Microbiology

Unit 5 · Lesson 1 · Physical and chemical control

The notes for Lesson 1 of Unit 5: chapters 5.1 to 5.2 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 1 of 2 | 2 chapters | Chapters 5.1 to 5.2

About these notes

These are the notes for **Lesson 1 · Physical and chemical control** of the University free n easy video lessons on Microbiology Unit 5, Control of Microorganisms. They are chapters 5.1 to 5.2 of the unit book, unchanged, so a page here is the same page in the full book.

5.1 Physical control: heat, filtration and radiation

Physical control means killing or removing microbes with energy or a barrier instead of a chemical: steam in an autoclave, a hot air oven, a membrane filter, a UV lamp or a gamma source. Every microbiology lab, hospital and food factory depends on it, because no culture, injection or canned food is safe unless the unwanted microbes are gone first. The skill this chapter builds is choosing the right method for the material and knowing how long it must run.

You need: the bacterial endospore and cell wall (Unit 2, Bacterial morphology and cell structure) and culture media (Unit 4, Nutritional requirements and culture media).

Words first: how much is "clean"?

Sterilization destroys or removes all living forms, including bacterial endospores. It is absolute: an object is sterile or it is not. **Disinfection** kills most disease-causing microbes on an object or surface, but not all spores. **Antisepsis** is disinfection of living tissue such as skin. **Sanitization** lowers microbial numbers to a level public-health rules accept, as in a restaurant dishwasher.

A suffix tells you what an agent does. **-cidal** agents kill (bactericidal, sporicidal, virucidal). **-static** agents only stop growth (bacteriostatic); remove the agent and growth starts again. Refrigeration

is bacteriostatic; autoclaving is cidal.

How microbes die: D-value and z-value

When a population is heated at a constant temperature, it does not die all at once. In each minute, the same fraction of the survivors dies. So the number of survivors falls by a constant factor per unit time, and a plot of $\log_{10}$(survivors) against time is a straight line. This is **first-order** (logarithmic) death:

$$N = N_0 \times 10^{(-t / D)} \qquad \text{or} \qquad \log_{10} N = \log_{10} N_0 - t / D$$

Here N_0 is the starting number of cells or spores, N is the number alive after time t, and **D** (decimal reduction time) is the time at a stated temperature that kills 90% of the population, that is, one log cycle. D is written with its temperature, for example $D_{121} = 1.5$ min.

The **z-value** is the rise in temperature that makes D ten times smaller. For many bacterial spores z is close to 10 °C, so the D at a lower temperature T is $D_T = D_{ref} \times 10^{((T_{ref} - T)/z)}$. The **F-value** is the total lethality of a process, expressed as minutes at a reference temperature; F_0 means minutes at 121.1 °C with z = 10 °C, and Chapter 5.5 uses it for canning. Older books use **thermal death point** and **thermal death time**; both depend on the starting number of cells, which is why D replaced them.

Killing never reaches exactly zero, so a process aims at a **sterility assurance level** (SAL); medical devices are usually sterilized to 10^{-6}, a one-in-a-million chance that an item carries a survivor. A dirtier load (bigger N_0) needs a longer run.

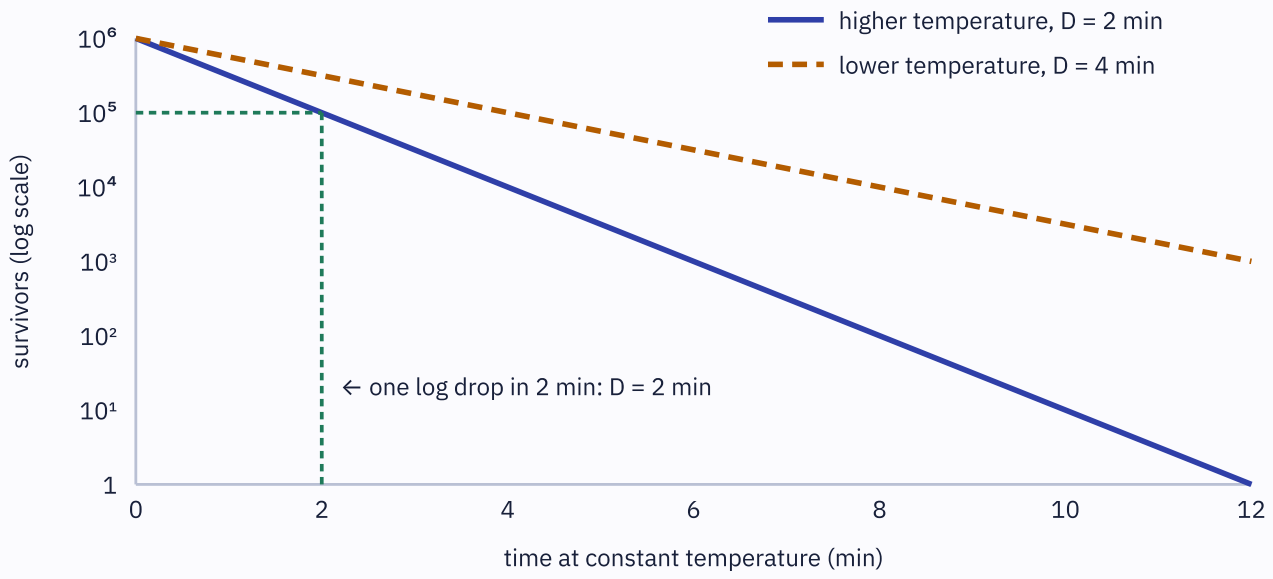


Figure 5.1.1 Survivor curves for 10^6 spores. On a log scale the deaths fall on straight lines; the time for one log drop is the D-value. Notice that the cooler process (dashed) needs twice as long for each log, and neither line ever touches zero.

Heat: moist and dry

Moist heat kills by denaturing and coagulating proteins, and water makes proteins unfold at lower temperatures. Steam also gives up its latent heat when it condenses on a cold object, so it heats the load fast. That is why steam at 121 °C works in minutes while dry air at the same temperature takes hours.

- **Autoclave**: saturated steam at about 15 psi (about 103 kPa) above atmospheric pressure, which raises the boiling point to 121 °C. Textbooks quote 15 minutes for small unwrapped loads; the US CDC guideline gives 30 minutes at 121 °C for wrapped instruments in a gravity-displacement autoclave, and 4 minutes at 132 °C in a prevacuum autoclave. Air must be driven out first, because an air pocket stays cooler than steam. Used for media, instruments and discarded cultures.
- **Boiling** at 100 °C for 10 to 30 minutes kills vegetative cells and most viruses, but not all endospores. It disinfects; it does not sterilize.
- **Tyndallization** (intermittent sterilization): steaming at 100 °C for about 30 minutes on three successive days. Spores that survive day one germinate overnight and the young vegetative cells die the next day. Used for media that 121 °C would spoil.
- **Pasteurization**: mild heat that kills pathogens and lowers spoilage organisms in milk and juice without cooking them. The batch (LTLT) method is 63 °C for 30 minutes; the flash (HTST) method is 72 °C for 15 seconds.

Dry heat kills mainly by oxidation and needs higher temperatures for longer. The **hot air oven** runs at 160 °C for 2 hours or 170 °C for 1 hour, for glassware, metal instruments, oils and powders that steam cannot reach or would damage. **Incineration** burns waste to ash, and **flaming** an inoculation loop red-hot sterilizes it in seconds.

A cycle is checked with indicators. Chemical indicator tape changes colour when heated, which proves the temperature was reached but not that spores died. A **biological indicator** is a strip of resistant spores, *Geobacillus stearothermophilus* for steam, incubated after the run; no growth means the cycle worked.

Filtration and radiation

Filtration removes microbes instead of killing them, so it suits heat-sensitive liquids such as antibiotic solutions, vitamins, serum and enzyme solutions. A **membrane filter** (cellulose ester or polycarbonate) with 0.22 µm pores holds back bacteria; 0.45 µm membranes are used to collect bacteria from water for counting. Viruses and some mycoplasmas pass through 0.22 µm pores, so a filtered liquid is not strictly sterile for them. Air is filtered by **HEPA** filters, which trap at least 99.97% of 0.3 µm particles; they supply laminar airflow hoods, biosafety cabinets and operating theatres.

Ionizing radiation (gamma rays from cobalt-60, electron beams, X-rays) knocks electrons off water molecules and makes hydroxyl radicals that break DNA. It penetrates packaging, so plastic syringes, catheters, gloves, sutures and Petri plates are sterilized after sealing, without heat; this is called cold sterilization. **Ultraviolet** light near 260 nm, where DNA absorbs most strongly, joins neighbouring pyrimidines into dimers (mostly thymine dimers) that block replication; germicidal lamps emit at 254 nm. UV does not penetrate glass, paper or thick liquid, so it only treats air, surfaces and thin films of water.

Cold, drying and high salt or sugar mostly stop growth rather than kill; they return as food preservation in Chapter 5.5.

WORKED EXAMPLE

Question (8 marks). One litre of medium carries 10^5 spores per mL. For these spores D_{121} = 1.5 min and z = 10 °C. (a) How long at 121 °C reduces the load to one surviving spore? (b) How long gives a sterility assurance level of 10^{-6}? (c) If the autoclave only reaches 115 °C, how long is needed for (b)?

1. Starting load: $N_o = 10^5$ spores/mL × 1000 mL = 10^8 spores, so $\log_{10}N_o = 8$.
2. (a) Reaching N = 1 (log 0) needs 8 log reductions. Time = 8 × D = 8 × 1.5 = **12 min**.
3. (b) SAL 10^{-6} means $\log_{10}N = -6$, so 8 − (−6) = 14 log reductions. Time = 14 × 1.5 = **21 min**.
4. (c) D at 115 °C: D_{115} = 1.5 × 10^((121 − 115)/10) = 1.5 × 10^0.6 = 1.5 × 3.98 = 5.97 min.
5. Time = 14 × 5.97 = **83.6 min**, about four times longer for a 6 °C drop.

These times start only when the centre of the flask reaches the set temperature, so large loads need longer cycles.

EXAM TRAPS

- Pressure does not kill; it only raises the boiling point so steam can reach 121 °C. An autoclave full of trapped air at 15 psi is cooler and fails.
- Boiling and pasteurization are not sterilization. Spores survive both.
- D is the time for a 90% kill, not 100%. A 99.9% kill is 3D.
- Filtration removes, it does not kill; and 0.22 µm filters let viruses through.
- UV has poor penetration; gamma rays penetrate. Do not swap them in "sterilization of disposable syringes" answers.

CHECK YOURSELF

– REMEMBER **State the standard conditions for a hot air oven and for an autoclave.** 2 marks

Hot air oven: 160 °C for 2 h (or 170 °C for 1 h). Autoclave: saturated steam at 121 °C, about 15 psi above atmospheric, 15 min for small loads (longer for wrapped or large loads).

– REMEMBER **Which organism's spores are used as the biological indicator for steam sterilization?** 1 mark

Geobacillus stearothermophilus.

– UNDERSTAND **Why does moist heat sterilize faster than dry heat at the same temperature?** 3 marks

Water helps proteins unfold and coagulate at lower temperatures, and condensing steam releases latent heat that warms the load quickly. Dry heat kills by slow oxidation and air transfers heat poorly.

– UNDERSTAND **Explain the principle of tyndallization.** 3 marks

Steaming at 100 °C kills vegetative cells but not spores. During overnight incubation surviving spores germinate into heat-sensitive vegetative cells, which the next day's steaming kills. Three cycles leave the medium sterile without exposing it to 121 °C.

– APPLY **A culture has 10^7 cells; D_{72} = 0.5 s. How many survive 15 s at 72 °C?** 3 marks

Log reductions = 15/0.5 = 30. $\log_{10}N = 7 - 30 = -23$, so $N = 10^{-23}$: effectively none survive.

– APPLY **Choose a method to sterilize (i) a vitamin solution, (ii) plastic Petri plates in sealed packs, (iii) glass pipettes.** 3 marks

(i) Membrane filtration through 0.22 µm. (ii) Gamma or electron-beam irradiation. (iii) Hot air oven, 160 °C for 2 h.

– ANALYZE **An autoclave run's indicator tape changed colour but the spore strip grew. Give two likely causes.** 4 marks

The tape only shows the surface got hot. Likely causes: air was not removed, so the load centre stayed below 121 °C; the cycle was too short for a large or wrapped load; or the load was packed too tightly for steam to reach it.

BEYOND THE SYLLABUS

Industry validates sterilization against international standards: ISO 17665 for moist heat and ISO 11137 for radiation sterilization of health care products, both built on the D-value and SAL ideas above. Heat-sensitive devices are often treated with hydrogen peroxide gas plasma, and UV-C LEDs are replacing mercury lamps for water disinfection. Search "sterility assurance level", "overkill method" and "bioburden method" to see how a cycle is designed. This is not for the exam.

5.2 Chemical control: disinfectants and antiseptics

Chemical control uses substances that kill or inhibit microbes on surfaces, instruments, water and skin, where heat cannot be used. The Dettol in a hospital mop bucket, bleaching powder in a village well, 70% alcohol on a vaccination swab and glutaraldehyde for an endoscope are all chemical agents chosen for one job. Each class attacks a different part of the cell, and each fails in a predictable way, which is what examiners ask about.

You need: kill kinetics and the D-value from Chapter 5.1; cell membranes and walls (Unit 2, Bacterial cell wall: Gram-positive and Gram-negative).

Terms and how to choose an agent

A **disinfectant** is used on non-living objects; it may be too harsh for tissue. An **antiseptic** is mild enough for skin and mucous membranes. A **sanitizer** brings counts down to a safe public-health level, as on food-contact surfaces. A **sterilant** (chemosterilant) kills spores too, given enough time. The general word **germicide** covers all of them.

Hospitals pick the level of treatment from what the item touches (the **Spaulding classification**). Critical items enter sterile tissue or blood, such as surgical instruments and needles, and must be sterile. Semicritical items touch mucous membranes, such as endoscopes, and need high-level disinfection. Noncritical items touch only intact skin, such as BP cuffs and bed rails, and need low-level disinfection.

Microbes are not equally easy to kill. Figure 5.2.1 ranks them. An agent that kills a group also kills every group below it, so an alcohol that kills mycobacteria also kills vegetative bacteria and enveloped viruses.

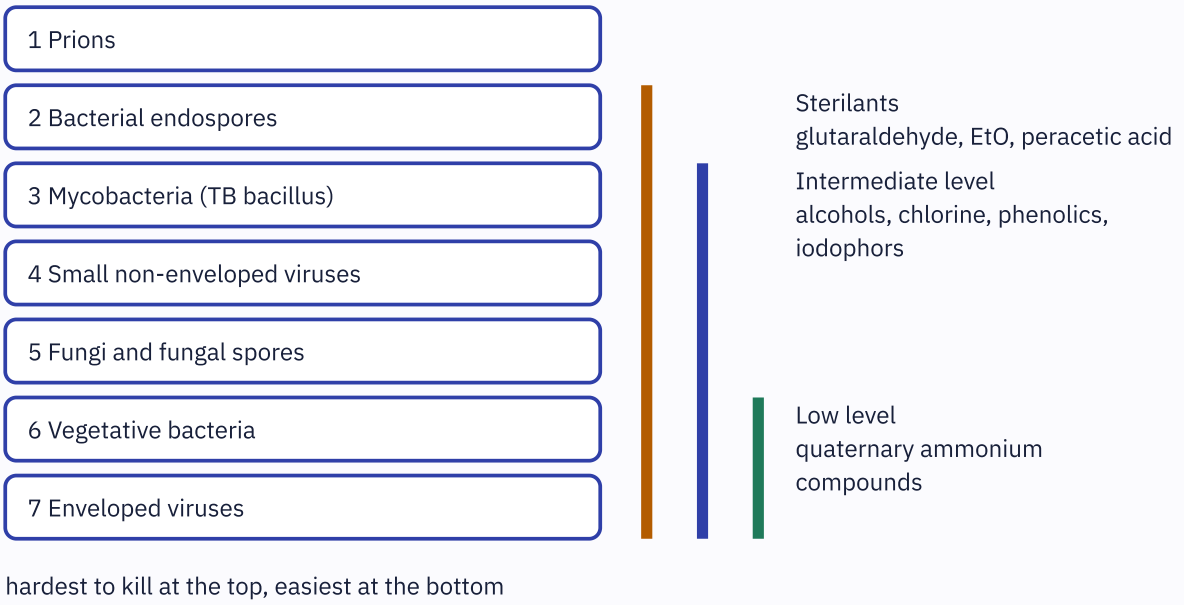


Figure 5.2.1 Resistance ladder for chemical agents. Each bar starts at the hardest group that class of agent can kill and covers everything below. Notice that only sterilants reach spores, and prions sit above every routine agent.

What affects a disinfectant

- **Concentration and contact time.** Killing follows the same first-order pattern as heat (Chick's law). Watson added the effect of concentration: $C^n \times t = K$, where C is concentration, t the time for a given kill, K a constant and n the **concentration exponent**. A large n means dilution weakens the agent sharply; for phenol n is about 6.
- **Organic matter.** Blood, pus, faeces and food residues react with chlorine, iodine and quaternary ammonium compounds and use them up. Clean first, then disinfect.
- **Temperature and pH.** Warmer usually works faster. Chlorine is far stronger at slightly acidic pH, because the killing form, hypochlorous acid (HOCl), turns into weaker hypochlorite ion (OCl^-) as pH rises.
- **Number and state of microbes.** More cells take longer; biofilms (Unit 6, Biofilms) and dried films are protected.

The main classes

CLASS	EXAMPLES	HOW IT ACTS	TYPICAL USE AND LIMITS
Phenolics	phenol, cresols, chloroxylenol (Dettol)	damage membranes, denature proteins	floors, discarded cultures; work in organic matter; irritate skin. Lister used phenol (carbolic acid) in surgery in the 1860s.

CLASS	EXAMPLES	HOW IT ACTS	TYPICAL USE AND LIMITS
Alcohols	ethanol, isopropanol, 60 to 90% in water	denature proteins, dissolve membrane lipids	skin before injection, hand rubs, thermometers; need water to work, evaporate fast, do not kill spores.
Halogens	sodium hypochlorite (bleach), bleaching powder, chloramines; tincture of iodine, povidone-iodine	oxidize proteins and other cell parts	drinking water, blood spills, surgical skin preparation; chlorine is used up by organic matter.
Biguanides	chlorhexidine	disrupt membranes	surgical hand scrub, mouthwash; Savlon combines it with cetrimide.
Quaternary ammonium compounds	benzalkonium chloride, cetrimide	cationic detergents that disrupt membranes	floors, food-contact surfaces; stronger against Gram-positives; inactivated by soap and organic matter; some *Pseudomonas* grow in weak solutions.
Heavy metals	silver nitrate, silver sulfadiazine, copper sulfate	bind thiol (–SH) groups of proteins	burn dressings, algicide in tanks; tiny amounts act (oligodynamic action).
Aldehydes	2% glutaraldehyde, formaldehyde	alkylate amino and other groups of proteins and nucleic acids	endoscopes, fumigation of rooms; slow, toxic, irritant.
Gas	ethylene oxide (EtO)	alkylates proteins and DNA	sterilizes heat-sensitive plastics and devices; penetrates packs; toxic, explosive, needs aeration.
Peroxygens	hydrogen peroxide, peracetic acid, ozone	oxidize via reactive oxygen	wounds (3% H_2O_2), sterilant for devices, water; catalase-producing bacteria resist weak H_2O_2.

Soap is not in the table because it mainly removes microbes: it lifts oil and dirt so that rinsing carries cells away, which is called degerming.

Testing how good a disinfectant is

The **phenol coefficient** compares an agent with phenol against a standard organism. In the **Rideal–Walker test**, the test organism is *Salmonella* Typhi; dilutions of the agent and of phenol are inoculated, sampled at fixed minutes and cultured. The phenol coefficient is the highest dilution of the agent that kills in a stated time divided by the same dilution for phenol. The

Chick–Martin test adds organic matter (yeast) to mimic real use. Because a single number hides the effect of dirt and surfaces, modern labs prefer the **use-dilution test**, where bacteria dried on steel carriers are dipped in the recommended dilution, and **in-use tests**, where samples from disinfectant buckets in the ward are cultured to see if anything survives.

WORKED EXAMPLE

Question (6 marks). In a phenol coefficient test against *S.* Typhi at 20 °C, phenol at 1:90 killed the organism in 10 minutes but not in 5 minutes. A new disinfectant X did the same at 1:400. (a) Find the phenol coefficient of X. (b) For phenol, n = 6. If a 1% phenol solution kills in 10 minutes, how long will a 0.5% solution take?

1. (a) Phenol coefficient = dilution of X ÷ dilution of phenol for the same result = 400 ÷ 90 = **4.4**. X is about four times as effective as phenol in this test.
2. (b) Watson: $C_1^n\, t_1 = C_2^n\, t_2$, so $t_2 = t_1 \times (C_1/C_2)^n$.
3. $C_1/C_2 = 1/0.5 = 2$, and $2^6 = 64$.
4. t_2 = 10 min × 64 = **640 min**, more than ten hours.

Halving phenol's strength makes it 64 times slower. This is why a ward that "saves" disinfectant by over-diluting it ends up with buckets in which bacteria survive.

EXAM TRAPS

- Disinfectant is for objects, antiseptic is for living tissue. The same chemical can be both at different strengths.
- Absolute (100%) alcohol is weaker than 70%; water is needed to denature proteins.
- A phenol coefficient above 1 means stronger than phenol, but it says nothing about performance in blood or on a dirty floor.
- Glutaraldehyde and ethylene oxide are sterilants; alcohol and quaternary ammonium compounds are not, because they do not kill spores.
- Do not write "chlorine is better at high pH". HOCl, the active form, dominates at slightly acidic pH.

CHECK YOURSELF

– REMEMBER **Name one disinfectant from each of: halogens, aldehydes, quaternary ammonium compounds.** 3 marks

Halogens: sodium hypochlorite or povidone-iodine. Aldehydes: glutaraldehyde or formaldehyde. Quaternary ammonium: benzalkonium chloride or cetrimide.

– REMEMBER **Which test organism is used in the Rideal–Walker test?** 1 mark

Salmonella Typhi.

– UNDERSTAND **Why must a blood spill be cleaned before it is disinfected with bleach?** 2 marks

Organic matter reacts with chlorine and uses it up, so little active HOCl is left to kill microbes; the protein also shields cells. Removing it first lets the bleach act.

– UNDERSTAND **Explain the Spaulding classification with one example of each class.** 4 marks

Critical items enter sterile tissue (scalpel): sterilize. Semicritical items touch mucous membranes (endoscope): high-level disinfection. Noncritical items touch intact skin (BP cuff): low-level disinfection.

– APPLY **An agent has n = 1. A 2% solution kills in 20 min. How long will 1% take?** 2 marks

$t_2 = 20 \times (2/1)^1 = 40$ min. With n = 1, halving strength only doubles the time.

– APPLY **Choose an agent for: (i) a heat-sensitive plastic catheter in its pack, (ii) skin before a blood draw, (iii) a village well.** 3 marks

(i) Ethylene oxide gas (or gamma radiation). (ii) 70% alcohol. (iii) Bleaching powder (chlorination).

– ANALYZE **A hospital finds *Pseudomonas* growing in its benzalkonium chloride bottles. Explain how and suggest a fix.** 4 marks

Quaternary ammonium compounds are weaker against Gram-negatives, whose outer membrane slows entry; over-dilution, topping up old bottles and organic contamination let resistant *Pseudomonas* survive and grow. Fix: fresh solutions at the right strength, no topping up, clean containers, and in-use testing.

BEYOND THE SYLLABUS

Regulators now test disinfectants with European EN standards and US EPA methods instead of the phenol coefficient, and they demand claims per organism and contact time. Triclosan was withdrawn from many consumer soaps after concerns about safety and resistance, and quaternary ammonium compounds are watched for the same reason. Hydrogen peroxide vapour is used to decontaminate whole hospital rooms and pharmaceutical cleanrooms. Search "EN 1276 suspension test" and "hydrogen peroxide vapour decontamination" to read further. This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Sterilization	removal or killing of all life, including endospores	5.1
Survivor equation	$N = N_o \times 10^{(-t/D)}$	5.1
D-value	time at a stated temperature for a 90% (one log) kill	5.1
z-value	temperature rise that cuts D tenfold; $D_T = D_{ref} \times 10^{((T_{ref} - T)/z)}$	5.1
Autoclave	saturated steam, 121 °C, about 15 psi above atmospheric; 15 min small loads, longer for wrapped loads	5.1
Hot air oven	160 °C for 2 h or 170 °C for 1 h	5.1
Watson's law	$C^n \times t = K$; n is the concentration exponent (about 6 for phenol)	5.2
Phenol coefficient	dilution of agent ÷ dilution of phenol giving the same kill	5.2

Glossary (terms used in this lesson)

Antibiotic A microbial product, or its derivative, that inhibits or kills other microbes at low concentration.

Antiseptic A chemical mild enough to reduce microbes on living tissue.

Autoclave	Pressure vessel that sterilizes with saturated steam, usually at 121 °C.
Biological indicator	Resistant spores used to prove a sterilization cycle worked.
D-value	Time at a given temperature to kill 90% of a population.
Disinfectant	A chemical that kills most pathogens on non-living objects.
MIC	Minimum inhibitory concentration: lowest drug level that stops visible growth.
Pasteurization	Mild heat (63 °C for 30 min or 72 °C for 15 s) that kills pathogens in milk without sterilizing it.
Phenol coefficient	Effectiveness of a disinfectant compared with phenol in a standard test.
Sterility assurance level	Probability that an item still carries a live microbe after sterilization, often 10^{-6}.
Sterilization	Destruction or removal of all life, including endospores.
Tyndallization	Steaming at 100 °C on three successive days to kill spores after they germinate.
z-value	Temperature change that alters the D-value tenfold.

The full unit book

Every chapter of Unit 5, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 5 · Control of Microorganisms (one file, works offline).

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: chapters on control of microorganisms, antimicrobial chemotherapy and food microbiology (from memory; check).
- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Ananthanarayan and Paniker's *Textbook of Microbiology*, Universities Press: sterilization, disinfection and antibiotic chapters (from memory; check).
- Jay, Loessner and Golden, *Modern Food Microbiology*, Springer: intrinsic and extrinsic factors and the water activity limits in Figure 5.5.1 (from memory; check).

- Frazier and Westhoff, *Food Microbiology*, McGraw-Hill: spoilage by food group (from memory; check).

Open web resources

- US CDC, Guideline for Disinfection and Sterilization in Healthcare Facilities (2008), Table 7: minimum cycle times for steam sterilization, cdc.gov. Source of the 30 min (121 °C, wrapped, gravity) and 4 min (132 °C, prevacuum) figures.
- Antimicrobial Resistance Collaborators, "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis", *The Lancet* 2022;399:629–655, open text at PubMed Central. Source of the 1.27 million and 4.95 million figures.

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