Keeping things clean

The secret to successful vector production for gene therapy

Indiana University in Indianapolis, in the US, boasts a vector production facility that manufactures a critical component for gene therapy: engineered viruses specially designed to carry the code to correct faulty genes or to treat cancers. It is a highly specialised process that demands strict controls to ensure there is no contamination of the products. Under the guidance of its director, Dr Daniela Bischof, the Indiana University Vector Production Facility (IUVPF) boasts standards that exceed the strict requirements for the safe production of these vectors.

Gene therapy is an exciting and ever-growing field of medicine. As more and more therapies are discovered to cure diseases that have hitherto evaded our best efforts, demand is increasing for a critical component to that end: engineered viruses. It is one of medicine's more remarkable ironies that something that can harm people can now be 'trained' to treat diseases.

Diseases – whether inherited or acquired – that emerge because of a failure in the body's genetic makeup can, theoretically, be cured by correcting the defective gene. The problem is getting the appropriate genetic material (DNA) into human cells. Evolution has the answer. Few organisms are better equipped at breaking into cells than viruses. Scientists can now strip viruses of their disease-causing structures and use them as carriers – or vectors – to deliver the correct DNA. Once the human cells are exposed to the vector, the viruses are designed to set in motion a cure instead of a disease. Until relatively recently, this would have been science fiction, but this wondrous reality demands the strictest care in manufacturing these vectors safely.

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The Indiana University Vector Production Facility (IUVPF), which is part of the university's School of Medicine, is dedicated to producing retroviral and lentiviral vectors for use in Phase I/II clinical trials. The facility was founded in 1995, but growing demands for vectors for clinical trials, a shift in priorities, developments in the science of vector manufacturing, and changing safety regulations led to the university building a new facility. According to the facility's director, Dr Daniela Bischof, through smart design, the new facility boasts standards that exceed the strict requirements for the safe production of these vectors.

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SPECIFICATIONS AND REGULATIONS

In the pharmaceutical sector, there is no such thing as a generic cleanroom. Instead, cleanrooms are classified according to their cleanliness, including the surfaces and quality of circulating air – the temperature, humidity, airflow, and air filtration. The International Organization for Standardization, or ISO, classification of cleanrooms ranks rooms in degrees of cleanliness from ISO class 1 to ISO class 9; ISO 1 being the 'cleanest' and usually reserved for nanotechnology or ultra-fine particulate processing. The manufacturing of vectors demands ISO class 7 and ISO class 8 facilities and adherence to good manufacturing practice (GMP) — the minimum standard any medicines manufacturer must adhere to in their production processes.

When Indiana University was designing its new Vector Production Facility, it had to adhere not only to ISO and GMP specifications but also to Food and Drug Administration regulations and National Institutes of Health guidelines for the safe handling of vector products, which included Biosafety Level 3 (BSL3) protocols necessary for containing agents or toxins that could cause serious or potentially fatal disease. This presented a significant shift in the safety protocols for the facility. While BSL3 labs are usually designed to prevent biological materials from escaping, the IUVPF facility needed to be able to keep contaminants *out*. The pressurisation of the cleanroom facilities was designed to meet BSL3 containment protocols, but the airflows are reversed in comparison to standard BSL3 labs to minimise the entry of contaminants.

The result is a state-of-the-art, secure facility purposefully built to meet the growing demand for viral vectors – specifically for retroviruses and lentiviruses. It achieves this status through the clever use of surfaces which are resistant to microorganisms and easy to clean, by carefully managing air pressure, filtration, and circulation, and by controlling the movement of personnel and materials.

MINIMISING CONTAMINATION RISK

At the heart of the IUVPF is about 400m^2 of ISO class 8 cleanroom space, containing four ISO class 7 production suites. Each production suite has ISO class 5 biosafety cabinets in which the work is performed. The cleanroom space is bordered on one end by a pre-production area that includes a storeroom and media prep room. At the other end is a post-production area that hosts a

Photo Credit: Alisha Moore

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freezer room for storing the manufactured vectors, a space for cryogenically storing the certified master cell banks that are used to manufacture the vectors, a sink to dispose of decontaminated liquids, and a large pass-through autoclave for decontamination of solid waste – waste is inserted on the cleanroom side and decontaminated waste removed from the autoclave outside of the cleanroom.

Minimising the possibility of contamination is critical in maintaining the integrity of the vectors produced. Central to this is ensuring a unidirectional flow of materials and personnel. Access to the facility is through a dedicated personnel entry anteroom divided into a 'dirty' and 'clean' side. Before crossing over to the clean side, personnel must dress using full sterile cleanroom-grade gowning. Personnel accessing any of the production suites need to prepare accordingly in the pre-production area. Once they have completed the necessary work in one of the production suites, they exit into the post-production area. It is also possible for personnel to access the post-production area from the pre-production using a separate corridor without going through a production suite – this is on the far left of the facility, linking areas 2 and 7 on the floor plan above.

Each production suite is isolated and has its own negatively pressurised entrance and exit anteroom. Should personnel move from an ISO class 8 room to an ISO class 7 production room, they must don additional gowning and sterile gloves.

CONTROLLING THE AIR

Adjoining the facility, but separate, is the mechanical room, which houses the specialised air-handling system for the cleanroom area. Air coming into the facility is high efficiency particulate air (HEPA) filtered and then HEPA filtered again before going out; it is not recirculated. Supply vents in the ceilings and exhaust vents close to the ground ensure the maximum flow of air in and out of the cleanroom.

Air pressurisation in the cleanroom is critical to reducing the possibility of contamination. At the IUVPF, the production suites are kept at a higher pressure than adjacent rooms. This reduces the possibility of contaminated air entering the production suites. Differential air-pressure monitors are located at all doors within the cleanroom space. These allow personnel to ensure that differential air pressures remain within acceptable limits. There are also monitors at the facility's main entrance and exit points. Regarding the air-pressure differences and the air quality, the IUVPF has managed to maintain levels stricter than those required.

However, clean air won't help if surfaces become contaminated, so the IUVPF follows a similarly strict cleaning regimen. The walls and ceilings of the cleanrooms are solid and seamless, and in the production suites, the walls are encased in vinyl to minimise potential contamination by microorganisms. Nevertheless, the facility employs specialised, daily, weekly, monthly, and semi-annual cleaning procedures to ensure the facility retains its strict levels of cleanliness. Constant environmental monitoring ensures the facility stays in a state of control.

LESSONS LEARNED

The IUVPF's success in ensuring it exceeds the strict requirements for the safe production of viral vectors has, ironically, highlighted a limitation in the facility's design. Given the growth in demand for the vectors the facility is producing, Bischof admits that, in hindsight, they should have considered ensuring adequate space to accommodate the growing number of staff, processes, and storage required. Cleanrooms are, by need and nature of design, contained units, and keeping them shielded requires dedicated structures. You can't grow such a facility by knocking down a few walls and raising prefabricated plasterboard room dividers.

Bischof has also learned that ensuring the IUVPF's strict cleanliness levels is associated with high costs. Specialised cleaning and cleanroom maintenance are expensive, and although the facility is not a commercial enterprise it still needs to retrieve these costs when charging for its manufactured products.

Indiana University's School of Medicine should be justifiably proud of its Vector Production Facility. Bischof and her team have ensured not only that they exceed the strict standards demanded of such a facility, but they are actively contributing to the search for cures for some of the world's most resilient diseases.

Behind the Research

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Research Objectives

Dr Bischof oversees the Indiana University Vector Production Facility, dedicated to the development and production of gene therapy technologies.

Detail

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Bio

Dr Daniela Bischof is Assistant Professor in the Department of Medical and Molecular Genetics, Indiana University School of Medicine. She is also Director of the Indiana University Vector Production Facility (IUVPF). The IUVPF is dedicated to the development and production of gene therapy technology (lentiviruses and retroviruses).

References

Bischof, D, Cornetta, K, (2022) Indiana University Vector Production Facility (IUVPF). In: Gee, AP, (eds) *Cell Therapy*. Springer, Cham. doi.org/10.1007/978-3-030-75537-9_20

Personal Response

Where do you see the IUVPF in ten years' time?

"Our focus will remain on producing lentiviral and retroviral vectors so that investigators can initiate Phase I/II human gene therapy clinical trials. The demand for vectors is high, and since currently we are not using our cleanroom to full capacity, we are working on increasing our output over the next few years."

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