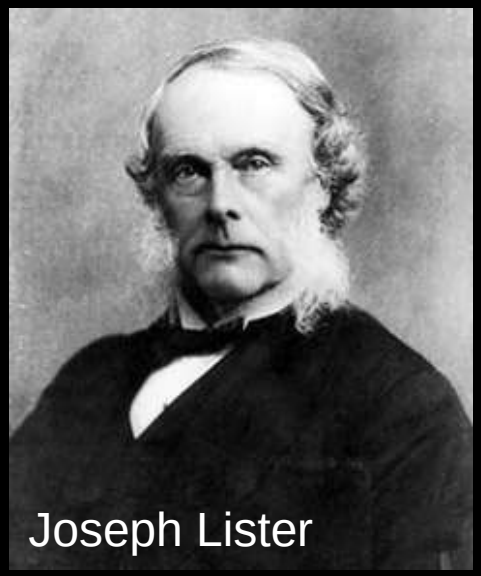
Disinfection and Sterilization Techniques, Methods of Sterility Control.

Karel Holada
Institute of Immunology and Microbiology
1st Faculty of Medicine

Aim:
Destruction / Removal of Harmful Microorganisms

- Foundation of modern medicine
- Prevention of nosocomial infections
- Prevention of epidemics
- Lister 1865 – carbolic acid (phenol) – antiseptic surgery


Joseph Lister

Ignaz Semmelweis (1818-1865)

- Vienna Maternity Clinics (1846)
- Incidence of death from puerperal fever:

1st clinic ~13% mortality (teaching of students)

2nd clinic ~ 2% mortality

- Hand washing with calcium hypochlorite
- Equal mortality in one year

Crises X Progress

- Major developments connected with severe health care crises

- 1970 – Hepatitis B: wearing of gloves, single use needles and syringes
- 1980 – AIDS epidemic: testing of blood and blood donors
- 2000 – prevention of vCJD spread (prions)

Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

William A. Rutala, Ph.D., M.P.H.[1,2], David J. Weber, M.D., M.P.H.[1,2], and the Healthcare Infection Control Practices Advisory Committee (HICPAC)[3]

https://www.cdc.gov/infectioncontrol/guidelines/disinfection/index.html

Definitions

- Cleaning – process which removes contamination but does not destroy micro-organisms
- Disinfection – using an agent that destroys harmful (pathogenic) microbes, usually referred to chemicals that kill the vegetative forms but not the resistant spores of bacteria
- Antisepsis – disinfection on living tissue
- Sterilisation – process that will destroy all forms of life, including bacterial, fungi, spores, and viruses (prions)

Methods

- **Physical**
  - Heat
  - Filtration
  - Irradiation
  - Quarantine
- **Chemical**

- Choice of method depends on purpose
- Different levels of object disinfection
  - CRITICAL
  - SEMICRITICAL
  - NONCRITICAL
- Cleaning precedes any method

Requirements

- CRITICAL - objects which enter normally sterile tissue or blood should be sterile

  (*surgical instruments; cardiac catheters; implants*)
- SEMICRITICAL - objects that touch mucous membranes or skin that is not intact require high-level disinfection – HLD (kills all microorganisms but high numbers of spores)

  (*respiratory therapy equipment; GI endoscopes*)
- NONCRITICAL - objects that touch only intact skin require low-level disinfection

  (*bedpans; EKG leads; furniture; walls; floors*)

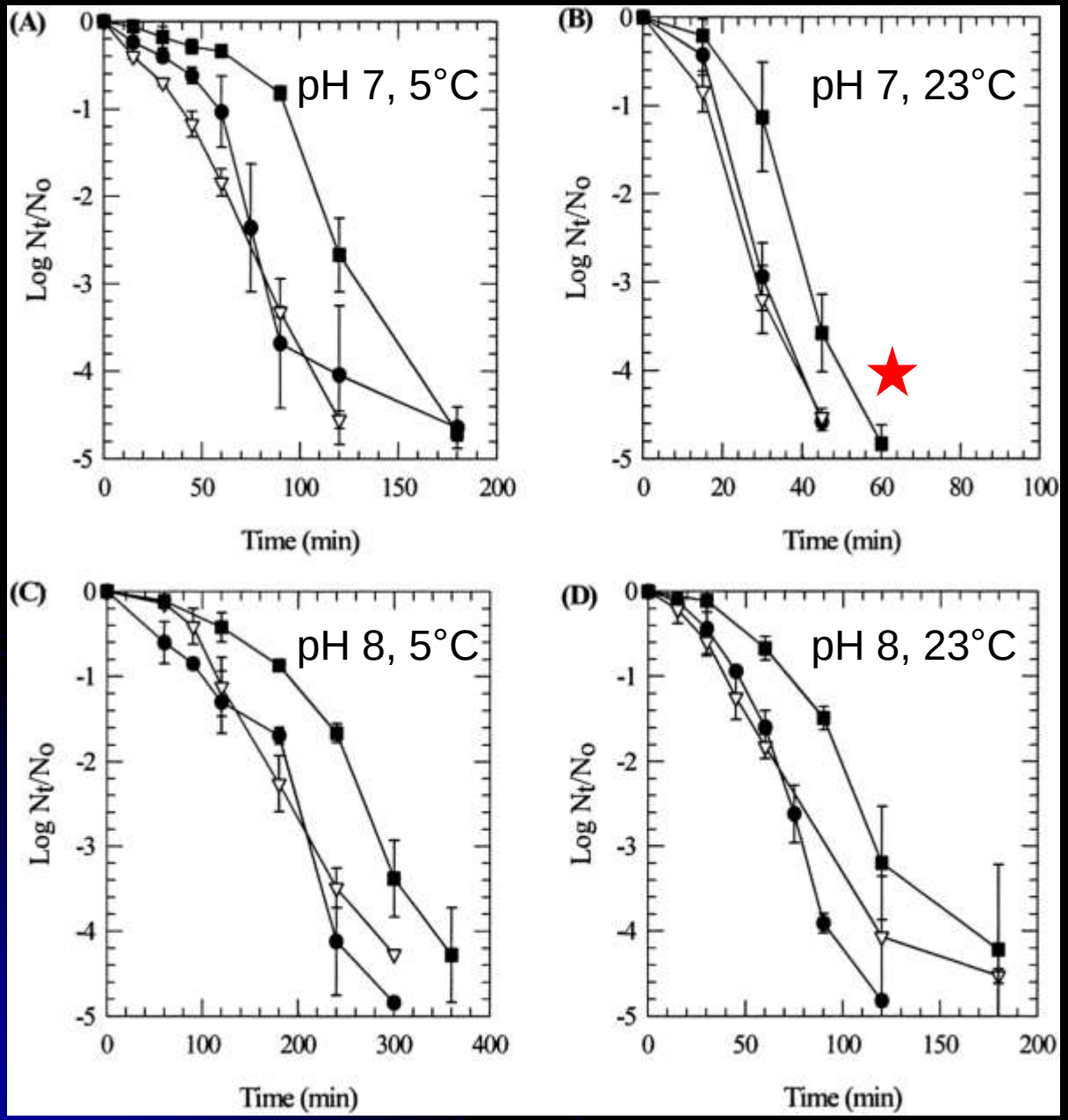
The Ideal Disinfectant

- Broad activity (against different pathogens)
- Not poisonous (or otherwise harmful)
- Penetrating (to pathogens)
- Not damaging to materials
- Stable and resistant to inactivation
- Easy to work with
- Otherwise not unpleasant

… difficult to fulfill

Factors influencing performance

- Strength of the disinfectant (concentration)
- Length of the exposure (time)
- Temperature of environment
  - rate of microbe death doubles with every 10˚C rise in temp.
- Substrate effect (contamination)
  - level of contamination e.g. by blood or soil
- Number of microbes to be killed
- Type of infectious agent



Inactivation of *Bacillus* species spores exposed to 2.0 mg/liter free chlorine under different conditions:

	min.
(A) pH 7, 5°C:	150
(B) pH 7, 23°C:	60
(C) pH 8, 5°C:	350
(D) pH 8, 23°C:	180

Symbols:

B. anthracis
B. cereus
B. Thuringiensis

Decreasing order of resistance to disinfectants / sterilants

Prions

Spores

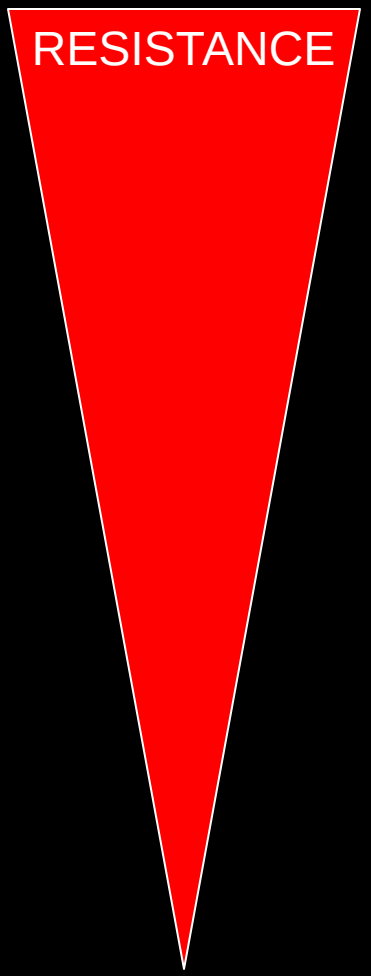
Mycobacteria

Non-Enveloped Viruses

Fungi

Bacteria

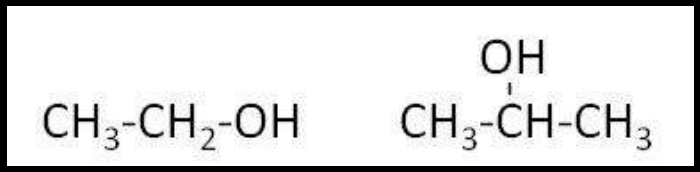
Enveloped Viruses



Chemical disinfectants / sterilants

- Alcohols (ethyl alcohol, isopropyl alcohol)
- Halogens (iodophors and chlorine compounds)
- Aldehydes (formaldehyde, glutaraldehyde)
- Ethylene oxide
- Peroxides (hydrogen peroxide, peracetic acid)
- Phenolics
- Detergents (soap, quaternary ammonium comp.)
- Heavy metals (silver, copper)
- Acids
- Hydroxides (sodium hydroxide)

Alcohols



- Ethanol, Isopropanol
- Disrupt membranes, denature proteins
- Kill vegetative bacteria in 30 seconds
- Inactive against spores, fungi
- Correct dilution (~ 70%)
- Inflammable

<u>Practical uses:</u>

- Skin antisepsis
- Hand disinfection
- Surface disinfection

NaClO $Ca(ClO)_2$

Halogens

$I_2 + KI$ PVP-I

oxidizing agents, damage membranes

Hypochlorites, chlorine

- Activity: bacteria, fungi, spores, viruses, prions
- Unstable if diluted (0.1- 2 %)
- Easily inactivated
- Corrosive for metals

Practical uses:

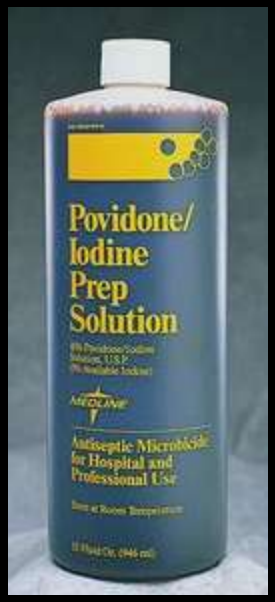
- Disinfection of water
- Surface disinfection (household bleach ~5 %)

Iodophors, iodine

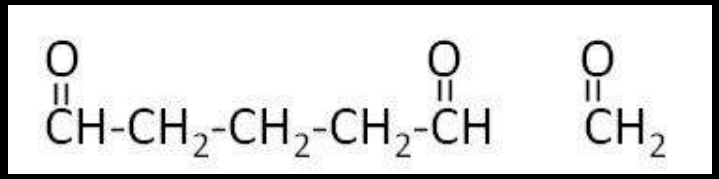
- Intermediate activity
- Good penetration
- Iodophors; + solubilizator e.g. povidone-iodine
- Easily inactivated
- Stain skin, irritant

Practical uses:

- Skin antiseptic
- Surgical scrub



Aldehydes



- **Glutaraldehyde, formaldehyde**
- Alkylating agents
- Broad activity: bacteria, fungi, spores, viruses
- Irritant and Toxic ! Washing !!!
- Unstable – freshness and pH critical
- ***ortho*-phthalaldehyde** – less irritating

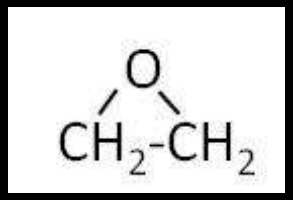
Practical uses:

- HLD or sterilization at low temperature
- Disinfection of endoscopes
- Fumigation of rooms

Colonoscop - HLD

Washing of endoscope after HLD

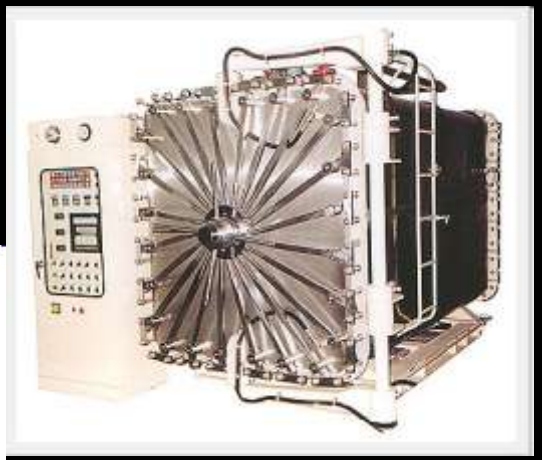
Ethylene oxide



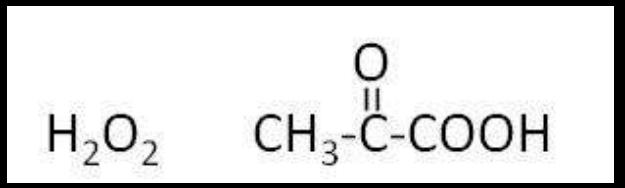
- colorless gas, alkylating agent
- Broad activity (not prions)
- Good penetration even in plastic
- Flammable, explosive and toxic !!
- Lengthy procedure, costly, danger

Practical uses:

- Sterilization of moisture and heat sensitive critical items



Peroxides

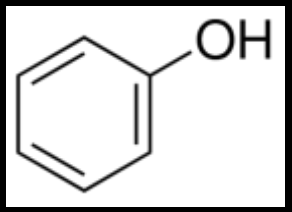


- **Hydrogen peroxide, peracetic acid**
- Oxidize organic molecules
- Broad activity: bacteria, fungi, spores, viruses
- No odor or irritation issues, nontoxic waste
- Effective in the presence of organic matter
- Can damage eye and skin, material compatibility

Practical uses:

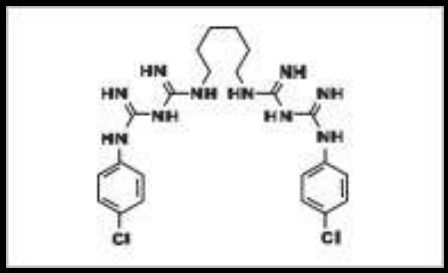
- HLD or sterilization at low temperature
- Disinfection of endoscopes
- Antiseptics (3% H_2O_2), contact lenses

Phenolics



- **Phenol, phenol derivatives**
- Precipitate proteins, damage membranes
- Intermediate activity, not sporicidal
- Use: disinfectants on soiled surfaces

Detergents



Chlorhexidine

- **Soap**, **quaternary ammonium compounds**
- Damage membranes, enzymes
- Low to intermediate activity
- Use: disinfectants for noncritical objects, skin

Heavy metals

- **Silver, copper**
- Oligodynamic effect, inactivate enzymes
- Use: $AgNO_3$ prophylaxis of newborn conjunctivitis, silver coating of catheters, Cu fungicides

Acids and Hydroxides

- **Sodium hydroxide**
- Strong disinfectant, alkalic hydrolysis
- Not inhibited by organic contamination
- Use: destruction of prions (1M NaOH, 60 min.)

General disinfection rules

- Choose appropriate disinfectant, rotate (!)
- Use correct concentration (follow instructions)
- Do not use old solutions (> 24 h)
- Do not top up old solutions
- Correct length, temperature and pH of treatment (instructions)
- Control sterility of disinfectant itself (e.g. *Pseudomonas sp*. in QAS)

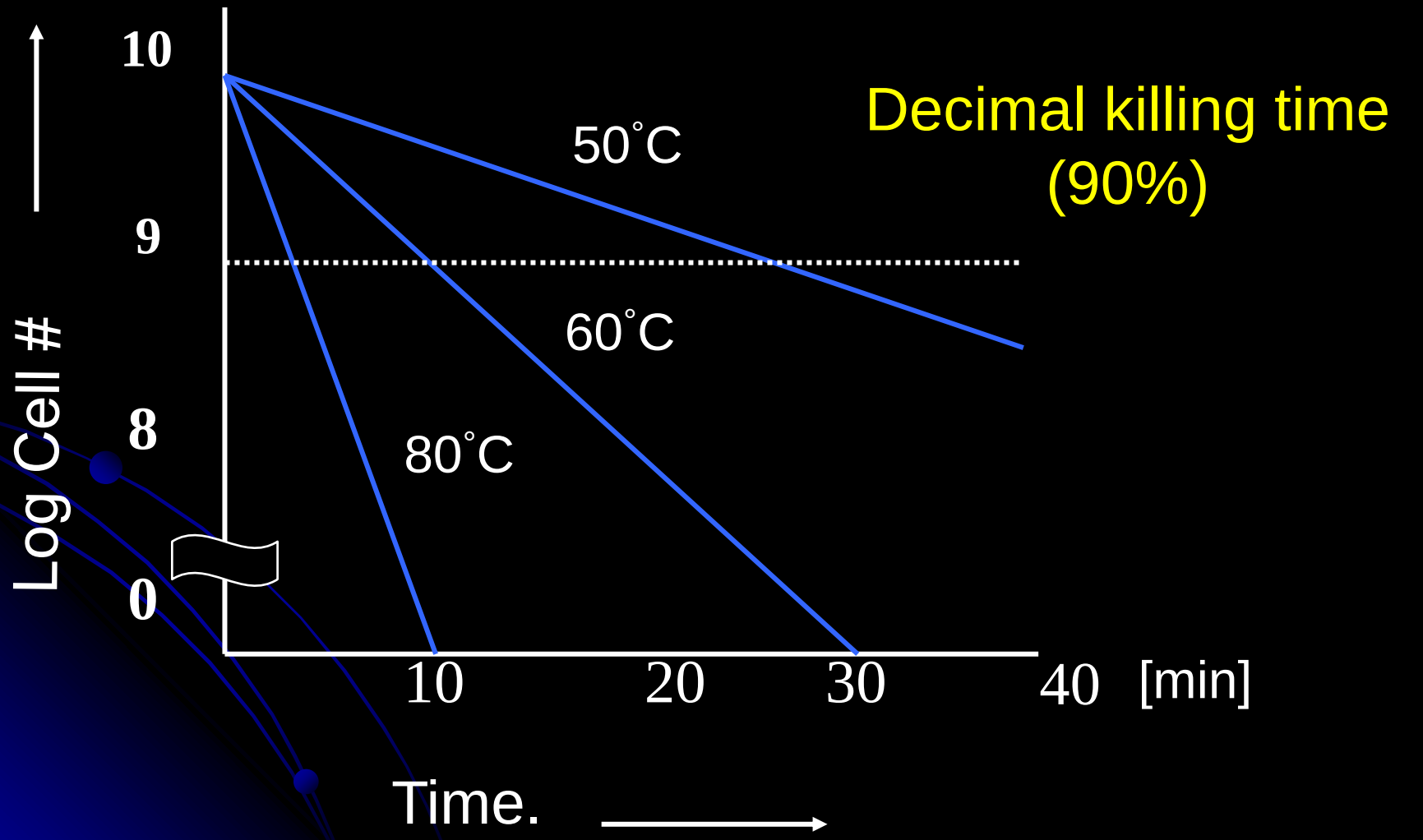
Physical methods

- Heat - dry
  - moist
- Filtration - liquids
  - gases
- Irradiation - UV
  - gamma
- Quarantine / Cover

Heat

- Nontoxic, quick, cheap
- Only for heat resistant items
- Pasteurization – kills vegetative bacteria
  (e.g. 72^{o}C, 20 sec.)
- Boiling
- Flaming (microbiology loops)
- Incineration – the ultimate sterilization
  (medical and biohazardous waste)

Logarithmic lethality curve



Dry heat sterilization

- **Hot air sterilizer**
- Oxidation of cellular components
- For moist sensitive materials (e.g. oils, powders, sharp instruments)
- Non corrosive
- 150-170^{o}C (150 - 60 min)

Steam sterilization

- **Autoclave**
- Denature enzymes and proteins
- Saturated steam applied under pressure
- Direct contact with items
- Nontoxic, rapid, cheap and dependable
- Exclusion of air from the chamber

Gravity displacement autoclave (121^{o}C, 20 min.)

High pre-vacuum autoclave (121^{o}C, 4 min.)

Autoclaves

Autoclave large

Autoclave for space ship

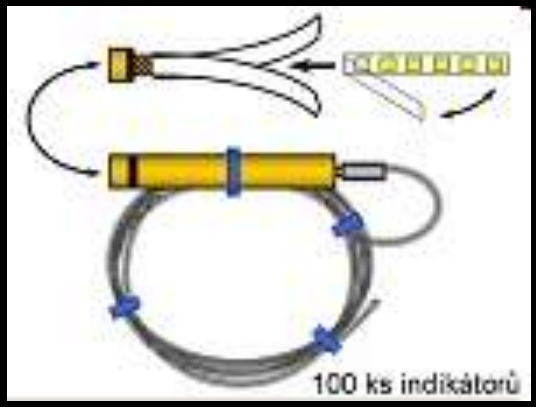
Pressure cooker

Autoclaving

- Controlled cycle (temperature, pressure, time)
- Typical settings (gravity displacement):

  121°C 200 kPa 20 min

  134°C 300 kPa 10 min (prions 60 min)
- Monitoring of effectiveness
  - temperature & pressure charts
  - chemical indicators (e.g. Bowie-Dick test)
  - biological indicators (spore tests, *B. stearothermophilus*)
- Documentation (store 15 years)



Filtration – liquids & gases

- Ultrafiltration of liquids – pore size e.g. 0.2 μm
- Labile fluids

- Gases (Air) – HEPA or ULPA filters
- Biosafety cabinets
- Infectious disease labs (e.g. BSL-3)
- Infectious disease clinics

Irradiation

- UV irradiation (220 – 330 nm)
- Causes DNA damage (254 nm), germicidal
- Disinfection of surfaces, water

- Gamma irradiation (^{60}Co, 25 kGy - high energy)
- Causes DNA damage
- Only in specialised centres
- Used on disposable plastics in sealed packs

Low temperature plasma

- Hydrogen peroxide gas plasma
- Generated using radio frequency or microwave energy – reactive particles, free radicals
- Cycle ~ 60 min. at 45^{o}C
- Broad activity including spores (and prions??)
- Nontoxic
- For thermo-labile materials (compatible with >95 % of medical devices)

Sterilization summary

CHEMICAL METHODS

- Glutaraldehyde (> 2.0%)
- Hydrogen peroxide (7.5%)
- Peracetic acid (0.2%)
- Ethylene oxide

PHYSICAL METHODS

- Heat
- Filtration
- Irradiation

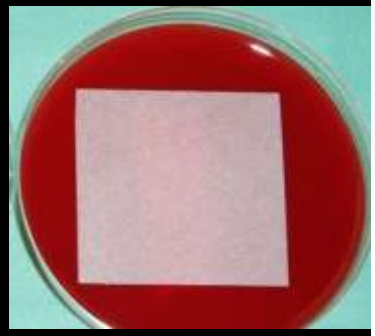
NEW METHODS

- Low temperature plasma

Pre-sterilization: cleaning (washing), drying, wrapping

Environmental microbiological control

- **Surfaces** - swabbing, printing
- **Air** - sedimentation
- **Liquids** - direct cultivation
- **Materials** - rinsing, immersion



Cultivation detection, molecular biology, immunomethods..

FIGHT GERMS BY WASHING YOUR HANDS!

1 Wet your hands

2 Soap

3 Lather and scrub - 20 sec

4 Rinse - 10 sec

5 Dry your hands

6 Turn off tap

DONT FORGET TO WASH:

- between your fingers
- under your nails
- the tops of your hands

www.lung.ca

THE LUNG ASSOCIATION
L'ASSOCIATION PULMONAIRE