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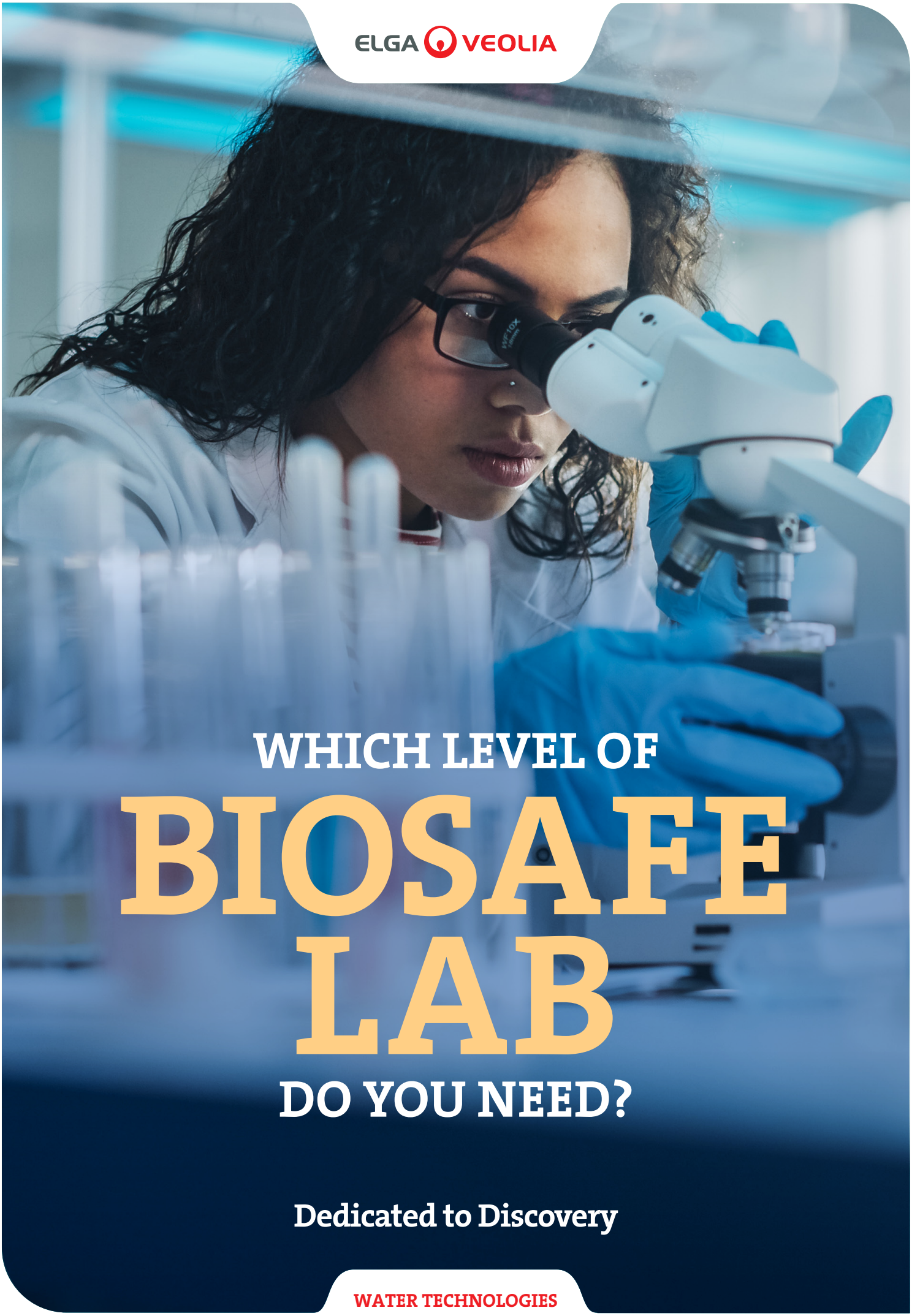


WHICH LEVEL OF BIOSAFE LAB DO YOU NEED?

Dedicated to Discovery

WATER TECHNOLOGIES

Inside

INTRODUCTION

The right biosafe lab

...for your needs

Understanding the principles of biosafety is key to minimizing the chance of laboratory-associated infections and contamination of the environment. This means working at the appropriate safety level for a given organism or toxin, and also encompasses the use of well-executed risk assessments, the adherence to defined microbiological practices, and effective containment for the level concerned.

This paper outlines the nature of the risk assessments you might be expected to conduct ahead of any given project, either in setting up, upgrading or running a biosafe lab. It goes on to examine best laboratory practices at the different biosafety levels, and ends with a brief look at the safety aspects of a selection of disease organisms.

Risk criteria

ESTABLISHING THE CORRECT LEVELS OF CONTAINMENT

The primary risk criteria used to define the four ascending levels of containment, referred to as Biosafety Levels (BSL) 1 through to 4, are: infectivity, severity of disease, transmissibility, and the nature of the work being conducted. Each level of containment has its associated microbiological practices, safety equipment, and facility safeguards to help protect non-laboratory occupants of the facility, the public health, and the environment. Before we go into more depth, here is a quick summary of the different levels.

Biosafety Level 1 (BSL-1) is the basic level of protection and is for labs working with defined and characterized strains of viable biological agents that are not known to cause disease in immunocompetent adult humans.

Biosafety Level 2 (BSL-2) is for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure.

Biosafety Level 3 (BSL-3) is for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections, and that are indigenous or exotic in origin.

Biosafety Level 4 (BSL-4) is for exotic (non-indigenous) agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available are restricted to high containment laboratories.

Using the most infectious agents also means extensive security measures are in place, not only because of their virulence but also because of their potential for use in bioterrorism. **Examples** are the Ebola virus, the Lassa virus, and any **agent** with unknown risks of pathogenicity and transmission.

An outline guide to the facility requirements and practices for the different biosafety levels is given in Table 1, and our associated white paper gives a more detailed description of the steps to consider when building biosafe labs.[1]

BS Level >	BSL-1	BSL-2	BSL-3	BSL-4
Agents	Well-characterized agents that do not consistently cause human disease and present minimal potential hazard to lab personnel and their environment e.g. *E. coli*	Agents associated with human disease that pose moderate hazards to personnel and environment e.g. *H. influenzae, Borellia spp, (Lyme disease) HIV*	Indigenous or exotic agents that may cause potentially lethal disease on exposure via inhalation e.g. *M. tuberculosis*	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted lab infections and potential fatal diseases for which there are no vaccines or treatments, or which have an unknown risk of transmission e.g. *Ebola virus*
Special practices	Standard microbial practices	Limited access; occupational medical services including risk assessment; training on procedures including decontamination	Limited access; viable material removed in primary and secondary containers; opened only in BSL-3 labs, all procedures in BSC	Clothing change on entry; daily inspections of containment and life support systems; all wastes decontaminated before removal from lab; shower on exit
Primary barrier and personal protective equipment	No primary barriers required; protective lab clothing, protective face and eyewear	BSCs (biological safety cabinet) or other primary containment device used for experiments that may cause splashes or aerosols; protective clothing, other PPE and respiratory protection as needed	BSC for all procedures with viable agents; solid front gowns; scrubs; gloves; eyewear and respiratory protection	Physical separation from access corridors; access via two consecutive self-closing doors; hands-free sink near exit; sealed windows, ducted air ventilation system with negative airflow into lab; autoclave, preferably in lab
Facilities (secondary barrier)	Lab doors; sink for handwashing; lab bench; windows fitted with screens; autoclave available.	Self-closing doors; sink located near exit; windows sealed or with fitted screens; autoclave available.	Physical separation from access corridors; access via two consecutive self-closing doors; hands-free sink near exit; sealed windows, ducted air ventilation system with negative airflow into lab; autoclave, preferably in lab.	Entry sequence; entry through airlock with airtight doors; walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system; double door, pass-through autoclave.

Table 1. Summary of the different biosafety levels, from BSL-1 to BSL-4

Risk management

IN BIOSAFE LABS

Laboratory-associated infections (LAIs) are relatively infrequent, representing a mere 10s of incidents globally over several years, but it is critical that research and service lab communities remain vigilant.[2] The accidental release of microbial aerosols is a major cause of many LAIs.[3] Attention to work practices, safety equipment, and engineering controls are essential, alongside worker training and the ability to recognize potential hazards and correct unsafe habits: in short, there must be *adequate risk management*.

Risk management must also take account of the fact that **not all possible adverse incidents can be predicted**, so judgments and decisions about biosafe lab control measures sometimes need to be based on incomplete information. For example, clinical laboratories may not always know the content of a given sample, since they are typically looking to identify the causative agent for a medical diagnosis.

The initial factors to consider fall into two broad categories: **agent hazards and laboratory procedure hazards**. Following the assessment of the inherent risk, the Biosafety Level is determined.

Before implementation, the results of the risk assessment and the selected safeguards are reviewed, the integrity of safety equipment is checked, the proficiency of staff regarding safe practices is evaluated, and any training or competency gaps addressed. This process is repeated regularly and updated as needed, for example if a lab is looking to expand its sampling capabilities.

1.

ASSESSING AGENT HAZARDS

The inherent risk considers the principle hazardous characteristics of the agent. These include its capability to infect and cause disease in a susceptible host, severity of disease, and the availability of preventive measures and effective treatments. Possible routes of transmission of infection in the laboratory also need to be examined, as do its infectious dose (ID), stability in the environment, host range, whether the agent is indigenous or exotic to the local environment, and the genetic characteristics of the agent.[4]

The identification and assessment of hazardous characteristics of genetically modified agents are the same as those for the equivalent wild-type organism. It is particularly important to address the possibility that the genetic modification could increase or decrease an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult to complete because important information may not be available for a newly engineered agent.

In the lab, the most likely routes for transmission are:

- **Direct skin, eye or mucosal membrane exposure to an agent**
- **Parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and insect vectors**
- **Ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure**
- **Inhalation of infectious aerosols.**

An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent.[5]

It should also be remembered that the nature and severity of disease caused by a laboratory-associated infection and the probable route of transmission of the infectious agent in the laboratory **may differ** from the route of transmission and severity associated with the naturally acquired disease.[4]

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is obviously a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. ID and agent stability are particularly important in establishing the risk of airborne transmission of disease e.g. *Coxiella burnetii*, the causative agent of Q fever, has an incredibly low inhalation ID, which is estimated to be 10 inhaled infectious particles, and its resistance to environmental stresses enable it to survive outside of a living host or culture media long enough to become an aerosol hazard.[5]

2.

ASSESSING LABORATORY PROCEDURE HAZARDS

The principle hazards here are: agent concentration, suspension volume, equipment and procedures that generate aerosols and droplets, and the use of syringes and sharps. Procedures involving animals present additional hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

A careful operator will minimize the generation of aerosols. For example, where an operator in a hurry may use a sonic homogenizer with maximum aeration, a more careful operator will consistently use the device to ensure minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.[5]

Procedures and equipment that generate respirable particles will also generate larger size droplets that settle out of the air rapidly, contaminating hands, work surfaces, and possibly the mucous membranes of the persons performing the procedure. The respirable component is relatively small; in contrast, hand and surface contamination can be significant.[6] Therefore the potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

3.

ASSESSING FACILITY CONTROL HAZARDS

Consideration of facility safeguards is an integral part of the risk assessment, since they help prevent the accidental release of an agent from the laboratory.

For example, one facility safeguard is **directional airflow**, which helps to prevent aerosol transmission from a laboratory into other areas of the building. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow may compromize safe laboratory operation. BSL-4 containment facilities provide more complex safeguards that require significant expertize to design and operate.

Working at the different biosafety levels

Assuming we have ascertained the required biosafety level for our specific organism and sample type, let us now look at ways of working at each level. A list of organisms classified into recommended containment levels can be found here. Additional information relating more specifically to the building of biosafe labs at these different levels can be found in our white paper.

Note that the level of containment does not only depend on the organism itself, but also on the type of sample in which it is lurking. For example, BSL-2 may be adequate if a pathogen is in relatively low concentration. However, on enrichment, or depending on the precise nature of the experiment, BSL-3 may be required for the same organism.

BIOSAFETY LEVELS

basic classes of laboratory risks from low to high

BSL-1 BSL-2 BSL-3 BSL-4

BSL-1

BIOSAFETY LEVEL 1

Biosafety level one, the lowest level, applies to work with agents that usually pose a minimal potential threat to laboratory workers and the environment and do not consistently cause disease in healthy adults. Non-pathogenic *Escherichia coli, Bacillus subtilis, Naegleria gruberi,* infectious canine hepatitis virus, and exempt organisms under the NIH Guidelines are examples of the biological agents meeting these criteria.

BSL-1 represents a basic level of containment that relies on standard, microbiological best practices and procedures with no special primary or secondary barriers, other than a door, a sink for handwashing, and non-porous work surfaces that are cleanable and easy to decontaminate.

BSL 1 labs are not usually isolated from the general building. Training on the specific procedures is given to the lab personnel, who are supervized by a trained microbiologist or scientist. Standard microbiology practices include mechanical pipetting only (no mouth pipetting allowed), safe sharps handling, avoidance of splashes or aerosols, and decontamination of all work surfaces when work is complete e.g., daily.

Decontamination of spills is done immediately, and all potentially infectious materials are decontaminated prior to disposal, generally by autoclaving. Standard microbiological practices also require attention to personal hygiene, i.e., hand washing and a prohibition on eating, drinking or smoking in the lab. Normal laboratory personal protective equipment is generally worn, consisting of eye protection, gloves and a lab coat or gown. Biohazard signs are posted and access to the lab is limited whenever infectious agents are present.

BSL-2

BIOSAFETY LEVEL 2

Biosafety level two is required when working with agents associated with human disease: pathogenic or infectious organisms posing a moderate hazard. Hepatitis B virus, human immunodeficiency virus (HIV), *Salmonella*, and *Toxoplasma* are all examples.

Work done with any human, animal, or plant-derived specimens (e.g., blood, body fluids, tissues, or primary cell lines), where the presence of a biological agent or toxin may be unknown, can also often be safely conducted under conditions typically associated with BSL-2.[5] With good practices and procedures, these agents and toxins can generally be handled safely on an open bench, provided the potential for producing splashes and aerosols is low.

Because of their potential to cause human disease, care is taken to prevent percutaneous injury (e.g., needles, cuts), ingestion and mucous membrane exposures, in addition to the standard microbiological practices of BSL-1. Contaminated sharps are handled with extreme caution. Use of disposable syringe-needle units and appropriate puncture-resistant sharps containers is mandatory. Direct handling of broken glassware is prohibited, and decontamination of all sharps prior to disposal is standard practice. The laboratory's written biosafety manual details any needed immunizations (e.g., hepatitis B vaccine or TB skin testing) and whether serum banking is required for at-risk lab personnel. Access to the lab is more controlled than for BSL-1 facilities. Immunocompromized personnel, or those with increased risk for infection may be denied admittance at the discretion of the laboratory director.

BSL-2 labs must also provide the next level of barriers, including those at BSL-1. This might include a Class II biosafety cabinet (BSC) or equivalent containment device for work with agents, and an autoclave, or other suitable method for decontamination within the lab. A readily available eyewash station is also needed, and self-closing lockable doors and biohazard warning signs are required at all access points. Waste decontamination capabilities to reduce the potential of environmental contamination, and the separation of laboratory spaces from office and public spaces to reduce the risk of exposure to other personnel should be considered.

BSL-3

BIOSAFETY LEVEL 3

BSL-3 standard practices, safety equipment, and facility specifications are applicable to laboratories in which work is performed using indigenous or exotic biological agents with a potential for respiratory transmission and those that may cause serious and potentially lethal infection by simple inhalation of particles or droplets.

Mycobacterium tuberculosis, St. Louis encephalitis virus, and *Coxiella burnetii* are examples of the biological agents that meet these criteria. Working with these agents is strictly controlled and must be registered with all appropriate government agencies.

The primary routes of exposure to personnel working with these types of biological agents and toxins relate to accidental exposure via the percutaneous or mucosal routes and inhalation of potentially infectious aerosols. At BSL-3 more emphasis is placed on primary and secondary barriers to protect personnel, the surrounding community, and the environment from exposure to potentially infectious aerosols.

Add to all the BSL-2 practices and equipment even more stringent access control and decontamination of all wastes, including lab clothing before laundering, within the lab facility. Baseline serum samples are collected from all lab and other at-risk personnel as appropriate.

All procedures involving the manipulation of infectious materials are conducted within a BSC or other primary containment device. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other primary containment strategies are implemented, based on a risk assessment. When centrifuging, the loading and unloading of the rotors and centrifuge safety cups should take place in the BSC or another containment device.

Secondary barriers for BSL-3 laboratories include those previously mentioned for BSL-1 and BSL-2 laboratories. They also include enhanced ventilation strategies to ensure inward directional airflow, controlled access zones to limit access to only laboratory approved personnel, and may contain anterooms, airlocks, exit showers, and/or exhaust HEPA filtration.

Since extra BSL-3 precautions include entry via interlocked doors, filtered ventilation systems, and the need for easy decontamination, the plumbing of BSL-3 labs must be quite specific, as described in our white paper “Building a Biosafe Lab”. BSL-3 labs must also be equipped to decontaminate laboratory waste using an incinerator, an autoclave, and/or another method of decontamination, depending on the biological risk assessment.

BSL-4

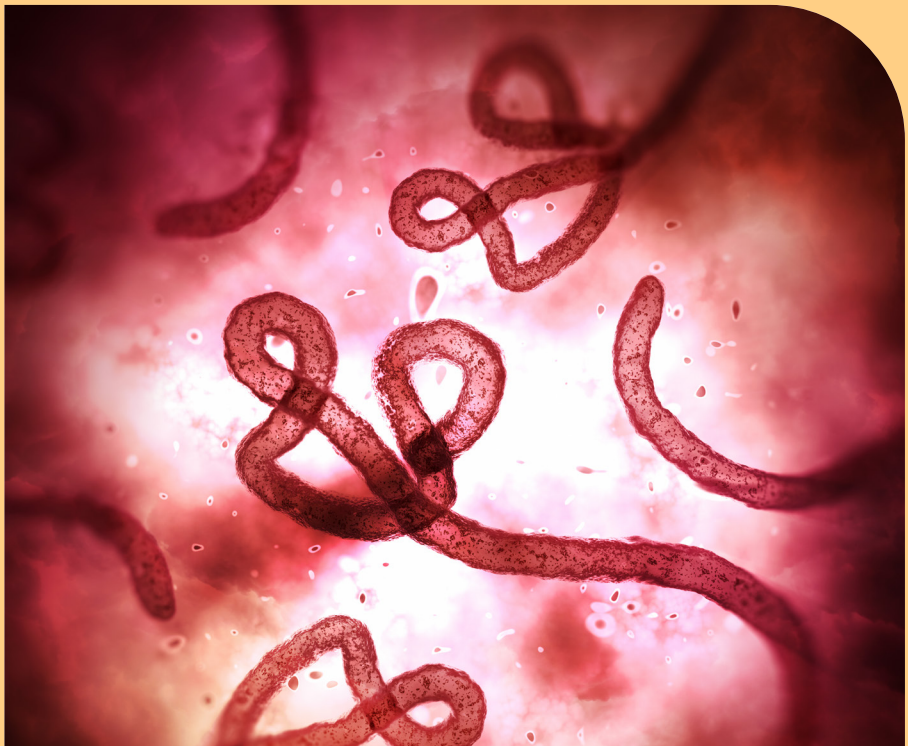
BIOSAFETY LEVEL 4

Biosafety Level 4 (BSL-4) standard practices, safety equipment, and facility specifications are applicable primarily for laboratories working with dangerous and exotic biological agents that pose a high individual risk of life-threatening disease that may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

Ebola, Lassa virus, Marburg virus and Congo-Crimean haemorrhagic fever virus are examples of the biological agents that meet these criteria. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level or to re-designate the level.

The primary routes of exposure to personnel working with these types of biological agents relate to accidental exposure via the percutaneous and mucous membrane routes and inhalation of potentially infectious aerosols. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a Class II BSC with a full-body, air-supplied positive-pressure personnel suit.

The laboratories incorporate all BSL-3 features, as well as additional safety features. Access to BSL-4 laboratories is carefully controlled and requires significant training. Additionally, the BSL-4 facility itself is often a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems, for both solid and liquid waste, to prevent the release of hazardous biological agents into the surrounding community. Complementary information on BSL-4 facilities can be found here.



Case studies

LABORATORY SAFETY AND CONTAINMENT RECOMMENDATIONS

Let us finish by looking at the biosafety levels needed for a selection of disease-causing microbes to get a feel for how the risk assessments and precautions described in this paper are put into effect when working with these organisms in the lab.

Tuberculosis

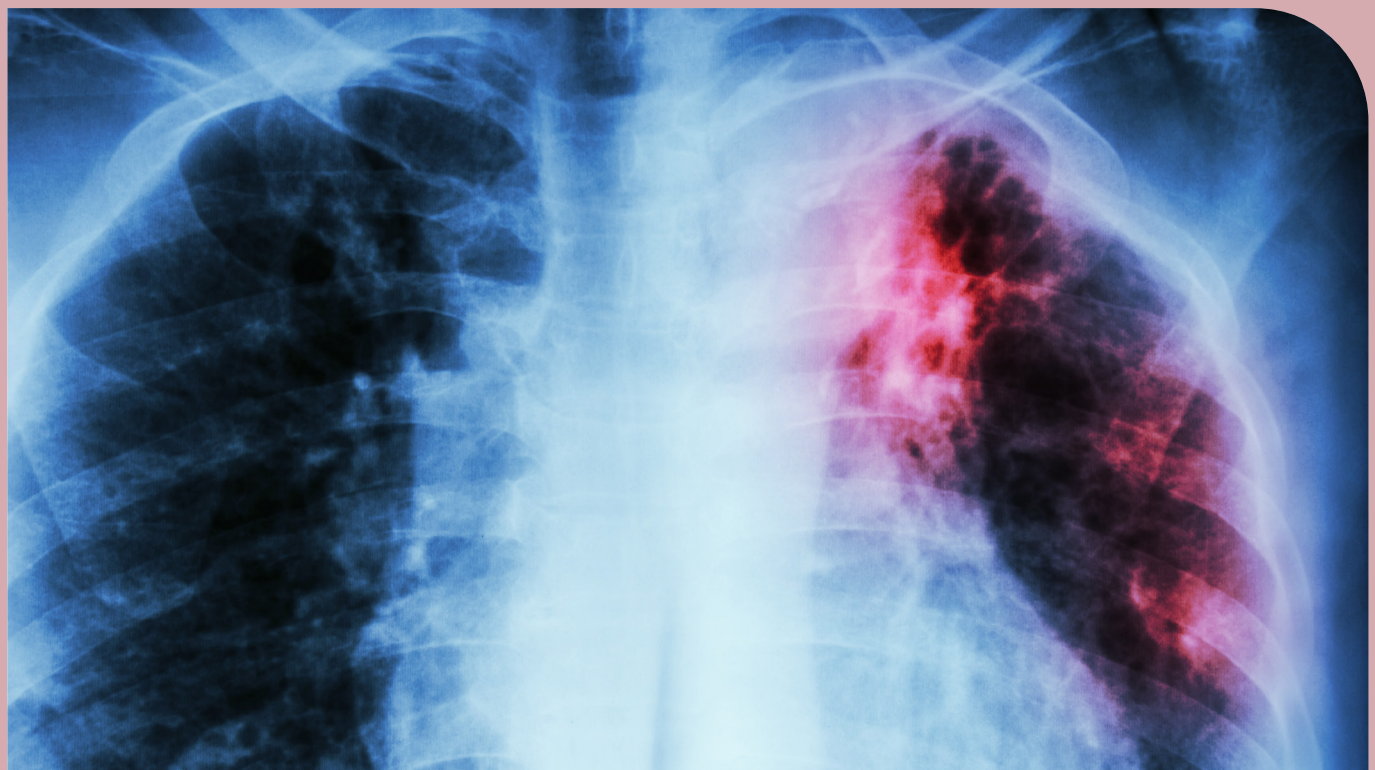
CASE STUDY / BSL-3 AND BSL-2

***Mycobacterium tuberculosis* is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide.**

Infectious aerosols produced by coughing spread disease from person to person. The primary focus of infection is the lungs. HIV infection is a serious risk factor for the development of active disease.

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebrospinal fluid gastric lavage fluids, cerebrospinal fluid (CSF), urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important laboratory hazard encountered. Tubercle bacilli can also survive in heat-fixed smears and be aerosolized in the preparation of frozen tissue sections. Because of the low infective dose of *M. tuberculosis* (<10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis should be considered potentially infectious when handling. Mycobacteria can resist disinfection and may survive on inanimate surfaces for long periods.

BSL-3 practices, containment equipment, and facilities are recommended for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. **BSL-2** practices and procedures, containment equipment, and facilities are recommended for non-aerosol-producing manipulations of clinical specimens.



SARS and MERS

CASE STUDY / BSL-3 AND BSL-2

Several human coronaviruses have been identified that can be broadly classified into low and high pathogenicity.

High pathogenic coronaviruses include SARS and MERS-CoV. SARS is a viral respiratory illness caused by SARS-associated coronavirus (SARS-CoV) within the family *Coronaviridae*. SARS is thought to be spread primarily through droplets and aerosols. The natural reservoir for SARS-CoV is unknown. Healthcare workers are at increased risk of acquiring SARS or MERS from an infected patient. For the latest biosafety recommendations regarding work with SARS Coronavirus 2 (SARS-CoV-2) please consult the CDC COVID-19 website.

SARS and MERS coronaviruses may be detected in respiratory, blood, urine, or stool specimens. SARS and MERS coronavirus propagation in cell culture and the initial characterization of viral agents recovered in cultures of clinical specimens must be performed at **BSL-3**. Respiratory protection should be used by all personnel. Activities involving manipulation of untreated specimens should be performed in **BSL-2** facilities using **BSL-3** practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection should be used. In clinical laboratories, respiratory specimens, whole blood, serum, plasma, and urine specimens should be handled using Standard Precautions at **BSL-2**. Work using intact, full-length genomic RNA should be conducted at **BSL-2**.

Hepatitis

CASE STUDY / BSL-3 AND BSL-2

Hepatitis B has been one of the most frequently occurring laboratory-associated infections, and laboratory workers are recognized as a high-risk group for acquiring such infections.

Hepatitis C virus infection can occur in the laboratory as well. The prevalence of the antibody to hepatitis C (anti-HCV) is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that HCV is spread predominantly by the parenteral route. These viruses are naturally acquired from a carrier during blood transfusion, injection, tattooing, or body piercing with inadequately sterilized instruments. Non-parenteral routes, such as domestic contact and unprotected (heterosexual and homosexual) intercourse, are potential modes of transmission.

HBV may be present in blood and blood products of human origin, in urine, semen, CSF, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards. The virus may be stable in dried blood or blood components for several days. HCV has been detected primarily in blood and serum, less frequently in saliva, and rarely or not at all in urine or semen. It appears to be somewhat stable at room temperature on surfaces or equipment.
Viral infectivity is sensitive to repeated freeze-thawing.

BSL-2 facilities with additional primary containment and personnel precautions, such as those described for **BSL-3**, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. **BSL-2** practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Personnel working with HBV, HCV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.

Anthrax

CASE STUDY / BSL-2 AND BSL-3

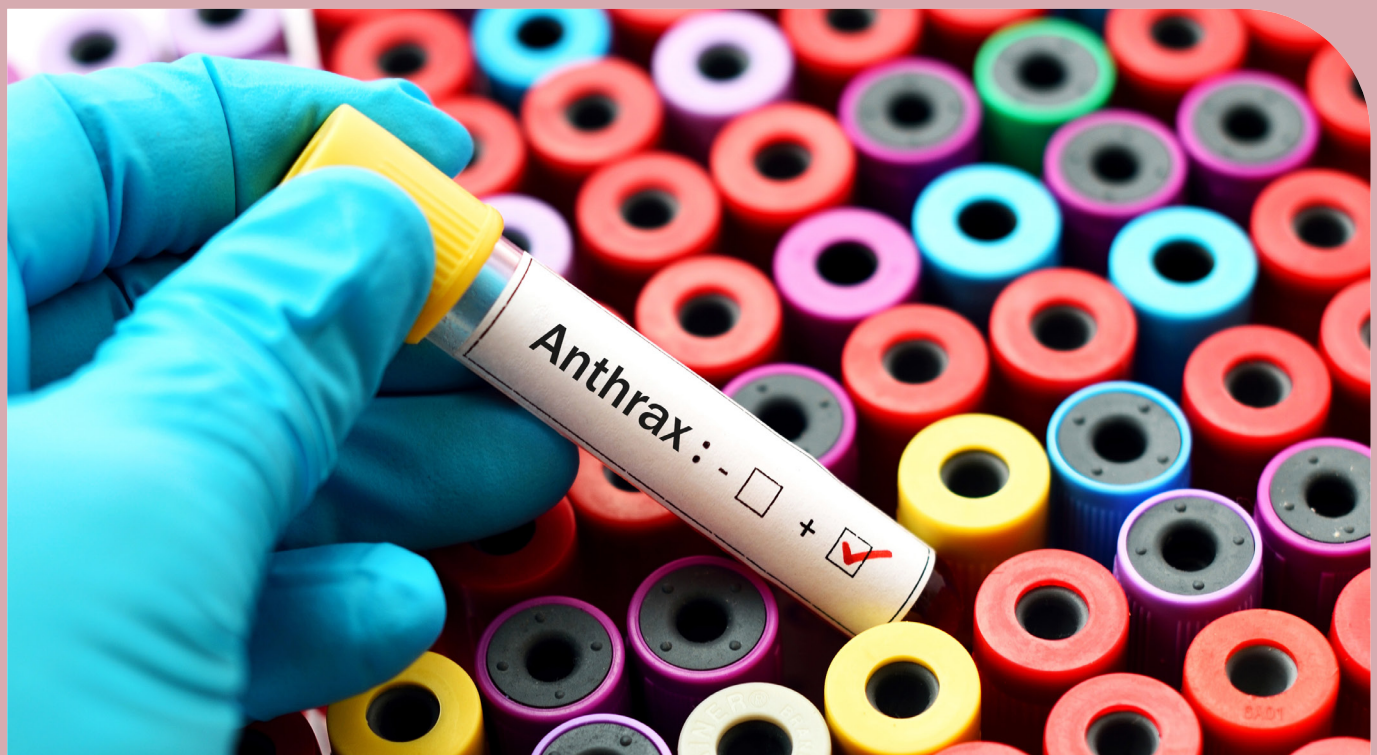
***Bacillus anthracis*, a gram-positive, non-haemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease among wild and domestic mammals, including humans. *B. anthracis* can produce spores that allow the organism to persist for years, withstanding heat and drying.**

This ability to produce spores coupled with significant pathogenic potential in humans means that this organism is considered a serious biowarfare or bioterrorism threat.

The median lethal dose (LD50) is likely within the range of 2,500–55,000 spores. It is believed that very few spores (ten or fewer) are required for cutaneous anthrax infection. Mortality rates can be 80% or more for inhalation anthrax. Cutaneous anthrax makes up > 95% of human cases worldwide and is readily treatable.

B. anthracis may be present in blood, skin lesion exudates, CSF, pleural fluid, sputum, and rarely, in urine and faeces. Primary hazards to laboratory personnel are direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Spores are resistant to many disinfectants and may remain viable on some surfaces for years.

As soon as *B. anthracis* is suspected, **BSL-3** practices are recommended for further culture and analysis. **BSL-2** practices, containment equipment, and facilities are recommended for primary inoculation of cultures from potentially infectious clinical materials. It is recommended that all centrifugation uses autoclavable, aerosol-tight rotors or safety cups that are opened within the BSC after each run. In addition, it is recommended to collect routine surveillance swabs for culture inside the rotor and rotor lid and to autoclave rotors before re-use.



ELGA LabWater and biosafety

THE ROLE OF PURE WATER IN BIO SAFE LABS

Now that you understand more about how biosafe labs are set up and run at different biosafety levels, it is time to consider the role of pure water in those experiments.

Whether you need a reliable source of Type III water for rinsing contaminated glassware, or Type I ultrapure water source for cell culture in a biosafe cabinet, find out here which ELGA Veolia LabWater purification system is the best fit for your biosafe lab.

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