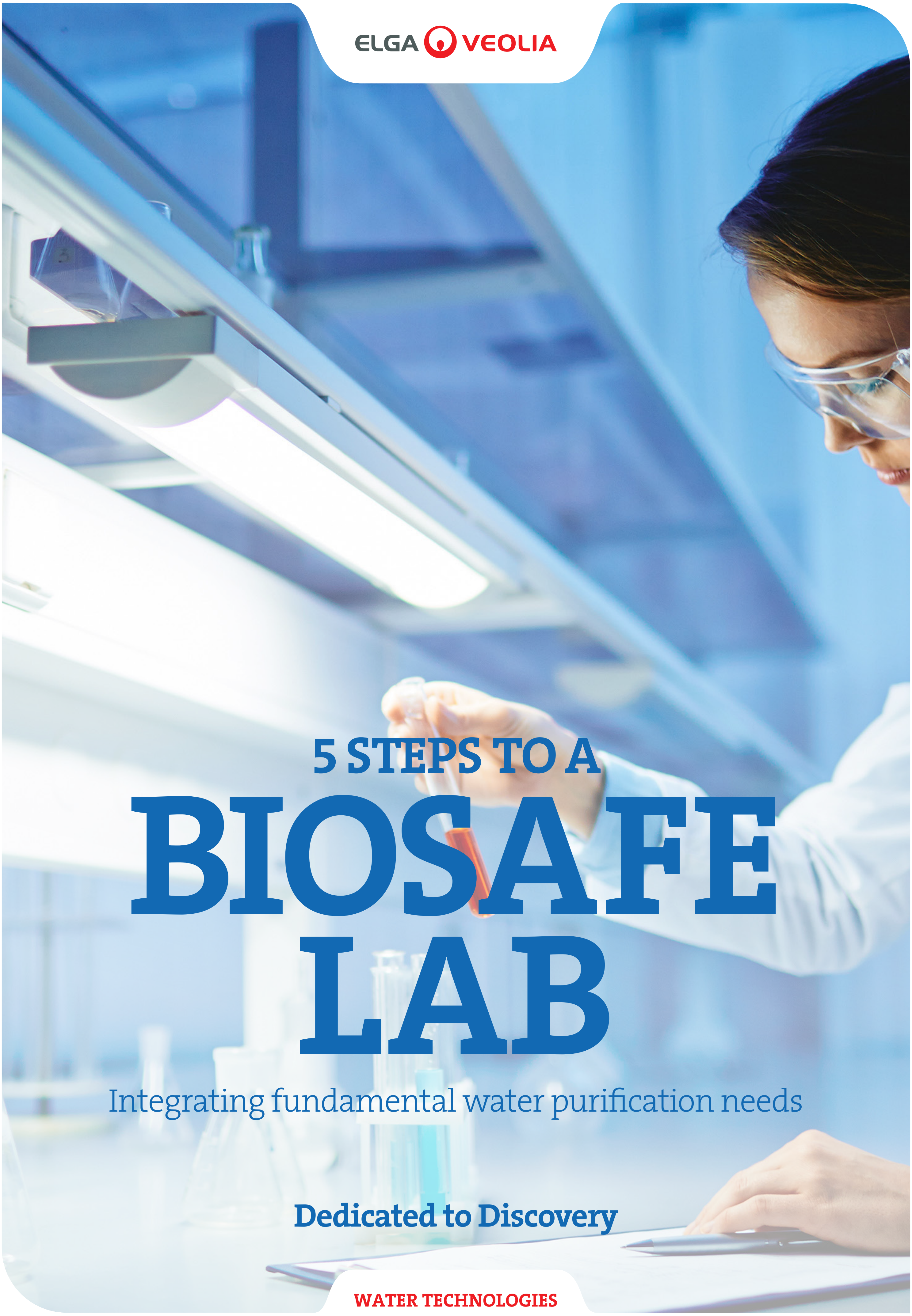


ELGA VEOLIA

5 STEPS TO A BIOSAFE LAB

Integrating fundamental water purification needs

Dedicated to Discovery

WATER TECHNOLOGIES

Inside

INTRODUCTION

Building biosafe labs

Key criteria

High-level biosafety laboratories are essential for public health, scientific research, and national biosecurity. This paper takes you through some of the key criteria to consider when designing, building and using biosafety laboratories at different levels, whether starting from scratch, or retrofitting your lab to be at a higher biosafety level than currently.

This is not an exhaustive document, nor is it intended as a regulatory guideline, but it aims to provide insights into some fundamental aspects of biosafety. It may also help you to better understand the broader needs to address with the decision makers involved in designing and using your lab, from ensuring a safe water supply to dealing with contamination and waste.

Biosafety levels

THE BASICS

Since the severe acute respiratory syndrome epidemic in 2003, China has constructed a primary network of high-level biosafety laboratories at different levels and established an expert team on laboratory biosafety.

China has also developed and published numerous laws, documents and research that together have enabled a new era of emerging infectious disease control and prevention, thus contributing to public health.[1,2]

Before we look at the challenges related to the building of different level labs and the current biosafety laboratory network in China, let us check out some of the basics (Table 1). There are four different biosafety levels, ranging from minimal containment for non-pathogens at Biosafety Level 1 (BSL-1), to the maximum level of containment provided by Biosafety Level 4 (BSL-4), for the deadliest pathogens. As seen in Table 1, the required level depends on the disease-spreading characteristics of the microbes being handled.

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Description according to biosafety risk	• No containment • Defined organisms • Unlikely to cause disease	• Containment • Moderate risk • Disease of varying severity	• High containment • Aerosol transmission • Serious, potentially lethal disease	• Maximum containment • Exotic, high risk agents • Life-threatening disease
Sample organisms (level also depends on sample type)	*E.coli*	*H. influenzae*, *Borrelia spp.* (Lyme disease), HIV	*M. tuberculosis*	Ebola virus
Pathogen type	Agents that present minimal potential hazard to personnel and the environment	Agents associated with human disease and pose moderate hazards to personnel and the environment	Indigenous or exotic agents that present a potential for aerosol transmission and cause potentially lethal disease	Dangerous and exotic agents that pose a high risk of aerosol-transmitted LAIs and life threatening disease
Autoclave requirements	None	None	Autoclave with bio-seal required in lab	Autoclave with bio-seal required in lab
Water purification needs	Type III to Type I ultrapure, in lab.	Type III to Type I ultrapure, in lab Possibly Type I in BSC.	Type III to Type I ultrapure, in lab. Supply for autoclave. Type I recommended in BSC. Specific defined plumbing requirements.	Type III to Type I ultrapure, in lab. Supply for autoclave. All types may be needed in BSC. Specialised plumbing avoids contamination.

Table 1: summary of the characteristics for each biosafety level. Abbreviations: BSC – biological safety cabinet; LAI – laboratory acquired infection.

China's biosafety laboratory network

THE CHALLENGE

At time of writing, over 20 BSL-3 laboratories are part of the Chinese Center for Disease Control and Prevention, including at least one mobile unit. In response to the COVID-19 pandemic in China, authorities in May "unveiled a plan... that requires every province to establish at least one BSL-3 lab, and major cities to have at least one BSL-2 lab."[2]

Low-level pathogenic infectious agents are tested, identified, and isolated in country-wide BSL-2 laboratories. Although a number of BSL-3 laboratories have been set up in the last decade, the government is making ongoing efforts to further improve this.[1]

Since 2003, China has successfully dealt with human infections of swine streptococcus, pandemic H1N1 influenza, the H7N9 avian influenza epidemic, and imported cases of Middle East respiratory syndrome, Zika virus disease, yellow fever, and Rift Valley fever, etc. High-level biosafety laboratories have played a significant role in the surveillance of, and basic research on these pathogens. In addition, the success of a series of large-scale events held in China, such as the 2008 Summer Olympic Games, has proven the long-term effectiveness of China's laboratory biosafety system.

The desire to expand the number and distribution of labs with higher levels of biosafety across the country brings challenges. The limited number of labs equipped to safely manage the most infectious and life-threatening of pathogens are few, and for a good reason. Building and maintaining a BSL-4 lab is not a minor project. Key issues are security, safety of staff, the community and the environment. However, building and maintaining labs of the highest biosafety will provide the fundamental support needed for a successful national and international response to disease control and prevention.

Let us now examine 5 key steps to building a successful biosafe lab, whichever level is needed, whether starting from scratch, or retrofitting an existing lab. These are:

1. Assess the biosafety risks correctly
2. Consider laboratory biosafety as more than a set of building requirements
3. Decide which facilities are needed, from BSL-1 to BSL-4
4. Ensure the right water supply where you need it
5. Standardize staff training and protocols.

1.

ASSESS THE BIOSAFETY RISKS CORRECTLY

Risk assessment is an important responsibility for directors and principal investigators. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians play a joint role.

"Risk assessment" in this context refers to the process used to identify the hazardous characteristics of a known infectious (or potentially infectious) agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a laboratory-associated infection (LAI), and the probable consequences of such an infection.

The information identified by risk assessment will then guide the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs. Laboratory directors and principal investigators should also use the risk assessment to alert their staff to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment.

Where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data is available. The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards.

In addition, the capability of the laboratory staff to control hazards must be considered. The need for regular training, and the keeping of training records forms an integral part of the maintenance of a particular biosafety level.

Finally, security and terrorist factors also join the list of risk factors that must be considered. For example, a BSL-4 lab could be a tempting target for extremist groups, with stolen materials potentially being weaponized to harm the general population, or damage to facilities posing a real threat of local contamination.

2.

CONSIDER LABORATORY BIOSAFETY AS MORE THAN A SET OF BUILDING REQUIREMENTS

It is easy to think of biosafety levels as being represented simply by the physical design and build of a lab, with a basic list of precautions that personnel need to take.

Laboratory biosafety is much more holistic than this: it describes the **application of specific practices, safety equipment and specially designed laboratories to create a safe environment, both within and outside the laboratory, for work conducted with infectious agents and toxins**. In addition, there must be monitoring for laboratory-acquired infections, and staff training, as mentioned above. Hence, laboratory biosafety consists of a **comprehensive and specific set of design, maintenance, monitoring and training guidelines for each level.**

The Biosafety Level designations BSL-1, BSL-2, BSL-3, and BSL-4 describe the minimum safe work practices, specially designed buildings, and safety equipment required to conduct work on infectious microorganisms and other biological hazards, with BSL-4 as the highest biosafety level, as seen in Table 1.

The appropriate BSL for a specific research or public health project is usually determined jointly by a team consisting of the principal investigator, the institutional biosafety professionals, and members of biosafety committees. There are extensive lists of internationally agreed standards for the different BSL levels, encompassed in directives such as those from the World Health Organisation, and the US Department of Health and Human Services.[3,4]

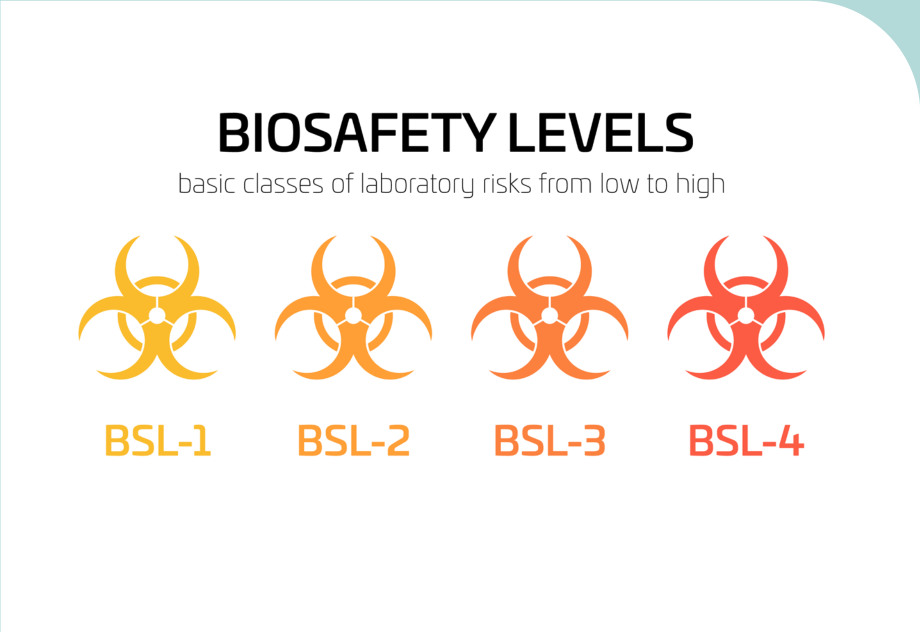
The BSL assigned to a project is based on a biological risk assessment that takes into account the nature of the infectious agent, toxin, or other biological hazard, including:

- **Its ability to cause disease and the way(s) in which it causes disease (transmissibility and pathogenicity)**
- **How much of it is required to cause disease (infectious dose)**
- **How many different organisms are susceptible to infection (host range)**
- **Its prevalence in the community (epidemiology)**
- **The specific laboratory activity or activities being performed, and**
- **The availability of preventive medical counter-measures (i.e. vaccines) or effective treatment (i.e., post-exposure vaccination and/ or use of antimicrobials, anti-virals and chemotherapeutic agents).**

The BSL assigned to a project determines the controls required to conduct the work safely:

- **Engineering controls, including facility construction and design elements in the laboratory space**
- **Safety equipment, including personal protective equipment (PPE) and biosafety cabinets**
- **Administrative controls, including specific access, training, and occupational medicine policies, and**
- **Laboratory practices and procedures workers use when working in the laboratory.**

Every biological laboratory, regardless of biosafety level, follows standard microbiological practices. Each biosafety level builds on the controls of the next lower level and has its own specific containment controls.



3.

DECIDE WHICH FACILITIES ARE NEEDED (1-4)

Most labs will likely be dealing with a variety of disease-causing organisms, from patient samples to pure cultures.

Most microbes will already have a recommended requirement for their safe handling, and it is likely that any existing or new lab set-up may well need any or all of levels BSL-1 to BSL-3, with BSL-4 in exceptional cases e.g. for newly-characterized microbes.

Let us now take a brief look at the basic design characteristics, safety equipment and laboratory practices required at each level.

STANDARD MICROBIOLOGICAL PRACTICES INCLUDE BUT ARE NOT LIMITED TO:

- Restricting lab access at the discretion of the laboratory director when experiments are in progress
- Decontaminating work surfaces once a day and after any spill of viable material
- Decontaminating liquid or solid waste before disposal
- Use of mechanical pipetting devices: mouth pipetting is prohibited
- Prohibiting of eating, drinking, smoking, and applying cosmetics in the work area
- Washing hands after handling viable materials and animals and before leaving the lab
- Minimizing the creation of aerosols by careful handling and processing of samples
- Wearing laboratory coats, gowns, or uniforms to prevent contamination or soiling of street clothes.

BIOSAFETY LEVEL 1 (BSL-1)

BSL-1 labs are used to study infectious agents or toxins not known to consistently cause disease in healthy adult humans or animals. Workers follow basic safety procedures, called standard microbial practices, and require no special equipment or design features.

Standard engineering controls in BSL-1 laboratories include easily cleaned surfaces that can withstand the basic chemicals used in the laboratory.

FACILITY CONSTRUCTION	SAFETY EQUIPMENT	LABORATORY PRACTICES
• A sink must be available for hand washing • The lab should have doors to separate the working space from the rest of the facility.	• PPE (lab coats, gloves, eye protection) are worn.	• Standard microbiological practices are followed • Work can be performed on an open lab bench or table • Pure lab water is generally available in the lab, from Type I to Type III.

BIOSAFETY LEVEL 2 (BSL-2)

BSL-2 laboratories are used to study moderate-risk infectious agents or toxins that pose a moderate danger if accidentally inhaled, swallowed, or exposed to the skin. Design requirements for BSL-2 laboratories include hand washing sinks, eye washing stations, and doors that close and lock automatically.

BSL-2 laboratories must also have access to equipment that can decontaminate laboratory waste, including an incinerator, an autoclave, and/or another method of decontamination, depending on the biological risk assessment.

In addition to BSL-1 considerations, BSL-2 laboratories have the following containment requirements:

FACILITY CONSTRUCTION	SAFETY EQUIPMENT	LABORATORY PRACTICES
• The laboratory has self-closing doors • A sink and eyewash are readily available.	• Appropriate PPE is worn, including lab coats and gloves, eye protection and face shields • All procedures that can cause infection from aerosols or splashes are performed within a biological safety cabinet (BSC) • An autoclave or an alternative method of decontamination is available.	• Access to the laboratory is restricted when work is being conducted • Pure lab water of all types is available in both the general lab area, and if possible, close to or inside any biological safety cabinet.

BIOSAFETY LEVEL 3 (BSL-3)

BSL-3 laboratories are used to study infectious agents or toxins that may be transmitted through the air and cause potentially lethal infections. Researchers perform all experiments in a biosafety cabinet. BSL-3 laboratories are designed to be easily decontaminated. As an additional safety measure, these laboratories must use controlled (“directional”) air flow, to ensure that air flows from non-laboratory areas (such as the hallway) into laboratory areas.

Other engineered safety features include a requirement for entry through two self-closing, interlocked doors, sealed windows, floors and walls, and filtered ventilation systems. 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.

In addition to BSL-2 considerations, BSL-3 laboratories have the following containment requirements:

FACILITY CONSTRUCTION	SAFETY EQUIPMENT	LABORATORY PRACTICES
• A hands-free sink and eyewash are available near the exit • Exhaust air cannot be recirculated, and the laboratory must have sustained directional airflow by drawing air into the laboratory from clean areas towards potentially contaminated areas • Entrance to the lab is through two self-closing and interlocked doors.	• Appropriate PPE must be worn, and respirators might be required • All work with infectious agents or toxins must be performed within an appropriate biosafety cabinet.	• An Occupational Health Program exists for medical surveillance of laboratory workers • Laboratory workers are under medical surveillance and may be offered immunizations for infectious agents or toxins they work with, if available • Access to the laboratory is always restricted and controlled • Pure lab water is generally available in the lab, as well as close to or inside any biological safety cabinets.

BIOSAFETY LEVEL 4 (BSL-4)

BSL-4 laboratories are used to study infectious agents or toxins that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease for which no vaccines or therapies are available. 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.

BSL-4 labs need procedures in place that will regulate access, inventory, and safeguarding of samples; strict rules for sample sharing; and security checks on all staff. Access to the high containment area needs to be strictly controlled and traceable through personal badges and security cameras, samples are stored inside the laboratory, and all aliquots must be accounted for, with audited records. Samples are shared on written request only between officially recognized BSL-4 laboratories.

And finally, the need for absolute containment and decontamination cannot be understated at this level. Whilst it is probably easier to decontaminate a BSL-4 lab than to decommission a nuclear reactor, it is still a major undertaking, requiring extensive planning, time, and cost.

There are two types of BSL-4 laboratories:

CABINET LABORATORY

All work with infectious agents or toxins is done in a Class III Biosafety Cabinet with very carefully designed procedures to contain any potential contamination. In addition, the laboratory space is designed to also prevent contamination of other spaces.

SUIT LABORATORY

Laboratory personnel are required to wear full-body, air-supplied suits, which are the most sophisticated type of PPE. All personnel shower before exiting the laboratory and go through a series of procedures designed to fully decontaminate them before leaving.

While the engineering controls required for BSL-4 cabinet and BSL-4 suit laboratories differ in some respects, the controls are extensive and supplemented by carefully designed procedures and practices in both types of laboratories.

In addition to BSL-3 considerations, BSL-4 laboratories have the following containment requirements (this list is not exhaustive):

FACILITY CONSTRUCTION	SAFETY EQUIPMENT	LABORATORY PRACTICES
• The laboratory is in a separate building or in an isolated and restricted zone of the building • The laboratory has dedicated supply and exhaust air, as well as vacuum lines and decontamination systems.	• All work with infectious agents or toxins must be performed within a certified biosafety cabinet, for work performed in the cabinet laboratory, or while wearing a full body, air-supplied, positive pressure suit.	• Change clothing before entering • Shower upon exiting • Decontaminate all materials before exiting • Whilst pure lab water is generally available in the lab, as well as inside biological safety cabinets, it requires separate, isolated plumbing system and feeds, to avoid any potential contamination issues.

4. ENSURE THE RIGHT WATER SUPPLY WHERE YOU NEED IT

The water types needed for the different biosafety levels will be broadly similar. They will each require water for general lab maintenance, for the manipulation of microorganisms in a sterile environment, and for carrying out different experiments when studying their component parts and their respective roles in disease e.g. viral-infected cell culture, or molecular biological techniques such as RT-PCR or electrophoresis. As such, most labs are likely to need most types of water at some point, be that Ultrapure Type I, Type II, or Type III.

However, the precise location of the different water supplies needed in the lab will change, depending on whether the lab is BSL-1, BSL-2, BSL-3, or BSL-4. These requirements will ideally need to be considered at the design stage of the laboratory.

BSL-1 labs will have the most freedom, with the **water purification system(s) able to be easily installed and maintained on the lab bench**, or wall-mounted, with laboratory personnel able to access it at all times, since minimal containment is required.

BSL-2 may require the use of biological safety cabinets, if working with agents that can cause infection from aerosols. This means that it would be useful to make provision for **sterile water to be either produced directly in the safety cabinet** or be easily accessible, close to the cabinet. If external, separate water sources might even be considered for separate cabinets, to avoid cross-contamination. Once experiments are complete, it should also be simple to dispose of any pathogen-containing solutions by autoclave, again not too distant from the safety cabinet.

At BSL-3, all experiments are carried out in biosafety cabinets. This means that the most convenient set-up for water that is designated for use directly in experiments would be to have the **water supply directly in the cabinet**. If this is not possible, then each cabinet would be recommended to have a separate supply located as closely as possible to it. 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.**[5]

To maintain biosecurity, water piping systems and equipment not serving BSL-3 facilities should not be located within containment areas i.e. water piping and service openings for systems serving other building areas should not require entrance into BSL-3 spaces as far as possible.

For decontamination, all plumbing system equipment, piping, seals, and components should be compatible with the anticipated fumigation method, and liquid disinfectant trap fluids.

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. The water supply used for these procedures must obviously be located inside the lab itself.

Finally, at BSL-4, there is the burden of extra decontamination to be taken into account when considering water supply, whether looking at fitting showers for personnel on entry to or exit from the facility, or at what happens to the waste resulting from experiments that have been carried out in such a restricted environment. **Water for BSL-4 experiments will ideally be produced and available as close to the cabinets as possible, as for BSL-3, with extra precautions taken to avoid cross-contamination pre- and post-experiment.**

5.

STANDARDIZE STAFF TRAINING AND OPERATING PROCEDURES

Although training and protocols have been mentioned in other sections, inadequate training and absence of standard operating procedures together form the biggest threat to the establishment of a successful national biosafety laboratory network.

In effect, it is relatively easy to build a lab to the required specification, from BSL-1 to BSL-4, but if there are no protocols for maintenance in place, and the staff are inadequately trained, the lab cannot be safely used, and catastrophic leakage of pathogenic organisms may result.

The accidental release of microbial aerosols is a probable cause of many laboratory-acquired infections, which demonstrates the importance of worker training and the ability to recognize potential hazards and correct unsafe habits. Attention to and proficient use of work practices, safety equipment and engineering controls are also essential. The expansion of biocontainment laboratories nationwide dramatically increases the need for training in microbiological practices and biosafety principles.

In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

Inadequate training in the proper use of personal protective equipment at any level may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervized by scientists competent in handling infectious agents and associated procedures.

Personal health status may also impact an individual's susceptibility to infection, or their ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counselling and guidance.

The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-3 and BSL-4 containment, ensuring they receive appropriate training in the practices and operations specific to the laboratory facility, as well as annual updates and additional training when procedural or policy changes occur.

Water in biosafe labs

If you can tick these five requirements, you will be well on the way to designing, building, and maintaining a successful biosafe lab, whatever the level required.

To guarantee that you will have the right type of water, in the right place, at the right time, contact your local ELGA representative, who will be able to help you design the appropriate water purification system for your biosafe lab.

To find your nearest ELGA representative, go to elgalabwater.com and select your country.

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