

Cleaning of Isolators and Bio-safety Cabinets

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1. Introduction

Isolators (Figures 1 and 2) and bio-safety cabinets³ (Figure 3) are well accepted as cost-effective, convenient and compact controlled environments to provide product and personnel protection. These devices consist of protective enclosures that physically isolate products from the background environment (the room outside the isolator), either because the product of interest is unsafe and therefore needs containment, or the product cannot tolerate contamination and therefore needs isolation. Collectively, they are known as “separative enclosures”, or more properly “separative devices”.



Figure 1 – Pharmaceutical Isolator (Photo courtesy Getinge, La Calhène)

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³ Also known as “safety cabinets”, “biocontainment isolators”, “containment isolators” or “barrier isolators”



Figure 2 – Compounding Aseptic Isolator (Photo courtesy NuAire, Inc.)



Figure 3 – Bio-safety Cabinet (Photo courtesy The Baker Company)

For this discussion, we will consider that isolators and Class II bio-safety cabinets (and associated transfer devices) in biotechnology, microbiology, pharmaceutical and pharmacy applications are used as ISO Class 5 devices in terms of air particle cleanliness and are maintained as sterile environments to a sterility assurance level (SAL) ranging from 10^{-3} to 10^{-6} depending upon application.

In order to maintain product quality and integrity, it is necessary to clean isolators and Class II bio-safety cabinets on a regular basis, with appropriate cleaning products, according to established and validated standard operating procedures (SOP's). This often entails manual cleaning with wipers, mops, cleaning agents and if necessary, disinfectants. Previous publications have addressed the general topic of cleaning of isolators. This paper, however, focuses on details such as wiper selection, wiping procedures, protocols and step by step guidelines for cleaning isolators and Class II bio-safety cabinets. The reader may also wish to refer to publications dealing with the cleaning of aseptic pharmaceutical environments for additional background information.

2. Optimum Cleaning Procedures

One could well ask, "Why bother with wiping? Why not just spray with alcohol to clean the surface?" The case for wiping as opposed to merely spraying was effectively made some years ago during a study of disinfection techniques for transfer of components into hospital pharmacy cleanrooms.

The wiping action puts the fabric in intimate contact with the surface, allowing the application of strong forces for the removal of contaminants such as bioburden. Wiping has a long and successful history for removal of contaminants from cleanroom surfaces. However, to be successful, the wiper must be used properly, such as shown in Figure 4 below.

Wiping Guide

1. Follow relevant site protocol (procedures for safety, contamination, etc.) and wear clean-room gloves.
2. Fold wiper in mid-air into quarter folds (Fig. 1A–1C). This will produce several clean surface areas and allow better contact with the surface to be wiped.
3. When wiping, hold the wiper so that the single-fold edge is outward toward the area to be wiped. Hold the selvages closer to your hand. (Fig. 2)
4. Use either a pre-wetted wiper or a dry wiper moistened with an appropriate cleaning agent.
5. Wipe in one direction, overlapping wiped area by 10% to 25%. Wipe systematically, for example, from top to bottom, far to near. (Fig. 2)
6. Wipe from cleanest to least clean regions of the surface being wiped.
7. Keep track of which surfaces have been cleaned and which wiper areas are unused.
8. Use the cleanest surfaces of the wiper. If re-wiping use clean wiper area, not used wiper area.
9. Dispose of wipers according to site procedures.

Wiping Wet Spills

1. Identify the spilled liquid. Follow the Material Safety Data Sheet (MSDS).
2. Choose wiper and gloves that will not be degraded by liquid.
3. For hazardous spills, wear two pairs of gloves and try to keep the gloves dry. Wear any other necessary protective gear.
4. Use dry wipers to wipe spills up immediately, then wipe slowly.
5. Dispose of wipers according to site procedures.



Figure 4 – Wiping Guide

3. Cleaning and Disinfection of Pharmaceutical Isolators

a) Overview

Pharmaceutical isolators are used to conduct aseptic filling operations, sterility testing, cell culturing and purification activities, among others.

It has been pointed out previously that there are no substantial differences between an isolator and a cleanroom as far as the cleaning of product contact surfaces are concerned. Isolators and cleanrooms need to be cleaned and disinfected and these processes should be thorough, consistent, convenient and validatable.

b) Wipers and Mops for Cleaning and Disinfection of Isolators

Much of the literature on isolator cleaning refers to the need for “low-linting” fabrics that do not shed. However, little guidance is provided as to which fabric types are best. The lint that is shed from wiping or mopping materials is made up of loose fibers that are not bound to the fabric surface or that have broken free during the cleaning process. Cleaning and disinfecting solutions can promote this linting or shedding activity if inappropriate fabrics are used.

Of the available wiper choices, only polyester knit fabrics have the requisite cleanliness, low particle and fiber counts, low endotoxin levels, low extractable residues, durability, and chemical compatibility that are needed for the cleaning and disinfection of isolators. Further, polyester knit fabrics can be sterilized by autoclaving or by gamma irradiation to an SAL of 10^{-6} without loss of structural stability. The characteristically low levels of releasable particles and fibers associated with polyester knit fabrics are especially important in aseptic applications since it is well known that particles are potential carriers of bacteria.

Sterile polyester knit wipers are used during production to clean up spills, wipe down gloves (when wetted with sterile 70% IPA) or to provide clean work surfaces. These wipers can be wetted with (i) detergents to clean the isolator, (ii) deionized water or 70% IPA⁴ to remove cleaning agent residues, (iii) disinfecting agents to disinfect the isolator and (iv) deionized water or 70% IPA to remove disinfectant residues. Pre-wetted sterile wipers, containing 70% IPA, are also available for these activities.

c) Cleaning and Disinfecting Frequency

Good contamination control practice would suggest that isolators and associated transfer devices be cleaned and disinfected after a production campaign has been concluded (referred to here as “post cleaning” which includes disinfection) and again before a new production campaign is begun (referred to here as “pre-cleaning” which also includes disinfection). This will avoid cross contamination. Decisions on cleaning/disinfecting frequency and procedures are the province of the Quality Supervisor.

d) Cleaning and Disinfecting Specifics

Since the isolator is most often cleaned and disinfected while closed, to maintain the sterility of the isolator, sterile cleaning and disinfecting consumables – wipers, pre-wetted wipers, mops, cleaning/disinfecting agents, water, 70% IPA, etc. – must be introduced through an appropriate transfer device. Even if a facility’s SOP calls for the isolator to be opened for cleaning and disinfection, the use of sterile wipers and pre-wetted wipers is recommended, since they can be introduced into the isolator for *in situ* cleaning needs. This also eliminates the confusion of having both sterile and non-sterile wipers on hand and eliminates the need to sterilize wipers prior to use within the isolator.

The usual sequence for cleaning and disinfection includes a cleaning step, a rinsing step, a disinfecting step, another rinsing step and if needed, a gaseous sterilization step and a cleaning validation step. Wipers can also be used to wipe down any hard surface articles that are introduced

⁴ All IPA solutions described in this article are assumed to be 70% IPA/30% water (v/v), where the “water” is either water for injection (WFI) or deionized water (DIW).

into the transfer device for use within the isolator. This will remove surface contaminants that might otherwise compromise disinfection or sporicidal treatments.

(i) Cleaning

To ensure that each production run will be conducted in a pristine environment, it is necessary to clean the isolator to remove any residues and soils produced from the prior run.

Typically, small flat surface mops known as isolator cleaning tools (Figure 5), wipers, swabs and detergents are most commonly employed for these cleaning applications. Detergent selection is based on the type of soil to be removed. The detergent is applied to the surface using quarter-folded wipers with linear overlapping strokes, wiping from clean areas to dirty, renewing the wiper surface after each stroke. Wipers are used for all surfaces within arm's reach. Isolator cleaning tools are used for surfaces beyond arm's reach.

Detergents also have the benefit of reducing the bioburden level on the surface; this lessens the task somewhat for the subsequent disinfection step.



Figure 5 – Isolator cleaning tool in use (Photo courtesy Getinge, La Calhene)

(ii) Rinsing Following Cleaning

After cleaning, detergent residues are removed from the surfaces with wipers or mops that have been wetted with sterile deionized water or sterile 70% IPA. IPA is a versatile cleaning agent and will remove many different types of soils. Rinsing will ensure that disinfectants have the opportunity to contact bare surfaces. Surfaces are considered clean

when devoid of visible surface contaminants. Verify visually that the last wiper used to wipe down the surface is also devoid of visible residues.

(iii) Disinfection

The same procedures are followed for disinfection, except that liquid disinfecting agents are substituted for detergents. Disinfecting agents can include phenolics and quaternary ammonium compounds⁵ (“quats”). Aqueous mixtures of IPA will provide some measure of disinfection, but they are ineffective against spores. Occasionally, liquid sterilants such as sodium hypochlorite (bleach), peracetic acid and hydrogen peroxide will be substituted for disinfectants when sporicidal activity is needed. These sterilants can be corrosive to surfaces and are therefore used intermittently.

(iv) Rinsing Following Disinfection

The same procedure is followed here as in Section (ii) above. Disinfecting agent residues are wiped from the surface with wipers or isolator cleaning tools that have been wetted with sterile deionized water or sterile 70% IPA. This will eliminate the buildup of residue deposits that become difficult to remove in subsequent cleaning operations, and that will cause staining of work surfaces.

v) Gaseous Sterilization

Once the cleaning and disinfection steps are completed, if required, the isolator can be sterilized, with a suitable sterilant such as Vaporized Hydrogen Peroxide (VHP).

vi) Cleaning Validation

Surface sampling with swabs to verify the absence of cleaning and disinfecting agents may be required after step (iv).

This constitutes the “post clean” described in the Cleaning Frequency Section above. The Quality Supervisor determines what cleaning and disinfecting steps are required for any given circumstance.

e) Recommended Products

Table 2 lists the various cleaning tasks and the products recommended for both pharmaceutical isolators and compounding isolators, since so many of the tasks are common to both. Those tasks that are unique to pharmaceutical isolators are shown in blue; those that are unique to compounding isolators are shown in red.

⁵ Use phenolics **or** quats, never both together.

Table 2. Cleaning Tasks for Pharmaceutical Isolators and Compounding Aseptic Isolators

Cleaning Task	Recommended Products
Post-clean or pre-clean of interior walls, ceiling and deck of closed isolators	Choose one or more of the following: 1. Isolator cleaning tool with polyester knit mop covers to reach all interior surfaces of the isolator. Sterilize the cleaning tool and mop covers before introducing them into the isolator. Dampen the mop covers with sterile WFI, sterile DIW, sterile IPA ⁶ , detergent cleaning solution, disinfectant solution or liquid sterilant as described in 3d above. 2. Sterile IPA-pretreated polyester knit wipers. 3. Sterile dry polyester knit wipers. Dampen the wipers with the solutions described in 1 above, as appropriate for the cleaning task at hand. 4. Swabs with polyester knit heads for cleaning hard-to-reach spaces, crevices, nooks, crannies, and isolator corners. These swabs can be dampened with one of the solutions described in 1 above.
Wiping down deck between CSPs	Sterile IPA-pretreated polyester knit wipers.
Cleaning up spills while isolator is in use.	Sterile dry polyester knit wipers for absorbing spilled liquid, then Sterile IPA-pretreated polyester knit wipers for removing surface contamination.
Wiping down gloves while isolator is in use. Wiping mating and sealing surfaces Wiping down articles placed in the transfer isolator	Sterile IPA-pretreated polyester knit wipers.
Validation of isolator cleaning	Total Organic Carbon (TOC) Cleaning Validation kit.

Text in black refers to tasks for both pharmaceutical isolators and compounding aseptic isolators. Text in blue refers to tasks unique to pharmaceutical isolators. Text in red refers to tasks unique to compounding aseptic isolators.

⁶ Again, IPA refers to 70% IPA, 30% DIW (v/v)

4. Compounding Aseptic Isolators(CAIs)

Compounding aseptic isolators are used in hospital pharmacies and other dispensing facilities to formulate (i.e. “compound”) individual prescriptions known as Compounded Sterile Preparations (CSPs) for patient care. To avoid cross contamination between CSPs, the accepted procedure for cleaning and sanitizing CAIs is to wipe the counter or “deck” of the compounding isolator with a wiper wetted with 70% IPA. Pre-wetted wipers are most convenient for this task. This will remove any residues from the work surface and will provide a measure of surface sanitization as well. Some residues may only be water soluble, so in those cases, wipers wetted with water for injection (WFI) should be used to remove the surface soils. A final wipedown with IPA will leave the surface clean for the next CSP. A second IPA-wetted wiper should be used to wipe down the gloves to guard against cross-contamination in the preparation of the next CSP.

If the CAI is used for compounding hazardous drugs, then swab sampling of the interior surfaces with subsequent analysis may be appropriate to prove that the compound of interest is not present at levels which would constitute an exposure limit danger.

Refer to Table 2 above for the various cleaning tasks and the associated products recommended for CAIs. Tasks relevant to CAIs are in black and red.

5. Summary

The manner in which pharmaceutical isolators, compounding aseptic isolators and bio-safety cabinets are used dictate the somewhat varied approach to cleaning and where applicable, disinfection practices. Good contamination control practices and dedicated adherence to established, written SOP’s will minimize cross-contamination surprises.

In order to meet the space requirements for this publication, this article is approximately one third of its original length. To request the complete document, including a list of 25 references or to request specific product recommendations for the cleaning of isolators and bio-safety cabinets, please contact the authors at hsiegerman@texwipe.com or kbonnell@texwipe.com.