 2017, researchers recreated an envelope protein that helped the HERV-T virus infect human cells by binding with the monocarboxylate transporter 1 (MCT1) receptor. Daniel Blanco-Melo, PhD, of The Rockefeller University in New York and colleagues published their findings in the *eLife* article "Co-option of an endogenous retrovirus envelope for host defense in hominid ancestors."

The HERV-T retrovirus spread among our primate ancestors for around 25 million years and became extinct roughly 11 million years ago. By analyzing the genetic remains of HERV-T in the genomes of humans and related primates, the team was able to recreate the ancHTenv envelope protein.

The group's analysis showed that the HERV-T provirus in hominid genomes includes an *env* gene (hsaHTenv) that has been uniquely preserved. Upon further investigation, they found that hsaHTenv caused the depletion of the MCT1 receptor. Because the MCT1 was not available to interact with the ancHTenv envelope protein, HERV-T was unable to enter the cells and they were not infected with the virus. These findings further illustrate the importance of the envelope/receptor relationship in viral transmission.

Assistance from Our Ancestors

In both these studies, viral envelope protein and receptor pairs associated with HERVs in our ancestral DNA were shown to affect immunity to ancient viruses. The results of both investigations demonstrated that the lack of either the viral envelope or the receptor blocked the viruses from entering and reproducing in cells.

These findings may have major implications for researchers testing for and developing disease treatments, especially for HIV and other viruses that integrate into host cell DNA, some cancers, and possible future pandemics.

Gina Wynn is a Thermo Fisher Scientific staff writer.

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It's Time for a Change

Sustainability is a word you hear often today, and companies are spending significant time and effort on this topic. Labs are notorious for using large quantities of plastic consumables and should be included in this discussion. Lab plastics are shatterproof, durable, lightweight, single-use products that can reduce cross contamination. They're easy to use and almost ubiquitous. Initiating changes in how they're used in a laboratory setting can be challenging. Standard operating procedures are commonplace, and scientists follow them day in and day out to maintain order and consistency.

Reduce, Reuse, Recycle

Sustainability related to plastic consumables generally defaults to a discussion about recycling. Can products be safely recycled and is this activity convenient? Although important, recycling is not the only option to consider.

Another issue is the amount of plastic we use, which can often be reduced from the start. As a part of its overall waste management strategy, the U.S. EPA developed a Waste Management Hierarchy: source reduction is the most preferred tool, followed by materials reuse, and finally, recycling. Simply put: reduce, reuse, recycle. The order here is important because the greatest impact on sustainability is through reduction in overall use.

Sample Collection Containers Are Leading the Way

One way of reducing overall plastic consumption in the lab is substituting flexible sampling containers for rigid plastic. Whirl-Pak sterilized sampling bags were developed over 60 years ago to transport liquid milk samples to the lab. Today, these flexible packages are preferred in many industries and across more than 75 countries. Not all industries, however, have embraced this sampling format.

One industry ripe for a sampling procedure change is the water industry. Of the hundreds of millions of water samples taken each year, the majority are collected in rigid, single-use plastic containers. On average, these containers use 5 to 10 times the amount of plastic in a comparably sized Whirl-Pak bag. While this more sustainable solution is readily available, initiating change has been difficult.

Evaluating Environmental Impact

Whirl-Pak Filtration Group conducted an evaluation of the environmental impact of using 100 mL and 500 mL sterile sampling bags instead of the rigid plastic containers currently used. The study used EcoImpact-COMPASS software, which is widely used for life cycle assessments in the packaging industry. A life cycle assessment characterizes the impact associated with sourcing, manufacturing, distributing, using, and disposing of a given product. It gives you an idea of which package may have a lower environmental footprint, allowing you to make better sustainability decisions.

The study compared Whirl-Pak bags made from linear low-density polyethylene (LLDPE) to rigid containers made from polypropylene (PP), polyethylene terephthalate (PET), or high-density polypropylene (HDPE). Environmental impact variables included

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fossil fuel use, greenhouse gas emissions, and water use. Both sizes of Whirl-Pak bags produced similar results; only results for the 100 mL size are discussed below.

The study assumed that one million containers were shipped a distance of 1,000 km. Data was segregated by material usage, manufacturing, transport, and end of life. In all product comparisons, the Whirl-Pak sterile sampling bags were significantly more sustainable than the three rigid containers commonly used in water collection, including less fossil fuel use, lower greenhouse gas emissions, less water usage, and an overall lower environmental impact throughout the product's life cycle.

Cost Considerations

Even when a sustainable alternative is identified, cost considerations can affect the decision to implement changes. Budgetary constraints can limit even a highly motivated lab manager. One must consider overall costs and also hidden savings associated with shipping, storage, and waste disposal costs. Savings may be magnified if shipping long distances is required.

A focus on small, yet meaningful changes can make a measurable impact. Replacing rigid plastic sample collection vessels with flexible solutions like Whirl-Pak bags can be the beginning of your journey toward a more sustainable work environment.

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First Plants Grown in Lunar Soil May Lead to Farming in Space

By Mark Miller

Researchers at the University of Florida Institute of Food and Agricultural Sciences are the first to successfully grow plants in lunar soil brought back by the Apollo space missions. The lab-grown garden—part of research supporting the ambitious Artemis program at the National Aeronautics and Space Administration (NASA)—marks an important step toward growing plants and food on the moon and possibly worlds beyond.

Tough Growing

Dirt from the moon is a challenging environment for plant growth. According to an article in *Science News*, lunar soil, known as regolith, is a fine powder full of metallic iron, which, unlike oxidized iron, isn't beneficial to plants. It also contains tiny shards of glass as a result of the moon's surface being continually struck by micrometeorites. What it doesn't contain much of is nitrogen, phosphorus, or other nutrients.

The research team chose thale cress or *Arabidopsis thaliana* to grow in this unwelcoming world. It's a relative of mustard greens and other cruciferous vegetables like broccoli and cauliflower. A small, hardy plant that grows easily, thale cress is also the model organism used in multiple studies of plant biology, which means scientists have a good understanding of its genetic makeup.

The team combined thale cress seeds and water with gram-sized samples of soil from the Apollo 11, 12, and 17 missions. These were placed in terrarium boxes under LED lights in a cleanroom. They also planted control samples in terrestrial volcanic material and added a nutrient to all of the samples daily.

"After two days, they started to sprout!" said Anna-Lisa Paul, PhD, a professor of horticultural sciences at the University of Florida and part of the research team, in a report from NASA. "Everything sprouted. I can't tell you how astonished we were! Every plant—whether in a lunar sample or in a control—looked the same up until about day six."

Better Plots and Plants

As Paul's reaction indicates, both sample sets did not grow equally well. After about a week, the growth of the lunar samples slowed. They had stunted roots and leaves and an unhealthy, purplish pigmentation.

To better understand what had happened, the team conducted genetic analysis by studying the ribonucleic acid (RNA) from the plants grown in the moon soil. RNA transforms information from deoxyribonucleic acid (DNA) into proteins that carry out many of a living organism's biological processes. Examination of the RNA sequence of the plants' cells revealed that the thale cress was reacting as it typically does when the environment contains too much salt or heavy metals.

The plants had the most trouble growing in the soil samples from Apollo 11, perhaps because these were drawn from the moon's Sea of Tranquility, according to an *NBC News* story. This region, according to the story, is an older surface of the moon and has experienced greater cosmic radiation and solar wind.

A correlation between the age of the lunar soil and the performance of the plants may help future moon gardeners know where—or where not—to collect their planting soil. It may also help to select plants better suited to moon dirt. "Maybe spinach plants, which are very salt-tolerant, would have no trouble growing in lunar regolith," wondered Paul in *Science News*.

One Small Step

Although the lunar samples didn't produce growth as well as the volcanic soil, the research is a dramatic initial success that significantly advances the role of plant life in space exploration and habitation.

Plants can be used as model organisms to help study gravity, radiation, and other biological phenomena in space, according to a paper the University of Florida team published in *Nature*. It points out that plants help us understand how biological organisms adapt away from Earth and could provide food and oxygen to support long-term habitation of space and even extraterrestrial surfaces. Using moon regolith could prove much more practical than transporting enough water to support hydroponic agriculture.

Returning to Space

The work done at the University of Florida is picking up where NASA left off. Over the course of their missions, Apollo crews returned to Earth with 842 pounds of moon rocks and soil. The plantings are making good use of these materials. "Here we are, 50 years later, completing experiments that were started back in the Apollo labs," said Robert Ferl, PhD, another member of the University of Florida team, in the NASA report.

The research plays a central role in the success of the Artemis program, which aims to return astronauts to the moon and establish a long-term presence there. Ultimately, its lunar missions will serve as a leaping-off point for human exploration of Mars.

Mark Miller is a Thermo Fisher Scientific staff writer.

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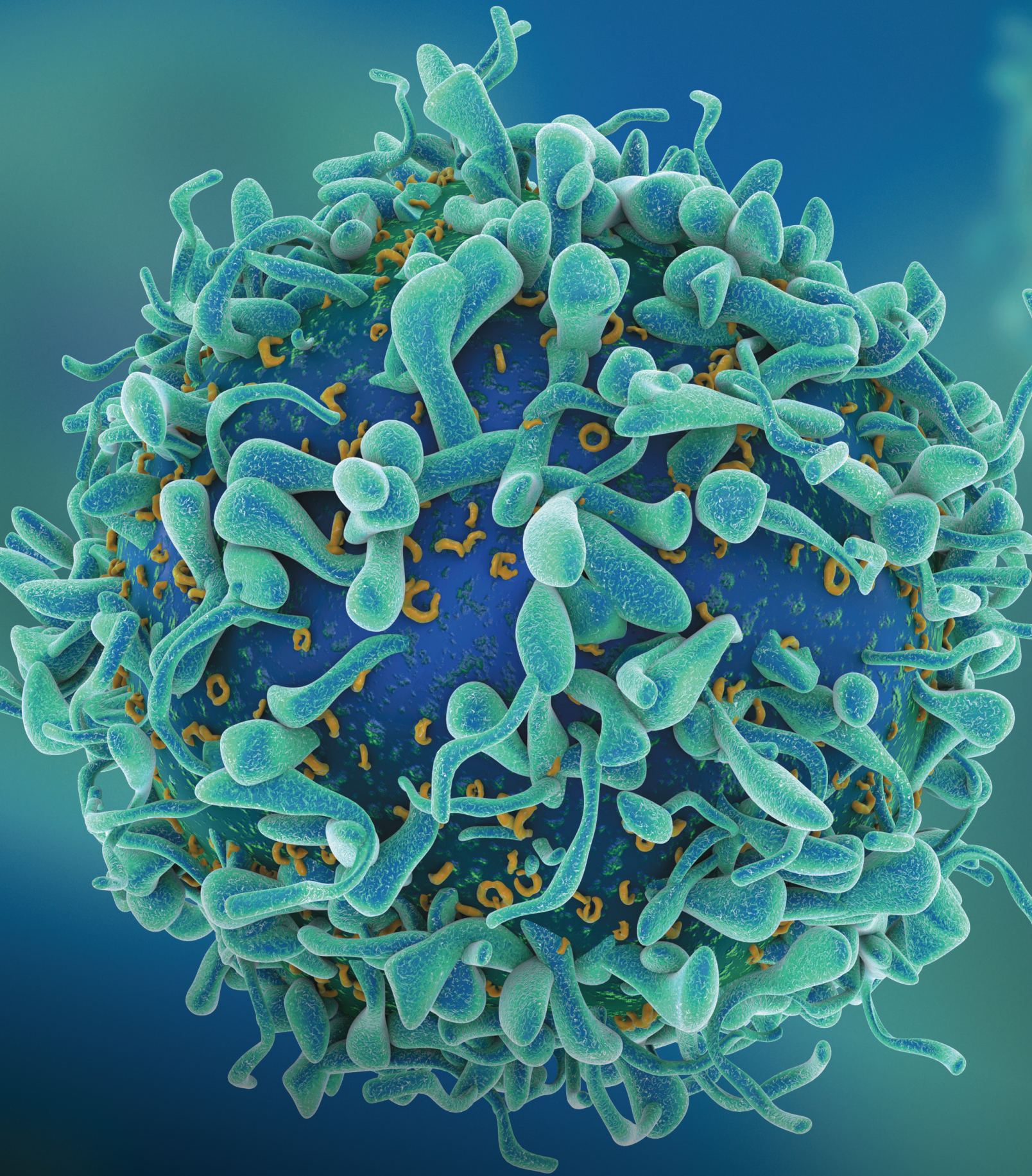

Successful Cell Therapy Manufacturing

By Iva Fedorka

Cell- and gene-based therapies are transforming cancer treatment. In the United States and Europe, cell therapies debuted with the use of chimeric antigen receptor (CAR) T-cell therapies, which have become the standard of care for some types of blood cancers.

Creating these therapeutics involves collecting T-cells from patients or healthy donors, genetically modifying, and then administering them to patients to rid the body of cancer cells.

However, producing these products presents new challenges. The first cellular therapies were developed without considering the regular production of these advanced therapy medicinal products (ATMPs).

The Manufacturing Process

Cell therapy manufacturing begins in an apheresis or clinical facility where the cells are collected and ends when the product is administered to the patient. Between those steps, a complex series of procedures takes place.

This process is very different from traditional pharmaceutical manufacturing. Most pharmaceutical products start with standardized materials and produce a fully characterized product. Cell therapy provides patients with personalized products based on their diseases, genetics, and medical histories, adding complexity and variability to their production.

Manufacturing Challenges

Manufacturers must produce a consistently safe and effective living product from highly variable living source material. They must produce consistent quality and performance; comply with regulatory and documentation requirements; maintain safety and quality control; manage supply chains and logistics; and develop bioprocessing scale-up and scale-out capabilities.

The success of producing an ATMP depends on the raw materials, manufacturing process controls, and safety and quality control procedures.

Using Appropriate Raw Materials

Perform a risk assessment of your raw materials to determine which ones may need to be altered or replaced. Choose raw materials that meet the standards and purity requirements for the end-product for all stages of development and manufacturing.

The responsibility for raw material quality and purity is shared by the supplier and manufacturer. Assess materials according to source and identity, purity, safety, and suitability as specified by USP <1043> Ancillary Materials (AM) for Cell, Gene, and Tissue-Engineered Products.

The Standard classifies materials as:

- Tier 1—low-risk, highly qualified, and regulated products
- Tier 2—low-risk, well-characterized, GMP, designed for use as AMs
- Tier 3—moderate risk, not intended for use as AMs
- Tier 4—high risk, biologically variable or contain harmful impurities and/or animal materials

As often as possible, choose Tier 1 and Tier 2 materials. Work with your supplier to identify alternative products for Tier 3 and Tier 4 materials, including substitutions or recombinant versions of animal components, additional material characterization testing, traceability documentation, and upgraded manufacturing methods like aseptic filling.

Finding Manufacturing Solutions

Producing cell therapies is labor-intensive, subject to contamination at multiple points, complex, difficult to monitor, and has zero failure tolerance. Verify that any equipment and instruments for "research use only" (RUO) or for in vitro diagnostics (IVD) are suitable for producing injectable therapeutics.

Look for ways to introduce modular or multifunctional equipment and instruments, closed systems, and automation to help address these issues. Some manufacturers have developed products specifically designed for cell therapy manufacturing, including direct replacements for RUO or IVD versions.

Characterization and Product Release

Cell therapy products have a short shelf life and cannot be fully characterized or purified pre-administration. However, some tests are essential for lot release and regulatory compliance for chemistry, manufacturing, and controls (CMC).

The U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) Office of Tissues and Advanced Therapies (OTAT) oversees CMCs. The FDA requires information for Investigational New Drug Applications (INDs), including safety, identity, quality, purity, and strength/potency testing.

Chain of custody and identity must also be maintained throughout the manufacturing process. Failure to properly document and control sample provenance can have serious patient consequences.

The Future of Cell and Gene Therapies

A new generation of CAR T-cell therapies has the potential to identify solid tumor cells in patients with prostate, breast, stomach, rectal, and other non-hematological malignancies. Stem cell therapies may one day be used to treat autoimmune, Alzheimer's, and Parkinson's diseases.

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Iva Fedorka is a Thermo Fisher Scientific staff writer.

Confocal Imaging and Analysis Using the Agilent BioTek Cytation C10

Since 2012, cells and tissues from many sources have been cultured in three dimensions (3D), enabling more complex biological models. The use of these 3D models has been increasing in medical research, precision medicine, disease modeling, and drug discovery efforts.

These 3D models are being used to simulate the native microenvironments found in organisms and are believed to provide a more accurate assay model in some instances. 2D models, consisting of a monolayer of cells, have been in use for decades and have provided extensive meaningful data.

However, 2D limitations became more apparent with the recent development of complex organoids, tissues, or tumoroids. These tissues, comprised of one or more cell types and often based on stem cells or patient-derived samples, have now become the focus of many studies.

The Cytation C10

The ability to perform a variety of assays on a single, compact instrument is advantageous, especially given the limited bench space in many laboratories. The Agilent BioTek Cytation C10 confocal imaging reader can perform microplate reading, widefield imaging, and confocal imaging, offering the means to gather data for today's complex studies.

The Agilent application note "Confocal Imaging and Analysis of Spheroids for Determination of Dose Response During Drug Treatment" by Peter J. Brescia describes the use of automated imaging to determine cell number in spheroids to evaluate drug dose responses.

Study Materials and Methods

The study discussed in the application note used CT116-H2B-GFP cells; the H2B-GFP construct provided a constitutively expressing nuclear marker for quantification by cell count for comparison to a nuclear stain during live cell analysis.

The cells were seeded at a relatively low density of 500 cells/well in a 96-well, round bottom ULA imaging plate (Cat. No. 15-100-173) and grown for three to four days to produce spheroids of roughly 100 µm in size.

Staurosporin, a potent, nonselective protein kinase inhibitor, was added to induce apoptosis. Propidium iodide (PI) was also added to monitor the kinetics to determine optimum timing (12 hours post-addition produced adequate apoptotic activity for analysis).

Study Imaging Procedure and Analysis

To minimize the data set, spheroids were sized to be captured in a single field of view (20X objective) and a beacon was used to correct for positioning variability in the wells. A 60 µm pinhole spinning disk provided a compromise between acquisition time and signal intensity for confocal imaging. Widefield images were captured in both GFP and TRITC channels to compare to confocal images.

The instrument was set for "Scan," followed by "Autofocus," which identified the approximate center of the spheroid along the z-axis. Each spheroid was captured as a z-stack using 11 steps at 10 µm, regardless of the z position in the well. Images were processed in several steps, including a z-projection for comparing single z-plane images at the approximate center of the spheroid and the entire z-stack for both imaging modes.

Automated cell counting was performed using confocal and widefield imaging mode and both single plane and z-stack projected images were analyzed.

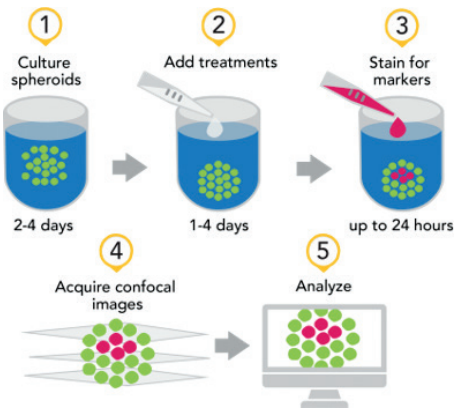

Figure 1. Spheroid assay workflow incorporating automated image acquisition and analysis (Agilent BioTek Cytation C10 confocal imaging reader and Agilent BioTek Gen5 microplate reader and imager software).

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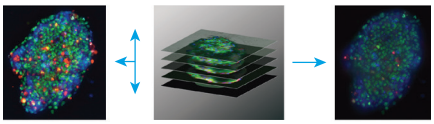

Figure 2. Spheroid captured as a z-stack using 11 steps at 10 µm intervals. Analysis performed on either a z-projection of all images or a single image representing a plane through the center of the spheroid.

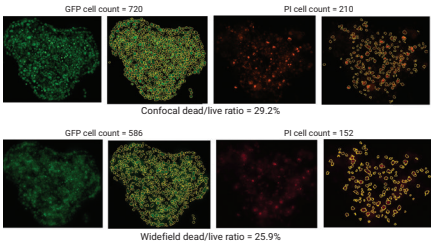

Figure 3. Examples of automated image acquisition and cell count analysis of spheroids by confocal and widefield fluorescent microscopy using z-projected images. Cell masking (yellow outlines) where GFP cell count indicates live cells and PI indicates dead cells.

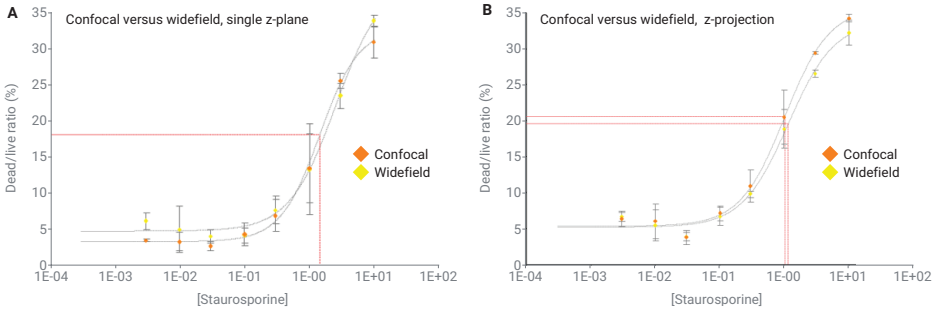

Figure 4. EC 50 determinations for each imaging mode, single versus z-projected images.

Since spheroids are increasingly used to represent the complex microenvironments of biological systems, the ability to automate image capture using multiple imaging modes and perform automated image analysis can increase throughput and enable comparative analysis.

The study results confirmed that confocal optics enable more accurate segmentation of labeled cells within spheroid samples than widefield optics. Cell count determination using a single z-plane image provided comparable results to methods relying on z-stack image acquisition and processing.

The study is just one example of the utility and flexibility of the Agilent BioTek Cytation C10 confocal imaging reader and Agilent BioTek Gen5 microplate reader and imager software to capture and analyze multiple parallel data sets.

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35 L/min.	1.5 torr	15 psig	16 × 7 × 9 in.	**13-880-22**

The Cost of Air and the Impact on the Laboratory

Engineered controls, like fume hoods, are an integral part of most laboratory facility planning.

Fume hoods have a significant impact on building infrastructure, are energy-intensive, and basically, remove conditioned air from the building. The extracted air then needs to be brought back into the building for the HVAC to adequately balance temperature, humidity, and pressure. Ducted fume hoods impact the building's need for larger air handlers, boilers, chillers, cooling towers, and exhaust fans due to the amount of air that needs to be processed.

Studies have calculated that laboratories consume an average of 66 percent or two-thirds of a building's total energy load while occupying only about 25 percent of the facility's usable space.

How can we address this issue, what are some possible solutions, and how do we address these very real problems while achieving carbon neutrality?

Alternative engineered control systems (ECS), including ductless filtering fume hoods, are one answer.

Cascade Effects

When a lab is designed with ductless filtering fume hoods (DFH) as the primary ECS, the impact includes carbon reduction. As we aim for carbon neutrality by 2030, the DFH plays a significant role in achieving this goal. Obvious savings can be found in reducing peak exhaust output, but that reduced exhaust air output allows buildings to achieve much more.

Let's look at the impact of an Erlab project at Bristol Community College in Massachusetts.

The Bristol high-performance laboratory was originally designed to include 22 ducted fume hoods, which had a combined intake and exhaust of 70,000 cubic feet per minute (CFM). It required three air-handling units (AHU), one with run-around loop heat recovery and one with enthalpy wheel energy recovery.

The proposed Erlab Zero Net Energy (ZNE) design replaced that equipment with:

- 13 filtered hoods
- 4 ducted hoods
- CFM: 24,000 intake and exhaust
- 2 AHUs
- Enthalpy wheel recovery

Along with the air reductions, the ZNE design included a combination of ground- and air-source heat pumps, enthalpy heat recovery wheels, fan coil units, centralized Indoor Air Quality (IAQ) monitoring, and natural ventilation. It reduced mechanical, electrical, and plumbing (MEP) to just 14 percent of the gross square footage (GSF). Additionally, the PV arrays, half of which would have supplied the 22 ducted hoods, would be used to recover energy to help achieve ZNE.

A Holistic Approach to Safety

Fume hoods and containment devices should be designed with safety as the priority. When considering ductless filtering fume hoods, chemical handling needs must be assessed to determine whether they are suitable for filtration. If so, a filter life cycle must be proposed based on analysis using AFNOR NF X 15 211, ANSI z9.5-2022, CSA Z316.5-2020, and NFPA 45-2023 edition standards.

The Bristol project considered many factors, including hood filtration performance. The hood's chemical listing booklet must provide the overall retention capacity of the filter prior to any release (under 1 percent) of the chemical threshold limit value (TLV). This reflects the life cycle of the filter and the maximum time between replacements.

Under normal operating conditions, a ductless filtering fume hood must also be able to guarantee user protection during two operating phases: detection and safety. One misconception about ductless filtering fume hoods is that they can be used for only small amounts of chemicals or for odor mitigation. In reality, Erlab hoods are approved for 80 percent of pharmaceutical, organic chemistry, agriculture, flavor and fragrance, and other common applications.

Safety does not stop with the fume hood but continues throughout the chemical life cycle. Proper controls must be in place to protect the breathing zones of people working in the lab. These are achieved through a combination of filtering storage, whole room air filtration, integrated filtration packs for safety cabinets, and filtering fume hoods.

The various safety aspects should be monitored: face velocity, filtration efficiency, and ambient air pollution. Continuous monitoring provides critical safety metrics and helps establish more effective lab safety protocols.

Conclusion

While we may be reluctant to change, we must consider what's best for the environment. Embracing new technologies can have a significant impact on the future. As we increase our understanding of the impact of our actions, we can make changes to improve safety and product performance.

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