



Regulatory Basics for Facility Design (WHO GMP): Biosafety Requirements



Main Topics of the Presentation

- BSL in Laboratories (WHO Requirements)
- Differences of BSL 1,2 and 3 in Laboratories and in Production Facilities
- Decontamination by Fumigation
- Air Supply Concept
- Utilities Distribution and Effluent Treatment
- Animal Facilities



Risk Group for Laboratory Work¹ (1)

- Risk Group 1: A microorganism that is unlikely to cause human or animal disease
- Risk Group 2: A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or environment
- Risk Group 3: A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another
- **Risk Group 4:** A pathogen that usually causes serious human or animal disease and that can be readily transmitted from on individual to another, directly or indirectly



Risk Group for Laboratory Work¹ (2)

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic – BSL 1	Basic teaching, research	Good microbiological techniques (GMT)	Open work bench
2	Basic – BSL 2	Primary health and diagnostic services, research	GMT + protective clothing, biohazard sign	Level 01 + BSC for potential aerosols
3	Containment – BSL 3	Special diagnostic services, research	Level 2 + special clothing, controlled access, directional airflow	BSC for all activities
4	Maximum containment – BSL 4	Dangerous pathogen units	Level 3 + airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave, filtered air



Requirements for Biosafety Level (BSL) in Laboratories

	BSL 1	BSL 2	BSL 3	BSL 4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation: - inward air flow - controlled ventilation system - HEPA-filtered air exhaust	No	Desirable Desirable No	Yes Yes Yes/No⁵	Yes Yes 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 - in laboratory room - double-ended	No	Desirable No No	Yes Desirable Desirable	Yes Yes 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.

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

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



Relevance of BSL for Production Facilities

For the production of BSL relevant products, only the
 BSL 1 – 3 have to be taken into account. The risk of an BSL 4 production facility is too high.



Risk Assessment for Determination of BSL

To provide the necessary level of safety for the specific work to be done, it has to be evaluated based on a risk assessment



Assignment of BSL

Consider...

- the used organism,
 - (To which risk group belongs the used organism?)
- the equipment,
 - (Is the used equipment operated as an open system or is it a closed containment?)
- and the procedure.
 - (Are open procedures carried out in a biosafety cabinet or not?)



Differences of BSL 1, 2 and 3 in Laboratories and in Production Facilities



Difference BSL 1: WHO and CBC Design Concept

Features in Facilities	wно	CBC Concept	Justification
Isolation ^a of laboratory	No	Yes	Automatically implemented because of the WHO GMP guidelines (Isolation of production rooms through separate airlocks, leading into separate corridors)
Room sealable for decontamination	No	Yes (if required)	To avoid cross-contaminations in a facility in which more than one product or strain is produced
Ventilation: - inward air flow (differential pressure) - controlled ventilation system (air recirculation) - HEPA-filtered air exhaust	No No (Possible) No	Yes/No (inward air flow) Yes No	 Depending on the risk of the product^c Air recirculation allow if HEPA-filtered and recirculated within the same bio-positive production rooms
Double-door entry	No	Yes	Automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Airlock	No	Yes	Automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Airlock with shower	No	No	
Anteroom	No	Yes	Automatically implemented (designed as airlocks, see above)
Anteroom with shower	No	No	
Effluent treatment	No	Yes	Because of the size of production volume and therefore also waste water volume (can be more than several 100 litres)
Autoclave: - on site - in laboratory room - double-ended	No	Yes Yes Yes	Double-ended autoclaves facilitate the process of handling contaminated goods / material. No wrapping to bring goods out of the bio+ area to the cleaning area. Cleaning area directly connected
Biological safety cabinets	No	Yes/No	Depending on the risk of the produced product ^d
Personnel safety monitoring capability ^b	No	Yes/No	Decide by the customer

^a Environmental and functional isolation from general traffic.

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

^c Differential pressure can either be positive, 0 or negative, depending on the risk, (e.g. if the risk of reverse mutation of the attenuated strain to the wild type strain exists, the building should have negative differential pressure to avoid the release of the virus into the environment)

^d If the risk of reverse mutation of the attenuated strain exists, BSCs should be installed



Difference BSL 2: WHO and CBC Design Concept

Features in Facilities	wно	CBC Concept	Justification
Isolation ^a of laboratory	No	Yes	Automatically implemented because of the WHO GMP guidelines (Isolation of production rooms through separate airlocks, leading into separate corridors)
Room sealable for decontamination	No	Yes	To avoid cross-contaminations in a facility in which more than one product or strain is produced. Decontamination before maintenance if pathogens are handled
Ventilation: - inward air flow (differential pressure) - controlled ventilation system (air recirculation) - HEPA-filtered air exhaust	Desirable Desirable (Possible ¹) No	Yes/No (inward air flow) Yes Yes	 Depending on the risk of the product^c Air recirculation allowed if HEPA-filtered and recirculated within the same biopositive production rooms Directly integrated on the clean room wall or centralized in the air handling unit system^d
Double-door entry	No	Yes	Automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Airlock	No	Yes	Automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Airlock with shower	No	No	Possible if emergency showers are needed
Anteroom	No	Yes	Automatically implemented (designed as airlocks, see above)
Anteroom with shower	No	No	
Effluent treatment	No	Yes	Because of the size of production volume and therefore also waste water volume (can be more than several 100 litres)
Autoclave: - on site - in laboratory room - double-ended	Desirable No No	Yes Yes Yes	Double-ended autoclaves facilitate the process of handling contaminated goods / material. No wrapping to bring goods out of the bio+ area to the cleaning area. Cleaning area directly connected
Biological safety cabinets	Desirable	Yes	Because of the large amount of the produced producte
Personnel safety monitoring capability ^b	No	Yes/No	Decide by the customer

^a Environmental and functional isolation from general traffic.

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

^c Differential pressure is either 0 or negative, for mAb facilities the differential pressure is positive

^d Consequence of centralized HEPA filter \rightarrow pipe system is also contaminated

^e if process can't be performed in an BSC, customer-made UAF with inward air flow are used. For mAb and genetically modified products not necessary



Difference BSL 3: WHO and CBC Design Concept

Features in Facilities	wно	CBC Concept	Justification
Isolation ^a of laboratory	Yes	Yes	
Room sealable for decontamination	Yes	Yes	
Ventilation: - inward air flow (differential pressure) - controlled ventilation system (air recirculation) - HEPA-filtered air exhaust	Yes Yes (Possible) Yes/No ^b	Yes (inward air flow) Yes Yes	 Negative pressure in bio-positive areas to avoid the flow of contaminated air into the clean bio-negative area of production Air recirculation allow if HEPA-filtered and recirculated within the same bio- positive production rooms Directly integrated on the clean room wall
Double-door entry	Yes	Yes	
Airlock	No	Yes	Automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Airlock with shower	No	Yes	Because of the large amount of produced product automatically implemented by the separation of the different clean rooms grades and the design of ALs between them
Anteroom	Yes	Yes	
Anteroom with shower	Yes/No ^c	Yes	Automatically implemented (designed as airlocks with shower, see above)
Effluent treatment	Yes/No ^c	Yes	On site liquid decontamination
Autoclave: - on site - in laboratory room - double-ended	Yes Desirable Desirable	Yes Yes Yes	Double-ended autoclaves facilitate the process of handling contaminated goods / material. No wrapping to bring goods out of the bio+ area to the cleaning area. Cleaning area directly connected
Biological safety cabinets	Yes	Yes	
Personnel safety monitoring capability ^d	Desirable	Yes	

^a Environmental and functional isolation from general traffic.

^b Dependent on location of exhaust.

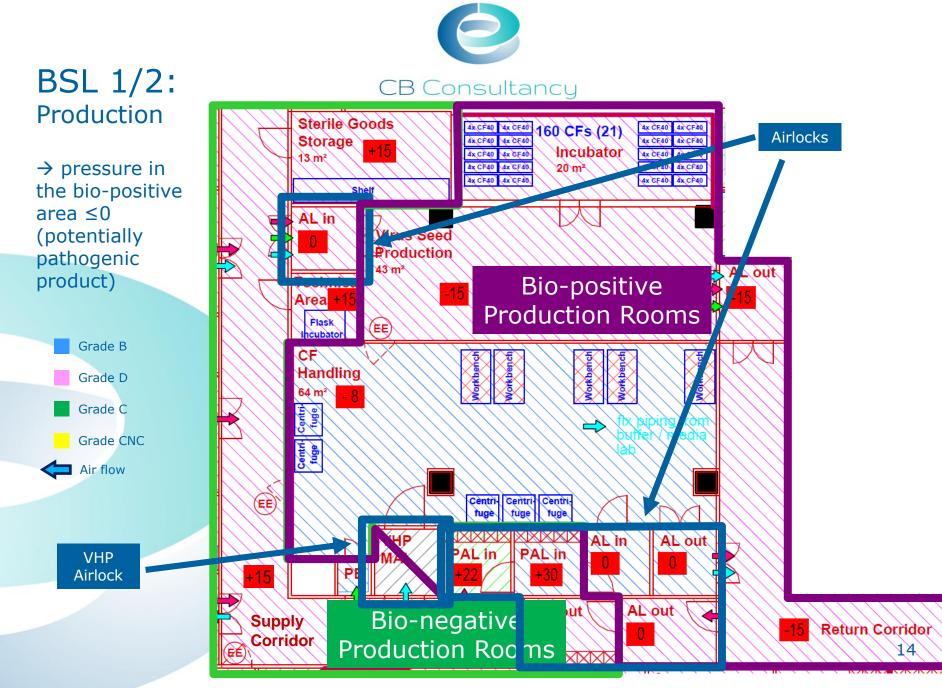
^c Dependent on agent(s) used in the laboratory.

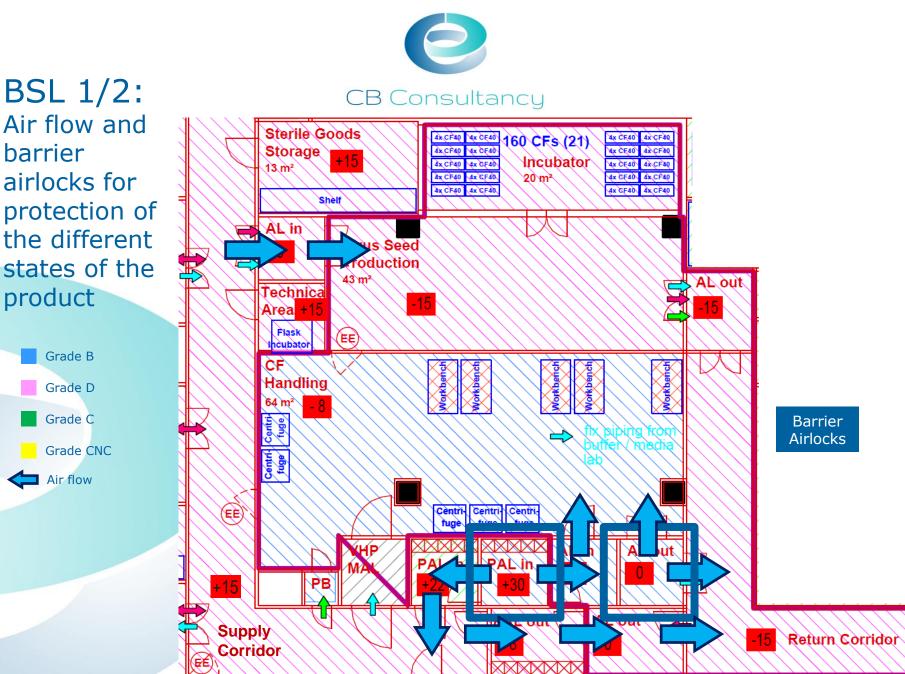
^d For example, window, closed-circuit television, two-way communication.



Conclusion for Facilities (BSL 1-3)

- Generally over-designed compared to laboratories because of the large amount of product which has to be handled
 - BSL 1 and 2: the **range of risks is huge** (depends on the product)
 - \rightarrow e.g. in Europe, genetically modified microorganisms are BSL 2 because of the risk of release to the environment, not because of pathogentic aspects
 - → On the other hand, if e.g. the risk of reverse mutation exists for attenuated virus strains, a BSL 1 production facility may need additional biosafety features normally not required for BSL 1 (e.g. the use of biosafety cabinets, etc.)
- At the end, critical BSL 1 and 2 production facilities may not be that different anymore compared to BSL 3 laboratories / facilities.

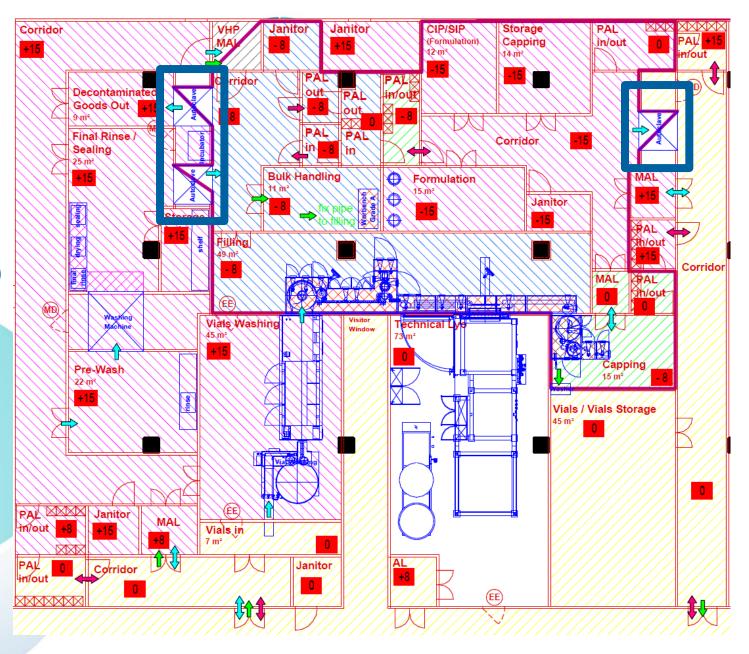




barrier airlocks for protection of the different states of the product



BSL 1/2: Doubleended autoclaves (into and out of the biopositive area)

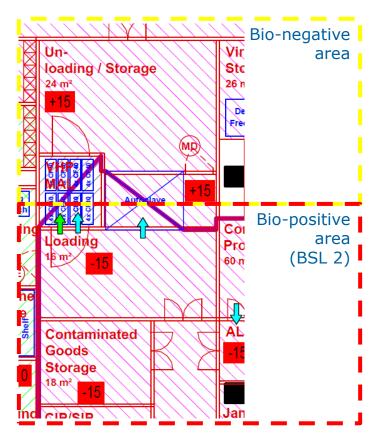




Decontamination of used Items



"All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse."



Grade D Grade CNC

WHO, Laboratory biosafety manual (3rd edition), chapter 3, Laboratory working areas, Point 3

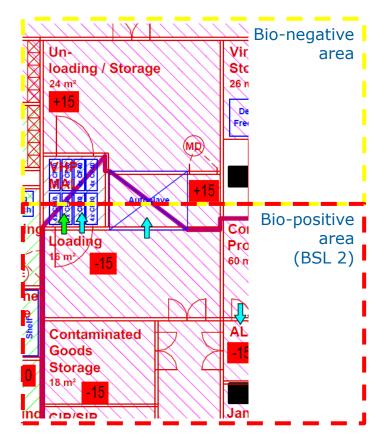


Decontamination of used Items

Use of VHP MALs (fumigation chamber) for...

- ...the surface decontamination of material from the bio-positive to the bio-negative area.
- ...for surface sterilization during the transport of material / product between different clean room grades.

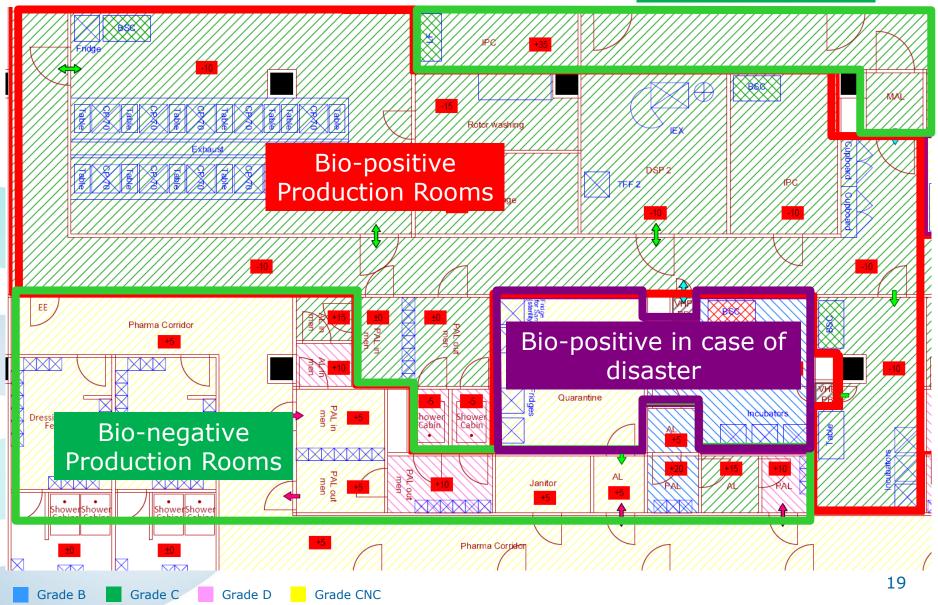
Further explanations will follow in few slides



BSL 3



Bio-negative Production Rooms





Bio-Positive in case of Disaster

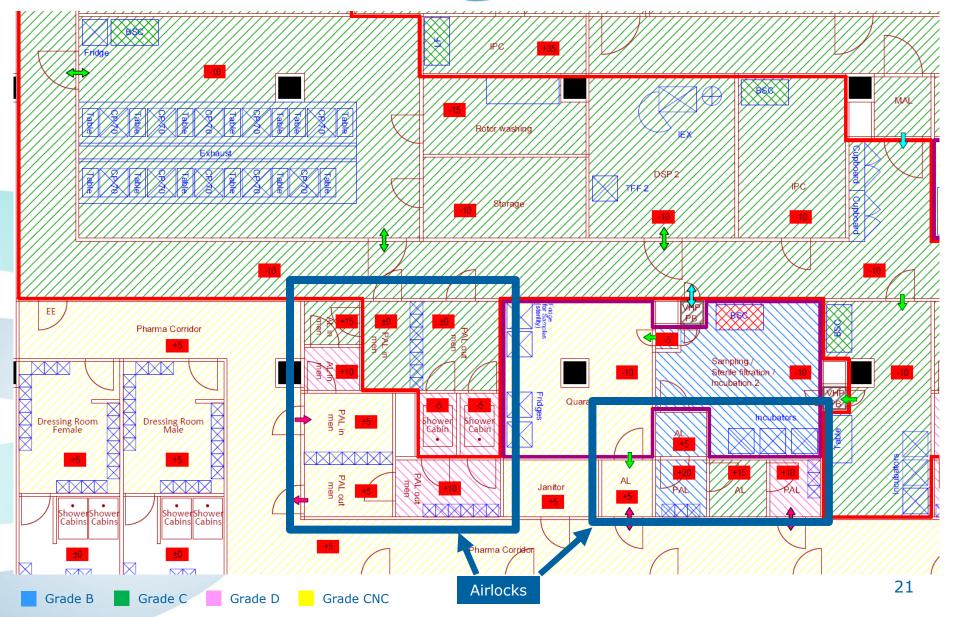
"Bio-positive in case of disaster" rooms are intended for quarantine purpose.

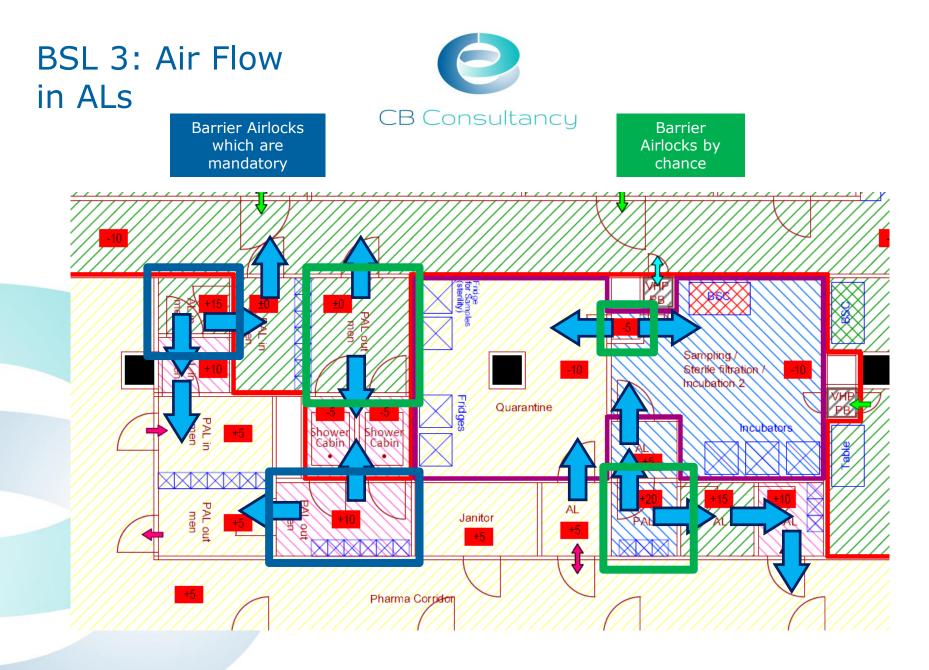
- Designed as bio-positive rooms but operated as bio-negative rooms
- In case of disaster with quarantined virus / bacteria solutions (during / after inactivation), the rooms become bio-positive and will be decontaminated appropriately afterwards

 \rightarrow Decontamination shall only be done with a mobile fumigation system (\rightarrow see VHP decontamination in few slides)

BSL 3: ALs







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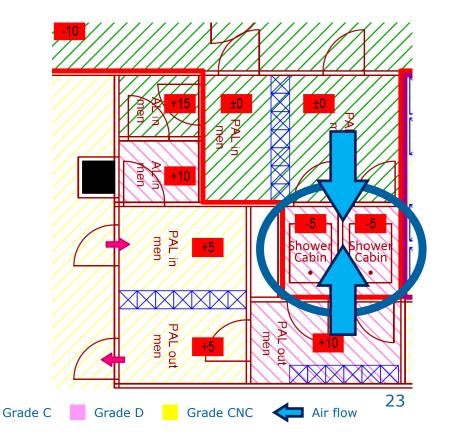
Airlocks with Shower in BSL 3 Facilities

WHO Requirement:

Anteroom with shower for BSL 3 laboratories required or not required (depending on agents).¹

CBC Design Concept:

- Airlock with shower available on the way out of the bio-positive production rooms
- Waste water is decontaminated (explained later in few slides)
- Shower designed as grade D because for this grade no monitoring is required for "in operation"





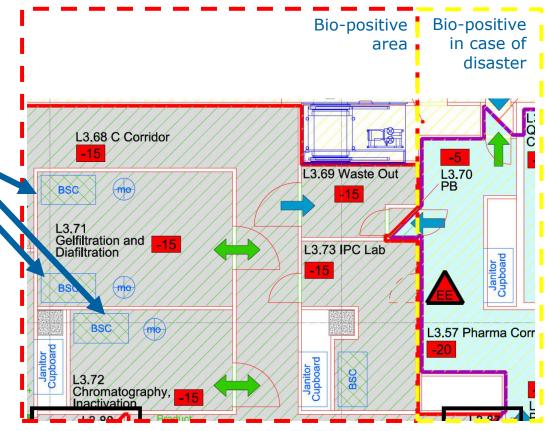
BSCs in Biosafety Relevant Production Facilities

WHO Requirement:

Special attention should be taken to processes causing safety problems, like formation of aerosols (centrifugation, opening of containers,...)

→ Use of Biological Safety Cabinets (BSC) (also for BSL 1 and 2 if necessary)

e.g. during the chromatography → if the different fractions of the product are **collected in open bottles**, this has to be **done in a BSC** (including the pooling). The chromatography column can be outside of the BSC (if sterile bags are taken instead, they can be connected aseptically and no BSC would be needed => **closed system**)





Decontamination by Fumigation





Decontamination by Fumigation

WHO requirement:

"The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination."¹

Remember! Air-tightness of clean rooms was the topic of the previous presentation

\rightarrow Usage of vaporized H₂O₂

- + less toxic than formalin and EtO (which are carcinogen)
- + not flammable or explosive in the used concentration
- + short fumigation cycles
- + decomposition into water and oxygen
- + no residues on clean room surfaces
- higher investment costs
- stronger precautions to avoid damages to the facility and the personnel



Mobile VHP Decontamination System

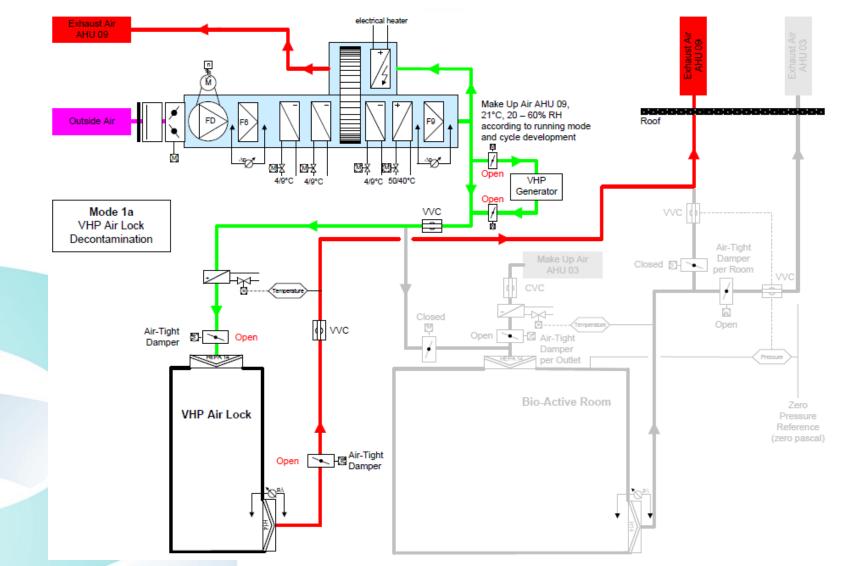
Example of an (schematic) mobile **VHP** airlock **VHP** decontamination CVC system for an VHP airlock (e.g. used for CA Heater -¥ air tight the transport between Air-Tight construction Damper bio-positive and bioerminal HEPA Filter negative area) Mobile VHP generator 18 | † VHP CVC Generator Air-Tight Damper -E

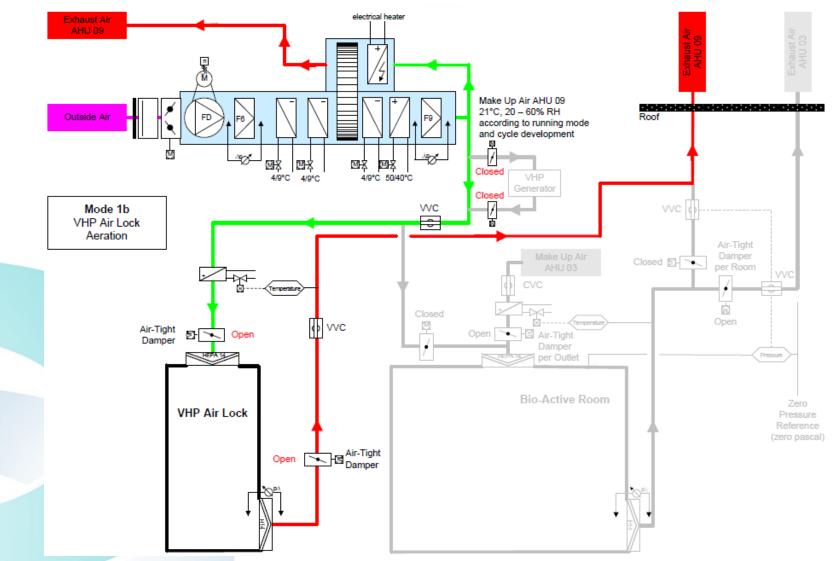


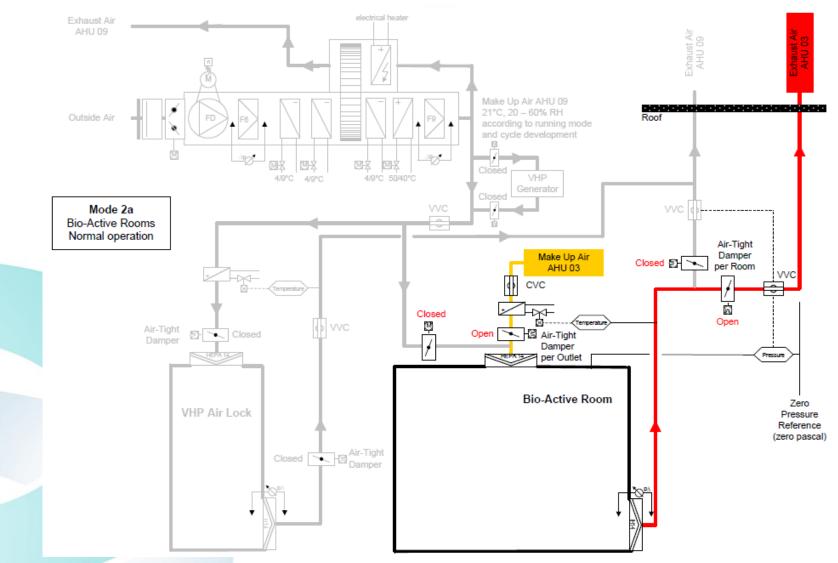
Centralized VHP Decontamination System for Entire Rooms or Facilities

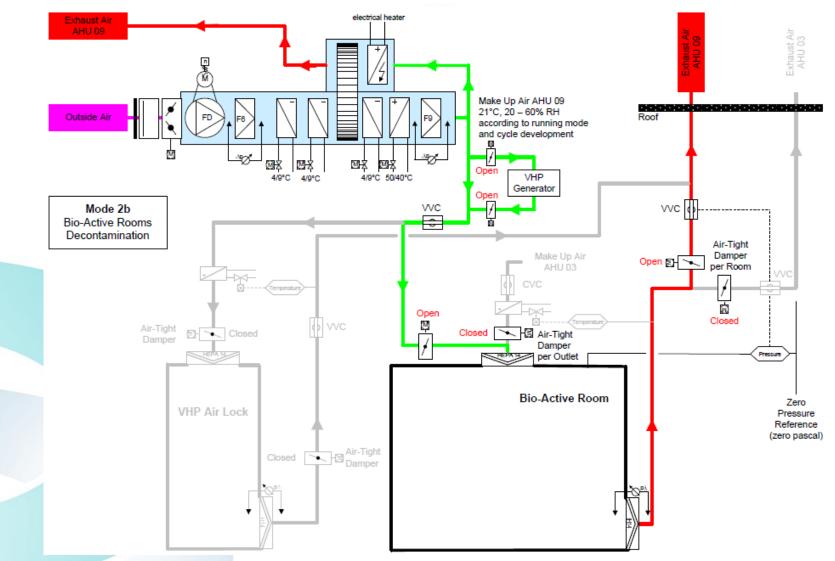


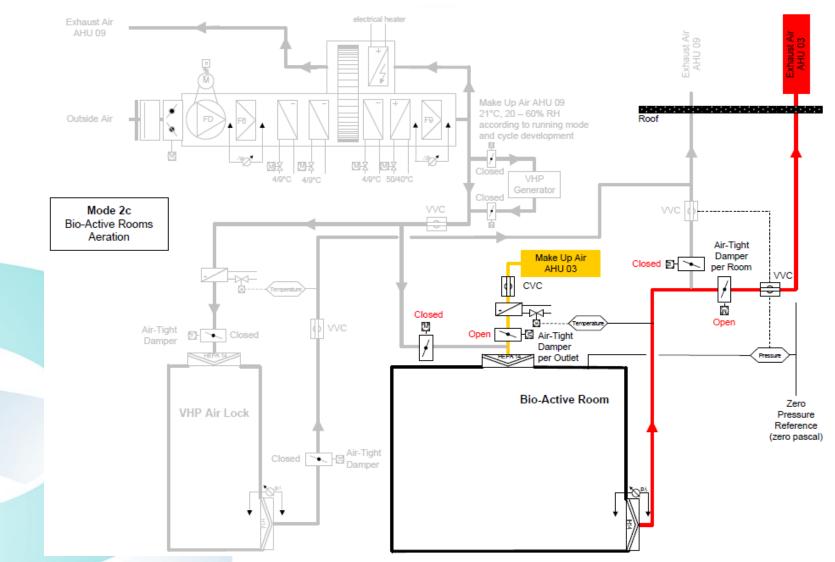
- Off-the-shelf, but still has to be integrated into the HVAC system which requires customized and high qualification effort
- Programming of fully automated cycles
- One fumigation cycle for a large facility
- Additional computerized control system is needed
- Higher price than mobile systems
- Higher safety risk than mobile systems













Air Supply Concept





Air Flow System

WHO Requirement for BSL 1 and 2:

"In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air **without** recirculation."¹

BUT for BSL 3:

"Air may be high-efficiency particulate air (HEPA) filtered, reconditioned and recirculated within that laboratory."² **So**....

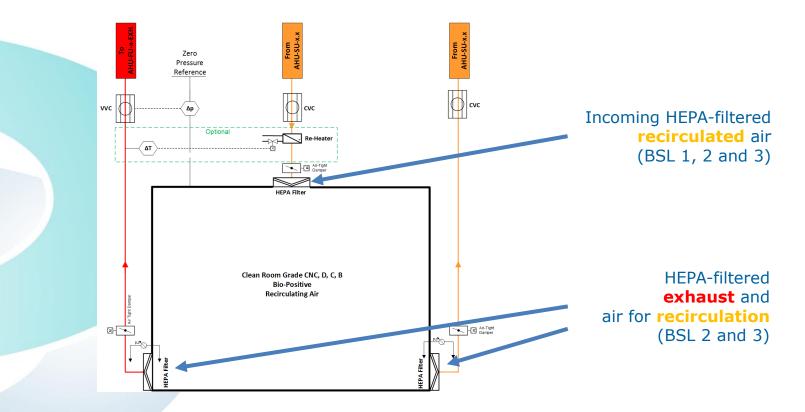


CBC Design Concept:

Recirculating air flow with HEPA filter for outflow (only in BSL 2 and BSL 3 rooms) and inflow (BSL 1, 2 and 3).



Implementation in the Design of the Bio+ Clean Rooms



¹ WHO, Laboratory biosafety manual (3rd edition), chapter 3, Design features, Point 15 ² WHO, Laboratory biosafety manual (3rd edition), chapter 4, Laboratory design and facilities, Point 8



Utilities Distribution and Effluent Treatment



General Requirements (1)

WHO Requirement:

- "A dependable supply of good quality water is essential. There should be **no cross-connections between sources of laboratory and drinking-water supplies**. An anti-backflow device should be fitted to protect the public water system." WHO, Laboratory biosafety manual (3rd edition), chapter 3, Procedures, Point 16
- 2. "Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment."

WHO, Laboratory biosafety manual (3rd edition), chapter 5, Laboratory design and facilities, Point 4

 "Effluents should be collected in closed vessels for further autoclaving and/or disposal."

WHO, Laboratory biosafety manual (3rd edition), chapter 12, Automated equipment (sonicators, vortex mixers), Point 2



General Requirements (2)

WHO Requirement:

4. "Water-treatment plants and distribution systems should be designed, constructed and maintained so as to ensure a reliable source of water of an appropriate quality."

WHO TRS 961, Annex 6, paragraph 12.6

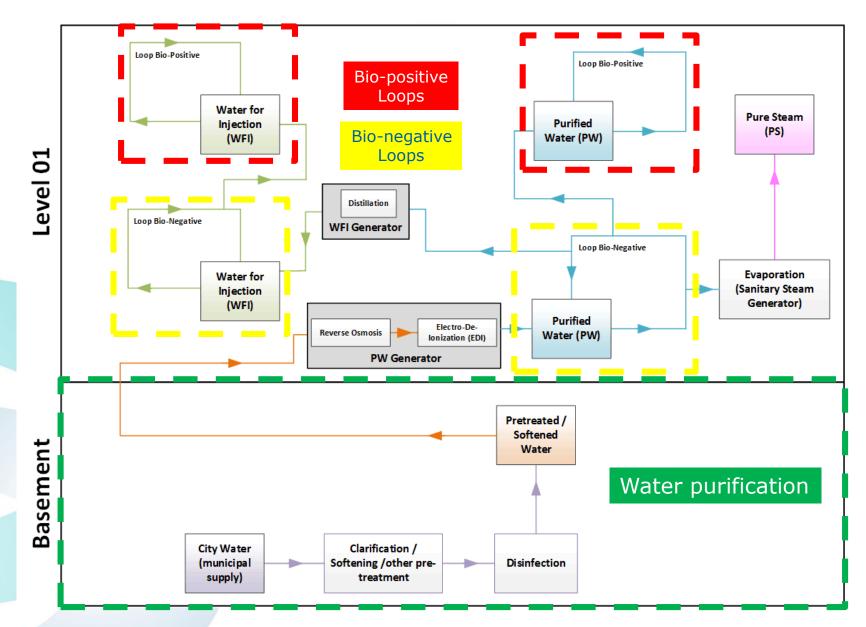
- 5. "An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled." WHO, Laboratory biosafety manual (3rd edition), chapter 3, Design features, Point 7
- 6. **"Backflow-precaution devices** must be fitted to the water supply." WHO, Laboratory biosafety manual (3rd edition), chapter 4, Laboratory design and facilities, Point 13



Conclusion for the CBC Design Concept

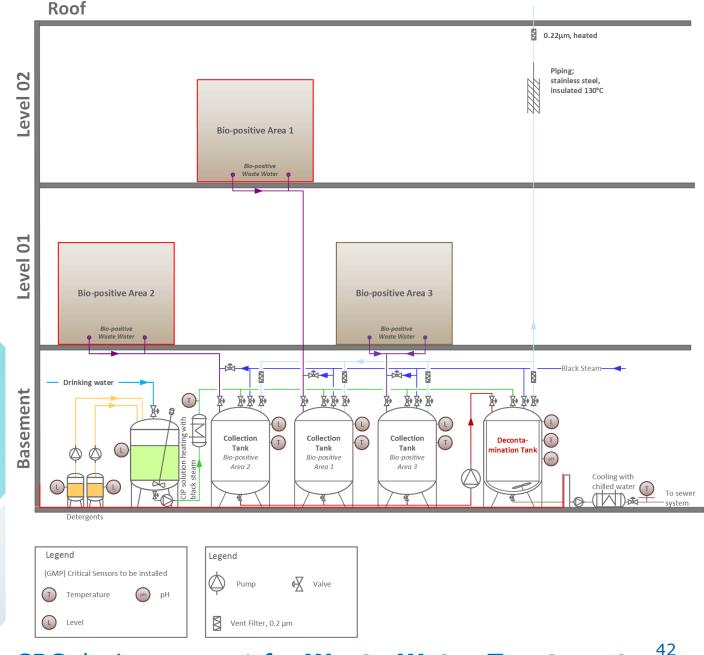
As a result of the above mentioned points (and other requirements¹), the CBC Design Concept implements the following points for clean room facilities:

- PW is generated out of pretreated/softened water by reverse osmosis and electrodeionization (EDI) => satisfies requirement 4 on the slide before
- WFI is generated out of PW by distillation => satisfies requirement 4
- Separate loops for bio-positive and bio-negative areas (for PW and for WFI) => 1/6
- Bio-positive loops are supplied by bio-negative loos (for PW and for WFI) => 1/6
- Each bio-positive area has its own bio-positive PW and WFI loop => 1/6
- An automatic decontamination system is proposed => 3/5
- Annotation: loop distribution system do normally not use backflow-prevention valves (would make no sense). Separation is achieved by separated loops / subloops as explained above



CBC design concept for **Utility Distribution (PW, WFI and PS)**

- Three different products (bio+ areas)
- No difference between BSL 1, 2 or 3 waste water
- Each bio+ area has its own waste water collection tank (permitted independence and precaution of crosscontamination)
- System allows continuous decontamination



CBC design concept for Waste Water Treatment



Animal Facilities





Animal Facility Containment Levels

Risk Group	Containment Level	Laboratory Practices and Safety Equipment
1	ABSL-1	Limited access, protective clothing and gloves
2	ABSL-2	ABSL-1 practices + hazard warning signs. Class I or Class II BSCs for activities that produce aerosols. Decontamination of waste and cage before washing
3	ABSL-3	ABSL-2 practices + controlled access. BSCs and special protective clothing for all activities
4	ABSL-4	ABSL-3 practices + strictly limited access. Clothing change before entering. Class III BSCs or positive pressure suits. Shower on exit. Decontamination of all wastes before removal from facility
APCI - animal facility biosofaty lovel		

ABSL = animal facility biosafety level



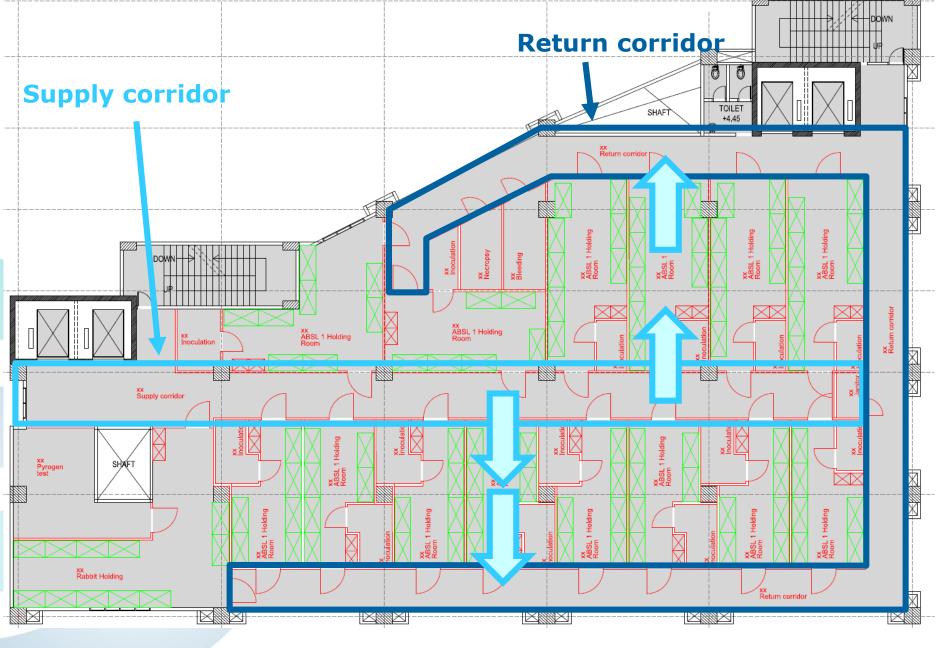
ABSL-1

WHO Requirement:

- For maintenance of most stock animals after quarantine
- For animals that are inoculated with agents (Risk group 1)
- Supportive operations related to the animal holding (cage washing, transport of material and equipment which were in contact with the animals, and transport of animals)

CBC Design Concept:

- Supply and return corridor (both ABSL-1)
- ABSL-1 animal rooms between the supply / return corridors
- Unidirectional path of material/personnel/animals
- Airlocks with lockers to put on dedicated garments for ABSL-1 area



ABSL 1



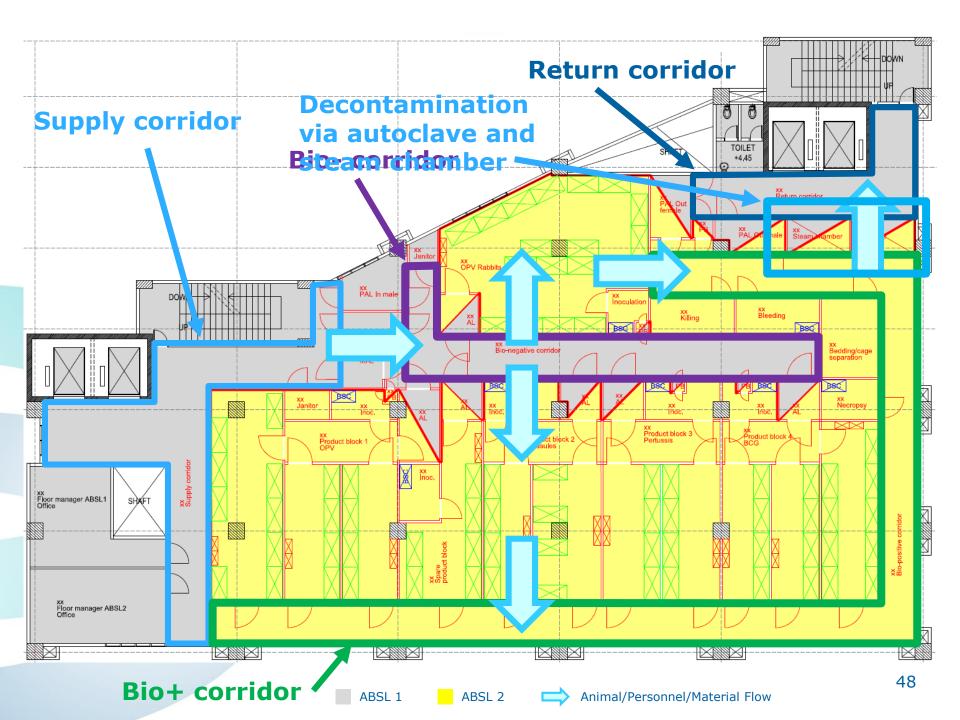
ABSL-2

WHO Requirement:

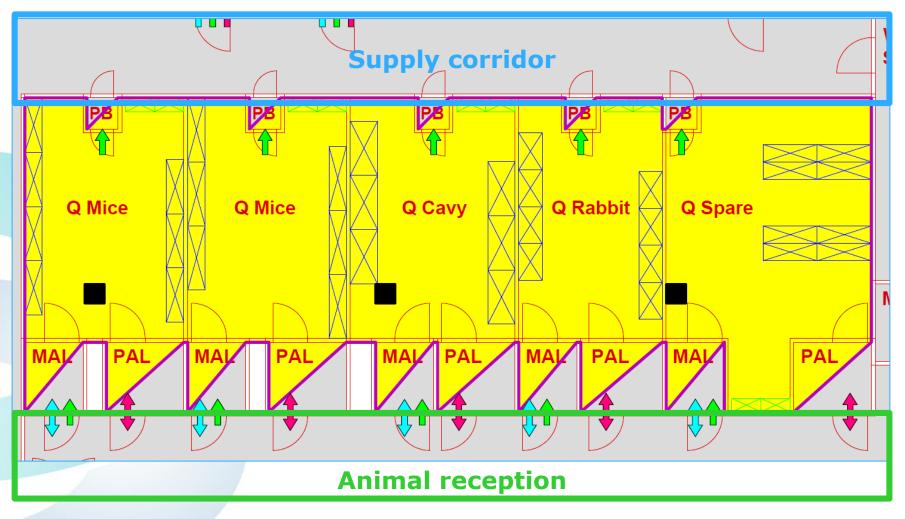
- For animals that are inoculated with agents (Risk group 2)
- Decontamination of waste material, bedding, animal cage by an autoclave
- BSCs for work that may generate aerosols
- Quarantine area for the animals

CBC Design Concept:

- Supply (ABSL-1), bio-negative (ABSL-1), bio-positive (ABSL-2) and return corridor (ABSL-2)
- ABSL-2 room between the bio-positive and bio-negative corridor
- Unidirectional path of material/personnel/animals
- Airlocks entering ABSL-2 rooms out of bio-negative corridor
- Quarantine is designed as ABSL-2 rooms
- Access through airlocks only with changing of garment
- Decontamination of all waste and materials which leaves the ABSL-2 area
- Use of BSC for work which may generate aerosols (inoculation, bleeding, etc.)







CBC design concept example for Quarantine Area

ABSL 1 ABSL 2 49



Animal Facilities: Design of Supportive Areas

Washing Area

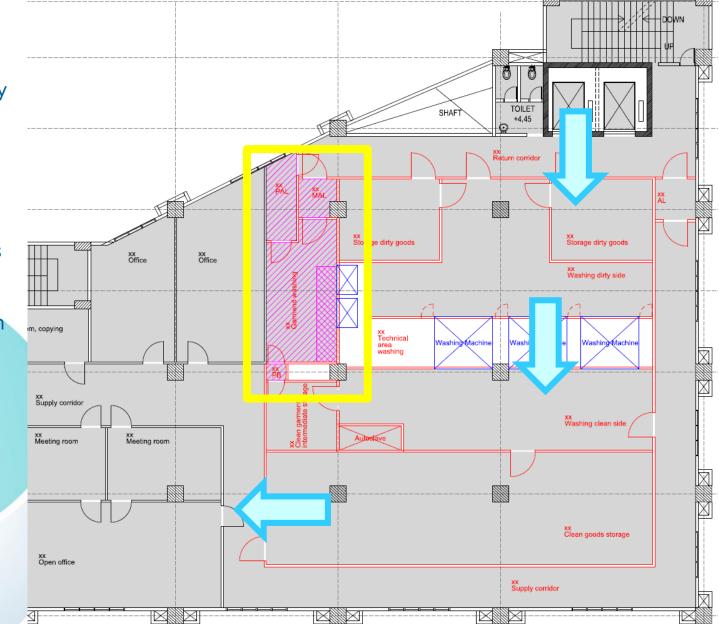
- After decontamination via autoclave (only out of ABSL 2 area), material is brought to the washing area (via return corridor)
- Pass-through washing machines wash the dirty material
- Clean material can be distributed in the facility again by the supply corridor

Grade D area because in this animal house other procedure were done in which clean room clothes were needed

ABSL 1

Grade D

Material Flow





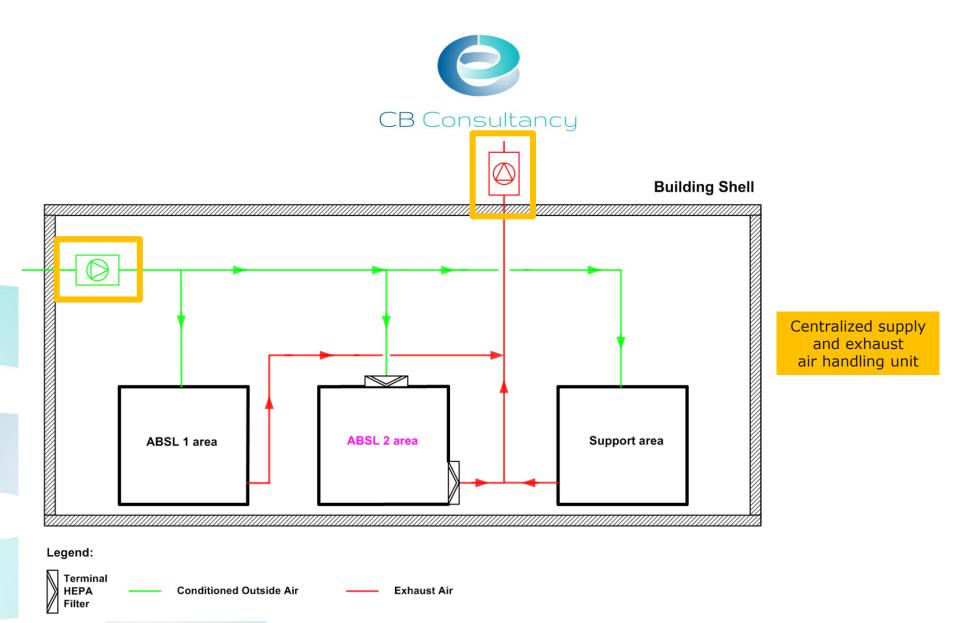
Similarities and Special Case

Similarities:

- As for BSL 2 area, waste water from ABSL-2 is collected and decontaminated
- As for BSL 1 and 2, ABSL-2 area has its own water supply loop
 - → ABSL-1 doesn't need a separate loop within the animal house, because inactive agents are handled. The ABSL-1 is needed because of the general handling of animals

Special Case:

- No recirculation of air (because of the smell of the animals)
 - → Centralized air handling units for conditioning of outside air, air supply to all areas of the building
 - \rightarrow Centralized exhaust air handling unit
 - → Segregation of ABSL-2 area from ABSL-1 and black (unclassified) area by terminal HEPA filters for supply and exhaust air in the ABSL 2 rooms
 - → Negative differential pressure in the animal holding rooms, ABSL-1 (smelly air) and ABSL-2 (biosafety containment)



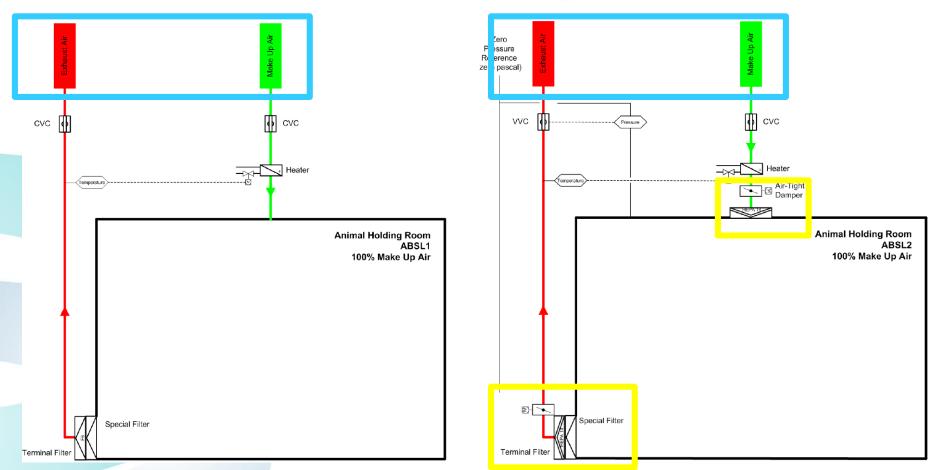
CBC design concept example for a Ventilation System



Terminal HEPA filter in ABSL-2 area

No air recirculation





Detailed Room Typicals for ABSL-1 and ABSL-2



Further Questions?