Cherwell
LABORATORIES

THE PHARMACEUTICAL LAB'S POCKET GUIDE TO

CLEANROOM DECONTAMINATION

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Introduction

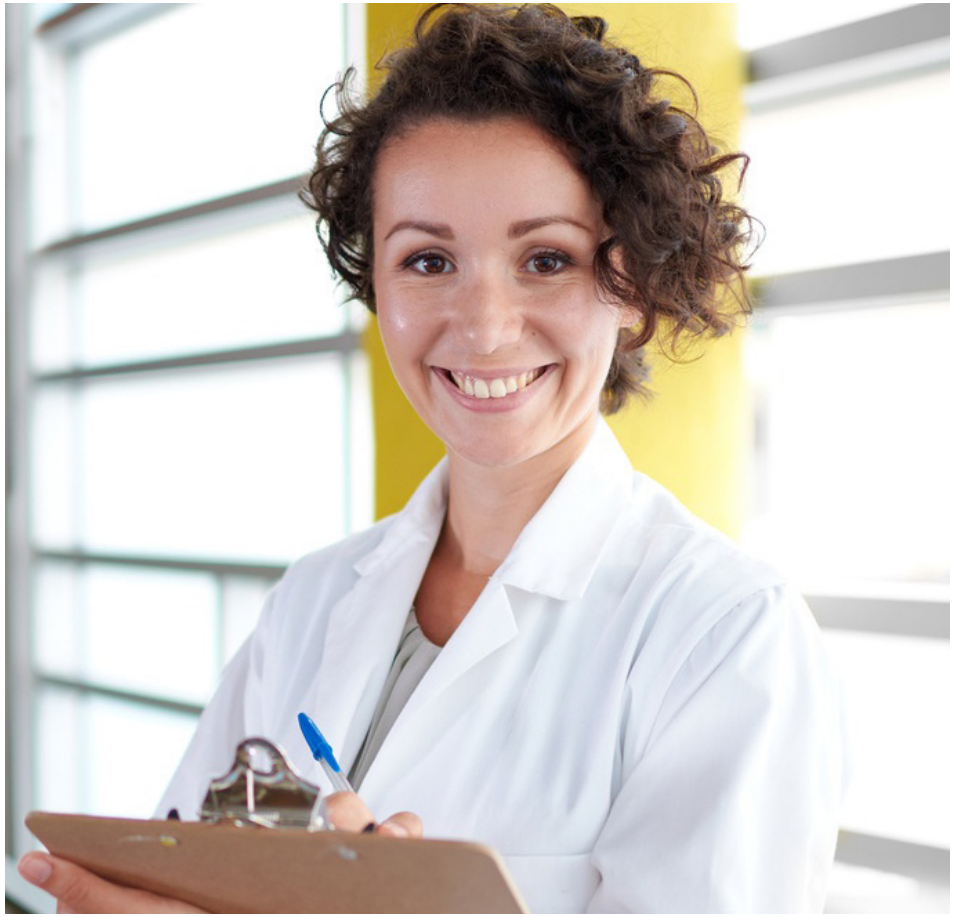
As the scientific knowledge of biocontaminants and the effects of exposure to various chemicals becomes more advanced, pharmaceutical cleanroom operators have to shoulder an increasing burden of complex protocols and heavily regulated procedures.

Fortunately for operators, cleanroom technology has kept up the pace, offering them a greater range of decontamination products, techniques and devices than ever before. This document has been developed to serve cleanroom operators with an overview of these technologies, including:

- Cleanroom Disinfectants
- Decontamination Techniques
- Decontamination Devices
- Systems that deliver quicker, safer cleanroom decontamination

Disclaimer:

Please be aware that the information in this resource is intended for use as a guideline only. At no time should this document replace existing documents established by your facility.



The Red Tape of Cleanroom Decontamination

Cleanroom Validation Standards

Validated cleanrooms are certified according to the specific class of cleanliness based on the ISO (International Standards Organisation) scale. Cleanliness can also be defined in terms of both particle counts (ISO 14644) and microbial counts (ISO14698), although ISO 14698 is not widely used and not quoted for in the EU GMP and USP and FDA GMP guidelines.

The purpose of the validation, or Performance Qualification (PQ), of a cleanroom is to demonstrate compliance with the user requirement specification (URS) of the cleanroom. Cleanroom PQ involves testing and monitoring the following aspects of the cleanroom environment:

- Airborne particulate levels
- Surface particulate levels
- Viable microbial particulates
- Relative humidity
- Differential pressure
- Temperature

ISO 14644-1 Cleanroom Standards

ISO Class number (N)	Maximum allowable concentration (particles/m³) for particles equal to and greater than the considered sizes, shown below[a]					
	0.1µm	0.2µm	0.3µm	0.5µm	1µm	5µm
ISO 1	10[b]	d	d	d	d	e
ISO 2	100	24[b]	10[b]	d	d	e
ISO 3	1,000	237	102	35[b]	d	e
ISO 4	10,000	2,370	1,020	352	83[b]	e
ISO 5	100,000	23,700	10,200	3,520	832	d,e,f
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7	c	c	c	352,000	83,200	2,930
ISO 8	c	c	c	3,52,000	832,000	29,300
ISO 9[g]	c	c	c	35,200,000	8,320,000	293,000

a. All concentrations in the table are cumulative, e.g. for ISO Class 5, the 10 200 particles shown at 0,3 µm include all particles equal to and greater than this size.

b. These concentrations will lead to large air sample volumes for classification. Sequential sampling procedure may be applied; see Annex D of BS EN ISO 14644-1:2015.

c. Concentration limits are not applicable in this region of the table due to very high particle concentration.

d. Sampling and statistical limitations for particles in low concentrations make classification inappropriate.

e. Sample collection limitations for both particles in low concentrations and sizes greater than 1 µm make classification at this particle size inappropriate, due to potential particle losses in the sampling system.

f. In order to specify this particle size in association with ISO Class 5, the macroparticle descriptor M may be adapted and used in conjunction with at least one other particle size (See C.7. of BS EN ISO 14644-1:2015.

g. This class is only applicable for the in-operation state.

While initial cleanroom validation will be undertaken during the setup phase, operators must demonstrate continual compliance by abiding by the following ISO testing schedule.

Required Testing (ISO 14644-2)

Schedule of Test to Demostrate Continuing Compliance			
Test Parameter	Class	Maximum Time Interval	Test Procedure
Particle Count Test	<= ISO 5	6 Months	ISO 14644-1 Annex A
	> ISO 5	12 Months	
Air Pressure Difference	All Classes	12 Months	ISO 14644-1 Annex B5
Airflow	All Classes	12 Months	ISO 14644-1 Annex B4

Standards for Cleanroom Operations

Once initial validation has been established, on-going **cleanroom operations** are regulated as defined by the EU GMP or the FDA guidelines. Guidance on how operators can implement these guidelines in their basic cleanroom protocols can be found in the ***Institute of Environmental Science and Technology's (IEST) recommended practices for contamination control and the ISO 14644 series of international standards for cleanrooms and associated controlled environments.***

Standards for Biocontamination Control

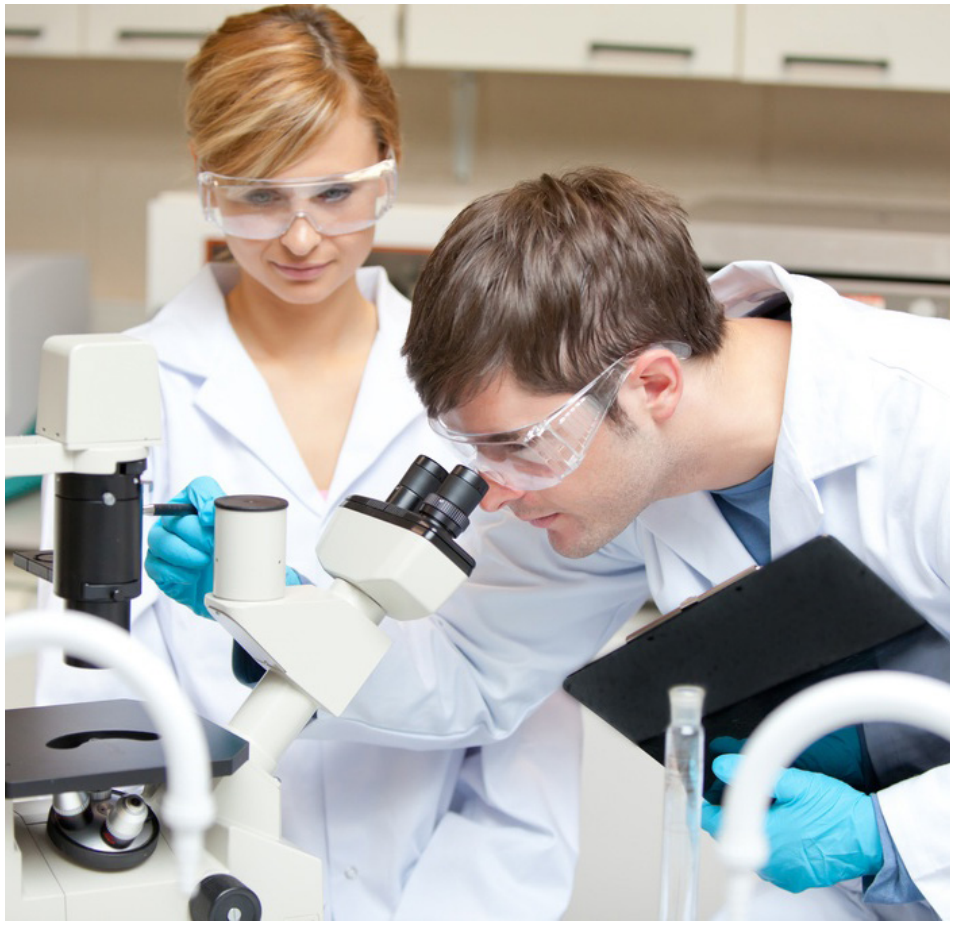
The ISO series also features two sets of standards on Biocontamination control to provide guidance to operators of critical areas. Please note: These standards are not lone-standing recommendations and always need to be used in conjunction with ***EU GMP Annex 1*** or USP chapter 1116.

- ***ISO 14698-1***, on general principles for zones of low or negligible risk. This standard describes the principles and basic methodology for a formal system to assess and control biocontamination, where cleanroom technology is applied, in order that biocontamination in zones at risk can be monitored in a reproducible way and appropriate control measures can be selected. In zones of low or negligible risk this standard may be used as a source of information.
- ***ISO 14698-2***, on the evaluation and interpretation of biocontamination data. This standard gives guidance on basic principles and methodological requirements for all microbiological data evaluation, and the estimation of biocontamination data obtained from sampling for viable particles in zones at risk, as specified by the system selected.

Decontamination Product Regulations

Most cleanroom disinfectants are regulated ***under the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012)*** and have to be approved as product type PT2, “Disinfectants and algaecides not intended for direct application to humans or animals” for use in a cleanroom or laboratory environment”. This regulation provides information on biocidal products that have been approved to be used to protect humans, animals, materials or articles against harmful organisms, like pests or bacteria, by the action of the active substances contained in the product. Article 95 of the guide lists the range of approved active substances and suppliers. For detailed information, you can download the European Chemicals Agency’s ***Practical Guide on Biocidal Products Regulation.***

Disinfectant products that are approved as sterilants for medical instruments are also suitable for use in pharmaceutical cleanrooms, although they are separately monitored by the Medicines and Healthcare products Regulatory Agency (MHRA). ***Minncare® Cold Sterilant (peracetic acid solution)*** and ***Actril® Cold Sterilant*** (a mixture of 0.08% peracetic acid and 1.0% hydrogen peroxide) are examples of products that fall in this category and are commonly used for cleanroom decontamination.



Maintenance of Cleanroom Standards

Maintaining the ISO standard of your cleanroom requires the enforcement of a rigorous decontamination routine as well as the institution of remedial decontamination protocols.

Routine Decontamination

To successfully enforce a routine decontamination policy, it is imperative that operators provide staff with disinfectant products and technology that enables them to execute cleaning protocols easily and efficiently.

In selecting disinfectants to stock up the pharmaceutical cleanroom, it is wise to have **at least two disinfectants available for routine use.** Ideally, **a third should be available in case a major contamination incident occurs.** Such a reserve disinfectant is especially valuable for scenarios of bioburden contamination build-up that have become resistant to routinely used disinfectants.

Remedial Decontamination:

Even when routine cleaning protocols are executed faultlessly, a major decontamination exercise will occasionally be required. Instances where remedial decontamination becomes necessary are, for example, following a bioburden outbreak, the failure of a HEPA filter, or where the cleanroom has to reopen after a period of being shut down.

While remedial decontaminations were traditionally performed by manual methods, (i.e. by operators using mops, buckets, and wipes); modern techniques include semi-automatic bio-decontamination solutions such as fumigation units and ***high tech dry fog systems.***

While the initial setup of semi-automatic systems might take some time and effort, it simplifies the process of recovering from cleanroom sterility issues or bioburden outbreaks to setting up the machines, then evacuating and sealing the room. Because the time needed to execute these solutions ranges from 5 to 30 hours, operators are advised to consider their options carefully before purchasing a new system. For more information on this topic, please see the final section of this guide.

Types of Decontamination Products, Techniques and Devices

Different Types of Cleanroom Disinfectants

The range of disinfectants approved for cleanroom decontamination is diverse, both in chemical composition and by modes of action they perform against microbial cells. Different chemicals target different areas within the microbial cell, including the cell wall, the cytoplasmic membrane (where the matrix of phospholipids and enzymes provide various targets) and the cytoplasm. Other chemicals target microbes on an intracellular level, entering the cell by disrupting or diffusing the membrane.

There are various approaches to categorising and sub-dividing cleanroom chemicals. Among these approaches, differentiation can be made according to its **chemical makeup, mode of activity, and also bacteriostatic and bactericidal effects on micro-organisms.**

The following classification model differentiates the range of disinfectants most commonly used for cleanroom decontamination according to three action modes: **non-oxidising disinfectants, oxidising disinfectants, and hand sanitisers.**

Non-oxidising Disinfectants

While most non-oxidising disinfectants take distinct modes of action against micro-organisms, the spectrum of activity is generally not as broad as with oxidising disinfectants.

Non-oxidising disinfectants commonly used in pharmaceutical cleanrooms:

- Alcohols, which disrupt the bacterial cell membrane and have a one-minute contact time.
- Aldehydes, which take a non-specific effect in the denaturing of bacterial cell proteins and can cause coagulation of cellular protein.
- Amphoterics that have both anionic and cationic character and possess a relatively wide spectrum of activity.

- **Phenolics**. Some phenols cause bacterial cell damage through disruption of proton motive force, while others attack the cell wall and cause leakage of cellular components and protein denaturation.
- **Quaternary ammonium compounds (QAC)**, which act on the cell membrane resulting in cytoplasm leakage and cytoplasm coagulation through interaction with phospholipids.

Oxidising Disinfectants

These disinfectants generally take non-specific modes of action against micro-organisms. The range of activities taken is broader than that of non-oxidising disinfectants, with most types able to damage endospores (these pose a greater risk to human health and require greater control).

Oxidising disinfectants commonly used in pharmaceutical cleanrooms:

- Halogens, such as iodine.
- Oxidising agents, such as peracetic acid. ***Minncare® Cold Sterilant*** is one such peracetic acid solution developed for use on reverse osmosis (RO) membranes and their associated distribution systems. As an effective decontaminant and sterilant for hard, non-porous surfaces (such as plastic, stainless steel or glass) it is commonly found in pharmaceutical cleanrooms.
- Chemicals containing oxygen deposits.
- Hydrogen peroxides.
- Combination products in this category include ***Actril® Cold Sterilant***, which contains a proprietary mixture of 0.08% peracetic acid and 1.0% hydrogen peroxide. It is effective against a broad spectrum of organisms, including bacterial spores, fungal spores, bacteria, mycobacteria, yeast, moulds and viruses.

Hand Sanitisers

There are many types of hand sanitisers available commercially, the most commonly used types being alcohol-based gels or alcoholic hand rubs. With hand sanitisers, the hand rubbing technique is required to ensure efficiency, as the disinfectant properties are activated by agitating it against your hands.

Decontamination Techniques and Devices

Various decontamination techniques are available to cater for different requirements, from routine cleaning to remedial cleanroom decontamination.

The most commonly used cleanroom decontamination methods are:

Manual Methods (foaming, mopping, full-immersion, soaking, and spray and wipe)

Manual methods involve hand-spraying surfaces, immersing or soaking equipment, or wetting a mop or disinfectant wipe and wiping surfaces **to physically remove and/or kill micro-organisms.**

Fumigation/Vapour Disinfection Devices

Disinfectants used in conjunction with vaporising equipment must be sporicidal (products that utilise active oxygen, such as hydrogen peroxide, peracetic acid). The use of these disinfectants requires extensive validation, using spore-forming micro-organisms such as *Geobacillus stearothermophilus*.

Devices for Spraying, Fogging, or Gaseous Decontamination

Fogging and spraying decontamination are highly efficient methods for facilities that are suffering from large-scale bioburden outbreaks, or where cleanroom operations have to resume after a shutdown, the occurrence of a natural hazard, a power failure, or a construction event.

While the majority of the systems used for fogging and gaseous decontamination automate the distribution of disinfectants, it is important to remove any soiling before fumigation as most systems cannot penetrate the layer of dirt. Pre-cleaning of surfaces is obviously not an option if the purpose is for an emergency decontamination.

Typical fumigation or gaseous decontamination methods are processes that can penetrate HEPA filters and effectively disinfect areas that are not easily reached by manual methods. These methods can also be validated and challenged with 10^6 biological indicators to confirm microbial kill.

Disinfectants Commonly used in Decontamination by Fumigation :

- While **formaldehyde** is an effective, low-cost fumigation agent, it is a human carcinogen and leaves a residue after the process.
- **Chlorine dioxide gas, is an excellent sterilant, but has a very low allowable** human exposure limit, making it critical that the space is evacuated and sealed-off before fumigation is initiated.
- **Hydrogen peroxide vapour** is increasingly used as an excellent alternative for large volume critical environments because it is easy to contain, is non-carcinogenic, leaves no residue, and is environmentally-friendly due to its decomposition into water vapour and oxygen.
- **Peracetic acid solutions.** Peracetic acid formulations like ***Minncare® Cold Sterilant*** have been successfully used at hundreds of locations on thin film composite membranes and cellulose acetate membranes. The combination of this highly effective chemical and a dry fog delivery system enables users to rapidly and safely decontaminate even the most complex areas.

Points to Consider when Selecting Decontamination Products, Techniques and Devices

A range of different factors needs to be considered during the selection process. These include the mode of action, efficacy, compatibility, cost and consideration of current health and safety standards.

When selecting cleaning agents, the aim should be to put together a supply of products in a range of different presentations to ensure that an appropriate cleaner is available for every scenario. The same rule applies to decontamination technology, where the operator must ensure preparedness to execute the required techniques by using the appropriate technology.

Smaller spillages and routine cleaning of smooth surfaces such as laboratory benches and process area surfaces can be adequately cleaned with sterile disinfectants in trigger spray form. Larger spills in more complex surface areas, on the other hand, will probably require the use of a concentrated solution, either ready-to-use or prepared by cleanroom operators by dilution. Decontamination of the entire cleanroom environment, from surfaces to air, will require a combined effort of surface cleaning and the use of an airborne decontamination system.

Particular points to consider include:

- **Time period needed to execute decontamination technique.** Large-scale decontamination by spraying, fogging, vapour or fumigation techniques will require the cleanroom to be shut down and sealed until the process has been completed and validated. As the cleanroom will be non-operational for the period, laboratory staff will have to plan accordingly.
- **Disinfectant contact time**. When minor cleaning is undertaken, the disinfectant used must be rapid in action. Ideally, a disinfectant should require a contact time of less than 10 minutes. Slower acting chemicals will require a longer contact time, and consequently a longer period where surfaces and articles are not available for use.
- **Health and safety.** Especially when sporicidal disinfectants are required, operators must take care to evaluate the health and safety considerations of particular products before purchasing. Main concerns are for the welfare of the operators and the impact on the environment.
- The combination of disinfectants used must cover a **wide spectrum of activity** in order to kill micro-organisms of varying types and in different physiological states.
- **Environmental conditions for disinfectant efficacy**. Some chemicals require a certain temperature and/or a pH range to function optimally. Where a coldroom is the area to be disinfected, for instance, careful consideration has to go into selection of an appropriate chemical.
- **Compatibility of chemicals.** Removing dirt particles from surfaces prior to disinfection as effectively as possible is essential. Where detergents are used for this pre-cleaning, operators must ensure their compatibility with the disinfectants used in the cleanroom. Inappropriate pairing could reduce the efficacy of decontamination.
- **Residues.** Where disinfectants are likely to leave a residue on surfaces, operators must consider the interaction with other disinfectant.
- **Compatibility of surface and chemical.** When incorrectly paired, the material being disinfected could suffer corrosion or reduction of its bactericidal properties due to absorption. Chlorine-based disinfectants in particular are known to respond aggressively on contact with certain types of surfaces, commonly causing discolouration and abrasion. Although surface corrosion building up over several years is unavoidable in many cases, the residue of more aggressive disinfectants might have to be wiped down using water or a less aggressive disinfectant.
- **Applicable validation standards.** Validation standards detail how to measure the bactericidal, fungicidal, sporicidal and virucidal activity of decontamination products and processes against a range of different surfaces. The standards applicable to your cleanroom will be in part undertaken by product manufacturers and also by your pharmaceutical organisation.
- **Aseptic areas.** Where a disinfectant is to be used in an aseptic filling area, it needs to be sterile-filtered or supplied sterile and wrapped in a suitable container.

The Key to Quicker, Safer Cleanroom Decontamination

Cleanroom operators who are looking for a quicker, safer way to achieve cleanroom decontamination are bound to look into high-tech devices and systems. While semi-automatic devices are certainly more precise and reliable than manual techniques, not all decontamination systems are created equal when it comes to speed and ease of use. Those who fail to do their homework could be in for a nasty surprise when they find that their newly acquired decontamination system leaves behind a residue or and takes 30 hours to execute.

Recommended Cleanroom Bio-Decontamination Solutions

By offering a combination of liquid, ready-to-use and airborne decontamination options, Cherwell can offer solutions that decontaminate the cleanroom in as little as 4-hours. Our recommended cleanroom decontamination products and devices are manufactured by Mar Cor Purification, a leading provider of decontamination products to the cleanroom market, including the following fogging systems, liquid disinfectants, and validation strips:

Minncare® Dry Fog 2 System

An easy to use, high technology solution for cleanroom and critical area fogging within pharmaceutical and other industries concerned with maintaining the highest levels of sterility. The combination of highly effective Minncare cold sterilant chemical and a state of the art Dry Fog delivery system, enables users to rapidly and safely deliver Minncare vapour, to even the most complex areas.

Minncare® Mini Dry Fog System

An easy-to-use solution for confined space fogging in critical areas within pharmaceutical and other industries concerned with maintaining the highest levels of sterility. The flexibility of the Mini Dry Fog nozzle allows for rapid vapour dispersion and ensures the entire space is exposed to dry fog.

Minncare® Cold Sterilant

An effective decontaminant and sterilant for hard surfaces commonly found in pharmaceutical cleanrooms. Minncare Cold Sterilant is a peracetic acid solution developed for use on reverse osmosis (RO) membranes and their associated distribution systems.

Actril® Cold Sterilant

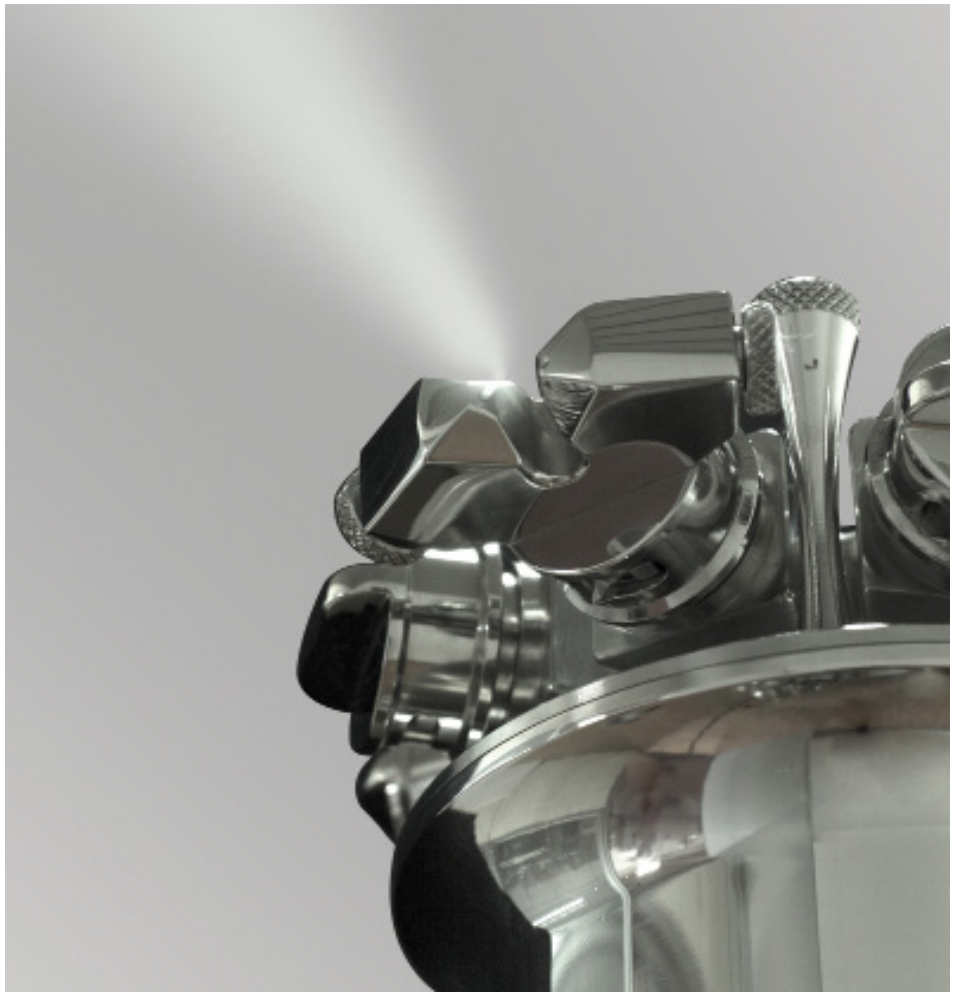
An effective, ready-to-use decontaminant and sterilant: hard surfaces in pharmaceutical cleanrooms including counter tops, walls and floors.

Minncare® Test Strips

Offering quick results, with easy-to-read indicators, Minncare 1% TS Test Strips confirm adequate sterilant concentration after dilution, whilst Minncare Residual Test Strips are used to verify residual levels after rinse-out.

Resources

- *European Chemicals Agency, Understanding Biocidal Product Regulations, https://echa.europa.eu/regulations/biocidal-products-regulation/understanding-bpr*
- *Fritz, C & Polarine, Gaseous Decontamination for Critical Environments, published in Controlled Environment Magazine, 1 October 2009, http://www.cemag.us/article/2009/10/gaseous-decontamination-critical-environments*
- *Muchemu, David, Principles of Cleanroom Validation, published in Controlled Environment Magazine, 6 November 2011, http://www.cemag.us/article/2011/11/principles-cleanroom-validation*
- *Professional and Technical Services (PTS), Cleaning & Disinfection Protocol for Cleanrooms, http://info.virox.com/hubfs/Cleanroom_Protocol_Final_Version.pdf*
- *Sandle, Tim (Microbiologist), Choosing disinfectants, published on Cleanroom Technology website, 15 September 2010, https://www.cleanroomtechnology.com/news/article_page/Choosing_disinfectants/55594*
- *Sandle, Tim (Microbiologist), Cleaning cleanrooms, published on Cleanroom Technology website, 24 November 2010, https://www.cleanroomtechnology.com/technical/article_page/Cleaning_cleanrooms/57871*



About Cherwell Laboratories

ENVIRONMENTAL MONITORING & PROCESS VALIDATION SPECIALISTS

Cherwell Laboratories was founded by Lawrence Whittard in 1971 as a veterinary diagnostic laboratory and over the years has evolved to where it is today – specialist suppliers of products for environmental monitoring, cleanroom bio-decontamination and process validation for healthcare, pharmaceutical and industrial applications. From our site in Bicester, we manufacture Redipor® Prepared Media, our own range of microbiological media products which has been developed to meet the specific needs of our customers. We are also the UK distributor for SAS microbial air samplers, ImpactAir® slit-to-agar microbial sampler and cleanroom bio-decontamination products from Mar Cor Purification.

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