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Safety precautions in laboratory work with microorganisms

So far this century there have been over 4000 victims of infections acquired in laboratories. About 200 of them have died as a result. But there have been very few infections among workers in industries (e.g. pharmaceutical and biotechnology) which use microorganisms. Nor have there been any infections as a result of genetic manipulation experiments.

Classification of infectious agents on the basis of hazard

Scientists who investigated infections among laboratory workers noted that some microorganisms were more likely to cause infections than others. This led them to classify the organisms into four Risk Groups on the basis of hazard to the workers. Group I contains the "harmless" organisms and Group IV the most dangerous.

Table 1

Classification of infective microorganisms by Risk Group

Risk Group I (low individual and community risk).
A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.

Risk Group II (moderate individual risk, limited community risk).
A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group III (high individual risk, low community risk).
A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual to another.

Risk Group IV (high individual and community risk).
A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

Table 1 shows the classification adopted by the World Health Organization. Those of individual states are similar. The Groups are based on:

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* C.H. Collins (DSc, MPhil, FRCPath, CBiol, FIBiol) jest pracownikiem Microbiology Department, University of London; do roku 1985 pracował w Public Health Laboratory Service. Pełni funkcję: eksperta WHO ds. bezpieczeństwa w mikrobiologii, sekretarza Brytyjskiego Konsultacyjnego Komitetu Mikrobiologicznego ds. Bezpieczeństwa w Mikrobiologii oraz jest członkiem Grupy Roboczej ds. Bezpieczeństwa w Biotechnologii Europejskiej Federacji Biotechnologicznej. Jego aktywność w zakresie tematyki prezentowanej w niniejszej pracy obejmuje kilkadziesiąt artykułów, kilka pozycji książkowych, wiele ekspertyz oraz liczne wykłady w ponad 10 krajach. Obecnie pracuje (jako edytor i współautor) nad przygotowaniem kolejnej książki dotyczącej bezpieczeństwa w biotechnologii.

W artykule omawiane są zagrożenia występujące w laboratoriach mikrobiologicznych, drogi infekcji, zasady klasyfikacji drobnoustrojów chorobotwórczych oraz laboratoriów do pracy z nimi, a także zasady i sposoby przeciwdziałania tym zagrożeniom. Prezentowana klasyfikacja patogenów opracowana została przez Światową Organizację Zdrowia w oparciu o nieco inne kryteria aniżeli te, które przyjęła Europejska Federacja Biotechnologiczna (patrz raport A. Chmiela w tym numerze).

1. Pathogenicity.
2. Mode of transmission (presence of a vector etc.).
3. Presence in the community.
4. Available prophylaxis – immunization, public health measures.
5. Available therapy.
6. Routes of infection in the laboratory.

Classification of laboratories for work with organisms in the Risk Groups

The most hazardous agents require the most stringent "containment", that is the kind of precautions necessary to protect the worker and other people. There are four Levels of Containment corresponding to the four Risk Groups. These are shown in Table 2.

Table 2

Risk groups in relation to category of laboratory

Risk group	Laboratory classification	Examples of laboratories	Examples of organisms
I low individual risk and low community risk	Basic Level 1	Basic teaching	*Bacillus subtillis* *Escherichia coli* K12
II Moderate individual risk and limited community risk	Basic (with biosafety cabinets or other appropriate personal protective or physical containment devices when required) Level 2	Primary health services; primary level hospital; doctors' offices; diagnostic laboratories; university teaching; public health laboratories	*Salmonella typhi* Hepatitis virus B *Mycobacterium tuberculosis*[a] LCM virus[b]
III High individual risk and low community risk	Containment Level 3	Special diagnostic laboratories	*Brucella* spp. Lassa fever virus *Histoplasma capsulatum*
IV High individual risk and high community risk	Maximum containment Level 4	Dangerous pathogenes units	Ebola–Marburg virus Foot-and-mouth-disease virus

[a] When larger volumes or high concentrations are used, or when techniques may involve aerosol production these and other agents should be promoted to Risk Group III.

[b] Includes research laboratories at appropriate risk group level.

Routes of transmission of laboratory–acquired infections

There are three important routes:

Through the lungs: inhalation of airborne infectious particles;
Through the mouth: mouth pipetting; hand to mouth;
Through the skin: hypodermic needles, broken glass, abrasions.

Through the lungs

When bubbles are burst or thin films of liquids are broken very small droplets of liquid – aerosols – are dispersed. Many ordinary laboratory techniques may disperse aerosols containing bacteria or virus particles: these include pipetting, pouring, centrifuging, plating and subculturing, slide agglutination and catalase tests. Large droplets fall rapidly and contaminate benches, hands, etc. The smaller droplets remain suspended, the liquid evaporates and the organisms remain suspended as "droplet nuclei" and may be inhaled. If they are larger than about 5 μm they will be filtered off in the upper air passages, but smaller particles reach the alveoli and cause an infection. Dried material, e.g. lyophilized cultures is also a hazard, as are the spores of fungi, which may cause infection or allergy.

Table 3 lists some agents that may cause infection in this way.

Table 3

Some agents concerned in the air–borne transmission of infection in the laboratory

Known	Potential
Brucella abortus melitensis	Bacillus anthraci
Francisella tularensis	Neisseria meningitidis
Mycobacterium tuberculosis	
Pseudomonas mallei pseudomallei	
Yersinia pestis	
Coccidioides immitis	*Blastomyces hominis*
Histoplasma capsulatus	
Coxiella burnetii	
Rickettsia (most spp)	
Chlamydia trachomatis psittaci	
LCM virus	*Herpes viruses*
VEE virus	*Flu viruses, poxviruses*
	VS virus

Through the mouth

Pipetting by mouth, instead of using rubber bulbs or pipetting devices is dangerous because infectious material may be accidentally aspirated and swallowed. If infectious material is splashed or spilled on benches and equipment, or contaminated by aerosols, the organisms may be picked up on the fingers and transferred directly to the mouth or indirectly through food and cigarettes. Many infections have been caused in this way and some of the agents are shown in Table 4.

Table 4

Some agents concerned in infections acquired in the laborator by ingestion

Salmonella typhi	Clostridium botulinum (toxin)
Other *Salmonella* spp.	*Leptospira* spp.
Vibrio cholerae	*Campylobacter* spp.
Enteropathogenic	*Hepatitis* viruses A and B
Escherichia coli	*Poliovirus*
Brucella spp.	*Poxviruses*

Through the skin

Very few microorganisms can enter the body through unbroken skin. But the skin of the hands and exposed parts of the body is rarely unbroken: there are usually many very small cuts, scratches and abrasions. If the skin is contaminated the organisms can enter with ease. But the most important risk today is with hypodermic needles. Many infections have been caused by what we call "needle stick" but nowadays hepatitis B virus is present in so much of the blood that is tested in laboratories. The AIDS agent HIV may also be present, but the risk with this is less than with HBV. Table 5 lists some of the agents that have entered the body and caused infections by this route.

Table 5

Some agents concerned in infections acquired through the ski in the laboratory

Known	Potential
Brucella abortus	Bacillus anthracis
Corynebacterium diphtheriae	Clostridium botulinus (toxin)
Leptospira spp.	*Francisella tularensis*
Mycobacterium tuberculosis	*Mycobacterium leprae*
Other *mycobacteria*	*Neisseria meningitis*
Neisseria gonorrhoeae (cutaneous)	*Yersinia pestis*
Spirillum minus	
Staphylococcus aureus	
Streptobacillus moniliformis	
Streptococcus pyogenes	
Blastomyces hominis	
Coccidioides immitis	
Cryptococcus neoformans	
Histoplasma capsulatum	
Sporothrix schenkii	
Ebola fever virus	*LCM virus*
Hepatitis B virus	*Poliovirus*
Herpes virus B	*Rubella virus*
HT virus	VEE virus
WEE virus	EEE virus
Yellow fever virus	West Nile virus
Rickettsia rickettsi	Other *Rickettsia* spp.
Rickesttsia tsutsuqamushi	*Coxiella burnetii*
Plasmodium spp.	
Toxoplasma gondii	

Precautions against laboratory-acquired infections

Safety of the worker and other people in microbiology is ensured by placing "barriers" around the organisms, the worker and the whole laboratory. These are shown in the diagram – Fig. 1.

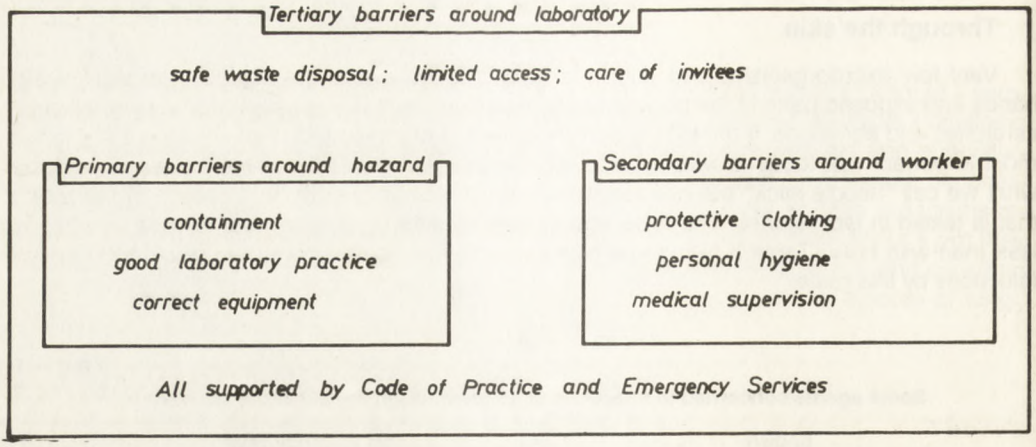

Fig.1. The barrier system to minimize laboratory acquired infections.

Primary barriers around the organisms

The most important of these is Good Microbiological Technique. This is achieved only by good training. Next, is correct equipment. Both are aimed at minimizing the production of aerosols and should include the use of microbiological safety cabinets (see below) for most of Risk Groups III and IV agents. Level 3 and Level 4 laboratories should be separated from other laboratories and require special ventilation. The design of a typical Level 3 laboratory is shown in Fig.2.

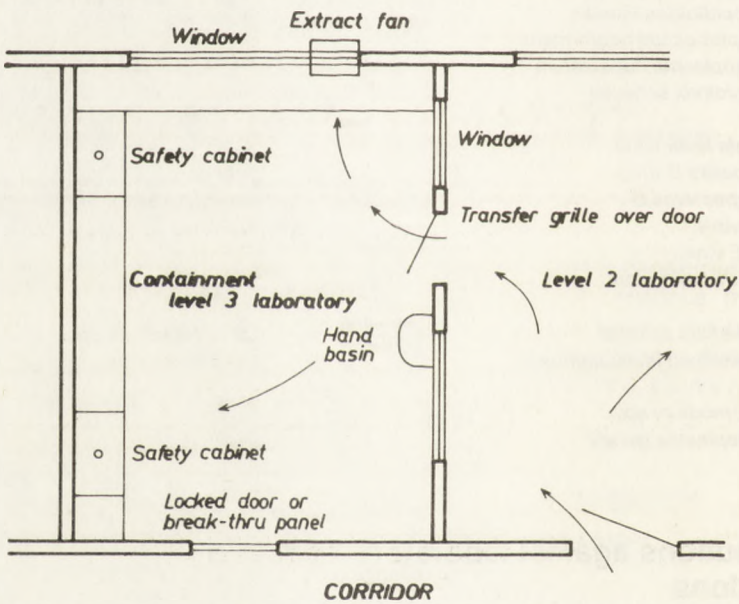

Fig.2. Airflows in biosafety level 2 and containment biosafety level 3 laboratories.

Secondary barriers around the worker

These barriers include protective clothing to avoid contamination of skin and personal clothing; good personal hygiene, particularly much washing of the hands; not touching mouth and eyes; not eating, drinking and smoking in the laboratory. There should also be good medical supervision of staff: e.g. immunization where indicated, care not expose pregnant ladies to certain viruses, and a reasonably high standard of health that helps protection against infection.

Tertiary barriers

These are placed around the whole laboratory and include efficient treatment of infected waste (see below), and the exclusion of people other than trained workers. They are particularly important for Containment Level 3 and Maximum Containment Level 4 Laboratories, where they are designed to prevent the "escape" of infectious agents into the community. Microbiological safety cabinets.

These cabinets are designed to protect the worker from inhaling any infectious particles that are released from his work. An extract fan takes air from the room into the cabinet, carries aerosols into the High Efficiency Particulate Air (HEPA) filter (HEPA), where they are removed. The clean air is then exhausted to the outside of the building. There are three classes of safety cabinets (Fig. 3).

Class 1. These protect the worker. All the air that passes through the cabinet and over the work space is exhausted to atmosphere. Class 1 cabinets are adequate for work with infectious agents in Risk Group III.

Class 2. These protect the worker and the work. The air that passes through the cabinet work space is divided. Some is exhausted through one HEPA filter; the rest is passed trough another HEPA filter and recirculated through the work space. The work is done in clean air.

Class 2 cabinets are used for work with infectious agents in Risk Group III and tissue culture work where it is important that the cells are not contaminated by room air.

Class 3. These cabinets are totally enclosed and hermetically sealed. Air enters only through a HEPA filter and is extracted through another HEPA filter. Class 3 cabinets are used only for work with Risk Group IV agents.

Microbiological safety cabinets will protect the worker only if they are well designed, correctly placed, satisfy stringent and frequent efficiency tests and are used with care and skill. Otherwise it is probably safer to work on the open bench.

Decontamination and disposal of infected laboratory waste

It is important that infected materials, e.g. cultures and specimens are made safe before they leave the laboratory. There are three methods of decontamination: chemical disinfection; sterilization by autoclaving; incineration.

Chemical disinfection

This is a temporary and internal laboratory procedure for materials used at the bench, so that they are safe until they are autoclaved or incinerated. Suitable disinfectants and their properties are shown in Table 6.

Autoclaving

This is a safe method if the autoclave is properly controlled. Autoclaves frequently fail to sterilize their contents if they are tightly packed so that heat cannot penetrate, and if the temperature in the chamber is too low and the time too short. The best temperature is 121°C and the time 20 minutes after this temperature has been reached in the chamber. Many autoclaves re-

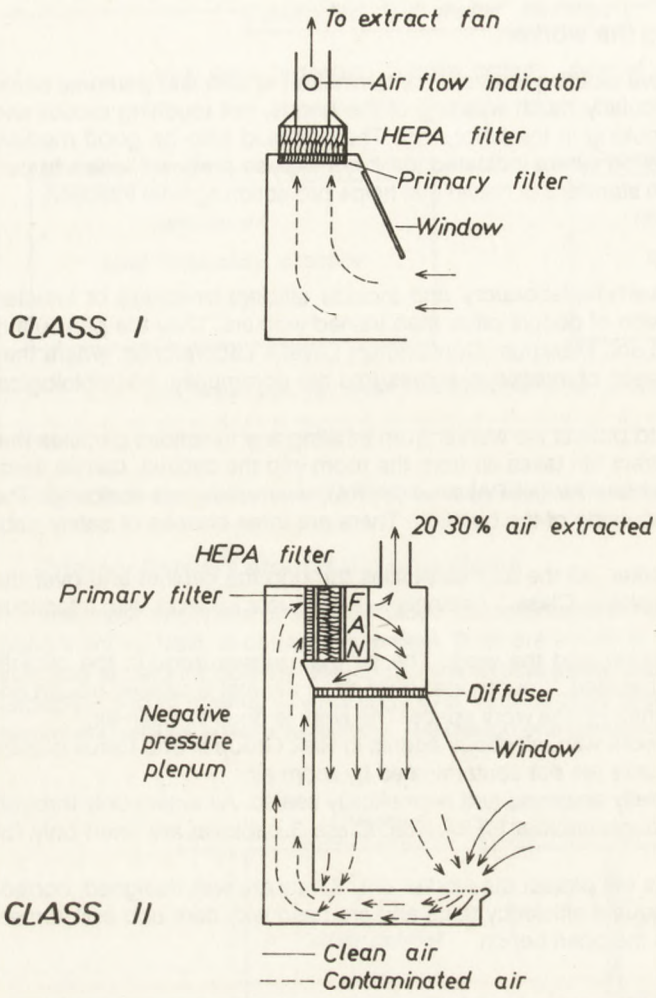

Fig.3. Classes of microbiological safety cabinets.

Table 6

General properties of some disinfectants

	Active against							Inactivated by					Toxicity	
	Fungi	Bacteria G+	G–	Myco-bacteria	Spores	Lipid viruses	Non lipid viruses	Protein	Natural materials	Man–made materials	Hard water	Detergent	Skin	Eyes
Phenolies	+ + +	+ + +	+ + +	+ +	–	+	v	+	+	+ +	+	C	+	+
Hypochlorites	+	+ + +	+ + +	+ +	+ +	+	+	+ + +	+	+	+	C	+	+
Alcohols	–	+ + +	+ + +	+ + +	–	+	v	+	+	+	+	–	–	+
Formaldehyde	+ + +	+ + +	+ + +	+ + +	+ + +[a]	+	+	+	+	+	+	–	+	+
Glutaraldehyde	+ + +	+ + +	+ + +	+ + +	+ + +[b]	+	+	NA	+	+	+	–	+	+
Iodophors	+ + +	+ + +	+ + +	+ + +	+	+	+	+ + +	+	+	+	A	+	+
QAC	+	+ + +	+ +	–	–	–	–	+ + +	+ + +	+ + +	+ + +	A(C)	+	+

+ + + good; + + fair; + slight; – nil; v. depends on virus; [a] above 40°C; [b] above 20°C; C – cationic; A – anionic

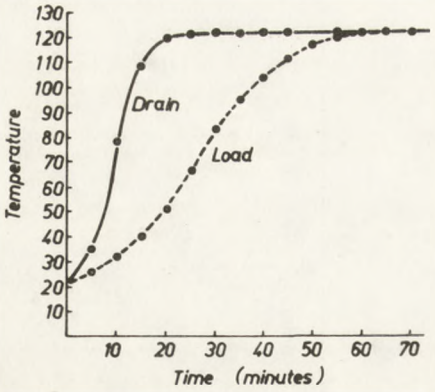

Fig.4. Different time–temperature relationships in the drain and the load in an autoclave.

cord the temperature in the drain, which is often much lower than that in the chamber. See Fig. 4. If autoclaves do not have thermocouples for reading the temperature in the chamber then chemical or bacteriological indicators should be included with the load

Incineration

Some incinerators do not burn everything. The air current through the furnace may carry infectious material up the flue and into the air before the flames reach it. Some material may not be burned but may be found afterwards unchanged among the ashes. The best kind of incinerator for laboratory and hospital waste is one that has an "afterburner" – another chamber where there is a secondary furnace to burn the smoke.

Decontamination and disposal procedures

Waste materials, discarded cultures and specimens should be collected in the laboratories in containers or bags that do not leak and sent to the utility or preparation room, where they can be sorted and then autoclaved and either cleaned and sterilized for re–use, or finally disposed of by incineration or collection by the domestic refuse organization. Flow charts for these are shown in Fig. 5.

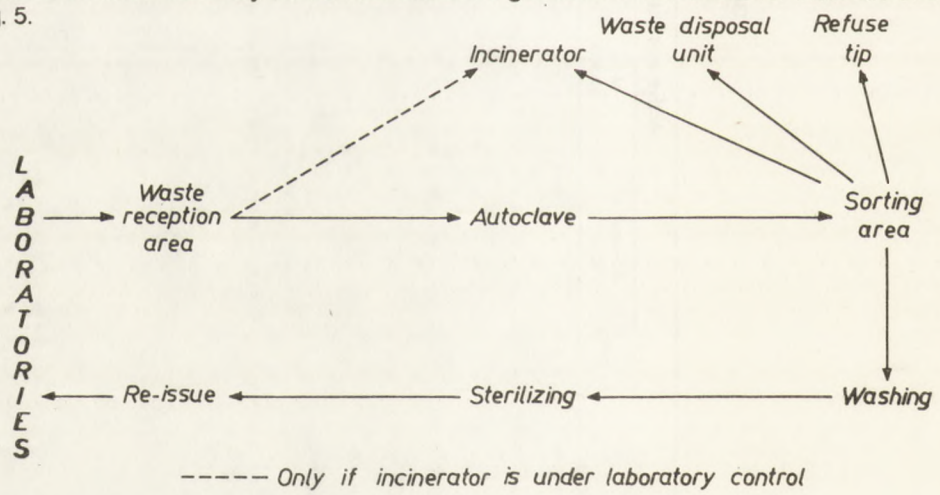

Fig.5. Flow chart for the disposal of infected laboratory waste and re–usable materials.

Safety management

It is good practice to have safety manuals – small instruction books, one for each worker. And to have a safety officer who has been trained and who possesses other books and sources of information. He should inspect, teach and advise and so assist the Director in keeping the laboratory free from job–associated infections.