

CONTROLLED ENVIRONMENTS IN A CLEANROOM SPACE

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Abstract

Recent biomanufacturing capacity expansions have led to an increasing demand for precise incubators and growth chambers that can meet a variety of cleanroom requirements. While there isn't a one-size-fits-all requirement, options now exist to accommodate a wide range of cleanroom needs.

Background

Cleanrooms are used within bioresearch and bioproduction applications, reducing the potential for culture cross-contamination due to airborne biologicals. These particulates can be suspended in general laboratory air, brought in on user shoes or clothing, directly shed by users and created by equipment or supplies within a workspace. Particulate reduction within the room is brought about by a combination of positive pressure filtered airflow, airlocks and entry barriers, special attire, elimination of materials that can shed fibers, and use of equipment designed for the application. As a further step, many bio cleanrooms are subjected to intensive cleaning protocols, which may involve the use of aggressive chemical compounds, to further reduce contamination risk. Some installations also move as much of the equipment mechanical components as possible outside of the room, shrinking exposed unit surface area to simplify cleaning protocols.

Cleanroom Design

Sterile product research and manufacturing starts with a clean entry area leading through airlocks to the clean working area. The working area is typically further subdivided by function into self-contained sub-units, each of which is usually equipped with its own airlock. Users checking into the facility will need to wear special garments designed both to retain any shed particulates (ie. skin or hair), and not to release any particulates of their own. Higher level cleanrooms require more complete personnel coverage, up to fully enclosed suits with respirators. Paper, such as process or product documentation, along with paper-based packaging for supplies, is usually avoided. Equipment should be designed so that it does not emit particles as part of general operation, or during regular maintenance. The cleanroom space must be completely sealed, with no openings that can allow ambient air within the space. This includes drop ceilings with interstitial spaces, and utilities access

points. Crevices and other difficult access areas should be minimized, to improve ease of cleaning, and prevent particulate retention. Pressurized HEPA or ULPA filtered air supply must be maintained, with a positive pressure differential between more and less critical areas. As the level of protection increases, unidirectional laminar airflow will become progressively more important, requiring that between 5 and 100% of the ceiling be covered with air handling ducts. Maintaining laminar airflow in the most critical environments also requires limiting air turbulence, such as that created by unit ventilation and general heat generation, within the space. Facilities often limit equipment and supply use within critical areas to only those units necessary to perform the requisite processes.

ISO 14644-1 Class	EU GMP	Cleanroom Application
4	-	Semiconductor nanofabrication, ultra-fine particulate processing
5	Grade A/B	High risk operations, including filling, making aseptic connections, preparation and filling. Grade A usually maintained in Biological Safety Cabinets
6		Medical device manufacturing, Computer chip fabrication
7	Grade C	Biomedical research, less critical sterile product manufacturing
8	Grade D	Less critical sterile product manufacturing
9		Offices, general laboratories

Due to the facilities and systems expense and complexity required to maintain ISO4 and ISO5/Grade A/B air quality levels, this is reserved for only the most critical applications. Performing these applications inside a specialized Class 4 or 5 enclosure located within a clean room space maintained at a lower “background” level of Class 6 or 7/Grade C is a common practice. ISO8/Grade D protection is the lowest level typically offered in a biological cleanroom.

Chemical Cleaning

Chemical cleaning agents are a supplemental tool to further reduce surface contamination risk within a cleanroom environment. While common alcohol and quaternary ammonium-based laboratory cleaning agents are extremely effective against vegetative bacteria, fungi, and most viruses, they are typically ineffective against bacterial or fungal spores, as well as certain types of viruses. For certain applications, many users rely on more aggressive cleaning solutions. These may include liquid or vaporized hydrogen peroxide, and acetic & peracetic acid.

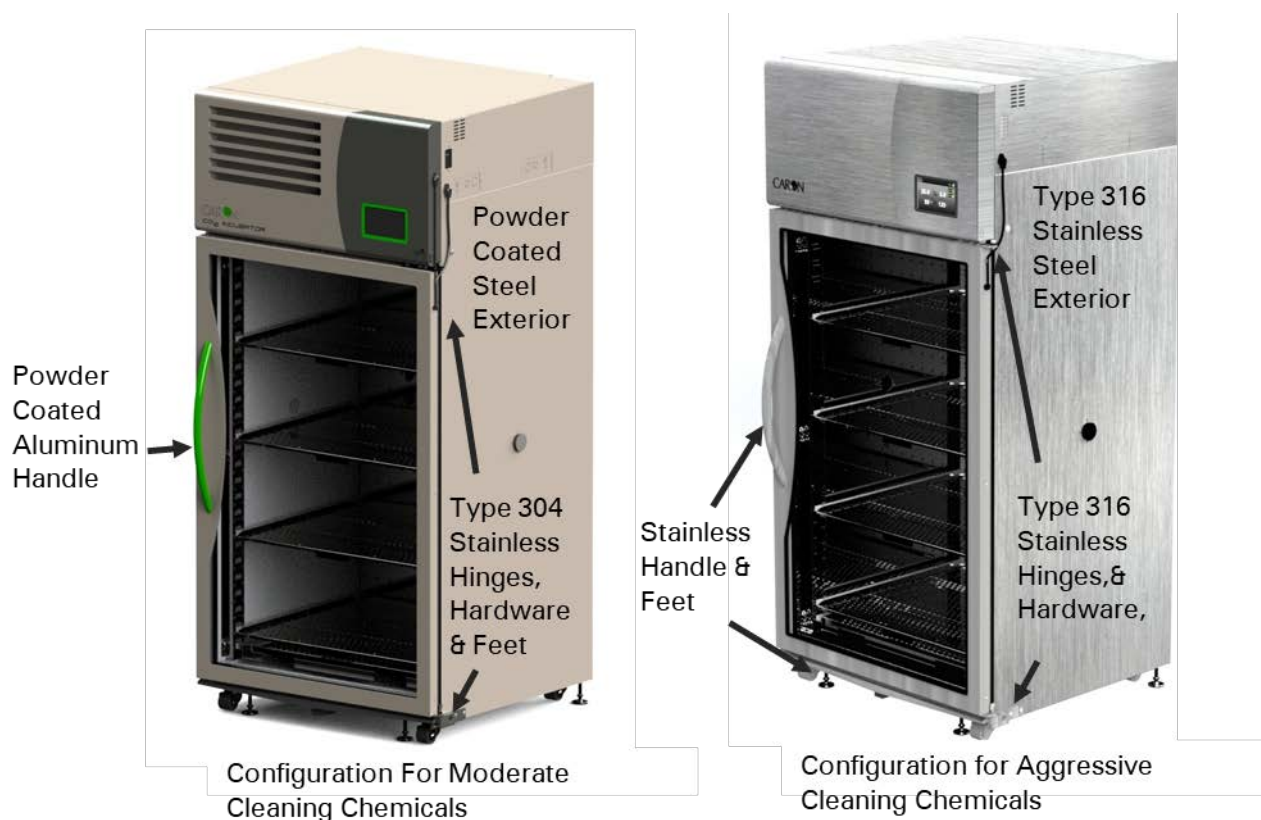
Category	Common Trade Names	Efficacy	General Materials Compatibility
Alcohol (Ethyl & Isopropyl)	N/A	Vegetative bacteria, fungi, & lipid-based viruses.	Very good
60-90% concentration. Often paired with other disinfectants and used as post-disinfection rinse. Ineffective against spores or hydrophilic viruses.			
Quats (Quaternary Ammonium)	Lysol No-Rinse®, Vesta-Syde®	Vegetative bacteria, fungi, lipid-based viruses	Very good
0.4-1.6% concentration. Can be left on surfaces, short required dwell time, usually combined with alcohol. Ineffective against spores or hydrophilic viruses			
Phenolics	Vesphene II®, LpH	Most vegetative bacteria, fungi, most viruses	Very good
0.4-1.6% concentration. Ineffective against non-enveloped viruses & spores			
Hydrogen Peroxide (H₂O₂)	HyProx®	Highly effective against vegetative bacteria, fungi, viruses, & spores	Good
0.5-7% concentration. Effective against spores in high concentrations. Limited materials compatibility issues (select plastics).			
Idophors (Iodine)	Clean Front	Vegetative bacteria, most fungi, viruses	Poor
Under 0.5% concentration. Not effective against certain fungi in lower concentrations, not effective against spores without prolonged contact times. Discolors many materials.			
Acetic & Peracetic acid	Spor-Klenz®, Nu-Cidex®	Highly effective against vegetative bacteria, fungi, viruses, & spores	Poor
10-15% concentration. Often combined with hydrogen peroxide. Attacks steel, aluminum, & most stainless without immediate & extensive post-dwell sterile H ₂ O or alcohol rinse.			

Cleaning protocols that pair frequent disinfection with extended disinfectant dwell time on surfaces can lead to surface corrosion on many types of metallic equipment components. This is particularly the case for wear parts, such as door hinges, door hinge pins, and door latch strike surfaces. These components are usually made of high-carbon cold-rolled steel, and corrode as their surface coatings are worn away. For this type of cleanroom usage, these parts can and should be upgraded to high-grade type 304 stainless steel.

Material	Corrosion-Inhibiting Components	Chemical Resistance
Cold-Rolled Steel, uncoated (ANSI 2021, DN 1.0402)	None	Alcohols – <i>A</i> , Mild Acids – <i>C/D</i> , H ₂ O ₂ – <i>D</i>
Type 440 Stainless	18% Chromium, 0.3% Nickel	Alcohols – <i>A</i> , Mild Acids – <i>B/C</i> , H ₂ O ₂ – <i>A</i>
Type 304 (“A2”) Stainless	18% Chromium, 8% Nickel,	Alcohols – <i>A</i> , Mild Acids – <i>A/B</i> , H ₂ O ₂ – <i>A</i>
Type 316 (“A4”) Stainless	18% Chromium, 10% Nickel 2% Molybdenum	Alcohols – <i>A</i> , Mild Acids – <i>A</i> , H ₂ O ₂ – <i>A</i>

A: No effect – Excellent, B: Minor effect – Good, C: Moderate effect – Fair, D: Severe effect – Not recommended

Some chemicals and chemical regimens, however, particularly those that using acid-based solutions, can also attack non-wear parts, such as powder coated steel casework, handles, and coated fasteners. For these aggressive cleaning regimens, it may be necessary to upgrade all exposed metallic surfaces to a very high grade stainless, such as type 316L.



Note that solutions containing sodium hypochlorite (bleach) in even very low concentrations are incompatible with all types of laboratory equipment. While an extremely effective sporicidal agent with a short required dwell time, bleach aggressively attacks even high-grade stainless steel. The resulting corrosion is often immediate, and impossible to repair. Always follow manufacturer's cleaning recommendations for laboratory equipment, which may include other model-specific cleaning chemical or protocol limitations.

Some facilities have also moved from liquid chemical disinfection to gas-based processes, most commonly vaporized hydrogen peroxide. While this is usually a highly effective and efficient alternative, manufacturer resources should always be consulted before implementation, to confirm that all equipment and instrument components are compatible with the sterilant.

All equipment must also be designed to not retain liquid cleaning chemicals or provide paths of ingress. For this reason, vents and ducts should be minimized, seams filled, and control panels and other electronics sealed to prevent component damage.

In-Room Versus Recessed Equipment

Equipment situated within a cleanroom can greatly increase the time and effort needed to thoroughly decontaminate the space. Time required to manually disinfect a room is directly related to its surface area, and chambers installed within the space can easily double that number.

Total surface area of a representative large single-door incubator requiring disinfection (*2 sides + top + bottom + front + back*) = 100ft²

Equivalent wall space area without incubator = 22ft²



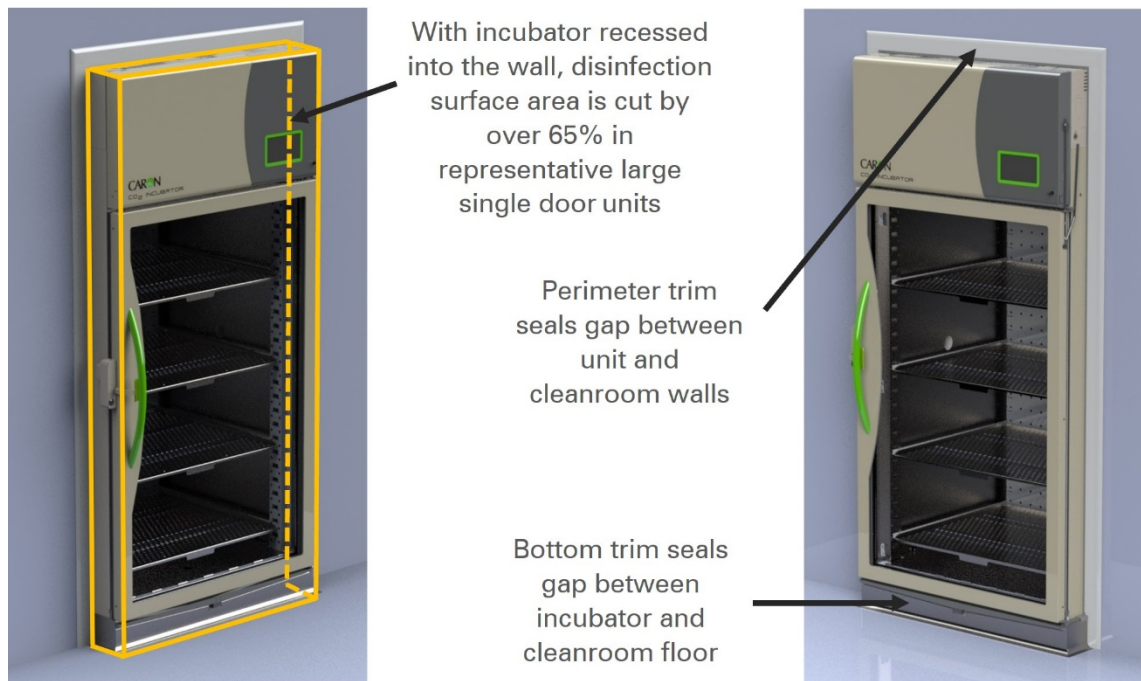
Equipment also complicates cleaning regimens, due to inherent crevices that can trap contaminants and difficult to reach surfaces that are easily missed. Regular maintenance on equipment located inside the cleanroom is very time-consuming, requiring that technicians and their tools go through full gown-up procedures every time they access the units, and potentially restricting user access to surrounding units.

Equipment electrical components, such as fans, power supplies, circuit boards, and motors also generate heat, which will be exhausted to the room. In higher level cleanrooms, this can cause turbulence issues, preventing true laminar airflow.

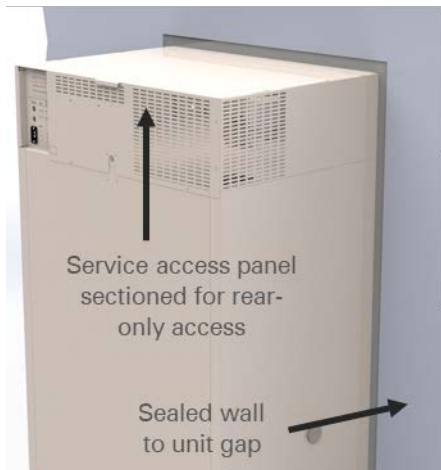
Recessed in-wall mounting may be an attractive alternative to placing equipment in the room. Openings are built into the cleanroom walls, and then sealed up around the unit fascia. All unit surfaces other than the fascia are located outside of the room, only slightly increasing cleaning surface area beyond that in the empty space. While not an option for equipment with a more three-dimensional user interface, it can work well with controlled-environment chambers. There are tradeoffs, however, since this approach requires external climate-controlled utility areas, to permit equipment service outside of the cleanroom, and by leaving gaps in the room casework, complicates site construction and commissioning.

For facilities interested in the recess-mount option, equipment with pre-engineered wall interface components can greatly reduce installation cost and complexity. Important

features include a perimeter trim kit that securely fills the gap between unit and wall opening, and a lower trim piece that fits between the bottom of the chamber and the floor, taking into consideration the casters, levelling feet, and door drip trays that are usually located in this area. The latter piece, in particular, can add to project cost and stretch out installation timing if must be developed and fabricated on a custom basis by a third party.



Designed-in unit serviceability is also a key in-wall installation requirement. Typical front or top access panels cannot be removed in this configuration without moving the unit or creating a path through the unit mechanical compartment to the utility space behind, either of which will open up the cleanroom to unfiltered ambient air and contaminate the space. In-wall mounting systems should include special rear or rear/top access systems, permitting regular maintenance and service without breaking the cleanroom seal. Product contamination risk and wasted technician time would result if touch areas like the power switch, access ports, electrical and plumbing connections were located inside the cleanroom space.



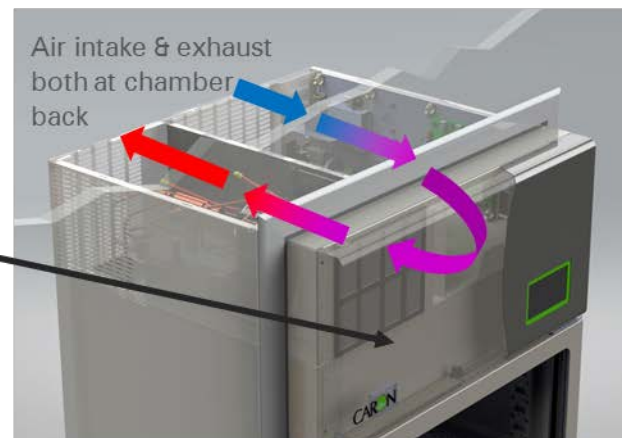
Rear of in-wall incubator, viewed from outside of cleanroom space



Finally, in-wall equipment should be designed to only ventilate its mechanical compartments to outside of the cleanroom space. This is even more important if the application requires cooling, since the mechanical compartment now also contains heat extracted from inside the chamber.



Solid fascia prevents air intake and exhaust inside Cleanroom Space



Front and cutaway views of incubators with in-wall installation.

Units cannot draw air from inside the cleanroom and must instead draw cool air from the utility area, and force hot air back into that same space. Simply blocking off the front air vents of a unit not designed for this required airflow path will lead to overheating and result in performance issues and even premature component failure.

Conclusions

While bio cleanrooms are a complex engineered space, new incubator options can greatly simplify equipment integration. Caron offers a range of offerings that combine features tailored to every level of cleanroom protection, but without the high cost and long lead times generally associated with product customization. Contact Caron for more details, or to discuss the full range of solutions available for your cleanroom incubator installations.

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