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editorial

Dear Colleagues

I would like to say how delighted I am that the *Industrial Pharmacy* also known as the *IP* Journal has been relaunched. It is now available to all pharmaceutical, life science and healthcare practitioners.

This new edition is particularly dedicated to the memory of John D.R. Jolley who sadly passed away last year. John, a former editor of the *IP* journal was an Industrial Pharmacist, Qualified Person, Colleague and Friend – this is for you John!

This issue begins with an overview of phage therapy by David Browning. Phage therapy could be one of the answers to the growing problems of antimicrobial (AMR) resistance and this article looks at the possibilities and gives examples of their therapeutic use as an unlicensed medical product in clinical practise. Our second article by Harald Flechl stresses the importance of air handling systems in cleanrooms and the importance of instrumentation and control systems to ensure proper registration and control of the technical data.

Professor Chris McConville provides the background to a novel drug delivery system and the difficulties of securing the necessary funding for the research and development and the ability to eventually proceed to phase 2 clinical trials. Our last article again revisits the important subject of cleanrooms. Nigel Lenegan and Tim Eaton detail a scientific approach to determine the air supply rate required to attain specified concentrations of airborne particulate and microbial contamination, in non-unidirectional airflow cleanrooms.

The relaunch of *IP* has been made possible by the sponsorship of the Precision Health Technologies Accelerator (PHTA) with the aim of fostering collaboration and knowledge exchange between scientists and medical experts. The *IP* Journal will be made available to anyone across the globe to facilitate innovation and problem solving – as they say a problem shared is a problem solved!

In that vein, the Editorial Board have and will continue to compile a broad spectrum of articles which should hopefully appeal to many readers and so stimulate *ideation* – e.g. we have highlighted the re-emergence of bacteriophages as a weapon to tackle antimicrobial resistance and the trials and tribulations of developing a novel drug delivery to treat brain tumours by CRISM therapeutics. The Editorial Board is open to receiving articles so please feel free to submit these articles to the following email: admin@phta.co.uk

We hope you find the new IP Journal useful and of interest.

Here is to an exciting 2025.

Best wishes

Professor Gino Martini FRPharmS
Editor, *Industrial Pharmacy*

CALL FOR ARTICLES

Dear Colleague

We hope you enjoy* Industrial Pharmacy *and find it both useful and informative. We are currently seeking new articles for future issues of the journal and would like to invite you to contribute an article or review paper on any aspect of* industrial pharmacy *to the journal. We are also pleased to receive letters on any aspect of pharmacy or with respect to any article published in the journal. All issues of* Industrial Pharmacy *are indexed by both Scopus and Embase and thus are available through the listings for all other scientists internationally.

Obituary

John Jolley

We are very sorry to report that John Jolley, a former editor of the IP journal, passed away on 26 August 2024, aged 79 years. As well as his valuable contribution to the journal John was a respected Fellow of the RPS.

John Jolley obtained a BSc Honours degree in pharmacy and a research fellowship at Boots, where he worked on formulation development, during which time he published several technical papers. John then joined Beecham Products UK as a management trainee and was later appointed general manager of a Beecham subsidiary in Ireland. He then returned to Beecham headquarters in London, where he worked as special products adviser to the divisional managing director for Beecham Products.

Later, John joined Boehringer Ingelheim UK as technical director and remained there for 15 years before leaving to establish Pharma Consult, an International technical and training consultancy working with associates in the United States, as well as countries across Latin America and Asia.

During his varied career, John held positions with responsibility for clinical research, product registration, manufacturing, distribution, quality assurance and general management. He was a good supporter of the local Slough branch of the Royal Pharmaceutical Society of Great Britain (RPSGB) and several meetings of the branch were held at Boehringer Ingelheim when he was running the factory.

As chair of the Industrial Pharmacists Group of the then RPSGB, John was its delegate to the European Group (EIPG) and acted as the meeting chair of European Industrial Pharmacists Group for the 2009 General Assembly. John was awarded Fellowship of the Royal Pharmaceutical Society (RPS) and, in 2003, was elected to serve on the RPS council as its treasurer. In 1998, he became consultant lead assessor of the Chartered Quality Institute. In 2015, he was invited to join the worshipful Society of Apothecaries and granted freedom of the City of London.

John was a popular warm-hearted personality and a great champion for Industrial Pharmacy and in particular for the role of the Qualified Person working in Industry. John was a well-respected member of the Industrial Pharmacists Group, the European Industrial Pharmacists Group and a popular figure in the Euromed Community being the Editor for the Industrial Pharmacy Journal.

Jane Nicholson and Gino Martini

Bacteriophage: Translation into clinical practice

by David Browning

New tools are required to address the significant and growing threat of antimicrobial resistance (AMR). This article provides a background on bacteriophages (phages), highly effective and naturally occurring microbial agents. It also provides an overview of recent progress in using phages to tackle bacterial challenges in human health, animal health and the environment. It concludes with a summary of some of the key challenges in phage adoption into clinical practice with a call to action for pharmacists to be at the heart of an exciting new ecosystem to facilitate better clinical outcomes and help address AMR.

David Browning applies 40 years comprehensive life sciences knowledge with an extensive industry network to support entrepreneurs, translating scientific discoveries to deliver global market impact, including Entrepreneur in Residence, Institute of Cancer Research, strategic advisor to multiple growth companies and organisations including Precision Health Technologies Accelerator and Chair, Executive Advisory Board, Clinical immunology Services.

David's work experience includes NHS Biochemistry, Johnson & Johnson clinical diagnostics innovator, Philips Personalised Medicine lead, MD MediCity / SVP Innovation BioCity (Pioneer Group). CEO roles include Fixed Phage (NexaBiome) focusing on bacteriophage-based products, Oxford Cancer Biomarkers and Oxonica Healthcare.

Introduction

We are entering an exciting new healthcare era, with pharmacists at the heart of a unique opportunity to harness and combine bacteriophages (phages), natural anti-bacterials, either alone or in combination with antibiotics, to address the rapidly increasing challenges presented by drug-resistant bacteria. Phage represents an opportunity to improve standards of care through a precision health approach.

Background

Phage are an incredibly ubiquitous type of virus, which are present in all natural environments. Whilst they are harmless to plants and animals, including humans, phage infect and kill bacteria with high specificity. Numbering around 10^{31}, phage represent the most abundant life-form on Earth[1], with over one trillion phages in existence for every grain of sand[2]. However, until recently, their importance and potential to improve both health and the environment have been significantly overlooked in the Western world.

First observed by English physician Edward Twort in 1915, phages were independently identified by Felix d'Herelle, a French-Canadian microbiologist who identified phage as a new type of virus that infected bacteria and named them bacteriophage, based on the Greek for 'bacterial eater'[2].

Figure 1 provides a schematic of a phage, showing the head that contains the phage's nucleic acid and the tail fibres that attach to a bacterium with (in the majority of cases) an extremely high degree of specificity. When the phage binds to a bacterium, the tail and surrounding proteins injects the phage's nucleic acid. The bacterium then becomes a viral factory, being forced to produce more (typically tens to hundreds) of phage virions. These phage, upon bacterial lysis, infect more bacteria of the same species as the host. The phage represented in **Figure 1** is a T4 phage that infects *Escherichia coli* bacteria. It is of note that it has been estimated that more than 99% of the types and structures of phage have not yet been discovered[1].

Building on d'Herelles work, microbiologists have gone on to recognise phage as incredibly important biological entities that have been at the heart of advancing molecular biology. For example they were used to show that DNA is the heredity agent of life, that three nucleotides constitute an amino acid, and are utilised within CRISPR gene-editing technology.

Phage have been successfully deployed in Eastern Europe for more than 100 years to successfully treat a wide range of bacterial infections, from cholera to infected wounds. The George Eliava Institute of Bacteriophages, Microbiology and Virology in Tbilisi, Georgia, continues to practice phage therapy, both within their outpatient clinic and remotely, treating patients from all corners of the globe, noting that it can

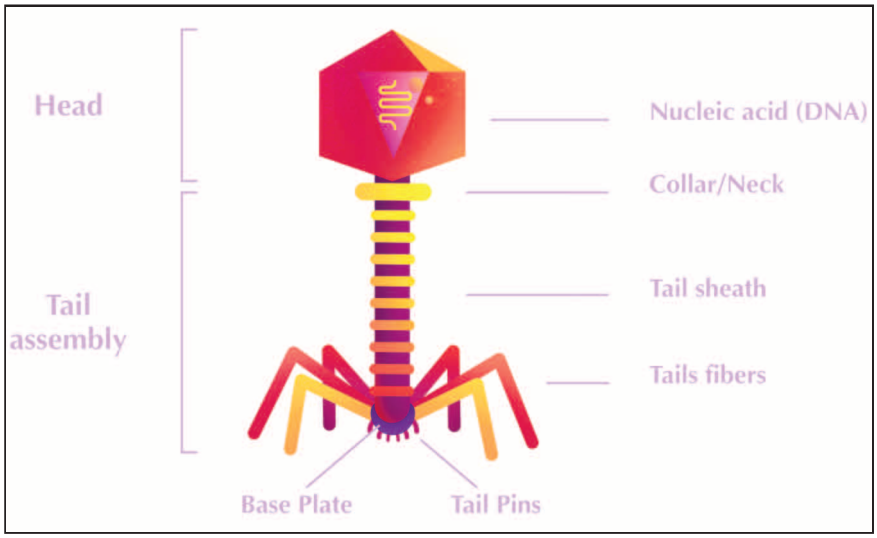

Figure 1: Phage structure, courtesy of the George Eliava Institute.

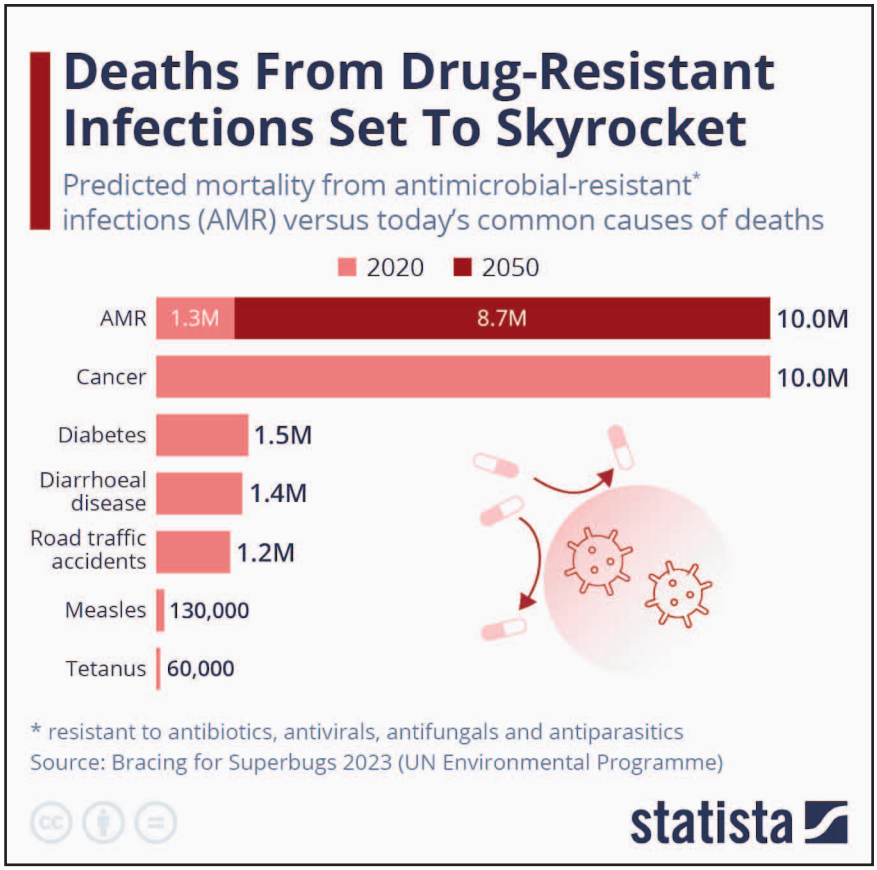

Figure 2: Predicted mortality from AMR infections versus today's common causes of deaths. Statistica, 2023. https://www.statista.com/chart/3095/drug-resistant-infections/

be challenging for people who may be very sick to travel long distances for treatment. In these cases, clinical samples are sent to the Eliava Institute by the patient's clinician and the Institute then despatches bespoke phage cocktails[3].

Alongside other leading phage centres, the Eliava Institute's current focus incorporates tackling the highly important and rapidly increasing global healthcare challenge presented by antimicrobial resistance (AMR). *The Lancet* estimates that by 2050, there will be almost two million annual deaths attributable to AMR globally and over eight million annual deaths associated with AMR[4].

Figure 2 highlights the magnitude of the AMR problem in terms of causes of death. Furthermore, AMR can impact common procedures, from caesarean sections to hip and knee replacements and cancer treatment. According to the UN 2023 report "Bracing for Superbugs: Strengthening environmental action in the One Health response to antimicrobial resistance" unless drastic action is taken to tackle the problem, AMR could also lead to a GDP shortfall of $3.4 trillion annually in the next decade and push 24 million more people into extreme poverty[5].

It is interesting to note that bacteria have become extremely efficient in overcoming antibiotics. This is because they have billions of years of experience in developing resistance to phage (and phage also develop mechanisms to overcome bacterial resistance), creating continuous evolutionary cycles. Phage resistance is usually metabolically disadvantageous to bacteria[6] and consequently once the bacteria have been put in check by phage, the bacteria and phage generally revert back to their previous forms. This means that there is always a balance between phage and bacteria[6]. However, antibiotics do not naturally evolve and therefore provide a continuous, homogeneous challenge to

target bacteria, which can result in the bacteria ultimately becoming not only resistant to the antibiotic, but in some cases more pathogenic (super-bugs)[7].

There is currently a unique opportunity for the UK to be at the forefront of the translation of our growing understanding of phage into clinical practice in order to help address the AMR burden and improve standards of care for a wide range of bacterial infections.

Whilst phage therapies have traditionally been developed using traditional and well-proven microbiological techniques, including bacterial culture and fermentation, UK centres of excellence have started to incorporate additional technologies into their work. For example Professor Martha Clokie's team at the Becky Mayer Centre for Phage Research at Leicester University, include imaging (TEM, Atomic Force Microscopy), host-infection ranges analyses and stability assays in their research. This is complemented by sequencing and analysis of bacteriophages genomes, viromics; the isolation of both viral (phage) DNA and RNA for metagenomic analysis and mathematical modelling to explore how bacterial populations are controlled by phages in complex spatial-temporal environments.

A key recent trigger which has galvanised interest in phage therapy was the January 2024 publication of the findings of the UK Government Science, Innovation and Technology Committee "The antimicrobial potential of bacteriophages"[8]. This important forum highlighted that safety of phage has been well established mainly on the basis of observational evidence drawn from specific clinical interventions. However, as with all medicines, robust clinical trial data is important to provide and develop assurances around all aspects of patient safety, including the long-term impact of phages, especially their interaction with human immune systems, such as anaphylaxis and auto immune response.

Barriers to clinical adoption include the necessary knowledge of the selection of appropriate phage for clinical applications. Purification and testing are also highly important. Collaboration with experts with deep experience, for example, the Eliava Institute will play an important role in addressing this. Additionally, phage can lack robustness to elevated temperatures, for example during transportation. Strategies are being developed to overcome this challenge too, including the selection of robust phage types, the use of cold-chain freight and phage-stabilisation technologies, such as immobilisation onto carrier substrates, such as those pioneered by the British phage company NexaBiome.

A significant additional barrier towards clinical trials successful adoption in the UK is the requirement for phage produced within the UK to be manufactured to Good Manufacturing Practice (GMP) standards. Currently, suitable specialised facilities do not exist in the UK, although a small number of phage manufacturing centres have been established or are under construction in other countries, including the U.S., Canada and Australia.

The Precision Health Technologies Accelerator in Birmingham (PHTA.co.uk) is currently assessing the potential to develop part of its cleanroom-designated area to provide GMP phage. This would leverage a number of key partnerships. The first focus would be on phage production for clinical trials, followed by translation into practice, commencing with Compassionate Use and building through precision health into broader application across the NHS. The PHTA team are building a business case to secure funding for this important potential initiative to break the key UK impasse; having clinical trial data to justify investment in phage infrastructure development (clinical trials require phage infrastructure and data).

Figure 3 summarises opportunities for collaboration to harness significant phage and clinical knowledge within the UK to translate phage-based therapies into practice. Example organisations are shown although it is anticipated that other phage-focused organisations and companies will also play key roles.

The range of potential applications for phage is significant. UK clinicians are expressing significant interest in phage, for example in the treatment of diabetic foot infections and in cystic fibrosis. This growing demand has been driven by AMR. Whilst the mass-production of antibiotics ranks as one of the twentieth century's greatest scientific achievements, saving millions of lives, the Director-General of the World Health Organization recently warned that "the world is on the brink of losing these miracle cures [antibiotics]" and that "in the absence of urgent

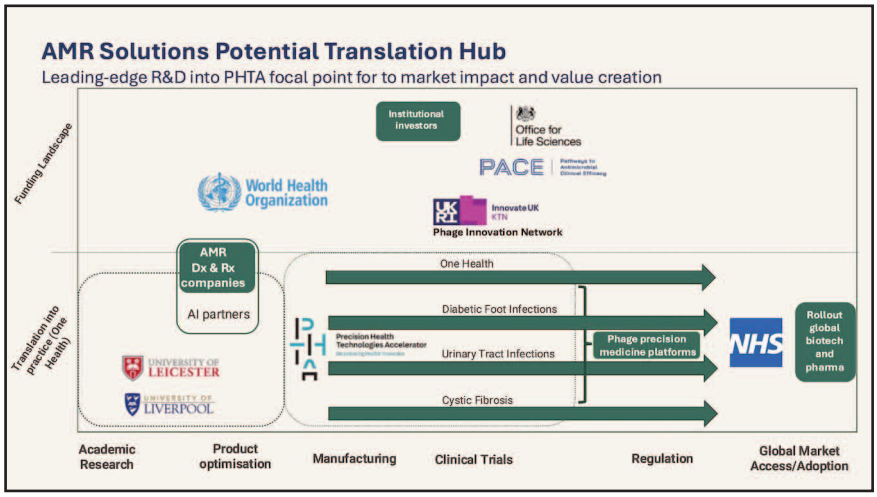

Figure 3: Potential UK ecosystem facilitating phage translation into clinical practice; example organisations. Courtesy of Paragreen Associates Ltd.

corrective and protective actions, the world is heading toward a post-antibiotic era, in which many common infections will no longer have a cure and, once again, kill unabated"[9].

In the United Kingdom (UK), phages are classed as a biological medicine. Although no phages are licensed for UK use, they may be used as unlicensed medicinal products where licensed alternatives cannot meet a patient's clinical needs. In the last two years, a small number of patients in the UK have received phage therapy, with growing clinical interest. Currently, clinical phage provision in the UK is *ad hoc* and relies on networking with international sources of phages[10].

Phages also have the scope to be fundamental to precision medicine-based approaches to tackle bacterial infections and AMR. State-of-the-art clinical diagnostics will be able to rapidly identify the bacterial species and enable the selection of a specific phage cocktail (together in some cases with specific antibiotics) to provide a personalised intervention. The patient would then be monitored and if bacterial resistance detected, the phage cocktail could then be adjusted to maintain efficacy.

Urinary Tract Infections (UTI), which are a leading cause of sepsis, represent an excellent near-term target. Tools for specific bacterial identification such as Llusern Scientific's near-patient molecular diagnostics system, Lodestar DX, can identify specific bacteria causing UTI. Diabetic Foot Infection represents another good target. The ultimate objective will be to be able to highlight specific antibiotic and phage combinations which best-address the infection within a particular patient, enabling targeted and effective therapy whilst minimising the risk of adding to the AMR problem.

UK phage translation projects are also starting to attract significant funding. For example, the Becky Mayer Centre for Phage Research at Leicester University have recently received a £4 million gift from entrepreneur Jimmy Mayer following the death of his wife, Becky, last year. The centre is currently establishing a large-scale phage bank which includes phages that target pathogens of medical and agricultural evidence. They have significant expertise in acquiring and characterising this essential biological resource and are well positioned to provide appropriate scientific knowledge and input to ensure the most appropriate phages are selected and developed.

Professor Jo Fothergill's team at Leicester University are progressing CF-TRAILFINDER, funded by the Cystic Fibrosis Trust and LifeArc, which aims to bring phage therapy closer to people with Cystic Fibrosis that may benefit from this approach. The team are performing translational preclinical research, supporting access to named patient basis and supporting innovation through the development of optimised phage cocktails and supportive methodology in the manufacturing process.

Pharmacists have the potential to play a central role in prescribing precision phage therapies, combining traditional approaches with cutting-edge science. The patient journey could be as follows: Following clinical assessment, the infecting bacteria would be identified using molecular diagnostics, potentially at the point-of-care. Genomics would be performed on the infecting bacteria and a clinician would then prescribe a phage cocktail comprising a selection of phage selected from a formulary held at the local pharmacy. The pharmacist would then combine these and issue them to the patient. The patient would then be regularly monitored and the cocktail adjusted in collaboration with

phage experts and clinicians if bacterial resistance begins to develop. This approach could also be extended to include antibiotics where appropriate.

It is interesting to note that the Eliava Institute prescribes phage over the counter for many bacterial conditions, including skin and gastro-intestinal conditions. This resonates with the outcomes from multiple studies demonstrating that phage are free of side-effects by invasive routes of administration (e.g., intravenous, intra-articular) and in immunocompromised patients[10]. In contrast, antibiotic-associated adverse drug events are comparably common; one 2017 study reported that 20% of patients had antibiotic-associated adverse drug events. It has been reported that some patients have suggested that, given the choice, they would prefer to try phage therapy before intravenous antibiotic[10].

The UK Government Science, Innovation and Technology Committee[8] also highlighted the opportunity for the UK to take a global-lead in deploying phage to address One Health[11] challenges. The potential ranges from addressing bacterial pathogens in farm animals, such as *Salmonella*, which can present serious health issues for humans (particularly where emerging strains may present with significant antibiotic resistance), to environmental challenges including contamination of drinking water with pathogenic bacteria.

Conclusions

By taking a central role in phage and antibiotic stewardship, there is a timely opportunity for British pharmacists to work with phage experts and clinicians in addressing AMR, incorporating a pro-active One-Health consideration as phage becomes available within the formulary. There will be multiple opportunities for collaboration, from leading academic centres and the PHTA to clinical trials and adoption into NHS care pathways. The Phage Innovation Network also provides a useful resource[12].

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Chair, Precision Health Technologies Accelerator

References

1. *The Good Virus, The Untold Story of Phages: The Most Abundant Life Forms on Earth and What They Can Do For Us.* T Ireland, 2023. London: Hodder Press.
2. A century of phage research: Bacteriophages and the shaping of modern biology. EC Keen, *Bioessays*. 2015, 37(1):6-9. doi: 10.1002/bies.201400152PMCID: PMC4418462 NIHMSID: NIHMS679685 PMID: 25521633
3. https://eliava-institute.org/en
4. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050 GBD 2021 Antimicrobial Resistance Collaborators. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(24)01867-1/fulltext
5. Deaths From Drug-Resistant Infections Set To Skyrocket. A Fleck, *Statistica*, 2023. https://www.statista.com/chart/3095/drug-resistant-infections/
6. Phage Therapy in the Resistance Era: Where Do We Stand and Where Are We Going? T Luong, A-C Salabarria, DR. Roach. *Clinical Therapeutics,* 2020, 42(9):1659-1680. https://www.sciencedirect.com/science/article/pii/S0149291820303489
7. Advances in Phage Therapy: Targeting the *Burkholderia cepacia* Complex P Lauman. JJ Dennis. *Viruses*, 2021, 13(7):1331. doi: 10.3390/v13071331 https://pmc.ncbi.nlm.nih.gov/articles/PMC8310193/
8. https://committees.parliament.uk/publications/42708/documents/212244/default/.
9. Phage Therapy: Concept to Cure. EC Keen. *Front Microbiol.* 2012, 19(3):238. doi: 10.3389/fmicb.2012.00238 https://pmc.ncbi.nlm.nih.gov/articles/PMC3400130/
10. The Future of Clinical Phage Therapy in the United Kingdom. JD Jones, C Trippett, M Suleman, MR J Clokie, JR Clark. *Viruses*, 2023, 15(3):721. doi: 10.3390/v15030721
11. https://www.who.int/health-topics/one-health#tab=tab_1
12. https://iuk-business-connect.org.uk/programme/phage-innovation-network/

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Monitoring of Air Handling Systems of Cleanrooms and Clean Areas

by Harald Flechl

The quality-relevant data of the air handling system must be defined based on risk analyses as described in the Contamination Control Strategy (CCS). It is the task of the instrumentation and controls system to register and control the technical data.

Harald Flechl is a freelance technical expert in the field of cleanroom technology. He has more than 35 years of professional experience in cleanroom engineering for pharmaceuticals, electronics and healthcare. He has assumed various functions in the areas of planning, implementation (project management) and maintenance. His main areas of activity are media supply, ventilation and air cooling systems. Currently he is working as a freelance technical expert in the field of cleanroom technology and as author/co-author for the GMP Compliance Adviser.

Purpose of pharma monitoring

The term "monitoring" refers to the continuous or periodic systematic observation and acquisition of data regarding procedures, systems and processes by means of technical aids or "manual" observation procedures. In the pharmaceutical manufacturing process, the purpose is to determine whether the process is taking the specified course and whether the tolerance limits and environmental conditions defined in URS for this process are being adhered to, in order to be able to intervene in a controlling manner.

The FDA Guidance for Industry *Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice* refers to a written monitoring plan to ensure consistent product and environmental quality. The timing, frequency, and location of sample collection should be carefully selected based on the context of the operation being performed. The FDA does not define specific limits for monitoring. Samples should be collected from all classified areas of the aseptic processing facility (e.g. aseptic corridors, gowning rooms) using scientifically sound sampling procedures. The scope of the sampling should be sufficient to keep the detection of environmental contaminants within the required limit and to detect changes in a timely manner.

According to the EU GMP Guide Annex 1, monitoring is intended to ensure that cleanrooms and clean areas (e.g. clean workbenches, unidirectional air flows, RABS, etc.) are monitored in such a way that the conditions established during qualification are demonstrated and meet the requirements of the authorities and the original design specifications. Requalification also serves as a periodic check. However, as an indicator of possible asepsis, monitoring should not look at individual parameters in an isolated manner, but rather evaluate the overall picture that results from the individual elements of the monitoring system (e.g., total particles, Colony Forming Units (CFU), room pressure cascade).One must be aware that the determined values are only a snapshot and thus only a time-limited view of the actual conditions of the cleanroom. Rather, monitoring is suitable for proving that the conditions in the cleanroom are not deteriorating compared to the conditions during the Performance Qualification (PQ) and that they remain comparable to them. The subject of monitoring is strictly separated from qualification in the new version of Annex 1 and therefore requires separate consideration.

Contents and implementation of pharma monitoring

Pharma monitoring programs include the aspects of particle counts and microbiological burdens as defined in accordance with the new Annex 1 (2022) in the Contamination Control Strategy (CCS) and incorporate both environmental monitoring and process monitoring in a meaningful trend observation procedure. Pharma monitoring is to be based on a risk assessment that also takes into account worst-case conditions that may occur during activities in the cleanroom. In the process, unnecessary risks of an additional source of contamination caused by monitoring are to be avoided.

In this article, only the environmental monitoring of cleanrooms and the room air supply is discussed.[1]

Physical and microbiological parameters

Buildings and their infrastructure systems that are used for the production of sterile drugs, for example, require pharma monitoring of both physical and microbiological parameters. Devices and systems are already available on the market that monitor and evaluate phy-sical and microbiological parameters by means of computerised systems. Only they are capable of also being the basis for automatic alarms. When using particle counters that can also record "viable" particle concentrations[2], the following must be observed:

- The reported particle count always indicates the sum of all detected particles (non-viable AND viable).
- "Viable" particles also include those that do not show growth on culture media and do not form countable colonies (VBNC – viable but non- culturable) are also recorded as part of the total.
- With the CFU counting method, it is irrelevant whether a colony was formed by one or more microbes, whereas the particle counters with the fluorescence method detect each individual microbe.

This explains why the results of the classical microbiological methods (microbe collectors, settle plates, contact plate tests etc.) do not correspond numerically to the newer measuring methods mentioned above.

Risk-based design of monitoring plans

The entirety of the environmental monitoring should be the result of a risk assessment in which the individual parameters are defined. For example:

- **Parameters to be recorded** (e.g. particles, CFU, room air flow, room pressure levels, filter integrity. Air temperature and humidity are essential if they are considered relevant for the process/product, monitoring would not be necessary for "personnel comfort")
- **Sampling locations** (which can be considered critical from the result of the qualification)
- **Frequency of sampling** (a meaningful trend observation should be taken into account)
- **Method** (in order to avoid any risk of contamination of the process or product by the measurement)

The *sampling location* should be representative of the highest contamination risk. The selection of the measurement site and the location of the measurement equipment should be justified in the CCS and be suitable for obtaining reliable data from the critical zones.

The *frequency of sampling* and the volume of the samples should be defined in the CCS in such a way that all interventions and exceptional situations of limited duration or any reduction in the system's performance are recorded. This should be combined with an alarm trigger as soon as alert limits are exceeded.

For particle monitoring, the sample volume used for particle measurement does not have to correspond to the volumes used during qualification. The *sampling volume* to be defined depends on the system used (often $0.1ft^3$/min = 2.83l/min) and the frequency of sampling in the CCS.

Particle monitoring

In grade A areas, compliance with the aseptic process environment must be demonstrated during critical operations. Critical operations also include equipment assembly and operator interventions – whether process- related or in the event of an incident. Airborne particles ≥0.5µm and ≥5.0µm shall be monitored throughout the duration of critical activities to ensure that system degradation is detected as soon as possible. Any potential deviation approaching or exceeding alarm values should be detected in this manner in time to react accordingly.

It is accepted that during filling activities the concentrations of air-borne particles ≥5.0 m in the filling area cannot always be maintained, e.g. if product particles or product droplets are released in the process, which do not in themselves represent a contamination. The measurement frequency and strategy must then be adapted accordingly, and additional measurements may have to be carried out during the simulated process in order to better assess possible risks.

Microbiological monitoring

Microbiological monitoring in areas with aseptic activities comprises a combination of different methods (e.g. air sampling, settle plates) on different objects (e.g. gloves, clothing, and surfaces). These different methods should be justified in the CCS and demonstrated to have no adverse effects on grade A and grade B airflows.

Microbiological monitoring should be performed in cleanrooms, adjacent rooms, and on surfaces (such as tables, equipment, etc.) even after a production process has been completed or outside of production activities. Certain points are specifically mentioned:

- After disinfection
- Before start of the production
- After completion of a batch
- After a shutdown period

Adjacent rooms are to be included to identify any influence on possible contamination of the cleanroom. These areas shall also be identified in the CCS.

In grade A and grade B areas the species of the microorganisms are to be identified, and the possible influence on the product quality is to be evaluated down to the batch level.

When action limits or alert levels are exceeded in grade C and D areas, consider identifying microorganisms at sufficient frequency to provide an up-to-date overview of the typical flora of these areas. This is especially true after isolation of organisms that indicate loss of control or deterioration of cleanliness or are difficult to eliminate, such as spore-forming microorganisms and molds.

When performing active airborne microbial sample collection and passive measurement with settle plates and contact plates, secondary contamination by the method or personnel is always possible. The significance of the passive measurement result by means of settle plates is also particularly difficult to assess, since these only cover a small proportion of the surface and can only be impacted "by chance" by airborne particles. Here there is a great dependence on physical parameters, such as the air flow direction at the measuring point, the inertia of the particles with their mass and the resulting sedimentation speed.

The snapshot assessment of a CFU count is further complicated by the maximum exposure time (usually 4 hours) and by the time delay during incubation (up to 7 days for the detection of microorganisms such as yeasts and molds).

Contaminants found at multiple locations in an area within a single sampling period may indicate an increased risk to the product and should be carefully evaluated. However, the near simultaneous occurrence of a contaminant at multiple locations could also be the result of poor sampling (methodological error). However, resampling an environment several days after contamination is not very useful because conditions during sampling cannot be duplicated exactly.

Table 1. Maximum permissible action limits for microbiological monitoring according to Annex 1 from 2022 to the EU GMP Guide

Cleanroom Grade	**Maximum total particle concentration – monitoring** ≥ 0,5 µm/m^3		≥ 5,0 µm/m^3	
	At rest	In operation	At rest	In operation
A	3520	3520	29	29
B	3520	352000	29	2930
C	352000	3520000	2930	29300
D	3520000	n. p.	29300	n. p.

n. p. – not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data where applicable.

Note 1: The particle limits for the at rest state should be achieved after a short "clean up period" (guidance value 20 minutes) after the end of production and without personnel, as checked during qualification. The difference between the clean up period of Annex 1 and the recovery time according to ISO 14644 is described in Chapter 3.I.10.3 Clean up period and recovery time.

Note 2: Occasional elevated counts of macro-particles (≥5.0µm) within grade A is often due to "false counts", e.g due to measuring equipment lines that are too long (e.g. "surge detachments" of

- deposited particles),
- stray light, or also|
- electronics malfunctions.

Consecutive or periodic counts of low levels of macro-particles may indicate possible contamination and should be investigated.

Limits

The maximum permissible limits for particulate matter and CFU in environmental and personnel monitoring are redefined in the Annex 1 from 2022 (**Tables 1 and 2**). These limits differ from the limits in Annex 1 from 2008, which does not specify separate values for monitoring. For plant revisions, conversions and the planning of new facilities, the values of the new version of Annex 1 from 2022 should already be taken into account in the CCS.

More restrictive limits may be derived from qualification, from review of trend histories, or from the nature of the process, and may be specified in the CCS.

The United States Pharmacopoeia (USP) does not specify absolute values for microbial monitoring for aseptic processes. The recovery rate method is recommended regardless of colony count (Source: USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments).

In the FDA Aseptic Guide, only "in operation" conditions are considered. Therefore, the same limit values apply for monitoring and qualification as set for "in operation" (**Table 3**).

An aseptic protective clothing qualification program should assess a cleanroom worker's ability to maintain the quality of protective garments after gowning. As part of this assessment, microbiological surface tests should be conducted at various locations on the garment (e.g., gloves, face mask, forearm, chest). The selection of sites and the testing frequency should be justified, which can be done as part of the CCS.

Table 2. Maximum permissible action limits for microbiological monitoring according to Annex 1 from 2022 to the EU GMP Guide

	Action limits for microbial monitoring			
Cleanroom Grade	Air sample	Settle plates Ø 90 mm	Contact plates Ø 55 mm	Gloves incl. 5 fingers on both hands
	CFU/m^3	CFU/4h[a]	CFU/plate[b]	KBE/glove
A	No growth(c)			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

a) Settle plates in grade A and B should be exposed for the entire duration, including set-up of the system, and changed after 4 hours. The exposure time shall be validated to exclude a negative effect on the growth support capacity of the medium (e.g. drying out). For Grades C and D, the exposure time should be specified based on QRM (quality risk management). Individual plates may also be deployed for less than 4 hrs.

(b) The potential uses for contact plates in grade A and B areas are dependent on equipment, room, and garment surfaces. Routine garment monitoring is generally not required in grades C and D.

(c) In grade A, any growth detected shall be investigated.

Note 1: The monitoring methods given in the tables are to be considered as examples. Alternative methods are permitted if they can provide the essential information for the entire critical process where the product could be contaminated.

Note 2: The microbial limits are given in CFU. If other or new technologies are used that provide different results compared to the CFU count, the limits determined from these must be scientifically justified and, if possible, a correlation with the CFU values derived from them.[3]

Table 3. Limits for classification and monitoring according to FDA Aseptic Guide

Cleanroom classification according FDA	ISO class	Particles/m^3 ≥ 0.5µm	Action limit for airborne microbiological contamination	
			Air sampler (CFU/m^3)	Settle plates Ø 90 mm[a] (CFU/4h)
100	5	3520	1[b]	1[b]
1000	6	35200	7	3
10000	7	352000	10	5
100000	8	3520000	100	50

a) Optional

b) This cleanliness class should generally not contain any microbiological burden.

Only in the totality of all parameters and trend observations do the results of monitoring help to confirm the reliability of the operation and the system they monitor. Therefore, careful consideration is warranted before drawing conclusions about a possible lack of control.

Monitoring of air handling systems

In the context of monitoring pharmaceutical manufacturing processes, it is also necessary to monitor and record room air quality parameters. A distinction must be made between:

- quality-critical parameters that have a direct impact on product quality and safety and whose compliance must therefore be documented as part of the batch documentation, and
- technical parameters that have no direct effect on product quality and safety, the monitoring of which, however, is necessary for the proper maintenance of operation of the air handling system.

The data for which monitoring is relevant to process and product quality and safety must be defined in the CCS and incorporated into the pharmaceutical monitoring plan of the air handling system. On the other hand, the instrumentation and control system (I&C system) for the air handling system and, where available, the building management system (BMS system) are responsible for acquiring, recording and managing the technical data.

The control units of the air handling systems and the higher-level building management systems have always been classified as unsuitable for validation. This strict separation of tasks led to monitoring and I&C being designed as separate systems that perform their tasks independently of each other. This was intended to rule out any mutual influence. Separate systems also have the advantage of being able to "check" each other – i.e.deviating or drifting sensor values are more likely to be detected.

Not least due to legally required energy efficiency efforts, this approach is increasingly being called into question. Integrated planning and networking of individual technical components of the monitoring and BMS system in the cleanroom is a decisive efficiency factor and essentially determines the investment and staffing needed to operate such a system and the resulting energy needs. One factor for efficient operation of the overall "cleanroom" system results from multiple use of the sensor technology. Multiple use of this so-called integrated building technology is possible according to the current state of the art and can be augmented to form a complete system by adding further modules (see **Figure 1**).

The requirement to validate and calibrate is upheld without significant additional expense. The parallel recording of other disturbances (such as door openings) can enormously reduce the time required to troubleshoot and process deviations and make them traceable. The number of instruments requiring calibration remains the same, the difference is that they are used more than once.

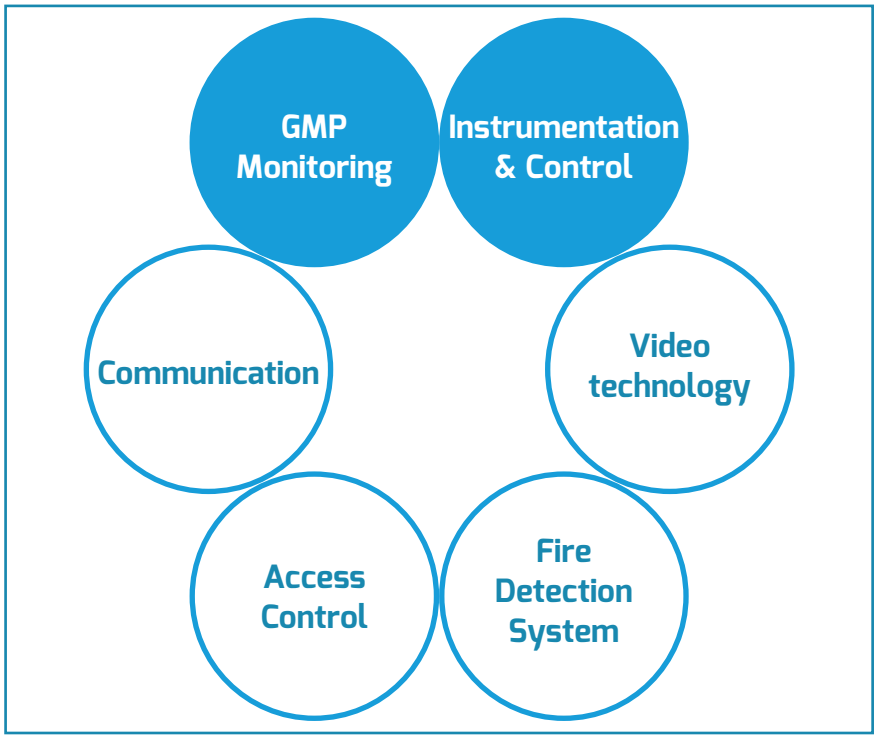

Figure 1. Example of integrated building technology.

GMP-Verlag Peither AG specialises in providing current GMP information. GMP-Verlag Peither serves as a platform for GMP knowledge and has become a trusted partner for all GMP-related questions and publications. The main product is the GMP Compliance Adviser (https://www.gmpcadviser.com). This paper is an extract of the extensive advice provided in GMP Compliance Adviser.

References

1. The PDA (Parenteral Drug Association) has issued a revision of Technical Report 13 – Fundamentals of an Environmental Monitoring Program in 2022.
2. A laser-induced fluorescence emission probe allows to distinguish between inert and biologically active particles.
3. This note also applies to particle counters with biofluorescence measurement. Here, each individual "viable" particle is detected and not the "colony", which can arise from one or more germs.

CRISM Therapeutics

by Chris McConville

This article recounts the trials and travails of securing the necessary and vital funding for UK based research ideas. Here the research is based on a novel drug delivery technology, using very small cylindrical implants called ChemoSeed. The author describes how the research ideas developed and how the necessary funding was eventually and successfully raised ensuring that the work can now proceed to Phase 2 clinical trials.

Dr Chris McConville is Chief Scientific Officer of CRISM Therapeutics and Professor of Biomedical Innovation, Ulster University. He is active in the field of drug formulation and delivery, with a focus on medical devices and combinational products. He works with the pharmaceutical industry, academia, and clinical partners to drive innovation from proof-of-concept and feasibility through clinical trials and onto commercial registration. His recent work has focused on the development of an implantable device for the local delivery of chemotherapy to the brain (ChemoSeed®) as well as a device for the delayed and localised delivery of chemotherapy to the pancreatic cancer margin (ChemoPatch).

Introduction

The initial research that resulted in the formation of CRISM Therapeutics Corporation started more than ten years ago and involved work that I was carrying out at the University of Birmingham, where I was Associate Professor in Pharmaceutics, Drug Formulation and Delivery. I was working on the development of implantable drug delivery technologies using hot-melt extrusion, 3D printing, injection moulding and other novel techniques using biocompatible materials.

I'd previously worked successfully on the delivery of anti-HIV drugs using replaceable vaginal rings. When I took up my first independent academic post I started to research other areas where localised drug delivery could bring significant benefit to patients. Given the huge unmet medical need, I focused on the potential treatment of solid tumours through the local delivery of chemotherapy.

Drug Delivery Systems

Drug delivery can play a key role in making a drug more effective clinically and avoiding negative side effects. With traditional systemic routes of administration, such as orally or by intravenous injection, the amount of drug that reaches the target can be quite small and the potential to increase the dosage is often limited by the side effect profile. The drug itself is effective, it's just not getting to the site in sufficient quantity where it will truly benefit the patients.

This is particularly true when seeking to introduce drugs to the brain, which is protected by the blood brain barrier, and also when treating solid tumours such as pancreatic cancer where a stroma develops around the tumour hindering the delivery of chemotherapy agents.

In an effort to find and expedite solutions to this problem, with support from private investors I founded Extruded Pharmaceuticals in 2016 with the objective of developing implantable drug delivery technologies. We were particularly focused on solid tumours, based on the belief that local administration of chemotherapy directly into cancerous tissue could result in improved patient outcomes through increased efficacy and reduced toxicity.

Using hot melt extrusion and injection moulding techniques, we developed a very small, cylindrical implant called ChemoSeed, which is 6mm in length and 2mm in diameter (**Figure 1**). Constructed from a polylactic-co-glycolic acid polymer, ChemoSeed is biocompatible and bioresorbable and is designed to sustain the delivery of a single drug or a combination of drugs (**Figure 2**).

Glioma Treatment

Our chosen disease area at Extruded Pharmaceuticals, and the lead programme at CRISM Therapeutics today, was high-grade glioma (HGG), a type of cancerous brain tumour originating from non-neuronal glial cells which support and

Figure 1. ChemoSeed – 6mm in length, 2mm in diameter

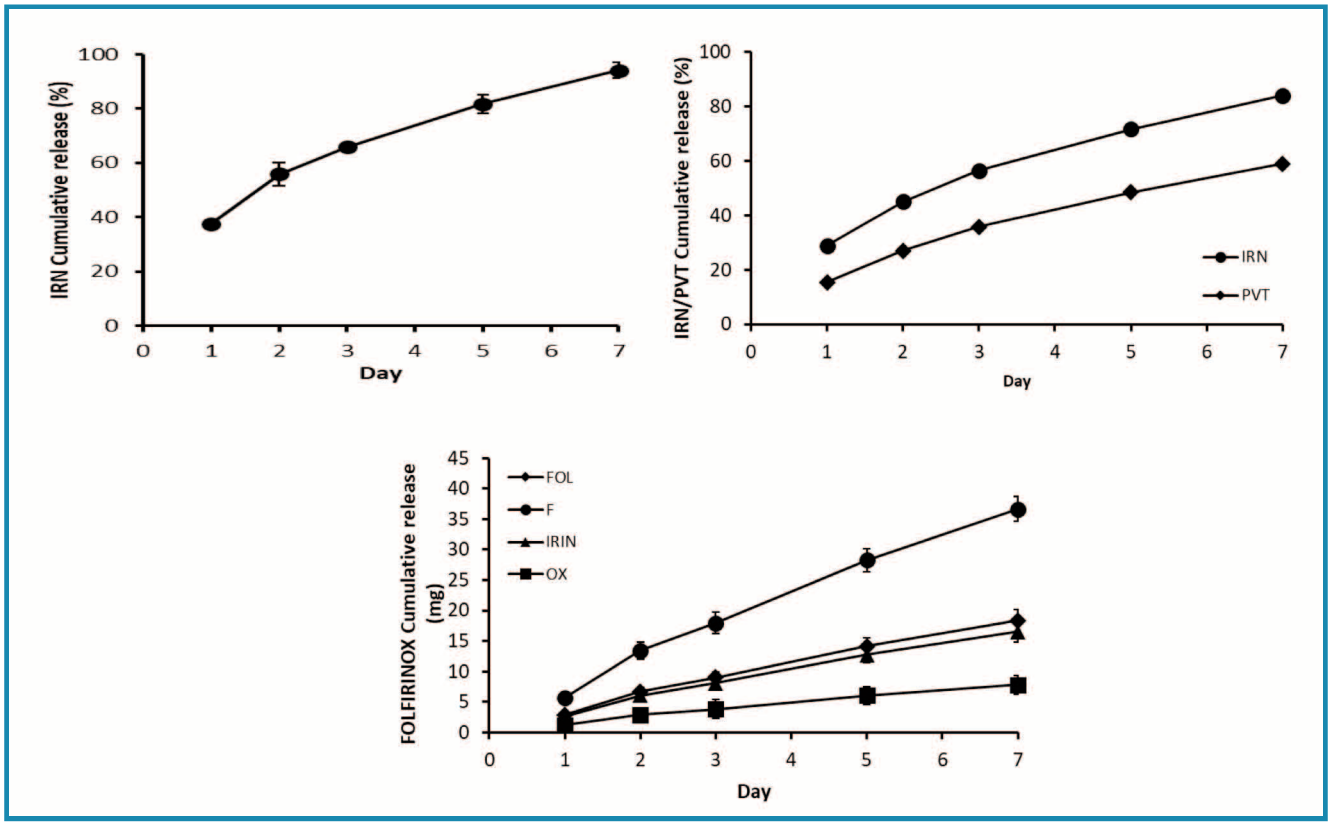

Figure 2. In vitro release of the single drug IRiNotecan, dual drug combination of IRiNotecan and PitaVastaTin and the 4 drug combination FOLinic acid, 5-Flurocil, IRINotecan and Oxaliplatin.

protect neurons in the brain. Brain tumours, particularly HGG, are the leading cause of cancer-related deaths among children and adults under the age of 40. The most aggressive form of glioma is glioblastoma (GBM) and the median overall survival for patients with GBM is just 14.6 months, making GBM an area of major unmet medical need. New treatment options for HGG have the potential to be granted orphan designation.

The current standard treatment for GBM is surgical resection followed by radiotherapy and subsequent systemic delivery of temozolomide, a chemotherapy drug. More than 80% of tumours recur after this treatment as GBM is highly infiltrative, and therefore extremely difficult to fully remove surgically whilst the effectiveness of systemic temozolomide is limited by the blood brain barrier and dose-limiting side effects.

ChemoSeed represents a new approach to the treatment of GBM.

Each ChemoSeed contains 7.2mg of the topoisomerase inhibitor irinotecan, a chemotherapy agent initially approved in the US in 1996 and included in the World Health Organisation's List of Essential Medicines. Interestingly, camptothecin, the compound on which irinotecan is based, was initially isolated from a tree, *Camptotheca acuminata*, used in traditional Chinese medicine.

ChemoSeed is designed to be implanted into the resection margin using a ventricular catheter with each seed able to deliver irinotecan for up to 35 days. For GBM, we believe that 10 to 30 seeds would need to be implanted in the resection margin dependent on the tumour (**Figure 3**). ChemoSeed is designed to remain *in situ* at the resection margin, which we've demonstrated through the use of a 0.6 per cent agarose gel model, and then biodegrade over a period of three to six months.

This sustained, localised irinotecan delivery, which bypasses the blood brain barrier as ChemoSeed implantation takes place during surgery, aims to address the problem of tumour recurrence through continuously attacking the remaining malignant cells for a week or more following resection surgery.

To test the safety and efficacy of ChemoSeed, and to identify the most suitable irinotecan dose for use in the first human trial of ChemoSeed, we carried out a number of preclinical studies including the use of a U87 cell line mouse model of

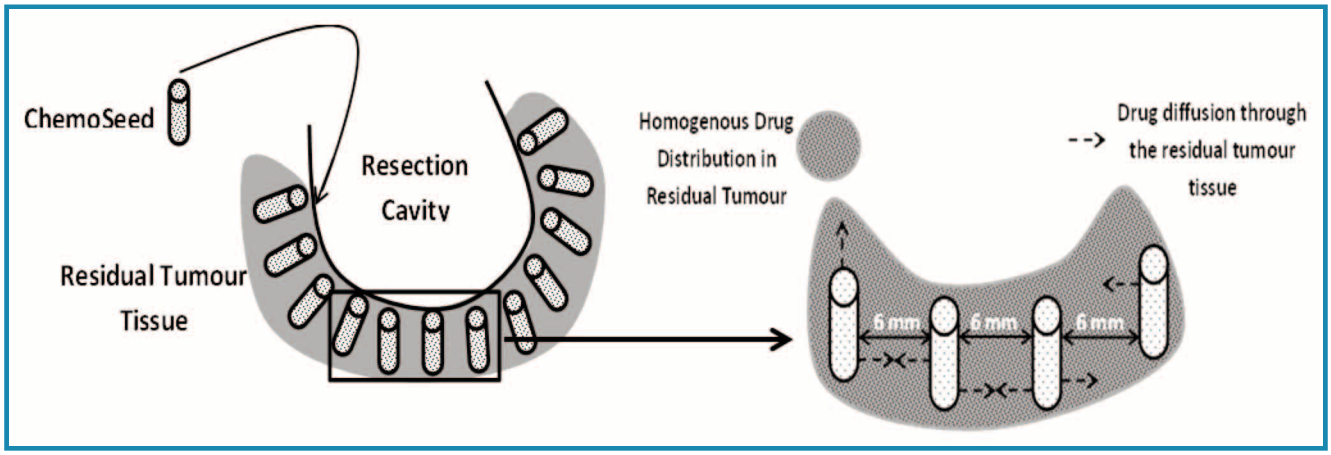

Figure 3. Schematic of the implantation of ChemoSeed into the resection margin of a high-grade glioma tumour during surgery.

GBM and a patient-derived xenograft (PDX) mouse resection model of GBM.

We collected extensive data from the preclinical studies, including data in the murine PDX study showing that, with 30 per cent irinotecan-loaded ChemoSeed, no tumour was detectable 148 days after resection surgery and implantation in all five mice in that arm of the study. Four of the mice lived to 148 days, the ethical cut-off of the study; the fifth mouse died at day 46, with poor recovery from surgery as the most likely cause of death. No tumour was detectable upon brain imaging of the surviving mice at 148 days. Mice in the control cohorts were all dead within 32 days and had detectable tumour recurrence in the brain (**Figure 4**).

We had reached the very exciting point where we knew we had a potential clinical candidate and that we were ready to conduct a Phase II clinical trial in patients with GBM.

We also have strong intellectual property (IP) in that the IP and know-how in the manufacturing of the ChemoSeed technology is owned by CRISM Therapeutics and patents have been filed in all key commercial territories.

Clinical development is expensive and, up to this point, we had received support from grant funding and early-stage finance from individual investors. However, we mostly funded the development of ChemoSeed from service contracts, designing drug-releasing implants for third-party pharmaceutical and biotechnology companies. We continue to offer these services at CRISM Therapeutics and, in July last year, signed a new agreement with a UK biotechnology company to formulate a novel ethylene vinyl acetate implant so that it can deliver a specific, customised dose of a synthetic hormone each day for up to a year.

Development Funding

Andrew Webb, an experienced life sciences entrepreneur, was one of the individual investors in Extruded Pharmaceuticals and became Chief Executive Officer in 2020, focusing on the strategic development of the company and on attracting funding. He said: "I invested £130,000 in Extruded Pharmaceuticals back in 2019. I was very impressed by Prof McConville's expertise and passion for drug formulation, by the scale of unmet medical need and by the risk profile of the business, specifically in terms of using existing, approved drugs to mitigate development risk."

Since he became Chief Executive Officer, Andrew has been closely involved in raising finance for the company.

He said: "We're profoundly grateful for the support we've received from the Precision Technologies Health Accelerator (PHTA) at the University of Birmingham, from grant support and from our early-stage investors but we needed access to larger amounts of capital to take the company towards, and fund, a clinical trial of ChemoSeed in GBM. To achieve this funding, we decided to float the company on the London Stock Exchange, which we did successfully in June 2024 and at the same time we changed the name from Extruded Pharmaceuticals to CRISM Therapeutics Corporation.

"We secured some £1.95 million in cash through the flotation and this is a key amount of money for the

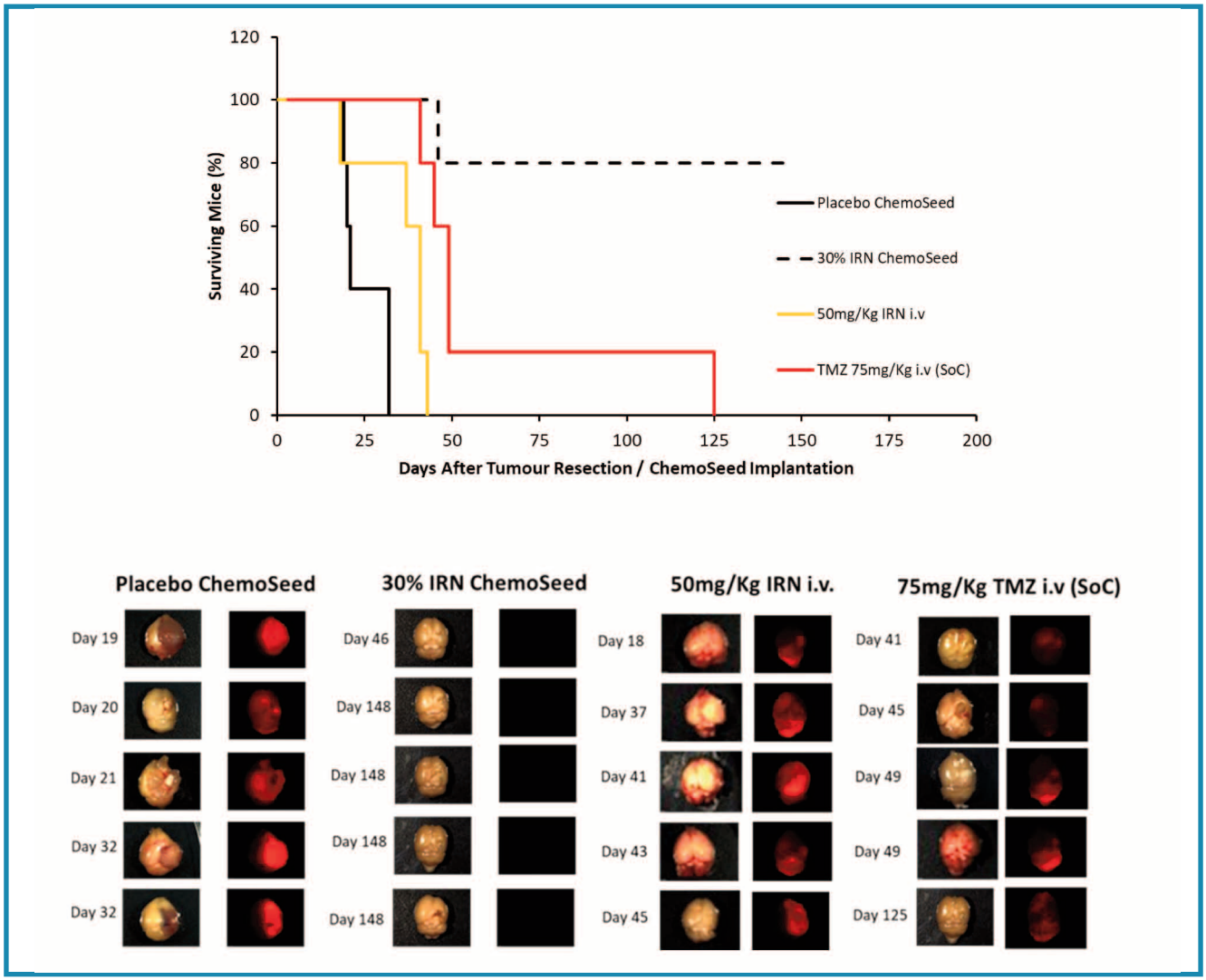

Figure 4. Kapplan-Mayer survival curves from the mice in the PDX study. All mice in the IRN i.v. and SoC groups had died by day 43 and 125 respectively. The 30% IRN mice had an 80% survival at day 148 when the surviving mice were euthanized and their brains removed and imaged there was no sign of tumour (red) present in the ChemoSeed group, while all other groups had tumour present.

company because, given the quality of the data we have, we have line of sight to a Phase II clinical trial, which ideally will start at the end of 2025. Financially, we are in a great position because so often highly promising technology gets through the preclinical phase but then enters the 'valley of death' in trying to transition to the clinical phase where regulatory, manufacturing and other costs rise steeply. We have sufficient funding to take us through to the start of the Phase II clinical trial ahead of which we will explore further funding options.

"We've made substantial progress since becoming a quoted company. We've been awarded an Innovation Passport by the MHRA, which aims to accelerate the time to market and facilitate access to novel treatments for patients in areas of unmet medical need. We're in talks with the MHRA regarding preparedness for the Phase II trial in GBM and we expect to make our Clinical Trial Authorisation Application in the near future."

Conclusion

Andrew Webb concludes with this thought: "We're at the start of an amazing journey. If we can show that ChemoSeed works in GBM, then we have a platform that could work in other indications and with other chemotherapy drugs and drug combinations. There's much we can do if we can prove the first indication."

Determining the required air supply rate to maintain airborne cleanliness levels in pharmaceutical cleanrooms using a scientific approach

by Nigel Lenegan and Tim Eaton

This paper details a scientific approach to determine the air supply rate required to attain specified concentrations of airborne particulate and microbial contamination, in non-unidirectional airflow cleanrooms. It applies the methodology described in published technical papers and compares this with the more traditional approach based on typically utilised air change rates.

Nigel Lenegan is Managing Director at Energy & Carbon Reduction Solutions Ltd and has worked in the building services sector for over 25 years. He has a first class honours degree in Building Services Engineering. In 2006 he co-founded the Global Sustainable Facilities CoP (Communities of Practice) for ISPE and in 2008 he established Energy & Carbon Reduction Solutions. His ground-breaking experiments with AstraZeneca and GSK on airflow reduction in sterile manufacturing have led to a successful consultancy in low energy cleanrooms and laboratories. He was a member of the BSI committee which wrote BS 8528:2013, the basis for ISO 14644-16:2019 – Energy efficiency in cleanrooms and clean air devices. Nigel is subject matter expert for cleanroom HVAC energy reduction and regularly provides training on the subject.

Tim Eaton is a process chemist with over 35 years experience of steriles manufacture. During this time he has held extensive roles in technical support, production management and specialist activities for aseptically prepared and terminally sterilised products. He has had responsibilities for the design, construction, start up and validation of multimillion pound aseptic manufacturing facilities and has managed the introduction, technical transfer and scale up activities for a number of sterile products. He has published a number of papers relating to cleanroom activities and has also presented at various industry forums in Europe, the US and Asia. He sits on LBI/30, the British Standards Committee for Cleanroom Technology.

Introduction

Over recent years, the pharmaceutical industry has attempted to reduce the energy consumption and related carbon dioxide emissions associated with its manufacturing activities. It is generally accepted that a significant proportion of this energy is used by heating, ventilation, and air-conditioning (HVAC) systems to supply conditioned air into manufacturing cleanrooms to ensure that the required airborne cleanliness levels are achieved.

Although demand has grown for low energy cleanrooms, many users and designers still calculate the required air supply flow rates for non- unidirectional airflow (non-UDAF) based upon the approach developed during the 1950s relating to the environmental controls parameters utilised for United States Air Force (USAF) aerospace facilities.

One of the first USAF controlled environment facilities was designed to manufacture World War II bomb guidance gyroscopes and had an average room air change rate of 20 per hour and this value seems to have become blindly engrained into cleanroom design air supply recommendations.

The US FDA Aseptic Processing Guide 2004[1] includes this value of 'at least' 20 air changes per hour (ac/hr) for Class 100,000 (ISO 8[2], EU Grade C[3] in-operation) supporting rooms with 'significantly higher air change rates' for rooms requiring better levels of cleanliness. Other documents, such as the WHO[4] GMP guidance for non- sterile facilities, cite 6 to 20 air changes per hour and although many other such documents do not state minimum values, the expectation is that a suitable supply of filtered airflow is utilised. Although this approach has historically been shown to be successful, it does not consider the over-control that may result and the associated higher energy usage and carbon dioxide emissions.

Consequently, a more scientific approach, that is based on articles written by Bill Whyte with the authors of this article and other co-authors, considers the correct factors that relate to the control

of airborne contamination. This approach, which can actually determine the required airflow to meet a specified cleanliness level, is desirable and is reported in this paper.

2. The Scientific Approach

2.1 Calculation of airborne contamination concentrations

Equations used to calculate the airborne concentration of particles and microbe-carrying particles (MCPs) in the build-up, steady-state and decay conditions in non-UDAF cleanrooms have been previously discussed[5]. When a cleanroom is empty and no machinery running, the airborne concentration of particles and MCPs is practically zero, but as personnel enter and machines are switched on, the concentration builds up to a 'plateau' or 'steady state' that is maintained during manufacturing. There will be some variation in the steady-state concentration owing to activity, but the average airborne concentration can be calculated by the **Equation 1**.

Equation 1

$C = D / Q_s$

Where,

C = airborne contamination concentration in the steady-state condition

D = total dispersion rate of airborne contaminant from sources in the cleanroom

Q_s = air volume supply rate to a cleanroom

This equation assumes the supply air passing through the cleanroom's high efficiency air filters contains few airborne particles, especially the larger-sized MCPs and almost all the airborne contamination is dispersed from sources within the cleanroom. Also, because cleanrooms are pressurised, it is assumed that no background contamination enters from adjacent areas when the doors are closed.

Equation 1 applies to small particles, such as particles ≥0.5µm, as these do not deposit, by gravity, onto cleanroom surfaces in sufficient numbers to noticeably reduce the airborne concentration. However, larger particles such as those ≥5µm, and MCPs, which are dispersed skin particles that have micro-organisms attached, will deposit by gravity onto surfaces, thus increasing the contamination decay rate, which translates into increasing the apparent air change rate[5]. The deposition velocities are related to the particle size and the cleanroom airborne cleanliness levels and for particles ≥5µm[6] and MCPs[7], are shown in **Table 1**.

Equation 2

$C = D / (Q_s + V_D \cdot A)$

Where,

V_D = deposition velocity of particles in room air

A = area of surface deposition (normally the floor area).

By rearranging **Equations 1** and **2**, the airsupply rate required for a given concentration of small particles can be calculated as shown in **Equations 3** and **4**.

Equation 3

For ≥0.5µm particles,

$$Q_s = \frac{D}{C}$$

Equation 4

For MCPs,

$$Q_s = \frac{D}{C} - V_D \cdot A$$

The average air change rate value is the number of times (per hour) that the volume of the air in the room is replaced by the supply airflow and is determined by dividing the rate of volume air supply by the room volume. However, it can be seen clearly from **Equations 1** and **2** that the airborne concentrations do not involve the room volume and therefore the airborne cleanliness in a non-unidirectional cleanroom is directly related to the air supply rate and not the room air change rate.

2.2 Dispersion rates of airborne contamination

In order to determine the required airflow rate, the total dispersion rate of airborne contaminant from sources in the cleanroom must be known. The airborne contamination in cleanrooms is dispersed from machinery and personnel, and the total emission rate (TER) can be calculated using **Equation 5**.

Equation 5

TER

= (ER per person x no. of personnel)

+ ER of machinery

(ER = emission rate).

There will be a small amount of re-dispersion of contamination deposited on the floor when personnel walk around the cleanroom, but this has been shown in a cleanroom to be typically less than 1% of the particles on the floor[8] and can

Table 1: Deposition velocities for particles ≥5µm and MCPs

Particles ≥5µm			MCPs		
ISO 14644-1 Class	**Class limit particles ≥5µm/m³**	**Deposition velocity (m/s)**	**EU GGMP Annex 1 Grade**	**Grade limit MCPs/m³**	**Deposition velocity (m/s)**
5	Not stated[a]	0.0105	A	<1	>0.0161[b]
7	2,930	0.0037	B	10	0.0073
8	29,300	0.0022	C	100	0.0033
9	293,000	0.0013	D	200	0.0026

a. Sample collection limitations for both sizes of particles in low concentrations and sizes greater than 1µm make classification at this particle size inappropriate, due to potential particle losses in the measurement system.

b. The deposition velocity increases as the airborne concentrations of MCPs become less[6]. In these circumstances, **Equation 2** gives a more accurate result.

therefore be ignored without significant loss of accuracy in the calculation of air supply rate.

2.2.1 Dispersion rates of airborne contamination from personnel - dispersal chamber

Information regarding dispersal rates, from personnel and machinery, within cleanrooms is available but this relates to the airborne concentrations that have already been subjected to the controls of the cleanroom air supply system within the cleanroom. Consequently, the actual, at source, uncontrolled dispersal rates, are more appropriate to utilise and dispersal chambers have been used to measure the airborne dispersion rate of total airborne particles and MCPs from a person. The following has been reported:

a. Dispersion rates vary between individuals, and from day-to-day. The average of dispersal rates taken from 25 males and 30 females when exercising in a dispersal chamber and wearing their own indoor clothing has been measured[8] and shown in **Table 2**.

b. Wearing cleanroom clothing reduces the dispersion of airborne contamination, and shown in **Table 2** is the effect of wearing a full set of cleanroom clothing over the indoor clothing used by the same 55 people[9]. The full set of cleanroom clothing consisted of a one-piece coverall manufactured from woven polyester fabric, hood, mask, latex gloves and over boots. The pore diameter of the clothing fabric, as determined by IEST Recommended Practise 3[10], was 26µm.

Table 2: Average dispersion rates from personnel in a dispersal chamber

Clothing worn	Particle Dispersal Rate (no./s)		
	≥0.5µm	≥5µm	MCPs
Normal indoor clothing	35,500	5,533	40
Full set cleanroom clothing (fabric pore diameter 26µm)	17,000	621	2.8
Reduction in dispersal rates, cleanroom clothing compared to indoor clothing	2.1	8.9	14.2

The following points should also be noted:

a. The dispersion rate varies according to the design of cleanroom clothing. The more of a person's body that is covered, the lower the dispersion rate. Gowns are less effective than full sets of cleanroom clothing owing to the lack of control of body emissions from below the gown.

b. The more effective the filtration properties of the fabric, such as obtained by tighter weaves of cloth, the more effective the clothing is in preventing particle and MCP dispersion, and new fabrics are more effective than used fabrics[11].

c. The fabrics used to manufacture cleanroom garments are more effective in filtering larger diameter particles (see **Table 1**), and consequently, more effective fabrics give a greater reduction of concentrations of MCPs than of smaller ≥0.5µm and ≥5µm particles.

d. Garments worn under cleanroom clothing influence the dispersion rate of particles, as the higher the number of particles dispersed from underclothing, the higher the number that will pass through outer cleanroom clothing. Fabrics that break up easily, such as cotton and wool, emit more particles than poly-cotton clothing, which in turn emit more particles than garments made of polyester or nylon.

Individuals who wore a cotton track suit under cleanroom clothing dispersed about 10 times more particles than when wearing their own indoor clothing under the same type of cleanroom clothing[12].

However, as the main source of MCPs is skin, and not fabrics, underclothing has a small effect on the dispersion rate of MCPs due to the relatively large equivalent pore diameter that allows MCPs to pass through. Nonetheless, undergarments that have a small enough equivalent pore diameter will influence the filtration effectiveness against skin particles.

e. The greater the activity of personnel, the greater the dispersion rate and the dispersion rate of particles is about 20 times more when exercising than when standing

2.3 Particle dispersion from machinery

The emission rates of MCPs from machinery can be assumed to be zero in normal conditions, and only in rare and accidental circumstances will machines emit MCPs. The emission rate of particles from machinery may be available from the manufacturer but it may be necessary to obtained it experimentally as shown in **Table 3**.

Table 3: Examples of particle dispersion rates from machinery and equipment

Type of machine	Emission rate, particles ≥0.5µm (no./s)
Vial filling machine A	3.3×10^4 [13]
Vial filling machine B	5×10^2 [13]
Blow-fill-seal	Between 10^2 and 10^7 depending on type of BFS machinery[14]
Six-axis robot, unmodified	4×10^3 [15]
Six-axis robot, modified to reduce emissions	0.3[15]

3. The ventilation effectiveness of supply air in cleanrooms

Studies of operational system ventilation effectiveness of the air supply in non-unidirectional airflow cleanrooms have reported imperfect mixing of the air supply and room air, and hence different concentrations of airborne contamination throughout the cleanroom[6,16]. The ventilation effectiveness (VE) at a locations in a cleanroom is the same as the Local Decay Rate (LDR) and also the Local Air Change (LAC) rate, and can be determined using **Equation 6**[16].

Equation 6

Ventilation Effectiveness (VE)

$$= \frac{\text{measured decay rate at a location}}{\text{average decay rate in cleanroom}}$$

$$= \frac{\text{N@ location}}{\text{N average}}$$

The decay rate at a location is obtained by measuring the decay of test particles and the use of **Equation 7**. This is also known as the Recovery Rate test as described in ISO 14644-3[17]. The location may be a location of specific significance to the user, a location that is considered to be representative of the whole of the cleanroom, or a location that is associated with the highest contamination concentrations, or several locations around the cleanroom, may be chosen.

Equation 7

$$N = -\frac{1}{t} \ln \frac{C}{C_i}$$

where,

N @ location = decay rate of particles at the test location

N average = The cleanroom average decay rate (this is the same as the average air change rate, determined by dividing the total air supply rate by the cleanroom volume)

C = airborne concentration of particle contamination after a given decay time,

C_i = initial airborne concentration of particles,

t = time of decay from C_i to C

To provide a good understanding of how well the cleanroom is ventilated and the ventilations system's overall effectiveness, multiple locations are evaluated. If the cleanroom air is perfectly mixed, the VE will have a value of 1 at all locations in the cleanroom, i.e. an even distribution of concentrations of airborne contamination throughout the cleanroom. If the VE is greater than 1 in some locations, this suggests that this location receives more clean air supply, or has more effective mixing and dilution of the supply air with the contaminated air, than the average. Other locations will therefore have VE values less than 1 to compensate for this and so the VE value can be used to identify cleanroom locations where poorer mixing occurs and, if required, improvements can be made.

From completed experimental work[18], typical VE values for some locations in non-UDAF cleanrooms are between 0.7 and 0.9 and at other locations in the cleanroom, the VE value will be better than that. These cleanrooms have been designed to provide good clean airflow

introduction and mixing throughout the cleanroom using effective diffusers and contaminated air removal by low level extraction.

Some cleanrooms have critical locations for product or process protection, or there are locations where higher contamination emissions are seen and, at these locations, better VE values may be required. This could be achieved, for example, with a localised UDAF using perforated plate diffusers or simple filter face outlets to increase the VE value at those critical locations. Critical products or processes are often protected by separative devices which provide localised higher protection, typically using UDAF. Any separative devices in the cleanroom, such as Class 2 microbiological safety cabinets, restricted access barrier systems (RABAS) and isolators, may emit air into, or extract air from, the surrounding cleanroom and so influence the airflows and mixing of the air in the cleanroom which may increase the VE values within a cleanroom.

Other diffuser performance experiments[19] showed that various diffusers mix and dilute the air differently resulting in different VE values. Swirls and louvre faced diffusers (office type solutions) provide strong sideways airflow direction which induces airflow upwards to the centre of the diffuser and spreads airborne contamination around the cleanroom before mixing and removing it into the extracts. Perforated plate, downward displacement diffusers provide good, localised contamination control but leave pockets of poorly ventilated and lower cleanliness in the zones in between. Cleanroom diffusers that deliver a mixture of both sideways and downward airflow generally provide better mixing of the supply air with the cleanroom air throughout the, relative to the swirl and louvre types. At the cleanroom design stage, this can be predicted using computational fluid dynamics.

By considering the ventilation effectiveness values, the previous **Equation 3** (for small particles not influenced by gravity) and 4 (larger particles influenced by gravity, such as MCPs) can be modified to provide a more accurate determination of the required air supply rate for a specified airborne cleanliness level, as shown in **Equations 8** and **9** respectively. This could provide the necessary cleanroom airflow rate in order to achieve the required conditions at the location where the VE was measured or, the airflow applicable for the whole of the cleanroom, if this location is considered to be representative of the whole cleanroom.

Equation 8

$$Q_S = \left[\frac{D}{C} \right] / VE$$

Equation 9

$$Q_S = \left[\frac{D}{C} - V_D \cdot A \right] / VE$$

4. Air supply volume to ensure required airborne concentration is unlikely to be exceeded

The previous sections describe a method used to calculate the air supply rate required for a non-UDAF cleanroom that is based on average dispersion rates, and therefore predicts the 'average' concentration of airborne contamination. The VE values indicate how well the ventilation system dilutes the contamination throughout the cleanroom and provides location specific levels of contamination. However, in some situations this may not be satisfactory as ISO 14644-1, for example, classifies cleanrooms by specifying the 'maximum' airborne concentrations of particles that should not be exceeded. Nevertheless, there is always a chance of a concentration recorded to be higher than the 'maximum' that is specified, and it may therefore be more appropriate to specify a concentration that should not be exceeded, except on a small and defined proportion of occasions. This concentration could relate to the actual cleanroom classification limit, or it could relate to an alert level that is set at a value which is less than the classification limit to provide an early indication of drift from established operational concentrations.

Two non-UDAF cleanrooms that are known to the authors are used as examples of the frequency distribution of airborne concentrations for particles ≥0.5µm. Shown in **Figure 1** is the frequency distribution measured in **Cleanroom 1** during operation. Here the distribution has a standard deviation that is approximately half of the mean. Shown in **Figure 2** is the equivalent frequency distribution measured in **Cleanroom 2** which is better controlled. Here the distribution has a standard deviation that it is approximately twice the mean. These distributions are typically found in non-UDAF cleanrooms and have a positive skew, with an

extended tail containing high concentrations.

Figure 2 shows a situation that is likely to occur in high-quality cleanrooms where many low counts are registered. Distributions of airborne counts in cleanrooms are routinely considered to conform to a Normal (Gaussian) distribution but in well controlled environments, they may actually conform better to a log-normal, Poisson, or negative-binomial distribution.

In a frequency distribution of the airborne counts, an Upper Confidence Limit (UCL) can be chosen that defines a given percentage of counts that should fall below the UCL. In a cleanroom, the low airborne counts can be disregarded and a one-sided frequency distribution assumed. In a one-sided normal distribution, a UCL of 97.7% will occur at a value of two standard deviations above the mean.

Any suitable UCL can be chosen, and the number of standard deviations that correspond to the UCL i.e. the Z-value, for a one-sided normal distribution, can be readily obtained. If an appropriate UCL is chosen, then the required increase in the air supply to control at a 'maximum' concentration instead of at an 'average' concentration can be determined using **Equation 10**.

Equation 10

$$N_S = 1 + C_V \cdot r$$

where,

N_S = number of times air supply rate to be increased to control at a 'maximum' concentration for a chosen UCL.

C_V = coefficient of variation

r = number of standard deviations above the mean that corresponds to the chosen UCL e.g. 1.65 standard deviations contain 95% of results, 2 contain 97.7% and 3 contain 99.9%

The coefficient of variation (C_V) is a statistical measure of the dispersion of data points in a data series around the mean

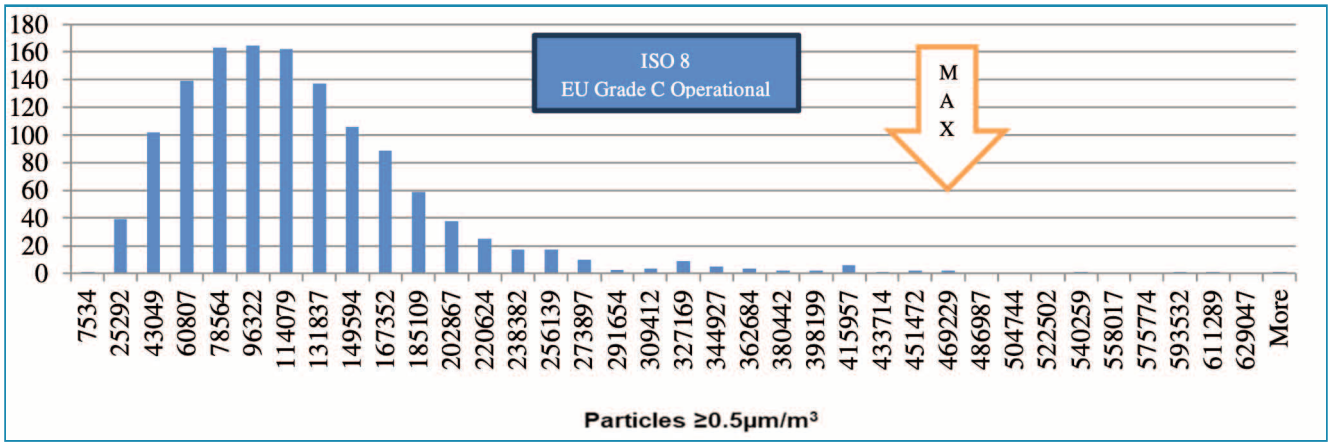

Figure 1: Cleanroom 1 – Distribution of airborne counts of particles ≥0.5µm/m³ in cleanroom 1 (mean 114,971, standard deviation 73,234).

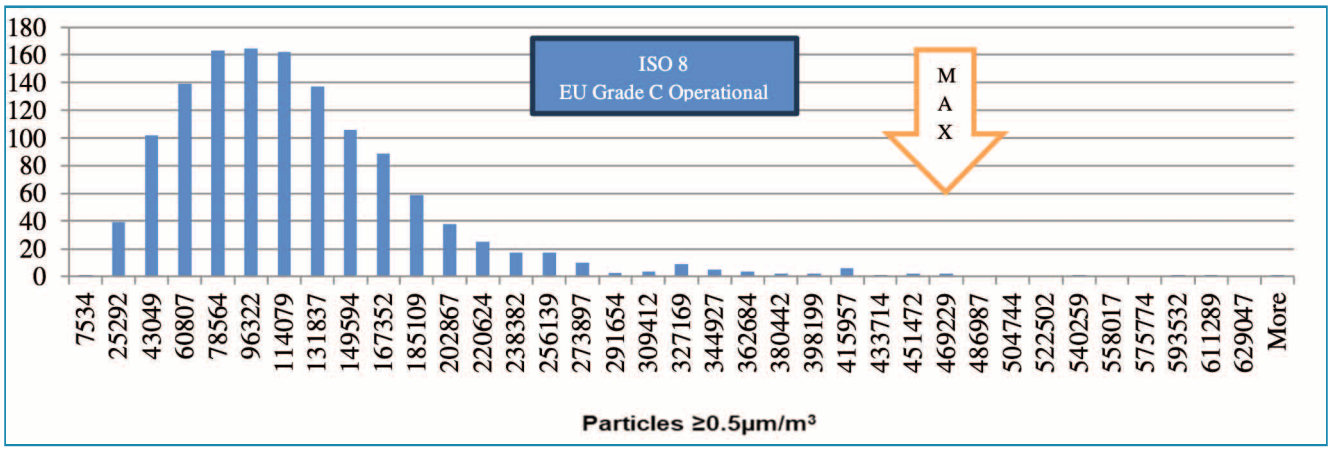

Figure 2: Cleanroom 2 – Distribution of airborne counts of particles ≥0.5µm/m³ in cleanroom 2 (mean 3027/m³, standard deviation 5208).

and the higher the coefficient of variation, the greater the dispersion level around the mean. The coefficient of variation (C_V) is calculated using **Equation 11**.

Equation 11

$C_V = \sigma/\mu$

where,

σ = standard deviation of the count frequency distribution
μ = mean of the count frequency distribution

Sampling in cleanrooms has shown that the coefficient of variations value can vary from about 0.5 to 2, with 1 being a common value[18]. Shown in **Table 4** are the calculations of the C_V values for the distributions in **Cleanrooms 1** and **2** shown in **Figures 1** and **2**, using **Equation 11**. Also included are examples of the number of times the supply airflow rate should be increased to obtain a 'maximum' concentration instead of an 'average' concentration for a defined standard deviation and the associated upper confidence limit for various C_V values.

The increased air supply rate, to achieve control against a specified concentration with a chosen UCL, is known as the assured air supply rate, Qs (assured), and can be calculated from **Equation 12**.

Equation 12

Q_S *(assured)* = Q_S x N_S

where,

Q_S = air volume supply rate to a cleanroom (to control contamination to a 'maximum' concentration)

N_S = number of times air supply rate to be increased to control at a 'maximum' concentration for a chosen UCL.

5. Examples of traditional and scientific approaches to the calculation of supply airflow rate for a required airborne concentration of contamination

Consider an EU Grade C (US Class 100 000, ISO 8) non-UDAF cleanroom that has a floor area of 52.3m^2 and is 3m high, and so has a volume of 157m^3. It is occupied for 8 hours per day by 5 operatives all gowned in EU Grade C garments with a pore size 26µm and there are no areas of exposed skin. The ventilation system has well distributed air supply terminals that each have H14 HEPA filters, which have been successfully integrity tested and utilise swirl diffusers, and there are low level air extraction ducts on all of the cleanroom walls. The cleanroom is controlled at a positive pressure with respect to adjacent rooms and the external environment and has no uncontrolled leak paths. There are no emissions from equipment used in the cleanroom and no emissions from the product or processes, as they are completed in enclosed workstations within the cleanroom and therefore the only emissions are from personnel. The required supply airflow rate is considered using non-scientific and scientific approaches.

5.1 Traditional approach

This is aligned with the US FDA Aseptic Processing Guide 2004 recommendations of a minimum 20 air changes per hour for this class of cleanroom. As stated in section 2.1, the cleanroom air change rate is determined by dividing the rate of volume air supply by the room volume. Therefore, the air supply rate can be determined from **Equation 13**.

Equation 13

Q_S = air change rate x room volume

= 20 x 157

= 3140m^3/hr (0.87m^3/s)

Table 4: Statistically derived requirements for increased air supply rate to control to a specified maximum

Cleanroom	Standard Deviation (s)	Mean of Count Frequency (µ)	Coefficient of Variation (C_V)	Standard Deviations above the Mean (r)	Upper Confidence Limit (UCL) (%)	Air Supply Rate Increase (N_S)
1	73,234	114,971	0.64	1.65	95	1 + (0.64 x 1.65) = 2.1
				2	97.7	1 + (0.64 x 2) = 2.3
				3	99.9	1 + (0.64 x 3) = 2.9
2	5,208	3,027	1.72	1.65	95	1 + (1.72 x 1.65) = 3.8
				2	97.7	1 + (1.72 x 2) = 4.4
				3	99.9	1 + (1.72 x 3) = 6.2

5.2 Scientific approach

The maximum concentrations for total particles ≥0.5µm and ≥5µm and MCPs for this cleanroom are shown in **Table 5.**

The emission rates for total particles ≥0.5µm and ≥5µm and MCPs per person, obtained from **Table 2**, are shown in **Table 6**, together with the total calculated emissions.

Although the average ventilation effectiveness (VE) value of the cleanroom is 1, there will be some locations where the VE value will be less than 1 and so it is important to utilise a conservative value to ensure that the required airflow for all areas of the cleanroom can be determined. This can be determined using CFD or estimated based on cleanroom design information. With swirl diffusers, which are well spaced out and low- level extraction on all four walls, this suggests the VE will be reasonably good and a value of about 0.75 and may be assumed and used for all particle sizes. A coefficient of variation of 1 is a reasonable value to utilise and the required air supply to control the emissions to the maximum particle concentrations can be calculated using **Equations 9** and **10** and are shown in **Table 7** for particles ≥0.5µm and ≥5µm and MCPs. Also included are the additional calculated assured air supply rates, Q_S *(assured)*, to ensure that the particle concentrations do not exceed the respective maximum concentrations with a 97.7% UCL.

Table 5: Maximum concentrations for total particles ≥0.5µm and ≥5µm and MCPs, EU Grade C cleanroom

Particle	Reference Document	Maximum Concentration (no./m³)
≥0.5µm	EU Annex 1, FDA Guide, ISO 14644-1	3,520,000
≥5µm	EU Annex 1, ISO 14644-1	29,300
MCP	EU Annex 1, FDA Guide	100

6. Discussion

It can be seen that the calculated air supply rate (0.097m³/s), required to control the airborne contamination within the maximum concentration is 11.2% of that associated with a supply rate (0.87m³/s) resulting from 20 air changes per hour. The calculated air supply rate is determined to control the specified maximum concentration level with a 97.7% upper confidence limit (UCL). This is a reasonable confidence limit to utilise, but greater limits could be utilised if more control was assessed to be required. However, 97.7% relates to 2 standard

Table 6: Cleanroom emissions for total particles ≥0.5µm and ≥5µm and MCPs

Particle	Emissions Rate per Person (no./s)	Number of People	Total People Emissions (no./s)	Total Equipment and Machine Emissions (no./s)	Total Emissions (no./s)
≥0.5µm	17,000	5	85,000	0	85,000
≥5µm	621	5	3,150	0	3,150
MCP	2.8	5	14	0	14

Table 7: Calculated air supply rates required to control emissions to maximum concentration levels with 97.7% confidence

Particle	Maximum Concentration C (no./m³)	Total Emissions D (no./m³)	Deposition Velocity VD (m/s)	Cleanroom Floor Area A (m²)	VD x A (m³/s)	VE	Air volume supply rate Qs (m³/s)	Standard Deviations above Mean [97.7% UCL]	Qs (assured) (m³/s)	Equivalent Ai Changes (ac/hr)
≥0.5µm	3,520,000	85,000	Not significant for particles this size			0.75	0.032	2	0.097	2.22
≥5µm	29,300	3,150	0.0022	52.3	0.115	0.75	-0.010	Not applicable*		
MCP	100	14	0.0033	52.3	0.173	0.75	0.043	Not applicable*		

*The maximum airborne concentrations of particles ≥5µm and MCPs will be controlled by gravitational deposition and no additional air supply is required

deviations from the mean value and for comparison with the air supply rate of 0.87m^3/s, associated with 20 air changes per hour, the corresponding standard deviations (calculated using **Equation 11**) would be around 5 which provides an UCL of 100.0%.

Overall, the scientific approach more accurately matches the required air supply rate with the defined airborne cleanliness levels with a significantly reduced air supply rate compared to that derived from the arbitrary application of 20 air changes per hour.

It is accepted that using arbitrary air-change rates as a basis for determining supply airflow rates is simple, easy and quick, but this solution may result in over-design or, conversely, may result in insufficient supply air, project delay and risk to product quality and patient safety.

It is also accepted that the scientific approach is more complicated, takes longer and requires data for particle emissions from people, equipment and process, which are experimentally derived although this data is becoming increasingly available. Additionally, some assumptions must be made regarding other key parameters, such as ventilation effectiveness and coefficient of variation, but reasonable assessments can be provided by suitably experienced designers and complemented by other technologies such as computational fluid dynamics. Moreover, all assumptions should be carefully considered and suitably safe values agreed to ensure all control requirements can be achieved.

7. Conclusion

A scientific approach, based upon the correct parameters associated with effective control of airborne contamination control, is more appropriate than assigning an unconsidered cleanroom air change rate when determining the required air supply rate for non-UDAF cleanrooms. With the appropriate expertise and experience, this will help to ensure that over-design or, conversely, a lack of air supply, are avoided and this presents the opportunity for considerable energy savings whilst maintaining appropriate environmental control. Correlations of this scientific approach with the actual resultant cleanroom performance would also be useful in order to endorse the methodology.

References

1. Guidance for industry sterile drug products produced by aseptic processing – Current good manufacturing practice. *US Food and Drug Administration*, 2004
2. ISO 14644-1. Cleanrooms and Associated Controlled Environments– Part 1: Classification of air cleanliness'. *International Organization for Standardization,* Geneva, Switzerland. 2015.
3. European Commission Guide to Good Manufacturing Practice, Annex 1: 2022. *European Commission, Enterprise Directorate-General,* Brussels.
4. WHO Technical Report Series, No 961 2011 Annex 5 "Supplementary Guidelines of Good Manufacturing Practice for HVAC for Non-Sterile Pharmaceutical Dosage Forms.
5. Whyte W, Whyte WM and Eaton T. The application of the ventilation equations to cleanrooms; Part 1: The equations. *Clean Air and Containment Review* 2012; Issue 12, pp. 4-8.
6. W Whyte W, T Eaton, WM Whyte, N Lenegan, S Ward and K Agricola. Calculation of air supply rates and concentrations of airborne contamination in non-UDAF cleanrooms. *European Journal of Parenteral & Pharmaceutical Sciences* 2017; 22(4): 126-138.
7. Whyte W and Eaton T (2016) Deposition velocities of airborne microbe carrying particles. *European Journal of Parenteral and Pharmaceutical Sciences,* 21(2): 45-49.
8. Whyte W, Whyte WM, Blake S and Green G. Dispersion of microbes from floors when walking in ventilated rooms. *International Journal of Ventilation* 2013; 12(3): 271-284.
9. Whyte W and Hejab M. (2007). Particle and microbial airborne dispersion from people. *European Journal of Parenteral and Pharmaceutical Science,* 12(2): 39-46.
10. IEST RP-CC003.5 Garment systems considerations for cleanrooms and other controlled environments, 2023
11. Whyte W and Bailey PV: (1985). Reduction of microbial dispersion by clothing. *Journal of Parenteral Science and Technology,* 39(1): 51-61.
12. Whyte W and Bailey PV: (1989). Particle dispersion in relation to clothing. *Journal of Environmental Sciences*, 32: 43-49.
13. Hejab M. Prediction of airborne contamination in conventionally ventilated cleanrooms. PhD Thesis, University of Glasgow, Scotland. 1992.
14. Sundstrom S, Ljungqvist B and Reinmuller B (2007). Some observations on airborne particles in blow-fill-seal filling rooms. *PDA Journal of Pharmaceutical Science and Technology*, 61(3): 147-153.
15. Hnatek, ER (1995) Integrated circuit quality and reliability – Second edition. CRC Press.
16. Whyte W, Ward S, Whyte WM and Eaton T (2014). The decay of airborne contamination and ventilation effectiveness in cleanrooms. *International Journal of Ventilation,* 2014, 13(3): 1-10.
17. Cleanrooms and associated controlled environments – Part 3: Test methods. Geneva, Switzerland, International Organization for Standardization, 2019.
18. Whyte W, Whyte WM, Eaton T and Lenegan N. Calculation of air supply rates for non-unidirectional airflow cleanrooms. *European Journal of Parenteral and Pharmaceutical Science,* 2014; 19(4): 121-129.
19. Lenegan N (2014), Diffuser performance in cleanrooms, *Clean Air and Containment Review,* Issue 18. pp. 8-14.

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Book Review

Industrial Pharmaceutical Microbiology - Quality Control

Edited by Edward Tidswell and Radhakrishna Tirumalai
Reviewed by **Tim Eaton**

Published by Euromed Communications and is available via this link: https://euromedcommunications.com/collections/pharmaceutical-sciences-manuals/products/industrial-pharmaceutical-microbiology-quality-control

The editors, Edward Tidswell and Radhakrishna Tirumalai, are both based in the US and are recognised for their pharmaceutical microbiology expertise with numerous publications relating to sterility assurance, aseptic and sterile manufacturing, sterilisation and patient risk and safety. Both have experience as participants on the United States Pharmacopeia (USP) Expert Committee for Microbiology and have extensive knowledge of microbiological testing. With the book made up of twenty chapters, covering a wide range of different topics, they are the main authors for seven chapters, with the remainder written by other recognised experts, including some based in Europe.

The book is intended for pharmaceutical, biotechnological and healthcare applications and although the information is directed at the QC Microbiologist, other functions with microbiological interests would find the contents to be extremely informative and useful. It is aimed at providing a comprehensive overview and an understanding of compendial and pharmaceutical related microbiological laboratory QC methods to enable better developed and more compliant procedures to be utilised. The chapters provide an impressive reading list and the micro-biological topics are wide ranging and applicable to both sterile and non-sterile items.

As expected, there is a lot of detailed information regarding the different microbiological tests, procedures and associated issues. The first three chapters are especially very useful, particularly for the novice or non-microbiologist, as they provide an introduction and overview of the types of qualitative, quantitative and identification microbiological testing, used for finished drug products, excipients and drug substances. With a focus on, and references to, the USP microbial tests throughout the book, this is initially reflected with a definitions table, for commonly used terms such as sterile, aseptic, bioburden, and so on, that utilises and expands on the USP definitions. A useful inclusion is an explanation of the legal recognition of the USP and also some of the microbiological ambiguities and the relationship with legislative requirements. The fundamentals and science of the colony forming unit (CFU), growth on, and requirements for agar-based medium, colony discrimination and evaluation are well explained and is a useful up-front reminder of the limitations and pitfalls of this methodology. One later chapter of note is dedicated to the role of water activity (a_w) in microbiological contamination and looks at a number of aspects, including the relationship between a_w and the growth of microorganisms. Below a certain a_w value, microbial growth is not possible and although widely used within the food industry, it can often be overlooked for other applications. Measurement of a_w can be used to effectively optimise a manufacturing process to reduce the risk of microbial proliferation that can lead to contamination.

Appropriate testing of laboratory procedures and management requirements to enable adequate compendial pharmacopeial testing is included and a further chapter provides information on laboratory assessments and case

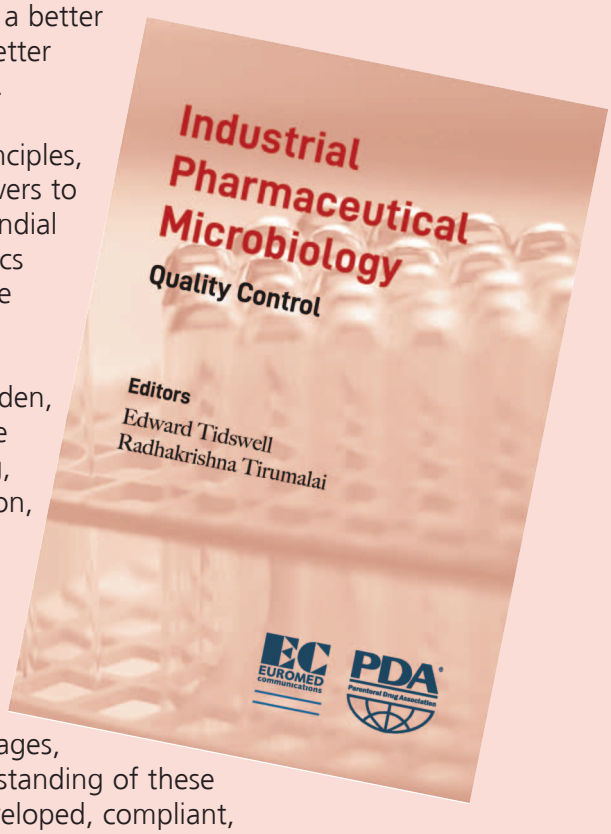
studies for application to laboratory practices. Detailed information is provided for all aspects of the compendial Sterility Test (USP<71>), including the statistical analysis that illustrates how unlikely that a non-sterile unit would be detected from this testing. Information regarding the challenging Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach (USP<1071>), is also discussed. This is becoming increasingly important due to the rapid emergence of short-life products such as cell therapies. The difficulties of applying the USP <71> legacy testing to these types of products is well explained. Enumeration testing and testing for the presence of certain types of specified and objectionable microorganisms provides an enhanced insight for the application to non-sterile products. Other chapters cover, in similar levels of detail, bioburden (microbiological load) testing, microbial identification, reference strains, the development of a compendial test method for the resistant *Burkholderia cepacia* complex and bacterial endotoxin testing. Testing requirements to determine the effectiveness of antimicrobial preservatives, and the process for the qualification and validation of disinfectants, are thoroughly covered in separate chapters. There is an important chapter for medical device testing and there are final chapters on environmental monitoring, different bulk waters used for manufacturing, and biological indicators.

The book is of an excellent standard and is well illustrated throughout with numerous tables, clear diagrams and the occasional photograph, and each chapter is concluded with a comprehensive reference section. With so many subjects and varying aspects included throughout, the addition of a comprehensive index would be beneficial for the reader searching for specific information. Overall, the book provides a rich and unique source of information, in a single location, and is fully recommended for the novice or non-microbiologist, as well as for the established practitioner.

Industrial Pharmaceutical Microbiology: Quality Control provides an in-depth comprehensive survey of QC pharma methods in the microbiology laboratory, to enable a better understanding of these methods, and to ensure better developed, more compliant, expedited procedures.

The reference text brings together the hitherto unavailable background, fundamental science, principles, development, intended purpose, and specific answers to questions of execution and qualification of compendial and related microbiological test methods. Key topics include the types of microbiological tests, reference strains and culture collections, and equivalence of reference strain.

Test methods and subject matter include bioburden, microbial enumeration, specified and objectionable microorganisms, antimicrobial effectiveness testing, endotoxins and sterility tests, microbial identification, biological indicators, water activity, disinfectant efficacy, and water. It also covers a critical, stimulating look into the topic of Environmental Monitoring.



In summary, ***Industrial Pharmaceutical Microbiology: Quality Control*** with its 20 chapters, 19 international authors and over 500 pages, enables the practitioner to have a complete understanding of these microbiological methods, and to ensure better developed, compliant, appropriate procedures and accurate meaningful data.

bottled brown

The Ascent of AI

Dr Malcolm E. Brown has degrees in natural and social science research and elected a Fellow of the Royal Pharmaceutical Society (FRPharmS) for distinction in the profession of pharmacy. He has worked in senior positions as a production manager (big Pharma) and in the British NHS including at director level — and as a community pharmacist. He has tutored many future pharmacists and trained the "entire" sales force of a major international company on selling medicines to the NHS. He was the keynote speaker at an international multidisciplinary conference at the Hague. Dr Brown is an award-winning writer with over 160 publications.

In 1964, while I calculated using logarithms, the science fiction writer Arthur C Clarke predicted intelligent machines. Science fiction has become fact.

In 1997, a machine beat a chess grandmaster. Go is an immensely more demanding game; AI beat a top human player in 2016.

An AI (*AlphaFold*) in 2021, predicted the structure of *200 million* proteins: immensely valuable for drug discovery. AI can match such large molecules with structures from known chemical libraries to suggest novel drug candidate molecules for synthesis and testing in the real world. Previously, identifying just one protein folding had demanded three years of research by one PhD student. Humans *can* do it but they do not live long enough. Demis Hassabis and John Jumper, *AlphaFold's* developers, shared a Nobel Prize in 2024. The tsunami of novel medicines in the pipeline will need GMP, including QPs, during manufacture.

ChatGPT 4: o ("free") and competitors astonish me. China's open-access "free" DeepSeek cost, reportedly, less to develop. These are wake-up calls for all those in the pharmaceutical industry who use words, such as the authors of cGXP Standard Operating Procedures.

AI software can create text quicker and cheaper than a human author. This has far-reaching implications for the pharmaceutical industry workforce: adapt or risk losing your jobs to AI. The message is clear: up your game or be left behind.

One ability of AI — superhuman pattern recogniser — is to trawl worldwide, gargantuan GMP data sets including validation, documentation, personnel training, facilities, equipment and risk-based inspections. New patterns may emerge that make humans seem blind.

As AI advances, some jobs will change. Some will go. New jobs will appear. Some may consider resisting or even sabotaging the brilliant, dogged machines or their electricity supply, akin to the 19th century Luddites smashing mechanised looms or a pedestrian waving a red flag walking before a motor car.

But all protests will probably fail. The pace of technological progress appears relentless: Moore's "Law" (doubling computing "speed" about every two years) has long been outpaced and appears to be increasing *exponentially*. Future exponential growth is not guaranteed. Niels Bohr, Nobel Laureate, quipped, "Prediction is very difficult, especially if it's about the future!" But, if "exponential" is not "hype" and does occur, implications are awesome.

By 2033, it may be too late to halt the effect of "non-ethical" human actors and AI's self-improvement loop; abilities may *emerge* (using the formal systems' term) that mismatch those of humankind. Leading experts do not know with certainty exactly what is happening inside AI's black boxes and have asked for more regulation. They have self-imposed voluntary guardrails. It bears mention that, to luminary enthusiasts in Silicon Valley, AGI (Artificial General Intelligence mimicking the full range of human intelligence) is not a question of if but when; Geoffrey Hinton, another 2024 AI Nobel Laureate, suggests about ten years.

Some people are anxious about AI as a possible existential threat to humans.

But biologists generally presume that all species become

extinct, eventually; that includes *Homo sapiens*. By inventing AI, humans may have created our successors. Mustafa Suleyman (CEO, *Microsoft* AI) suggests possibly perceiving AI as a "new digital species".

But we are where we are. We cannot uninvent AI. Pandora's metaphorical box, containing hopes and troubles, has been opened. Such ponderings are well above my pay grade.

I am less concerned about AI than how humans use AI. I suggest that for GMP and the pharmaceutical industry benefits outweigh harm. Human health should improve.

To thrive in this new era of GMP, adapt. It seems prudent for pharmaceutical GMP professionals to invest in lifelong learning in STEM subjects. Learn about programming, use AI as a brainstorming partner and study for, say, a Master's in machine intelligence that many universities offer. Be alert for updated guidance from regulatory authorities. Work with AI companies already offering enhanced tools for validation, review and interactions with regulatory authorities.

Cultivating uniquely human skills, such as interpersonal relationships is equally important. Network with other humans preferably face-to-face. While AI can replicate human writing, it cannot replace the emotional intelligence and social skills advantageous in many roles, such as managing teams. Such investments should help you keep your edge.

Hippocrates emphasised that health required proper nourishment and exercise; that remains sound advice for our future living with AI. Cosmetic procedures or a dab of perfume or aftershave, pleasant to any manager nearby, may give you the edge over the unscented, spotlessly clean GMP bot — but, for some pharmacists and pharmaceutical scientists, be steps too far.

More seriously, for crucial activities, remember that AIs still hallucinate. But humans also suffer countless biases and imperfections. Instead of fighting AI or resenting its widespread disruption (including to the pharmaceutical industry), build upon the strengths of AI. AI-enabled humans may then climb to new heights of excellence.

Malcolm E Brown

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