

## **Bio-Burden Reduction in Biological Laboratories**

In biological laboratories samples worked with can harbor microorganisms that could be pathogens. Work is also done with microorganisms that are known pathogens. In addition to the use of proper and appropriate aseptic techniques when handling samples/microorganisms, it is vitally important for the safety of all laboratory personnel that the facility and laboratory rooms are maintained clean, clutter free, and organized.

It is important to be knowledgeable about how to establish a required level of cleanliness for the task that is being performed. This will prevent contamination of the specific sample/microorganisms/cell cultures that are worked with, and also prevent contamination of the room and personnel who are working with the material. To prevent contamination is also essential for the successful completion of any research work involving living microorganisms.

All personnel should be trained in processes that are set up to maintain a required level of cleanliness. The greatest source for contamination is the people who work in the laboratories and the subjects of their research. It is critical that protocols to maintain a required level of cleanliness are established, personnel are trained, and processes/techniques that are established to achieve the necessary cleanliness are documented and recorded.

### **Ways to reduce bio-burden**

The bio-burden is defined as the degree of microbial contamination or microbial load; or the number of microorganisms contaminating an object. There are different ways to reduce the level of bio-burden. Areas/surfaces/utensils/equipment can be cleaned, sanitized, disinfected, or sterilized. The key difference between all these ways to receive cleanliness is the level of bio-burden reduction. The lowest level of bio-burden reduction is achieved by cleaning, then sanitizing, followed by disinfection, and finally by sterilization.

Cleaning is general removal of debris, dirt, and spills from floors, laboratory table tops, and equipment. Cleaning reduces the amount of organic matter that contributes to proliferation of microorganisms.

Sanitation, as it applies within the food industry, means the adequate treatment of food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance and in substantially reducing numbers of other undesirable microorganisms. In laboratory settings, hand sanitizing is something that needs to be done each and every time a person enters or leaves the laboratory.

Disinfection is defined as a bio-burden reduction of 4 to 5 logs, or reducing the number of microorganisms by 99.99% (4-log) to 99.999% (5-log). Disinfection removes most organisms

present on surfaces that can cause infection or disease by killing most bacteria, many types of viruses, some fungi, but not prions.

Sterilization is defined as the statistical destruction of all microorganisms and their spores. This is referred to as a 6-log kill or 99.9999% reduction of the bio-burden.

The setting and type of work that will be done typically dictate the best method to reduce the level of bio-burden. For example, if one is working in a vivarium one should expect the instruments that are used for surgery to be sterile, but it may be sufficient for the surrounding areas to be disinfected daily.

Each level of bio-burden reduction can be achieved in many different ways. Disinfection or sterilization, however, cannot be achieved without pre-cleaning. Since organic material diminishes the effect of disinfectants, bio-burden must first be reduced for processes to be effective. Therefore, the first step is to remove visible soil from the surface/equipment. It is important to avoid organic material drying on surfaces by rinsing or soaking. When dealing with instruments, disconnecting or separating instrument parts may be necessary.

### **Disinfection**

Typically disinfection is done with various chemicals and applied by hand. [Environmental Protection Agency \(EPA\)](#) registered products can be selected as disinfectants to achieve the desired level of disinfection. It is important to follow the manufacturer's recommendations to avoid damage to surfaces. Use the correct dilution (more is not better!), correct contact time, and correct temperature! Personal protective equipment (PPE) needs to be used when mixing chemicals or disinfecting surfaces/equipment (long sleeved gowns/lab coats, eye protection, such as chemical goggles, and gloves).

### **Sterilization**

Sterilization requires a higher level of technology than disinfection. To achieve sterilization, dry heat, steam, peracetic acid, or gases such as ethylene oxide, vaporized hydrogen peroxide, chlorine dioxide, or formaldehyde can be used. In order to achieve a sterile room/surface environment in any setting, you will need to introduce some type of gas/vapor, as it is impractical to use heat in an open environment. These processes involve mechanical controls of the air handling system, and appropriately trained personnel or contractor to apply them in a safe and effective manner. Steam sterilization using an autoclave achieves rapid heating and penetration. The exposure times are short (when autoclave is loaded correctly) and there are no toxicity effects to workers. The method is inexpensive but can damage some delicate instruments. Items to be sterilized must be clean and free of protein (e.g., blood) or other organic material and packaged so that the steam can penetrate.

## **Monitoring**

Monitoring of environmental cleaning processes can simply be done by visual inspection. The use of ultraviolet (black) light is also an easy method. It detects fluorescent gels (chemical markers fluorescence if not removed during cleaning) and is an excellent training tool, but fails to measure the actual removal of biological matter. Another technique is microbiological testing; microbiology tests give the most quantitative, specific results for pathogens or bacteria on a surface, but results are slow and tests are expensive. The bio-burden of surfaces can be monitored in real time using adenosine triphosphate (ATP) bioluminescence technology. ATP tests detect all organic matter, which includes microbiological material, and deliver those results quickly in 15 seconds. Though ATP testing cannot replace microbiological testing for the identification of pathogens and specific microorganisms, ATP testing can serve as an indicator for potential microbiological matter.

## **Validation**

Any process that is used to achieve either disinfection or sterilization should be validated. Validation can be done in several different ways. Swab samples or contact plates done prior to the chemical application and then after the application will show what was present prior to and then after the process. In addition, biological indicators populated with various spores to a known minimum level (4-log, 5-log, or 6-log) can be placed in the area before a process is performed and then incubated to show total destruction of the spores.

## **Developing a Decontamination Plan**

A decontamination plan should be a part of every biological laboratory's safety plan. The decontamination plan should include all surfaces and equipment that can reasonably be expected to be contaminated by microorganisms. Responsibilities and frequency for cleaning and disinfecting equipment and surfaces need to be defined. Cleaned/disinfected items should also be recognizable (e.g., labeled with date/time when decontamination was done). Appropriate PPE needs to be available for personnel performing the task.

When evaluating and choosing a decontamination method, some factors that should be considered are:

- Will it be effective in killing the microorganisms used in the laboratory/contaminants entering the laboratory area?
- Will it reach all surfaces, cracks, and crevices?
- Will it be safe on equipment and surfaces, without causing any damage to them?
- Will it be able to be performed within time constraints?
- Will it be environmentally friendly?



