3P innovation

BRINGING GMP COMPLIANCE

INTO CELL & GENE THERAPY PRODUCTION

THE QUEEN'S AWARDS FOR ENTERPRISE: INNOVATION 2020

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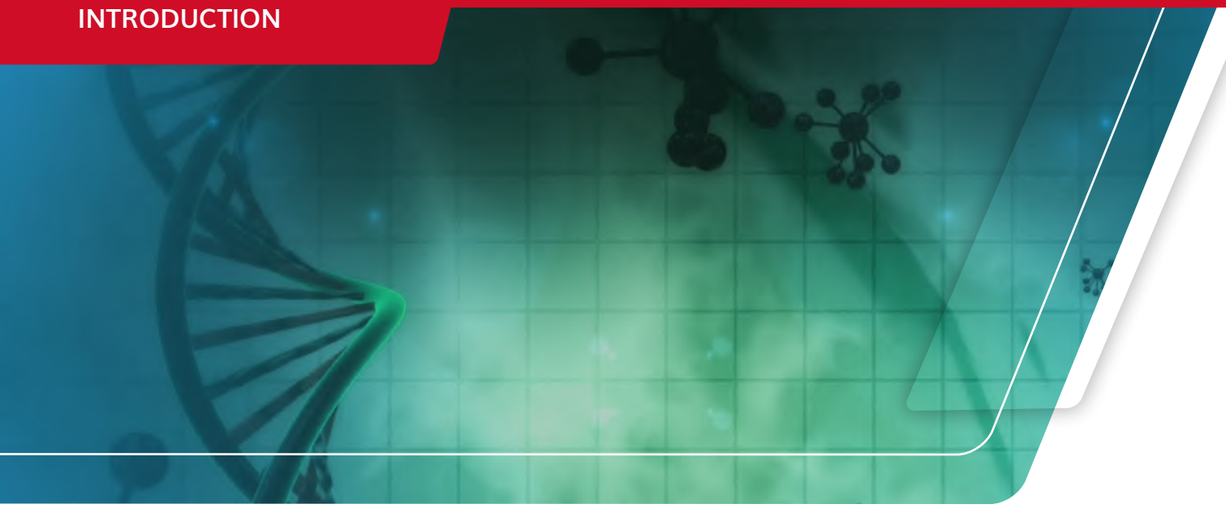


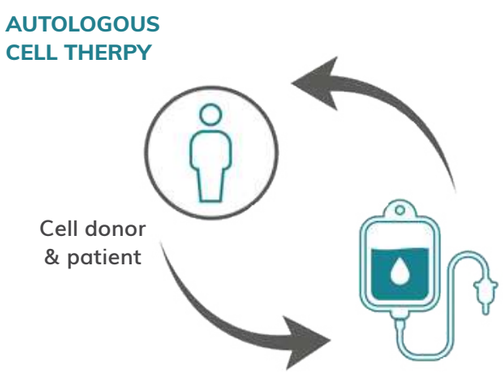
Cell and Gene therapies hold the promise of cure for a wide range of life limiting disease. Until recently these therapies could only be found in academic laboratories being produced on laboratory equipment. They are now being commercialised at pace often with equipment with a laboratory heritage. This nascent sector is being developed in parallel regulatory frameworks and guidance documents. This whitepaper investigates the features required from equipment to meet the regulatory framework and in particular references the recently published Annex1 of EU Good Manufacturing Practice (GMP), "Manufacture of Sterile Medicinal Products". It describes some of the considerations when selecting equipment that complies with GMP's for Cell and Gene Production. Some of the differences between commercial GMP and laboratory equipment are highlighted. The author is an equipment designer such that the paper provides insights into GMP compliant production equipment design.

WHAT ARE CELL AND GENE THERAPIES?

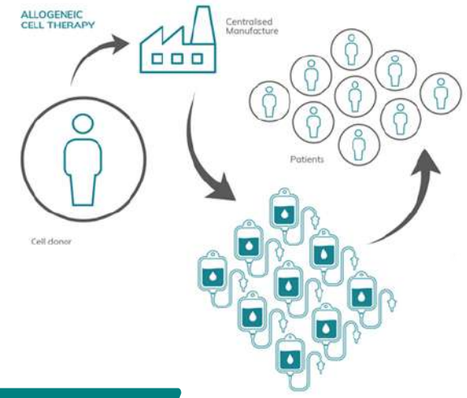
Advanced Therapy Medicinal Products (ATMPs) as they are known in Europe, or cellular and gene therapies (C&GT) products in the US, offer innovative treatments for a variety of complex diseases and conditions. Many of these nascent products can be administered as one-off treatments, offering life-long benefits, or curing a potentially life limiting disease.

At this moment in time there are two main modalities for these therapies; **Autologous and Allogenic.**

Autologous: Also referred to as "vein to vein". The start of the process is to extract material from the patient. The starting material might be blood containing stem cells or a solid tumour containing T cells. The target cells are harvested and then they are typically genetically modified to provide the efficacy. These modified cells are then expanded over many days, prior a final harvest, and then infused back into the patient.



Allogenic: The start of the process is to extract material from a donor. As per Autologous therapies, cells are then genetically modified and expanded. In this case the cells are cell banked typically using cryopreservation. Unlike autologous therapies allogenic therapies are available to many patients.



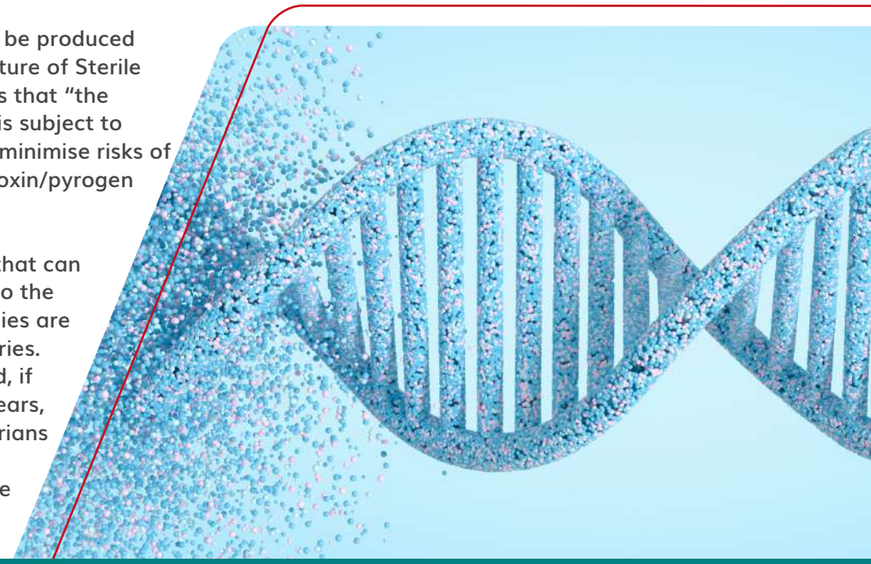
WHY ARE THESE NEW THERAPIES SO CHALLENGING AND COSTLY TO MANUFACTURE?

When compared to conventional solid oral dose or injectable therapies ATMP's are significantly more complex to produce. The variability and fragility in starting material and the overall number of processing steps presents significant opportunity for error. Overlaid upon this are further complications around manual processes that were recently developed in academic laboratories and that now need to be carried out aseptically. These products are typically delivered as infusions or injections and being cellular in nature they cannot be terminally sterilised. This adds an additional layer of complexity, namely the need to ensure that the product is not contaminated by any viable or non-viable particulate. Unlike with orally delivered therapeutics, the body struggles to defend against contaminants delivered into the blood stream via injected therapeutics: poisoning a patient with a viable particulate into the blood-stream is not an option!

These products should therefore be produced according to Annex 1, "Manufacture of Sterile Medicinal Products" which states that "the manufacture of sterile products is subject to special requirements in order to minimise risks of microbial, particulate and endotoxin/pyrogen contamination".

Unlike solid oral dose therapies that can trace their manufacturing back to the mid 1800's, cell and gene therapies are barely out of academic laboratories. Biotech isn't of itself new. Indeed, if you stretch back thousands of years, the ancient Egyptians and Sumerians were known to use fermentation to make bread and cheese. These were almost certainly accidental discovers with no underpinning knowledge. We are now able to manipulate cells using the underlying principles of biology. What we recognise as modern biotech kick-started when Watson and Crick solved the structure of DNA in 1953. It then took a couple of decades before humankind figured out how to transfer DNA between organisms using a technology termed recombinant DNA. Recombinant DNA (rDNA) is a technology that uses enzymes to cut and paste together DNA sequences of interest. The first publications describing the successful production and intracellular replication of recombinant DNA appeared in 1972.

The recombined DNA sequences can be placed into vehicles called vectors that ferry the DNA into a suitable host cell where it can be copied or expressed. These vectors are usually viruses.



It was the discovery of rDNA that led to the formation of Genentech in 1976 (now part of Roche). By 1977 Genentech had produced the first human protein somatostatin (a growth hormone inhibitor) in bacteria and shortly afterwards human insulin which revolutionised diabetes treatment when licensed to Eli Lilly in 1982. Fast forward to around 2010 when an increasing number of cell and gene therapies started to be approved for a mix of rare genetic disorders and cancer treatment.

Most of the equipment used to manufacture cellular therapies has a laboratory heritage. These systems were designed to appeal to researchers in academic laboratories rather than meeting the strict requirements of Good Manufacturing Practice (GMP) and the recent Annex 1 guidelines for their manufacture.

For ATMP's early production has typically been performed by highly skilled/trained laboratory technicians in PPE working in bio safety cabinets (BSC's). This is usually referred to as using aseptic technique. Aseptic technique means using procedures to prevent contamination from pathogens and is similar to the procedures used in operating theatres. Unless you have attempted it personally, it is difficult to appreciate how difficult it can be to perform manual operations in BSC's whilst wearing full aseptic PPE. Despite all the precautions, the human operator remains the biggest risk to the patient in terms of accidental contamination of the product. Numerous articles have demonstrated that human operators are the main contamination risk in cleanrooms, particularly through the shedding of particles from personal clothing and skin, exacerbated by movement. A typical person sheds around a billion skin cells every day and 10% of them have viable micro-organisms on them. If this wasn't bad enough, we humans need to breathe and microorganism loaded liquid droplets are released from our mouths and noses.

When it comes to meeting the needs of Annex 1 there are technical decisions to be made that involve inevitable trade-offs. To protect therapies from the risks outlines above has led to a trend towards what is generally termed "functionally closed" and "closed" systems and also to the implementation of robots.

FUNCTIONALLY CLOSED SYSTEMS

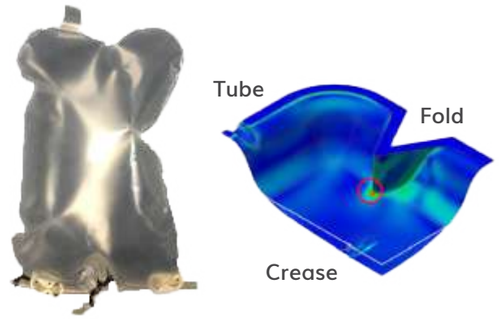
Many of the therapies have elected to use what are known as "functionally closed" systems. So called "bag-sets" are complex arrangements of welded bags and tubes that are pre-sterilised. A number of vendors have developed their own ecosystems of disposable processing containers and bags containing costly reagents to be used within their equipment. They use an inkjet cartridge model whereby the equipment is sold as a means to lock-in clients to high margin consumables.

Despite the high cost of consumables, the advantage of these systems is that they can operate in a relatively low-grade clean room (grade C – the grading of cleanroom can be found in ISO 14644-1:2015) whilst maintaining sterility inside the tube set. The end-user has a "one stop shop" for equipment and reagents. This can provide significant value added for a developer especially during development. There is, however, a significant risk of becoming "locked-in" to one vendor's equipment and consumables for commercial manufacture. The automation is relatively simple in that it typically involves the sequencing of peristaltic pumps and pinch valves to move liquids from bags into processing chambers within tube sets.

At the same time the equipment accurately controls temperature and nutrients supplied to the cells. Another downside is that these machines spend most of their time on one unit operation – cell expansion. The machine acts as an incubator for much of its life, and spends a small amount of time on the other value added and specialist unit operations such as cell separation, activation and transduction. As a result, more lab space and equipment is required than necessary from the perspective of a "cycle-time" analysis. Within automation of complex products there is the concept of matching "Takt" time. Takt time is the time needed to ensure all production unit operations match the needs of production. From a classical automation perspective functionally, closed systems make no sense. The use of these systems drive-up the cost of goods for these therapies. The authors are aware of frustration from end users at being tied into relatively inflexible systems, tube sets and reagents. Some believe an "open source" machine and, or "tube-set" is likely to appear.

The primary drug container is often a small number of bags, and the equipment retains a laboratory feel (colourful epoxy coated steel rather than the stainless-steel clad machinery that most pharmaceutical factories are equipped with). These therapies are often cryogenically stored to ensure they survive the journey between a centralised processing "hub" and the patient administration centres, the "spokes". Cryogenically stored bags are very fragile since the plastic becomes very brittle, especially around crease lines which are naturally created when a bag is formed from two flat layers of plastic film and then filled with a liquid. These bags are normally placed in protective cradles during cryogenic freezing and transport: what is known as "bag and shell". It is usual to expand more cells than necessary. These additional cells are filled into spare bags. This is to protect the patient from the potentially life-threatening failure of a bag by providing back-ups.

It is likely that in the near future regulators will insist that ATMP manufacturers move from the use of operators in BSC's to closed systems. The product equipment is increasingly becoming less lab like with design principles lifted directly from conventional aseptic fill-finish equipment. This has also led to the use of more conventional primary drug containers in the form of vials. These vials are typically formed from cyclic olefin plastics such as COC or COP rather than glass in order to survive cryogenic temperatures. Vials are naturally "open" systems during filling, and this has led to an increasing trend away from BSC's and towards closed systems.



A finite element analysis of bag inflation carried out by the authors to explain the high localised stress caused by creasing during bag inflation which can lead to catastrophic bag failure at cryogenic temperatures.

CLOSED SYSTEMS

An alternative to a functionally closed system is a closed system. This is where the production is carried out within a closed box or isolator.

Isolator technology can be traced back to handling of radioactive materials during WWII, but it was Willis Whitfield who invented the modern-day cleanroom in 1962 and the use of heap filtered air to create a 1000-fold reduction in particulate.

He led the introduction of:

- Highly filtered air to continuously wash away/dilute any impurities in the room.
- A linear air speed almost undetectable to operators (the current speeds were originally introduced for operator comfort as much as to avoid turbulence).
- Unidirectional down flow to move particulate in a controlled way away from critical zones.

Before production can begin isolators rely upon decontamination of all surfaces, typically with a hydrogen peroxide vapour (some operators also use peracetic acid). Isolators can be “hard-wall”, constructed from stainless steel with glass windows and glove ports or “soft-wall”, essentially a welded clear plastic bag welded together that fits over a space frame. A slight positive pressure is maintained in the isolator in order to ensure that particles from the clean room will not enter the isolator and potentially contaminate the product in the case of a small leak.

The processing area is fed from above by HEPA filtered unidirectional air to continuously “wash” the processing area. Such systems can have features to enable product to be safely passed into and out of the process area. Sophisticated viable and non-viable monitoring assures that the production batch has been produced aseptically. They have an energy advantage over functionally closed systems in that Annex 1 states they may operate in a grade D background.

Arguably Annex 1 can be charted back to Whitfield’s innovations. Isolators were introduced to the pharmaceutical industry in the early 1980s. They drew heavily upon Whitfield’s pioneering cleanroom technology, whilst also separating the operator from the process. Isolators with glove ports were utilised to protect operators outside of the isolator against the risk of exposure to a toxic drug, and to protect sterile products inside the isolator against contamination from operators in the clean room.

By the end of the 20th century, containment solutions were significantly developed to handle the use of complex technologies and equipment, such as robotic arms and powder dispensing systems. Custom automation in this space is one of 3P innovation’s core competencies and has led directly to projects in the ATMP space.

A 3P innovation aseptic isolator under test at 3P innovation’s UK facility

THE NEED FOR GMP COMPLIANT BENCHTOP SYSTEMS

If anything has been learned from over 150 years of pharmaceutical machinery development, the cell and gene sector will inevitably trend away from manual processes on essentially laboratory equipment towards automated process on GMP production equipment. What does this actually mean for producers and users of equipment? Fundamentally the equipment needs to be designed from the ground up to comply with the various standards and guidelines that cover pharmaceutical machinery. Guidelines are often interpreted in subtly different ways within User Requirements Specifications (URSs) of producers who are mainly big pharma. This means that equipment producers need to ensure their equipment is to the highest standards. In particular the relatively new version of Annex 1 for sterile manufacture will increasingly be applied to cell and gene therapies.

As one would expect from a producer of equipment with a heritage in commercial pharmaceutical equipment, 3P innovation has a comprehensive set of internal engineering standards that ensure equipment complies with these external standards and guidelines. It may seem trivial but even the seals used within such equipment are important and need attention to detail.

WHAT'S IN A SEAL?

Automation usually comprises end effectors that move linearly or rotary (or both). In conventional machines the prime mover (motors etc) is often next to the product and cross contamination is not an issue. Within pharmaceutical equipment the prime movers need to be kept away from the product in a technical area. How does one then connect the prime mover to the end effector? For aseptic processing the norm is to connect them with ground stainless steel rods.

Typically machines have a base plate through which these rods pass. Isolators need to be pressure tested and to avoid contamination the technical space needs to be isolated from the processing area. One solution is to employ bellows, however these are very challenging to clean. An alternative and preferred solution is an elastomeric seal. These simple components are however challenging to engineer for this environment. There are a limited number of materials that can be used around parenteral drugs. The material needs to be inert to the sterilant. The surface finishes and dimensions of both the steel rod and the seal need to be to very high standards. This ensures that the seal actually acts as a seal. The fit between the seal and the shaft cannot be too loose otherwise it may leak. It can't be too tight as this can lead to audible squeaking, high loads for the driving motors or shedding of particulates.

3P innovation has internal standards specifically around such seals which it views as valuable intellectual property. This provides insight into the attention to detail required and some of the differences between laboratory and GMP equipment.

The seals are just one of many areas that need to be considered. 3P innovation also has standards for the materials of construction and their surface finish. Care is needed to consider direct contact parts which are those which touch the product (like filling needles) and indirect contact parts. Indirect contact parts touch the parts that touch the product so could transfer contamination: these might be bowl feeder parts that touch the stoppers which will then touch the drug substance.

There are very limited number of materials that are accepted as contact and indirect contact parts. These are typically 316 stainless steel and PEEK. Full traceability of the production of these parts is required including what are termed Mill Test Certificates (MTC). Each MTC provides information on the original dimensions and weight of material, the mill where it was originally produced and it also verifies the chemical and physical properties. Overlaid upon this are certificates for the process of converting a block of material into a finished part – for example cutting fluid is to be avoided and must be BSC/TSE free. Guarantees are often required to ensure that cutting tools have not been used on ferrous materials for fear of cross contamination that can lead to localised corrosion. This isn't the norm for laboratory equipment. There is similar rigour around certificates for any welding.

WHAT'S IN A BOLT?

When it comes to hygienic design even the fasteners holding the equipment together need to be considered. There can be air leakage past a conventional bolt. The heads of a conventional bolt are also relatively rough and sharp, making them difficult to clean with the potential to tear a glove – a torn glove in an isolator is a breach of the aseptic containment.

A hygienic bolt (left) compared to a conventional one – note that the hygienic bolt has a highly polished surface, is sculpted to ease wipe down and includes a small elastomeric seal to prevent leakage.

As with the fasteners, cleanability of the whole machine is very important. For this reason sharp corners are avoided at the design stage. Engineers familiar with industrial automation are often taken aback by the cost and complexity of parts for the pharmaceutical world. Much of this is driven by the need to be able to easily clean parts with a wipe-down, to seal the technical area from the process zones and to provide certification of the materials of construction.

A benchtop fill-finish system – note the use of:

- Hygienic fasteners.
- Sculpted features to ease cleaning.
- Lack of sharp corners.
- Low profile of parts above the process line.
- Height of the process from the machine bed.
- Seals around moving shaft and the surface finish of parts.

Notwithstanding the complexity of the mechanical design, there also needs to care when selecting electrical parts. Some of this is again due to sealing. It is not uncommon to find leakage paths within conventional electrical parts or the use of materials incompatible with sterilants. Special care is needed to design the cable routes to ensure there aren't leak paths from the process to technical area. Space is always at a premium in a clean room meaning that compact electrical control parts are preferable. This has driven much of 3P innovation's "standard" electrical component library.

The way software is written is also important. Traceability is necessary to ensure an audit log is available of who logged onto the machine. When changes are made these need to be recorded. Electronic records are covered in a standard known as 21CFR11 which pharmaceutical equipment typically needs to comply with to be saleable. The software needs to be designed, written, and tested as per another guideline called GAMP5 (GAMP stands for Good Automated Manufacturing Practice and the latest version from the ISPE is 5). GAMP follows what is known as the V model for validation.

Which leads nicely to validation. The FDA defines validation as "establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes." At the core of 3P innovation's ways of working is pharmaceutical validation. There is an expectation that all our equipment will be validated. The internal systems that manage the design process ensure that equipment can be easily validated. This means we consider how a machine can be efficiently validated during design, fabrication, and most importantly during the testing phase. This systematic approach ensures validation activities are completed efficiently to the delight of our clients around the globe.

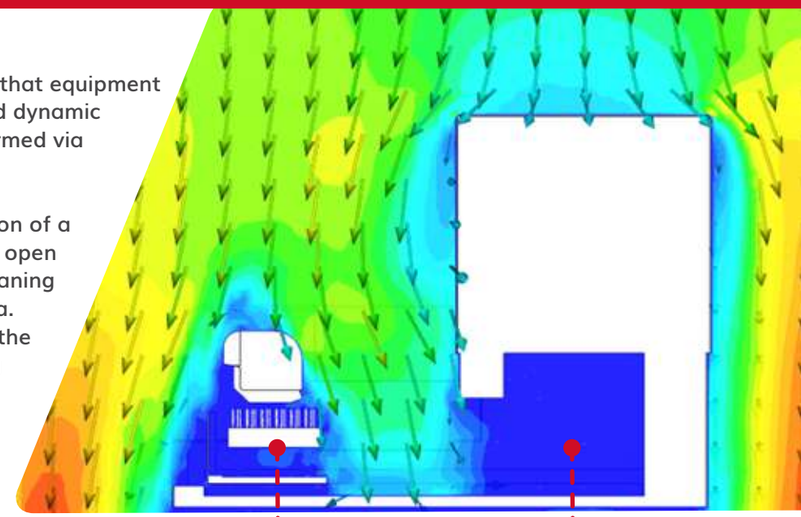
One final consideration when it comes to Annex 1 if the principle of "first air". As mentioned earlier it was Willis Whitfield who discovered that "washing" a system with HEPA filtered air significantly reduce viable and nonviable particulate within an aseptic field. The "first air" principle refers to the concept that any air that comes into contact with the product must not touch anything else prior to leaving the HEPA filter. For this reason, cross flow isolators are losing favour. In a cross flow isolator air leaves the filter and then can flow over many surfaces (potentially picking up contamination) prior to contacting the product.

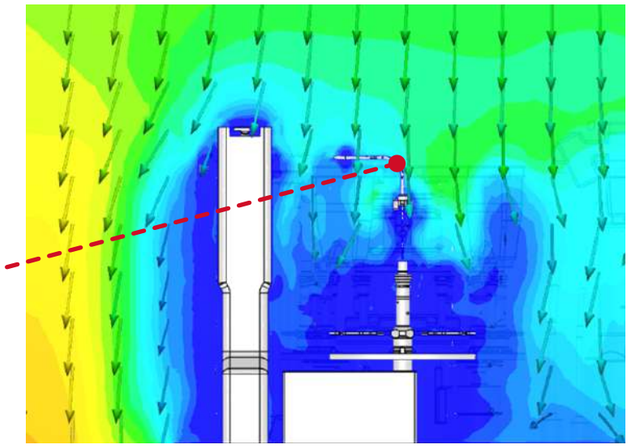
Annex 1 states "Grade A: The critical zone for high-risk operations (e.g. aseptic processing line, filling zone, stopper bowl, open primary packaging or for making aseptic connections under the protection of first air). Normally, such conditions are provided by a localised airflow protection, such as unidirectional airflow workstations within RABS or isolators. The maintenance of unidirectional airflow should be demonstrated and qualified across the whole of the grade A area" and "Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the working position, unless otherwise scientifically justified in the CCS. Airflow visualisation studies should correlate with the air speed measurement."

There is an expectation within Annex 1 that equipment is subjected to both computational fluid dynamic (CFD) simulation of air flow to be confirmed via smoke studies.

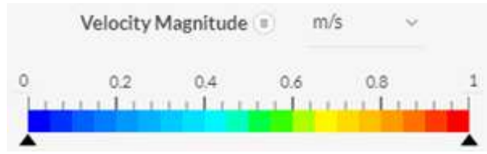
The images (right) show a CFD simulation of a laboratory cryovial filler. The filling and open container zones are solid navy blue meaning there is no air flow over the critical area. Worse than that the air has contacted the frame of the instrument before passing down to the filling zone.

By contrast the industrial design filler has air flow above and near the critical filling zone and first air principle is maintained.





The image (left) shows a CFD simulation for the commercial-designed cryovial filling platform. By contrast, the commercially designed cryovial filling platform has airflow above and near the critical filling zones where the blue dead zones are now beneath the process line. This shows that the first air principle is complied with.



This paper has provided an insight into what good manufacturing practice (GMP) means for equipment intended to produce sterile injectable products. In particular it focuses on the production of cell and gene therapies and how they comply with the latest international regulations and industry body guidance. GMP compliance is essential for the successful development and commercialisation of cell therapy products. By understanding and adhering to GMP principles, manufacturers can mitigate risks, optimise processes, and ultimately deliver innovative therapies that meet the highest standards of safety and efficacy.

REFERENCES

Annex 1 is available at: https://health.ec.europa.eu/system/files/2020-02/2020_annex1ps_sterile_medicinal_products_en_0.pdf

GAMP 5 is available at: https://ispe.org/publications/guidance-documents/gamp-5-guide-2nd-edition

ISO 14644-1:2015 "Cleanrooms and associated controlled environments, Part 1: Classification of air cleanliness by particle concentration" is available at: https://www.iso.org/standard/53394.html

Code of Federal Regulations Title 11 part 11 "PART 11—ELECTRONIC RECORDS; ELECTRONIC SIGNATURES" can be found at: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-11

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