
TOYOTA


BIOHAZARD

http://f-labor.mkt.bme.hu/

Education/Biosafety2 .pdf

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What is bio(logical)safety?

- every biomaterials are potentially pathogen and polluter/contaminant
- (direct)biosafety is focusing on infections and toxic effects
- goal: to avoid the getting out of living materials in to the nature

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Laboratory infections

- **1949 - Sulkin és Pike**
  - 222 viral infections (21 fatal/deadly)
  - only 27% was connected to known accidents (the reasons of teh rests are unknown)

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Laboratory infections

- **1951,1965, 1976 - Sulkin and Pike**

Made a survey on laboratory binded infections

- Involved more than 5000 laboratory
- Registred 3921 infections
- In less then 20 % was observed the infections reasons
- Infective aerosol was probably the reason in more then 80%
- Most frequently :

Bacterial	viral
brucellosis	hepatitis
tularemia	venezuelai equine encephalitis
tubercolosis	**HIV**
tiphus	**EBOLA**

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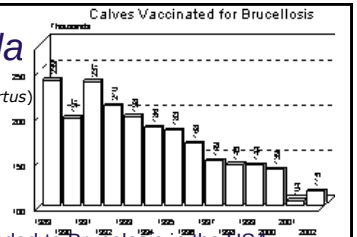
Brucella

(*Brucella abortus*)
proteobacter



- 74 laboratory infections were binded to Brucelosis in the USA [Meyer és Eddie, 1941]
- Originally this is the disease of domestic ruminants, like buffalo, deer, praire-dog, opossum, moose
- occurence: mediterran lands, middle east, west asia, Africa, Asia, and full America, Australia, for Example: in Kosovo!
- vacccination program (domestic animals): USA, Canada, Australia
- With milk or via epithelial damage it can spread to humans, too. (cyprus fever, Gibraltar fever) **ABORTIVE DISEASE!!!**
- Long term (over month)fever, headache, muscle and joint ache, lost of wight + superinfection

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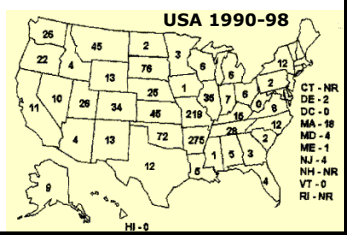
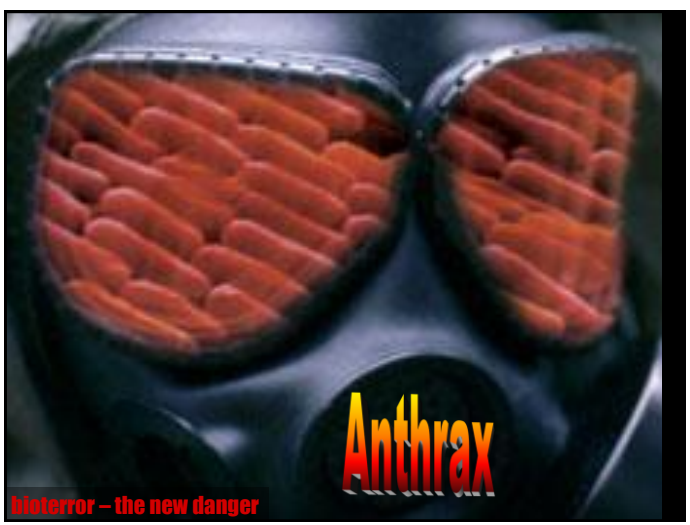
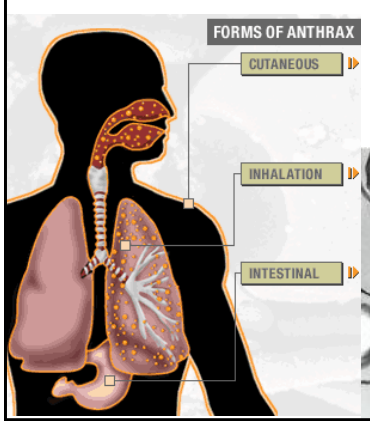
Tularemia

- *Francisella tularensis* bacteria
- Host:rabbit vector: tick
- Can infect stillwater, too (like. Death of beaver)
- 1966-67 Sveden: 600 infections via inhalations (hover dust of straw)
- Bio-weappons: 1942 - Stalingrad, soviets against germans – so many victims, that the offensive was stopped, BUT they infected themselves too (10 000 infections/yr-> jumped to 100 000)
- It is a potential tool for bioterror

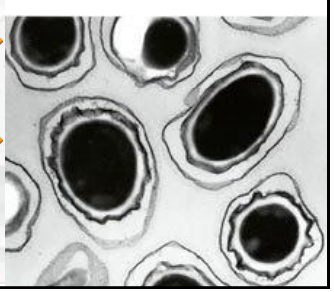




Anthrax



Spores of Bacillus anthracis



The risk of Infection

- infectious dose (ID) : this the number of microorganism, which is nessecary for appearing the disease, or which is enough to reproduce cells in a host resulting **mesurable effect**:
  - Appear of symptoms
  - Titer (i.e. conc.) of antibodies
  - Incorporation of nucleic acid
- Problems with determination of ID:
  1. Entry way – 4 order of magnitude differences between oral and subcutan administration/introduction
  2. Difference between hosts results 6 order of magnitude ID differences!

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The risk of Infection

3. Way of entry differs between host individums, too.
4. After antibiotic treatment (i.e. no gut-microbiota) of mouses they become 5 order of magnitude more sensitive against oral entry
5. Mouse and human ID's are hardly comparable (for example: in case of *Salmonella enteridis* 3 order of mgnitude difference)
6. Virulence of the pathogeni is variable: regionally (=in populations) and also with time

- Lethal Dose (LD) :
- More exact detection
- Not measurable for humans

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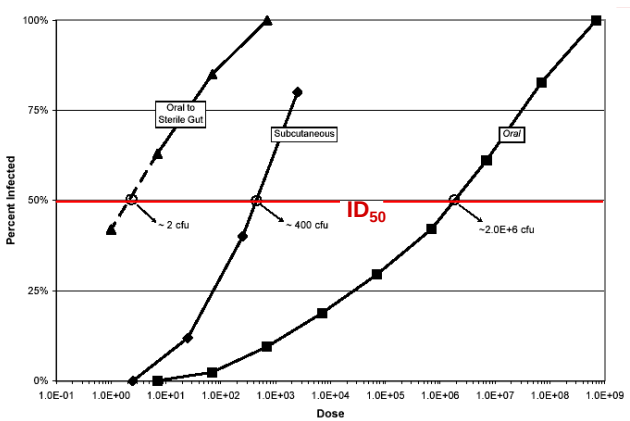
The risk of Infection



The risk of Infection

LD_{50} of *B. anthracis* for different hosts		
	injection LD_{50} [spores]	**inhalation** LD_{50} [spores]
Cynomolgus majom	-	$4.1x10^3$
Rhesus majom	$3x10^3$ spores	$5.3x10^4$ - $7.6x10^5$
mouse	5	$1,4x10^4$
rat	10^6	$2,6x10^4$
pig	10^9	$2,7x10^7$
dog	$5x10^{10}$	$1,8x10^7$
human	not given	ID: $6x10^2$-$2,2x10^3$

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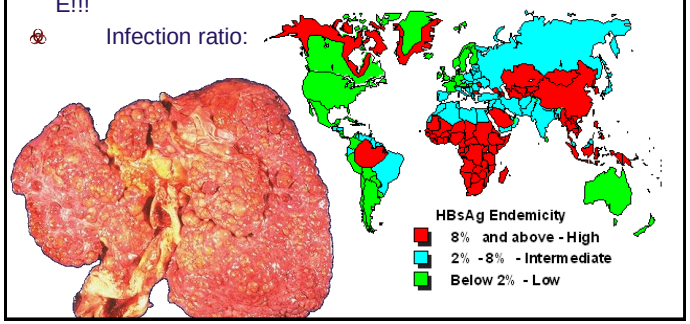
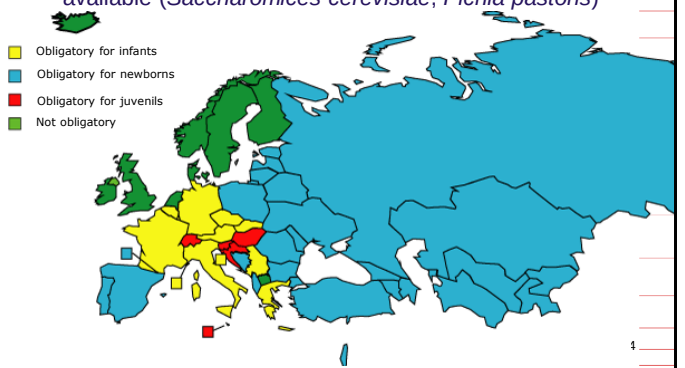
Hepatitis B

- Infectius inflammation of liver
- virális infection, lifelong effect,, cirrhosis, liver cancer, death
- Blood products are screened for HpA and B, but not for C,D, E!!!
- Infection ratio:



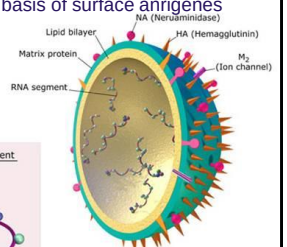
Hepatitis B

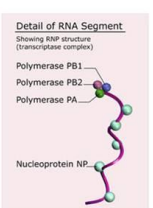
- There's no cute! But recombinant vaccination is available (*Saccharomices cerevisiae*, *Pichia pastoris*)



Bird flu

- Flu viruses are classified (class A, B, C) based on their capsid proteins:
  - „A" pathogenic for animals and humans
  - „B" and „C" not infective for animalls and rarely for humans
- Inside a class subgroups are formed on the basis of surface anrigenes (proteins) :
  - hemagglutinines – H
  - neuraminidases – N
  - type H and N can freely recombinated
  - >140 subtypes

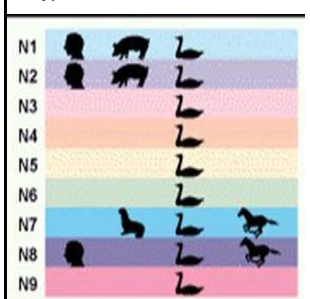


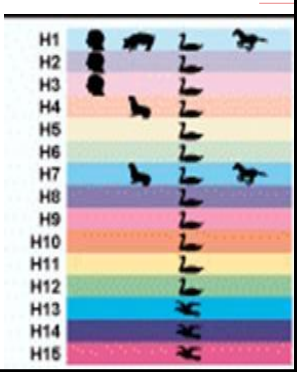


Kamarudin Isa, Aziz Mangkat, Rozanah Asmah, Abd. Samaddan, Asiah Naina, Mohd Alim: JANGKITAN HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI): RISIKO KEMASUKAN KE MALAYSIA

Bird Flu

- Birds can infected by any type H virus => they mainten high varaiblity virus population
- Birds are manily infected by H5 and H7 type





Bird Flu

- Primarly water birds but other poultry are infected (pigeons are not sensitives only collared doves)
- Bird can be a carier without symptoms, but its faeces are still infective (that's why waterbirds are so sensitive): the virus can survive for 1-2weeks in lakes and rivers)
- Danger occure, when wild water birds and other poultry are breeded together
- Syptomes in birds: 1-3 days of latency, than respiratory syptomes, sometimes hemorrhagic lessions, diarrhea
- Prevention: controlled (closed) livestock – Southeast Asia and USA do not
- Last decades more than 12 epidemmcs on Hungary, last time in 70's (before the recents)

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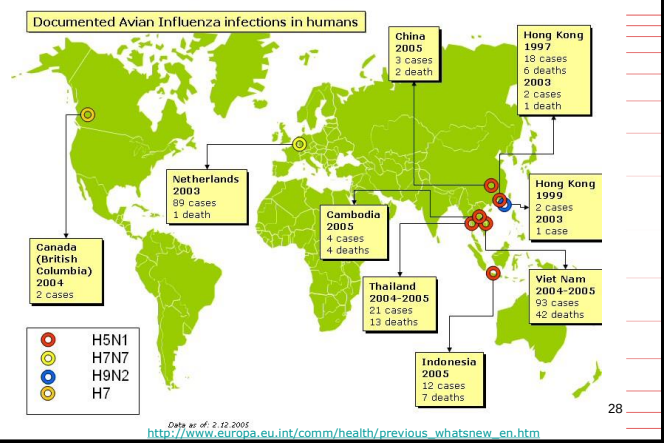
Bird Flu



http://www.europa.eu.int/comm/health/previous_whatsnew_en.htm

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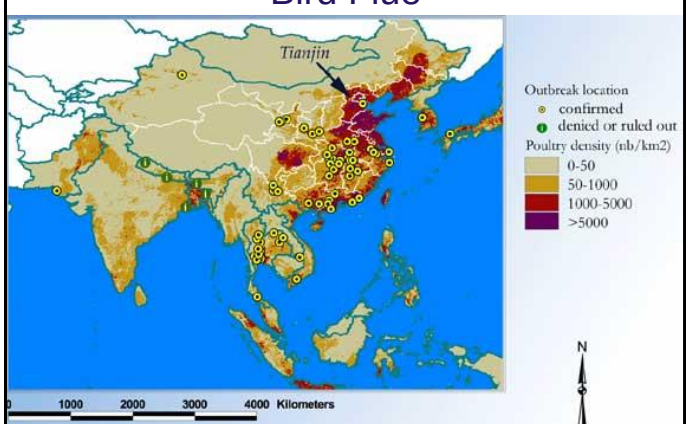
Bird Flue



http://www.fao.org/ag/againfo/subjects/en/health/diseases-cards/map1202.html

Bird Flu

- Animal's flu viruses are noot infecting directly humans, only rarely in high amount:
  - From 2003 ca 140 millions of bird infections, 137 human infections, 70 known death!
  - 2003 Netherlands: 89 human infections, 1 death
- Bird flu is not infective from huaman to human
- Risk: a human flue (infective from humans to humans) and at the same time a bird flu infectin ->recombination, supervirus formation

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Bird Flu

9 December 2005 - WHO

	Cambodia	China	Indonesia	Thailand	Viet Nam	Total
	cases/deaths					
2003	0/ 0	0/0	0/0	0/0	3/3	3/3
2004	0 /0	0/0	0/0	17/12	29/20	46/32
2005	4 /4	5/2	13/8	5/2	61/19	88/35
Total	4 /4	5/2	13/8	22/14	93/42	137/70

Total number of cases includes number of deaths.
WHO reports only laboratory-confirmed cases.

- human flu: 5-15 %-infected of world populations
- casue 250-500.000 death

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Bird Flu

- Rapid death of the virus above 60°C: cooked /baked infected birds become un-infective: the consumption of poultry is safe
  - Effect of press: in Hungary consumption decresed by 40%
- Defense:
  - Blood serum: contains antibody, gives passive defense
  - vaccination: contains antigens, gives active immunity
- Pilot production of vaccinca in Hungary:
  - A H5N1 virus was isolated in Vietnam from a human, and sent to 50 different countries
  - The vaccine can defense against only this type =>not economical to apply it neither for humans nor for birds
- Real defense: annihiliate infected animals

- **1951,1965, 1976 - Sulkin and Pike**

Made a survey on laboratory binded infections

- Involved more than 5000 laboratory
- Registred 3921 infections
- In less then 20 % was observed the infections reasons
- Infective aerosol was probably the reason in more then 80%

641cases/100.000 employed while
0,08 cases/100.000 citizen
=>8000x higher risk

- **2009 Weinstein, Singh**

Table 1. Ten most frequently reported laboratory-associated infections worldwide.

Disease	No. of cases	No. of deaths
Brucellosis	426	5
Q fever	280	1
Hepatitis	268	3
Typhoid fever	258	20
Tularemia	225	2
Tuberculosis	194	4
Dermatomycoses	162	0
Venezuelan equine encephalitis	146	1
Psittacosis	116	10
Coccidioidomycosis	93	2

NOTE. Data are for the years 1976 [3] and 1978 [4].

Table 2. Laboratory-associated infection and relative risk of infection, compared with the risk among the general population.

Organism	No. of cases of infection	Relative risk of infection
Shigella species	15	1
Brucella species	7	8012.5
Salmonella species	6	0.08
Staphylococcus aureus		
All	6	NA
MRSA	5	NA
Neisseria meningitidis	4	40.8
Escherichia coli O157:H7	2	8.6
Coccidioides species	2	1.1
Clostridium difficile	1	0.03

NOTE. Data are for the years 2002–2004 [11]. MRSA, methicillin-resistant *S. aureus*.

In 33% of participant lab at least one accident

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Brucellosis:
- Most frequent, 24% of total lab infections
- Still casued by aerolsols, BUT
  - -also direct contacts (like out of safety cabinet)
  - -the spreading way is often unknown
  - -no cases from humans to humans (only once, a spouse)
  - -assistant staff and visitors too!!

N.meningitis (2005)
- Forum and email alarm chain:
- 16 labor accident, 50% deadly(!), mostly B and C serotype
- Most probably by aerosol
- All of them clinical microbiologist
- 43x relative risk compared to the population
- Vaccination exists, but not 00% of defense, and not effective aginst B…
- Preention: Rifampicin or. Ciprofloxacin (=antibiotics)

Shigella
- Enterobacter (~Salmonella)
- More virulent ->less is enough to make infections

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Observation of biological risk

Every coutry have to classified microorganism with consideration of:

- a pathogenity of microbe
- Methode of infection and host organism
  - Immunity of the human population, their migrants, their density, hygienic conditions
- Tools for defense in the coutry
  - passive immunisation, hygienic rules, registration of animal infections
- Tools for treatment of the disease in the coutry
  - passive and post infection immuisation, antiviral agents

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The 4 level of Biosafety EüM 61/1999 (according to WHO)

- 1. level - basic biological risk

  that biological agent, which is not able to casue human disease
- 2. level – low risk

  that biological agent, which is able to casue human diseae, therfore it is dangerous for employee, but its spread is not probably, becasue effective treatment exist, or mild infection is casued
- 3. level – infection risk

  can cause serious human disease, and can have the oppurtinity to spread among the human population, but generally effective tretments exists, or can prevent.

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A biológiai biztonság 4 szintje EüM 61/1999 (WHO alapján)

- 4. szint – Emphasised infection risk

  that biological agent, which can casuse seriius human disease, and has high risk of spread among human population, becasue we either do not have any effective treatments or prevenntions.

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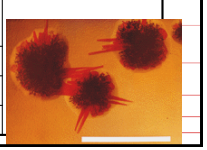
Bacteria

BSC 1	BSC 2	BSC 3	BSC 4
Escherichia coli K12	*Chlamidia pneumoniae*	*Bacillus anthracis*	*Mycoplasma mycoides* AIDS, Gulf syndrome, rheumatoid arthritis, etc. Between viruses and bacteria – can reach all tissue even into the brain
Lactobacillus sp.	*Clostridium butulinum*	*Coxiella burnetii*	
	Clostridium tetani	*Mycobacterium tuberculosis*	
	Corynebacterium dyphtheriae	*Rickettsia akari*	
	Escherichia coli	*Salmonella thyphi*	
	Haemophilus influenzae	*Yersinia pestis*	
	Klebsiella sp		
	Legionella sp.		
	Vibrio cholerae		



Fungi

2009: accident (cut wounds)<spores inhalation (dimorph) (lifting the cover)

BSC 1	BSC 2	BSC 3	BSC 4
	Aspergillus fumigatus	*Paracoccidioides brasiliensis*	
	Candidaalbicans	*Histoplasmacapsulatum*	
	Penicilliummarneffei	*Blastomycesdermatitidis*	

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Viruses

BSC 1	BSC 2	BSC 3	BSC 4
Baculovirus (pl. transgenic insect tissues)	cytomegalovirus (CMV) genus *Lymphocryptovirus*	Creutzfeldt-Jacob disease	*Middle eu. encephalitis (inflammation of brain) virus*
cattle papilloma virus	Hepatitis	Hantaan (corean haemorrhagic fever)	Congo Crimean haemorrhagic fever TBE (1999 Volgograd, 32case)
hamster leukemia	Herpes simplex	HIV	Ebola virus
Flu strains for vaccination	Influenza virus A-C	West-Nile fever virus	Marburg virus
	measles virus	Yellowfever virus	
	poliovirus		

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Biosafety – rational considerations

- the methods limiting the escape of microbes from a system are the same, than those, which limits the entry of a system:

  These provide the sterlity of a process, too, therefore they are required for appropriate products as well.

- "not the size of the essence" :

  Methods are the practically the same.

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Biosafety cabinet vs. Laminar box

- **Biosafety Cabinet**: infection-sure shut off from the environment.
- **Laminar box**: a laminar airflow prevent the materials from getting in/out microbes – only prevent a the processed **material**
  - •There exists horizontal and vertical airflow box.
  - •The airspace of laminar box can sterilized with a filter. Its surfaces can be sterilized with disinfectant agents and/or UV lamp.
  - •Before use, surfaces must be disinfected and irradiated by UV lamp for at least 15-30 minutes. No wet surface is allowed.
  - •Keep order in the box!

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Biosafety cabinet

- Prevent users, laboratory and the materials from aerosols formed during the job.
- aerosol < 5 µm and microdrop 5-100 µm – invisible with native eyes
- HEPA (high-efficiency particulate air) filter: filter off particles smaler than 0,3 µm (micron) with 99,99% safety. Only microbe free air can leave the cabinet.
- Classification: I, II and III class cabinets and furthermore IIA1, IIA2, IIB1, IIB2

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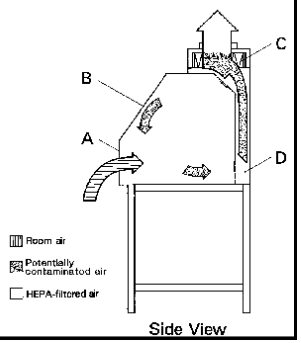
I class biosafety cabinet

- At least 0,38 m/s air inlet from room air (A)
- Outgoing air pass through HEPA filterinto the room or into the environment
- Does not fully prevent the used material

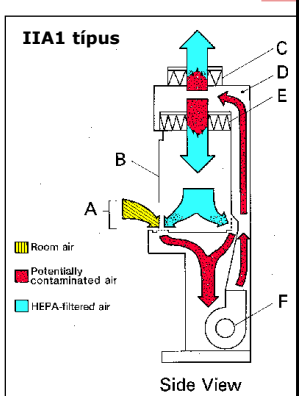
Figure 1.
Class I Biological Safety Cabinet.

A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum

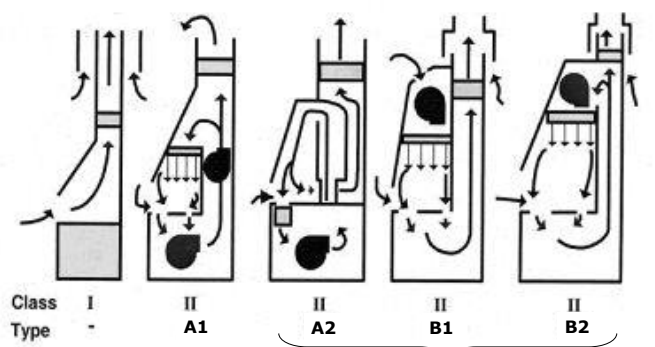


II class biosafety cabinet

- Prevent both user and material: filtered air (HEPA) meet the target material
- 2nd and 3rd risk group microbes can also be manipulated under this
- 4th risk group can onlly be manipulated under this cabinet in pressurized protective clothing
- IIA1 : 30% of air outlet is released into room, 70% is recycled into workplace



II class biosafety cabinets



Air release out of the working place (~chimney)

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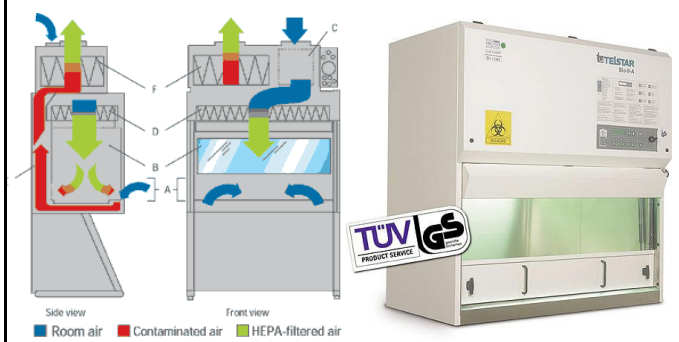
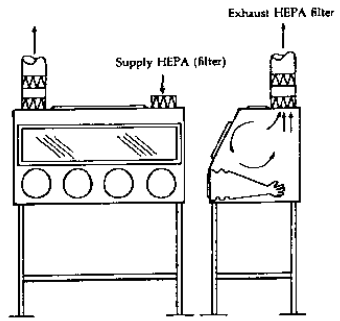
IIB2 biosafety cabinet



III class biosafety cabinet

- For handling 3rd and 4th risk level microbes
- Every connections are gastight
- Outgoing air pass through double HEPA filters
- There are a weak negative preassure in the cabin
- It may connected to a double doored autoclave for waste treatment
- Working is done with rubber gloves connected gastightly to the cabinet



III. Biosafety cabinet

Biosafety cabinets (summary)

Class	Innlet air velocity	Ratio of recycled air %	Ratio or released air %
I	0,36	0	100
IIA1	0,38-0,51	70	30
IIA2	0,51	70	30
IIB1	0,51	30	70
IIB2	0,51	0	100
III	0	0	100

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Application of biosafety cabinet

protection	Biosafety cabinet
employee, against risk level 1-3	Class I-III.
employee, against risk level 4	Class III.
employee, against risk level 4, with pressurized protective clothing	Class I, II.
Material protection	Class II and III, if laminar airflow is applied
Volatile radioactive/chemically toxic protection, small amount	IIB1 and IIA2, if air outlet goes to the environment
Protection of volatile radioactive/chemically toxic maaterials	Class I, IIB2 and III.

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Protection levels according to risk levels

- **Intorduction od protective levels:**
  - Personal conditions
  - General rules
  - Specific rules
  - Safety equipments (primary defense line)
  - Attribution of laboratory (secondary defense line)

First protection level

- **Personal conditions**:
  - **The head of laboratory** has general lab practice and good proffessional background
  - **Laboratory technician:** have to take part on specific trainings according to his/her working place.
- **General rules**
  - The **entry is limited** during work..
  - It is **obligatory to wash hands after taking off the gloves, and before leaving the lab.**
  - It is prohibited in the lab area: **to eat, to drink, to smoke, to purify eyelens.** Protective glasses are recommended. Food have to stored outside the working area in separated fridge.

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First protection level

- **General rules**
  - Pipetting with mouth is forbidden! (Griffin-ballon, piston pipette, pipettor, automatic pipette)
  - **Carefull working**, to avoid splashing or forming aerosol. (Appointed workplace according to the task)
  - Every working place (surface) should at least daily once disinfected, specially after working with living organism. (registred and controlloed cleaning and disinfection)
  - Wastes have to be decontaminated with one of the below recommended processes: heat (121ºC, 30min), chemical (alcohol, H_2O_2, formaldehyde etc.), radiation (UV)
  - **Insect and rodent controlling system have to be operated.** (cross contaminations)

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First protection level

- **Safety equipment (primary defense line)**
- Generally any special infection limiting tool or equipment is not required at this level.
- **Recommended** to wear jacket, gloves or uniform.
- **Gloves** are recommended, if skin is injured or pimply.
- **Protective glasses** are nessecary in case of rik of splash.

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First protecction level

- **Specification of Laboratory (or plant) (=secondary defense line) :**
  - **Eye wash bottles** are required in every labs.
  - **Surface of workplace** must be water-repellent, acid-, base-, and solvent-fast, and partly heat resistant.
  - If windows can be opened, than mosquito net have to be applied..
  - Lab Furniture should be durable. Betwwen worktables, cabins and equipments must be leaved **enough place for easy cleaning**.

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Second protection level

Personal conditions:

- **Head of Laboratory:** must be known his/her responsibility, must have specific knowledge on pathogens.
- **Labor technician:** Must have appropriate practice with pathogens under the supervision of a competent leader.

- **General rules**
- No additive requirements to Level 1.

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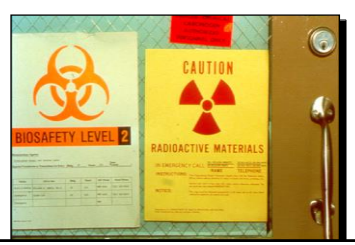
Second protection level

- **Special rules**
  - The Head of Lab **decide on any entry** with full responsibility (beside mechanic or electronic registration).
  - If infectious agent is used, there are some requirements for entry (like vaccination/immunization)
  - The **biosafety board have to be appeared** on thee door, including the name of the infectious agent, the safety responsible person and contacts, and the special requirements of entry.



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Second protection level

Special rules

- A lab-specific safety handbook have to be prepeared or adapted a ready protokoll.
- Staff have to be inform about hte special risks and danger. It is a basic requirement, that they have to read and follow the Handbook. Staff have to receive yearly refreshing or supplementory education on changes of applied procedures.
- Every **sharp tools** must be used and handled very carefuly: injection needle, slide glasses, pipette, capillars, scalpel etc. Plastic dishes have to be used.
- Single use tools!!!
- Cultures and tissues have to be **stored in appropriate dishes**, to prevent any release during harvesting, storing, or transporting. Before an **equipment is sent to service**, it must be disinfected!.

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Second protection level

- **Special rules**
  - If any agent is gushed/overflowed, or any accident occur resulting risk of infections evidently, it must be reported immediatly to the head of the lab. Medical examination have to be granted, and report have to be kept.
- **The application of biosafety cabinet is required:**,
  - A) if during the operation aerosol formation is expected.
  - B) if high amount of infectious material is handled
- **Face protection**: if during any operation outside of the biosafety cabinet aerosol formation may occur. (protective glasses, mask)
- **jacket or uniform** is obligatory. These cloaths have to be leaved in the lab. It is forbidden to take home, their cleanig must be soved on site.
- Single use gloves have to be weared, if infectious animal or material is used. Gloves have to be taken off after the job, and throw out into infection safe bin/store..

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Second protection level

- **Specification of the laboratory (secondary defense line):**

- Must be ready **disinfection and lab waste disposal method.** (like: autoclave, chemical disinfection, waste incineration furnance)

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Third protection level

- **Personal conditions/requirements**
  - **Head of laboratory**: inspect the employee, must be expert both theoretically and practically
  - **Laboratory staff**: Have to take part on specific education prepaering them to the work with pathogenic or lethal agents.
- **General rules**

No additive requirements to Level 1.

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Third protection level

Special rules

- The entrance of the lab must **be kept closed** if any experiment is running.
- The head of the lab **control the entries and limit it** to that people, who are absolutly nessecary to carry out the given task.
- **Biosafety boards** have to be placed on every entries of lboratories and animal houses.
- It is the responsibility of the head, that before start every work with 3rd class agents, **staff have to testify** their competency in the practice, and operating of lab equipments.
- Any interventions having infection risk must be done in **cabinets/boxes**.
- Every **single use materials** (gloves, jackets) have to be decontaminated/disinfected, before bringin out from the lab.
- **If infectious material flow out,** specific staff have to be called to first disinfect than stop its spread.

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Third protection level

- **Safety equipments**
  - **Out of the cabinets staff have to be continously defending themselves.** (special protecting clothes, mask, gloves, face-protection or gasmask), in combination of appropriate accessories (incl. containment area for animals).
  - **Gasmaks** have to be applied, if the potentially formed aerosol can not be kept back in the boksz, and in the rooms infected animals are present.
  - **Resusable protective clothes** have to be disinfected before taking to laundry.

Third protection level

- **Specification of teh laboratory**
  - The **lab have to be separated** from other frequent traffic places in the building. (negative pressure)
  - Basically required, that **two automatic doors** have to separate the lab from the other parts of the buildings. The **dressing room** (with disinfection possibility) must be placed in the way of passage.
  - **Ventillation** have to be one directed, the exhaust air can not be reused at other parts of the buildings, and must be filtered.
  - The outgoing wastes (even waste water) have to be collected and disinfected separatelly.
  - The **walls, the ceylon and the floor** have to be water-repellent for easy cleaning.
  - **Windows** always have to be kept closed.
  - Every tools and equiipments with **potential aerosol formation** must be kept in the cabinets.
  - **Vaccum lines** must have disinfection trap and HEPA filters. These have to be mainetned and replaced regulary and carefully.

(industrial GMO lab is similar...)

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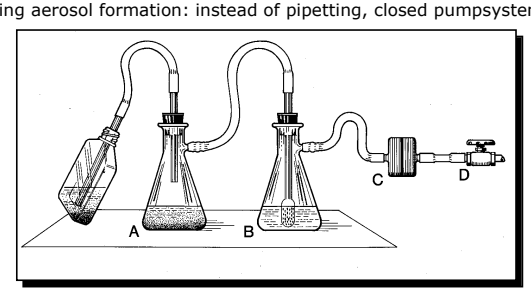
Avoiding aerosol formation: instead of pipetting, closed pumpsystem must used



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Required tools

~ recommended

Biosafety level	1.	2.	3.	4.
Isolation of the lab	-	-	~	+
Possibility for hermetic close	-	-	+	+
Venting	-	~	+	+
Common venting	-	~	+	-
Separate venting	-	~	+	+
Outgoing HEPA filter	-	-	~	+
Double-door entrance	-	-	+	+
Air-lock with shower	-	-	-	+
Foreground dressing room	-	-	+	-
Foregground with shower	-	-	~	-
Efluent treatment	-	-	~	+

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GMO

Legal regulation

Emphasized parts:
2004. XI.
• Risk assesments of workingplaces can only be done with appoved persons or companies
• regulate education of safety, and the requirements for administrations safety representatives have to be elected
• while the modified law is expanded to woorking higienic questions, **but not include biosafety**

EüM 61/1999 decree
• health protection of employee working with biohazard agents
• it effets to regular employment and any other type of employment, too, where biological agents and their effects are present.

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GMO

Törvényi szabályozás

1. At least yearly **risk assesment** have to be done, examining the real accidents and expositions to biological agents.

2. Risk level must be decresed by
   -decrese the number of exposed staff
   -control and inspect of procedures
   -prevent the spread of biological agents
   -applying warnings
3. Action plans have to be pprepeared for bio-accidents and detection of these agents.
4. Appropriate waste managment tools have to be provided

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GMO

Törvényi szabályozás

5. Risk assesment and Accident Pprevention Plans have to be sent to National Public Health and Medical Officer Service (ÁNTSZ)

6. Employers have to give information to emplyee about risks, and provide safe
7. Accident Action plan must be available and information to responsible person.

8. Staff working in risk must be recorded and registred.

9. Medical supervision have to be provided!

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Examples – for Tests

- What are the differences between II. and III. class biosafety cabinets?
- What is the difference between laminar box and biosafety cabinets?
- What is ID_{50}?
- What is ID_{50} depending on?
- What is closed system applicartion?
- What are „A” and „B” type applcations of GMO’s, and what is the difference of their legal regulations?
- Please list at least 3 problems of transgenic plant pplications!
- Please list at least 2 methodes to decrease the ecological risk of applying malware resistant plants!

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