PHCbi

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Mitigating Contamination in the Cell Culture Incubator

APPLICATION NOTE

Contamination control begins from the inside out. Incubator engineering, materials and prudent operating techniques combine to minimize the propensity for surface and airborne contamination.

MCO-170AICUVL-PA

INTRODUCTION TO CELL CULTURE INCUBATORS

The cell culture incubator is designed to artificially replicate *in vitro* the conditions essential to *in vivo* physiology typical of human and animal models. Cell growth outside of a natural environment presents a myriad of challenges associated with exposure to microorganisms that are not present in the *in vivo* state. Depending on the type of cell cultures being managed, several operating parameters must be carefully controlled with accuracy, repeatability and flexibility in setpoint choices. These include temperature, gas control and relative humidity.

- Cell culture incubators are designed to establish and maintain a controlled, stable environment by regulating temperature at a typical setpoint of 37°C or over a range from ambient to points above 37°C.
- Incubator gases typically include CO_2 and/or O_2. CO_2 is controlled at a precise setpoint to maintain desired pH in the cell culture media, whether liquid or gel. The CO_2 concentration in the incubator functions as a critical pH buffer. Some biological materials may require different pH levels. Desired CO_2 setpoint concentrations may differ. Most media contain an indicator which helps detect the change in pH.
- Optimal cell culture environments must include humidification to prevent desiccation of cell culture media. While some incubators have internal humidification systems with heated water reservoirs, most incubators include simplified, removable humidity pans designed to hold sterile distilled water which evaporates to naturally increase the vapor pressure within the chamber.
- For assured repeatability, incubators must provide a sterile environment through contamination control methods that ensure protection from microorganisms introduced during door openings.

The incubator systems that create these operating conditions, along with other considerations, require periodic maintenance to minimize contamination and to ensure good laboratory practices in the cell culture equation.

Types of Cell Culture Contamination

Contamination of a cell culture *in vitro* is usually caused by the inadvertent introduction of one or more organisms that can damage or destroy the cell culture in progress. These organisms include:

- Bacteria (including Thermophilic Bacteria) and Mycoplasma
- Molds and Yeasts
- Viruses

Other contaminants include dust, VOCs from adjacent instrumentation or processes, cross contaminants from other cultures in a shared incubator environment and particulates found in the natural environment. Regardless of the contaminant or its source, prudent laboratory techniques can help avoid the recurrence of contamination.

The Incubator Bubble

Unlike closed systems, such as hollow-fiber substrates, stirred tank or airlift bioreactors, the typical cell culture incubator is a conditioned chamber with a door that closes against a soft gasket. When the door is closed, the incubator creates an ideal environment for the cell culture process based on user-defined setpoint parameters for temperature, CO_2 and O_2. Humidification is naturally evaporative from the humidity pan, and positive vapor pressure is sufficient to eliminate desiccation, especially in microplates with small media volumes. Some larger cell culture incubators use immersion heaters to supplement the natural humidification process.

When the incubator door is opened, however, the conditioned bubble is lost. Accessing cell culture labware for transport to a biological safety cabinet (BSC) or other processes is a normal part of laboratory workflow. Opening the door exposes the incubator interior walls, shelves, humidity pan water and culture vessels to ambient conditions that carry the potential for contamination from molds, yeasts, fungi or other microorganisms such as mycoplasma and viruses. In a practical sense, unless the incubator is installed in a clean room, this exposure cannot be avoided. Proper technique can reduce the potential. The first consideration is to understand basic incubator systems how they can harbor contamination.

Incubator Design Prerequisites

The first step in managing cell culture contamination is to consider the incubator design, specifically, the interior. All interior components exposed to the high humidity atmosphere should be constructed on high quality stainless steel and should be easily removable (preferably without tools) for manual cleaning or autoclaving. These include shelves, shelf brackets, plenums, floors, humidity pans, blower wheels, sensor housings, inner door gaskets and anything present in the chamber during cell culture. Control probes are often protected by stainless steel sheath housings. These must be cleaned according to the manufacturers' instructions.

Components manufactured from copper enriched or copper supplemented stainless steel contain an inherent germicidal property that resists airborne organisms introduced to the chamber during door openings. Such materials are considered "passive" contamination control insofar as the inability of organisms to sustain growth on these surfaces.

Installation, Location and Clearances

There are many factors to consider when determining the permanent location of the cell culture incubator. It is desirable to locate the unit where there is minimal foot traffic and where air disturbance is of little consequence. This reduces the volatility of outside air entering the incubator during a door opening.

Avoid installing the incubator near window air conditioners, ceiling or floor HVAC air diffusers and return air intakes, all of which are sources of airborne contamination.

It is important to consider the function of the biological safety cabinet when planning for incubator contamination mitigation. If possible, locate the incubator as close to the biological safety cabinet as possible. This limits the exposure when removing or replacing cell cultures for processing. Improper use of the BSC, wrong sash height, blockage of downflow slots and use of instrumentation or equipment on the BSC work surface can create pathways for contaminants to attach to the cell culture labware when working in the hood. These contaminants are then returned to the incubator where they can migrate to other cultures via cross contamination or to interior surfaces exposed to a conditioned atmosphere ideal for cell growth.

While BSCs are usually equipped with HEPA filters designed to trap particulates of 0.3 microns (0.12 microns for ULPA filters), smaller viruses can easily pass through these barriers. Although the cell culture lab may normally be under positive pressure, this can change to neutral or even negative pressure when a BSC is operating, especially when the BSC has an exhaust transition connected to or over the exhaust filter.

Other laboratory equipment such as centrifuges, stirrers and shakers and robotic plate readers can aggravate an otherwise calm air environment to create aerosols that are easily airborne.

Because some components must be removed from the incubator for thorough cleaning, it is important to establish clearances adjacent to and behind the incubator. This space is required to provide easy access to gas supply tubing, tubing filters, gas input ports, pass-thru ports and blanking plugs and any interior components such as blower motors, fans or sensors that must be removed for maintenance.

Most CO_2 cylinders, for example, contain an industrial grade CO_2 supply in liquid form wherein the CO_2 gas evaporates and moves through the two-stage pressure regulator as a gas. It exits the regulator at a pressure of approximately 20 PSIG, sufficient to prevent the introduction of contaminants into the gas system. The CO_2 itself, however, often contains microscopic particles that may provide surfaces for contaminants. Thus, it is recommended that the final CO_2 supply tubing be fitted with a 0.3-micron filter prior to passage into the incubator solenoid controls. A relatively long section of laboratory grade tubing (i.e. Tygon®) from the regulator to the incubator input offers an easy and non-invasive method for pulling the tubing from behind the incubator to visually check the 0.3-micron filter cartridge and to replace it if discolored.

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Cleaning and Decontamination Methods

Most incubator manufacturers recommend a solution of 70% ethanol and manual cleaning prior to initial start-up and regularly thereafter. The 70% ethanol solution is intentionally diluted to give the ethanol time to kill the contaminant before the ethanol evaporates.

Why is 70% ethanol better than 100% ethanol in bacterial inhibition? 100% ethanol coagulates and dehydrates proteins so quickly that a layer of relatively impermeable denatured protein forms in the exterior parts of the bacterial cell (in and under the cell wall), and this prevents further diffusion of the alcohol into the cell. This protects the core of the cell from denaturation. With 70% ethanol, the process is slower and the alcohol manages to diffuse throughout the cell denature proteins.

In addition to conventional manual wipe down using 70% alcohol, the incubator may be equipped with a sterilization cycle such as a high heat (180°C) system or a hydrogen peroxide vapor H_2O_2 system. The cycle should be performed prior to first use.

If commissioning and cGMP criteria are in place, all contamination control efforts must be in compliance with previously approved best practices and facility protocol.

Instrumentation and Equipment Interface

Cell culture apparatus such as orbital or reciprocal shakers, rockers, cell bottle rollers, magnetic stirrers and other devices are commonly used in the cell culture incubators. These must be free of contaminants before they are placed in the incubator. If possible, an H_2O_2 decontamination process provides a thorough decontamination of exposed surfaces. In some cases, the instrumentation can be placed in the incubator chamber during the H_2O_2 process before the incubator is put into service and cell cultures are introduced.

Cell culture vessels usually include flasks with and without vent caps, Petri dishes, roller bottles and multi-well plates. These are usually prepackaged and sterilized by gamma radiation prior to shipment. They should be opened only in a BSC to preserve the integrity of the sterilization.

Other labware returned from a central sterilization room must be considered a source of contamination if exposed to ambient air during cart transit and shelf storage.

Summary Contamination Sources

The following contamination points must be included in a regular schedule for cleaning *in situ* or removal and cleaning manually or by autoclaving.

Inside the Incubator

- Walls
- Ceiling
- Floor
- Chamber corners
- Ductwork and plenums
- Humidity pan
- UV light housing if so equipped
- Temperature control probe and probe housing
- Probe wire to control panel

Incubator Cabinet

- Inner door gasket and feather surfaces
- Inner door latch
- Inner door glass
- Inner door hinges and fasteners
- Cool spots where condensation may accumulate due to insufficient cabinet insulation

Gas System

- CO_2 or O_2 sensor
- Sensor housing and connectors
- Injection tubing from control solenoid(s)
- Air pump
- Filters and housings
- Blower wheel, shaft and seal

Humidity Reservoir

- Humidity pan
- Water level contacts
- Water level float
- Water level inject

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Best Practices and Good Laboratory Technique

The most obvious approach to contamination-free incubator operation is to keep the incubator clean. A combination of manual cleaning and automatic (if equipped) decontamination processes managed on a regular schedule help protect cultures *in situ* and minimize loss of work due to contamination and downtime.

Predictive maintenance is analogous to preventive maintenance, whereby cleaning processes can be documented for standardization and compliance, scheduled in advance and assigned to laboratory staff as required.

There is no substitute for aseptic technique when handling cell cultures. Both personal and laboratory hygiene are essential to a holistic contamination management program.

ACTIVE VS. PASSIVE DECONTAMINATION

Active decontamination, whether by manual wipe down, high heat sterilization, H_2O_2 vapor or other method, must be initiated by the user. Design attributes inherent to a properly engineered cell culture incubator offer an additional layer of protection by working in the background to inhibit and destroy contaminants as they occur.

Active Decontamination

- High Heat. A high heat process utilizes time and temperature, typically 160°C to 170°C for a two-hour period, for a proven method of decontamination. The PHCbi brand, new thermal decontamination system operates at a higher temperature. It is the fastest and most effective active method of decontamination in a cell culture incubator reaching 180°C for a two-hour dwell before returning to ambient. To minimize downtime, total cycle time is less than 12 hours. This energy-efficient process does not require the removal of the CO_2 sensor and UV light in the PHCbi brand incubator.
- Hydrogen Peroxide (H_2O_2) Vapor. PHCbi brand incubators permit the use of active hydrogen peroxide (H_2O_2) vapor decontamination with complete safety and zero impact on the surrounding environment. Hydrogen peroxide starts in aqueous form and is converted to vapor using a nebulizer; this exposes all interior surfaces to the H_2O_2 vapor which ultimately resolves to benign water at less than 1 ppm when catalyzed by a UV lamp.

Passive Decontamination

- Copper Enriched Stainless Steel (marketed as inCu-saFe® under the PHCbi brand) is a stainless steel and copper composite alloy that forms a germicidal barrier to prevent growth of organisms on surfaces. All interior surfaces, shelves and brackets are comprised of the inCu-saFe composite. This material is a hybrid of Type 304 stainless steel. It is 100% corrosion-proof and will not corrode or discolor like conventional C100 copper surfaces.
- Ultraviolet Light (marketed as SafeCell™ UV under the PHCbi brand) consists of a concealed UV lamp that creates a serial exposure of 257.3 nm wavelength sufficient to destroy DNA of any organism passing through the airflow system as well as surface water contaminants in the removable humidity pan. The UV lamp initiates automatically upon a door opening/closing event. SafeCell UV inhibits the growth of mycoplasma, bacteria, molds, spores, viruses, yeasts and fungi without costly HEPA filter air scrubbers which accumulate contaminants in the filter media.
- Additionally, the UV lamp can be programmed for a timed 100% ON cycle for a supplemental chamber decontamination process when all interior components are removed.

CONCLUSION

Management of contamination in the cell culture incubator starts with good incubator design. Materials, temperature and gas control methods, interior component orientation and good laboratory technique are essential to minimize the risk of contamination and cross contamination in the cell culture environment. Regularly scheduled cleaning and maintenance help to avoid downtime and loss of work *in situ*.

For More Information

PHC Corporation of North America offers a cell culture product line that represents more than 50 years of innovation and successful application throughout the life science community. If you have persistent or unexplained contamination issues with your cell culture incubator we can help. Contact your PCH Area Sales manager listed at www.phchd.com/us/biomedical/contact-us.

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Printed in USA | 03 | 17 | 2019 | OW11687.1 | vF