



S U N C O M B E
CIP, BIOWASTE & PROCESS SOLUTIONS

Biosafety Levels (BSL)

An introduction – Biosafety levels 1-4

- Safety standards to be followed by labs working with biological material that can infect humans.
- Levels 1-4 based on potential harm and treatability.
- Many agencies around the world have matching standards.
- Defined by Centers for Disease Control/National Institute of Health, World Health Organisation, Health and Safety Executive.
- All agree on safeguards to be put in place.

Biosafety Level 1

- For agents not known to cause illness in healthy humans.
- (No or low individual and community risk).
- Example: *Bacillus subtilis* (A gut bacteria).
- Safety measures involve washing hands and not eating in the lab.

Biosafety Level 2

- For agents that cause human disease of varying severity by ingestion or mucous membrane exposure but for which treatments are available.
- (Moderate individual risk; low community risk)
- Example: Hepatitis B, Salmonella
- Safety measures include appropriate lab PPE, hand washing sinks, lockable doors, biohazard signs and air recirculation separated from the main building system. The lab could also include waste decontamination facilities such as an autoclave

Biosafety Level 3

- For agents with a known potential for aerosol transmission and that may cause serious and potentially lethal infections but for which treatments are available.
- (High individual risk; low community risk)
- Example: Tuberculosis, Hepatitis C, West Nile fever
- Safety measures include appropriate lab PPE, hand washing sinks, 2 door entry, biohazard signs, Biosafety Cabinets and HEPA filters on extracted air. Air pressure in the lab must be kept negative relative to atmosphere. The lab must also include available waste decontamination facilities such as an autoclave and/or liquid waste decontamination system

Biosafety Level 4

- For agents that may cause serious and potentially lethal infections and for which no treatments are available.
- (High individual and community risk)
- Example: Ebola, Smallpox
- Safety measures include appropriate lab PPE, hand washing sinks, Biosafety Cabinets/Positive Pressure Suits and dual HEPA filtered extract air and single filtered input. Air pressure in the lab must be kept negative relative to atmosphere. The lab must also include available waste decontamination facilities such as an autoclave and/or liquid waste decontamination system

Summary – WHO guidelines

Table 3. *Summary of biosafety level requirements*

	BIOSAFETY LEVEL			
	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No ^b	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No ^c	No
Effluent treatment	No	No	Yes/No ^c	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability ^d	No	No	Desirable	Yes

^a Environmental and functional isolation from general traffic.

^b Dependent on location of exhaust (see Chapter 4).

^c Dependent on agent(s) used in the laboratory.

^d For example, window, closed-circuit television, two-way communication.

Summary – HSE guidelines

Containment measures		Containment Levels		
		2	3	4
1	The workplace is to be separated from any other activities in the same building.	No	Yes	Yes
2	Input air and extract air to the workplace are to be filtered using HEPA or equivalent.	No	Yes, on extract air ¹	Yes, on input and double on extract air
3	Access is to be restricted to authorised people only.	Yes	Yes	Yes, via airlock key procedure
4	The workplace is to be sealable to permit disinfection	No	Yes	Yes
5	Specified disinfection procedure.	Yes	Yes	Yes
6	The workplace is to be maintained at an air pressure negative to atmosphere.	No	Yes ¹	Yes
7	Efficient vector control, eg rodents and insects.	Yes, for animal containment	Yes, for animal containment	Yes
8	Surfaces impervious to water and easy to clean.	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
9	Surfaces resistant to acids, alkalis, solvents, disinfectants.	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
10	Safe storage of biological agents.	Yes	Yes	Yes, secure storage
11	An observation window, or alternative, is to be present, so that occupants can be seen.	No	Yes	Yes
12	A laboratory is to contain its own equipment.	No	Yes, so far as is reasonably practicable	Yes
13	Infected material, including any animal, is to be handled in a safety cabinet or isolator or other suitable containment	Yes, where aerosol produced	Yes, where aerosol produced	Yes
14	Incinerator for disposal of animal carcasses.	Accessible	Accessible	Yes, on site

1 In the table, COSHH Schedule 3 - Part II, the requirement for several containment measures at CL3 is risk based, eg the need for HEPA filtration of extract air and the provision of an inward airflow is dependent on the ability of the biological agent to be transmitted via the airborne route. The Approved list of biological agents helpfully identifies, with an asterisk, which biological agents are not normally transmitted via an airborne route. This information should be used in the first instance to inform the risk assessment. The actual specifics of the contained use then need to be considered to make a final decision on the extent to which it is necessary to protect workers from exposure to airborne biological agents, eg propagation of blood-borne viruses such as Hepatitis B virus is unlikely to require room air to be extracted through a HEPA filter or an inward airflow into the room, but would require the use of a microbiological safety cabinet. However, other containment requirements will still necessitate the laboratory being designated as CL3.

Source [<https://www.hse.gov.uk/biosafety/management-containment-labs.pdf>]

Summary - Waste Disposal

- BSL1 – Waste decontamination not required
- BSL2 – Waste decontamination recommended but not required by regulation
- BSL3 and BSL4 – Waste decontamination required by regulation
- These requirements also hold true for animal research waste

Regulation regarding animals

There are also Biosafety Levels specific to animals/agriculture. These are labelled BSL1-Ag through BSL4-Ag. They follow the exact same form as the levels previously listed with specific provisions for animal cages and enclosures. Waste handling requirements are identical.

In UK and mainland Europe the equivalent used is the 'Specified Animal Pathogens Order' (SAPO).

Effluent Decontamination System (EDS)

- The systems used to treat waste from BSL labs are not specified in documentation.
- The system must simply be able to demonstrate appropriate sterilisation ability.
- f_0 is a useful number when evaluating this.

The F Zero Factor (f_0)

- A measure of sterilisation strength
- A sustained 121°C (250 °F) for 1 minute gives 1 f_0 point.
The f_0 value of a sterilisation process can be increased linearly with time or exponentially with temperature.
- $f_0 = t \cdot 10^{\left(\frac{T-121}{10}\right)}$ t=time (mins), T=temp (°C)

If a given process requires an f_0 of 30 then this can be achieved at 121°C for 30 mins or at 130°C for 3.7 mins

Different microbes require different f_0 values to be reliably killed.

D-Values

- Pathogens with a D-value of 1min will be reduced by $1 \log_{10}$ for every f_0 point.
- So a pathogen culture numbering 10^4 individuals with $D=1$ heated at 121C for 1 minute will now contain 10^3 individuals

Useful links

- <http://www.hse.gov.uk/biosafety/management-containment-labs.pdf>
- <https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>