E3

1 Basic microbiology

1.1 What are microorganisms?

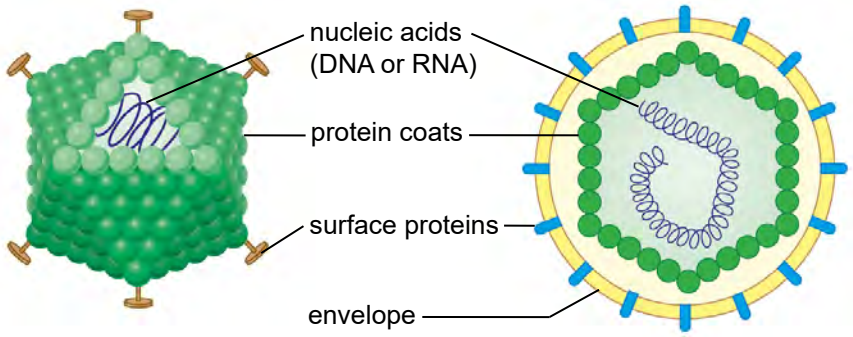
1 **Microorganisms** (微生物) (also called **microbes**) are very small organisms. Most of them can only be observed under a microscope. **Bacteria**, certain **protists** and certain **fungi** are microorganisms.

2 **Microbiology** (微生物學) is the scientific study of microorganisms.

3 **Viruses** are not classified as organisms, but they are also extensively studied in microbiology due to their small size and their close relationship with living hosts.

1.2 Viruses

1 General structures of viruses:



Non-enveloped virus
(裸露病毒)

Enveloped virus
(囊膜病毒)

2 **Multiplication** (增殖) of viruses:

a **Attachment**: A virus recognizes and **attaches** to a host cell by binding its surface proteins to specific receptors on the host cell.

b **Penetration**: The **viral nucleic acid enters** the host cell.

c **Synthesis of viral components**: The viral nucleic acid **directs the host cell's machinery** to produce many copies of the viral nucleic acid and proteins.

d **Assembly**: Viral nucleic acids and proteins **assemble** into viruses.

e **Release**: Once the resources in the host cell have been exhausted, the newly formed viruses **burst the host cell** and spread to infect nearby host cells.

3 Attachment can only take place when the surface proteins of a virus bind to specific receptors on the surface of a host cell. Thus, viral infection is often **specific**.

4 Viruses stay **inactive** and **non-infective** when they enter a **latent period** (潛伏期).

- Their viral nucleic acid may be **integrated into the host cell's DNA** and **replicated** along with the host cell's DNA during cell division.
- **Most viral genes** are **not expressed** and the **host cell** can still **function normally** until the viruses are **reactivated**.

1.3 Diversity of microorganisms

1 Features of different groups of microorganisms:

	Bacteria	Protists		Fungi	
		Protozoans	Algae	Yeast	Moulds
Cell type	Prokaryotic	Eukaryotic		Eukaryotic	
Unicellular or multicellular	Unicellular	Unicellular	Unicellular or multicellular	Unicellular	Multicellular
Cell wall	Present (with peptidoglycan)	Absent	Present (with cellulose)	Present (without cellulose)	
Mode of nutrition	Saprophytic, autotrophic or parasitic	Heterotrophic (a few are parasitic)	Autotrophic	Saprophytic or parasitic	
Reproduction	Asexual (binary fission)	Asexual (binary fission), sexual	Asexual (binary fission, fragmentation (斷裂生殖)), sexual	Asexual (budding (出芽生殖))	Asexual (spores in spore-forming bodies)

2 Some bacteria can form an **endospore** (內孢子) in order to **survive adverse conditions**.

- It has a **tough wall** that can resist heat, low temperatures, dryness and many toxic chemicals.
- It can remain **dormant** for many years. It will germinate and grow into a bacterial cell when the conditions become favourable again.

3 Moulds have thread-like **hyphae**, which can secrete digestive enzymes to penetrate the material on which the moulds grow and carry out **extracellular digestion** (胞外消化).

1.4 Growing microorganisms

1 The growth requirements of microorganisms:

Conditions	Description
a Supply of nutrients	
- Carbon	- Autotrophic microorganisms use **carbon dioxide** to produce organic compounds. - Some heterotrophic microorganisms obtain carbon in an organic form by **feeding** on other microorganisms, or by decomposing **dead organic matter**.
- Nitrogen	- Through nitrogen fixation, certain species of bacteria can convert **atmospheric nitrogen** into ammonium compounds. - Some bacteria and algae obtain nitrogen from **inorganic nitrogenous compounds** such as nitrate and ammonium salts. - Some bacteria and fungi obtain nitrogen from **organic nitrogenous compounds** such as proteins and nucleic acids.
b Temperature	- Each species of microorganisms grows within a **specific temperature range**. - **Many** microorganisms favour **moderate temperatures** (25–45 °C), whereas others are **cold-loving microorganisms** or **heat-loving microorganisms**.
c pH	- Each species of microorganisms grows within a **specific pH range**. - **Many** microorganisms grow best **near neutral pH** (pH 5.5–7.9), whereas others grow best in **more acidic** conditions or **alkaline** conditions.
d Oxygen availability	- **Obligate aerobes** (專性需氧生物) can grow only in the **presence of oxygen**. - **Facultative anaerobes** (兼性厭氧生物) can grow **with or without oxygen**. - **Obligate anaerobes** (專性厭氧生物) can survive or grow only in the **absence of oxygen**.
e Water availability	- The availability of water for microorganisms depends on both the **amount of water** and the **amount of solutes** (e.g. salts and sugars) dissolved in the water. - **Most** microorganisms live under **hypotonic or isotonic** conditions. - A few microorganisms can grow well under **hypertonic** conditions.

2 Microorganisms are usually grown in a **culture medium** (培養基) in a laboratory to study their growth and metabolism. A culture medium can be a liquid or a solid.

	Liquid culture medium	Solid culture medium
Preparation method	Dissolve nutrients in water	Solidify a liquid culture medium using a gelling agent (e.g. **agar** (瓊脂))
Use	For growing large populations of microorganisms	For observing the appearance of **colonies** (菌落) of microorganisms
Example	**Nutrient broth** (營養液體培養基)	**Nutrient agar plate** (營養瓊脂平板)

3 **Risk assessment** (風險評估) for an investigation involving microorganisms includes:

- **identifying potential hazards** of contamination.
- **assessing** the **possibilities and severity** of the hazards.
- **designing precautions** to minimize the risk of contamination.
- **making contingency plans** (應變計劃) in case of accidents.

4 **Aseptic techniques** (無菌操作) must be employed before, during and after any investigation involving microorganisms. These techniques serve two purposes:

- They help **avoid contamination of a microbial culture** with unwanted microorganisms from handlers and the environment.
- They help **avoid contamination of handlers and the environment** with microorganisms in a culture.

5 Examples of aseptic techniques:

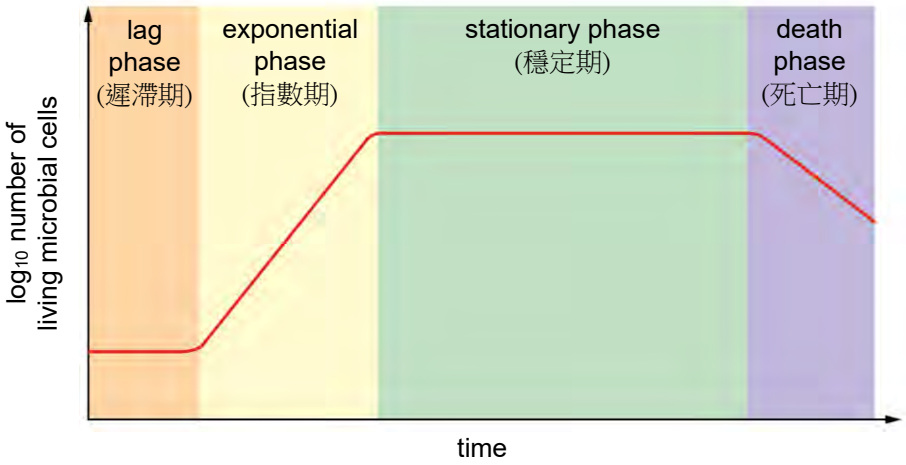
Before the investigation	- Clean all work surfaces and interior surfaces of the equipment involved with **disinfectant** (消毒劑) (e.g. 70% ethanol). - **Sterilize** culture media and apparatus in an **autoclave** (高壓滅菌器).
During the investigation	- **Sterilize** the apparatus used to transfer microorganisms before and after use. For example, **flame** the **inoculating loop** (接種環) until it is **red hot** using a Bunsen flame. - **Flame** the **mouths of culture tubes and flasks** before and after use.
After the investigation	- Clean work surfaces and equipment with **disinfectant**. - Collect any apparatus contaminated with microorganisms in **special containers**. The apparatus should then be **autoclaved** or **incinerated**.

1.5 Microbial growth

1 In microbiology, **growth** often refers to an **increase in the number of microbial cells** in a population. In **bacteria**, its number **doubles** with each binary fission.

- The time required for a bacterial population to double its size is called the **doubling time** (倍增時間) (or the **generation time**).
- The number of bacteria in a population increases exponentially. This pattern of population growth is called **exponential growth** (指數生長).

2 For microorganisms growing in a **batch culture** (分批培養) (which is a **closed system** with no renewal of nutrients and no removal of metabolic waste), the growth curve is **S-shaped**, which typically shows four phases:

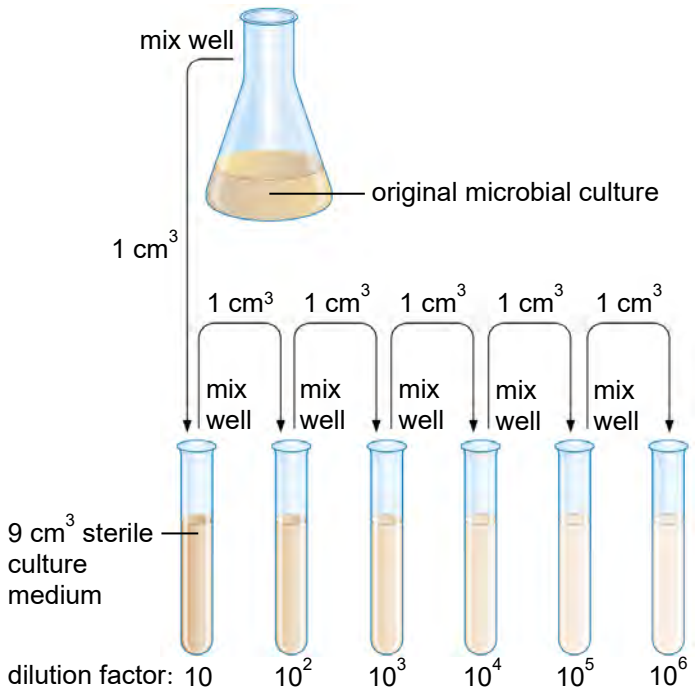


	Lag phase	Exponential phase	Stationary phase	Death phase
Population growth	No growth or little growth	Growth at its maximum rate	No growth	Negative growth
Rate of cell formation vs death	Rate of new cell formation > Death rate	Rate of new cell formation >>> Death rate	Rate of new cell formation = Death rate	Rate of new cell formation < Death rate
Explanation	The cells are **metabolically active**. They are **adapting** to the new environment and **preparing** for growth.	The supply of **nutrients is adequate** and other conditions are **favourable**.	**Depletion of nutrients** and **accumulation of toxic waste** make the environment less favourable for growth.	**Further decrease in** the amounts of **nutrients** and **further accumulation of toxic waste** make the environment harsh for growth.

3 Methods of measuring microbial growth:

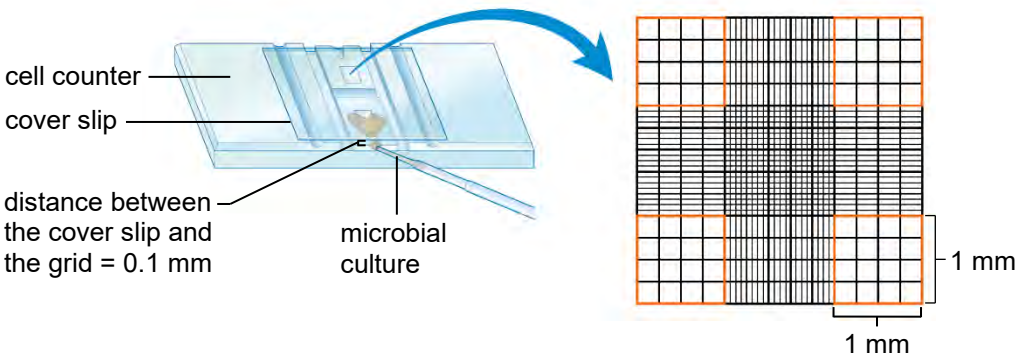
Method	Parameter measured	Result obtained
Total cell count (細胞總數計算) (using a **cell counter** (細胞計數器) and a **light microscope**)	Number of cells in a known volume of liquid sample	Estimation of the number of **both living cells and dead cells**
Viable cell count (活細胞計算) (**spread plate method** (塗佈培養法))	Number of **colonies** on an agar plate	Estimation of the number of **living** microbial **cells** only
Biomass (filtering a sample, drying and weighing the cells)	**Dry mass** of cells	Estimation of the number of **both living cells and dead cells**
Turbidity (混濁度) (using a **spectrophotometer** (光譜儀))	**Turbidity** of the sample (expressed in **optical density** (光學密度) (OD) units)	Estimation of the number of **both living cells and dead cells**

4 If a microbial culture contains too many cells, we can prepare a series of diluted cultures using a technique called **serial dilution** (連續稀釋). One of the diluted cultures may then have an appropriate cell density for cell counting. The **dilution factor** (稀釋因子) should be taken into account when calculating the cell count.

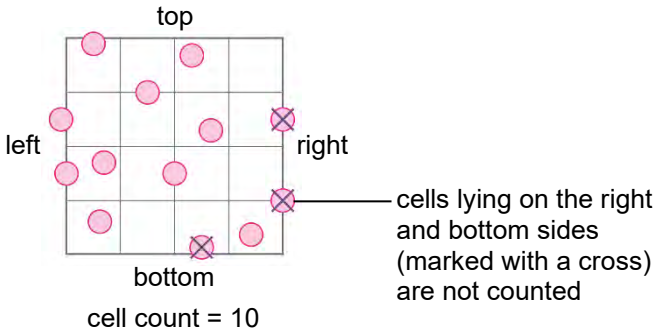


5 Main steps in **total cell count**:

❶ Transfer microbial culture to a cell counter.



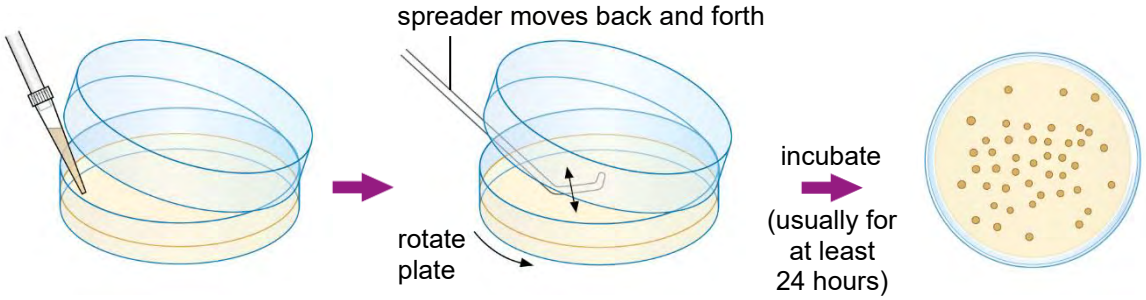
❷ Count the cells in the four large squares at the corners under a light microscope and obtain an average number.



❸ The total cell count of the culture is calculated by:

$$\text{number of microbial cells (per cm}^3\text{ of culture)} = \frac{\text{average number of microbial cells in a large square}}{\text{volume of sample covering a large square (mm}^3\text{)}} \times 1000$$

6 Main steps of the **spread plate method** (塗佈培養法) in **viable cell count**:



❶ 0.1 cm^3 of a diluted culture is added to a nutrient agar plate.

❷ The diluted culture is spread evenly over the agar surface.

❸ The number of colonies on the most appropriate agar plate (a plate with 30–300 colonies) is counted.

❹ The viable cell count of the original microbial culture is calculated by:

$$\text{number of living microbial cells (per cm}^3\text{ of culture)} = \text{number of colonies on the plate} \times \frac{1}{0.1} \times \text{dilution factor}$$

7 Advantages and limitations of different methods of measuring microbial growth:

Method	Advantages	Limitations
Total cell count	- **Quick**	- **Cannot distinguish** between **living cells and dead cells** without the use of special stains - **Cell debris** is easily **mistaken** for microbial cells - **Not** suitable for microbial cells that are **too small** to be observed under a light microscope - **Not** suitable for microbial cells that are **motile** - **Not** suitable for microbial cells that grow **in chains or clusters** - **Not** suitable for a microbial culture with a **low cell density**
Viable cell count	- **Highly sensitive** - **Particular types of microorganisms in a mixed culture** can also be counted using selective culture media and growth conditions	- **Time-consuming** because incubation is involved - **Not** suitable for **filamentous microorganisms** - Microbial growth is **underestimated** when some microorganisms **grow in chains or clusters**, or a **short incubation time** is used - Can be subject to **errors** due to cell clumps in the culture, contamination of agar plates, etc.
Biomass	- Suitable for **filamentous microorganisms**	- **Cannot distinguish** between **living cells and dead cells** - **Time-consuming** because it may involve repeated drying and weighing - **Not** suitable for **slow-growing microorganisms**
Turbidity	- **Easy** and **quick** - Does **not destroy** the sample	- **Cannot distinguish** between **living cells and dead cells** - Microbial growth is **underestimated** for cells that form **clumps** or **films** on the sides of tubes - **Not** suitable for microbial cultures with a **low cell density** because such cultures allow almost 100% of light transmission - **Not** suitable for microbial cultures with a **high cell density** because the light scattered away by one cell may be re-scattered by another back to the detector