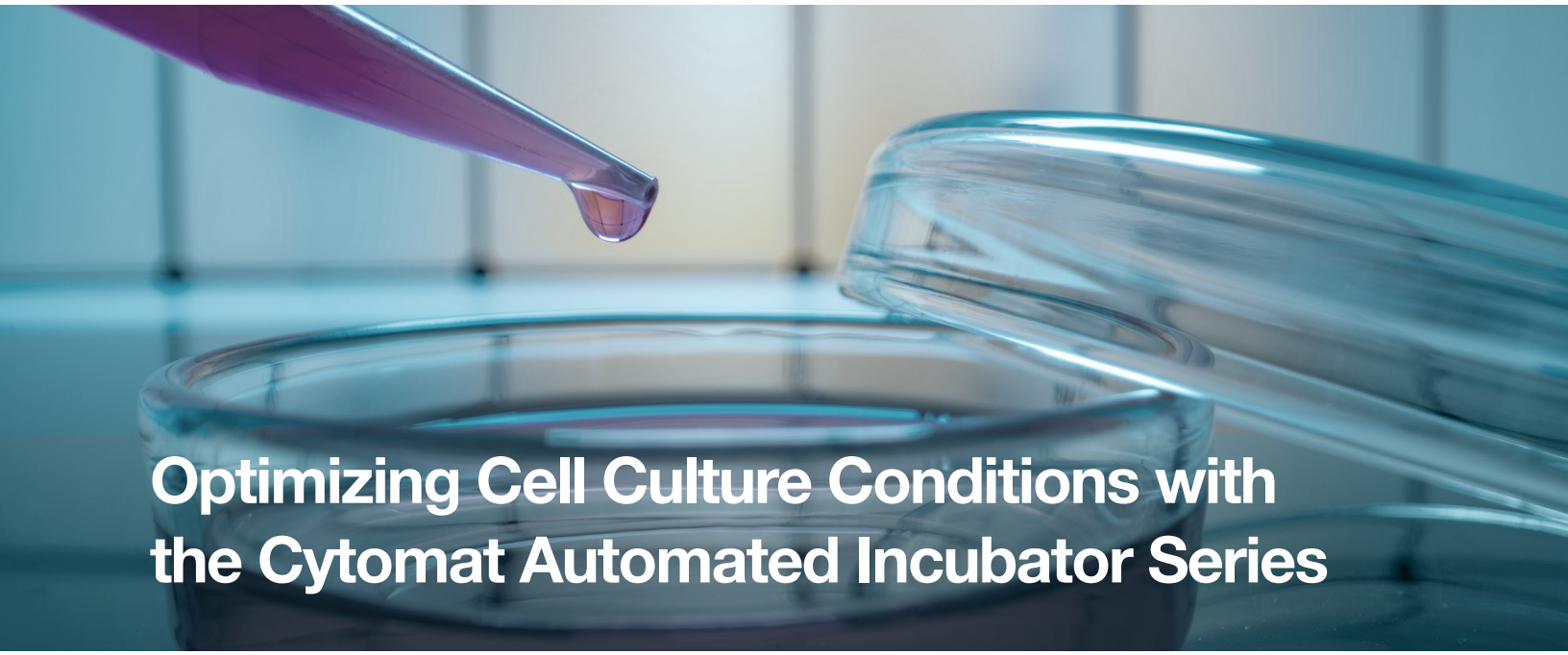
thermo scientific



Optimizing Cell Culture Conditions with the Cytomat Automated Incubator Series

The capability for cells in culture to represent complex living systems is wholly dependent on the environment they are grown in. The goal of any type of cell culture therefore is to keep cells growing in conditions that most closely mimic their native environment, while still allowing them to be modified to suit experimental objectives. At the same time, it is paramount to protect cells from contamination and chemicals that may cause adverse effects.

Automated incubators have revolutionized cell-based experiments by increasing productivity, data quality and reducing the risk of cell culture contamination. The Thermo Scientific™ Cytomat™ series offers the broadest set of incubation and storage capabilities for all applications. In this guide we discuss the importance of the cell culture environment for ensuring robust, reproducible and scalable results, and outline the product features unique to the Cytomat series that enable operational flexibility and reliable sample protection.

Figure 1. *Cytomat 10 Automated Incubator Series*

"Our research involves protein engineering with E.Coli-expressed protein. We have three applications, one is the incubation of these cells, to grow the cells, and express the protein. Then we lyse the cells within these Cytomat devices by adding lysis buffer, and evenly shake them to open the cells and release the protein of interest for our screening assay. Finally, we capture and store the lysate at 4 degrees in a dedicated Cytomat." – Dr Mark Doerr, Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, University of Greifswald.

Creating the Optimal Cell Culture Environment

The tiniest environmental perturbations can affect biological behaviors of cell samples and adversely affect experimental outcomes. Hence, it is essential to maintain uniform culture conditions while plates are being accessed, treated, during sample removal, or during analysis. It is also vital that the ideal culture conditions are achieved for the specific type of cells being studied – whether the goal is to culture bacteria, yeast, or mammalian cells. Generally, most cultured cells like to be in an environment that is 37°C, with > 90% relative humidity, and have carefully controlled pH by CO_2. When considering how best to protect your samples, these are the key factors that need to be considered.

Temperature

Cells are extremely sensitive to temperature. Although they may be happy in incubation at lower temperatures, exposure to high temperatures are much more detrimental. Cells cannot withstand more than a few hours at a temperature just 2°C above normal physiological levels.

It is also crucial that the temperature is uniform across the incubator and is maintained during experiments. Conventional incubators have suffered from “hot spots” caused by direct sources of heat and cooler spots where cells are being grown near the door, for example. Simple operations like opening the incubator door can profoundly affect cell growth as the temperature can change once the door is opened.

Cytomat incubators have features that virtually eliminate temperature recovery times. The entry and exit of microplates occur in a matter of seconds, through a small “gate”, significantly minimizing disruption of the controlled environment and ensuring the optimal temperature (red line) displayed in figure 2.

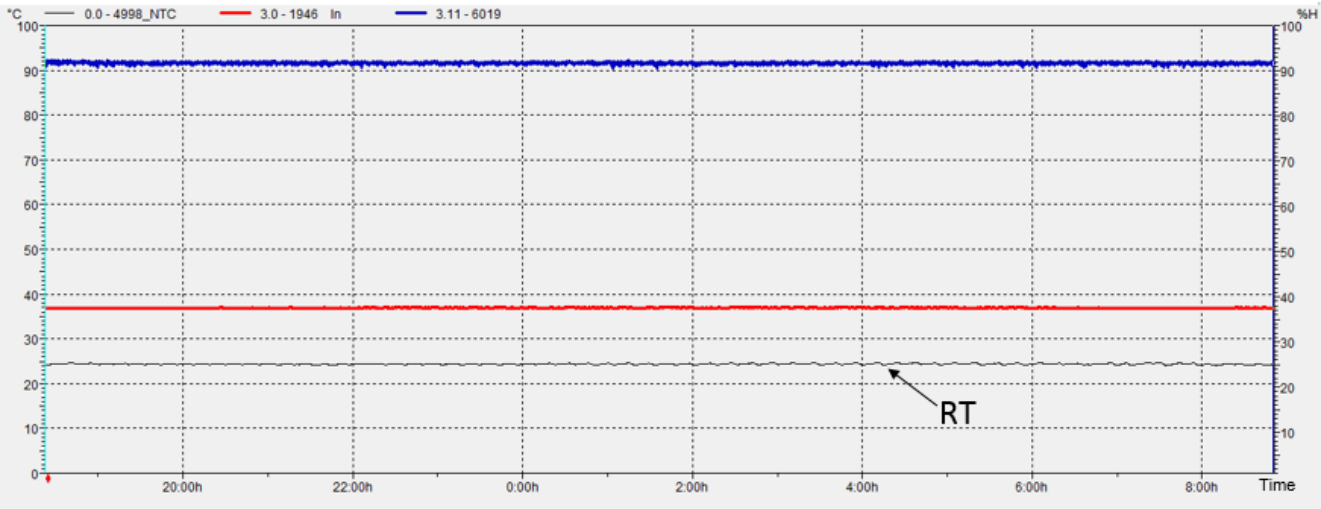


Figure 2. Cytomat temperature fluctuations over time. Temperature (37°C) and humidity (92% r.h.) with gate opening every 30 seconds for 10 seconds during a duration of 12 hours.

Temperature is also maintained in the incubator by convection, there is no direct internal heat source. Other types of automated incubators use a direct heat introduction to the chamber, to distribute the temperature. However, this can cause “edge effects” where cells at the outer edge of the plate dry out. By contrast, convection-based heat distribution achieves even temperatures (± 0.2°C) across the chamber.

Humidity

Cell culture environments need to be humid to prevent evaporation. Evaporation results in concentrated levels of salts and minerals that are toxic to cells and can cause cell lysis. However, it’s important that condensation is prevented, as it can lead to biological contamination. Maintaining the right level of humidity is critical for the growth of cells.

Optimal humidity for most cells is 90% relative humidity (rH)[1]. Water evaporation inside the wells is four times faster at 80% rH than at >90%. Cytomat incubators have features that enable precise control of humidity to meet the requirements of different cell-based assays even during transfer of the plate. See figure 2 (blue line).

Some incubators use at least two or more nebulizers which produce large water drops. While other incubators use a heating plate set at 145°C which generates humidity where water is dropped onto the hot plate. The result is comparable to an infusion in a sauna that causes significant temperature deviations to occur inside the chamber – due to difference between the chamber temperature (37°C) and the "steam" (95°C).

Cytomat uses its Hydra-Smart technology, an ultrasonic vaporizer to precisely control the humidity. This controlled humidity avoids issues associated with temperature deviation as a fine mist is produced rather than large water droplets, and therefore minimizing the risk of contamination through water and avoiding temperature fluctuations or "hot spots". Sterilized water is supplied to the Cytomat incubator via an external water reservoir (tank) that includes an externally accessible microfilter technology, maximizing convenience and minimizing contamination risk.

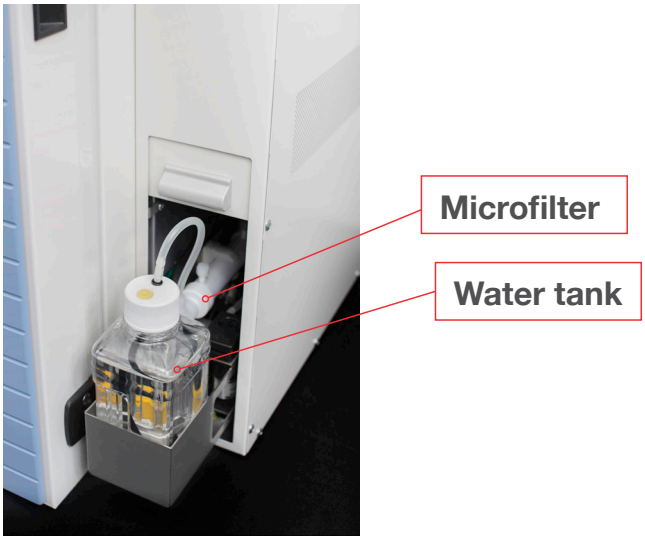


Figure 3. *External water tank and microfilter (Cytomat 2-LiN Automated Incubators series)*

CO_2

In the incubator atmosphere, the growth medium controls the pH of the culture and buffers the cells in culture against changes in pH. For mammalian cells, the target pH is usually between pH 7.0 and 7.4. CO_2 gas works with sodium bicarbonate (or another buffer) in the cell culture medium to maintain a constant pH. Usually, this buffering is achieved by including an organic (e.g. HEPES) or CO_2-bicarbonate based buffer. As the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO_2) and bicarbonate (HCO3–), changes in the atmospheric CO_2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO_2 when using media buffered with a CO_2-bicarbonate based buffer. Especially, if the cells are cultured in open dishes or transformed cell lines are cultured at high concentrations. While most researchers usually use 5–7% CO_2 in the air, 4–10% CO_2 is common for most cell culture experiments[2].

CO_2 control is a standard feature in Cytomat models, rather than being an "optional extra". Accurate and reliable measurement of "true" CO_2 levels in the incubator is also crucial. While a variety of CO_2 sensors are available, Cytomat incubators are equipped with modern infrared (IR) sensors designed by Thermo Fisher Scientific. Currently the IR sensors are already in use in hundreds of thousands of HERAcell incubators featuring a silicone light source. IR sensors reliably maintain strength after intense use. Finally, the ability of the Cytomat models to maintain the internal chamber environment, even with repeated access, is seen relative to CO_2 levels as well. This minimizes the length of time that cells are exposed to suboptimal conditions.

Decontamination

Contamination of cell cultures is among the most common challenge encountered in cell culture laboratories. Cell culture contaminants can be divided into two main categories: chemical contaminants such as impurities in media, sera and water, and biological contaminants such as bacteria, fungi, viruses and mycoplasma. Cross contamination by other cell lines is also a concern[4,5]. You can carry out a sterilization by heat or gases to eliminate contamination entirely.

Contaminants in a cell culture incubator originate mainly from opening and closing the main incubator door. For example, as soon as the main door is opened, the incubator interior is at risk from users' normal flora on their hands, breath, hair, and the room environment.

Contaminants can impact your cells in many ways – from killing your cells leading to the destruction of an entire culture, to introducing foreign nucleic acid materials into your sample which compromise their experimental validity. It is imperative that measures are in place to prevent contamination, however, in the event that conditions become compromised it is crucial to have efficient processes for decontaminating the incubator in place.

As a result, the Cytomat series is designed to help to prevent contamination. The external water reservoir reduces the risk of contamination as you can avoid opening the chamber for refilling. In addition the microfilter cleans the water before it is used to humidify the chamber. The chamber also has the optional feature to be manufactured from solid copper (not copper coating as others). Solid Copper (99.9%) has significant properties compared with other materials such as copper alloy or stainless steel.

In addition, Cytomat's automated decontamination routine ContraCon, which was adapted from the HERAcell incubator, involves a simple moist heat process (at 90°C) that is safe, non-toxic, and non-corrosive (figure 4). This comes as a standard feature in most of the Cytomat series of automated incubators.

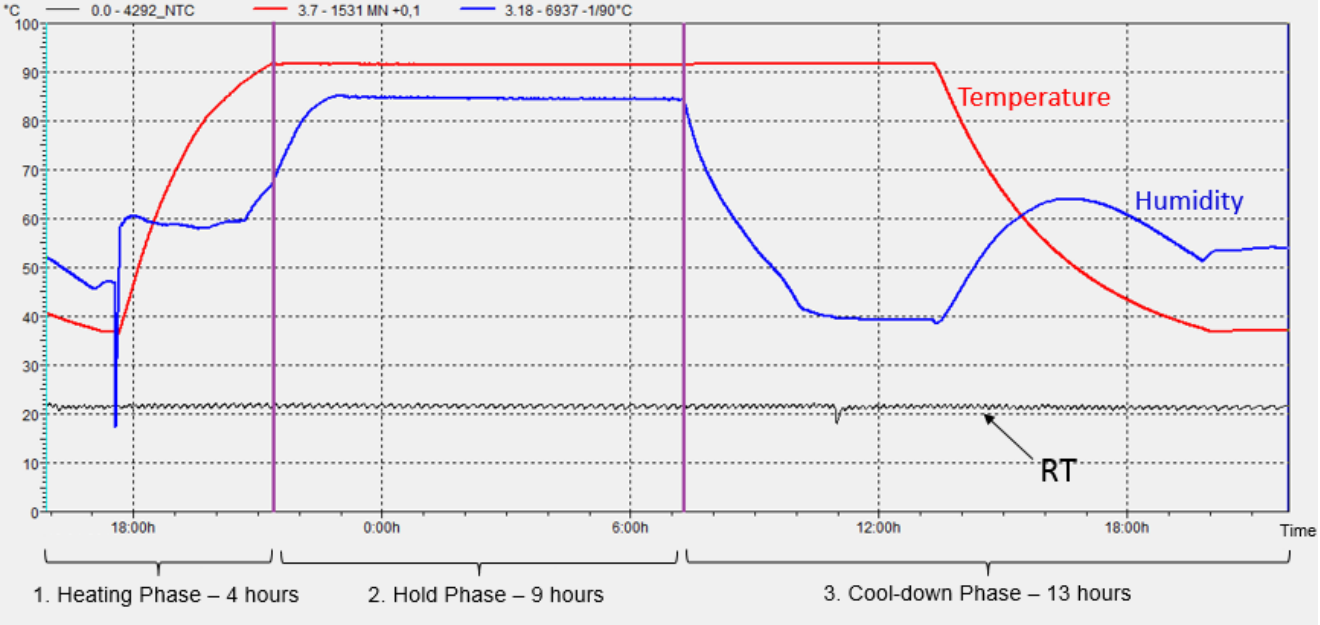


Figure 4. *Cytomat 10 decontamination routine using ContraCon. Duration of 26 hours with measuring point in the center of the chamber, no cycling, stable RT 22°C.*

To enable a sterilization procedure, optional ports are available and are upgradeable in the field to connect a Hydrogenperoxid (H_2O_2) generator from various suppliers.

Other decontamination processes recommend chlorine dioxide. This process can result in the formation of hydrochloric acid due to a shifting of the tight required temperature distribution in the chamber. Hydrochloric acid is not only highly toxic, but it is also highly corrosive to the internal components of the device and requires dedicated intensive safe sealing of the device.

Process Stability

Cells are profoundly affected by small variations in environmental conditions and will respond to these changes, altering their biology and affecting the outcome of carefully designed experiments.

It is therefore crucial to maintain a consistent temperature, gas concentration and humidity to minimize variation in cell responses. An automated incubator must provide uniform conditions, to minimize variation in cell responses and maximize consistent data quality. For these reasons, uniformity from top to bottom and side to side in all areas of the incubation chamber is critical.

Based on the 20+ years of experience in building automated incubators and 50+ years in building incubators with its ideal design the Cytomat series ensures consistent environmental conditions (below 1% fluctuation) during gate opening. Plate access won't disturb inner chamber conditions as all Cytomat models are equipped with a small gate through which microplates enter and exit in a matter of seconds (see also figure 2).

Figure 5. *Examples of possible gate locations*

"We use four Cytomat incubators for cultivation of these bacterial cells and are very happy with the consistency of the growth. It is very important for us that the growth is very even. Three of the incubators are equipped with true orbital shaking units which, combined with the homogeny of the moisture in the atmosphere, helps us to achieve consistency." – Dr Mark Doerr, Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, University of Greifswald.

Various gate positions (up to 12 located in the back, left and right side; depending on the device) are available based on the laboratory space requirements and restrictions.

Cytomat incubators also include an internal barcode reader which allows users to conduct an inventory scan without disrupting the environmental conditions.

Shaking and Agitation

Factors such as cell type and experimental protocol can affect the shaking and agitation required for the culture plates. Agitation is important in achieving sufficient oxygenation of samples (e.g. bacterial cells) to support optimal, rapid growth. This is especially important where cell number is crucial for scaling up production of proteins or metabolites. It is also important during in situ hybridization protocols or for ensuring that cells are fully interacting with certain compounds (e.g. potential drugs) during an experiment.

Cytomat offer different shaking and agitation options for cell growth. With linear agitation, the turntable is rotated back and forth and is ideal for gentle cell dispersion and mixing applications. This might be applicable in application areas where large dimension/volume wells, or squared wells are being used.

Cytomat automated incubators also have the unique capability of providing true orbital shaking. With the true orbital shaking technology, the stand-alone shaking principle is adapted for automation inside a tower Shaker stacker. It is ideal for applications requiring highly consistent sample agitation and cells that need to be kept in suspension like yeast or microbial cell culture. Synchronized unique magnetic drive systems, located at the top and bottom of each tower shaker, ensure that consistent shaking performance can be achieved. This also guarantees that every plate is subject to identical shaking conditions regardless of its placement in the incubator.

True orbital shaking is provided by individual Tower Shaker Stackers which have true orbital shaking from top to bottom within the Cytomat with its synchronized dual magnetic drive design where other devices in the market are using the bottom drive shaking principle. The Cytomat approach provides consistent growth within and between plates and is ideal for applications requiring sample agitation and cells that need to be kept in suspension like yeast or microbial cell culture (see figure 6 for differences in growth).

The individual tower shaker stacker technology also provides the opportunity to run two different applications in parallel with different agitation speeds from 100–1200 rpm. As the system contains a synchronized and unique magnetic drive system, it ensures that shaking performance is consistent even when the load is unbalanced. There is also no process disruption in the other tower shaker during plate handling due to the individual tower technology.

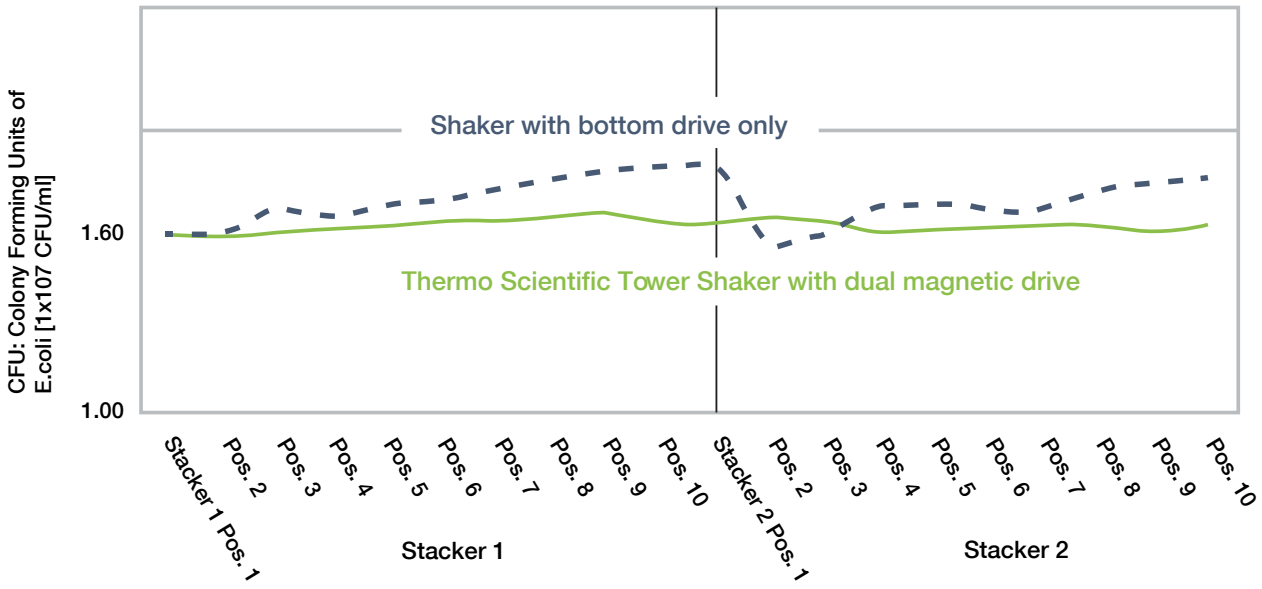


Figure 6. *Bacterial growth consistency over the entire two Stackers (bottom to top) of the Tower Shaker.*

Product features of the Thermo Scientific Cytomat Series

Robust and Reliable Design

Developed by the inventors of microbiological incubators and taking advantage of more than two decades of unequalled experience in automation.

Unrivalled Speed

Fastest mean access time in their class.

Flexible Design

Suitable for bench top and under bench with its "one body fits all" design, with different location options for loading/unloading plates, and options to mix-and-match plate types and increase capacity with an additional stacker position.

Ease of Use and Integration

Glove-friendly display with intuitive operation, read-out messaging and warning alerts.

A Product Range for Many Applications

Various Cytomat models are available to match different application and temperature requirements. Flexible designs include benchtop, under bench, carousel and linear.

References

1. Thermofisher.co.nz. (2019). Dependable Sample Protection. [online] Available at: http://www.thermofisher.co.nz/Uploads/file/Scientific/Scientific-Instruments-Automation/Cytomat_Series_Brochure_Fast_and_Precise_Plate_Delivery_Thermo_Fisher_Scientific.pdf [Accessed 27 Aug. 2019].
2. Thermo Scientific Technical Reference Library, Cell Culture Basics. Available: https://www.thermofisher.com/uk/en/home/references/gibco-cell-culture-basics/cell-culture-environment/ph-co2-levels.html
3. Thermo Scientific blog. Available: https://www.thermofisher.com/blog/cellculture/optimal-cell-growth-incubator/
4. Thermo Scientific Technical Reference Library, Cell Culture Basics. https://www.thermofisher.com/uk/en/home/references/gibco-cell-culture-basics/biological-contamination.html
5. Lab Manager, 2011. Cell Culture Contamination. Available: https://www.labmanager.com/laboratory-technology/2011/05/cell-culture-contamination#.XUFgdndFycw
6. Michels HT et al. From Laboratory Research to a Clinical Trial: Copper Alloy Surfaces Kill Bacteria and Reduce Hospital-Acquired Infections. HERD. 2015; 9: 64-79. doi: 10.1177/1937586715592650.