



Clinical
Microbiology

生物安全介紹-以COVID 19為例

病理檢驗部 微生物科 黃采菽

生物安全-以COVID 19為例

Outline

背景介紹


新冠病毒

傳染途徑

負壓病房

配戴口罩

-2-

- ▶ 背景介紹：
- ▶ 病原體：
 - ▶ SARS-CoV-2 介紹(含變種病毒 **NEW**)
 - ▶ 實驗室診斷方法簡介
- ▶ 傳染途徑：傳染病檢體包裝及運輸 
- ▶ 設施設備：負壓實驗室/負壓病房、HEPA原理
- ▶ 個人防護：口罩



生物安全V.S.生物保全

Outline

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- ▶ 生物安全(保護人)
 - ▶ 經由防護原則、技術及操作以預防病原體、毒素暴露或溢出之意外
- ▶ 生物保全(保護病原體)
 - ▶ 持有病原體的機構或個人防範菌株遺失、失竊、不當使用、誤用、或意圖散播

Chain of Infection

Outline

背景介紹

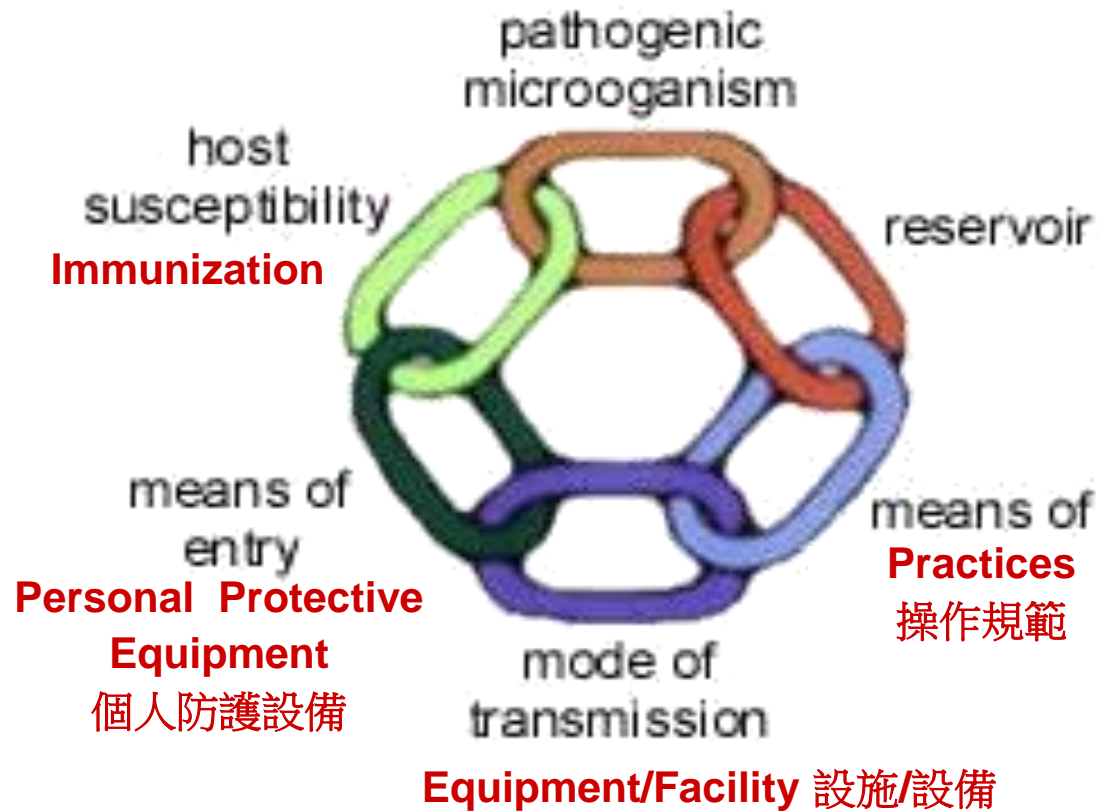
新冠病毒

傳染途徑

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配戴口罩

4-



Primary and Secondary Barrier

Outline

背景介紹

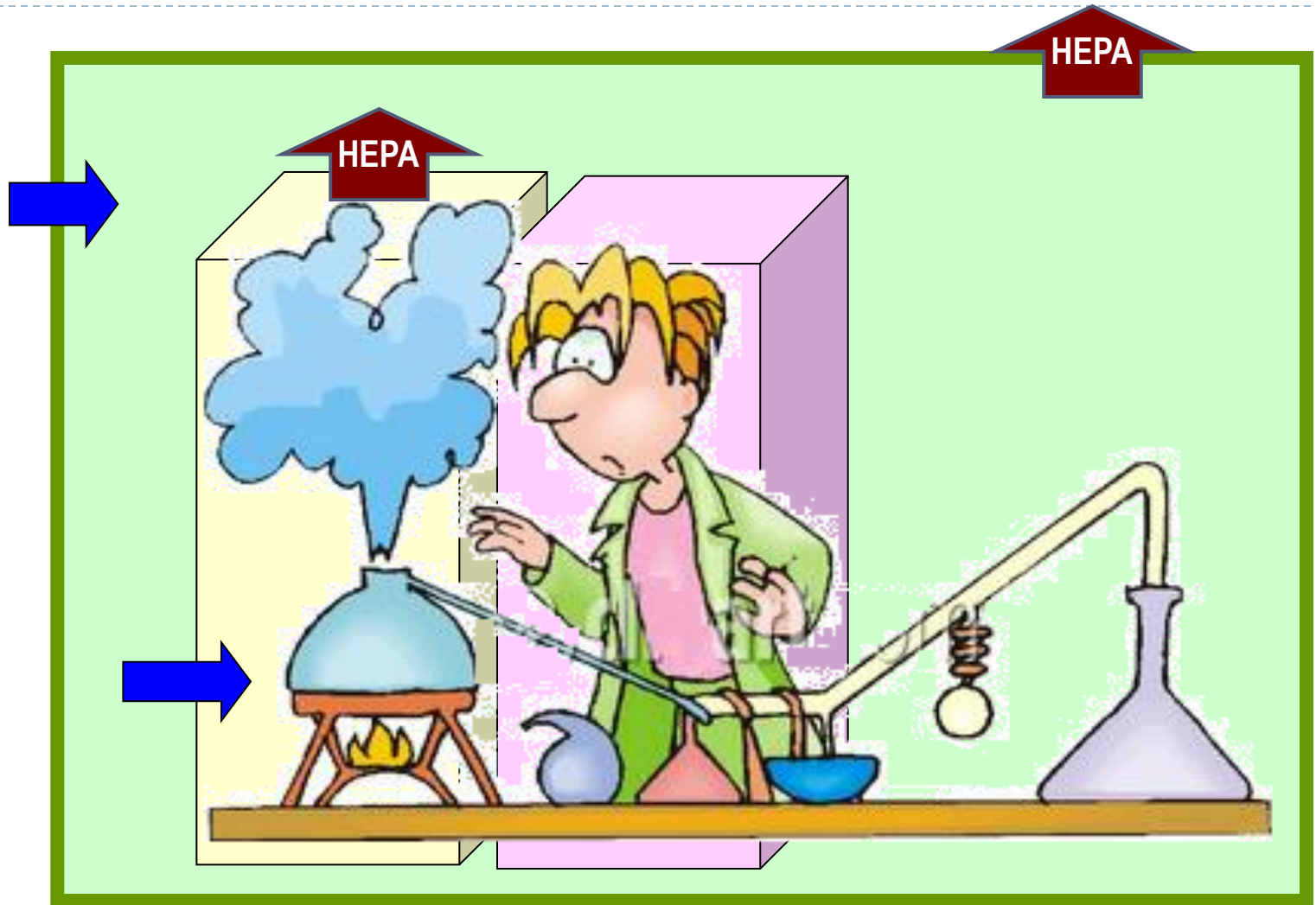
新冠病毒

傳染途徑

負壓病房

配戴口罩

5-



Chain of Infection-COVID 19

Outline

背景介紹

新冠病毒

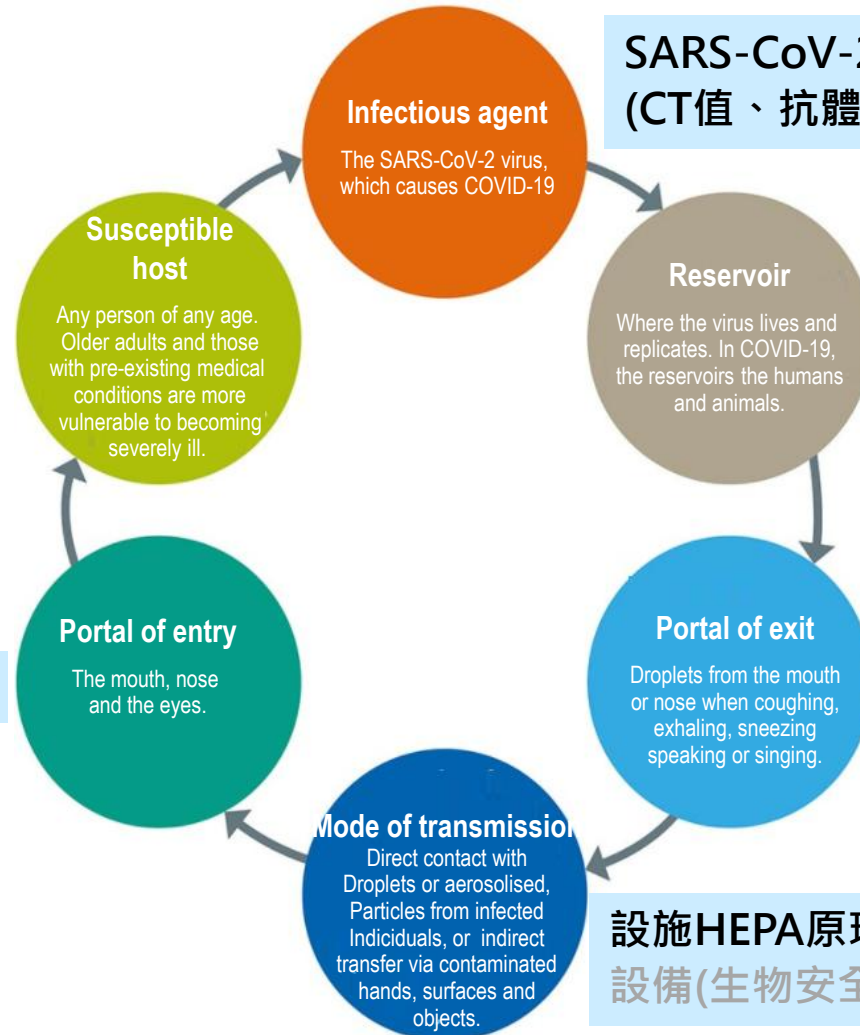
傳染途徑

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配戴口罩

6

口罩原理及應用



SARS-CoV-2簡介
(CT值、抗體、變種病毒)

傳染途徑

設施HEPA原理、應用(負壓實驗室/病房)
設備(生物安全櫃)

相隔253天，再現本土病例

12月22日

案例 765 ②

紐西蘭籍機師染疫關鍵時間點



密切往來

#771 30多歲女性
(本土案例) ③

匡列 167 接觸者

同班機

#760 台籍機師 ①
#766 日籍機師

匡列 89 接觸者

國內疫情通報 | 2020年 12月22日

累計確診
770 移除案 530

累計死亡
7

解除隔離
632

新增確診案例

4

本土個案：1例
境外移入：3例

境外移入 (詳見另一篇新聞)

本土個案

案 771 為今年 12/20 公布之案 765 友人 (30 多歲女性)，由於案 765 疫調時表示無法回想確切活動史，且並未提及曾與案 771 接觸，12/21 經警政單位調查案 765 之活動軌跡，發現案 765 曾於 12/7 至 12 日間與案 771 密切往來，故列為接觸者，同日由衛生單位安排採檢，於今日確診。

為釐清案 765 (外籍機師) 於 12/8 至 12 日間之活動史與接觸者，經衛生、警政單位密切調查個案活動軌跡發現，個案於上述期間曾有多處公共場所活動史 (詳見新聞附件)，提醒曾於附件所列時段出入相關場所的民眾，請自主健康管理，12/25 前如出現相關症狀，應戴上口罩前往全國指定社區採檢院所，經醫師評估是否採檢。

目前衛生單位已掌握案 771 接觸者共 167 人，其中 13 人列居家隔離，154 人列自主健康管理，將全數安排採檢 (21 人檢驗中，146 人待採檢)，衛生單位已針對案 771 工作單位等公共區域進行消毒；另針對航空器群聚案，已採檢 87 人，其中 3 人確診 (案 760、765、766)，78 人檢驗陰性，6 人檢驗中，10 人待採檢。



確診女機師**感染源找到了**！紐西蘭籍同事咳嗽未戴口罩

2020-12-20 15:45 聯合報 記者黃惠群、邱宜君／台北即時報導

今日新增境外移入案765、766為前日確診國籍女機師案760的同事，中央流行疫情指揮中心發言人莊人祥表示，**因案765最早發病，認為其是感染源**。案765於機上工作時**已有咳嗽症狀且未配戴口罩**，入台後曾外出3至4日，外界疑竇其中是否有制度上的疏漏。

莊人祥表示，**CT值24.9**，目前**血清抗體**亦為陽性，估計是12日還在發病十天內，有傳染力。衛生單位已匡列接觸者8人並安排採檢，3人檢驗結果陰性，其餘檢驗中。

莊人祥證實，案765當時在機上沒有戴口罩還咳嗽，而**國籍女機師案760、日籍機師案767**全程都有戴，通常國人和日本人會比較小心一點，有些外國人會覺得戴口罩有文化差異的問題，將要求國籍航空要求所有機組員都遵守規定。

至於採檢的結果，莊人祥表示，這起航空器群聚共有89人密切接觸，都需要採檢，目前已採檢54名，19名陰性，2名陽性，41名檢驗中，至於**血清抗體**採檢55名，**只有一例陽性**。

【武肺變種】首例入侵！陳時中證實：發燒少年驗出英變種病毒 後天起再次封境

2020-12-30 14:45 聯合報 記者陳婕翎、黃惠群／台北即時報導

該名10多歲少年是在12月27日自倫敦搭機返台，下機後發燒達攝氏39度送醫，陳時中曾說，個案搭機報到時是36.5度、登機前36.6度，下機後發燒39度，其Ct值僅15.7，顯示剛發病不久，如今經病毒基因序列比對已確認是變種病毒。

除該發燒少年確認感染英國變種病毒，當天同班機其他2位確診者的病毒基因序列比對結果如何？指揮中心發言人莊人祥說，同班機其他2人的Ct值都在30以上，比較難比對，不過已另外送驗13株病毒，預計明日會有結果。



SARS-CoV-2

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-10-



▶ Credit: Cognition Studio Inc.

Human virus relative size

THE RELATIVE SIZE OF PARTICLES

From the COVID-19 pandemic to the U.S. West Coast wildfires, some of the biggest threats now are also the most microscopic.

A particle needs to be 10 microns (μm) or less before it can be inhaled into your respiratory tract. But just how small are these specks?

Here's a look at the relative sizes of some familiar particles \blacktriangleright



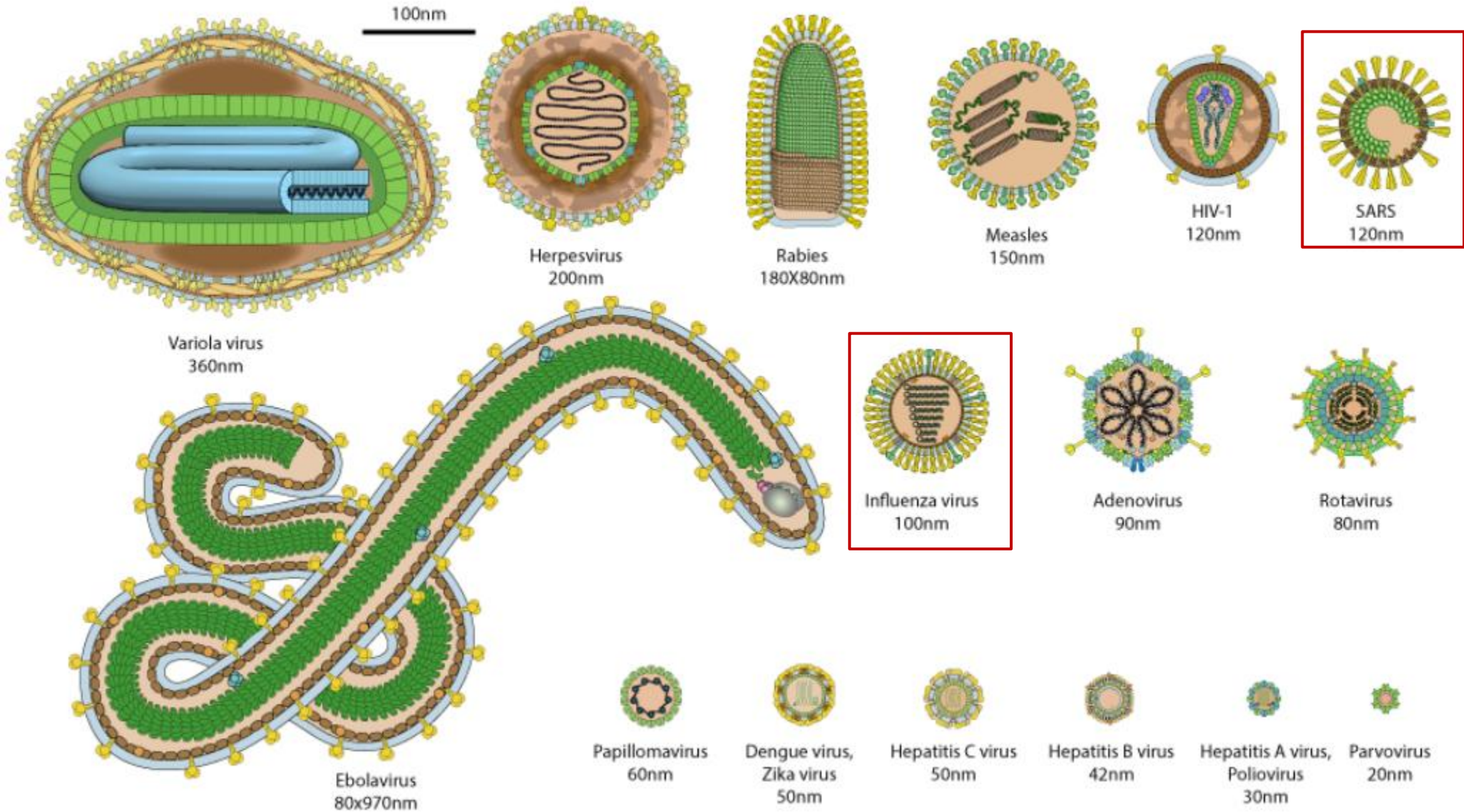
SOURCES: Clearstream, Doris Lowerley, EPA, Financial Times, News Medical, Science Direct, SCMP, Susan Sokolowski, Petroclear, U.S. Dept. of Energy

COLLABORATORS: RESEARCH + WRITING: Carmen Ang, Irina Ghosh | DESIGN + ART DIRECTION: Harriette Schell



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SARS virus relative size



Nomenclature-SARS-CoV-2

Outline

背景介紹

新冠病毒

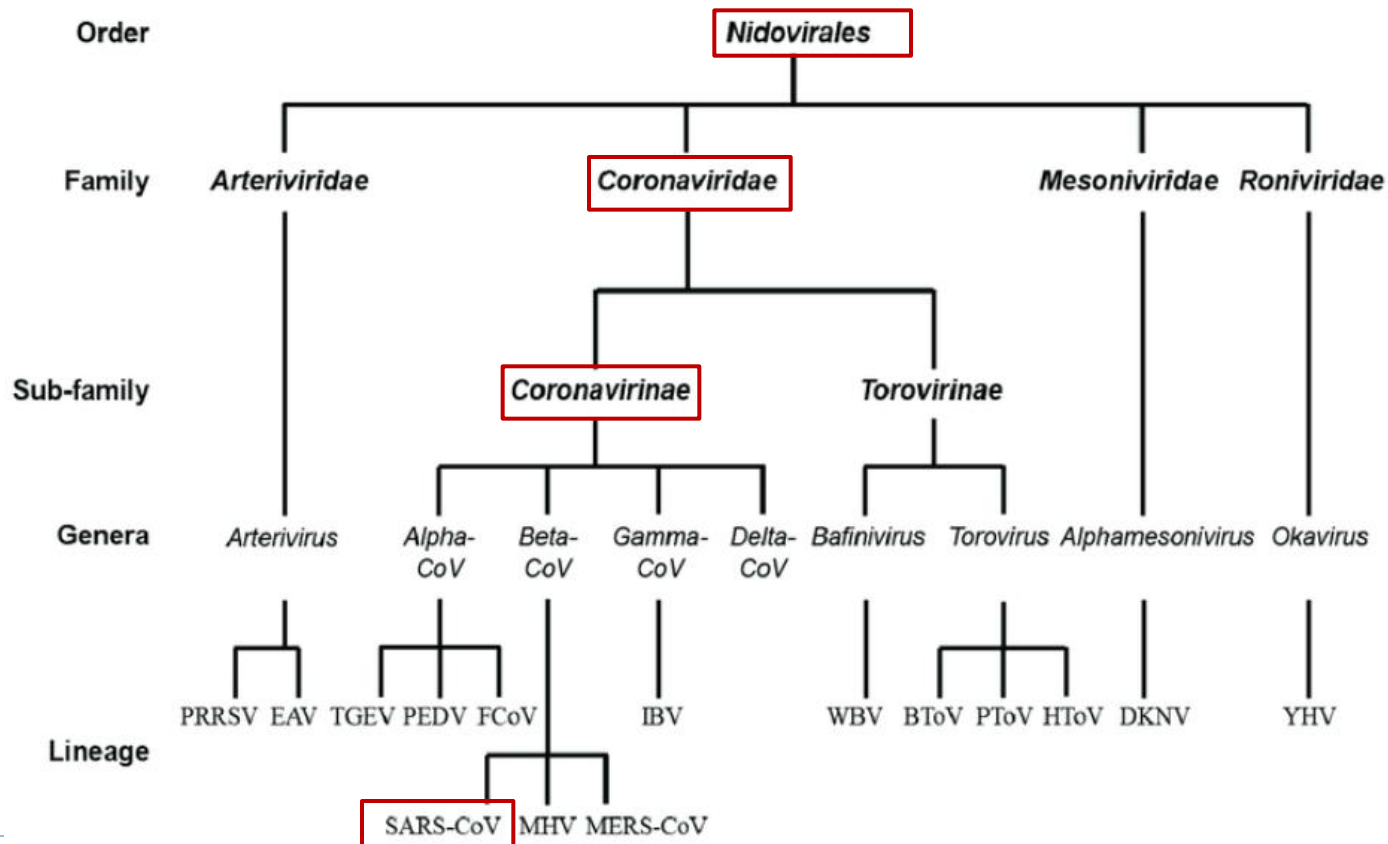
傳染途徑

負壓病房

配戴口罩

-13-

- ▶ Disease: **COVID-19** (coronavirus disease)
- ▶ Virus: **SARS-CoV-2** (severe acute respiratory syndrome coronavirus)
剛開始命名為2019-nCoV (2019 novel coronavirus)



- 域(Domain)
- 界(Kingdom)
- 門(Phylum)
- 綱(Class)
- 目(Order)
- 科(Family)
- 屬(Genus)
- 種(Species)

SARS-CoV-2

Outline

背景介紹

新冠病毒

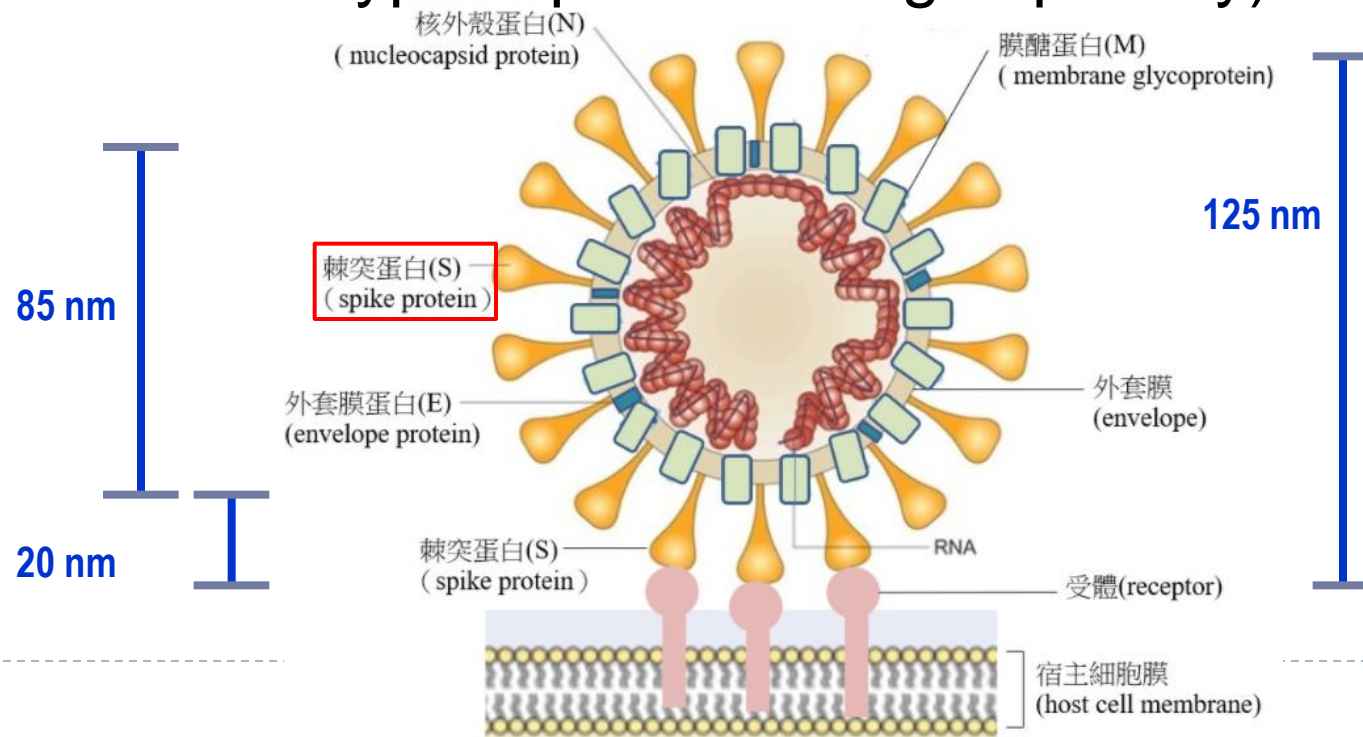
傳染途徑

負壓病房

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-14-

- ▶ Virus size: Diameter of virus particles: ~125 nm
- ▶ Genome size: 29.8 kb-29.9 kb
- ▶ Replication supercomplex (RdRp, nsp7, nsp8) interacts with nsp14 (exonuclease having the Nidovirales-typical proofreading capability)



英國變種病毒

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-15-

- ▶ **Lineage B.1.1.7:**
 - ▶ COVID-19 Genomics UK (COG-UK) Consortium
- ▶ **VUI – 202012/01**
 - ▶ Variant Under Investigation in December 2020
 - ▶ Public Health England
- ▶ **VOC-202012/01**
 - ▶ Variant of Concern 202012/01
 - ▶ Meera Chand and her colleagues in a report published by Public Health England on 21 December 2020
- ▶ **20B/501Y.V1**
 - ▶ US CDC



Dynamic nomenclature for SARS-CoV-2 lineages

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-16-

- ▶ Dynamic nomenclature proposal for SARS-CoV-2 lineages
 - ▶ **Tractable**: constraining the number and depth of hierarchical lineage labels;
 - ▶ Focusing on **active lineages** (delabelling inactive): genomic epidemiology
- ▶ Chronological order of their appearance
 - ▶ A1 lineage (co-occurrence of C8782T, T28144C) : 2020/1/5 Asia
 - ▶ **B1 lineage** (co-occurring G1397A, T28868C, G29742T) : 2020/1/18 Asia
 - ▶ C1 lineage (G26144T) : 2020/1/22 North America
 - ▶ D1 lineage (co-occurring C241T, C3037T, C14408T, A23403G): 2020/2/20 Italy, Europe
 - ▶ E1 lineage (T514C): The Netherlands, Europe.

Phylogenetic network analysis

Outline

背景介紹

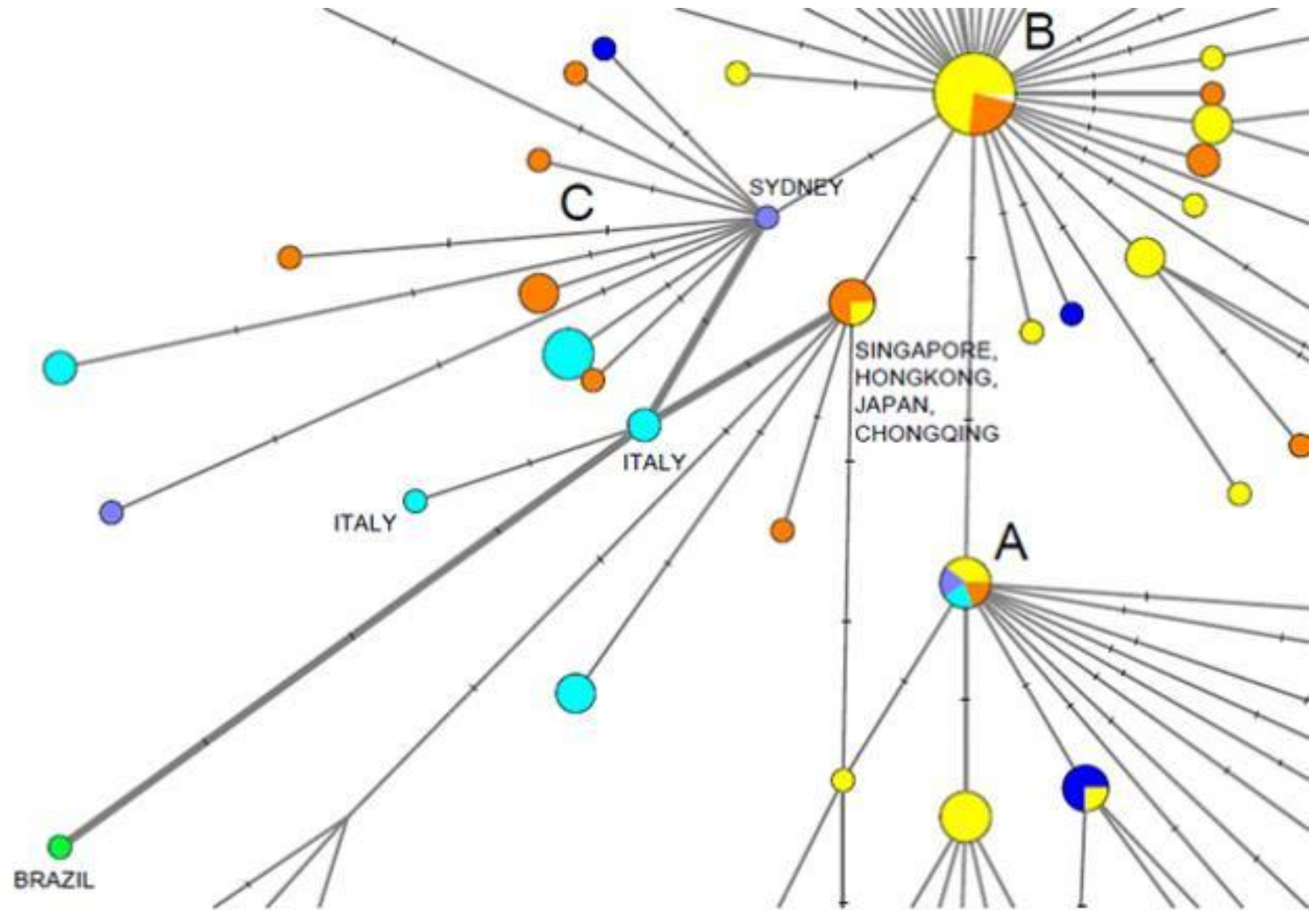
新冠病毒

傳染途徑

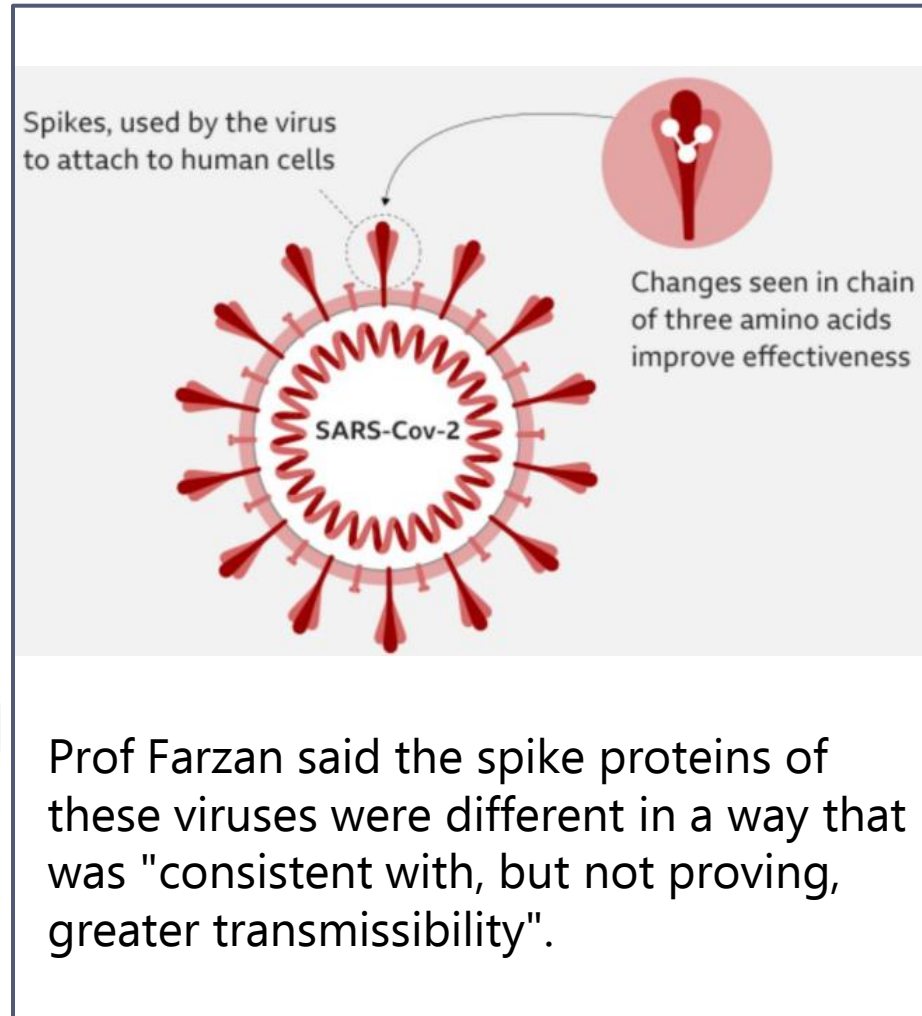
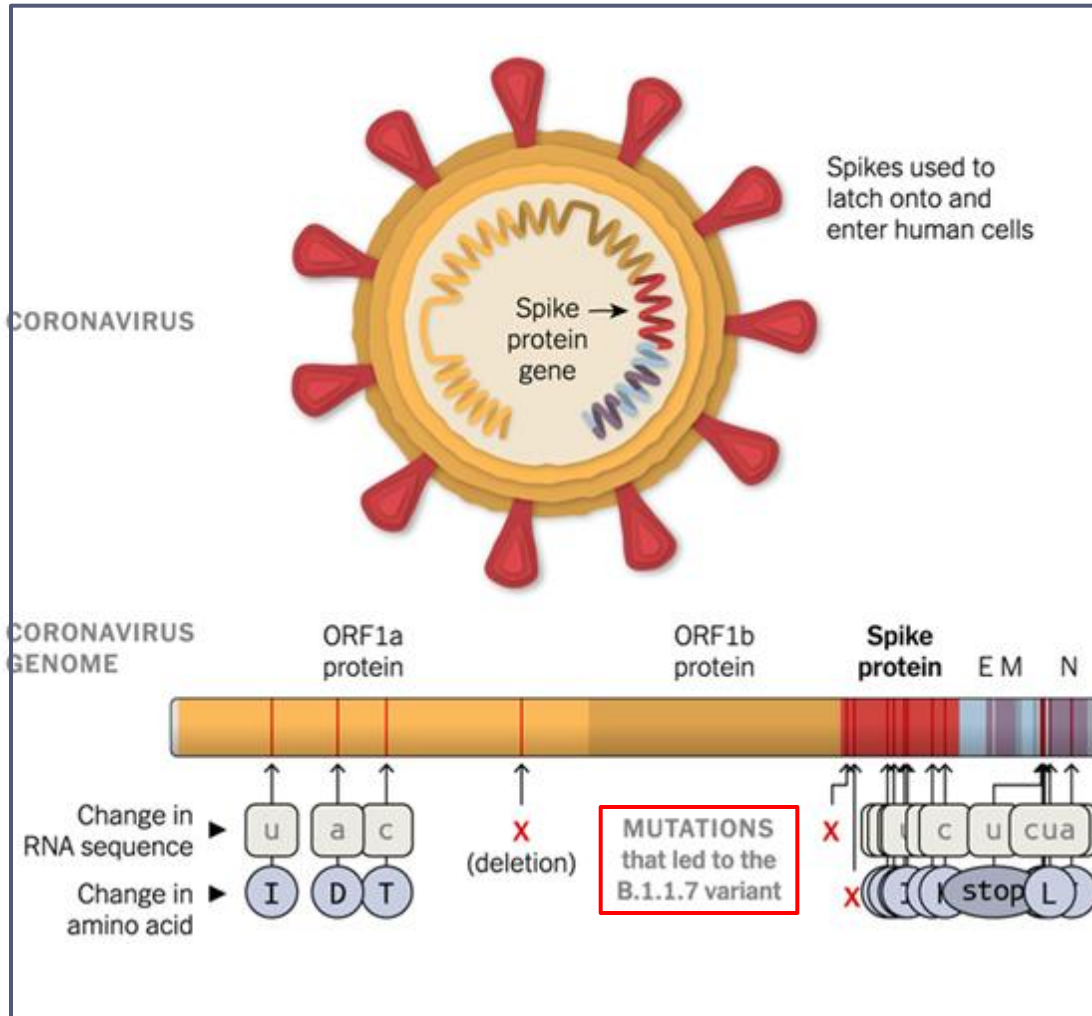
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-17-



B.1.1.7 lineage



Amino Acid Changes in Spike Protein

Outline

背景介紹

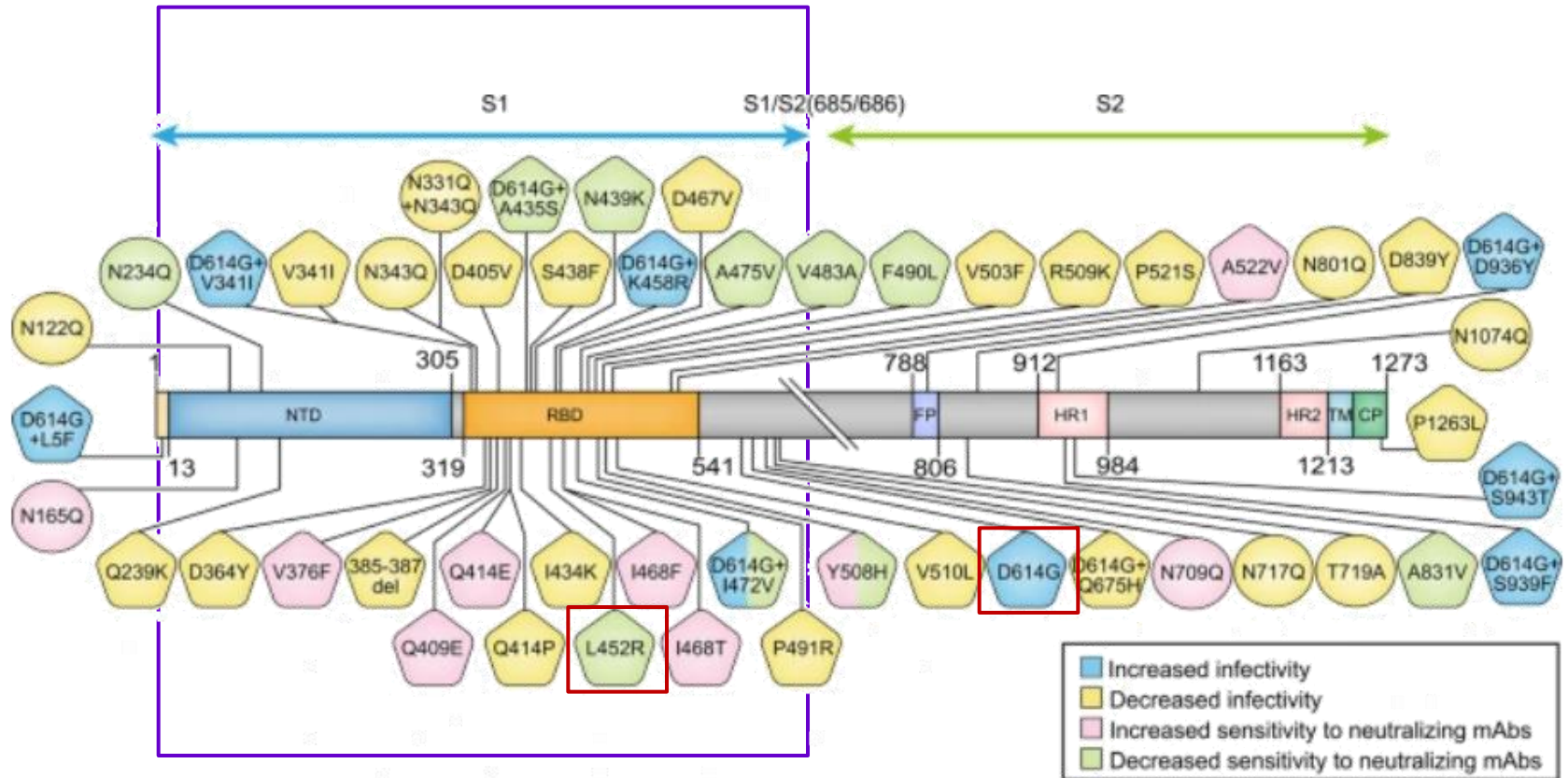
新冠病毒

傳染途徑

負壓病房

配戴口罩

-19-

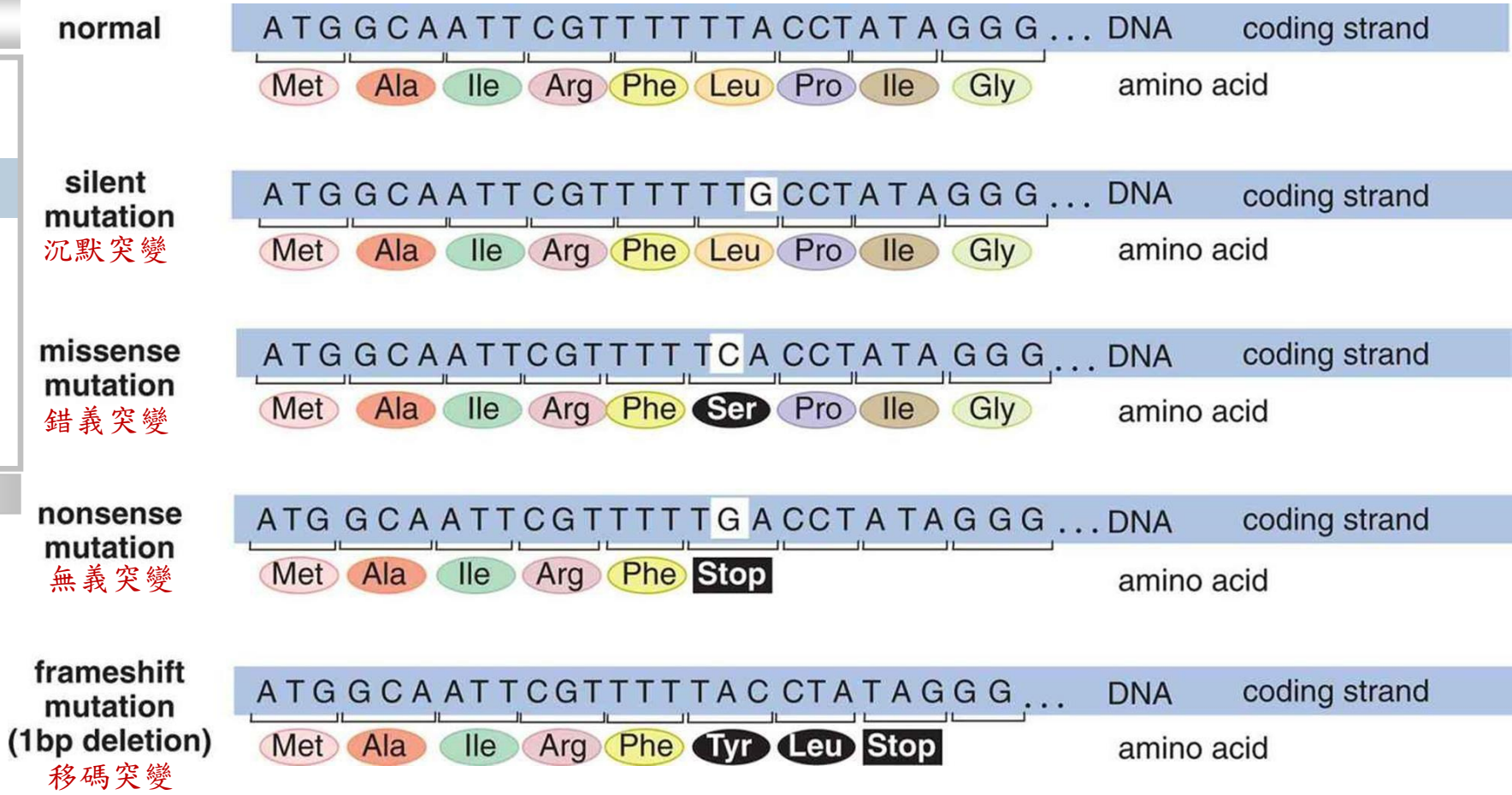


Schematic illustration of amino acid changes in spike protein. Mutations yielding at least fourfold changes in infectivity and neutralizing reactivity to mAbs are shown in the figure

突變 (Mutation)

D (Aspartic acid) D614G (Glycin) G
L (Leucine) L452R (Arginind) R

Outline
背景介紹
新冠病毒
傳染途徑
負壓病房
配戴口罩



B.1.1.7 Strain: Scientific Updates

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-21-

- ▶ Diagnosis: S gene target failure (E, R, N detected in Taiwan)
- ▶ Higher Secondary attack rate? **YES**
 - ▶ 121,072 (12.7%) for all contacts; B.1.1.7 (14.7%) v.s. Wild type (11%)
- ▶ Immune escape? **NO evidence**
 - ▶ Pfizer vaccine:
 - ▶ Do not affect recognition by antibodies resulting from natural immunity or from immunisation
 - ▶ Moderna's vaccine:
 - ▶ Full-length Spike protein is 1,273 amino acids long
 - ▶ B1.1.7 (up to 8 amino acid changes in spike protein) < 1%
- ▶ Importance of IgA antibodies
 - ▶ IgAs may be better at neutralising virus and controlling the infection (Science Translational Medicine 07 Dec 2020 eabd2223)
- ▶ **Biosafety guideline changed? NO**

- ▶ Investigation of novel SARS-CoV-2 variant. Variant of Concern 202012/01 Technical briefing 3. Public health England.

Entering and Replicating in a Host Cell

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-22-

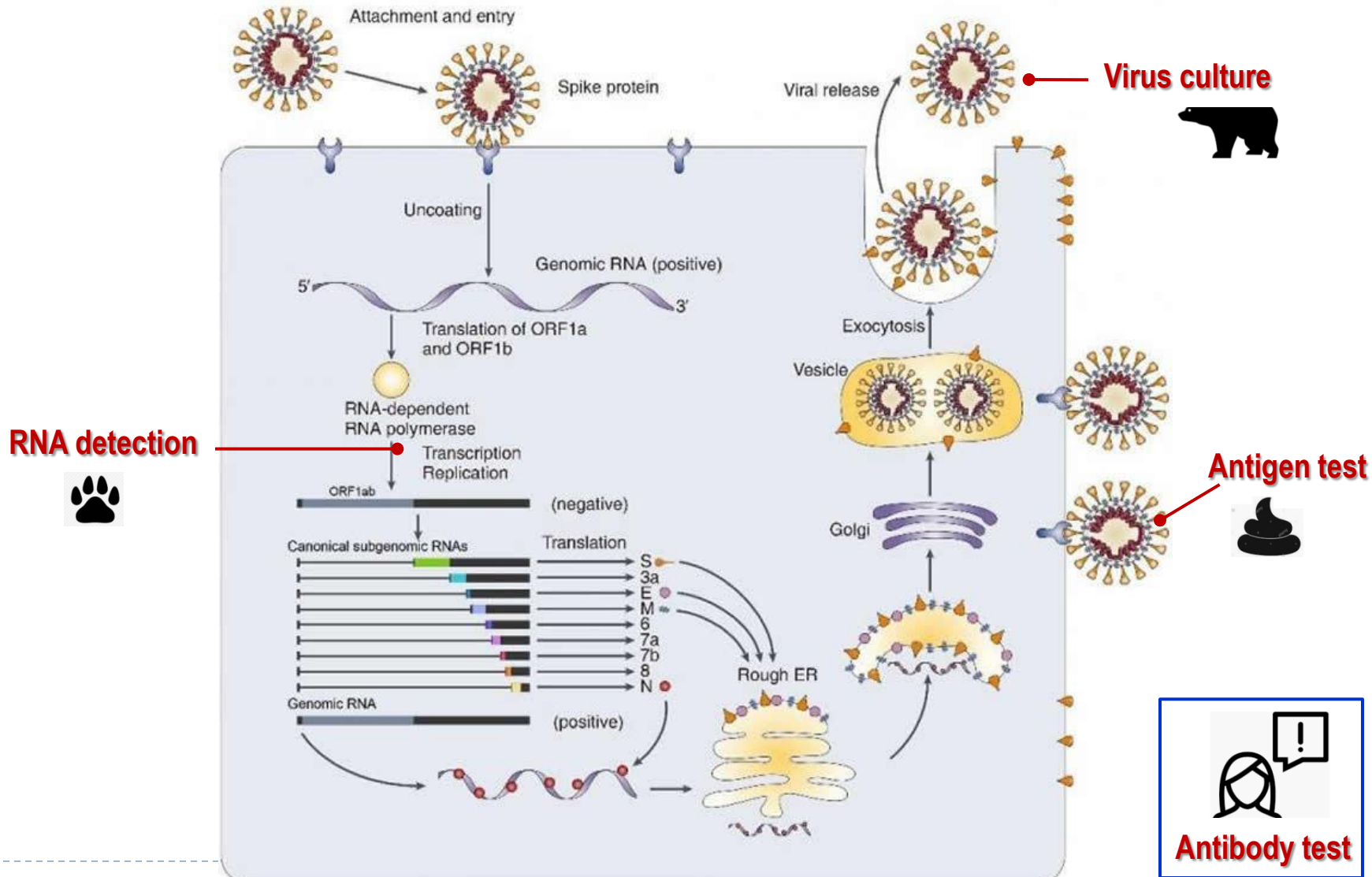


Figure: <https://neurosciencenews.com/coronavirus-genetic-mapping-16130/>

Time to Positivity for COVID-19 Tests

Outline

背景介紹

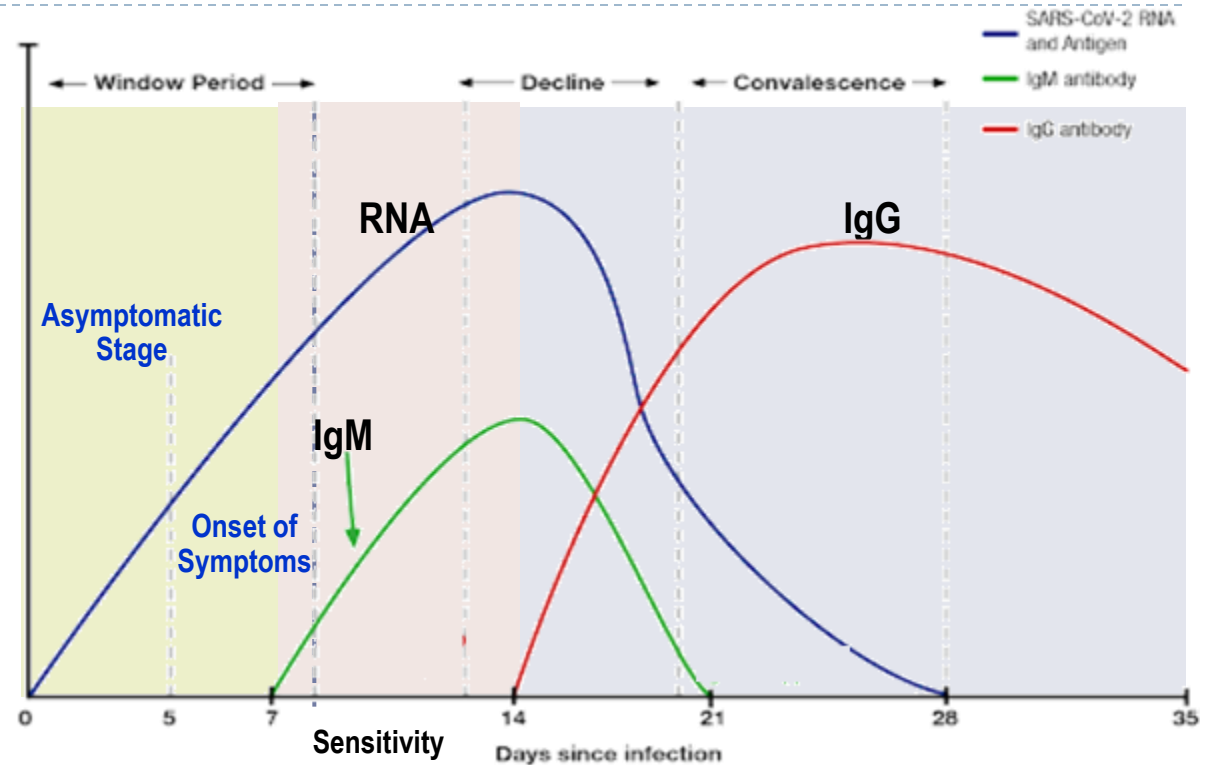
新冠病毒

傳染途徑

負壓病房

配戴口罩

-23-



	Time to pos.	0-7 days	8-14 days	≥ 15 days
Real-time PCR	~2 days	>95%	>95%	>95%
IgM Antibody	~7 days	10.8%-46.0%	19.0%-75.6%	50%-100%
IgG Antibody	~14 days	7.1%-40.5%	33.3%-71.8%	87.5%-100%

▶ Fig: <http://rensimer.com/covid-19-antibody-testing-available/>.

Li C, et al. Laboratory diagnosis of coronavirus disease-2019 (COVID-19). Clinica Chimica Acta 2020; 510:35-46,

PCR Reaction

Outline

背景介紹

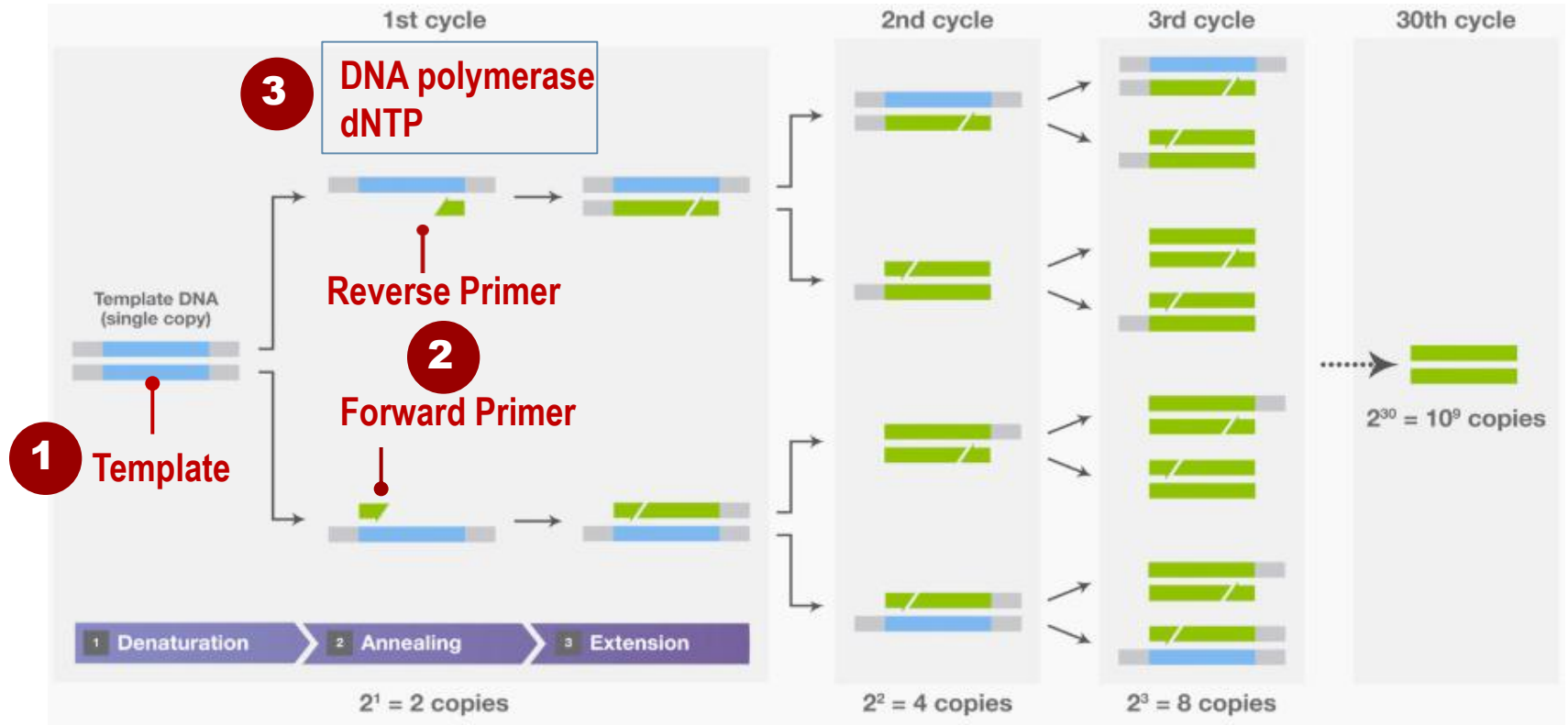
新冠病毒

傳染途徑

負壓病房

配戴口罩

-24-



4

Buffer (Ca^{2+} , Mg^{2+} ...),

Real-Time PCR

Outline

背景介紹

新冠病毒

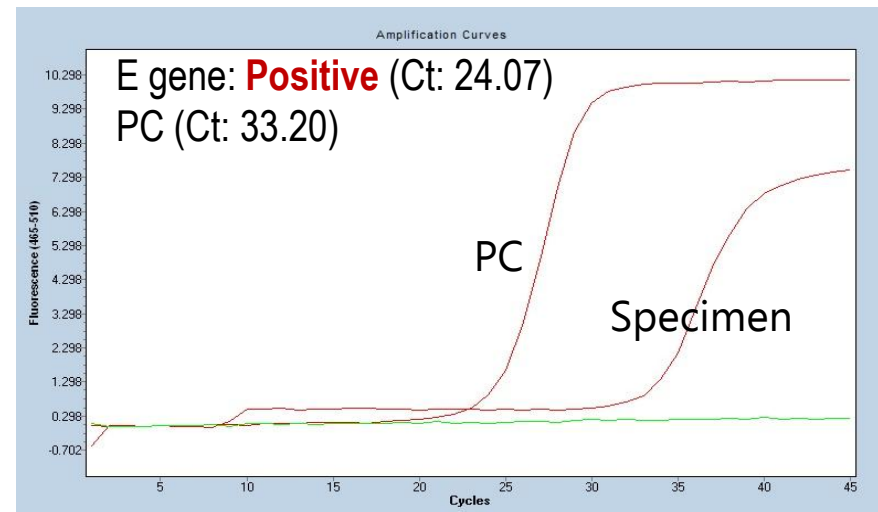
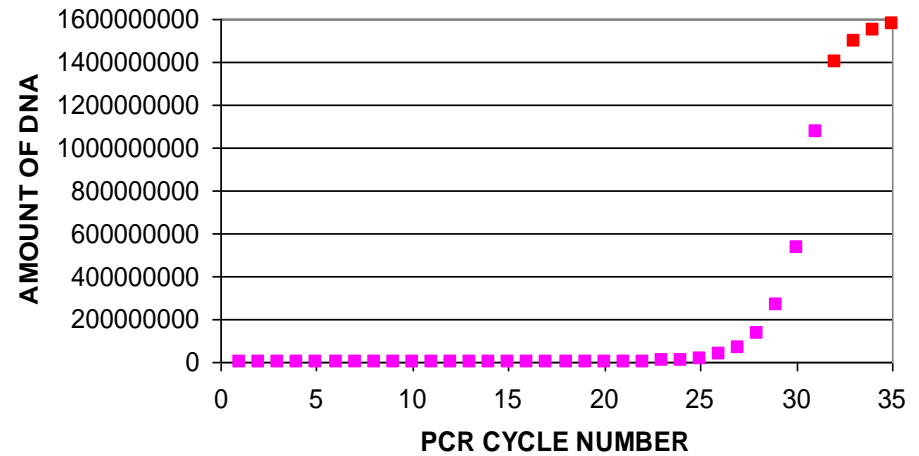
傳染途徑

負壓病房

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-25-

CYCLE NUMBER	AMOUNT OF DNA
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
11	2,048
12	4,096
13	8,192
14	16,384
15	32,768
16	65,536
17	131,072
18	262,144
19	524,288
20	1,048,576
21	2,097,152
22	4,194,304
23	8,388,608
24	16,777,216
25	33,554,432
26	67,108,864
27	134,217,728
28	268,435,456
29	536,870,912
30	1,073,741,824
31	1,400,000,000
32	1,500,000,000
33	1,550,000,000
34	1,580,000,000



TaqMan Probe

Outline

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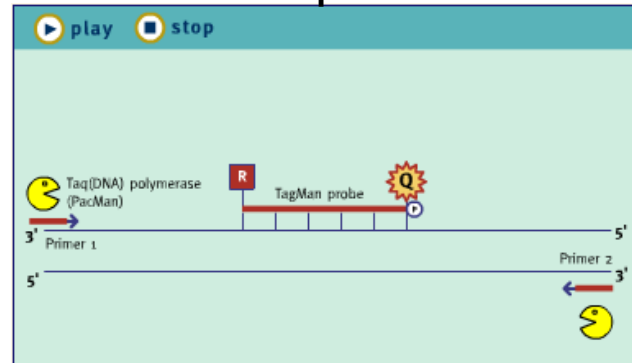
傳染途徑

負壓病房

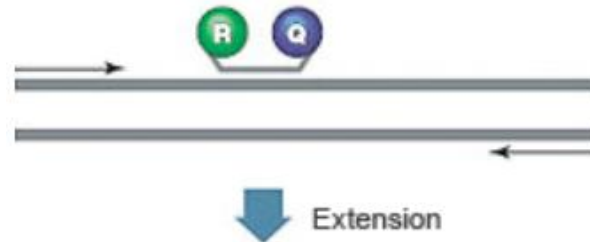
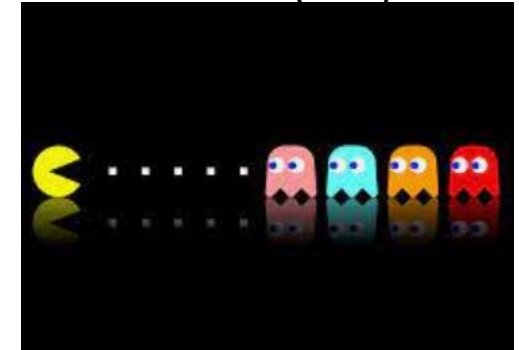
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-26-

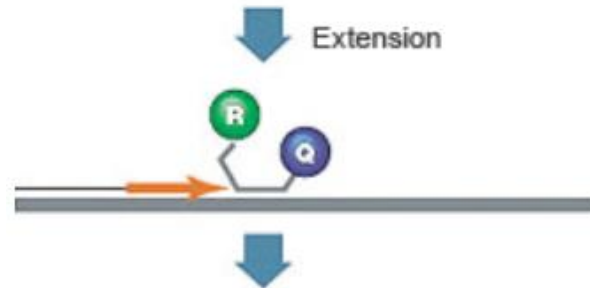
TaqMan



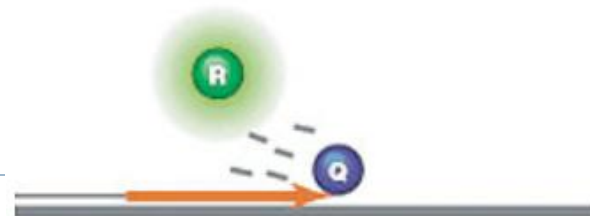
PacMan (1980)



During annealing, the hydrolysis probe binds to the target sequence



During extension, the probe is partially displaced and the reporter is cleaved. The free reporter fluoresces



R Reporter
Q Quencher

Cycle Threshold (Ct)

Outline

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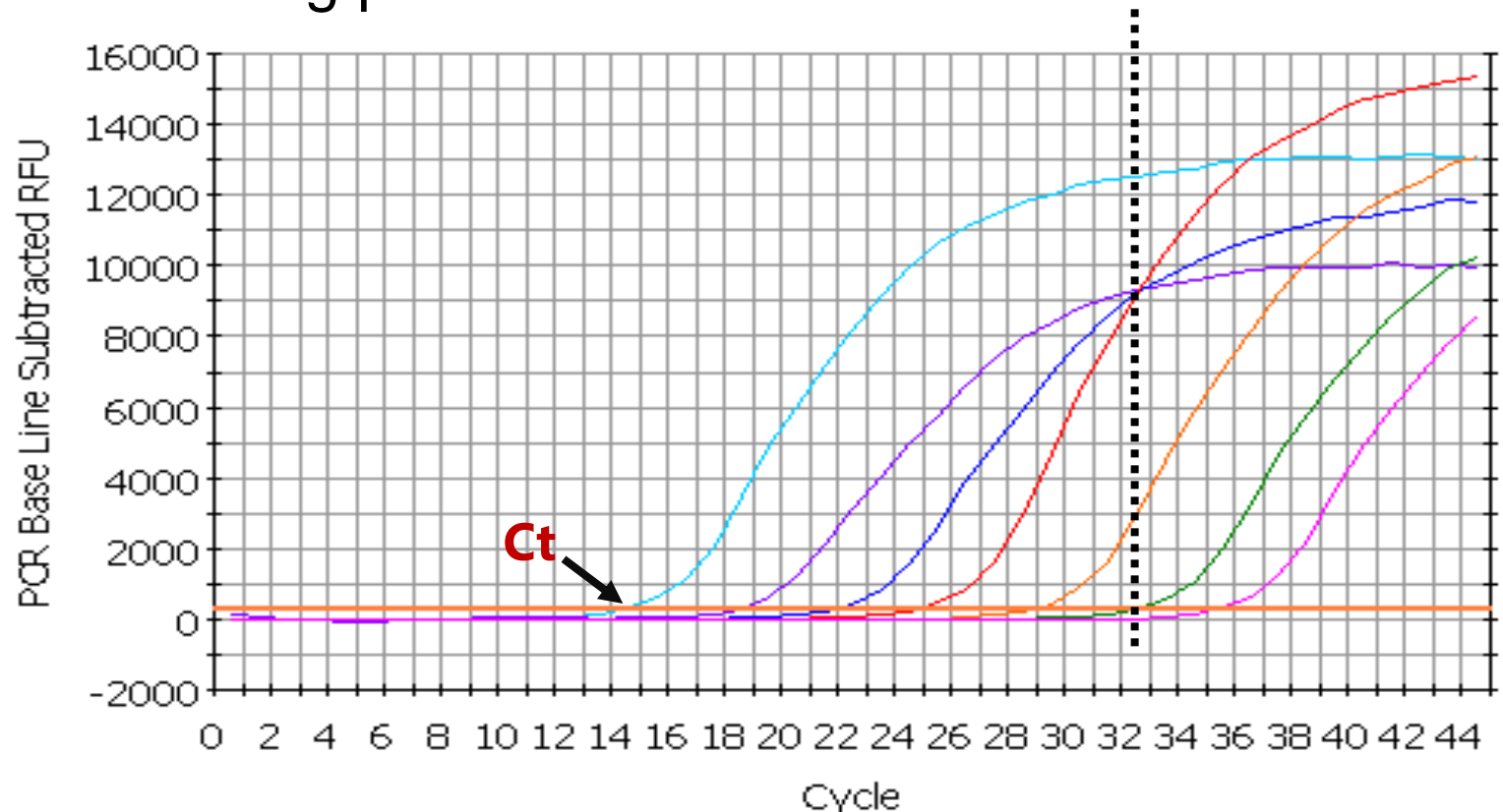
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負壓病房

配戴口罩

-27-

- ▶ Ct: (cycle threshold): the number of cycles required for the fluorescent signal to cross the threshold
- ▶ CP: Crossing point



Amplicon Target

Outline

背景介紹

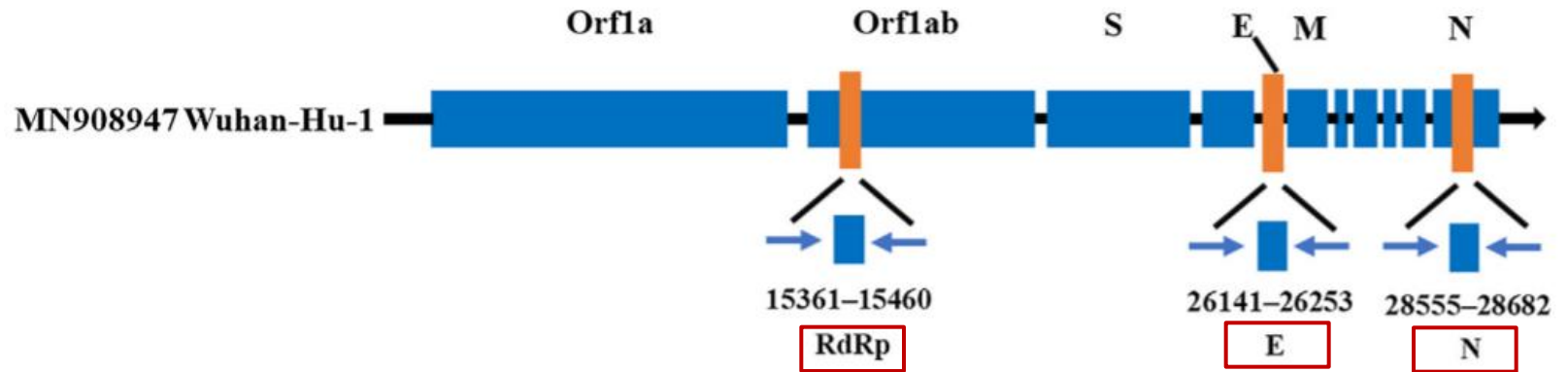
新冠病毒

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配戴口罩

-28-



Component (ul)	SARS-CoV2 E gene			SARS-CoV2 RdRp gene			Human Rnase P (Specimen control)	
	Patient	Pos. Cont.	Neg. Cont.	Patient	Pos. Cont.	Neg. Cont.	Patient	Neg. Cont.
RNA	Patient	E PC	—	Patient	RdRp PC	—	Patient	—
RT mix	+	+	+	+	+	+	+	+
Master mix	+	+	+	+	+	+	+	+
Primer-F, R	SARS-CoV2 E primers			SARS-CoV2 RdRp primers			Human Rnase P primers	
Probe	SARS-CoV2 E Probe			SARS-CoV2 RdRp Probe			Human Rnase P Probe	

Potential transmission routes for SARS-CoV-2

Outline

背景介紹

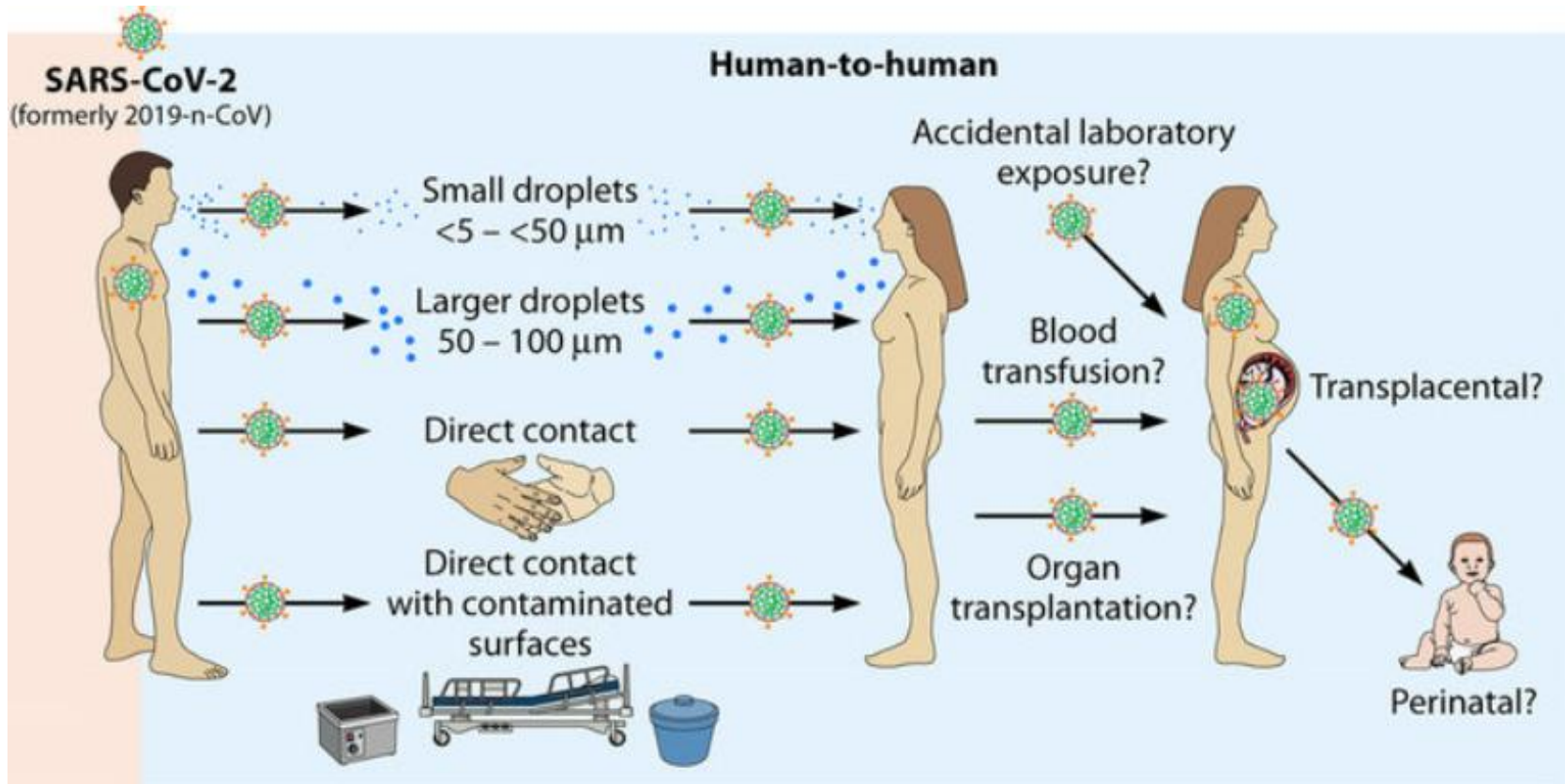
新冠病毒

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負壓病房

配戴口罩

-29-



第一、五類傳染病包裝要求

Outline

背景介紹

新冠病毒

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負壓病房

配戴口罩

-30-

類別	傳染病	病原體	RG	P620	P650
第一類	狂犬病	Rabies virus	2	V(培養)	V
	鼠疫	Yersinia pestis	3	V(培養)	V
	嚴重急性呼吸道症候群	SARS coronavirus (SARS-CoV)	3		V
	天花	Variola virus	4	V	
第五類	新型 A 型流感	Influenza virus H5N1, H7N9	3	V(培養)	V
	黃熱病	Yellow fever virus	3	V(培養)	V
	裂谷熱	Rift valley fever virus	2		V
	中東呼吸症候群	Middle East respiratory syndrome coronavirus [MERS-CoV]	3		V
	拉薩熱	Lassa virus	4	V	
	馬堡病毒出血熱	Marburg Virus	4	V	
	伊波拉病毒感染	Ebola virus	4	V	

感染性物質包裝與運輸規定

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-31-

▶ A類感染性物質(三層包裝, P620)

- ▶ 當運輸此類感染性物質，健康的人或動物若在運輸過程中暴露到這類感染性物質，**會導致人或動物產生永久性的失能或殘疾、引發具有生物命威脅或致死的疾病**
- ▶ 如Ebola virus, *Bacillus anthracis* (culture only)

▶ B類感染性物質(三層包裝, P650)

- ▶ 感染性物質，不符合A類感染性物質的定義、**未經醫學專家判定帶有病原體的可能性的**小。
- ▶ 如*Bacillus anthracis* (patient specimen); Highly pathogenic influenza virus (patient specimen)

▶ 豁免(三層包裝)

- ▶ 經醫學評估，被認為**帶有病原體的可能性很小**
- ▶ 如懷孕試驗、藥物篩檢試驗

▶ 例外(無包裝要求)

- ▶ 不含感染性物質或不會使人類或動物致病等

感染性物質包裝要求之差異比較

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-32-

要點附表六、感染性生物材料之三層包裝規定

項目	國際包裝規定	P620	P650	豁免
第一層（主）容器		有（防滲漏）	有（防滲漏）	有（防滲漏）
第二層容器		有（防滲漏）	有（防滲漏）	有（防滲漏）
外層包裝		有	有	有
第一層與第二層容器之間吸收性材料		有	有	有
1.2公尺落地測試（完整包裝）		—	必須通過	—
9公尺落地測試（第二層容器）		必須通過	—	—
7公斤穿刺強度測試（第二層容器）		必須通過	—	—
95 KPa壓力測試（第一層或第二層容器）		必須通過	必須通過	—

Negative Pressure Isolation Room

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-33-

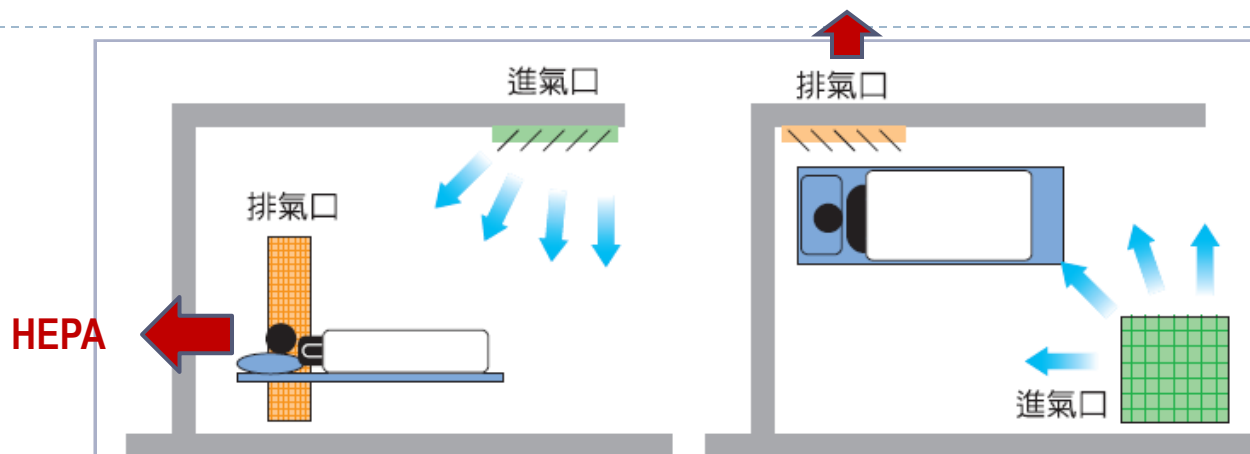


圖14 導向氣流之調整示意（單一進排氣口案例）

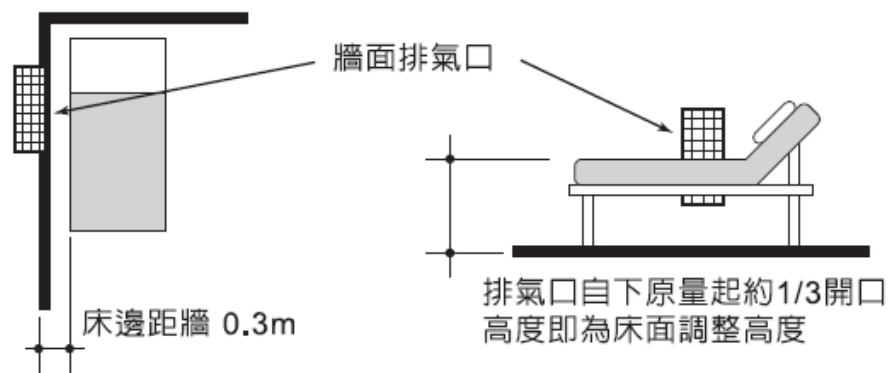


圖15 病床與排氣口之相對位置規劃案例

BSL-3 Laboratory

Outline

背景介紹

新冠病毒

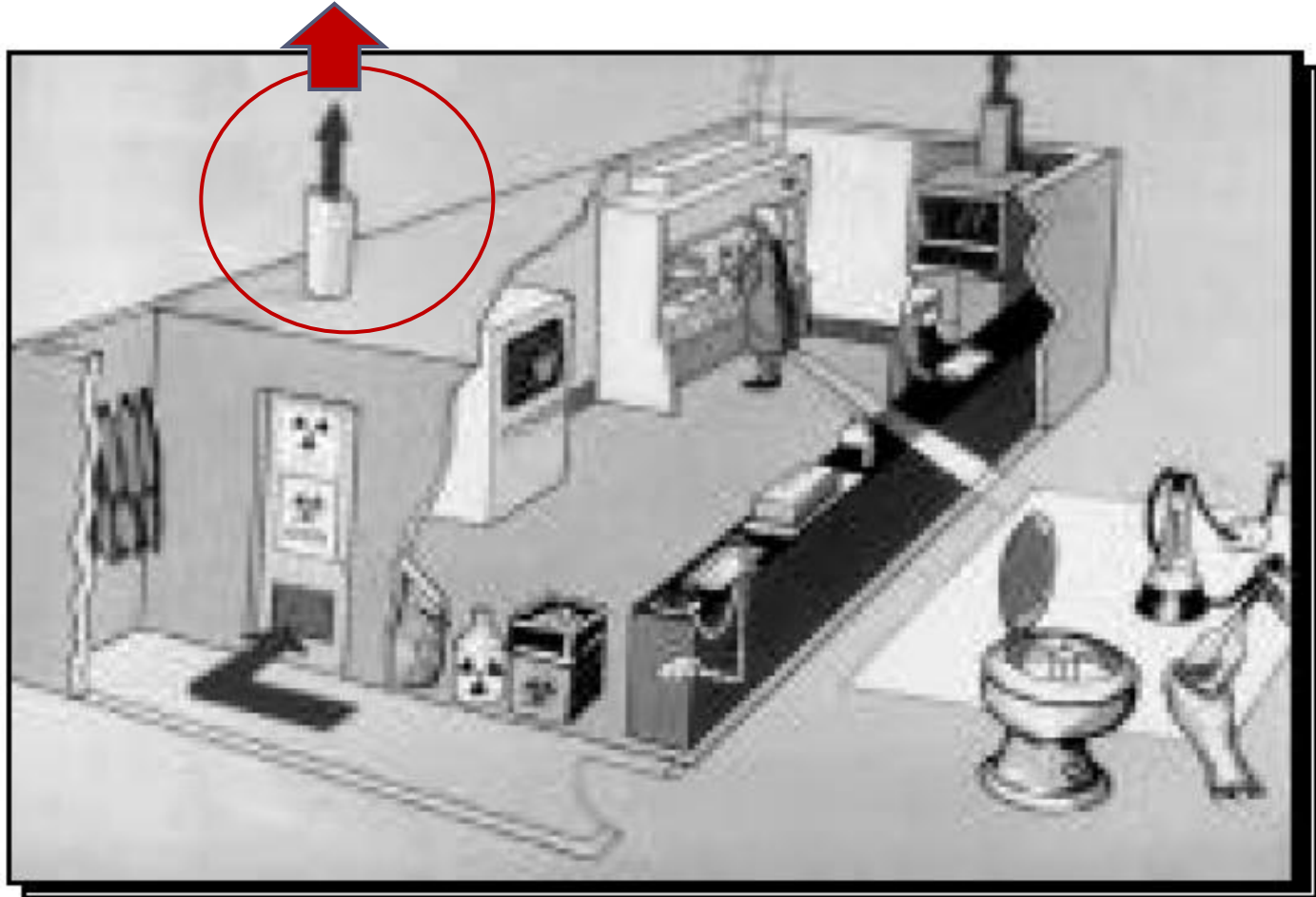
傳染途徑

負壓病房

配戴口罩

-34-

HEPA filter



HEPA (High Efficiency Particulate Air) Filter

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

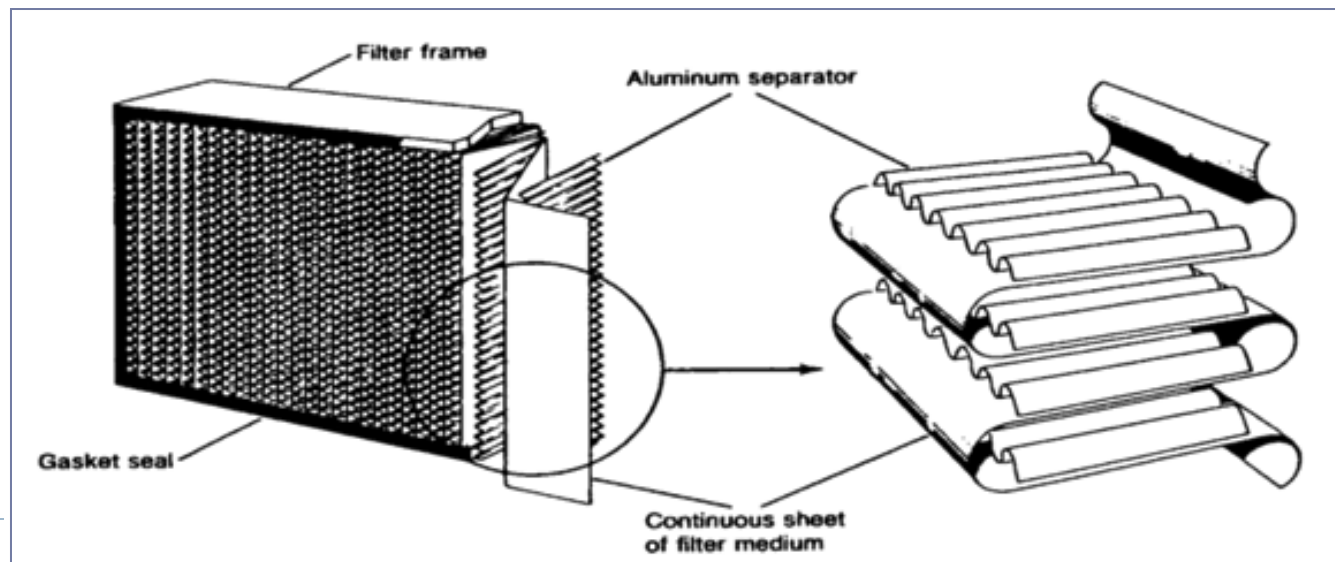
配戴口罩

- ▶ Traps particulates **only**
- ▶ Remove at least 99.97% of particles that have a size of 0.3 μm .

Average human hair (100 microns)

PM10 particulate (<10 microns) PM2.5 particulate (<2.5 microns)

HEPA-filter (0.3 microns)



HEPA Filter

Outline

背景介紹

新冠病毒

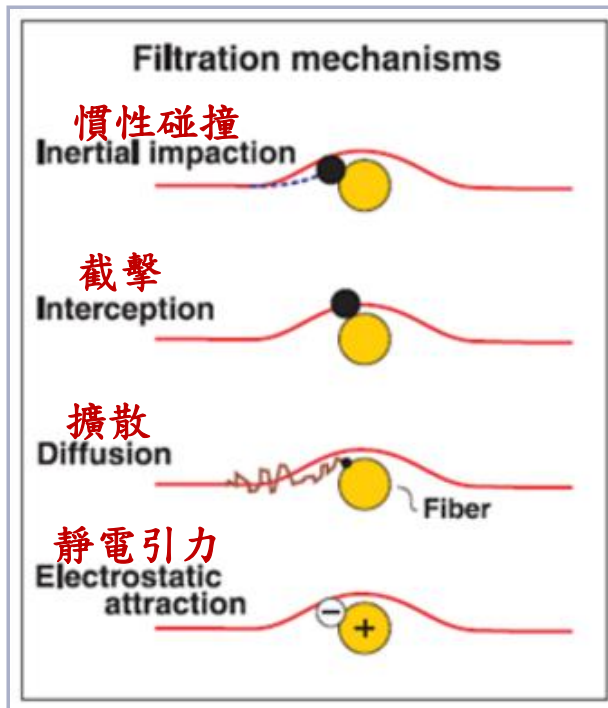
傳染途徑

負壓病房

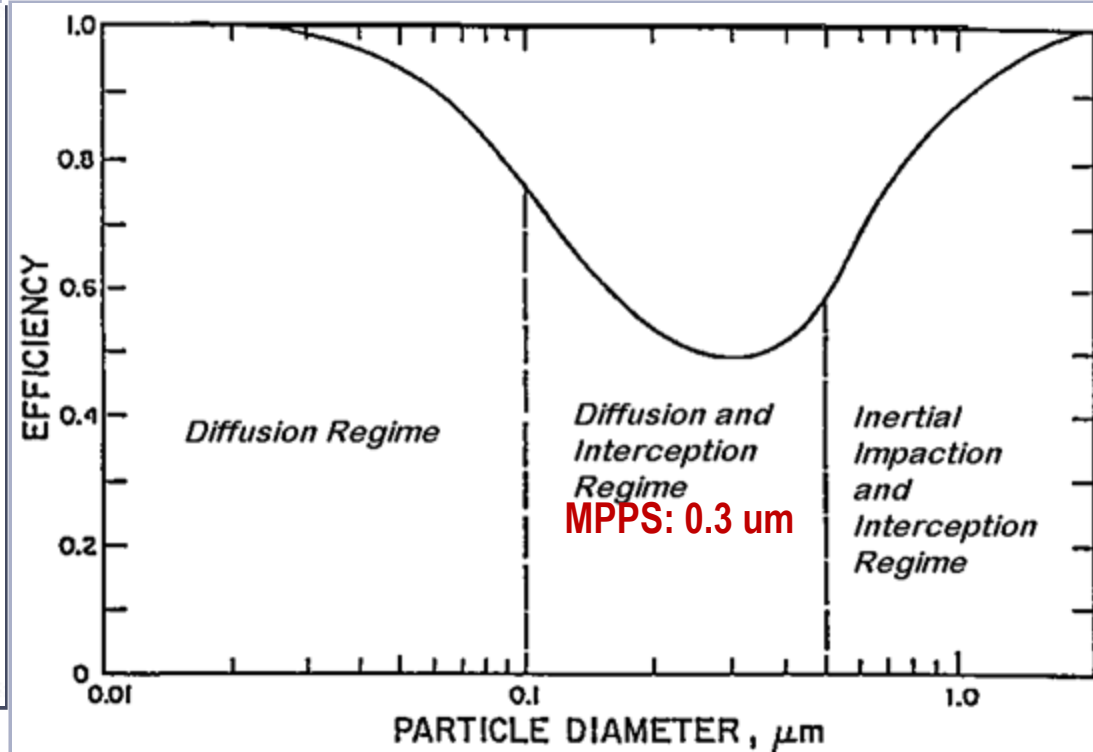
配戴口罩

-36-

- ▶ Remove (from the air that passes through) at least 99.97% of particles that have a size of $0.3 \mu\text{m}$.



MPPS: Most Penetrating Particle Size



Respirator v.s. Surgical mask

Outline

背景介紹

新冠病毒

傳染途徑

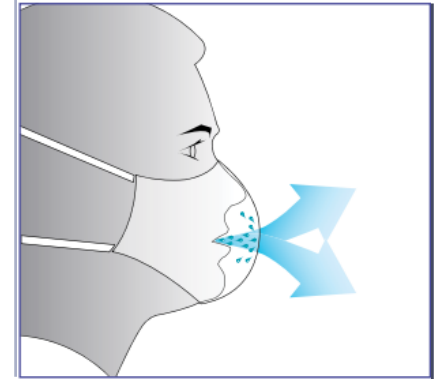
負壓病房

配戴口罩

-37-

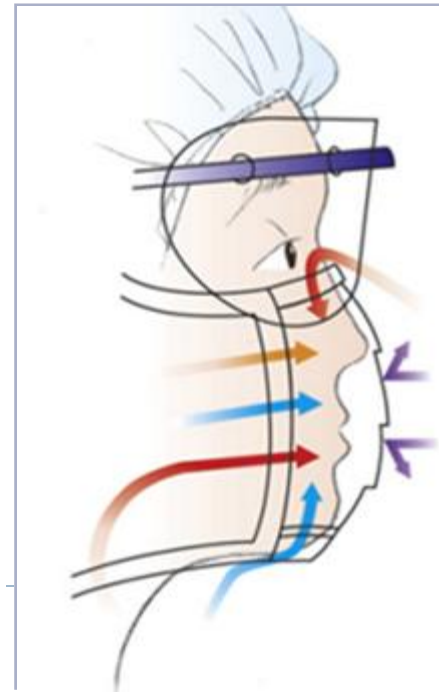
▶ Respirator

- ▶ Reduce the **wearer's** respiratory exposure to particles less than 100 microns (μm) in size
- ▶ Includes airborne particles that may contain biological material (mold, bacteria, viruses) or even PM2.5 / PM10



▶ Surgical mask

- ▶ Prevent contamination of the **work environment or sterile field** from large particles generated by the wearer (e.g. spit, mucous).
- ▶ Reduce the risk of **splashes or sprays** of blood, body fluids, secretions and excretions from reaching the wearer's mouth and nose.
- ▶ **NOT PPE** under the European Directive 89/686



Surgical Mask

Outline

背景介紹

新冠病毒

傳染途徑

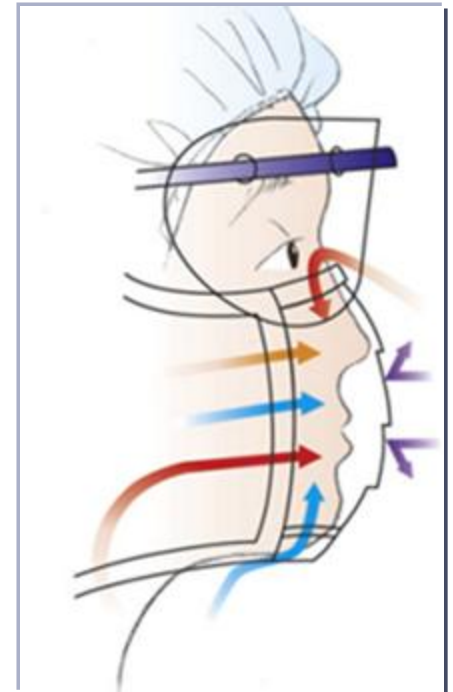
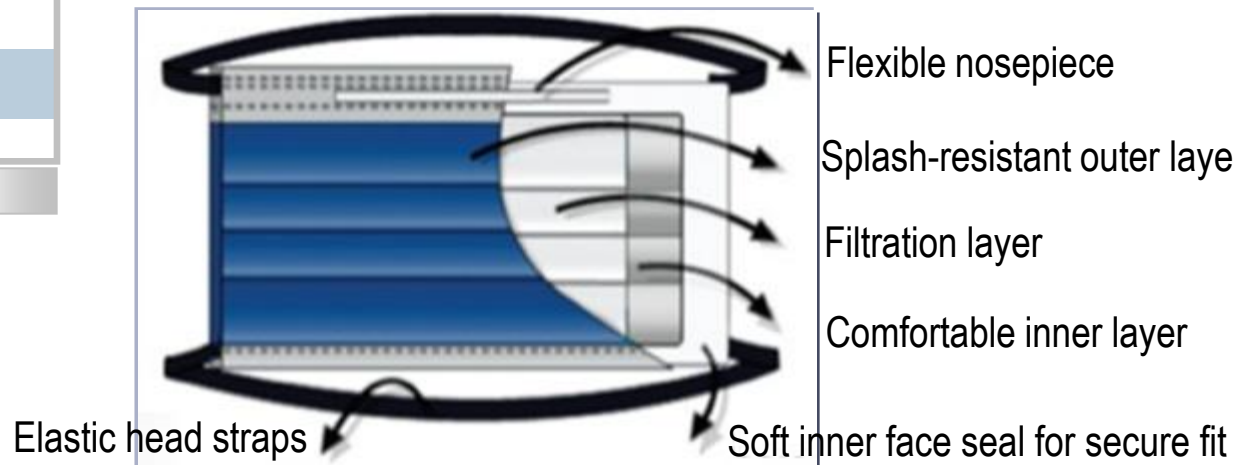
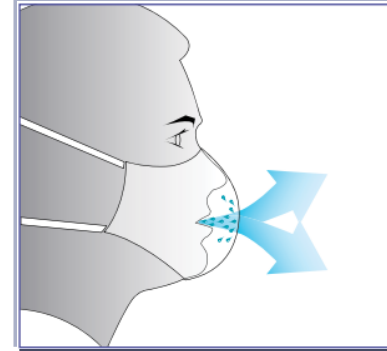
負壓病房

配戴口罩

-38-

- ▶ Prevent droplets being expelled into the environment
- ▶ Do NOT effectively filter small particles from the air
- ▶ Do NOT prevent leakage around the edge when inhales
- ▶ **NOT PPE** under the European Directive 89/686

*PPE: personal protective equipment (個人防護設備)



3-ply surgical mask.

Outline

背景介紹

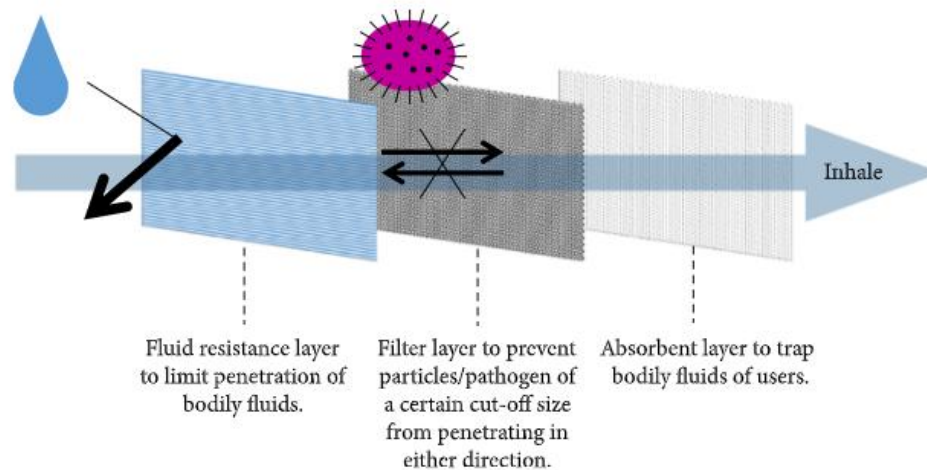
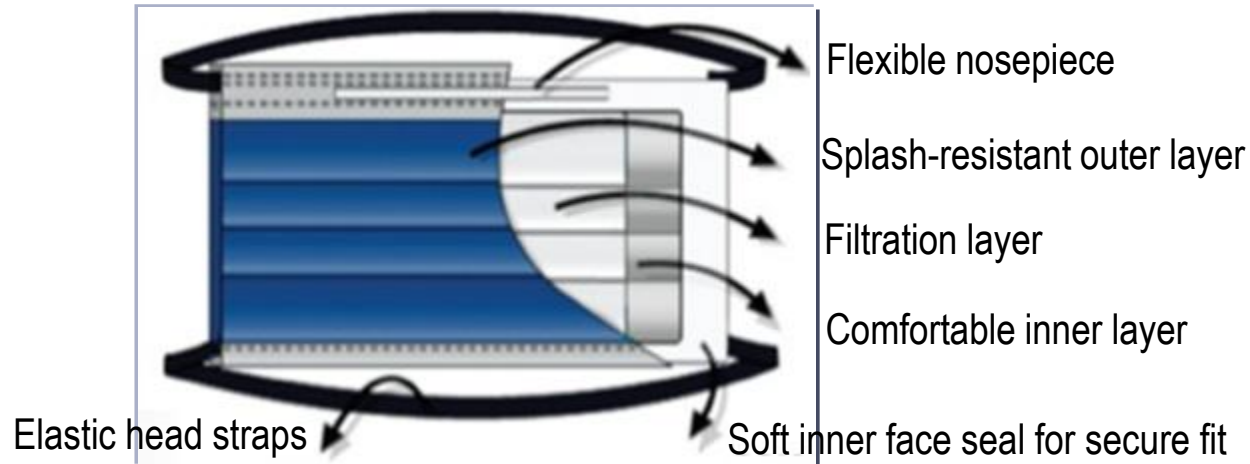
新冠病毒

傳染途徑

負壓病房

配戴口罩

-39-



Putting on Surgical Mask

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

40-



Tie the upper strings at the top of the head



Tie the lower strings at the back of the neck



Fix the metallic strip securely over the bridge of the nose.



Change mask if it becomes moist or damaged.



Ensure mask fully cover nose, mouth. Stretch over the chin & fit over face.

Important Features

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

- ▶ Capture the full range of hazardous particles
 - ▶ Typically within a wide range of sizes (<1 to >100 μm)
 - ▶ Over a range of airflow (approximately 10 to 100 L/min)
- ▶ Leakage must be prevented at the boundary of the facepiece and the face.
- ▶ Keep breathing resistance low

Respirators

Outline

背景介紹

新冠病毒

傳染途徑

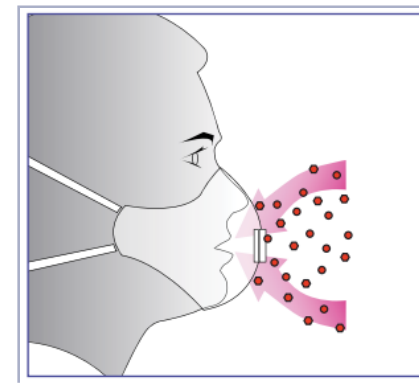
負壓病房

配戴口罩

42-

- ▶ Respirators are PPE, Reduce the potential exposure of the wearer to airborne hazardous contaminants.

*PPE: personal protective equipment (個人防護設備)



- ▶ Effective use of N95 respirators
 - ▶ Must be replaced if wet or damaged
 - ▶ Only wear the model and size of respirator that **fit tested**
 - ▶ If you experience medical signs or symptoms (difficulty breathing, dizziness...etc), leave the work area and remove the respirator when you are no longer exposed to the hazard. Seek medical attention
- ▶ Single use

Particle Filter Classification

▶ US: NIOSH 42 CFR 84

▶ Rating for filter

- ▶ 95: collect at least 95% of the challenge aerosols
- ▶ 99: collect at least 99%
- ▶ 100: collect at least 99.97%

▶ Level of protection against oil aerosols

- ▶ N: **N**ot resistant to oil
- ▶ R: **R**esistant to oil
- ▶ P: **P**roof resistant (oil **P**roof)

▶ EU/India: EN 149/India standard

- ▶ FFP1: efficiency 80%
- ▶ FFP2 : efficiency 94%
- ▶ FFP3 : efficiency 99%

*FFP: Filtering Face Piece

N-95, N-99, and N-100
R-95, R-99, and R-100
P-95, P-99, and P-100

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

43-

Wearing the N95 Mask

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-44-

Use BOTH hands



Step 1

Wash hands before putting mask.



Step 2

Select suitable N95 mask. That fits well.



Step 3

Hold with cupped hand. Place firmly over nose, mouth and chin.



Step 4

Position top band high at the back of head. Position bottom and under the ears.



Step 5

Press metal wire along upper edge of nose. Fit the mask nicely on your face



Step 6

Perform fit check by inhaling and exhaling. During exhalation check for air leakage around face.

Respirator Fit Checks

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

45-

- ▶ Perform both **every time** the respirator is used.
- ▶ Positive pressure fit check
 - ▶ Cup hands over the mask
 - ▶ Exhale normally
 - ▶ The respirator should expand slightly
- ▶ Negative pressure fit check
 - ▶ Cup hands over the mask
 - ▶ Inhale normally
 - ▶ The respirator should collapse slightly



N95 Respirator Limitations

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-46-

- ▶ Proper **fit**
- ▶ Filters **particulates** only, not protect from gases, vapors, chemicals
- ▶ May be **uncomfortable** if used for extended periods
- ▶ Can not be worn by individuals with facial hair that disturbs the respirator **seal**
- ▶ Must be replaced when **wet**, damaged, soiled or contaminated
- ▶ Cannot be used in an oxygen deficient atmosphere



Don'ts

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

47-

Do not pinch the nose clip with one hand



Do not fold



More is Better?

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

48-



Multiple surgical masks



N95 with surgical masks overlay



Should I wear a mask while exercising?

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

49-

- Even when you're in an area of COVID-19 transmission, masks should **not** be worn during vigorous physical activity because of the risk of reducing your breathing capacity.
- No matter how intensely you exercise, keep at least 1 metre away from others, and if you're indoors, make sure there is adequate ventilation.

How to put on and take off a medical mask

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-50-

- Before touching the mask, **clean your hands** with an alcohol-based hand rub or soap and water.
- Inspect the mask for tears or holes; do not use a mask that has previously been worn or is damaged.
- Verify which side is the **top** – this is usually where the metal strip is.
- Then, identify the **inside** of the mask, which is usually the white side.
- Place the mask on your face covering your nose, mouth and chin, making sure that there are **no gaps** between your face and the mask. Place the straps behind your head or ears. Do not cross the straps because this can cause gaps on the side of the mask.
- Pinch the **metal strip** so it moulds to the shape of your nose.
- Remember, **do not touch the front of the mask** while using it to avoid contamination; if you accidentally touch it, clean your hands.

How to take off a medical mask

Outline

背景介紹

新冠病毒

傳染途徑

負壓病房

配戴口罩

-51-

- Before touching the mask, **clean your hands** with an alcohol-based hand rub or soap and water.
- Remove the straps from behind the head or ears, **without touching the front of the mask.**
- As you remove the mask, lean forward and pull the mask away from your face.
- **Medical masks are for single use only**; discard the mask immediately, preferably into a closed bin.
- **Clean your hands** after touching the mask.
- Be aware of the condition of the mask; **replace it if it gets soiled or damp.**

Thank you

