正子製藥區
滅菌劑介紹
Part I

大綱

潔淨區定義

潔淨區汙染

如何清潔消毒潔淨區

清潔、消毒劑簡介

生產製造藥典中各個品項時，應在受到嚴格管制的環境中進行，以避免該品項受到微生物的污染 － 潔淨室

選擇適當的清潔劑、消毒劑或殺菌劑是維持良好製造環境的重要關鍵

Without Measurement
there is no Control

潔淨室

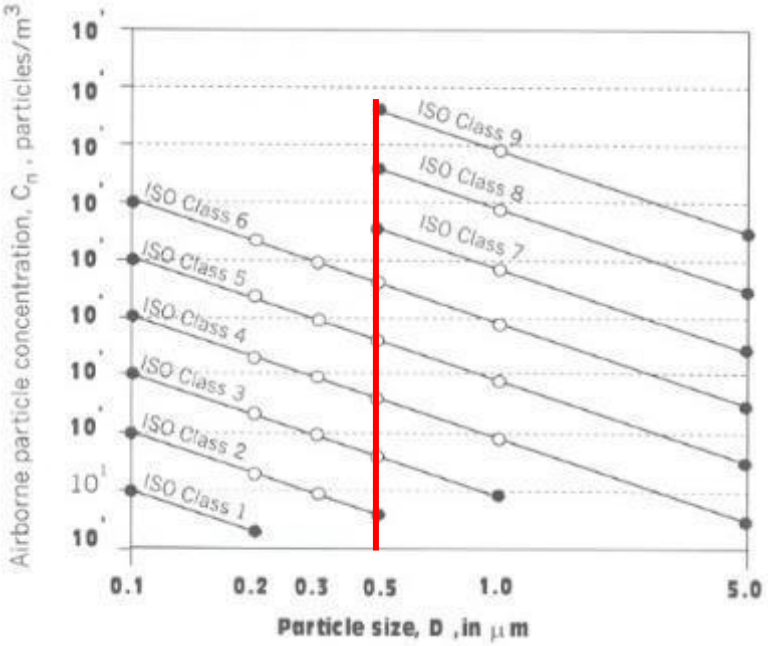
設定空氣品質的管制區域

空氣品質須受到管制的區域包括：

醫院手術室、藥局及生產無菌製劑的工廠

- 空氣中懸浮粒的管制
- 空氣流動方向、風量
- 高效率過濾裝置的功能及效率
- 溫溼度管制
- 清潔與消毒
- 工作人員的態度與更衣程序

潔淨室



空氣品質監測主要包括：

單位體積空氣中懸浮粒的最大濃度

單位體積空氣中活的微生物最大容許量

單位體積空氣中懸浮粒的最大濃度

ISO 14644 : 1999　　ISO 14644 : 2015

ISO Class number (*N*)	Maximum allowable concentrations (particles/m³) for particles equal to and greater than the considered sizes, shown below[a]					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
1	*10*[b]	d	d	d	d	e
2	100	*24*[b]	*10*[b]	d	d	e
3	1 000	237	102	*35*[b]	d	e
4	10 000	2 370	1 020	352	*83*[b]	e
5	100 000	23 700	10 200	3 520	832	29
6	1 000 000	237 000	102 000	35 200	8 320	293
7	c	c	c	352 000	83 200	2 930
8	c	c	c	3 520 000	832 000	29 300
9g	c	c	c	35 200 000	8 320 000	293 000

$$C_n = 10^N \times \left[\frac{0.1}{D}\right]^{2.08}$$

潔淨室取樣點數量

ISO 14644 : 1999　　ISO 14644 : 2015　　$N = \sqrt{A}$

潔淨室面積(m^2)≤	最小測定點數
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10

≥ 1000 m^2 ; $N = 27[\frac{A}{1000}]$

製藥廠所懸浮粒的規範

PIC/S Annex 1 PE009-12, 2015

Grade	Maximum permitted number of particles/m³ equal to or greater than the tabulated size			
	At rest		In operation	
	0.5μm	5.0μm	0.5μm	5.0μm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	not defined	not defined

PIC/S Guide to GMP (PE 009-5, 2006)

Particle measurement based on the use of a discrete airborne particle counter to measure the concentration of particles at designated sizes equal to or greater than the threshold stated. **A continuous measurement system should be used for monitoring the concentration of particles in the grade A zone**, and is recommended for the surrounding grade B areas. For routine testing the total sample volume should not be less than **1 m^3** for **grade A and B areas** and preferably also in grade C areas.

單位體積空氣中活的微生物最大容許量

ISO 14644 Air Classifications

Clean area classification (0.5 μm particle/ft^3)	ISO designation	> 0.5 mm particle/m^3	Microbiological active air **action level** (cfu/m^3)	Microbiological setting plates **action levels**(diam. 90mm; cfu/4hrs)
100	5	3,520	1	1
1,000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

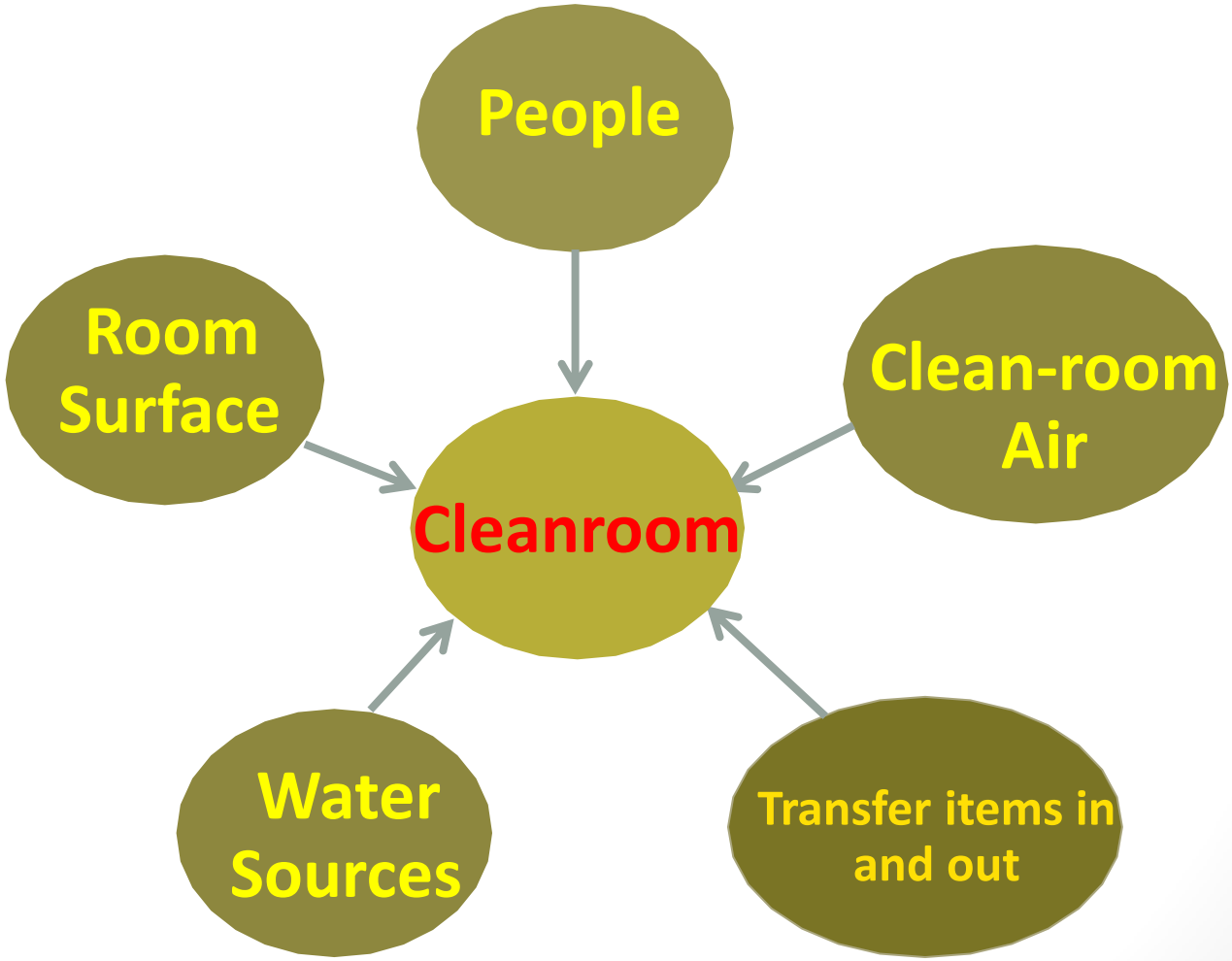
單位體積空氣中活的微生物最大容許量

PIC/S Annex 1 PE009-12, 2015

	Recommended limits for microbial contamination			
Grade	Air sample (cfu/m^3)	Settle plates (dia. 90mm), cfu/4 hours	Contact plates(dia. 50mm), cfu/plate	Glove print 5 fingers cfu/glove
A	<1 (1)	<1 (1)	<1	<1
B	10 (1)	5 (1)	5	5
C	100 (10)	50 (5)	25	-
D	200 (100)	100 (50)	50	-

潔淨室為何會被汙染?

污染源



潔淨室為何會被汙染?

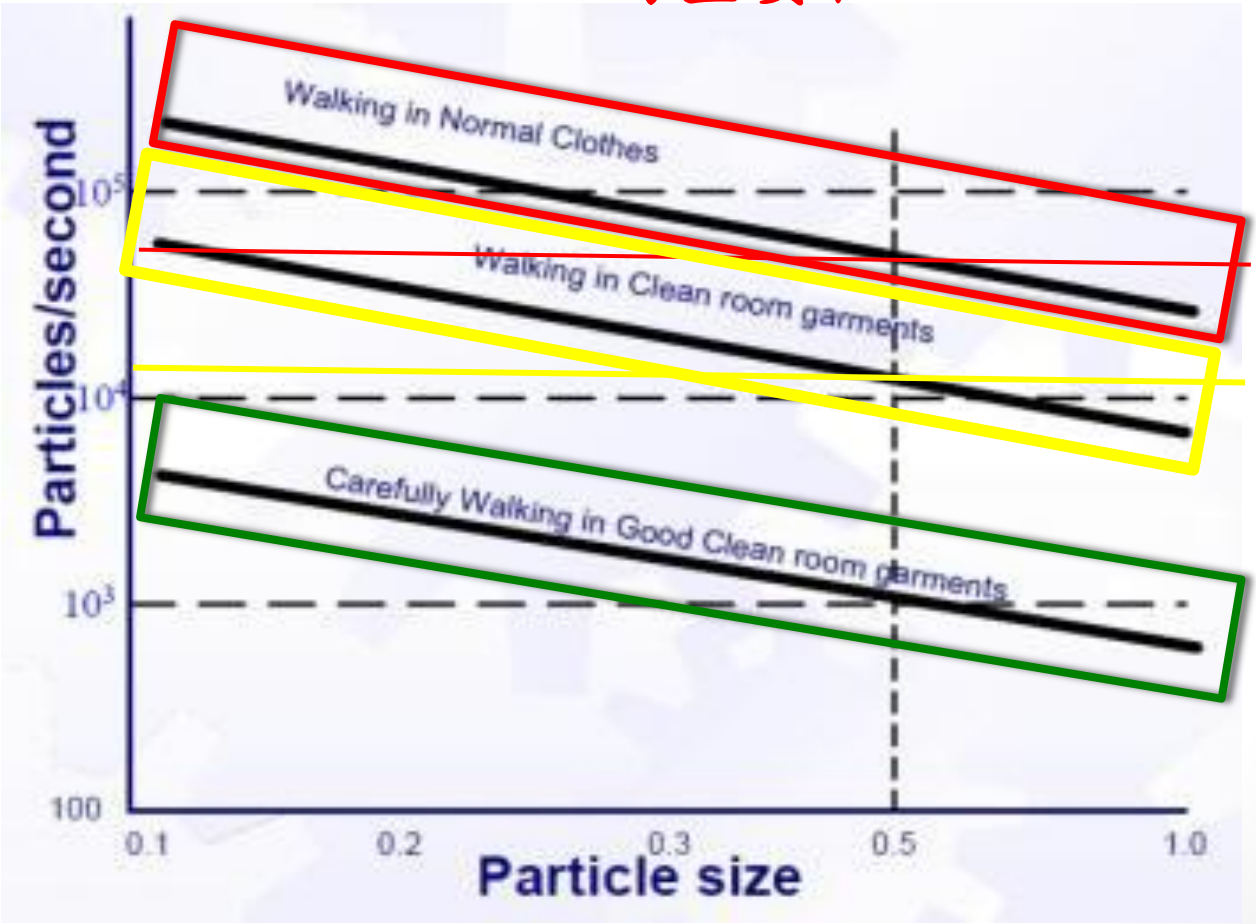
- **People**

Particulate Propagation Rate ≧ 0.3 μm/min	
Motionless – sitting or standing	100,000
Motion – head, neck or arm	500,000
Motion – head, neck, arm and foot	1,000,000
Motion – standing up or sitting down	2,500,000
Walking at 2.0 mph	5,000,000
Walking at 3.5 mph	7,500,000
Walking at 5.0 mph	10,000,000

潔淨室為何會被汙染?

- People

正確的衣著及適度動作的重要性!!!



潔淨室為何會被微生物汙染?

- **People - Microorganisms released from**

Area	Number of Microorganisms/cm^2
Scalp	$1x10^6$
Saliva & Nasal fluid	$1x10^7$/gm
Back	100
Groin	$1- 2x10^7$
Forehead	100 - 1000
Hand	$1x10^4 – 1x10^5$
Armpit	$1-1x10^6$
Feet	$1x10^6$

The microbiome was first coined "*to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease*"

Human Microbiome Project

人們的皮膚每天會脫落約$1x10^9$的皮屑，相當於每分鐘有$3\text{-}4x10^4$皮屑產生，10%的這些皮屑上帶有微生物

Microbial Carrying Particles

潔淨室常見的微生物：

人皮膚上大約有19門1000多種的微生物，主要可分為四大類

- **Actinobacteria(放射菌門)**
  Micrococcus, Propionibacteria, Corynebacteria,
- **Firmicutes(厚壁菌門)－24％**
  Staphylococcus, Clostridia, Bacillus
- **Proteobacteria(變形菌門)－17％**
  Escherichia, Salmonella, Vibrio, Helicobacter
- **Bacteriodetes(擬桿菌門)－6％**
  G(-) nonospore-forming, anaerobic, rod-shaped bacteria

潔淨室常見的微生物：

Categories	Micococcus (微球菌)	Propionebacteria (座瘡丙酸桿菌)	Corynebacteria (棒狀桿菌)
Kingdom	Bacteria	Bacteria	Bacteria
Phylum	**Actinobacteria**	**Actinobacteria**	**Actinobacteria**
Genus	Micrococcus	Propionibacterium	corynebacterium
Species	M. luteus	P. acnes	C. diphtheriae
distribution	Human skin, animal and dairy products and beer	Skin, sweat glands, sebaceous glands	Microbiota of animal (including human)

潔淨室常見的微生物：

Categories	Staphylococcus (葡萄球菌)	Clostridia (梭狀桿菌)	Bacillus (芽孢桿菌)
Kingdom	Bacteria	Bacteria	Bacteria
Phylum	**Firmicutes**	**Firmicutes**	**Firmicutes**
Genus	Staphylococcus	Clostridium	Bacillus
Species	S. aureus	C. tetani	B. anthracis
distribution	Human skin, mucous membrane, soil microbial flora	Found in soil, the intestinal tract of animal, including human, lower reproductive tract	Found in soil, also occur in extreme environments

潔淨室常見的微生物：

皮膚的生物化學性質會影響到微生物的分布，如溫度、濕度、油脂量及酸鹼度

- 乾燥皮膚：G(+) cocci(micrococcaceae)出現在四肢；G(+) rods出現在軀幹
- 潮濕皮膚：Staphylococcus, Corynebacteria and some G(-)bacteria
- 油脂性皮膚：Propionibacteria, Staphylococcus

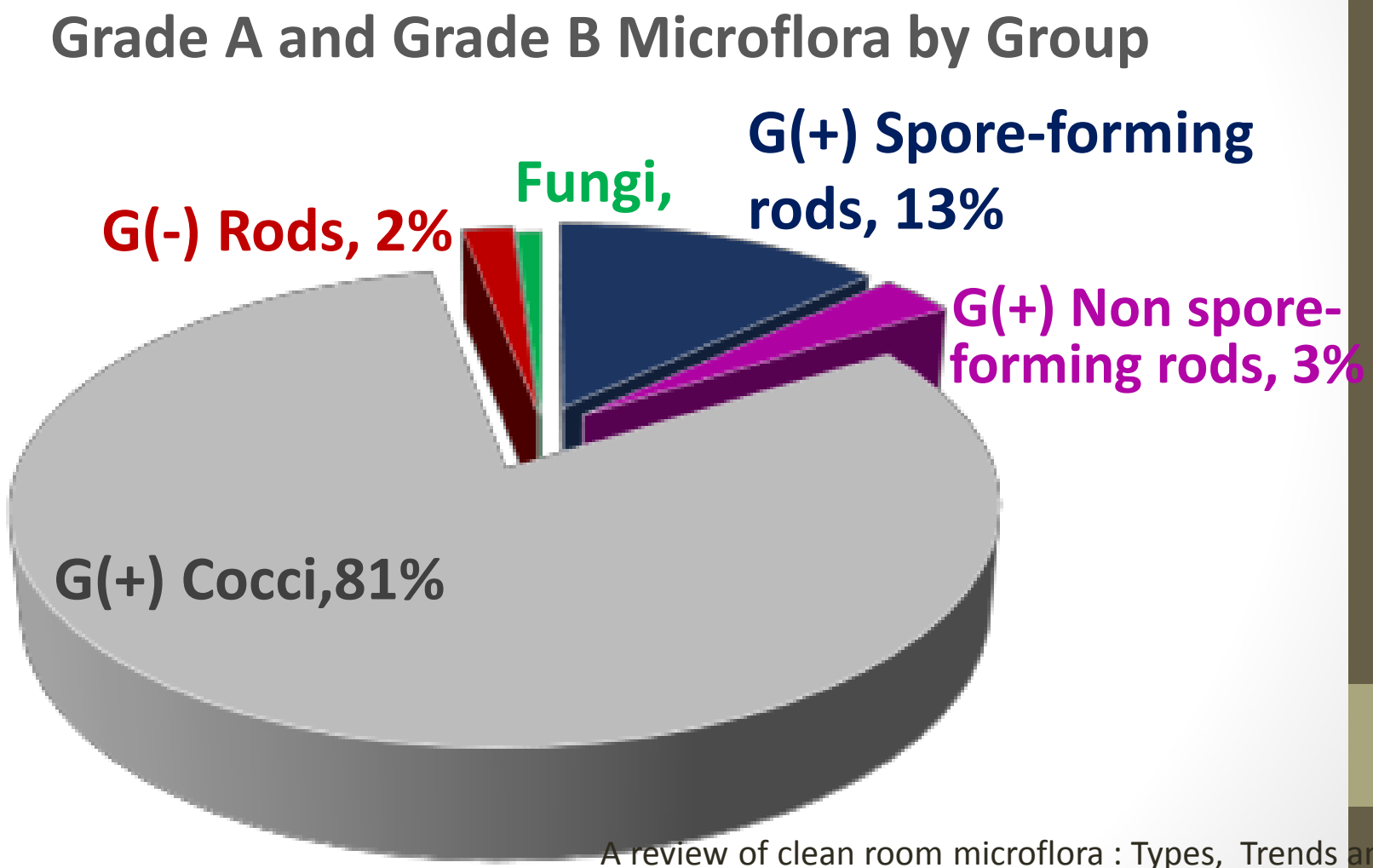
潔淨室常見的微生物：

Clean room microflora

- 了解人體不同部位微生物菌落的分布情況，將有助於評估潔淨室內菌落來源

- 觀察潔淨室內的微生物群落數量、種類及類型的變化，其結果有助於瞭解潔淨室存在的菌落是否產生抗藥性?或是清潔、消毒的程序是否不確實？並可作為環境管制的依據

潔淨室常見的微生物：



A review of clean room microflora : Types, Trends and patterns by Tim Sandle
PDA J Pharm Sci & Tech: 2011, 65:392-403

潔淨室常見的微生物：

Sample Type (Number of Isolates)	G(+) Cocci	G(+) Non Spore-forming Rods	G(+) Spore-forming Rods	G(-) Rods	Fungi
Surface samples (N=340)	60% (204)	28% (97)	6% (21)	4% (14)	1% (4)
Air samples (N= 1564)	82% (1277)	3% (44)	13% (199)	6% (99)	<1% (12)

A review of clean room microflora : Types, Trends and patterns by Tim Sandle
PDA J Pharm Sci & Tech: 2011, 65:392-403

潔淨室常見的微生物：

Sample Type (Number of Isolates)	G(+) Cocci	G(+) Non-Spore-forming Rods	G(+) Spore-forming Rods	G(-) Rods	Fungi
Finger dab samples (N=424)	68% (290)	7% (28)	16% (28)	7% (29)	2% (9)
Suit plates (N= 362)	69% (246)	7% (27)	16% (58)	7% (27)	<1% (4)

A review of clean room microflora : Types, Trends and patterns by Tim Sandle
PDA J Pharm Sci & Tech: 2100, 65:392-403

潔淨室常見的微生物：

Genus	A/B (6729)	C/D (2500)
Micrococci (and related)	38%	40%
Staphylococci	21%	11%
Bacillus (and related)	13%	10%
Pseudomonas (and related)	<1%	8%
Corynebacterium (and related)	3%	5%
Rhodococci	<1%	N/A
Fungi	N/A	3%

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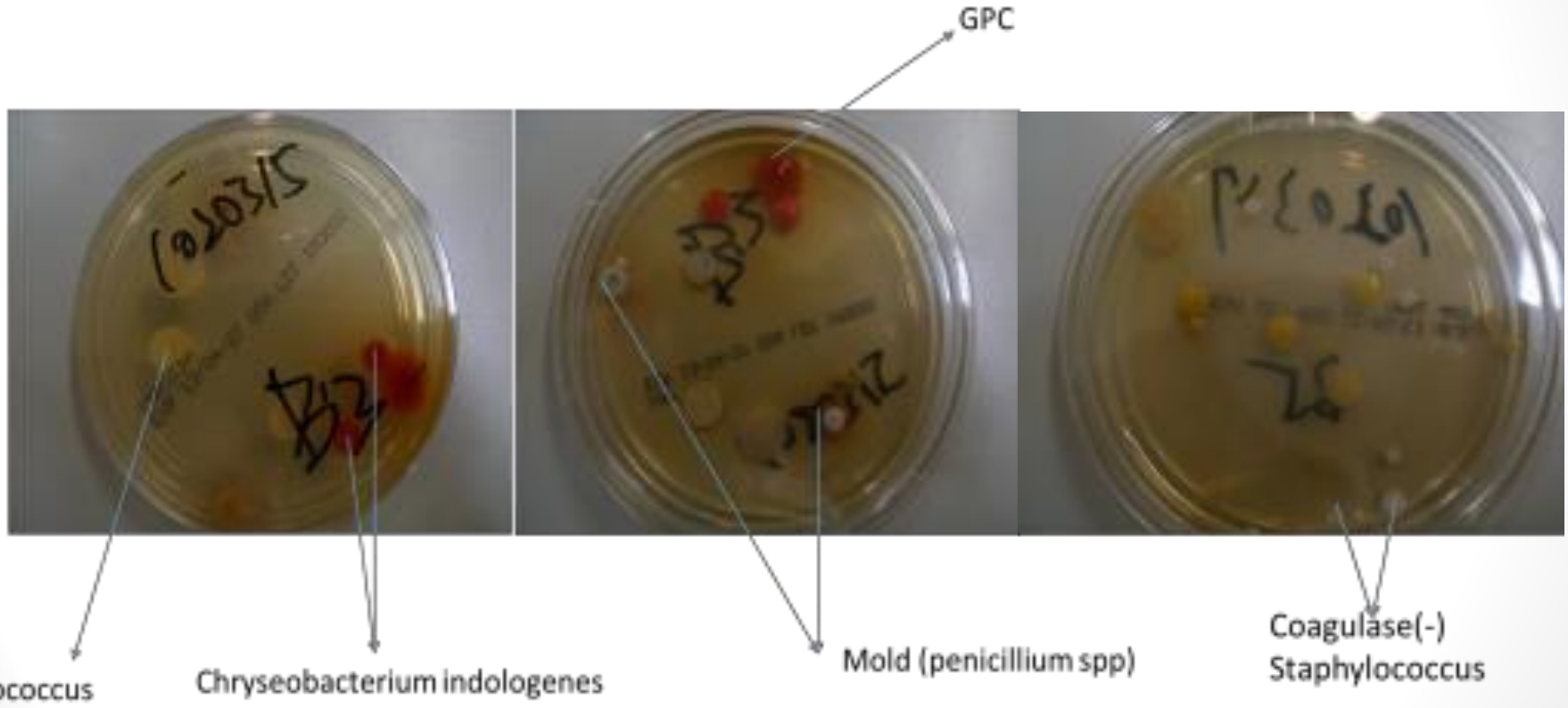
潔淨室常見的微生物：

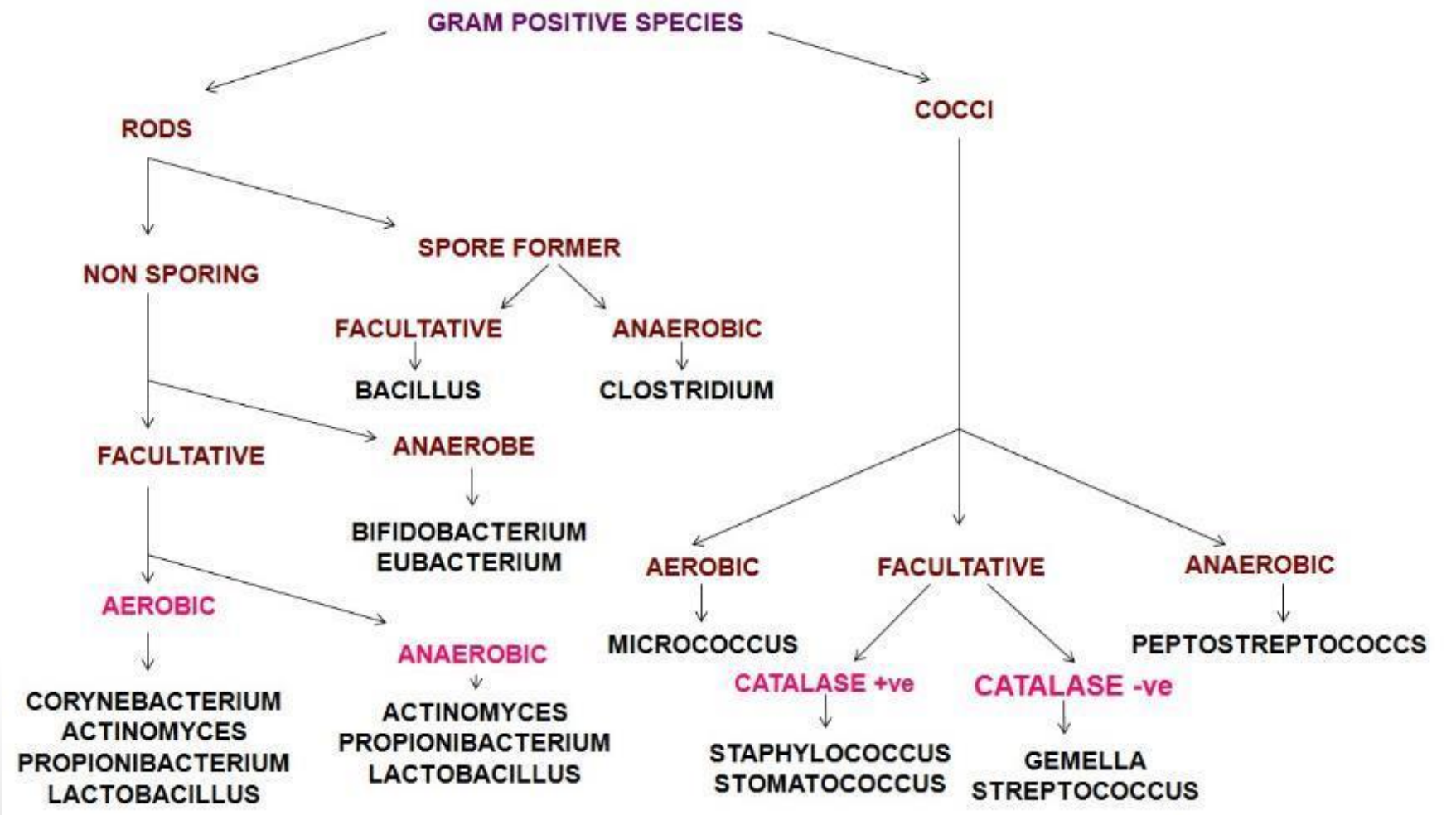

GPC
ococcus
Chryseobacterium indologenes
Mold (penicillium spp)
Coagulase(-)
Staphylococcus

潔淨室常見的微生物：

- 來自於人身體皮膚的皮屑：G(+) Coccus : Micrococcus, Staphylococcus
- 來自土壤，食物等：G(+) Rods : Bacillus spp 出現在潔淨室的黴菌包括：Aspergillus, Penicillium, 及Trychophyton

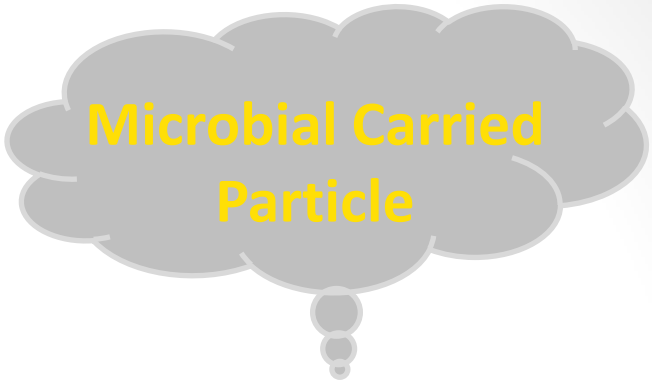
A review of clean room microflora : Types, Trends and patterns by Tim Sandle
PDA J Pharm Sci & Tech: 2011, 65:392-403

潔淨室常見的微生物：


GRAM POSITIVE SPECIES
RODS
COCCI
NON SPORING
SPORE FORMER
FACULTATIVE
ANAEROBIC
BACILLUS
CLOSTRIDIUM
FACULTATIVE
ANAEROBE
BIFIDOBACTERIUM
EUBACTERIUM
AEROBIC
ANAEROBIC
CORYNEBACTERIUM
ACTINOMYCES
PROPIONIBACTERIUM
LACTOBACILLUS
ACTINOMYCES
PROPIONIBACTERIUM
LACTOBACILLUS
AEROBIC
FACULTATIVE
ANAEROBIC
MICROCOCCUS
PEPTOSTREPTOCOCCS
CATALASE +ve
CATALASE -ve
STAPHYLOCOCCUS
STOMATOCOCCUS
GEMELLA
STREPTOCOCCUS

保持潔淨室乾淨的方法：

- 清潔
- 消毒
- 滅菌



清潔：

阻隔

衣著及更衣程序是否真能防止身體上的皮屑釋出至潔淨室

- 使用的潔淨室專用的內衣，且須經過驗證，能提供有效阻隔，尤其是身體較潮溼的部分
- 外層的無塵衣應能包覆整個身體，包括額頭
- 更衣程序的訓練及考核
- 無塵衣更換頻率
- 潔淨室內空氣的換氣次數
- 無塵衣使用期限

清潔：

清除場所或器物表面的汙物

- 場所、設備的清潔主要的目的在移除存在於場　所、設備表面的灰塵及異物
- 應慎選清潔用具，包括拖把、抹布等
- 清潔的順序：由上而下，由後而前，由清潔區而後骯髒區
- Clean-in-Place(CIP)

清潔：

清潔劑的選擇

Cleaning agent - 用來移除設施或設備上的殘留物的化學物質，這些物質會影響到清潔劑 效果或微生物的殘留

- 可與不同類型的水(硬水或軟水)
- 可與消毒劑配合使用
- 不會傷害物體表面
- 不會起泡沫
- 有效地清除不同形式的汙垢，如灰塵，油脂，鐵鏽，蛋白質等

清潔：

清潔劑的選擇 – Cleaning Agents

依清潔劑的化學類型可分為：

- 酸性清潔劑：用來清除無機物質，如水漬。常用的酸性清潔劑包括，鹽酸，醋酸及硫酸。
- 鹼性清潔劑：用來清除油脂，脂肪及含蛋白質的汙物。包括氫氧化鈉，氫氧化鉀，漂白水及氨水，加入螯合劑可用於除鏽
- 中性清潔劑：中性肥皂，陰離子清潔劑等

如何保持潔淨室的清潔：

Aseptics are designed to be used for reducing or destroying microorganisms on **tissues**

消毒劑的選擇

Disinfectant

在物質表面使用化學物質或物理方法來消滅或移除有害的活菌，但不用於殺孢子

- Type of contaminating microorgainsm
- Degree of contamination
- Amount of proteinaeous material present
- Presence of organic matter and other compounds may neutralize some disinfectants
- Chemical nature of disinfectant
- Contact time, temperature, and pH
- Residual activity and effects on the surface
- Toxicity to environment and operator
- Cost

如何保持潔淨室的清潔：

消毒劑的作用模式(Modes of Action)

- Action on the external membrane of the bacterial wall
- Action on the bacterial wall
- Action on the cytoplasmic membrane
- Action on energy metabolism
- Action on the cytoplasm and nucleus
- Action on bacterial spores

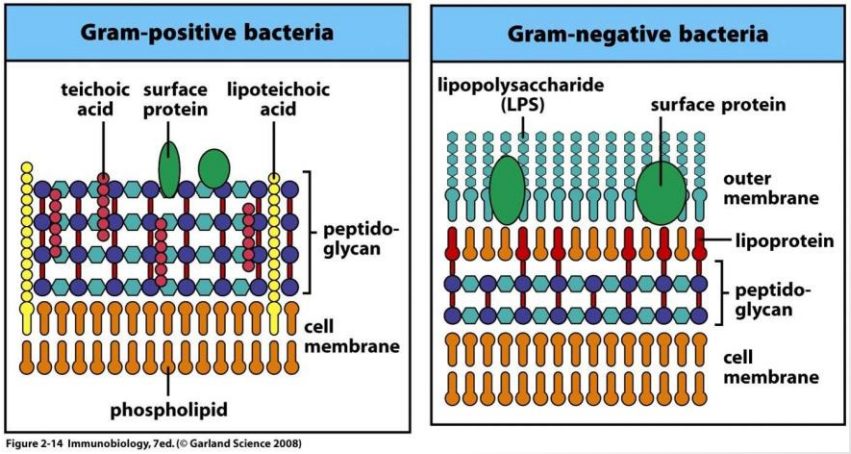
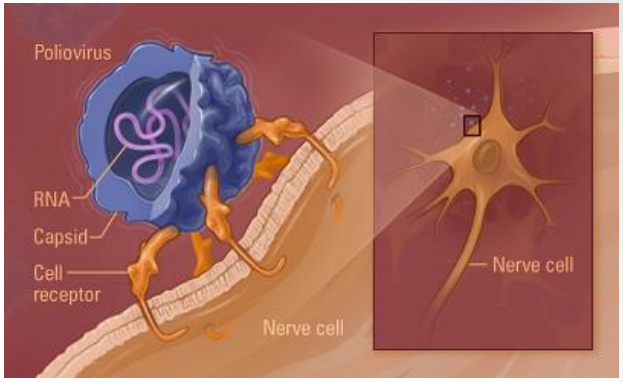


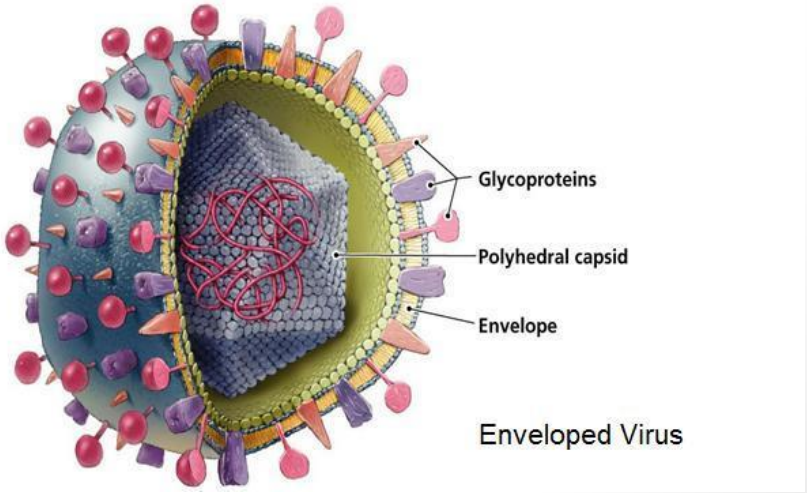
Figure 2-14 Immunobiology, 7ed. (© Garland Science 2008)

依消毒劑的效力

- Low level disinfection
  - Kill most vegetative bacteria and some fungi as well as enveloped viruses. Do not kill mycobacteria or bacterial spores. For cleaning of environmental surfaces.
- Intermediate level disinfection
  - Kill vegetative bacteria, most viruses and fungi, but not for bacterial spores
- High level disinfection
  - Destroy bacteria, mycobacteria, fungi and enveloped and non-enveloped viruses, but not for bacterial spores. Also called as chemical sterilant, if the contact time is extended.



- Non-enveloped viruses – Capsid protein
  - HAV
  - Rotavirus
  - Adenovirus
- Enveloped viruses – Outer lipid membrane
  - Influenza
  - Ebola
  - HIV
  - HBV
  - Rabies
  - SAR virus



依消毒劑的化學性質可分為：

非氧化類消毒劑 (Non-oxidizing Disinfectants)

氧化類消毒劑 (Oxidizing Disinfectants)

消毒劑的選擇

依消毒劑的化學類型可分為：

- Aldehydes (醛類)
- Alcohols(醇類)
- Halogens(鹵素類)
- Peroxides(過氧化物)
- Quaternary ammonium compounds(四級氨化合物)
- Phenolic compounds(酚類化合物)

非氧化類消毒劑 (Non-oxidizing Disinfectants)

Alcohols

- Intermediate disinfection
- 70% Ethanol, IPA, denatured alcohol
- Mode of action :
  - Protein denaturation
  - Damage outer membrane of bacterial cell membrane
- Hard-surface disinfection and skin antisepsis
- Ethanol(hydrophilic) is effective against viruses, IPA(lipophilic) is more efficacious against bacteria
- Optimal concentration : 60-80%, water content increases the efficacy of disinfection.
- The effectiveness of alcohol as an anti-bacterial or antifungal disinfectant increase as the MW increases.

非氧化類消毒劑 (Non-oxidizing Disinfectants)

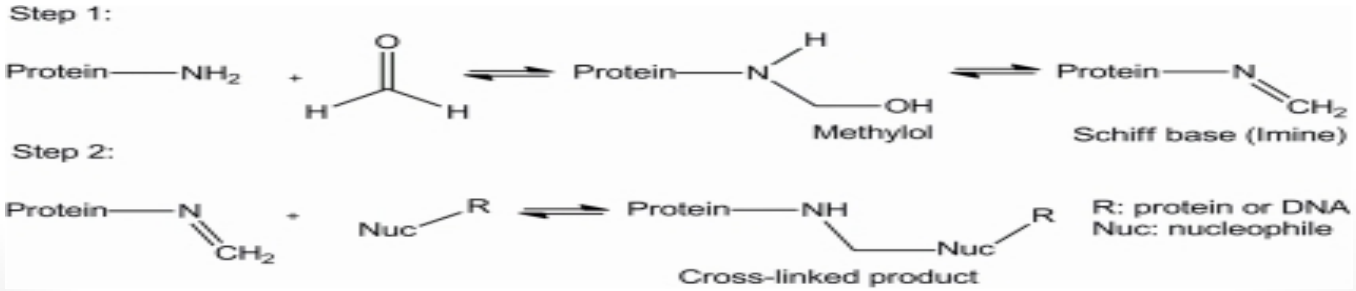
Alcohols

- Isopropanol –
  - Used as surface cleaning and aseptic
  - It is not effective at dehydrating living tissue and it is a better solution for disinfecting skin than ethanol
  - Isopropanol is converted into acetone in the liver
- Ethanol –
  - Used in pure surface-cleaning application
  - To dehydrate cells more readily
- Advantages : fast acting, leaves no residue, compatibility combined with other disinfectants, inexpensive.
- Disadvantages : flammable, interfered by organic matters, evaporates quickly and limits its effectiveness

非氧化類消毒劑 (Non-oxidizing Disinfectants)

Aldehydes

- High level disinfection
- Glutaraldehyde, O-phthalaldehyde(OPA)
- Denaturation of protein and nucleic acids, presented on the bacterial cell wall, by alkylating amino groups, sulfhydryl group etc.
- **The chitin** present in the fungal cell wall is its primary target site as a fungicidal agent.
- To form protein-DNA crosslinkage and irreversible changes in the viral capsid.



非氧化類消毒劑 (Non-oxidizing Disinfectants)

Aldehydes

- Contact time : 10 min. for disinfection; 10-12 hours for sterilization of medical devices.
- More active at **higher pH** (8), but stable at lower pH; **high pH** cause the release of alkaline phosphatase
- The presence of Mg^{++} increases the activity of glutaraldehyde by concentration of cell wall
- Low concentration(0.1%) inhibits spore germination, higher concentration (2%) are sporicidal
- Useful steriliant for heat sensitive instruments
- Glutaraldehyde is more stable than formalin; no carcinogenic effect
- The area where glutaraldehyde is used must be well **ventilated with >10 ACH** (limit in the air : 0.05 ppm)

非氧化類消毒劑(Non-oxidizing Disinfectants)
Phenolics

- Intermediate level disinfection
- Chlorocresol, Chloroxylenol (Lysol, Pine Sole)
- Acts on cell membrane and inactivates intracytoplasm enzymes by forming unstable complexes.
- Lipophilic phenols trapped by membrane phospholipids,
  - liberates cell constituents at low concentration;
  - inhibits permeases at high concentration, causing denaturation of bacterial protein and lysis of the cell membrane components and denaturation of protein and enzyme
- Phenolic compounds are not recommended for use on food contact surface

非氧化類消毒劑(Non-oxidizing Disinfectants)
Quaternary Ammonium Compounds

- Low level disinfection
- Cationic disinfectants, Benzalkonium chloride
- Irreversibly bind to the phospholipids in microbial cell wall.
- proteins of membrane, impairing permeability
- More active against G(+) bacteria than G(-) bacteria, effective against enveloped viruses, no effect against non-enveloped viruses, fungi and bacterial spores.
- Sporostatic, inhibits the outgrowth of spore not for germination
- QACs are commonly used in ordinary **environmental sanitation** of non-critical surfaces such as floor, furniture, and wall.

非氧化類消毒劑 (Non-oxidizing Disinfectants)
Biguanides

- Intermediate level disinfection
- Chlorhexidine (CHX)
- The primary site of action is the cytoplasmic membrane, modification of membrane permeability.
- At very low concentration, it is attached to the negative charged phospholipids in the cell wall or cell membrane and caused it to rupture and leakage of cytoplasm, lead to lysis and cell death.
- At high concentration, bactericidal effect is very fast due to coagulation of cytoplasm

非氧化類消毒劑(Non-oxidizing Disinfectants)

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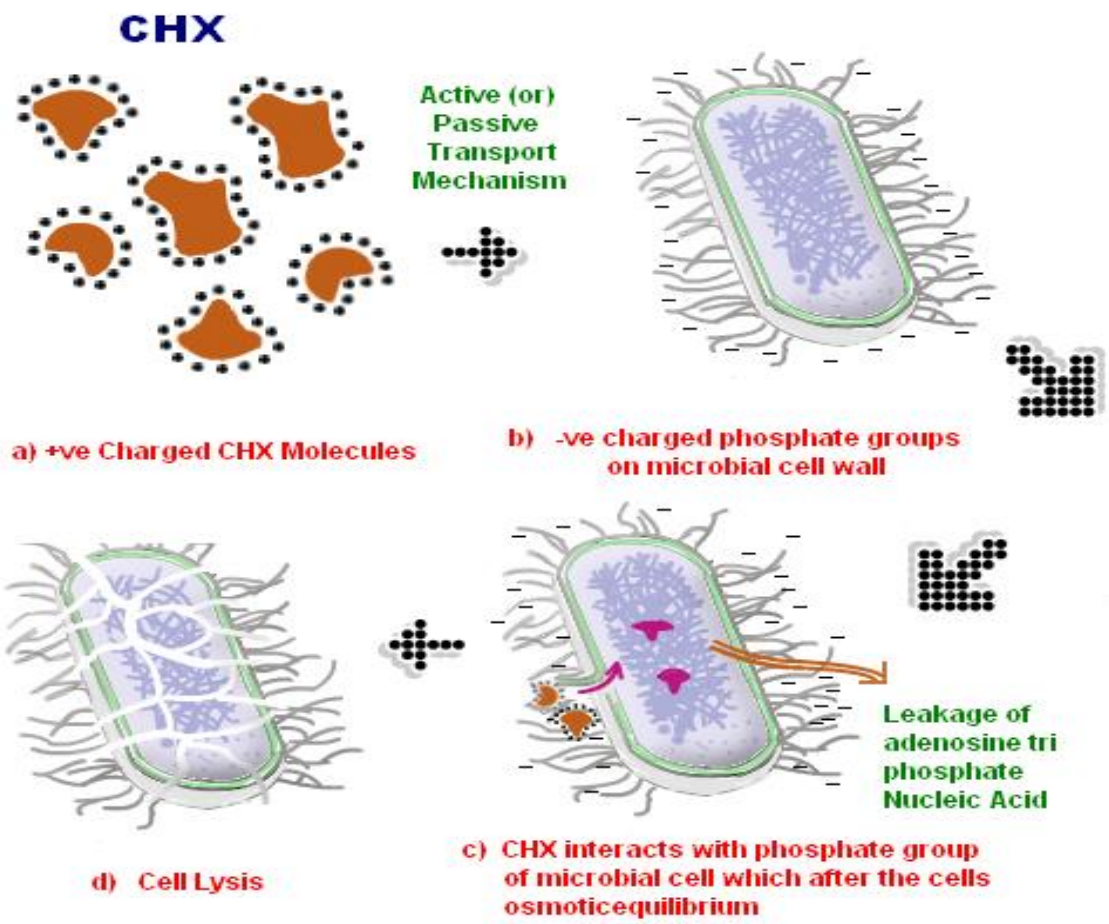

Mechanisms of CHX

氧化類消毒劑(Oxidizing Disinfectants) Halogens – Iodine and Iodophores

Intermediate to low level disinfection

- Betadyne, Povidone
- To penetrate the microbial cell wall quickly and disrupt protein and nucleic acid structure and synthesis.
- Interferes the respiratory chain of the microorganisms by blocking the transport of electron through electrophilic reactions with enzymes of the respiratory chain

氧化類消毒劑(Oxidizing Disinfectants) Halogens – Chlorine compounds

- Intermediate level disinfection
- Bleach Clorox(Sod. hypochlorite), Chloramine-T
- Oxidation of thiol group, disrupt SH groups of amino acids
- Attack the cell membrane leading to cytoplasm leakage and cytoplasm coagulation through interaction with phospholipids

氧化類消毒劑(Oxidizing Disinfectants)

Peracetic acid

- High level disinfection
- Oxidizes the outer cell membrane of microorganisms.
- Denatures protein, disrupts the cell wall permeability, and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites
- No harmful decomposed products and leaves no residue.
- It remains effective in the presence of organic matter.

氧化類消毒劑(Oxidizing Disinfectants)

Hydrogen peroxide

- High level disinfection
- It works by producing destructive hydroxyl free radicals that can attack membrane lipid, DNA, and other essential components.
- Disrupting the cell wall, causing cytoplasm and denature bacterial enzymes through oxidation (by nascent oxygen)
- Vopour phase is more potent than liquid phase

Part I