

# How Clean is Clean?

Understanding the various cleaning levels can help you identify an appropriate safety and decontamination plan for your facility.

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These days, issues of contamination control have been thrown into the daily spotlight, whether from the news reporting on a Norovirus outbreak on a cruise ship, a MRSA outbreak in the local hospital, or your fear of catching a cold from a co-worker or family member. These same issues exist in today's laboratory, research, and production environments as well. This article will focus on the various levels of cleanliness as it applies to the laboratory area, and the appropriate methods of biological contamination control.

## Cleanliness Defined

The various levels of biological cleanliness can be broken down into three categories: sanitization, disinfection, and sterilization. Sanitization and disinfection are both described as the destruction of "most" microorganisms on a surface, whether by heat or chemicals. When we take a closer look at the two, we are able to further define them quantitatively by the bio-burden reduction that each provides. The bio-burden is defined as the degree of microbial contamination or microbial load; or the number of microorganisms contaminating an object. To evaluate the bio-burden reduction, we start with a known number of spores and expose them to the agent and then evaluate if we have successfully destroyed all or

some of the spores. Typically we start with a population of 4 log or 6 log ( $10^4$  or  $10^6$ ) spores of a specifically known spore that is resistant to kill, such as *Geobacillus Stearothermophilus* or another similar spore.

**Sanitization** will offer a contamination reduction or bio-burden reduction of 99.9% or 3 log ( $10^3$ ). This means that we can expect that out of one million microorganisms, a sanitizer will destroy approximately 990,000 of the organisms leaving behind many viable microorganisms to reproduce. Sanitization is accomplished by utilizing chemicals and gels to achieve this level of cleanliness.

**Disinfection** will offer a bio-burden reduction of 99.99% and up to 99.999% or up to 5 log ( $10^5$ ). This means that we can expect that out of one million microorganisms, a disinfectant will destroy up to 999,990 of the organisms leaving behind very few, but still some, viable organisms. Disinfection is accomplished by utilizing many different chemicals or ultraviolet light.

**Sterilization** is the statistical destruction of all microorganisms and their spores. This is defined as 6 log ( $10^6$ ) or a 99.9999% reduction. Statistically, this definition is accepted as zero viable organisms surviving. Steriliza-

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tion is accomplished via several methods including ionized hydrogen peroxide or other hydrogen peroxide based solutions, high heat, ultraviolet light, ozone, radiation, and chemicals (chlorine, formaldehyde, glutaraldehydes, etc.).

Now we can apply these definitions to their applications in today's laboratory animal facilities. Here we find many types of surfaces, equipment, materials, people, and animals—all of which have contamination control challenges. You need to protect your lab workers, your animals, and, hopefully, the environment. To accomplish this, a detailed contamination control plan should be in place for all laboratory facilities.

## **Methods of Providing the Appropriate Level of Cleanliness**

### ***Sanitization***

Sanitization can be accomplished in a very easy and inexpensive manner. We've all been trained from the time we were small children to wash our hands before eating. Washing your hands often will help protect you from germs. The Centers for Disease Control and Prevention (CDC) recommends that when you wash your hands with soap and warm water, that you wash for at least 15 to 20 seconds. When soap and water are not available, alcohol-based disposable hand wipes or gel sanitizers may be used. If using gel, rub your hands until the gel is dry. The gel does not need water to work. The alcohol in it kills the germs on your hands. Washing your hands before entering or leaving a laboratory should be a key component of any contamination control plan. By doing this, you will protect both your work from any germs that may have been on your hands prior to entering the laboratory, as well as protecting you from taking any of the germs outside of the laboratory. Hand sanitizing should be done each and every time you enter or leave the laboratory or any other critical area.

### ***Disinfection and Sterilization***

Decontamination is any activity that reduces the microbial contamination of materials or surfaces to prevent inadvertent infection. The appropriateness of a decontamination procedure depends on your goal. Do you wish to disinfect or sterilize? Will you be using the disinfectant on hard surfaces, in a biosafety cabinet, on instruments, or waste? Disinfection results in destruction of specific pathogenic microorganisms and refers to the elimination of virtually all pathogenic organisms on inanimate objects and surfaces thereby reducing the level of microbial contamination to an acceptably safe level. When choosing a disinfectant, one should consider the organism, the item to be disinfected, and the cost and ease of use of the disinfectant. Disinfection should be performed on all work surfaces and high touch areas including benches, countertops, bench top equipment, door and cabinet knobs, work surfaces in biosafety cabinets, incubators, etc., and will provide a higher level of cleanliness than sanitization.

Microorganisms vary in their resistance to destruction by physical or chemical means. A disinfectant that destroys bacteria may be ineffective

against viruses or fungi. There are differences in susceptibility between various bacteria, and sometimes even between strains of the same species. Bacterial spores are more resistant than vegetative forms, and non-enveloped, non-lipid-containing viruses respond differently than do viruses which have a lipid coating. Information on the susceptibility of a particular microorganism to disinfectants and physical inactivation procedures can be found in the material safety data sheet (MSDS) for that agent. MSDSs provide additional details such as health hazards, containment requirements, and spill response procedures.

Direct contact between the disinfectant and microorganism is essential for disinfection. Microorganisms can be shielded within air bubbles or under dirt, grease, oil, rust, or clumps of microorganisms. Agar or proteinaceous nutrients and other cellular material can, either directly (through inactivation of the disinfectant) or indirectly (via physical shielding of microorganisms) reduce the efficacy of some liquid disinfectants. The majority of chemical disinfectants have toxic properties. Follow the manufacturer's directions for use and wear the appropriate personal protective equipment (e.g. gloves, eye protection, apron), especially when handling stock solutions.

Sterilization refers to the destruction of all microbial life, including bacterial endospores. For example, surgical instruments must be sterile, but what about your facility and equipment? From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million, or a reduction of the bio-burden by  $10^6$ . This is more commonly known as a six log reduction and often referred to as the "sterility assurance level."<sup>1</sup>

Traditionally, sterilization is best achieved by physical procedures such as steam autoclaving, which is the most practical option for the majority of laboratories for both sterilization and decontamination purposes of small equipment, supplies, and waste. However, steam sterilization is not practical in all applications. Instruments or materials which cannot withstand sterilization in a steam autoclave or dry-air oven can be sterilized with many different solutions, including ionized hydrogen peroxide or other gaseous sterilants. Ionized hydrogen peroxide is one of the newest technologies providing sterilization for rooms and buildings as well as providing excellent results for use on equipment, such as isolators, incubators, etc.

Hydrogen peroxide has long been known as an effective sterilant both in liquid and vapor forms. The process of aerosolizing and ionizing the aerosol positively charges the mist droplets causing them to disperse more like a gas than a non-ionized droplet because the droplets are now mutually repulsive, having the same polarity. These active droplets are attracted to negatively charged surfaces. Once the droplet attaches to the surface, the attachment point is no longer available for other droplets causing them to search for and attach to an unoccupied point. This process continues until the surface is uniformly covered by a very fine layer of sterilant, even covering hard-to-reach areas such as the underneath side of ledges and small cracks and crevices.

A second result of ionizing the fine mist is the breaking apart (disassociation) of the constituents of the hydrogen peroxide into reactive species such as hydroxyl radicals, reactive oxygen species (ROS), and reactive nitrogen species. Micro-organisms (proteins, carbohydrates, and lipids) are destroyed by these reactive species through a process called lysing, or disintegration of the cell wall causing the exposure and killing of the cell nucleus.

The ionized hydrogen peroxide process offers many advantages—there is no precipitate or any other chemical residue as the only byproducts of the process are oxygen and water, which both safely evaporate into the atmosphere, making it a truly environmentally friendly “green” choice.

### Developing a Decontamination Plan

If your laboratory is certified ABSL-3 or ABSL-4, you must pass several rigorous testing requirements as set forth by the CDC and the NIH. In order for you to meet those certification requirements annually, you will likely need to decontaminate your facility prior to the certification process. In addition to the annual certification, you may choose to decontaminate your facility in order to eliminate any risks of cross contamination between differing experiments, or have the need once a contamination problem has affected your work, animals, facility, or staff. Regardless of the reason, there will be a time when decontamination is required.

Part of your safety plan, contamination control plan, and laboratory certification will require you to have a decontamination plan in place. When evaluating and choosing a decontamination method, several factors should be considered:

- Will it be effective in killing the contaminants in the lab?
- Will it reach all surfaces, cracks, and crevices?
- Will it be safe on your equipment and surfaces, without causing any damage to them?
- Will it be able to be performed within your time constraints?
- Will it be environmentally friendly?
- And lastly, do you want to own your own equipment for this process, or contract the service to a competent company?

If you choose to purchase equipment, the purchase price should not be the only consideration. Maintenance costs and the training requirements should be considered. Do you have the appropriate personal protective equipment (PPE)? Is your staff appropriately trained to operate the equipment safely and effectively? Do you have the room to store this equipment?

If you choose to contract these services out to a competent supplier, consider the response time to an emergency (total time to perform the service and the technology utilized by the contractor). Be sure to confirm they have the appropriate insurances in place. Price should be an important factor, but decisions based on price alone usually turn out to be bad decisions.

It is always best to make these decisions prior to having an emergency need. Being prepared is a key component to a good safety plan and decontamination plan. Consult with a professional today so you can be prepared when the need arises, making an emergency or scheduled decontamination process easier to overcome.

### Reference

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