SAFETY FIRST
automating cell line development

Developing and maintaining cell cultures in a stable and sterile environment is of paramount importance to protect samples and increase overall success of drug discovery pipelines. Cell lines are extremely fragile and susceptible to contamination which can be catastrophic in terms of both time and money. Therefore, adequate environmental protection must be provided at all times in automation system enclosures. Here we examine what to look out for to ensure safe and successful automated cell line development.

Automation in drug discovery has become a fundamental driver of progress, enabling increased throughput and reduced reliance on labour-intensive workflows. One of the main drivers behind this is the need for increased ‘speed to clinic’ – investors and CROs are placing heightened importance on shorter cell line development times and the production of consistently high-quality recombinant products. As a result, many companies are developing automation strategies in order to meet these demands.

There are various processes in cell line development that are particularly labour-intensive. For example, transfection (gene knock in or knock out), which is a random process, can often create a major bottleneck. This is because multiple screens and selection panels are needed to ensure the best clones are identified. Cell maintenance and cell line stability are also time-consuming, involving selection of high-performing clones and culture and expansion over a range of volumes to achieve yields that can be moved on to systems for further development.

Of course, well-designed, comprehensive enclosed automation systems can accurately perform a wide range of tasks covering the entire cell line development workflow (Figure 1):

- Cell seeding
  - Dispensing cells into primary culture plates
  - Maintaining cell lines in water baths to avoid temperature shocking
- Dilutions to required cell concentration with fresh media
- Addition of growth supplements or inhibitors
- Automated incubation
  - Controlled CO₂ and temperature
  - Confluence measurement
  - Assess cell growth and clones
  - Automatic clone selection based on operator-set growth parameters
- Clone selection and cell maintenance
- Transfection of cells
  - Both chemical and viral transfection
- Clone selection
  - Stripping of adherent cell lines
  - Cell centrifugation
  - Transfer to expansion plates and fresh media
- Lead optimisation and target identification
- Phenotypic screening of suspension cells
- Clone selection for antibody production
- Hit picking

These automation solutions normally consist of robotic plate handlers, microplate stores, incubators and laboratory instruments controlled by scheduling software. These are housed in an enclosed system also known as a workcell or an enclosure that importantly monitors and controls the internal sterile environment. Automating these processes significantly streamlines the workflow,
not only saving time and money but also helping to eliminate human error and liberating operator resources.

However, caution should be exercised when planning to automate, to ensure adequate protection for cell lines, protection for operators and data handling. As well as ensuring that the best equipment is selected to complete the tasks at hand, it is also important to ensure that both the system and provider are equipped to meet safety requirements as well as being compliant with the most up-to-date standards.

### Safety classifications

Safety requirements are two-fold in automation systems; protection for the cell lines and protection for operators. The best way to ensure this is for workcells to meet class II requirements.

So, what exactly is the difference between class II requirements and biosafety level (BSL)-2? BSL-2 is a specification of a laboratory, which means that a system labelled as BSL-2 offers the same level of protection as if the user is performing the task on an open bench. In contrast, a workcell built in accordance to class II classification provides the same level of protection as if the scientist were working inside a biological safety cabinet – which is significantly safer for both the operator and the product.

The need to protect valuable cell lines is obvious – any sort of contamination could destroy months or even years of investment at great cost. However, operator protection is a growing concern, particularly for certain scenarios, and safety requirements are adapting to address this. One such scenario is if the workflow involves cells of human origin. Although cell lines should be pure, there is still a risk that various infectious agents can be carried by the cell lines that could be deleterious to human health.

Another reason to be vigilant about operator protection is transfection. There are various methods to insert an identifiable segment of DNA into the genome of a cell. This can be done chemically or via electroporation, but perhaps the simplest and most popular method is using viral vectors that have been edited with CRISPR/Cas9 or an equivalent technology. The modified virus can then be used to infect the cell, delivering the desired segment of DNA as it inserts its genome into the cellular genome.

The potential problem with viral vectors is that the most effective viruses are the ones that rapidly react to protect themselves and are therefore often highly infectious, such as Hepatitis B, HIV or the equine influenza virus. It is therefore clearly very important to protect operators against exposure to these biological agents, due to the risk of aerosol dispersion during dispensing or via a dropped plate.
Although the requirement to protect operators is not currently fully implemented in various laboratories using open class II hoods, it is gradually becoming more enforced and should be taken into consideration when automating the process.

Going beyond standards

Of course, to ensure a system has a reliable class II classification, it should be tested to comply with standards – in this case BS EN12469-2000 and ANSI/NSF49. These standards dictate the performance and testing criteria with which the systems should comply. Enclosures should produce a laminar airflow across the deck of the workcell. However, it is important to note that while an empty workcell may achieve this, the addition of robots and instrumentation will disrupt this significantly. While compliance with BS EN 12469-2000 is enough on paper, automation system providers should also understand this and test systems accordingly. It is also wise to look for systems certified by a nationally-recognised test laboratory such as ETLus in the US.

Systems are tested to BS EN 12469-2000 by measuring various air velocities, particle counting and tracer visualisation to meet the class II requirements. However, careful consideration of the location of equipment inside the system is also important (Figure 2). This is because, as mentioned, instrumentation such as stacked equipment can result in poor airflows and disrupt the laminar airflow potentially causing stagnant pockets of air – not ideal if these form over an unlined sample plate. To protect against this, systems should be tested with smoke trace profiling and for cleanliness using particle counting around the system.

Of course, contamination must be avoided at all costs and so it follows that cleanliness must be maintained within the workcell. Therefore, equipment layouts must also consider maintenance of equipment and spill points, etc. When working with multiple cell lines, the ability to chemically deep clean the system is critical. UV sterility offers minimal effectiveness within large automation systems due to the wavelength of UV compared to the size of the system and associated shadow effects. For this reason, some systems allow for vapourised hydrogen peroxide cleansing which is a valuable benefit.

Flexible designs that achieve security

It is clearly important then to select and install an automation system that will provide both product and operator protection. However, a system also needs to be easy to work with. Inevitably, there will be the need to access instruments inside the workcell when cells are in situ – for example to replace a seal on a plate sealer or reagents for a dispenser, or even just to add sample plates or additional labware. So there has to be adequate protection in place for this.

For example, it is not uncommon during cell line development for cells to be incubated for a month to six weeks to achieve the required outcome.

Figure 2
Placement of equipment inside workcells is an important consideration as it can disrupt laminar flows and cause pockets of stagnant air, risking contamination.
During this time the cells are continually cultured, taken out of the incubator, reformatted, expanded and returned to the incubator for continued growth. There is clearly a constant requirement to replenish consumables and reagents within the enclosure, which in turn increases the risk of contamination, resulting in the potential loss of six weeks’ work and perhaps significant clones. Any potential sources of contamination must therefore be avoided. A workcell must be safe, but also flexible enough to fit around a particular workflow and easy to use.

Workcells maintain safety and sterility via carefully-designed and monitored airflows. These airflows must be monitored with particular attention to the access doors and windows to ensure that contaminated air is prevented from being pulled from the system by the lab air conditioning or being drawn through large, full-height doors. An automated system should be designed with this in mind, reacting to opening of portals with continuous monitoring and adjustment of the airflow to ensure protection is maintained. An example of this would be sensor detection of door opening that automatically increases the downflow of air, providing protection of the internal environment. Such a set-up enables an operator to access instruments inside the system without risking contamination.

A key feature that delivers not only safety but flexibility is a recirculating laminar flow system (Figure 3). This draws in air from outside and recirculates it round the enclosure, passing through triple HEPA filters which provide class II protection in accordance with BS EN12469-2000. The air is then normally cleansed via another HEPA filter and exhausted at height – sometimes up to 2.5m – providing additional assurance that it is not vented on to operators.

The alternative to such a system would be a ducted cabinet which is plugged into an existing air extraction system within the building. However, this can be very restricting as the automation workcell has to be sited in a particular place to have access to the infrastructure. A recirculation system is an attractive alternative as it can be sited anywhere in the laboratory as long as the ceiling is tall enough.

As well as being safe and flexible it is also worth bearing in mind that the system should be easy to operate. Good system providers will have user-friendly scheduling software with an intuitive interface and will consider the workflow as part of the system build, ensuring easy access to instrumentation.

Protecting data and preventing bottlenecks

Another, often overlooked, consideration is that of data handling. While data handling is challenging for operations of every size, the increased volume
of data generated by automated systems can lead to bottlenecks if not collated in an effective way. This, in turn, compromises the whole workflow and the samples it contains – hold-ups expose precious cell cultures to additional risk.

The term data handling encompasses many tasks, from data generation and reformatting to suit individual requirements, through to communication with third party software programmes and databases. Full sample tracking and ease of operation is highly advantageous for efficient routine use, and each factor must remain flexible to fit around the great variety of demands of individual laboratories and their existing data infrastructure.

Often, due to regulatory requirements such as FDA approvals, stored data needs to be traced back to a corresponding sample, perhaps even a number of years after the initial experiment. Therefore, it is vital that effective data handling systems are in place to protect critical data. Without such automated data handling, resource-intensive data analysis can also create a bottleneck that undermines the efficiency benefits afforded by the automated set-up.

Database tracking functionalities ensure that a full history of each sample is securely stored and readily available to the operator. This can serve many purposes, such as tracing the origin of outliers when the results are reviewed. This capability also facilitates assays requiring the association of multiple plates, for example when cell culture supernatant is assayed on one plate, while the cells remain in culture on the second plate. The results from the first plate containing the supernatant must then be tracked to the corresponding cells, providing the position and barcode for easy location of the relevant sample.

It is equally important to be aware of the challenges involved in retrospectively organising such complex data acquired over months or even years of development. To place this into context, consider having to present data covering nine years of work, where five different technicians were involved in data handling. It can easily require months to organise the data, retrieving it from multiple network locations and unformatted spreadsheets. Some work might even need repeating if results have become lost over this length of time, and each file must be reformatted to provide directly comparable data across the entire project.

With data management systems in place, data is easily accessible and consistently formatted to streamline the reporting process.

It is important then to considering data handling as an integral part of the automated system build. This often-overlooked function is key to maintaining efficiency and also for reaching the system’s full capacity from the outset by protecting against bottlenecks. Build-only automation solutions may initially appear a cost-effective means to automation, however the hidden costs of this are later revealed when it comes to software and data handling – this can be extremely expensive in terms of time and cost when trying to integrate retrospectively. Integrating data handling into automated platforms from the outset delivers significant advantages, such as securing data and protecting samples by avoiding bottlenecks – which are crucial to consider, and detrimental when overlooked.

Summary

Automating cell line development makes a tremendous difference to drug discovery pipelines, speeding up processes, increasing reliability and reproducibility, reducing risk from human error while also saving time and money. Due to the extremely fragile nature of cultured cells, safety should be front of mind when looking to automate to protect investment and product. However, this should also extend to operator safety, particularly when working with human cell lines and potentially infectious agents. As a part of this, an automated workcell should comply with, and ideally go beyond, standards, and also provide flexibility, ease of use and, importantly, adequate data handling to circumvent issues down the line. Good solution providers will understand these requirements and have the necessary expertise and experience to guide the creation and installation of the best automation solution possible for the required workflow.

Jon Newman-Smith is Engineering Operations Director at PAAs. Jon’s background is in engineering, specialising in robotics and automated systems. He is also a certified Machine Safety Expert with 17 years’ direct experience at PAAs, where he leads the design, specification and delivery of bespoke automated systems for PAA’s Life Science and Personal Healthcare customers. His team of highly skilled engineers, scientists and project managers guide PAA’s customers through the project lifecycle to achieve a successful outcome for each customer. Jon has a passion for developing new ideas for PAA’s laboratory workcells and applying new concepts such as collaborative robotics to break down traditional barriers of automation.