# A COMBINED OPERATION: CELL CULTURE PRACTICES

***Cell–based research has contributed to many areas of groundbreaking science, thereby proving itself a valuable and essential research tool. The ability to study interactions at a cellular and molecular level using highly relevant in vitro models has enabled significant inroads into research across the entire life scientific community. The significance of cell-based studies across a broad range of applications is reflected by the annual multi-billion dollar market for cell culture technology.***

## PRACTICAL CONSIDERATIONS

As a valuable in-vitro model for many disease targets, the use of cell culture systems and cell lines that are highly representative of conditions in-vivo is increasingly in demand. However, any deviations in phenotype or interference by contaminants could have a significant impact on cell response and thus, resulting data. As such, the development of systems that can efficiently maintain cultures and ensure phenotypic stability and contamination control is a priority for both researchers and manufacturers of cell culture materials.

Researchers can optimise their chances for success in the cell culture laboratory by focusing on selecting high-quality equipment and materials (e.g. instrumentation, vessels and reagents); ensuring the proper use and maintenance of all equipment and materials; and enforcing precise handling of the cultures themselves and their derived products.

## COMBATING CONTAMINATION

There are numerous potential sources of contamination and many different contaminants, both biological and chemical [4], which pose a continuous threat to the cell culture laboratory. The use of improved materials in the manufacture of disposable single-use products – such as culture vessels, pipettes, and vials – has greatly reduced the risks. However, glassware, which requires efficient washing and sterilisation, is still widely used for making media and reagents. The vessels themselves can contain residual compounds from inefficient cleaning and the water used can often present a source of contamination.

*The use of sterile, disposable, single-use products such as culture vessels, vials and pipette tips can also reduce contamination risks.*



## FIT FOR PURPOSE

Products developed for very specific applications or cell lines have significantly impacted the cell culture field. Vendors and researchers alike have been optimising culture techniques [1] and technologies so that today there is a broad selection of products suitable for cell culture of nearly any scale and scope. There has also been much focus on the cell culture micro-environment to ensure conditions more closely mimic those in vivo and promote more natural cell behaviour. As such, complementary to the optimisation of media formulations and supplements, the development of modified surfaces [2,3] and in vitro cell matrices has been an area of intense interest. Both can significantly affect cell viability, growth and differentiation, and are influential in holding cells to their natural phenotype, across numerous passages.

*Performing experiments within a biological safety cabinet can reduce risks from airborne contaminants such as aerosols, dust and microbes.*

Furthermore, water distilled for use in reagents or autoclaves can contain low levels of chemical contaminants that can accrue over time. Water purification and filtration systems that can effectively reduce these risks are available, and although many require proper maintenance to ensure consistent purification, there are now some new systems that provide reliable operation with minimal intervention.

Proper equipment maintenance and operational compliance can greatly impact on experimental success. Choosing robust and dependable equipment, e.g. biological safety cabinets [5], incubators, refrigerators and freezers, can substantially reduce contamination risks. Equally important is the way in which this equipment is used and maintained, a factor that can often dictate the degree of success.

## EXPERIMENTAL BEST PRACTICE

Modern incubators have been designed with features such as solid copper interiors and sophisticated built-in decontamination programs [6] to improve incubation sterility [7]. New tools for the continued decontamination of water reservoirs have also emerged. The advent of cold storage systems that incorporate removable, easy-to-clean components, along with biological safety cabinets offering improved functionality, recirculation and filtration power, further reduce risks of contamination.

The design of a cell culture laboratory and the positioning of equipment within it can also greatly affect contamination risk. Aerosols, dust and microbes are universal, and the use of equipment such as pipettors, centrifuges, shakers, refrigerators and freezers can disturb and elevate these particles through aspiration, vibration, heating, or user non-compliance. Performing experiments within a biological safety cabinet can help to reduce these risks.

**Author Details:**
Jeff Goldman,
Strategic Marketing,
Thermo Fisher Scientific


*Using a high-quality water purification system can effectively reduce contamination risks when making media and reagents.*

But even within a closed environment, culture manipulations must be undertaken with care. Although routine UV or liquid disinfection is effective, residual spores or cells can remain. Human error, spillages, and careless pipetting and vessel handling (that may lead to splashing within a plate or flask), can inadvertently provide micro-environments for foreign organisms to flourish and spread. To combat this, a thorough, ongoing cleaning process is essential. Choosing equipment with interior surfaces designed for ease-of-cleaning – e.g. with minimal cracks and corners where contaminants might accumulate – is a good way to mitigate this risk.

## TRACEABILITY

Reagent and material quality is essential, and in the past certain media and constituents (i.e. human/bovine sera and trypsin), have been identified as potential sources of contamination. Thus, quality control throughout product development and manufacturing is critical to ensure consistent products that can reduce experimental variables. Manufacturers have focused on sourcing the highest quality raw materials, which are sampled and tested. Products then undergo a large number of screens for product certification, and lots are coded to enable full traceability. Sterile plastics and culture ware are also designed, manufactured, validated and supplied with Certificates of Analysis.

The use of serum or animal-free media has increased significantly in recent years mainly to eliminate the safety/contamination hazard it can present in the production of biopharmaceuticals. Furthermore, a simplified composition allows tighter control of experimental conditions. However, some cell types are more reliant on the proteins in animal-based media, either for growth and substrate attachment or for protection from shear damage. Today, selected vendors can now supply a broad range of media products validated to ensure compliance with cGMP and ISO 9001 using both original and Animal Derived Component Free (ADCF™) formulations.

## FOCUSED EXPERTISE

Today there is an opportunity to source instrumentation, equipment, reagents and consumables of the highest quality from reputable suppliers and reap the benefits of their in-house expertise to obtain practical advice on experimental best practice, equipment performance, compliance and maintenance. The cell culture scientist can access these available resources to help overcome the daily obstacles of cell-based research.

*Disposable products are efficiently sterilised by the manufacturers using high intensity gamma or electron beam radiation, following sealing and packaging.*

## REFERENCES

*[1] Tissue Culture Techniques, An Introduction. Bernice M. Martin, Birkhauser, 1994, p.72*

*[2] The dependence of fibrillar adhesions in human fibroblasts on substratum chemistry. Nathalie Faucheuxa, Rumiana Tzonevab, Marie-Daniele Nagelc, Thomas Groth. Biomaterials 27 (2006) 234–245.*

*[3] Surface Treatments for Fastidious Cells, Maximising the Success of In Vitro Cell Growth through Novel Surface Coatings. GEN, May 1 2006, Vol.26, No.9.*

*[4] Cell Culture Contamination: Sources, Consequences, Prevention and Elimination. C.K. Lincoln and M.G. Gabridge. Methods Cell Biol, 1998, 57, 49-65.*

*[5] Ergonomics and safety in biological research cabinets. Dave Philips, Bioscience Technology, September 2007.*

*[6] $CO_2$ Incubator Disinfection Validation, Test Report from the Biosafety investigation unit of the CAMR, 1998.*

*[7] The Influence of Plumbing Material, Water Chemistry and Temperature on Biofouling of Plumbing Circuits with Particular Reference to the Colonisation of Legionella pneumophila (1993). ICA Project 437B.*