



生醫材料導論

Biosafety Level

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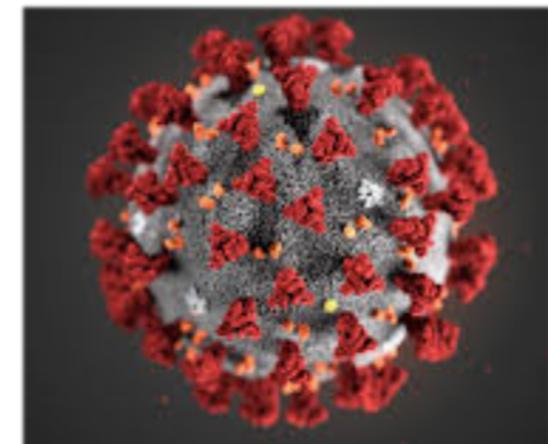
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COVID-19

SARS-CoV-2 came from an animal but finding which one will be tricky, as will laying to **rest speculation of a lab escape.**



The Wuhan Institute of Virology in China is at the centre of the claims. Credit: Hector Retamal/AFP/Getty



惡靈古堡

Biohazard - Resident Evil



惡靈古堡

4

Biohazard - Resident Evil

生化危機

Biosafety vs. Biohazard

- **Biohazard** is an organism, or substance derived from an organism, that **poses a threat to human health**.
- This includes medical waste, samples of a microorganism, virus or toxin (from a biological source) that can impact human health.

Protection :

- Workers
- Products
- Support personal
- Environment



Classification of infective microorganisms by Risk Group

Risk Group 1

(no or very low individual and community risk)

- A microorganism that **is unlikely to cause human or animal disease.**

yeast

一種不太可能引起人類或動物的微生物
疾病。
酵母

Risk Group 2

(moderate individual risk, low 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 of infection is limited.**

一種病原體，可引起人類或動物疾病，但不太可能對
實驗室工作人員、社區、牲畜或環境造成嚴重危害。
• 實驗室接觸可能導致嚴重感染，但有有效的治療和
預防措施，感染傳播的風險有限。

Staphyococcus, Candida

Classification of infective microorganisms by Risk Group

Risk Group 3

(high individual risk, low community risk)

- A **pathogen** that usually **causes serious human or animal disease**, but **does not ordinarily spread from one infected individual to another.**
一種病原體，通常會導致嚴重的人類或動物疾病，但通常不會從一個受感染個體傳播到另一個受感染個體。
• 有有效的治療和預防措施。
- Effective treatment and **preventive measures are available.**

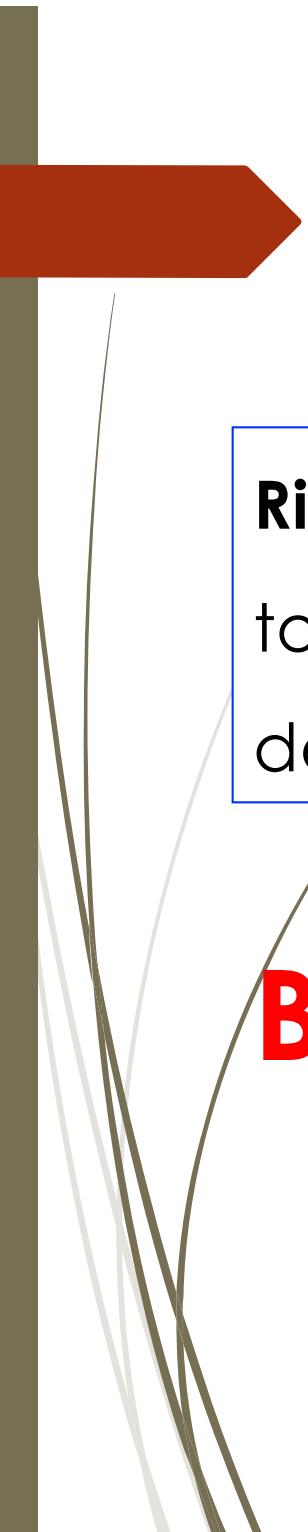
Hepatitis B virus, HIV

Risk Group 4

(high individual and community risk)

- A **pathogen** that usually causes **serious human or animal disease** and that can be **readily transmitted** from one individual to another, directly or indirectly.
- Effective treatment and **preventive measures are not usually available.**
一種病原體，通常會導致嚴重的人類或動物疾病，並且可以很容易地從一個人直接或間接地傳播給另一個人。
• 通常沒有有效的治療和預防措施

*Ebola Pox virus
COVID-19*



Biological Safety Level (BSL)

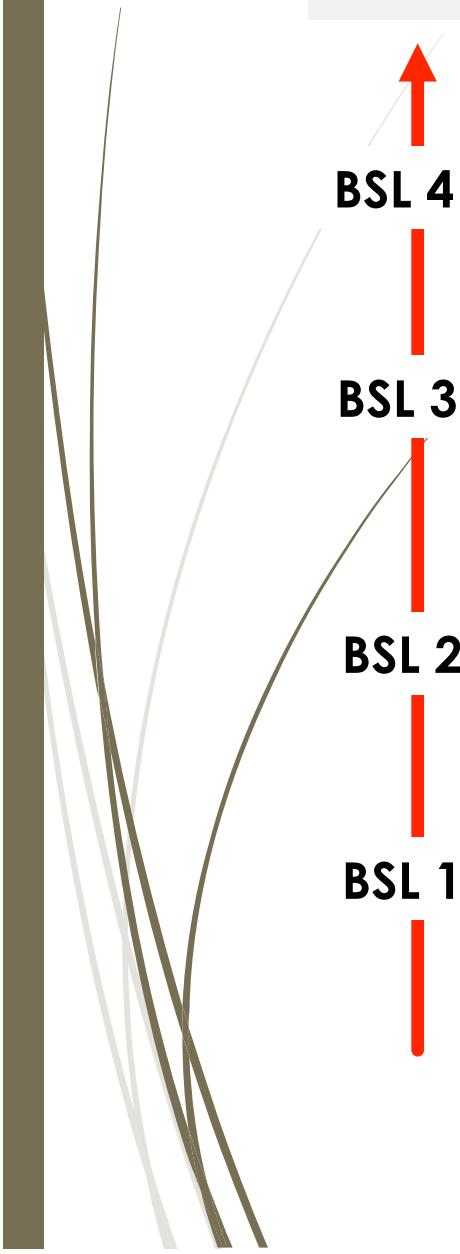
Risk groups (RG) relate to, but **do not “equate”** to the **biosafety level (BSL) of laboratories** designed to work with organisms in each RG

BSL =

- **RG Information**
- + **Practices & Techniques**
- + **Safety Equipment**
- + **Laboratory Facilities**



Classification of Biohazards



BSL 4

BSL 3

BSL 2

BSL 1

- The risk of the organism to humans, animals, plants and /or the environment
- The procedural and facility requirements
- The level of containment required
- The degree of protection for personnel, the environment and the community.



Why BSL Laboratory is needed?

- Contaminate and Spread Control
- Pressure Difference Control
- Dilute Contaminated Air
- Uni-direction Flow and Air Distribution
- Cleanliness, Temperature and Humidity Control
- Exhaust Filtration
- Compartmentalization

Basic laboratory-BSL1 & 2

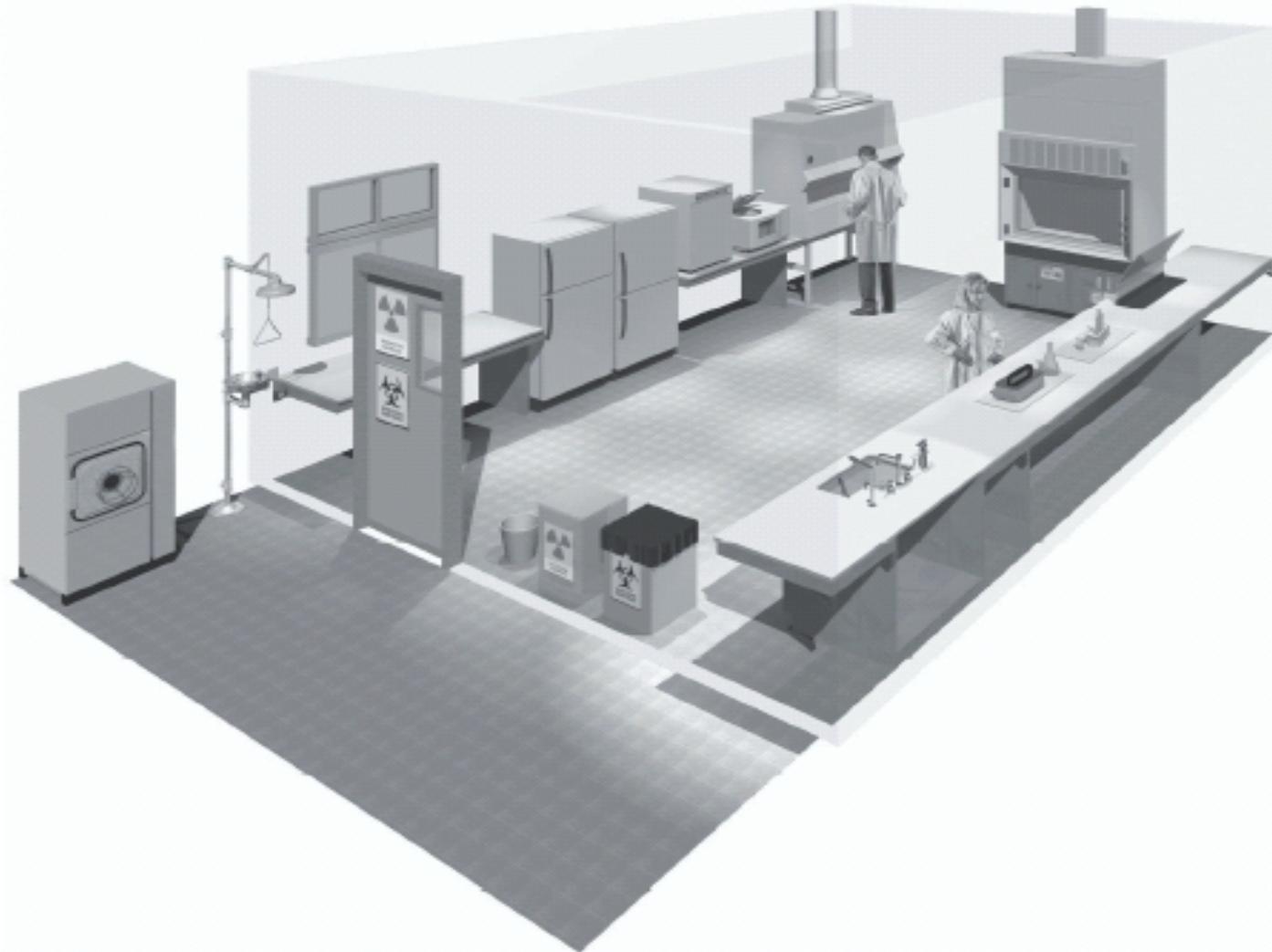


Figure 3. A typical Biosafety Level 2 laboratory

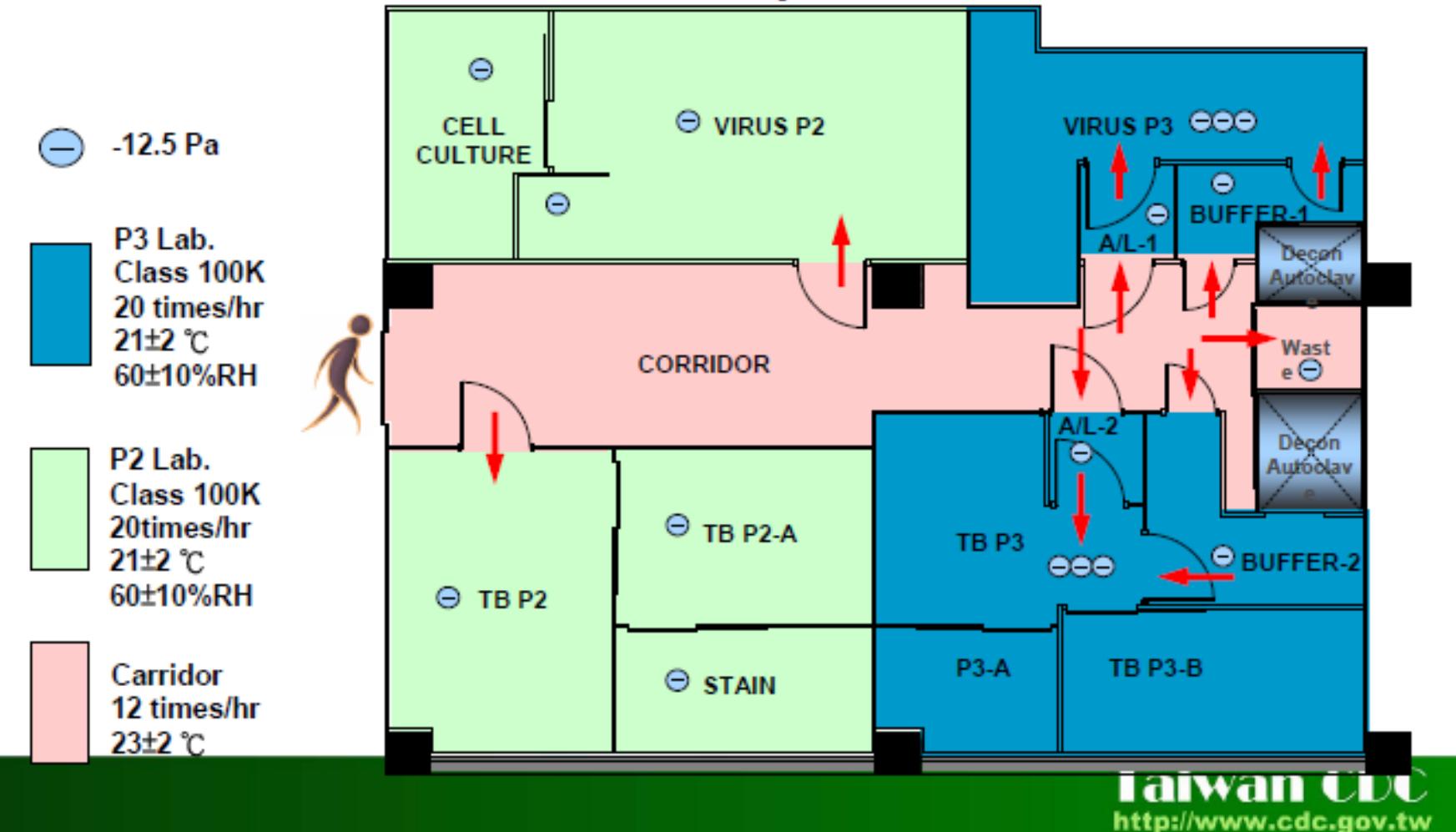
(graphics kindly provided by CUH2A, Princeton, NJ, USA). Procedures likely to generate aerosols are performed within a biological safety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream.



衛生署疾病管制局

Class 100: 1ft³ air has particles number <100,
(particles size ≥ 0.5μm)

Air Balancing & Pressurization Relationships (範例)



參考資料-負壓實驗室生物安全查核硬體之項目、技巧及判定/鄭詠仁博士/台灣生物安全協會
TBSA秘書長/工業技術研究院能源機械部經理

Equipment

For Cell culture

- a. Laminar flower (無菌操作台)
- b. Incubator (培養箱)
- c. Inverted microscope
(倒立式顯微鏡)
- d. Water bath (水浴槽)
- e. Centrifugation (離心機)
- f. -80/-20 °C Refrigerator
- g. N₂ (liquid) Tank (液氮桶)



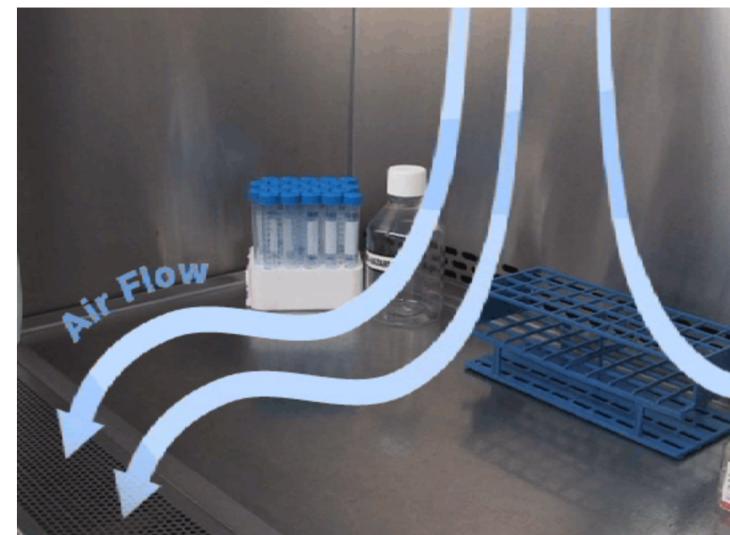
無菌操作台 (Laminar flow hood)

- The laminar flow hood **provides an aseptic work area** while allowing the containment of infectious splashes or aerosols generated by many microbiological procedures.
- Three kinds of laminar flow hoods, designated as **Class I, II and III**, have been developed to meet varying research and clinical needs.

超淨工作臺的工作原理是利用鼓風機驅動空氣通過高效濾器除去空氣中的塵埃顆粒，使空氣得到淨化。淨化空氣徐徐通過工作臺面，使工作臺內構成無菌環境。



Air-Flow Characteristics of Cell Culture Hoods



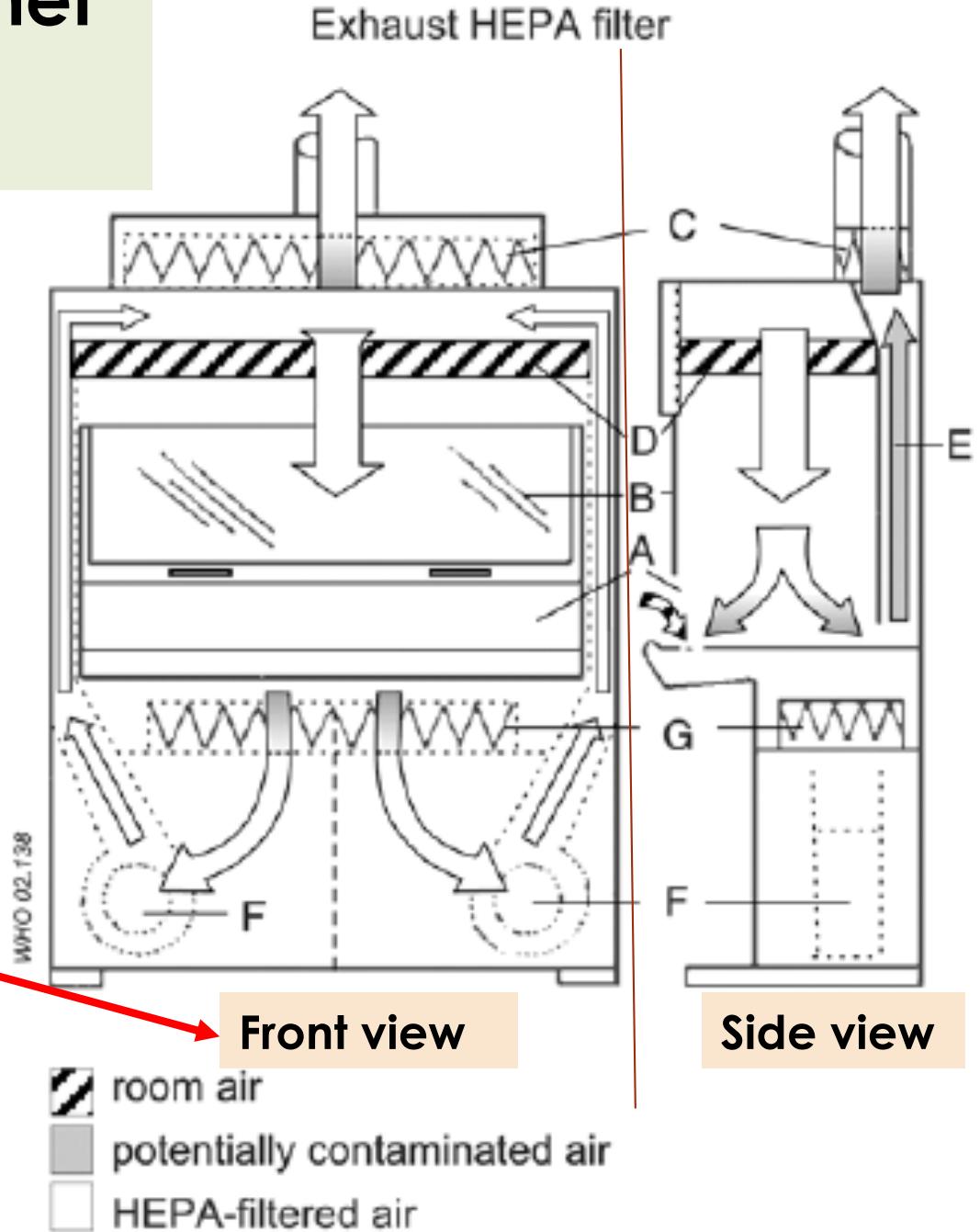
Biological safety cabinet (BSC) 生物安全操作台

Schematic diagram of a Class IIB2

HEPA: High-Efficiency
Particulate Air filter



LABORATORY BIOSAFETY MANUAL (SECOND EDITION,
REVISED), WHO/CDS/CSR/LYO/2003.4



Biological safety cabinet (BSC) 生物安全操作台

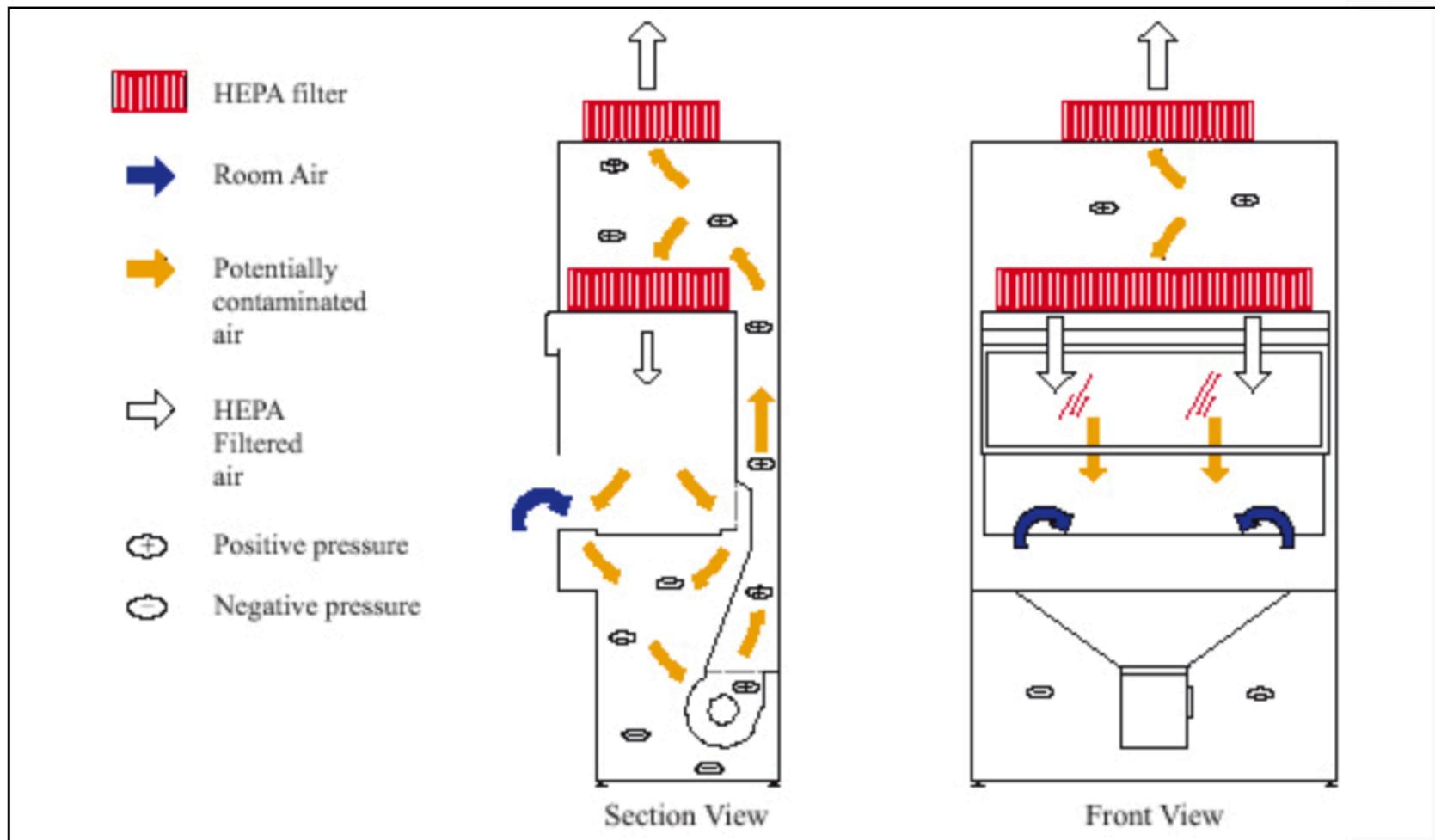


Fig. 2. CLASS II TYPE A1 BIOLOGICAL SAFETY CABINET (Can be room re-circulated or air gap type (thimble).)

Biological safety cabinet (BSC)

生物安全操作台

Table 6. Differences between Class I, II and III biological safety cabinets (BSCs)

| BSC | Face velocity (m/s) | % of air flow | | Exhaust system |
|---|------------------------|---------------|-----------|---------------------------------------|
| | | Recirculated | Exhausted | |
| Class I ^a | 0.36 | 0 | 100 | Hard duct |
| Class IIA1 | 0.38–0.51 | 70 | 30 | Exhaust to room or thimble connection |
| Class IIA2 ^a vented to the outside ^a | 0.51 | 70 | 30 | Exhaust to room or thimble connection |
| Class IIB1 ^a | 0.51 | 30 | 70 | Hard duct |
| Class IIB2 ^a | 0.51 | 0 | 100 | Hard duct |
| Class III ^a | NA ^b | 0 | 100 | Hard duct |

^a All biologically contaminated ducts are under negative pressure, or are surrounded by negative pressure ducts and plenums.

^b NA, not applicable.

Table 2. Relation of risk groups to biosafety levels, practices and equipment

| Risk Group | Biosafety Level | Laboratory type | Laboratory practices | Safety equipment |
|------------|---|---|---|---|
| 1 | Basic – Biosafety Level 1 | Basic teaching, research | GMT | None; open bench work |
| 2 | Basic – Biosafety Level 2 | Primary health services; diagnostic, research | GMT plus protective clothing, biohazard sign | Open bench plus BSC for potential aerosols |
| Risk Group | Biosafety Level | Laboratory type | Laboratory practices | Safety equipment |
| 3 | Containment – Biosafety Level 3 | Special diagnostic, research | As Level 2 plus special clothing, controlled access, directional air flow | BSC and/or other primary devices for all activities |
| 4 | Maximum containment – Biosafety Level 4 | Dangerous pathogen units | As Level 3 plus airlock entry, shower exit, special waste disposal | <u>Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air</u> |

GMT: good microbiological technique

BSC: biological safety cabinet

| 危險群等級 | 生物安全等級 | 實驗室類型 | 實驗室操作規範 | 安全設備 |
|-------|--------|----------------|----------------------------|--|
| 1 | 1 | 基礎教學、研究 | 優良微生物學技術 | 無，開放式工作檯 |
| 2 | 2 | 初級衛生服務、診斷服務、研究 | 優良微生物學技術加上防護衣、生物危害標誌 | 開放式工作檯加上防止氣霧外流之生物安全櫃 |
| 3 | 3 | 特殊診斷服務、研究 | 同第 2 等級加上特殊防護衣、進入管制及定向氣流 | 生物安全櫃及（或）其他所有實驗室工作所需要之基本防護裝備 |
| 4 | 4 | 具危險性之病原體 | 同第 3 等級加上氣密門、出口淋浴及廢棄物之特殊處理 | III級生物安全櫃或II級生物安全櫃並穿著正壓防護衣、雙門高壓蒸氣滅菌器（穿牆式）及經過濾之空氣 |



<https://www.alamy.com/stock-photo-centres-for-disease-control-microbiologists-in-biohazard-suits-before-84966848.html?imageid=7A56CC61-1A4C-4DB4-93F0-687A11841865&p=75935&pn=1&searchId=2c134 bdfa7200fa065350a4efb0b0bd&searchtype=0>

BSL Laboratory

Table 3. Summary of biosafety level requirements

| | Biosafety level | | | |
|-----------------------------------|-----------------|-----------|-----------|-----|
| | 1 | 2 | 3 | 4 |
| Isolation of laboratory | No | No | Desirable | Yes |
| Room sealable for decontamination | No | No | Yes | Yes |
| Ventilation: | | | | |
| – inward air flow | No | Desirable | Yes | Yes |
| – mechanical via building system | No | Desirable | Yes | No |
| – mechanical, independent | No | Desirable | Yes | Yes |
| – HEPA filtered air exhaust | No | No | Desirable | Yes |
| Double-door entry | No | No | Yes | Yes |
| Airlock | No | No | No | Yes |
| Airlock with shower | No | No | No | Yes |
| Anteroom | No | No | Yes | No |
| Anteroom with shower | No | No | Desirable | No |
| Effluent treatment | No | No | Desirable | Yes |

BSL Laboratory

| | Biosafety level | | | |
|-----------------------------|-----------------|-----------|-----------|---|
| | 1 | 2 | 3 | 4 |
| Autoclave: | | | | |
| – on site | Yes | Yes | Yes | Yes |
| – in laboratory room | No | No | Desirable | Yes |
| – double-ended | No | No | Desirable | Yes |
| Biological safety cabinets: | | | | |
| – Class I | No | Optional | Yes | No |
| – Class II | No | Desirable | Yes | Yes, in conjunction with suit laboratories |
| – Class III | No | No | Desirable | Yes, in conjunction with cabinet laboratories |

Conjunction with cabinet



Cleanroom Pass through Transfer Box

BSL Laboratory

△ Pressure to
neighbor room

| grade 名稱 | 潔淨度 等級 | Ventilation 換氣次數 (回/小時) | 與相鄰相通 房間的壓差 (Pa) | Temp 溫度 °C | Humility 相對濕度 % | Noise 雜訊 dB(A) | Light 照度 (lux) |
|-------------|-----------|---------------------------------|------------------------|------------------|-----------------------|----------------------|----------------------|
| 1 一級 | | 可自然通風 | | 16~28 | ≤70 | ≤60 | >300 |
| 2 二級 | 8~9 | 非實驗動物時 可回風≤50% 8~10 | -5~-10 | 18~27 | 30~65 | ≤60 | >300 |
| 3 三級 | 7-8 | 全外氣：10~15 主要保護環境： 可回風≤30% | -15~-25 | 20~26 | 30~60 | ≤60 | >500 |
| 4 四級 | 7-8 | 全外氣：>10~15 | -20~-30 | 20~25 | 30~60 | ≤60 | >500 |



Negative pressure
(compared with surround room)