MET Revised Basic Course Workshop in Medical Education Technology

Under Aegis of

NMC Regional Centre for Faculty Development
Sri Aurobindo Medical College & P.G. Institute,
Indore (M.P.)

At

People's College of Medical Sciences & Research Centre, Bhopal

6th to 8th, January 2022

Organized by: Medical Education Unit, PCMS & RC, Bhopal


LECTURE HALL
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At
Research Centre
January, 2022


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1. Desired outcome
2. Final End point
3. Destination
4. Specific & Fixed
5. ROLE
1. Part we need to play
2. Responsibility to achieve goal
3. Direction towards goal
4. Defined character


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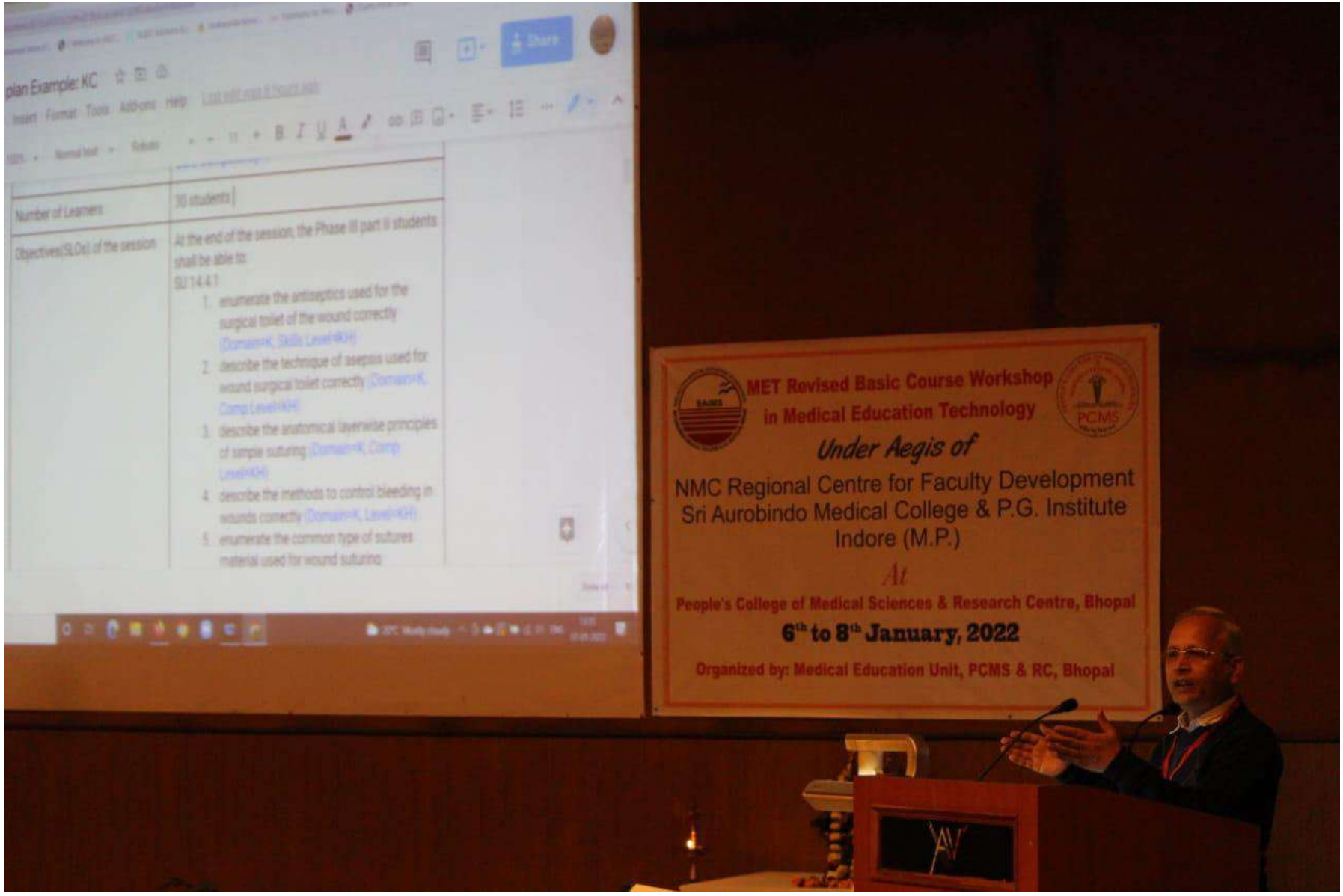
Share
plan Example: KC
Insert Format Tools Add-ons Help
Number of Learners
30 students
Objectives(SLOs) of the session
At the end of the session, the Phase III part II students shall be able to:
SLO 1 & 2
1. enumerate the antiseptics used for the surgical toilet of the wound correctly
2. describe the technique of asepsis used for wound surgical toilet correctly
3. describe the anatomical layerwise principles of simple suturing
4. describe the methods to control bleeding in wounds correctly
5. enumerate the common type of sutures material used for wound suturing
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LADIES
TOILET


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RBCW, 6 - 8th January 2022:

Video 2022-01-09 at 7.26.50 PM.mp4

Thank You for visiting and remembering the EVENT
RBCW 6-8th January 2022
Dr. Kailash Charokar
MEU Coordinator PCMS & RC, Bhopal

Glimpses and Memories


REGISTRATION


Revised Basic Course Workshop
in Medical Education Technology
Under Aegis of
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Sri Aurobindo Medical College & P.G. Institute,
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At
People's College of Medical Sciences & Research Centre, Bhopal
7th to 9th October, 2021
Organized by: Medical Education Unit, PCMS & RC, Bhopal


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in Medical Education
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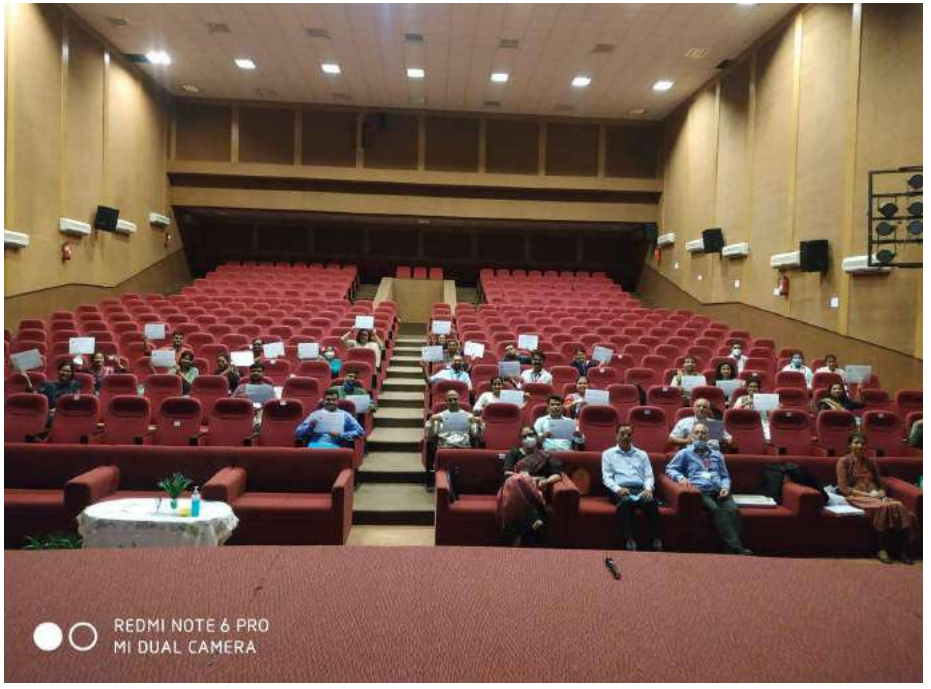

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Predetermined TARGET
Attends OUTCOME/
Each
Not time bound
Foreseen
Fact to be achieved
Perspective to LIFE
Non-Specific


The current class change –
Interactive lectures


1


Revised Basic Course Workshop
in Medical Education Technology
Dr. Arti Jain
Associate Professor
Dept. of Microbiology
PCMS & RC, Bhopal


AUDITORIUM


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Historical Development of Microbiology:

Spontaneous Generation

Germ Theory of Disease

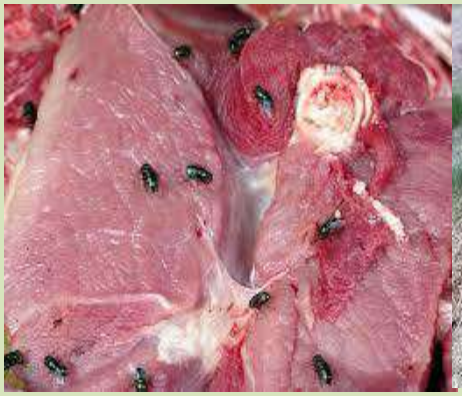
Dr. Kamal Uddin Zaidi

Aristotle (384-322 BC)

- Proposed the theory of spontaneous generation
- Also called abiogenesis
- Idea that living things can arise from nonliving matter.
- Noted several instances of the appearance of animals from environments previously devoid.
- Sudden appearance of fish in a new puddle of water
- Idea lasted almost 2000 years

Beliefs based on prior Observation

- If leaf land on water it becomes a fish.
- If bale of hay left in barn it produced mice.
- Muddy soil gives rise to frog.
- Meat hung out in the market is the source of flies



Van Helmont: (1600s) : He thought he could produce mice from wheat and a soiled shirt.

Recipe for Mice by Dr. Jan Baptista von Helmont


Dirty Shirt

+


Wheat

+

21 Days
Time

=

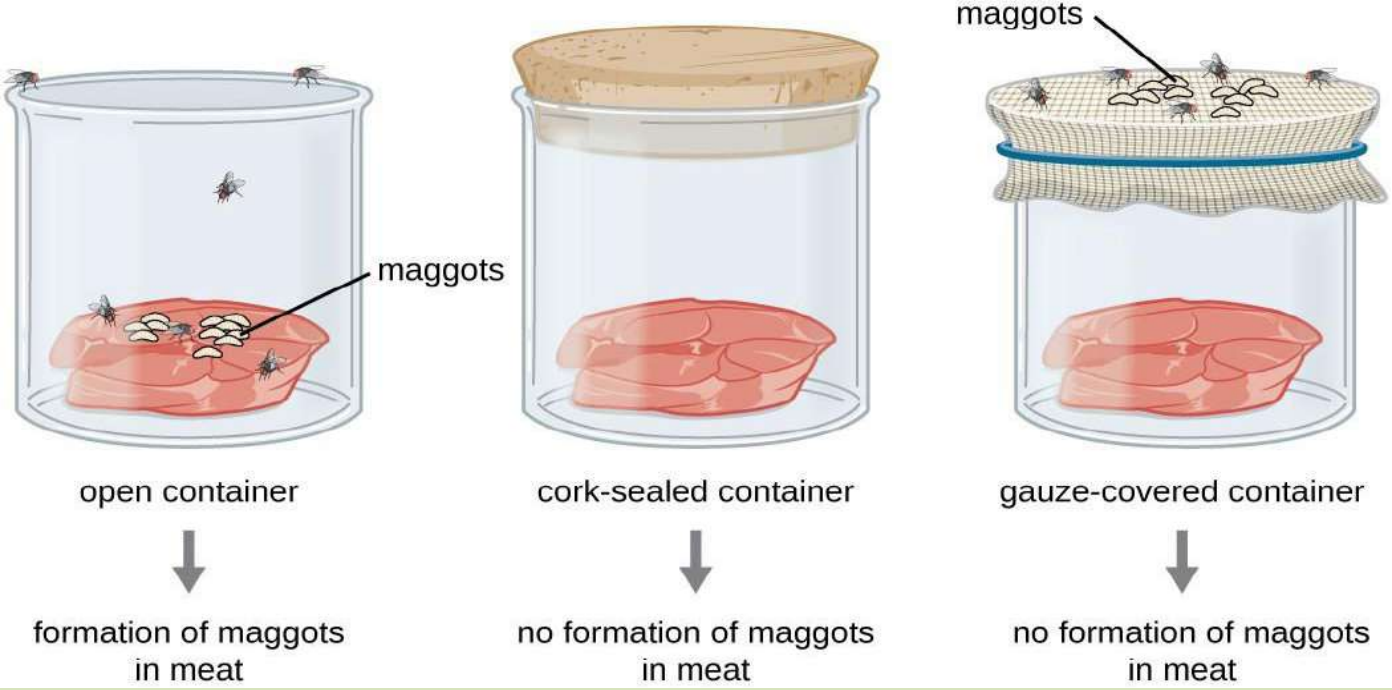

Mice

Francesco Redi's (1668)

Living organism could develop from Non living matter Spontaneously

Evidence against spontaneous generation:

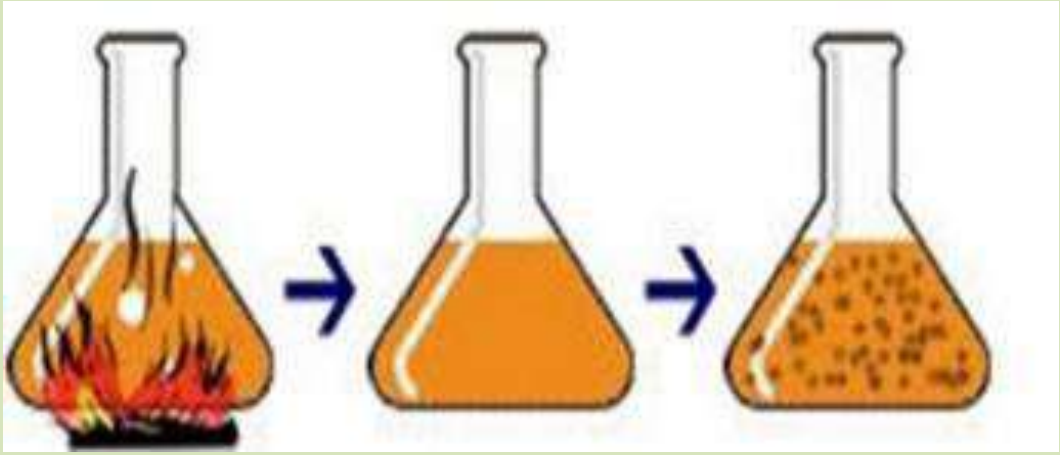
1. **Unsealed** – maggots on meat
2. **Sealed** – no maggots on meat
3. **Gauze** – few maggots on gauze, none on meat



John Needham 1745

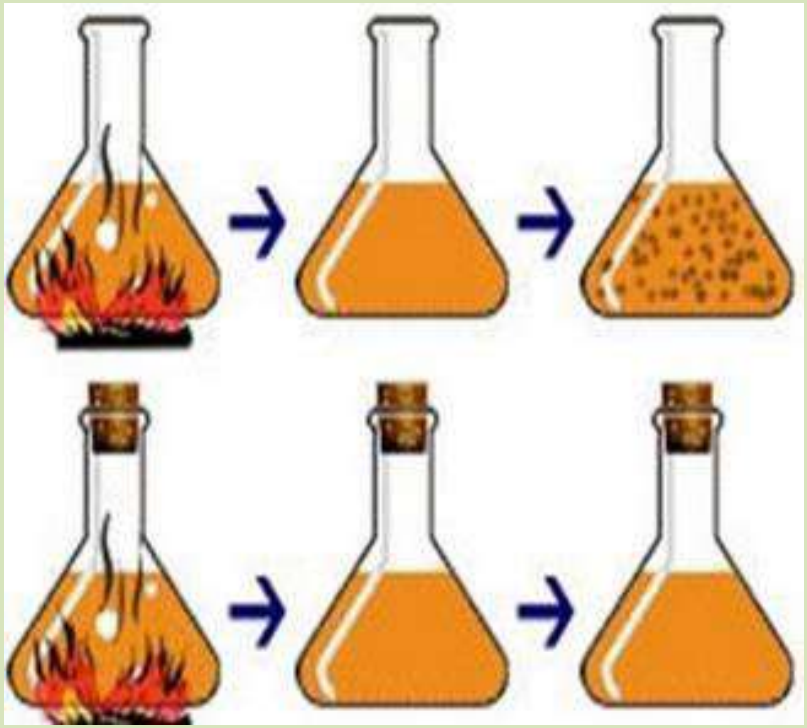
English scientist

- Tried to prove **spontaneous generation**
- To test the theory he boiled meat broth for several minutes in a loosely sealed flask.
- Immediately after boiling he saw under the microscope that the broth had no living things.
- After a few days he examined the flask and found microrganism.



Lazzaro Spallanzani (1765)

- Italian scholar Improved upon Needham's experiment.
- Boiled flasks of broth to kill life, sealed one jar, left other jar open.
- Open jar had living microorganisms, sealed jar did not.
- Concluded that broth did not produce life, organisms entered through the air.

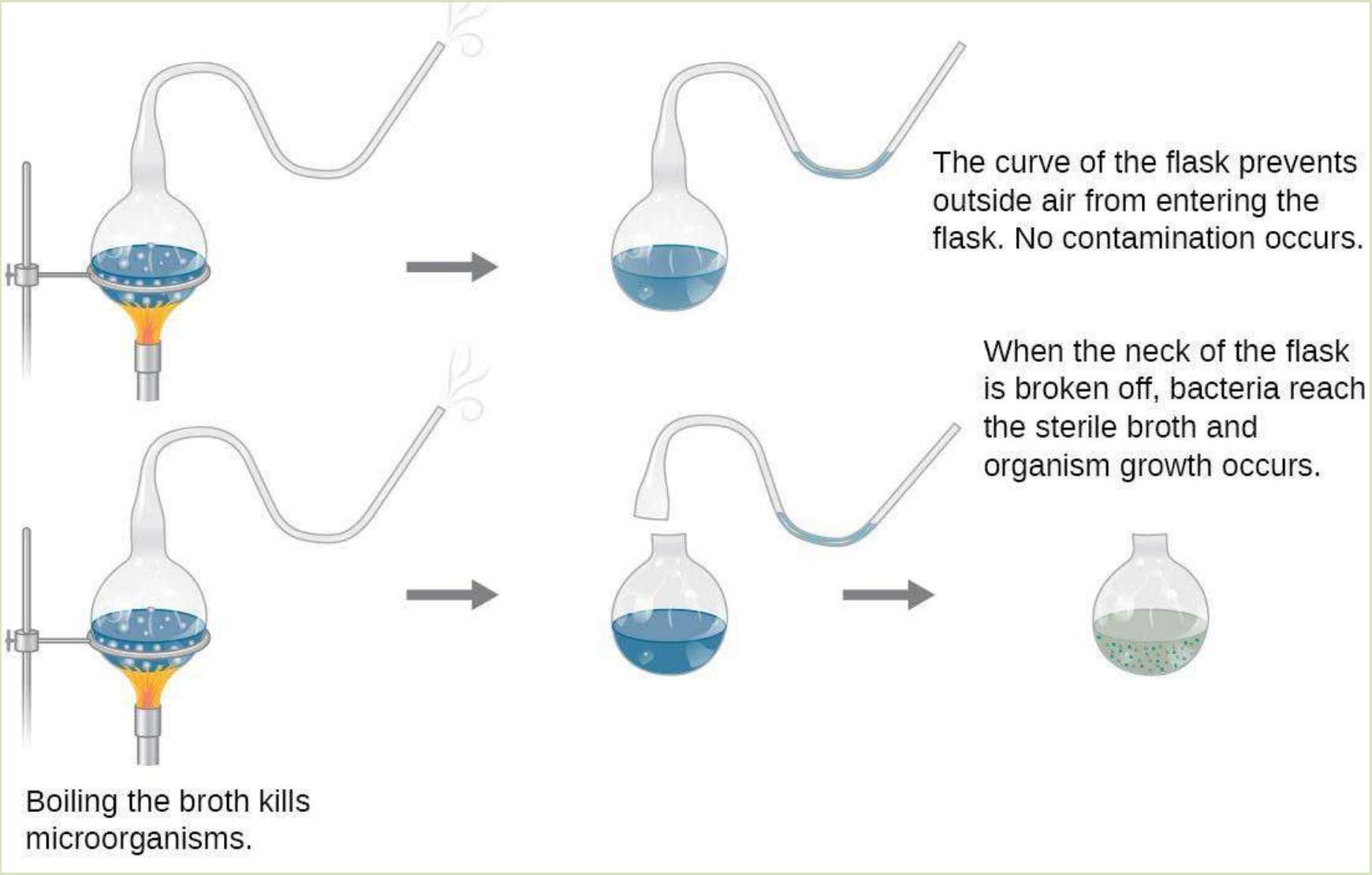


Pasteur's Experiment: (1822-1895)

Hypothesis: Microbes come from cells of organisms on dust particles in the air; not the air itself.

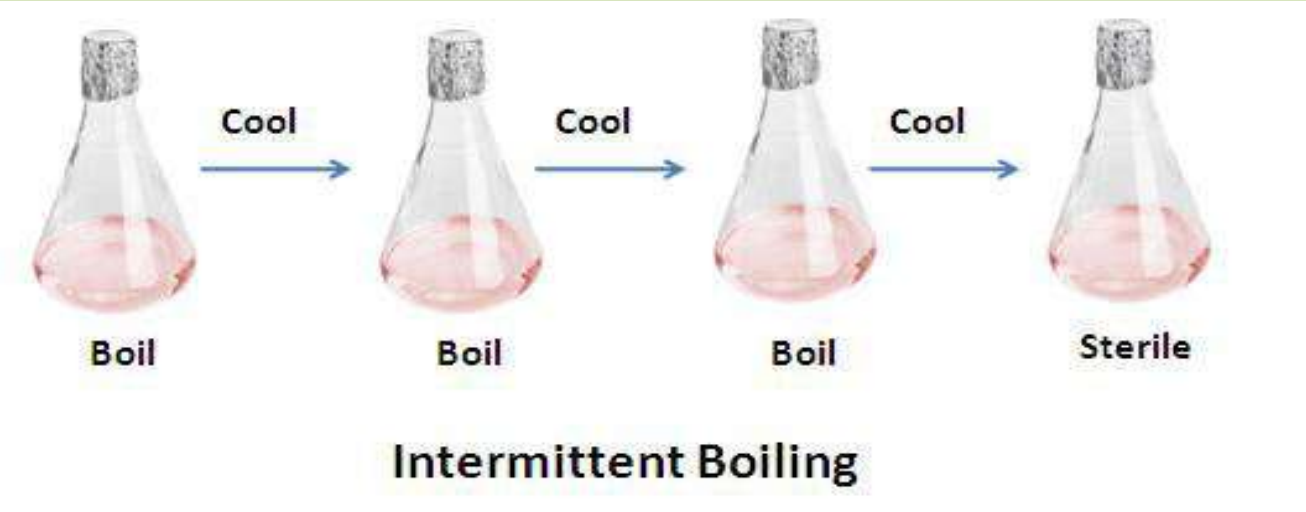
- Pasteur put broth into several special **S-shaped flasks**
- Each flask was boiled and placed at various locations.
- Did not turn cloudy
- Microbes not found
- Notice the dust that collected in the neck of the flask

The Theory of Biogenesis 1864



JOHN Tyndall theory (1820 - 1893).

- Support for Pasteur's findings
- He discovered highly resistant bacterial structure, later known as endospore.
- Prolonged boiling or intermittent heating was necessary to kill these spores, to make the infusion completely sterilized, a process known as Tyndallisation.



Germ Theory of Disease

- The germ theory of disease is the currently accepted scientific theory for many diseases.
- It states that microorganisms known as pathogens or "germs" can lead to disease.
- The theory that certain diseases are caused by the invasion of the body by microorganisms.
- This is based on three basic underlying principles.

1. Microbes can cause illnesses within the body.
2. Microbes can spread from one person to another.
3. A specific microbe exists for each illness which will always invoke the same illness.

Miasma theory
19th century

- The miasma theory was the predominant theory of disease transmission before the germ theory
- It is no longer accepted as a scientific theory of disease.
- It held that diseases such as cholera, chlamydia infection, or the Black Death were caused by a *miasma* ("pollution"), a noxious form of "bad air" emanating from rotting organic matter.

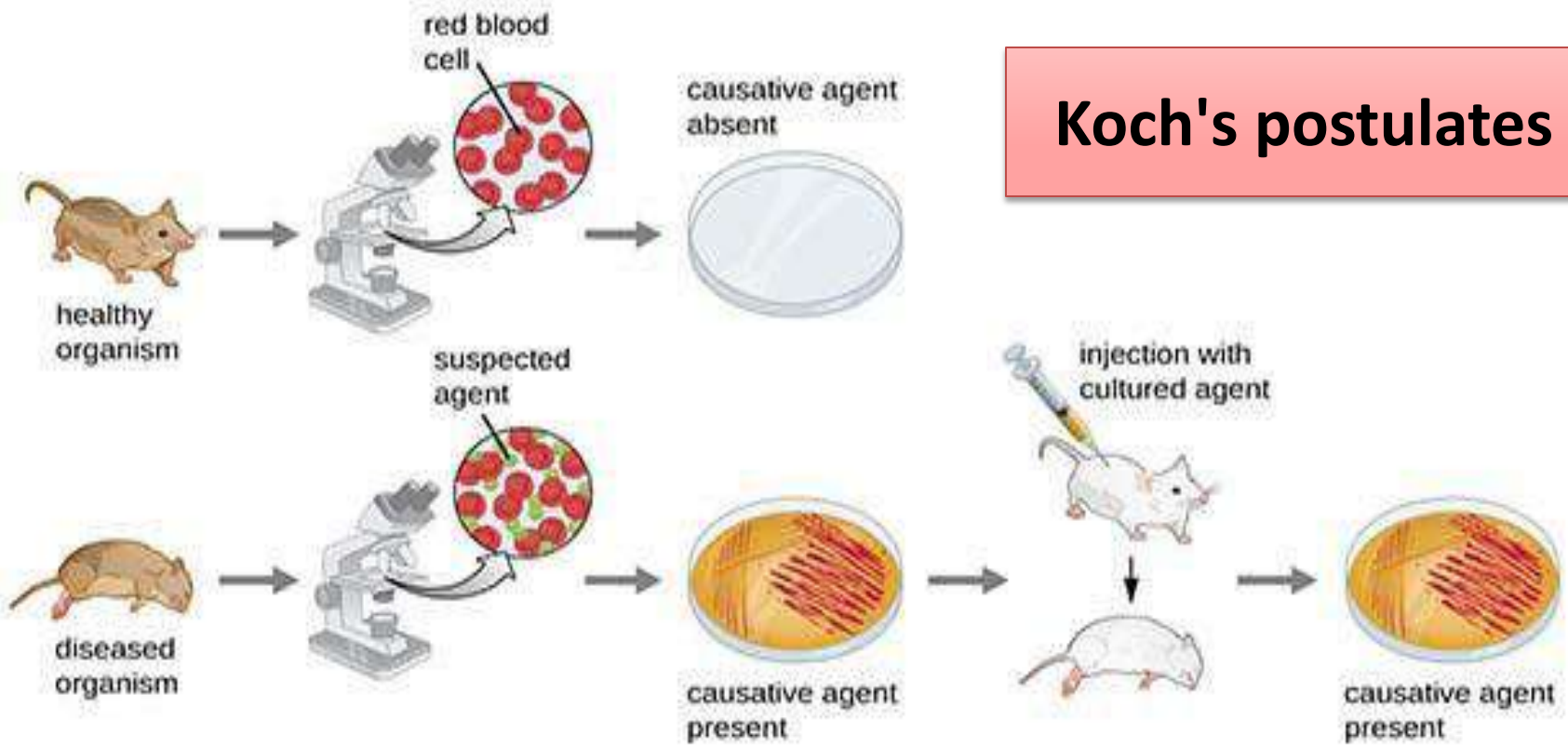
Louis Pasteur

- A French chemist who studied microbes in 1854.
- He noticed certain microbes cause food and drink to spoil but also that heat kill many of these microbes (pasteurization).
- He suggested that microbes (germs) could cause infectious diseases and were easily spread by people.
- He discovered the pathology of the puerperal fever and the pyogenic vibrio in the blood, and suggested using boric acid to kill these microorganisms before and after confinement.
- Pasteur discovered that another serious disease of silkworms, *pébrine*, was caused by a microscopic organism now known as *Nosema bombycis* (1870).

Robert Koch
1843-1910

- In 1876 he identified the microbe that caused anthrax, an infectious disease that was killing cows.
- He later identified the microbes that caused tuberculosis and cholera.
- Developed a way to prove that a specific microbe caused a particular disease
- He also developed agar, a gelatin like substance whish is used to grow microbe cultures in a petri dish

Koch's postulates



1

The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

2

The microorganism must be isolated from a diseased organism and grown in pure culture.

3

The cultured microorganism should cause disease when introduced into a healthy organism.

4

The microorganism must be re isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Exceptions to Koch's Postulates

- Microorganisms that are unable to be cultured on artificial media (example: *Treponema pallidum)*
- 2 or more organism work in synergy to cause a disease.
- Symptoms and diseases can be causes by any one of several microbes.

Early Discoveries

- **Lucretius**, a Roman philosopher (98-55 B.C.), and **Girolamo Fracastoro**, a physician (1478-1553) believed invisible creatures were responsible for disease
- **Franscesco Stelluti** observed bees and weevils using a microscope in the early 1600s
- **Antony van Leeuwenhoek** (1632 - 1723) was the first to report microorganisms (Royal Society) (Animalcules)
  - 50-300X magnification

Spontaneous Generation

The belief that life could originate from non-living or decomposing matter.

Supported by:

- **Aristotle** (384-322 BC) – Believed that simple invertebrates could arise by spontaneous generation.
- **John Needham** (1713-1781) – Concluded that bacteria originated from meat.
- **Lazarro Spallanzani** (1729-1799) - No growth in sealed flask after boiling, proposed that air was needed for growth of organisms.
- **Felix Archimede Pouchet** (1859) – Proved growth without contamination from air.

Spontaneous Generation

Disproved by:

- **Francesco Redi** (1626-1697) – maggot unable to grow on meat if meat was covered with gauze.
- **Schwann, Friedrich Schroder and von Dusch** (1830s) – Air allowed to enter flask but only after passing through a heated tube or sterile wool.
- **John Tyndall** (1820-1893) – Omission of dust → no growth. Demonstrated heat resistant forms of bacteria (endospores)
- **Louis Pasteur (1822 - 1895)**
  - trapped airborne organisms in cotton;
  - he also heated the necks of flasks, drawing them out into long curves, sterilized the media, and left the flasks open to the air;
  - no growth was observed because dust particles carrying organisms did not reach the medium, instead they were trapped in the neck of the flask; if the necks were broken, dust would settle and the organisms would grow; in this way Pasteur disproved the theory of spontaneous generation

Demonstrations that microorganisms cause disease

- **Agostino Bassi** (1773 - 1856) showed that a silkworm disease was caused by a fungus
- **M. J. Berkeley** (ca. 1845) demonstrated that the Great Potato Blight of Ireland was caused by a Fungus
- **Louis Pasteur** showed that the pébrine disease of silkworms was caused by a protozoan parasite.
- **Joseph Lister** (1827 - 1912)
  - developed a system of surgery designed to prevent microorganisms from entering wounds – phenol sprayed in air around surgical incision
  - Decreased number of post-operative infections in patients
  - his published findings (1867) transformed the practice of surgery

Demonstrations that microorganisms cause disease

- **Charles Chamberland** (1851 - 1908) identified viruses as disease-causing agents – Tobacco Mosaic Virus
- **Edward Jenner** (ca. 1798) used a vaccination procedure to protect individuals from smallpox
- **Louis Pasteur** developed other vaccines including those for chicken cholera, anthrax, and rabies
- **Ignaz Semmelweiss** (~1850) demonstrated that childbed fever (puerperal fever), caused by streptococcal infections, was transmitted to patients by doctor's hands
  - Pioneer of antisepsis in obstetrics
  - Women giving birth in hospitals by medical students and physicians were 4x more likely to contract puerperal fever compared to those by midwives

Demonstrations that microorganisms cause disease

- **Emil von Behring** (1854 - 1917) and **Shibasaburo Kitasato** (1852 - 1931) induced the formation of diphtheria tetanus antitoxins in rabbits which were effectively used to treat humans thus demonstrating humoral immunity
- **Elie Metchnikoff** (1845 - 1916) demonstrated the existence of phagocytic cells in the blood, thus demonstrating cell-mediated immunity
- **Robert Koch** (1843 - 1910)
  - using criteria developed by his teacher, Jacob Henle (1809-1895), established the relationship between *Bacillus anthracis* and anthrax;
  - his criteria became known as Koch's Postulates and are still used to establish the link between a particular microorganism and a particular disease:

Development of Culture Media

- **Fannie Hesse**, the wife of one of Koch's assistants, proposed using agar
  - Not digested by most bacteria
  - Melts at 100 degrees Celcius
  - Used today - ~2% in solid media
- **Richard Petri**, another of Koch's assistants, developed the Petri dish

Development of Vaccines

- **Edward Jenner** in 1796 discovered that cowpox (vaccinia) induced protection against human smallpox
  - Called procedure vaccination

- **Pasteur** and **Roux** reported that incubating cultures longer than normal in the lab resulted in ATTENUATED bacteria that could no longer cause disease
  - Working with chicken cholera (caused by Pasteurella multocida), they noticed that animals injected with attenuated cultures were resistant to the disease

Development of Vaccines

- **Pasteur** and **Chamberland** developed other vaccines:
  - Attenuated anthrax vaccine
    - Chemical and heat treatment (potassium bichromate)
  - Attenuated rabies vaccine
    - Propagated the virus in rabbit following injection of infected brain and spinal cord extracts
- Passive immunization work by **Emil von Behring** (1845-1917) and **Shibasaburo Kitasato** (1852-1931)
  - Antibodies raised to inactivated diphtheria toxin by injection different host (rabbit) with the toxin (a toxoid form)
    - Antiserum recovered
      - Contains antibodies specific for the toxin
      - Protection from disease when injected nonimmune subject

How Microorganisms Affect Their Environment

- **Louis Pasteur**
  - demonstrated that alcoholic fermentations were the result of microbial activity,
  - that some organisms could decrease alcohol yield and sour the product, and
  - that some fermentations were aerobic and some anaerobic;
  - he also developed the process of pasteurization to preserve wine during storage
- **Sergei Winogradsky** (1856 - 1953)
  - worked with soil bacteria and discovered that they could oxidize iron, sulfur, and ammonia to obtain energy;
  - he also studied anaerobic nitrogen-fixation and cellulose decomposition
- **Martinus Beijerinck** (1851 - 1931) isolated aerobic nitrogen-fixing soil bacteria (Azotobacter and Rhizobium) and sulfate reducing Bacteria
- **Beijerinck** and **Winogradsky** pioneered the use of enrichment cultures and selective media

Microorganisms in the 20th Century

- **George W. Beadle** and **Edward L. Tatum** (ca. 1941)
  - studied the relationship between genes and enzymes using the bread mold, *Neurospora*
  - Precursor→ ornithine → citrulline → arginine
  - One gene, one polypeptide hypothesis
- **Salvadore Luria** and **Max Delbruck** (ca. 1943) demonstrated spontaneous gene mutations in bacteria (not directed by the environment)
- **Oswald T. Avery, Colin M. MacLeod**, and **Maclyn McCarty** (1944)
  - Following initial studies by Frederick Griffith (1928) they provided evidence that deoxyribonucleic acid (DNA) was the genetic material and carried genetic information during transformation
  - Worked with Streptococcus pneumoniae (rough and smooth)
  - In the 1970s new discoveries in microbiology led to the development of recombinant DNA technology and genetic engineering

Communication Skills (MSC-108)

Lecture 2- Technical writing skills

by Dr. Dipanjana Ghosh

Date 22 Jan 2022

TOPICS TO BE COVERED

Technical Writing Skills:

- Types of reports
- Layout of a formal report
- Scientific writing skills
- Importance of communicating Science
- Problems while writing a scientific document
- Plagiarism
- Scientific Publication Writing
- Elements of a Scientific paper including Abstract, Introduction, Materials & Methods, Results, Discussion, References; Drafting titles and framing abstracts

Technical Writing Skills

Types of Scientific reports

1. Original article
2. Case report
3. Technical note
4. Pictorial essay
5. Review
6. Commentary
7. Editorial
8. Letter to the editor

General Layout of a formal report

1. Title
2. Abstract
2. Introduction
3. Materials & Methods
4. Results
5. Discussion
6. Conclusion (in some types of reports)

Technical Writing Skills

Layout of a formal report (by types)

Original Article

- This is the most important type of paper.
- It provides new information based on original research.
- This category of paper is usually prospective and is supported by in-depth statistical analysis.
- The conclusions should be supported by the data provided in the results.

Case Report

- This is a description of a single case with unique features.
- These unique features may consist of previously unreported observation of a recognised disease
- The unique use of imaging or diagnostic test to reveal a disease, previously unreported clinical condition, previously unreported treatment in a recognised disease, or previously unreported complication of a procedure.
- Case Reports are usually short and focused.

Singapore Med J. 2008 Jul;49(7):522-5.

Layout of a formal report (Contd.)

Technical Note

- Also known as Technical Innovation
- This type of article is a description of a specific technique or procedure, modification of an existing technique, or new equipment applicable to a branch of medicine.
- Discussion is limited to the specific message. There is often a prescribed limit to the number of figures and references.

Pictorial Essay

- This is a teaching article that relies on the quality of its images.
- The text is usually limited with much of the message contained in the figure legends.
- The message should however be current and practical, and does not introduce new information.
- Emphasis is placed on the teaching value of the article.

Singapore Med J. 2008 Jul;49(7):522-5.

Layout of a formal report (Contd.)

Review

- This is a detailed analysis of recent developments on a specific topic.
- It serves to highlight important points that have been previously reported in the literature.
- This type of paper does not introduce new information and does not include the author's opinion or personal experience.
- A large number of relevant references are expected.

Singapore Med J. 2008 Jul;49(7):522-5.

Layout of a formal report (Contd.)

Commentary

- This is a short article that describes an author's personal experience of a specific topic.
- The subject may be controversial and the author's perspective is provided.
- This type of paper does not introduce new information, and should outline the various viewpoints that exist.
- It may be based on a current hot topic or may be commissioned to accompany an original paper on the same topic.
- The number of references and illustrations should be limited to support the author's opinion.

Singapore Med J. 2008 Jul;49(7):522-5.

Layout of a formal report (Contd.)

Editorial

- This may take several forms, most often: a short review or critique of original articles accepted for publication in the same issue of the journal, a brief description of a subject that does not warrant a full review, or serve to draw attention to very recent innovations or subjects of general interest to readers.
- The number and types of editorials vary according to the editorial policy.
- Editorials are invited by the editor or written by the editor.

Singapore Med J. 2008 Jul;49(7):522-5.

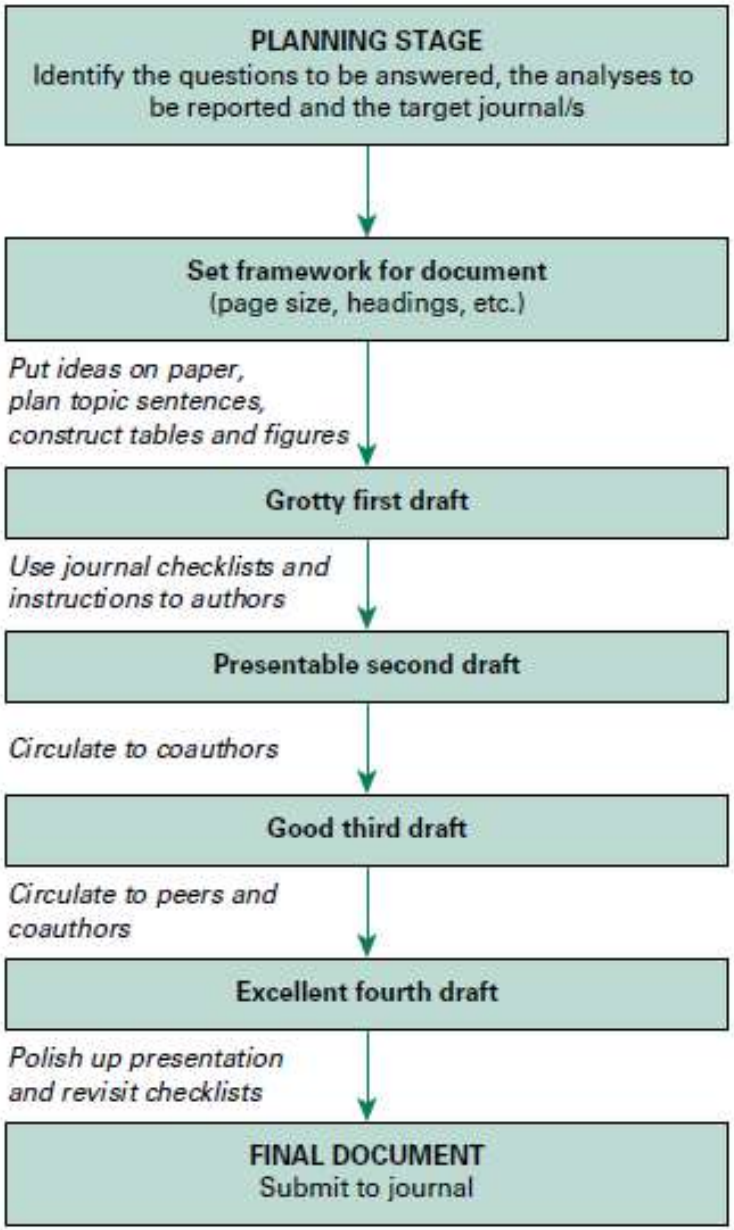
Layout of a formal report (Contd.)

Letter to the Editor

- Many journals have a Letters or Correspondence section.
- Letters are usually short and can be written on any subject of interest to the journal reader, including comments on previously -published articles.
- These comments should be objective and constructive.
- Authors of previously published articles commented on by the letter writer, are usually invited to make a written response (Author's Reply to Letter).
- This section may sometimes also be used for floating new hypotheses, and for drawing readers' attention to important hazards and points of interest or relevance to clinical practice.

Singapore Med J. 2008 Jul;49(7):522-5.

Scientific writing skills (Scientific Publication Writing)



Drafting titles and framing abstracts

Scientific writing, by Jennifer Peat, BMJ Books

Importance of communicating Science

- A scientific discovery is only as good as its communication; the key is to accommodate the multiple communication paths from that discovery.
- Scientists doing the work can see more connections themselves when they organize data in a different way.
- Colleagues will more easily replicate experiments.
- Technical writers would have more context when writing up the research.
- Reviewers can see more of the scientists' thought processes and paths during the peer-review process.
- Editors will better understand the papers' fit for the industry and publications.
- Journalists will present the research more accurately and provide a broader base.
- Readers—those thousands of readers that can be reached—will more easily comprehend, contextualize, and then build upon and spread the science.

Abraham, G. The Importance of Science Communication. Metallogr. Microstruct. Anal. 9, 3–4 (2020).

Problems while writing a scientific document

Everyone Struggles

- Writing a research manuscript is difficult on many levels.
- The structure of a scientific manuscript differs from undergraduate writing, and this structure takes time to learn.
- Data analysis can be challenging, particularly when results between studies are slightly inconsistent or if your current results show patterns that differ from patterns reported in the literature.
- Citing the work of others is also a challenge; knowing which articles are the most appropriate to reference in your given field requires experience.
- Finally, identifying your unique contribution to the literature can be challenging given all the previous research likely done on topics related to your manuscript.

Embrace Criticism

- Science improves through critical review

- Criticism is so important for improving one's writing, and there are many opportunities to seek out reviews from peers.

The Difficulties of Scientific Writing. Michael W. Kraus, 2009

Plagiarism

- Plagiarism is presenting someone else's work or ideas as your own, with or without their consent, by incorporating it into your work without full acknowledgement.
- All published and unpublished material, whether in manuscript, printed or electronic form, is covered under this definition.
- Plagiarism may be intentional or reckless, or unintentional.
- Under the regulations for examinations, intentional or reckless plagiarism is a disciplinary offence.
- Plagiarism is common and threatens the integrity of the scientific literature.
- Most manuscripts with plagiarized material were submitted from countries in which English was not an official language.

https://www.ox.ac.uk/students/academic/guidance/skills/plagiarism

Plagiarism (Contd.)

Why should you avoid plagiarism

- You have come to university to learn to know and speak your own mind, not merely to reproduce the opinions of others - at least not without attribution.
- At first it may seem very difficult to develop your own views, and you will probably find yourself paraphrasing the writings of others as you attempt to understand and assimilate their arguments.
- However it is important that you learn to develop your own voice.
- You are not necessarily expected to become an original thinker, but you are expected to be an independent one - by learning to assess critically the work of others, weigh up differing arguments and draw your own conclusions.
- Students who plagiarise undermine the ethos of academic scholarship while avoiding an essential part of the learning process.

https://www.ox.ac.uk/students/academic/guidance/skills/plagiarism

Elements of a Scientific paper including Abstract, Introduction, Materials & Methods, Results, Discussion, References

Section	Question to be answered	Purpose	Expected length with A4 paper, font size 10–12 and 1·5 line spacing
Introduction	Why did you start?	Summarise the context of your study and state the aims clearly	1 page
Methods	What did you do?	Give enough detail for the study to be repeated	2–3 pages
Results	What did you find?	Describe the study sample and use the data analyses to answer the aims	2–3 pages
Tables and figures	What do the results show?	Clarify the results	3–6 tables or figures
Discussion	What does it mean?	Interpret your findings in context of other literature and describe their potential impact on health care	2–3 pages
References	Who else has done important work in your field?	Cite the most relevant and most recent literature	20–35 references
Total document			**12–20 pages**

Scientific writing, by Jennifer Peat, BMJ Books

Elements of a Scientific paper

Authorship

Responsibilities of authors and coauthors

First author

Takes primary responsibility for all aspects of publishing the paper
Conducts or supervises the data analyses and interprets the results
Writes the paper in consultation with coauthors
Maintains ownership of the master document
Submits the paper to a journal and deals with the correspondence
Responsible for archiving and documenting all data and files

Coauthors

Make early decisions about the aims of the paper
Keep the paper on track in terms of the main messages
Make intellectual contributions to the data analyses
Contribute to the interpretation of the results
Review each draft
Take public responsibility for the content and results

Scientific writing, by Jennifer Peat, BMJ Books

References

- *Singapore Med J. 2008 Jul;49(7):522-5.*
- *Scientific writing, by Jennifer Peat, BMJ Books*
- *Abraham, G. The Importance of Science Communication. Metallogr. Microstruct. Anal. 9, 3–4 (2020).*
- *The Difficulties of Scientific Writing. Michael W. Kraus, 2009*
- *https://www.ox.ac.uk/students/academic/guidance/skills/plagiarism*

CULTURE MEDIA
&
CULTURE METHODS

By: Dr. Kamal Uddin Zaidi

Need for Culture media:

- Bacteria: mixed population in nature
- By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure culture for study
- Medium → Nutrients → support growth

Liquid medium:

- Diffused growth
- No characteristics for identification
- Difficult to isolate
- Earliest liquid medium: urine or meat broth used by Louis Pasteur

Solid medium:

- Distinct colony morphology
- Characteristics → easy to identify
- ***Colony*** – macroscopically visible collection of millions of bacteria originating from a single bacterial cell

- Earliest solid medium:
  Cooked cut potato by Robert Koch
- Gelatin - not satisfactory
  - liquefy at 24°C

Agar

- Frau Hesse
- Universally used for preparing solid medium
- Obtained from seaweed: *Gelidium* ⟶
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98°C & sets at 42°C
- 2% agar is employed in solid medium

Types of culture media

I. Based on their consistency
   a) Solid medium
   b) Liquid medium
   c) Semi solid medium
II. Based on the constituents/ ingredients
   a) Simple medium
   b) Complex medium
   c) Synthetic or defined medium
   d) Special media

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

III. Based on Oxygen requirement

- Aerobic media
- Anaerobic media

Solid media – contains 2% agar

- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

Liquid media – no agar.

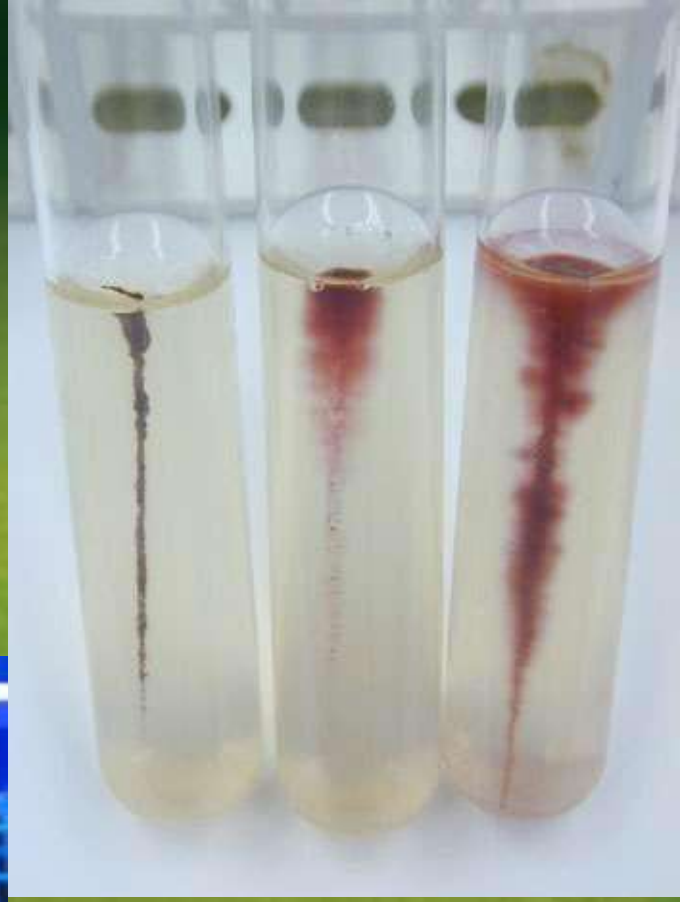
- For inoculum preparation, Blood culture, continuous culture.
- Eg: Nutrient broth

Semi solid medium – 0.5% agar.

- Eg: Motility medium

Solid medium

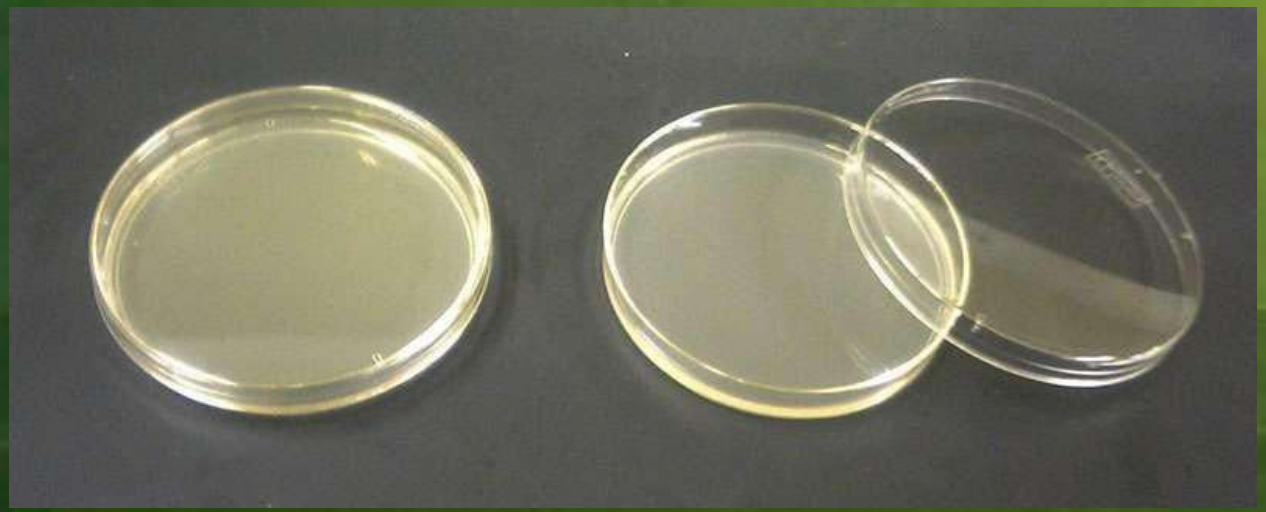
Liquid medium

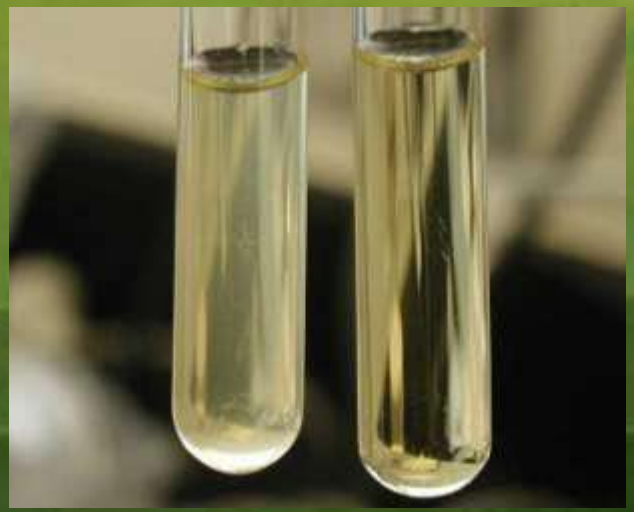


Semi-solid medium

Simple media / basal media:

- Most common in routine diagnostic laboratories
  Eg: Nutrient Broth, Nutrient Agar
- NB consists of peptone, meat extract, NaCl, water
- NB + 0.5% Glucose = Glucose Broth
- NB + 2% agar = Nutrient agar
- Agar conc. Reduced (0.2 - 0.5%) = Semi-solid medium





Complex media

- Media other than basal media.
- They have added complex ingredients such as yeast extract or casein hydrolysate, which consist of a mixture of many chemical species in unknown proportions
- Provide special nutrients

Synthetic or defined media

- Media prepared from pure chemical substances
- exact composition is known
- Used for special studies, eg. metabolic requirements
- Eg: Dubo's medium with Tween 80.

Enriched media

- Substances like blood, serum, egg are added to the basal medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- Eg: Blood agar, Chocolate agar and Loeffler's serum slope

←Blood agar

Chocolate agar →

Enrichment media

- Liquid media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium
  Inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism → increase in numbers of desired bacteria
- Eg:
  Selenite F Broth – for the isolation of *Salmonella*, *Shigella*
  Tetrathionate Broth – inhibit coliforms
  Alkaline Peptone Water – for *Vibrio cholerae*

Selenite F Broth

Tetrathionate Broth

Alkaline Peptone water

Selective media

- The Selective Agent /Inhibitory substance is added to a solid media

•Increase in number of colonies of desired bacterium

Eg:

- Deoxycholate citrate medium for dysentery bacilli
- Mac Conkey's medium for gram negative bacteria
- TCBS (Thiosulfate-citrate-bile salts-sucrose) for *V. cholerae.*
- LJ Medium (Lowenstein-Jensen) for M. Tuberculosis

Mac Conkey's medium

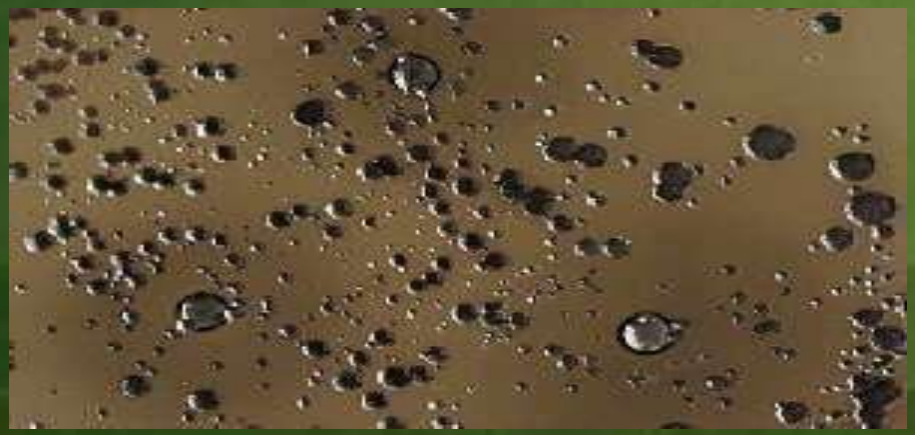
Thiosulfate citrate bile salts sucrose (TCBS) agar

Deoxycholate citrate agar

LJ media

Indicator media

- contain an indicator which changes its colour when a bacterium grows in them
- Eg:
  Wilson-Blair medium – *S. typhi* forms black colonies.
  (Bismuth sulphite and Brilliant green make this medium highly selective for salmonellae.)
  McLeod's medium (Potassium tellurite)– Diphtheria bacilli.



Wilson-Blair Medium

McLeod's medium

Urease producing bacteria

↓

Urease

↓

Urea → $CO_2 + NH_3$

↓

NH_3 → Medium turns pink

Urease medium

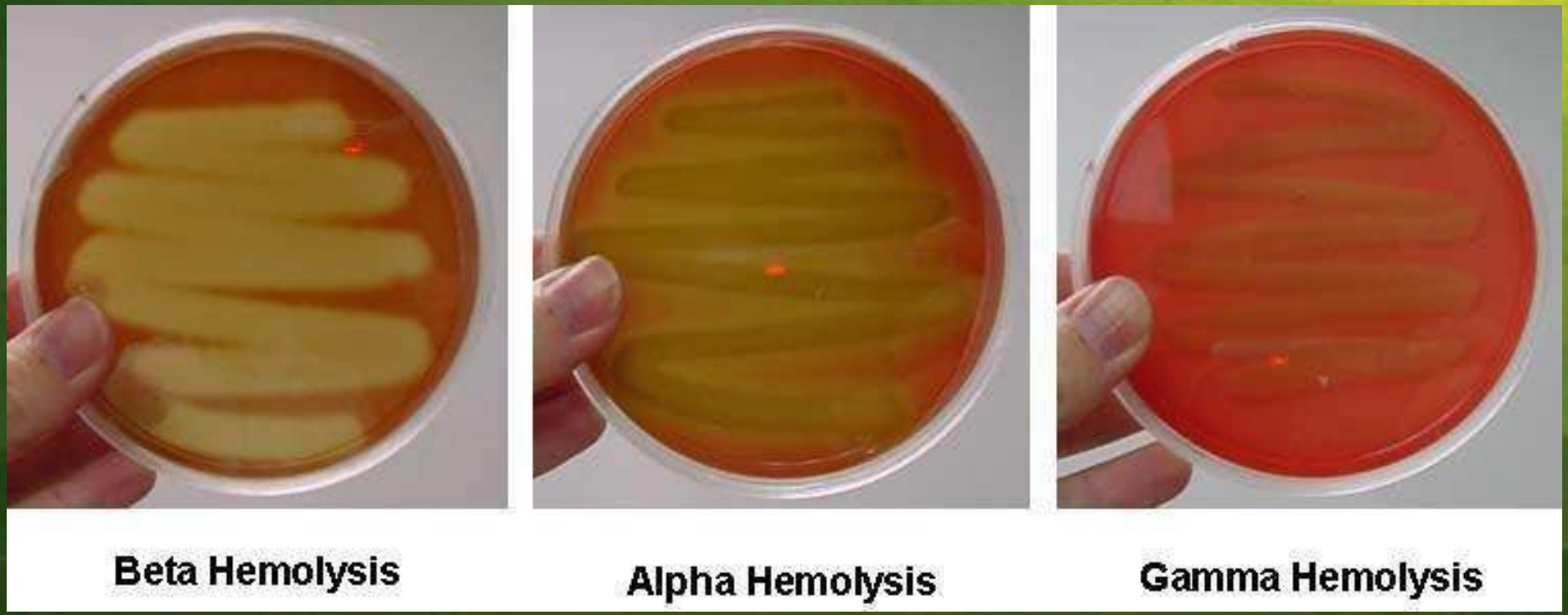
Blood agar:

shows three types of Hemolysis

α Hemolysis

β Hemolysis

γ Hemolysis



Differential media

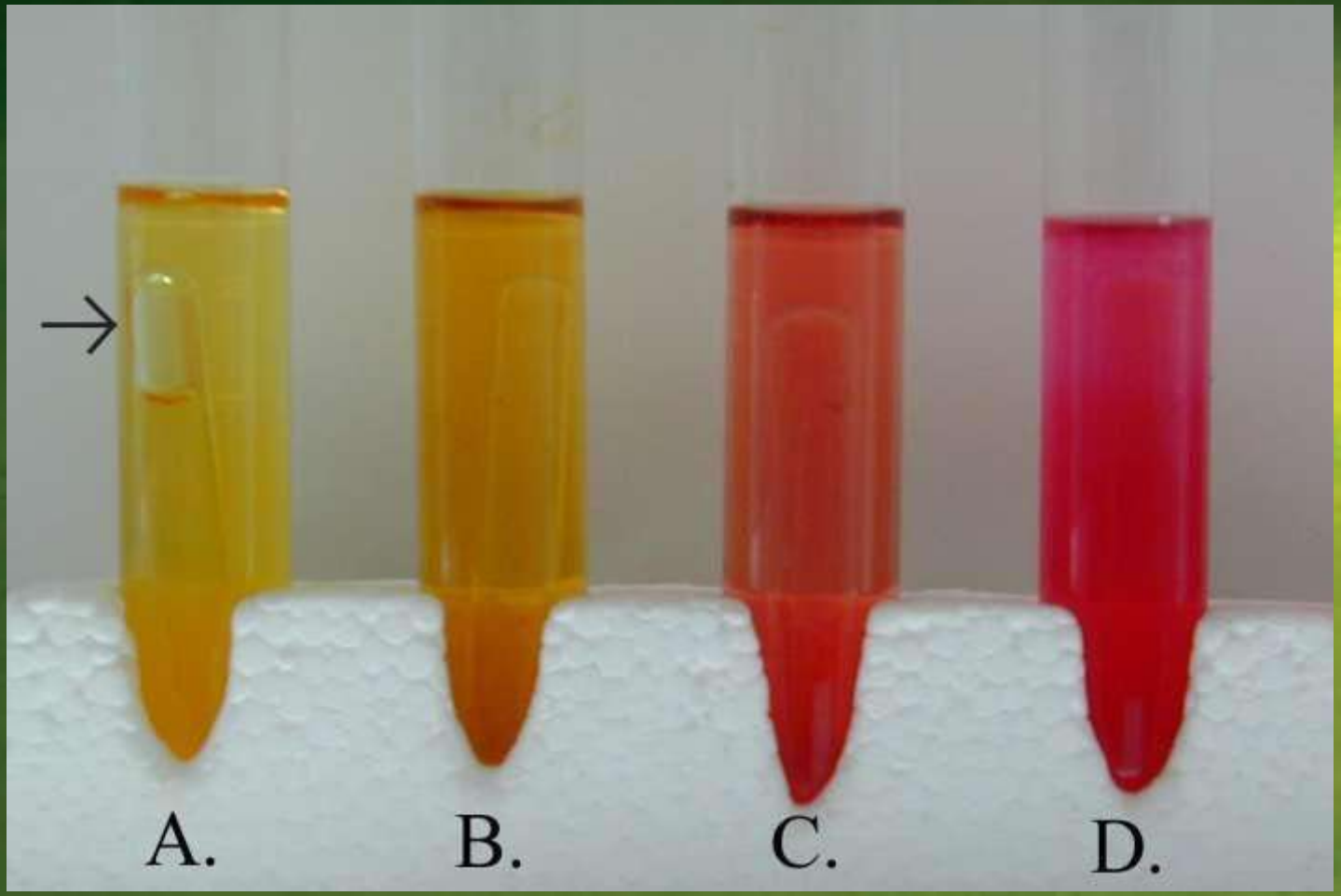
- Substances incorporated in the medium it permit to distinguish between bacteria.
- Eg: Mac Conkey's medium
  - Peptone
  - Lactose
  - Agar
  - Neutral red
  - Taurocholate
- Distinguish between lactose fermenters & non lactose fermenters.

MacConkey agar:

- Lactose fermenters – **Pink** colonies
- Non lactose fermenters – colourless colonies

Sugar media

- Media containing any fermentable substance
- Eg: glucose, arabinose, lactose, starch etc.
- Media consists:
  1% of the sugar in peptone water + Indicator
- Contain a small tube (Durham's tube) for the detection of gas production by the bacteria


A.
B.
C.
D.

Transport media

- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
  - Stuart's medium –is a non nutrient soft agar gel containing a reducing agent to prevent oxidation and charcoal to neutralize bacterial inhibitor.
    used for organism such as : *Gonnococci*
  - Buffered glycerol saline – enteric bacilli


LOT
LOT
UTM-RT

Anaerobic media

- These media are used to grow anaerobic organisms.
- Eg: Robertson's cooked meat medium, Thioglycolate medium.

CULTURE METHODS

Culture methods employed depend on the purpose for which they are intended.

Purposes:

- To isolate bacteria in pure cultures.
- To demonstrate their properties.
- To obtain sufficient growth for the preparation of antigens and for other tests.
- For bacteriophage and bacteriocin susceptibility.
- To determine sensitivity to antibiotics.
- To estimate viable counts.
- Maintain stock cultures.

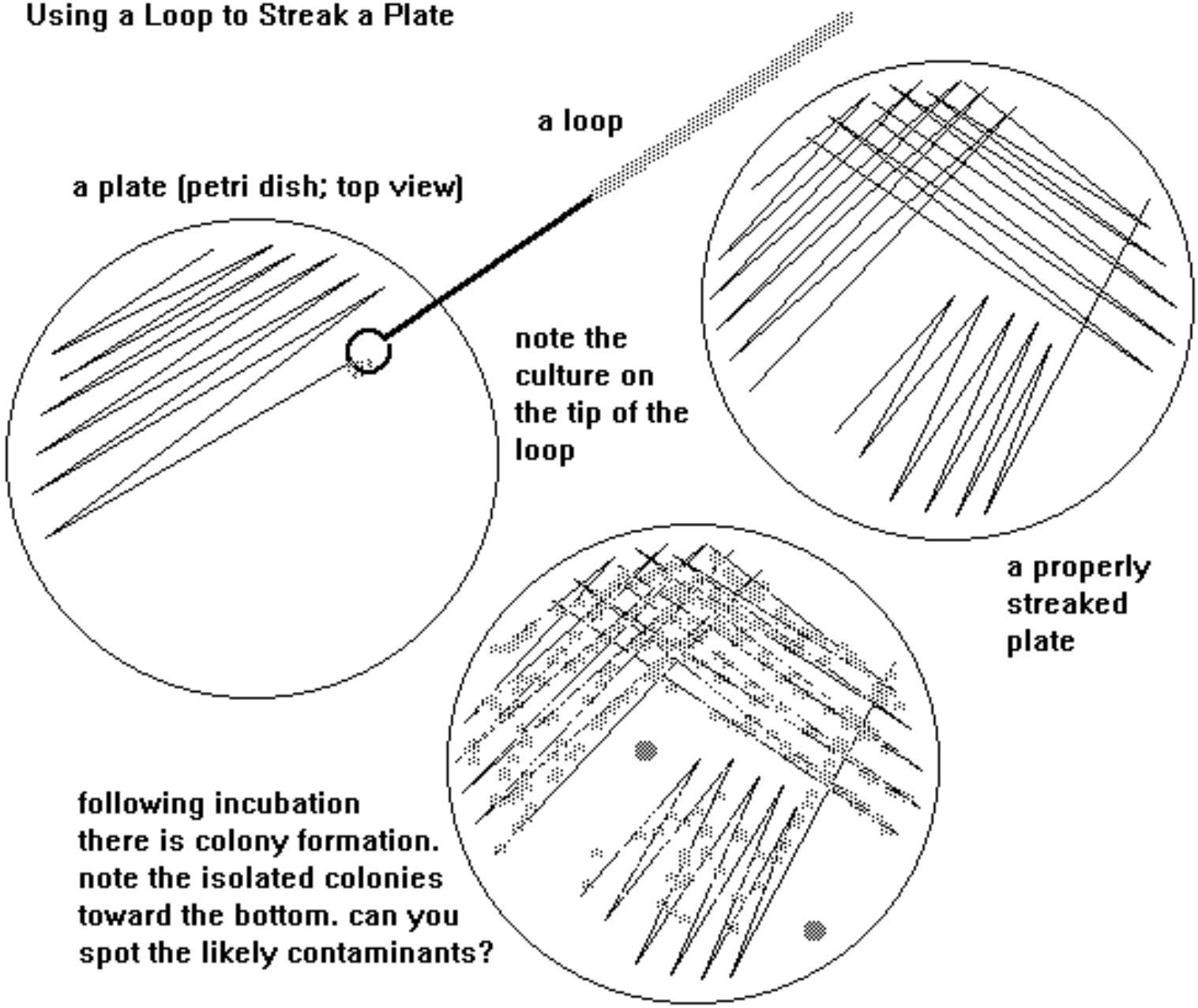
Culture methods include:

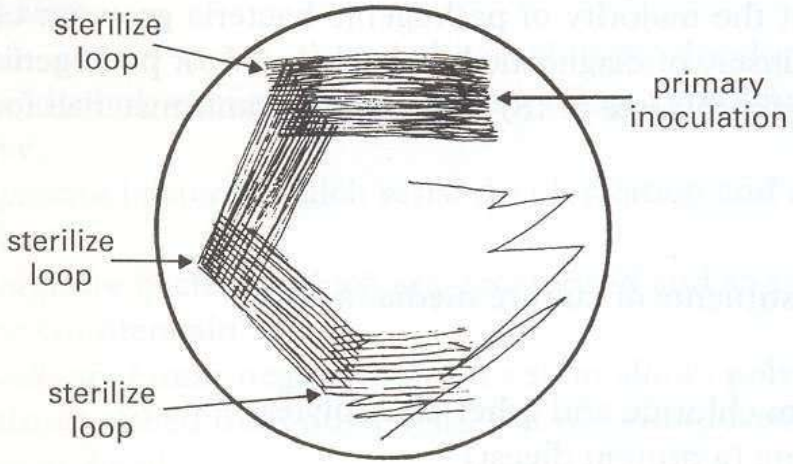
- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method
- Liquid culture
- Anaerobic culture methods

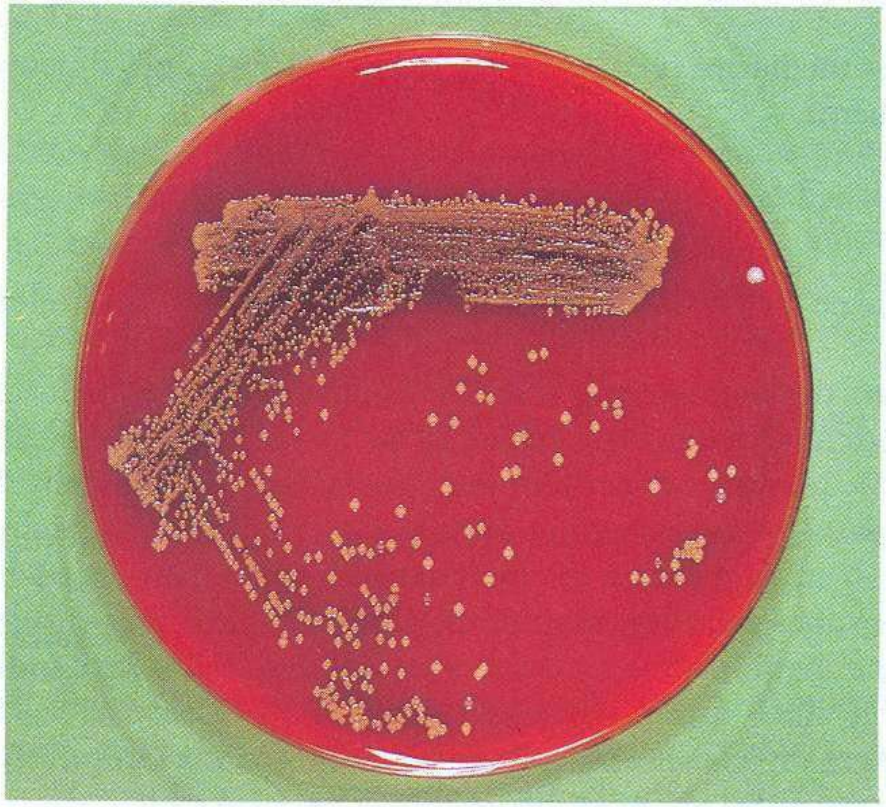
STREAK CULTURE

- Used for the isolation of bacteria in pure culture from clinical specimens.
- Platinum wire or Nichrome wire is used.
- One loopful of the specimen is transferred onto the surface of a well dried plate.
- Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks.

Using a Loop to Streak a Plate




sterilize loop
primary inoculation
sterilize loop
sterilize loop



LAWN CULTURE

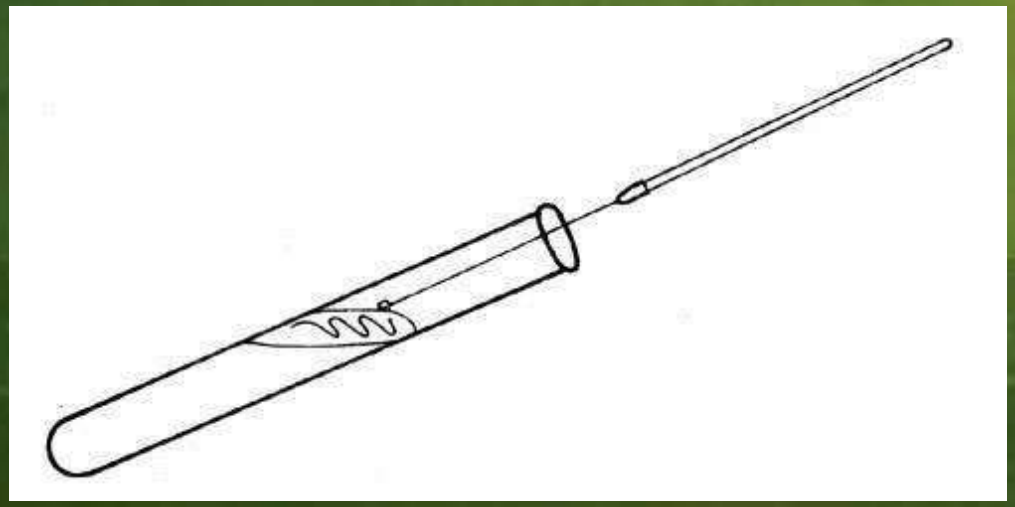
- Provides a uniform surface growth of the bacterium.
- Uses
  - For bacteriophage typing.
  - Antibiotic sensitivity testing.
  - In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.

Antibiotic sensitivity testing

STROKE CULTURE

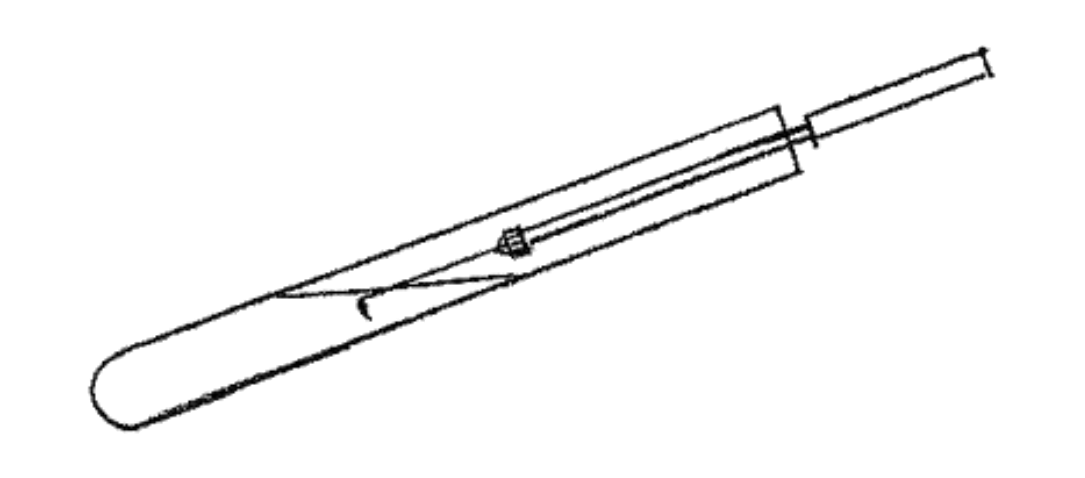
- Stroke culture is made in tubes containing agar slope / slant.

- Uses
  - Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.



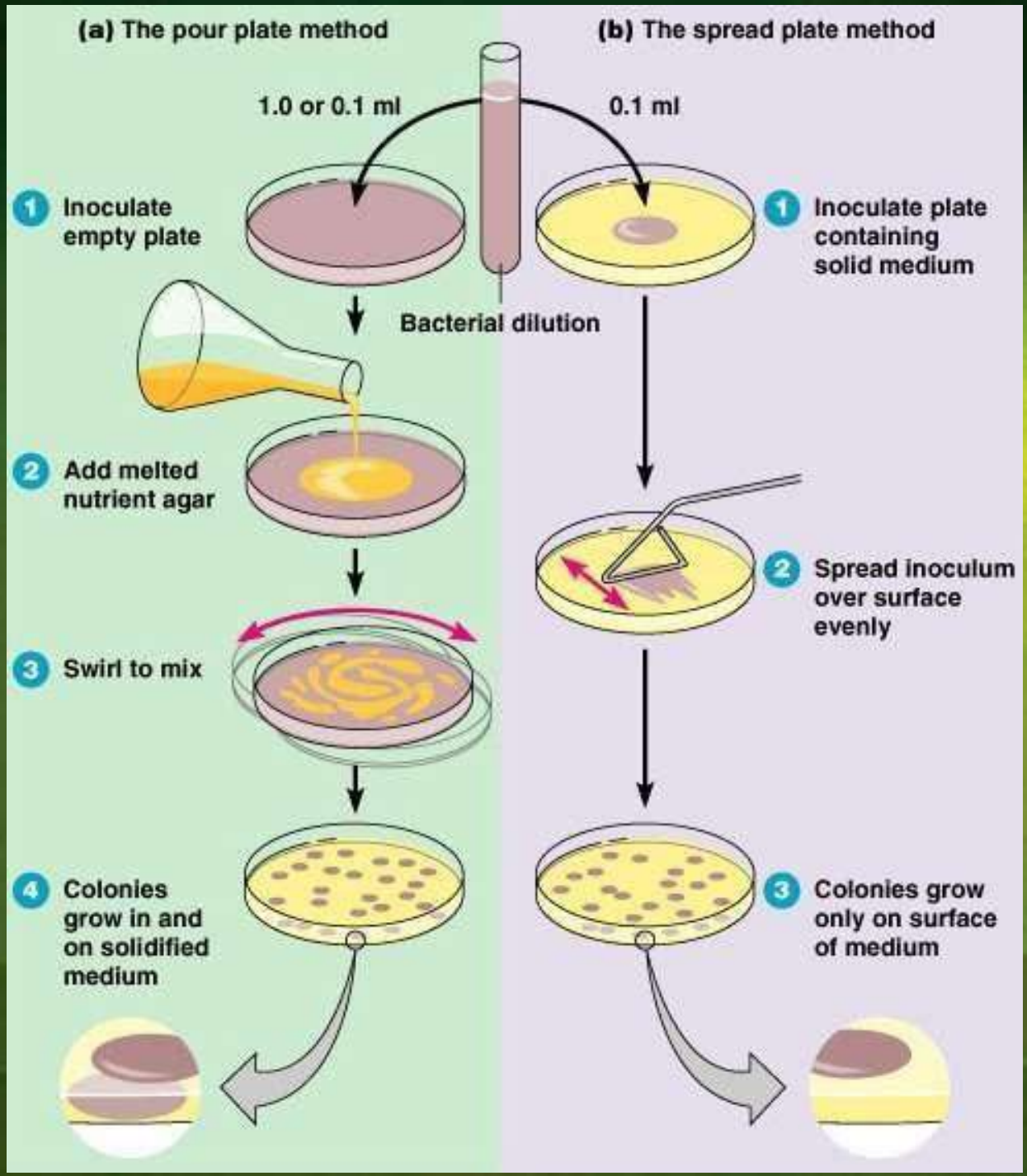
STAB CULTURE

- Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.
- Uses
  - Demonstration of gelatin liquefaction.
  - Oxygen requirements of the bacterium under study.
  - Maintenance of stock cultures.



POUR PLATE CULTURE

- Agar medium is melted (15 ml) and cooled to 45^{o}C.
- 1 ml of the inoculum is added to the molten agar.
- Mix well and pour to a sterile petri dish.
- Allow it to set.
- Incubate at 37^{o}C, colonies will be distributed throughout the depth of the medium.
- Uses
  - Gives an estimate of the viable bacterial count in a suspension.
  - For the quantitative urine cultures.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
1 Inoculate plate containing solid medium
Bacterial dilution
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

LIQUID CULTURES

- Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.
- Uses
  - Blood culture
  - Sterility tests
  - Continuous culture methods
- Disadvantage
  - It does not provide a pure culture from mixed inocula.

Blood culture bottles

ANAEROBIC CULTURE METHODS

- Anaerobic bacteria differ in their requirement and sensitivity to oxygen.
- *C. tetani* is a strict anaerobe
- **Methods:**
  - Production of vacuum
  - Displacement of oxygen with other gases
  - Chemical method
  - Biological method
  - Reduction of medium

Production of vacuum:

- Incubate the cultures in a vacuum desiccators.

Displacement of oxygen with other gases

- Displacement of oxygen with hydrogen, nitrogen, helium or CO_2.
- Eg: Candle jar

Chemical method

- Alkaline pyrogallol absorbs oxygen.

(Sodium Hydroxide and pyrogallol)

- Chromium and Sulphuric acid

McIntosh – Fildes' anaerobic jar

- Consists of a metal jar or glass jar with a metal lid which can be clamped air tight.
- The lid has 2 tubes – gas inlet and gas outlet
- The lid has two terminals – connected to electrical supply.
- Under the lid – small grooved porcelain spool, wrapped with a layer of palladinised asbestos.

Working:

- Inoculated plates are placed inside the jar and the lid clamped air tight.
- The outlet tube is connected to a vacuum pump and the air inside is evacuated.
- The outlet tap is then closed and the inlet tube is connected to a hydrogen supply.
- After the jar is filled with hydrogen, the electric terminals are connected to a current supply, so that the palladinised asbestos is heated.
- Act as a catalyst for the combination of hydrogen with residual oxygen.

Gaspak

- Commercially available disposable envelope.
- Contains chemicals which generate H_2 and CO_2 on addition of water.
- Cold catalyst – permits combination of Hydrogen and Oxygen
- Indicator is used – reduced methylene blue.
  - Colourless – anaerobically
  - Blue colour – on exposure to oxygen


methylene blue
indicator strip
anaerobe
jar
GasPak
BBL
GasPak Plus

Biological method

- Absorption of oxygen by incubation with aerobic bacteria, germinating seeds or chopped vegetables.

Reduction of oxygen

- By using reducing agents – 1% glucose, 0.1% Thioglycolate

Bibliography:

Ananthnarayan and Paniker's Textbook of Microbiology
C.P Baveja Textbook of Microbiology

Replica plating method (Lederberg, 1952)

- ✓ Colonies of bacteria were transferred from master plate, on to a number of other plates, using a velvet template.
- ✓ Relative position of all the colonies was retained.

- ✓ By replica plating on culture plates with and without bacteriophages, they were able to demonstrate that bacteriophage resistant mutants appeared without ever having had contact with the selective agent.

- ✓ This technique is used to detect auxotrophic mutants and wild type strains on the basis of ability to grow in the absence of amino acids.

- ✓ Also this test is used to demonstrate the presence of antibiotic resistance in bacterial cultures prior to exposure of antibiotic.

Procedure

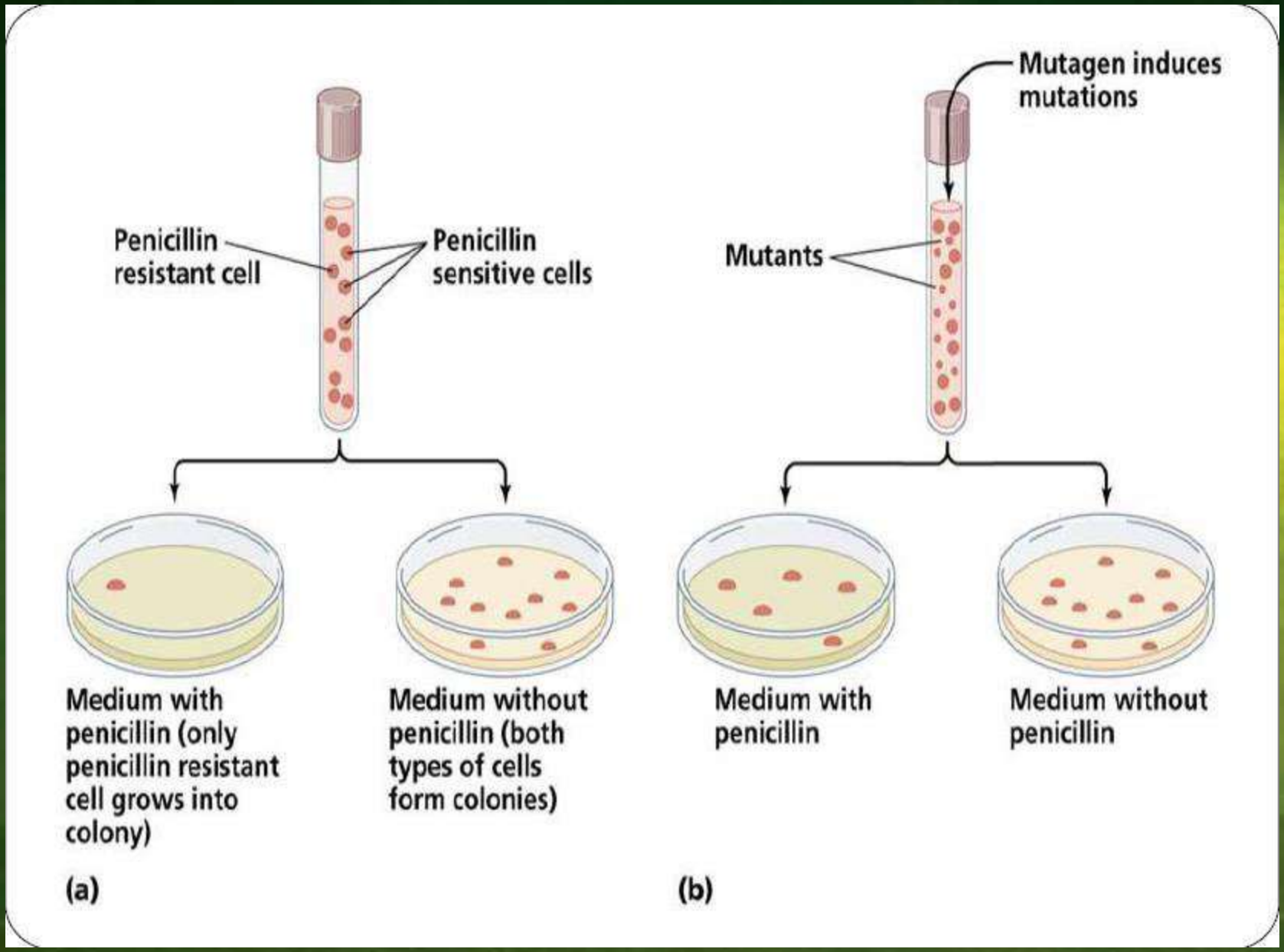
- ✓ Generate the mutants by treating a culture with a mutagen e.g. nitrosoguanidine.
- ✓ Inoculate a plate containing complete growth medium and incubate it at proper temperature.
- ✓ Both wild type and mutant survivors will from complete medium.
- ✓ This plate containing complete medium is called master plate.

- ✓ Prepare a piece of sterile velvet and gently on the upper surface of the master plate to pick up bacterial cell from each colony.
- ✓ As pressed the master plate, again gently press the velvet on the replica plates containing complete medium.
- ✓ Thus, the bacterial cells are transferred in replica plates in the same position as in master plate.

- ✓ Incubate the plates and compare the replica plate with master plate for bacterial colony not growing on replica plate.

Positive Selection

- Positive selection entails growing the culture on a medium that will allow for the growth of only the mutant colonies.
- If, for example, we want to find mutants that resistant to penicillin, we grow the culture on a medium that contains penicillin. Only those colonies that are resistant to penicillin will grow and we can identify them directly.


Penicillin resistant cell
Penicillin sensitive cells
Medium with penicillin (only penicillin resistant cell grows into colony)
Medium without penicillin (both types of cells form colonies)
(a)
Mutagen induces mutations
Mutants
Medium with penicillin
Medium without penicillin
(b)

Negative Selection:

Negative selection is used to identify mutants that have lost the ability to perform a certain function that their parents had.

Auxotrophic mutants, for example, are bacteria that have lost the ability to synthesize an essential nutrient.

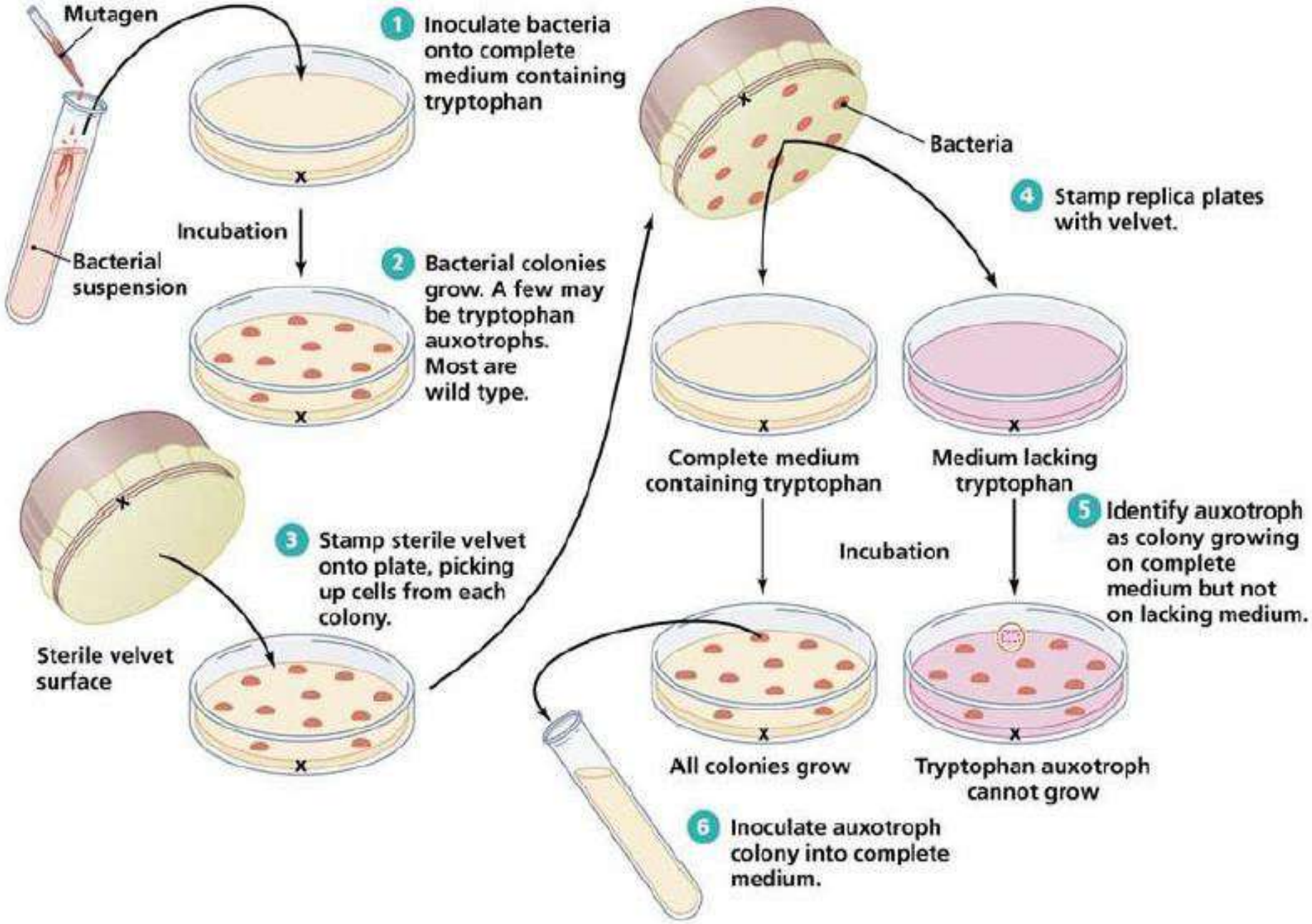
- Inoculate a tryptophan enriched medium with bacteria. Incubate so that cells can form colonies. This is the master plate.

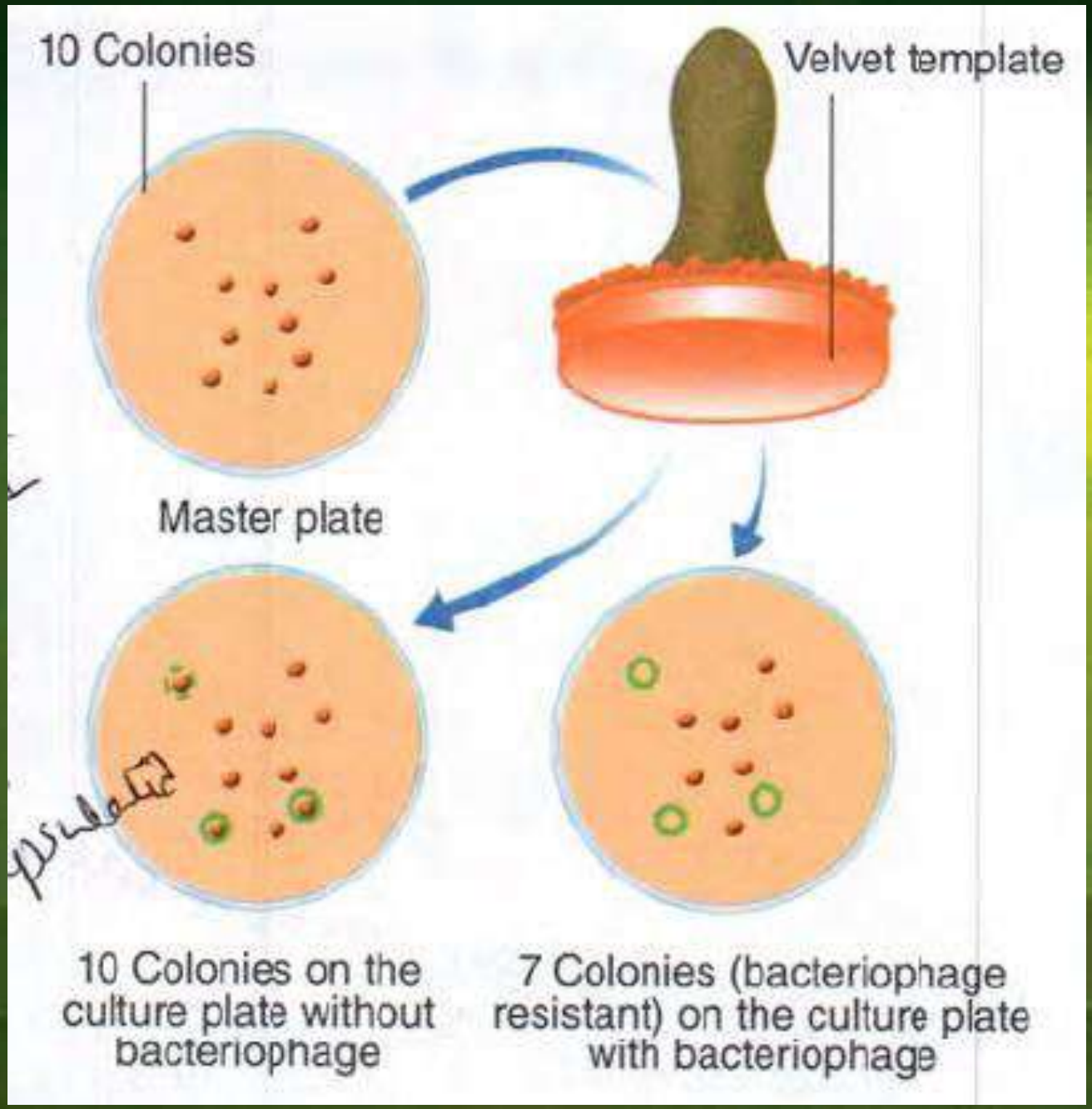
Press a sterile velvet surface into the colonies of the master plate. Some cells from each of the colonies adhere to the velvet.

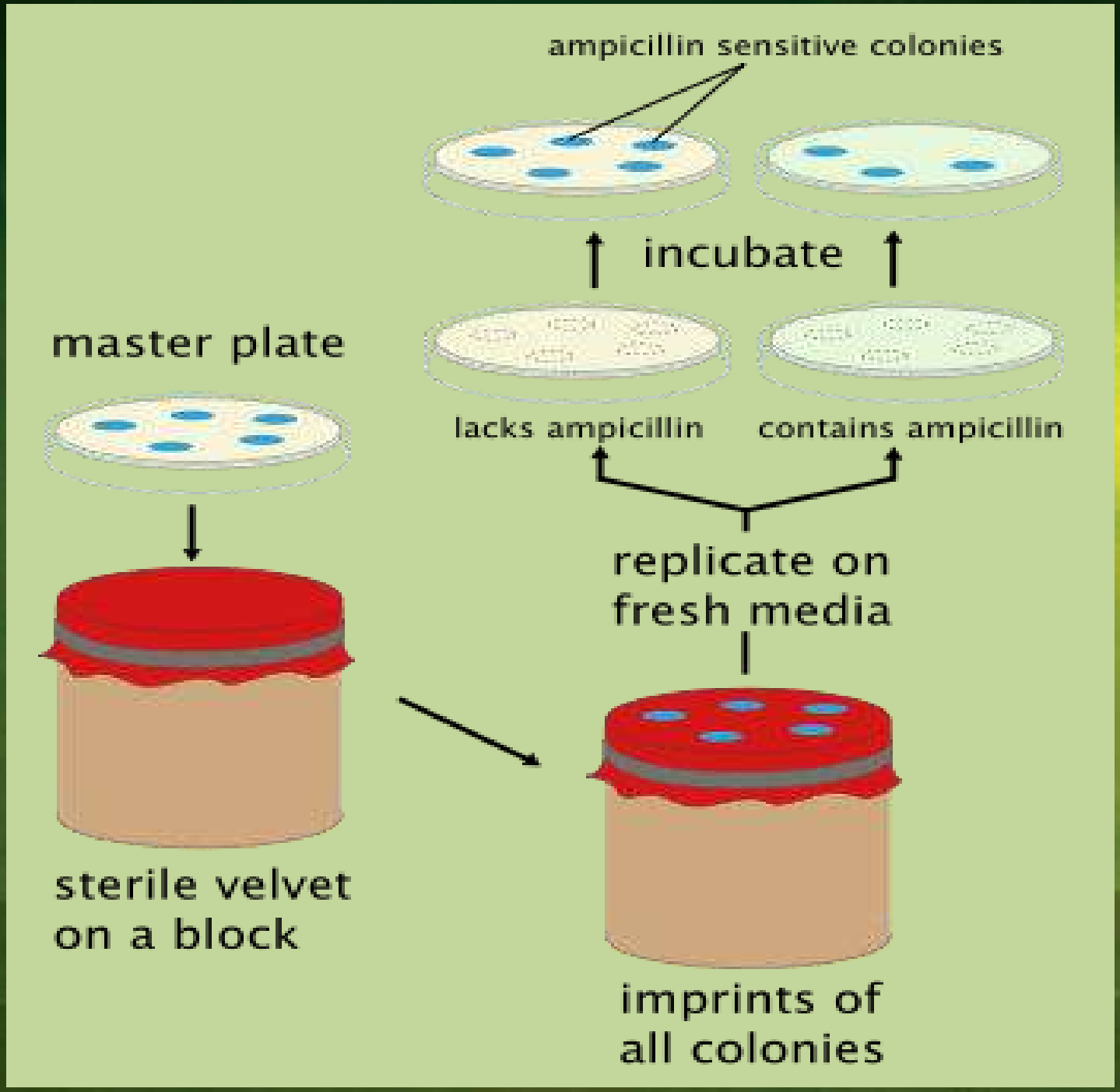
Prepare two mediums, one with tryptophan, the other without tryptophan.

Transfer cells from the velvet to each plate.

Compare growth on the two plates after incubation. Colonies that grow on the tryptophan enriched medium but not on the medium lacking tryptophan are Tryp mutants.


Mutagen
Bacterial suspension
1 Inoculate bacteria onto complete medium containing tryptophan
Incubation
2 Bacterial colonies grow. A few may be tryptophan auxotrophs. Most are wild type.
3 Stamp sterile velvet onto plate, picking up cells from each colony.
Sterile velvet surface
Bacteria
4 Stamp replica plates with velvet.
Complete medium containing tryptophan
Medium lacking tryptophan
5 Identify auxotroph as colony growing on complete medium but not on lacking medium.
Incubation
All colonies grow
Tryptophan auxotroph cannot grow
6 Inoculate auxotroph colony into complete medium.


10 Colonies
Velvet template
Master plate
10 Colonies on the culture plate without bacteriophage
7 Colonies (bacteriophage resistant) on the culture plate with bacteriophage


ampicillin sensitive colonies
incubate
master plate
lacks ampicillin
contains ampicillin
replicate on
fresh media
sterile velvet
on a block
imprints of
all colonies

THANK YOU

BIOCHEMISTRY AND METABOLIC DISORDERS
(MSc-110)

Lecture 8: Lipid metabolism disorders (contd.)

by Dr. Dipanjana Ghosh

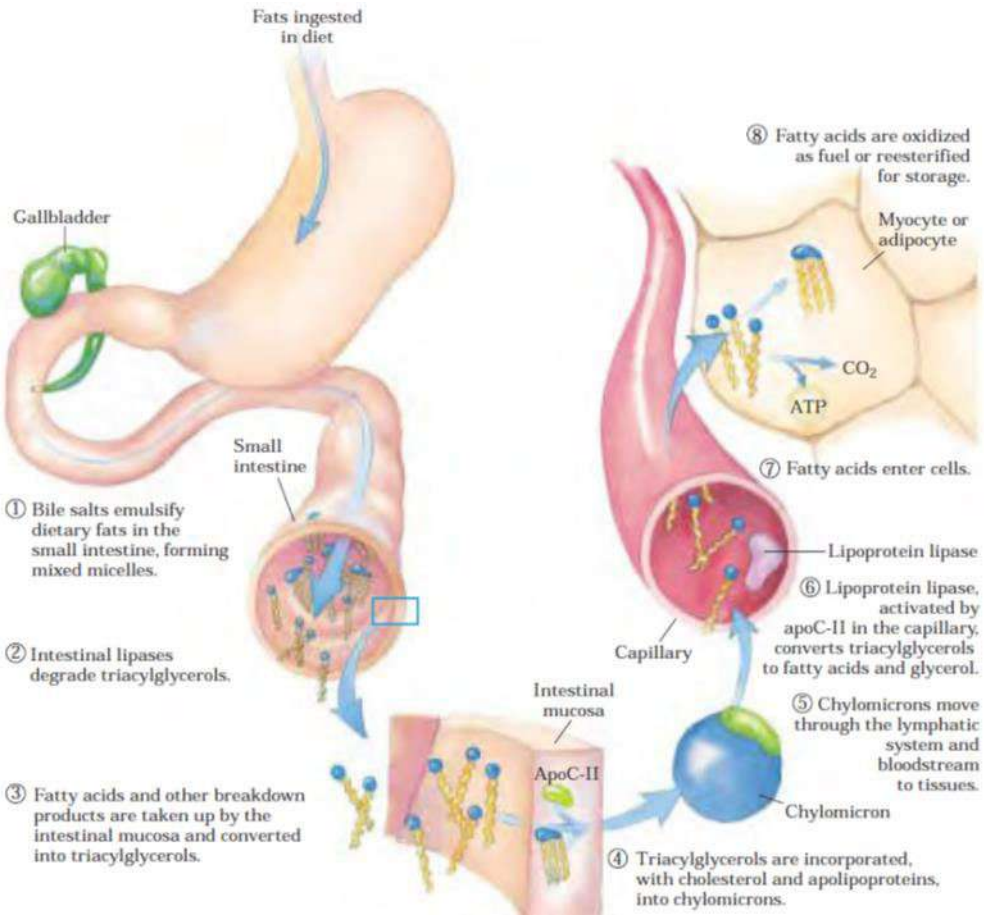
Date 15 Feb 2022

Topics to be covered

- Fatty acids, triglycerides, phospholipids, compound lipids, cholesterol, prostaglandins.
- Lipoprotein, HDL, LDL, VLDL, IDL, Chylomicrons.
- Structure, function and mechanism of actions of apolipoproteins
- Biosynthesis and degradation of lipids and cholesterol
- Lipid metabolic disorders (Contd..)

Maldigestion & Malabsorption

- An increase in the energy density of foods is often achieved by increasing the lipid content. However, in children with severe malnutrition, who are most in need of additional dietary energy, there are perturbations in lipid metabolism.



- The handling of different triglycerides (TAG) in the gastrointestinal tract is determined in part by the physicochemical properties of the individual constituent fatty acids (FA).
- No consideration has been given to this factor in the formulation of recovery diets. There is a need to know whether there is any clinical advantage in selecting one lipid source over another, especially during rapid catch up growth when a diet rich in lipid has been recommended to enable speedier recovery.

Maldigestion & Malabsorption

- Medium chain TAG, trilaurin was not handled differently to the longer chain unsaturated TAGs, triolein and trilinolein in the bowel.

- The possibility of further hydrolysis of TAG by bacterial lipases in the colon and stool should not be overlooked. Although bacterial lipases have been described, the relative contribution that lipases may have to hydrolysis in vivo within the colon has not been directly determined.

- Other studies have confirmed continuing TAG hydrolysis in collected and stored stool specimens.

- FA loss in excess of TAG suggests that the primary problem is either one of impaired absorption or possibly impaired TAG digestion with continued hydrolysis in the colon.

- Lipid in stool was largely attributed to malabsorption although impaired digestion could have been a contributory factor.

Ketone bodies

- In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the **"ketone bodies"**, acetone, acetoacetate, and D--hydroxybutyrate, for export to other tissues.

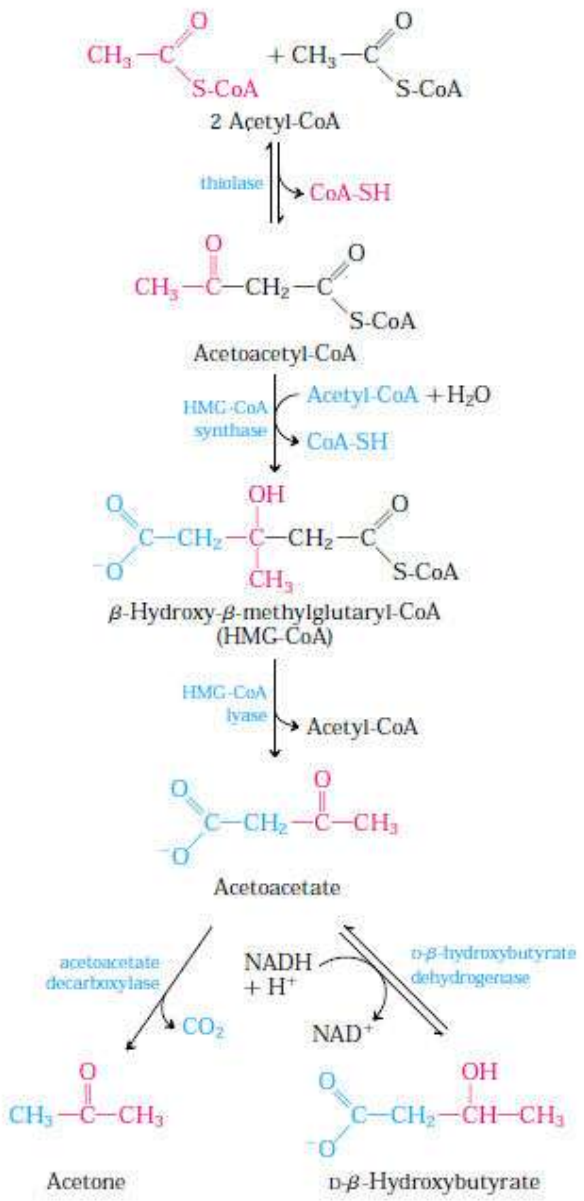
- Ketone bodies are quite soluble in blood and urine. Acetone, produced in smaller quantities than the other ketone bodies, is exhaled.

- Acetoacetate and D-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex.

- The brain, which preferentially uses glucose as fuel, can adapt to the use of acetoacetate or D-hydroxybutyrate under starvation conditions, when glucose is unavailable.

- The production and export of ketone bodies from the liver to extrahepatic tissues allow continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle.

Formation of ketone bodies from acetyl-CoA



- Healthy, well-nourished individuals produce ketone bodies at a relatively low rate.
- When acetyl-CoA accumulates (as in starvation or untreated diabetes, for example), thiolase catalyzes the condensation of two acetyl-CoA molecules to acetoacetyl-CoA, the parent compound of the three ketone bodies.
- The reactions of ketone body formation occur in the matrix of liver mitochondria.
- The six-carbon compound hydroxy-methylglutaryl-CoA (HMG-CoA) is also an intermediate of sterol biosynthesis, but the enzyme that forms HMG-CoA in that pathway is cytosolic.
- HMG-CoA lyase is present only in the mitochondrial matrix.

Ketone Bodies Are Overproduced in Diabetes and during Starvation

- Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid cycle intermediates, diverting acetyl-CoA to ketone body production.

- In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat.

- Under these conditions, levels of malonyl-CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to acetyl-CoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis.

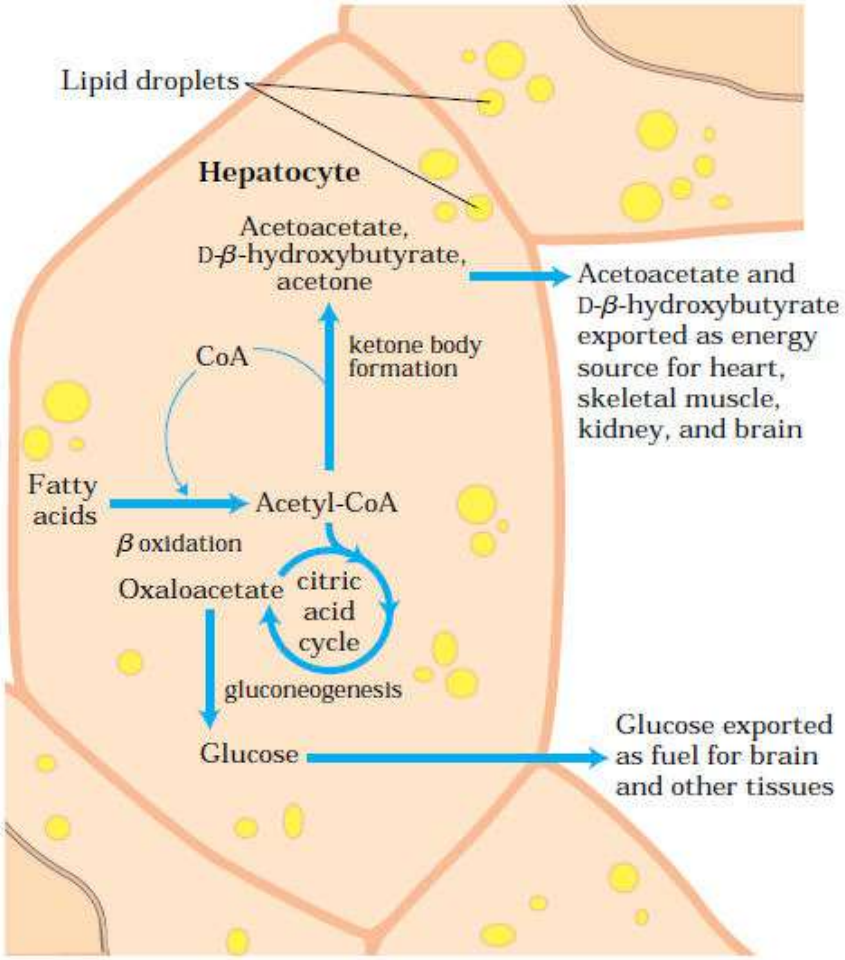
- The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them.

- The increased blood levels of acetoacetate and D-hydroxybutyrate lower the blood pH, causing the condition known as acidosis.

Ketone Bodies Are Overproduced in Diabetes and during Starvation (Contd..)

- Extreme acidosis can lead to coma and in some cases death.
- Ketone bodies in the blood and urine of untreated diabetics can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of 3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of 125 mg/24 hr). This condition is called ketosis.
- Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine.
- These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis).

Ketone Bodies formation & export from the liver



- Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate.
- The released coenzyme A allows continued oxidation of fatty acids.

Steatorrhoea

- The definition of steatorrhea is an increase in fat excretion in the stools. Steatorrhea is one of the clinical features of fat malabsorption and noted in many conditions such as exocrine pancreatic insufficiency (EPI), celiac disease, and tropical sprue.

- An increase in the fat content of stools results in the production of pale, large volume, malodorous, loose stools. Screening for steatorrhea may be carried out by examining stool samples for the presence of fat by Sudan III staining. However, quantitative fecal fat estimation is required to confirm the diagnosis.

- Among the macronutrients, digestion and absorption of fat involve a complex mechanism. Fat absorption requires bile acids, digestive enzymes, and a normally functioning small intestinal mucosa. Dietary lipids, mostly as triacylglycerols, are initially emulsified by bile acids and then hydrolyzed by the pancreatic lipases and colipases into free fatty acids and monoglycerides.

- In the proximal small bowel, these hydrolyzed lipids form micelles by the action of bile acids. The micelles are then absorbed across the intestinal villi and transported as chylomicrons via the intestinal lymphatics. Therefore, any defects in the availability or function of bile acids, pancreatic digestive enzymes, or absorptive villi will lead to steatorrhea.

Diarrhoea related to lipid metabolism

Chronic Fatty Diarrhea

- Steatorrhea implies the disruption of fat solubilization, digestion, or absorption in the small intestine. Evaluation of chronic fatty diarrhea is designed to distinguish maldigestion (inadequate luminal breakdown of triglycerides) from malabsorption (inadequate mucosal transport of the products of digestion)

Hyperlipoproteinemia

- Hyperlipoproteinemia is a common disorder. It results from an inability to break down lipids or fats in your body, specifically cholesterol and triglycerides. There are several types of hyperlipoproteinemia. The type depends on the concentration of lipids and which are affected.

- Hyperlipoproteinemia can be a primary or secondary condition.

- Primary hyperlipoproteinemia is often genetic. It's a result of a defect or mutation in lipoproteins. These changes result in problems with accumulation of lipids in your body.

- Secondary hyperlipoproteinemia is the result of other health conditions that lead to high levels of lipids in your body. These include:

  - diabetes
  - hypothyroidism
  - pancreatitis
  - use of certain drugs, such as contraceptives and steroids
  - certain lifestyle choices

Hyperlipoproteinemia

- Types of primary hyperlipoproteinemia
- There are five types of primary hyperlipoproteinemia:

- Type 1 is an inherited condition. It causes the normal breakdown of fats in your body to be disrupted. A large amount of fat builds up in your blood as a result.

- Type 2 runs in families. It's characterized by an increase of circulating cholesterol, either low-density lipoproteins (LDL) alone or with very-low-density lipoproteins (VLDL). These are considered the "bad cholesterols."

- Type 3 is a recessively inherited disorder in which intermediate-density lipoproteins (IDL) accumulate in your blood. IDL has a cholesterol-to-triglycerides ratio that's higher than that for VLDL. This disorder results in high plasma levels of both cholesterol and triglycerides.

- Type 4 is a dominantly inherited disorder. It's characterized by high triglycerides contained in VLDL. The levels of cholesterol and phospholipids in your blood usually remain within normal limits.

- Type 5 runs in families. It involves high levels of LDL alone or together with VLDL.

Hyperlipoproteinemia

Symptoms of hyperlipoproteinemia

- Lipid deposits are the main symptom of hyperlipoproteinemia. The location of lipid deposits can help to determine the type. Some lipid deposits, called xanthomas, are yellow and crusty. They occur on your skin.

- Many people with this condition experience no symptoms. They may become aware of it when they develop a heart condition.

- Other signs and symptoms of hyperlipoproteinemia include:

- pancreatitis (type 1)
- abdominal pain (types 1 and 5)
- enlarged liver or spleen (type 1)
- lipid deposits or xanthomas (type 1)
- family history of heart disease (types 2 and 4)
- family history of diabetes (types 4 and 5)
- heart attack
- stroke

Abetalipoproteinemia

- Abetalipoproteinemia (ABL) is a rare autosomal recessive disorder marked by low or absent levels of plasma cholesterol, low-density lipoproteins (LDLs), and very-low-density lipoproteins (VLDLs). It should not be confused with a deficiency in beta-lipoproteins. Hallmark symptoms include fat malabsorption, spinocerebellar degeneration, acanthocyte red blood cells, and retinitis pigmentosa.

- **Etiology**

Abetalipoproteinemia is caused by a homozygous autosomal recessive mutation in the MTTP gene. More than 33 mutations that cause the disease have been identified. The gene codes for microsomal triglyceride protein (MTP) that mediates intracellular chylomicron or VLDL assembly and transport in the intestinal mucosa and hepatocytes. Most of the signs and symptoms of the disease result from a severe deficiency of fats and fat-soluble vitamins, especially vitamin E. It usually presents in infants as failure to thrive, steatorrhea, and abdominal distension and results in spinocerebellar degeneration and retinitis pigmentosa.

- **Pathophysiology**

Beta apolipoproteins are very large apolipoproteins. They are critically important for the secretion and formation of chylomicrons (CMs) and VLDL. Abnormalities that impede this process result in abetalipoproteinemia and hypobetalipoproteinemia.

Abetalipoproteinemia

❖ **Role of MTP in Abetalipoproteinemia**

- MTP acts as a chaperone that facilitates the transfer of lipids onto apo B. MTP is found within the lumen of microsomes in the liver and intestinal mucosa and catalyzes the transfer of triglyceride, cholesteryl esters, and phosphatidylcholine between membranes. Lipid transport rates decrease in the order of triglyceride to cholesteryl ester to diglyceride to cholesterol to phosphatidylcholine. Unlike other lipid transfer proteins, MTP is a heterodimer containing subunits of molecular mass 58 and 97 kDa. The large 97-kDa subunit possesses the lipid transfer activity or confers lipid transfer activity on the complex. The large subunit of MTP may be missing in abetalipoproteinemia.
- Initial assembly occurs in the endoplasmic reticulum where apolipoproteins, cholesterol, phospholipid, and triacylglycerides are synthesized and incorporated into lipoprotein particles. The particles are subsequently transported to Golgi and secreted. Each lipoprotein is specific in its lipid composition and type of apolipoproteins it possesses.
- The two beta apolipoproteins are B-100 and B-48. ApoB-100 is carried on VLDL. ApoB-100, synthesized by the liver, is larger than apoB-48, which is made up of 4536 amino acids. Unlike apoB-48, apoB-100 contains the binding site essential for LDL uptake by hepatocyte LDL receptors. ApoB-48 is carried on CMs, is derived from the same gene as apoB-100.

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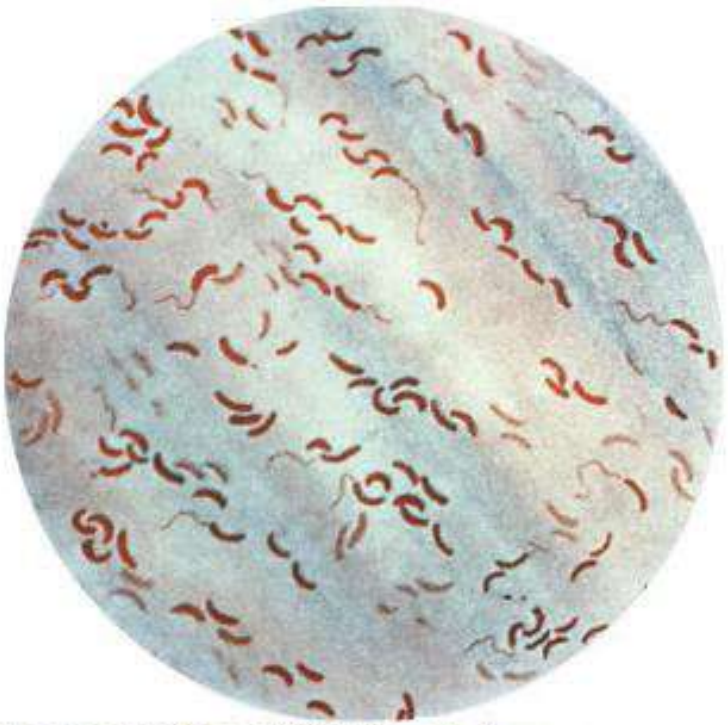
VIBRIO CHOLERAE

Dr. kamal Uddin Zaidi

Morphology

- *V. cholerae is a Gram negative, curved or comma-shaped* rod, non-sporing, non-capsulated, about 1.5 µm x 0.2-0.4 µm i n size (Fig. 1) .
- The organism is very actively motile with a single polar flagellum and movement is named as *darting motility.*
- Because of its typical *comma shaped appearance, it is also* named *Vibrio comma. S shaped or spiral forms may be* seen due to end to end attachment of two or more cells.
- In old cultures, they are frequently pleomorphic.
- In stained mucous flakes of cholera cases, the vibrios are arranged in parallel rows, described by Koch as the *fish in stream appearance.*



Gram staining of *Vibrio cholerae*

Culture

- *V. cholerae is strongly aerobic, growth being scanty and* slow anaerobically.
- It grows within a temperature range of 16°-40°C but optimum temperature is 37°C.
- It grows best in alkaline media , the optimum pH being 8.2 (pH range 7.4-9 .6).
- The organism is extremely sensitive to an acidic pH which kills it. *V. cholerae is a non-halophilic vibrio,* therefore, cannot grow in media with a concentration of sodium chloride more than 7%.
- However it can grow in media without sodium chloride.

Ordinary Media

(i) ***Nutrient agar***

After overnight incubation, the colonies are moist, translucent, round disks, 1- 2 mm in diameter, with a bluish tinge in transmitted light.

(ii) ***MacConkey's agar***

The colonies are colourless or pale at first, but become reddish or pink on prolonged incubation due to late fermentation of lactose.

(iii) ***Blood agar***

V. cholerae , classical biotype, does not produce hemolysis although some strains produce greenish discolouration around colonies which later becomes clear due to haemodigestion.

However, colonies of El Tor biotype produce haemolysis on blood agar.

Special Media

The special media are classified as follows:

(i) Transport or holding media

- *Venkatraman-Ramakrishnan (VR) medium*
- *Cary-Blair medium*

(ii) Enrichment media

- *Alkaline paptone water (APW)*
- *Monsur's taurocholate tellurite peptone water*

(iii) Plating media

- *Alkaline bile salt agar (BSA); pH 8.2*
- *Monsur's gelatin taurocholate trypticase tellurite*
- *agar (GITA) medium; pH 8.5*
- *Thiosulphate citrate bile sucrose (TCBS) agar; pH 8.6*

Biochemical Reactions

Catalase	Oxidase	Glucose	Lactose	Mannitol
+	+	A	–	+
Sucrose	Maltose	Mannose	Indole	NO_3 reduction
+	+	+	+	+
Urease	Gelatin	MR	VP*	
–	+	–	–	
Lysine	Ornithine	Arginine	Sheep RBCs haemolysis*	
+	+	–	–	

*In case of El Tor biotypes, all biochemical reactions are similar but VP and sheep RBCs haemolysis are positive.

Resistance

- *Vibrio chloerae is susceptible to heat and drying.*
- *It is* killed at 55°C in 15 minutes. It is sensitive to common disinfectants and a pH less than 5.
- It survives for months in sea water. In grossly contaminated water, such as the Ganges in India, the vibrios do not survive for any length of time perhaps due to the presence of large number of vibriophages in this water.
- ELTor vibrios are hardier and survive for longer duration than the classical biotypes.
- On fruits, they survive for 1- 5 days at room temperature and for a week in the refrigerator.

Antigenic Structure

- *V cholerae contains somatic 'O' and flagellar 'H' antigens.*
- The 'H' antigen is shared by all the strains. *Gardner and Venkatraman (1935) classified vibrios based on antigenic* structure (Fig. 34.2).

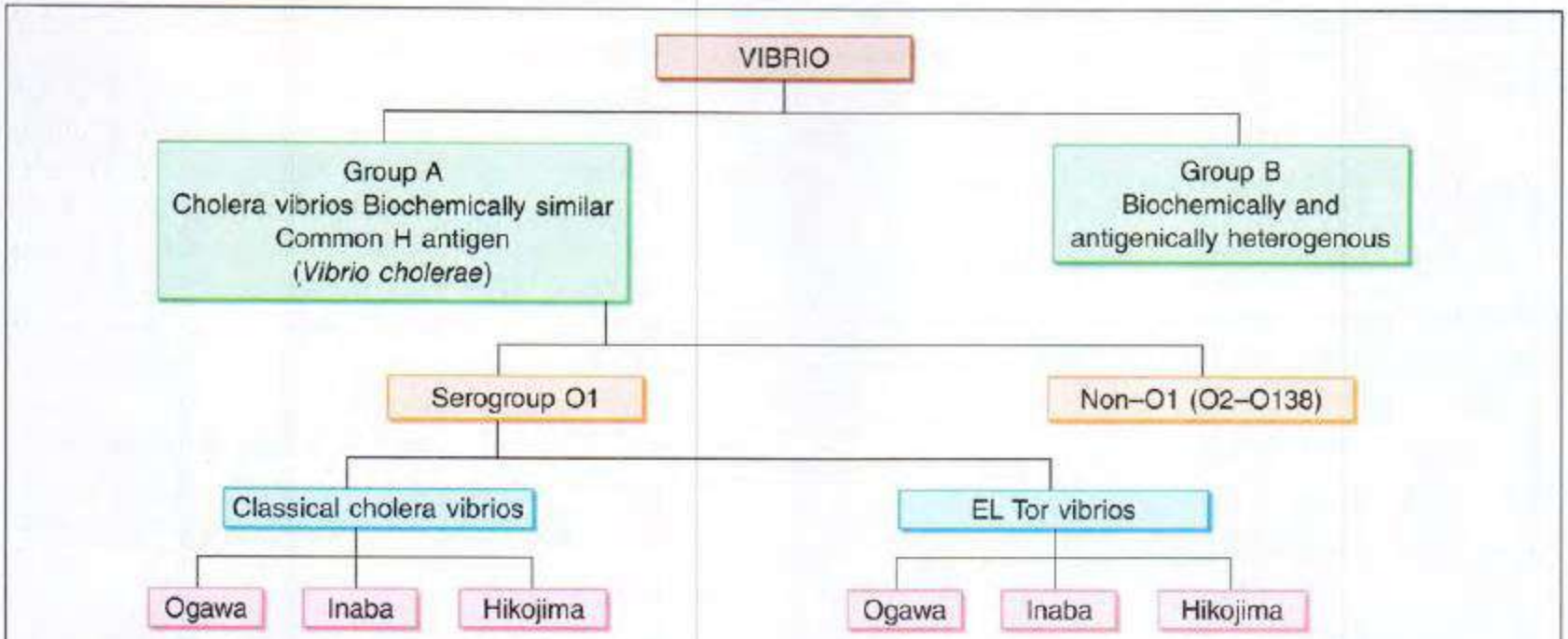


Fig. 34.2 Antigenic classification of vibrios (Gardner and Venkatraman, updated)

Antigenic Structure

- Group A vibrios have a common H antigen but distinct 'O' component. On the basis of O antigen, it is divided into subgroups (now named O serogroups or serovars).
- Both classical and ElTor biotypes belong to serogroups O 1 and are antigenically indistinguishable.
- These are referred as *V cholerae OJ. On the basis of* minor O antigens (A, B, C), *V cholerae 01 are subdivided* into three serotypes- Ogawa (AB), Inaba (AC) and Hikojima (ABC) (Table 34.1).
- At least 139 (01 to 0139) 0 serogroups are recognised. Serogroups 02 to 0138 are called non-O1 *V cholera.*
- Since these organisms were not agglutinated by 0 -1 antiserum, they were called non-agglutinating (NAG) vibrios.
- Though NAG vibrios are not agglutinable by 0-1 antiserum, they are agglutinated by their specific homologous antisera.

Table 34.1 Serotypes of Cholera Vibrios

Serotype	O antigen
Ogawa	AB
Inaba	AC
Hikojima	ABC

Pathogenesis

- ✓ *V. cholerae* (both 01 and 0139) causes an acute diarrhoeal disease known as cholera and it occurs only in man.
- ✓ The human infection occurs by ingestion of contaminated foods and drink.
- ✓ The ingested organisms pass through the acid barrier of the stomach and multiply in the alkaline medium of the small intestine.
- ✓ The vibrio,s do not penetrate deep into the gut and there is no bacteraemia.
- ✓ Vibrios become adherent to the epithelium by special fimbria such as the tox in co-regulated pilus (TCP).
- ✓ Once epithelial cell attachment occurs, V. cholerae produces enterotoxin and the disease.
- ✓ Mechanism of action of enterotoxin has been described earlier.
- ✓ Enterotoxin and TCP are regulated by the Tox R gene product, Tox R protein.

The massive loss of water and electrolytes (sodium and bicarbonates) by action of enterotoxin, leads to:

I. Dehydration causing haemoconcentration, anuria and hypovolaemic shock

2. Base-deficit acidosis

3. Muscle cramps due to hypokalaemia.

In untreated cases the mortality rate is 60- 70% due to renal failure.

Both the biotypes of *V. cholera* O1 produce equally severe disease but in El Tor strains, the incidence of mild and asymptomatic infections is more frequent.

The NAG vibrios may produce a clinical disease indistinguishable from cholera.

Epidemiology

- ✓ Cholera is an epidemic disease and man is the only natural host of *V. cholerae.*
- ✓ Cholera has been endemic in the Ganges and Brahmaputra deltas in Bengal and neighbouring parts of the Indian subcontinent.
- ✓ Before 1817, cholera was confined to these endemic areas.
- ✓ Between 1817 and 1923 cholera spread to all over the world in six pandemics.
- ✓ All these pandemics were caused by classical biotype of *V. cholerae*.
- ✓ Between 1923 and 1961, the disease remained confined almost to endemic areas except for one isolated epidemic in Egypt in 1947.

- ✓ Epidemic cholera has a seasonal distribution, the epidemic seasons are different in different areas.
- ✓ In India (Calcutta) the epidemic season is in the hot dry months of March to May and ends with onset of monsoon in June, while in Bangladesh the cholera season (November to February) follows the monsoon rains.
- ✓ Epidemic cholera has been associated with fairs, festivals during which sanitary conditions tend to be unsatisfactory.
- ✓ The only natural reservoir is man in the form of convalescent and chronic carriers.
- ✓ The transmission is maintained by a cycle involving the excretor (carrier) and the environment, particularly a water source.
- ✓ Since vibrios do not survive in water for a long period, so it should be repeatedly contaminated to act as a prolonged source of infection.
- ✓ In 2012, the WHO reported an outbreak of cholera in Sierra Leone, causing 280 deaths among 20,700 cases.

Laboratory Diagnosis

Specimens

(i) Watery stool

(ii) Rectal swab

Collection and Transport

Specimens should be collected preferably prior to start of antibiotics.

These should not be collected from bedpans due to risk of contamination.

Specimens should be immediately transported to the laboratory for processing.

- ✓ In case of delay, stool samples may be preserved in holding media such as VR fluid or Cary-Blair medium for long periods.
- ✓ If the specimen can reach the laboratory in a few hours, enrichment media such as alkaline peptone water or Monsur's medium may be used as transport media.
- ✓ When transport media are not available, strips of blotting paper may be soaked in watery stool and sent to the laboratory after proper packing in plastic envelopes.
- ✓ If possible, specimens should be plated at bedside and the inoculated plates sent to the laboratory.

Direct Microscopy

It is not a reliable method. For rapid diagnosis, the characteristic darting motility of the Vibrio and its inhibition by adding antiserum can be demonstrated under the dark field or phase contrast microscope, using cholera stool.

Culture

(i) Stool sample is directly cultured on following media.

(a) Selective media (BSA , TCBS or Monsur's GTTA) and non-selective media (blood agar and MacConkey's agar) are inoculated. These plates are incubated at 37°C for overnight.

(b) Enrichment media such as alkaline peptone water or Monsur's liquid media are inoculated. These media are incubated at 37°C for 6-8 hours before subculturing on to selective media.

(ii) When the specimen has been collected in holding medium, it is first inoculated into enrichment medium and incubated at 37°C for 6-8 hours before plating onto a selective medium.

(iii) The specimen collected in enrichment medium should be incubated for 6-8 hours including transit time before subculturing onto a selective medium .

Colony Morphology and Staining

- After overnight incubation , culture media are examined for typical colonies of *V. cholerae. On MacConkey 's agar,* colonies are pale and on Monsur's medium the colony has a black centre with a turbid halo around the colony.
- TCBS shows yellow colonies and on BSA, translucent colonies are present.
- Gram staining from colony shows typical Gram negative comma sh aped bacilli.
- These show darting motility on hanging drop preparation.
- Further confirmation is done by biochemical reactions and agglutination test.

Biochemical Reactions

- *V. chole rae ferments glucose, mannitol, sucrose maltose,*mannose with acid production. Lactose is usually not fermented.
- Catalase, oxidase and cholera red reactions are positive.
- The El Tor biotype is usually haemolytic , VP positive, agglutinate chick erythrocytes and is resistant to polymyxin B and group IV cholera phage.

Treatment

Oral Rehydration Therapy

- ✓ The most important is prompt water and electrolyte replacement to correct the severe dehydration and salt depletion.
- ✓ This can be achieved by oral rehydration therapy, either alone or supplemented by intravenous fluids.

Antibiotics

Antibiotics are of secondary importance. Tetracycline is useful in reducing the number of stool and it also shortens the period of excretion of vibrios.

Prophylaxis

General Measures

(i) Purification of water supplies.

(ii) Better provision for sewage disposal.

(iii) Infected patients should be isolated, their excreta disinfected.

(iv) Contacts and carriers are followed up.

Specific Measures

ACTIVE IMMUNISATION

Killed parenteral vaccine

- ✓ This vaccine contains 12,000 million *V cholerae per ml,* composed of equal number of *Inaba and Ogawa strains.*
- ✓ It is widely used for active immunisation. Two injections are given intramuscularly at an interval of four weeks.
- ✓ Degree of protection does not exceed 50- 60% and the duration of protection is only 3- 6 months.

- ✓ A single dose confers good protection in adults due to its action as a booster on top of prior natural immunisation.
- ✓ In children below five years of age, a single dose is ineffective.
- ✓ There is a good cross protection between classical and El Tor vibros.
- ✓ The aluminium hydroxide and phosphate adjuvant vaccines have been tried.
- ✓ These vaccines have induced a high degree of immunity, particularly in young children.
- ✓ Cholera toxoid has been tried as vaccine but without any success.

Killed oral vaccine

- ✓ *B subunit whole cell (BS- WC) vaccine has undergone a* field trial in Bangladesh.
- ✓ The vaccine contains cholera toxin B subunit, heat killed classical vibrio (both Ogawa
- ✓ and Inaba) and formalin killed ElTor vibrio (both Ogawa and Inaba).
- ✓ Degree of protection was 85% for one year and 60% for two years. This vaccine also conferred significant protection against diarrhoea due to *Esch. Coli* (LT- ETEC).

Live oral vaccine

- ✓ Recombinant DNA vaccine with expression of *V. cholerae in attenuated strain Ty2la of Salmonella typhi has been developed.*
- ✓ *This vaccine colonises Peyer's* patches and induces IgA response by local immunity.
- ✓ Live attenuated vaccine against O 139 is also available in some countries.

FUNDAMENTALS OF BIOCHEMISTRY

Lecture 2: Triglycerides, Oil wax

by Dr. Dipanjana Ghosh

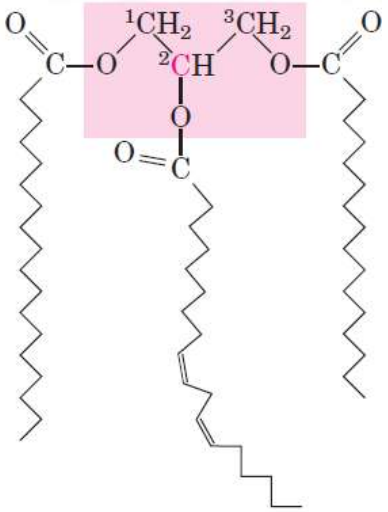
Date 22 Dec 2021

Triglycerides

- The simplest lipids constructed from fatty acids are the triacylglycerols, also referred to as triglycerides, fats, or neutral fats.

Glycerol

+ fatty acids



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol

- Simple triacylglycerols: Those containing the same kind of fatty acid in all three positions

- Named after the fatty acid they contain. Simple triacylglycerols of 16:0, 18:0, and 18:1, for example, are tristearin, tripalmitin, and triolein, respectively.

Triglycerides (Contd..)

- Most naturally occurring triacylglycerols are mixed; they contain two or more different fatty acids. To name these compounds unambiguously, the name and position of each fatty acid must be specified.

- Because the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages,triacylglycerols are nonpolar, hydrophobic molecules, essentially insoluble in water.

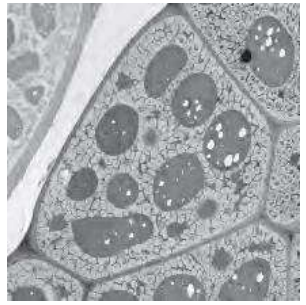
- Lipids have lower specific gravities than water, which explains why mixtures of oil and water (oil-and-vinegar salad dressing, for example) have two phases: oil, with the lower specific gravity, floats on the aqueous phase.

Triacylglycerols Provide Stored Energy and Insulation

- In most eukaryotic cells, triacylglycerols form a separate phase of microscopic, oily droplets in the aqueous cytosol, serving as depots of metabolic fuel.

- In vertebrates, specialized cells called adipocytes, or fat cells, store large amounts of triacylglycerols as fat droplets that nearly fill the cell

- Triacylglycerols are also stored as oils in the seeds of many types of plants, providing energy and biosynthetic precursors during seed germination



- Adipocytes and germinating seeds contain lipases, enzymes that catalyze the hydrolysis of stored triacylglycerols, releasing fatty acids for export to sites where they are required as fuel.

Why Triglycerides are better stored fuels than carb stores

- There are two significant advantages to using triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch.
  - The carbon atoms of fatty acids are more reduced than those of sugars, oxidation of triacylglycerols yields more than twice as much energy, gram for gram, as the oxidation of carbohydrates.
  - Triacylglycerols are hydrophobic and therefore dehydrated, the organism that carries fat as fuel does not have to carry the extra weight of water of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide).

- Humans have fat tissue (composed primarily of adipocytes) under the skin, in the abdominal cavity, and in the mammary glands. Moderately obese people with 15 to 20 kg of triacylglycerols deposited in their adipocytes could meet their energy needs for months by drawing on their fat stores.

- In contrast, the human body can store less than a day's energy supply in the form of glycogen. Carbohydrates such as glucose and glycogen do offer certain advantages as quick sources of metabolic energy, one of which is their ready solubility in water.

Triglycerides are insulators

- In some animals, triacylglycerols stored under the skin serve not only as energy stores but as insulation against low temperatures. Seals, walruses, penguins, and other warm-blooded polar animals are amply padded with triacylglycerols.

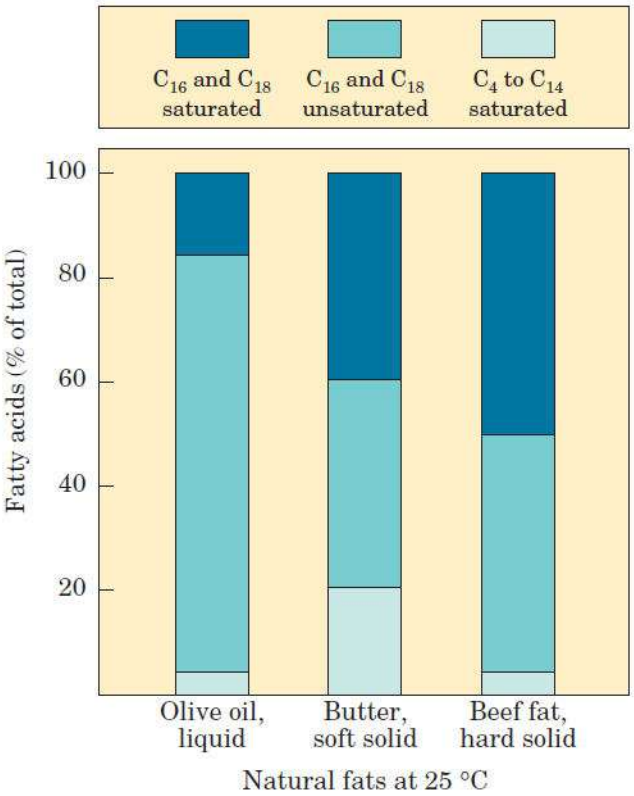
- In hibernating animals (bears, for example), the huge fat reserves accumulated before hibernation serve the dual purposes of insulation and energy storage

- The low density of triacylglycerols is the basis for another remarkable function of these compounds. In sperm whales, a store of triacylglycerols and waxes allows the animals to match the buoyancy of their bodies to that of their surroundings during deep dives in cold water

Many Foods Contain Triacylglycerols

Oil & Wax

- Most natural fats, such as those in vegetable oils, dairy products, and animal fat, are complex mixtures of simple and mixed triacylglycerols. These contain a variety of fatty acids differing in chain length and degree of saturation



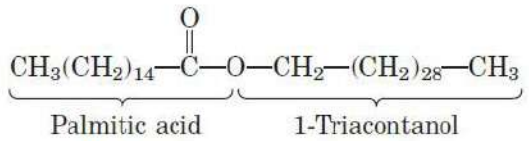
- Vegetable oils such as corn (maize) and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature.
- They are converted industrially into solid
- Fats by catalytic hydrogenation, which reduces some of their double bonds to single bonds and converts others to trans double bonds.
- Triacylglycerols containing only saturated fatty acids, such as tristearin, the major component of beef fat, are white, greasy solids at room temperature.

- When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become rancid. The unpleasant taste and smell associated with rancidity result from the oxidative cleavage of the double bonds in unsaturated fatty acids, which produces aldehydes and carboxylic acids of shorter chain length and therefore higher volatility.

Wax

Waxes Serve as Energy Stores and Water Repellents

- Biological waxes are esters of long-chain (C14 to C36) saturated and unsaturated fatty acids with long-chain (C16 to C30) alcohols.



(a)

(b)

Triacontanoylpalmitate, the major component of beeswax, is an ester of palmitic acid with the alcohol triacontanol.

- Their melting points (60 to 100° C) are generally higher than those of triacylglycerols. In plankton, the free-floating microorganisms at the bottom of the food chain for marine animals, waxes are the chief storage form of metabolic fuel.
- Waxes also serve a diversity of other functions related to their water-repellent properties and their firm consistency.
- Certain skin glands of vertebrates secrete waxes to protect hair and skin and keep it pliable, lubricated, and waterproof.
- Birds, particularly waterfowl, secrete waxes from their preen glands to keep their feathers water-repellent.

Wax (Contd..)

- The shiny leaves of holly, rhododendrons, poison ivy, and many tropical plants are coated with a thick layer of waxes, which prevents excessive evaporation of water and protects against parasites.

- Biological waxes find a variety of applications in the pharmaceutical, cosmetic, and other industries. Lanolin (from lamb's wool), beeswax, carnauba wax (from a Brazilian palm tree), and wax extracted from spermaceti oil (from whales) are widely used in the manufacture of lotions, ointments, and polishes.

Different types of lipids

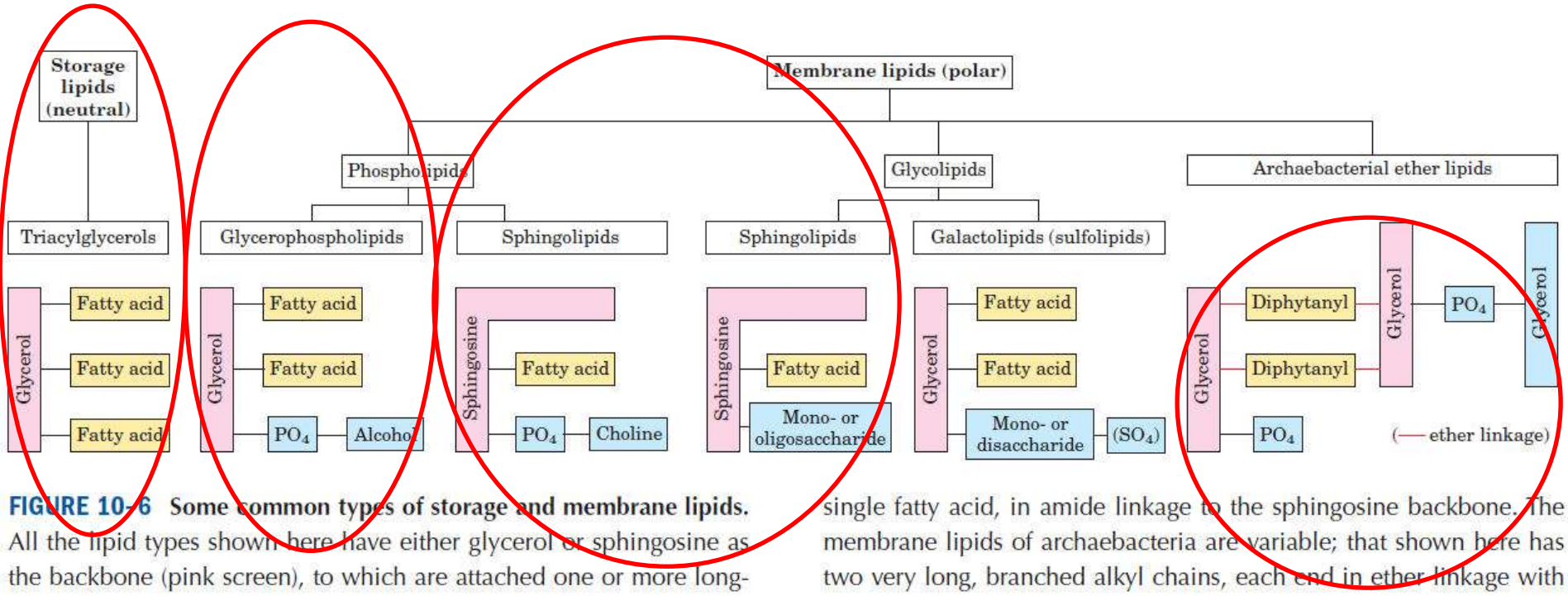
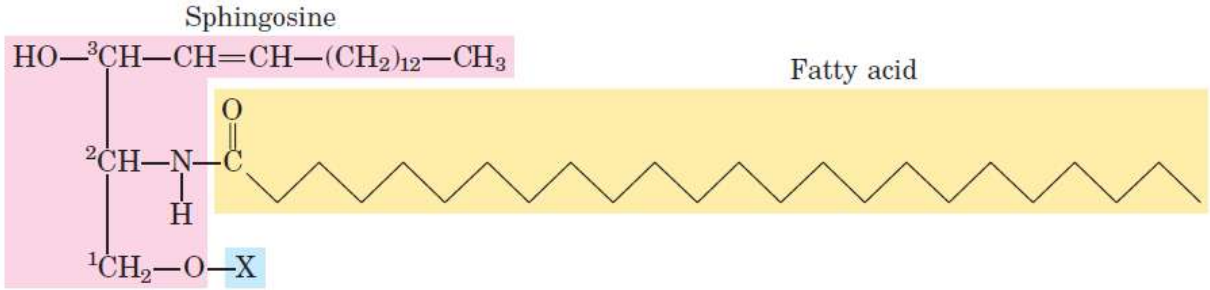
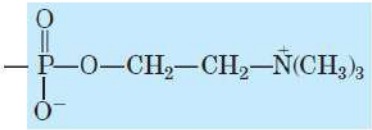
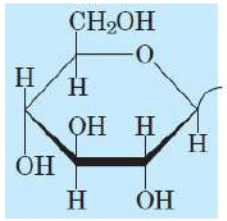
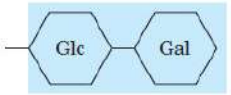
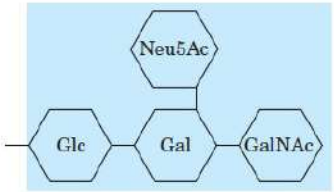


FIGURE 10-6 Some common types of storage and membrane lipids. All the lipid types shown here have either glycerol or sphingosine as the backbone (pink screen), to which are attached one or more long-chain alkyl groups (yellow) and a polar head group (blue). In triacylglycerols, glycerophospholipids, galactolipids, and sulfolipids, the alkyl groups are fatty acids in ester linkage. Sphingolipids contain a single fatty acid, in amide linkage to the sphingosine backbone. The membrane lipids of archaebacteria are variable; that shown here has two very long, branched alkyl chains, each end in ether linkage with a glycerol moiety. In phospholipids the polar head group is joined through a phosphodiester, whereas glycolipids have a direct glycosidic linkage between the head-group sugar and the backbone glycerol.

Sphingolipids and their derivatives

Sphingolipid (general structure)



Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Reference

Lehninger's Principles of Biochemistry, fourth edition

Genomics and Proteomics (MSC-301)

Lecture 5: Functional Proteomics

Proteomics

by Dr. Dipanjana Ghosh

Date 28 Jan 2022

TOPICS TO BE COVERED

- Protein Analysis:

1. Measurement of protein conc
2. Amino acid composition (by N-terminal sequencing)

- 2D gel electrophoresis of protein
- MALDI-TOF and Peptide mass fingerprinting
- LC- MS/MS for protein identification
- Functional proteomics: protein-protein interactions

Functional Proteomics

❖ Functional proteomics constitutes an emerging research area in the proteomic field whose approaches are addressed towards two major targets:

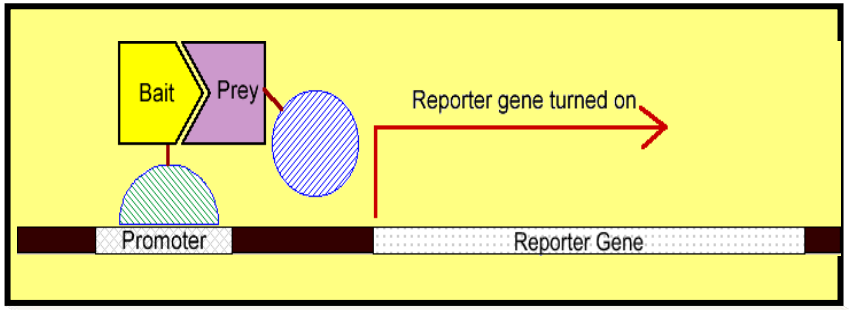
- ✓ The elucidation of the biological function of unknown proteins and
- ✓ The definition of cellular mechanisms at the molecular level.

- ❑ In the cells, many proteins display their biological functions through the rapid and transient association within large protein complexes.
- ❑ Understanding protein functions as well as unraveling molecular mechanisms within the cell then depend on the identification of the interacting protein partners.
- ❑ There are various strategies for unravelling protein-protein interaction. Yeast-two hybrid system, Co-immunoprecipitation, Protein-based microarray.

Functional Proteomics (Contd.)

High-Throughput Experimental Detection Method I

Yeast-Two-Hybrid



Yeast-Two-Hybrid (Y2H)

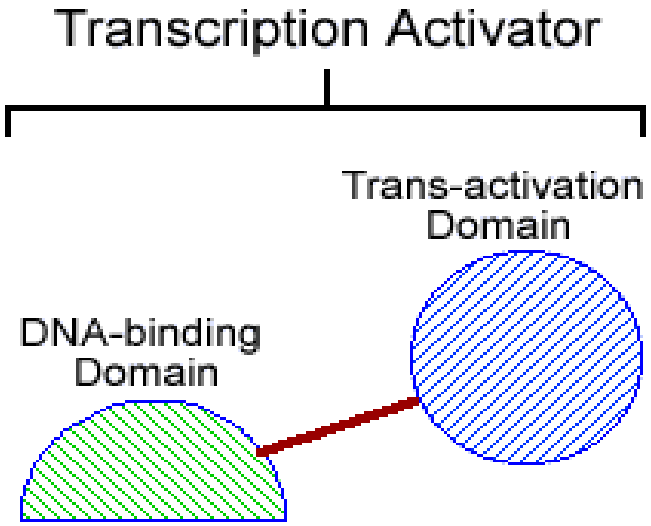
- Want to develop an *in vivo* system for detecting physical association between proteins
- First reported in 1989 by Fields and Song from State University of New York
- Use *yeast* as the *in vivo* platform for protein interaction detection

- *in vivo* : in a natural environment within a living organism
- *in vitro*: in an artificial environment outside a living organism (e.g. in a test tube)
- *in silico… ?*

Yeast-Two-Hybrid (Y2H)

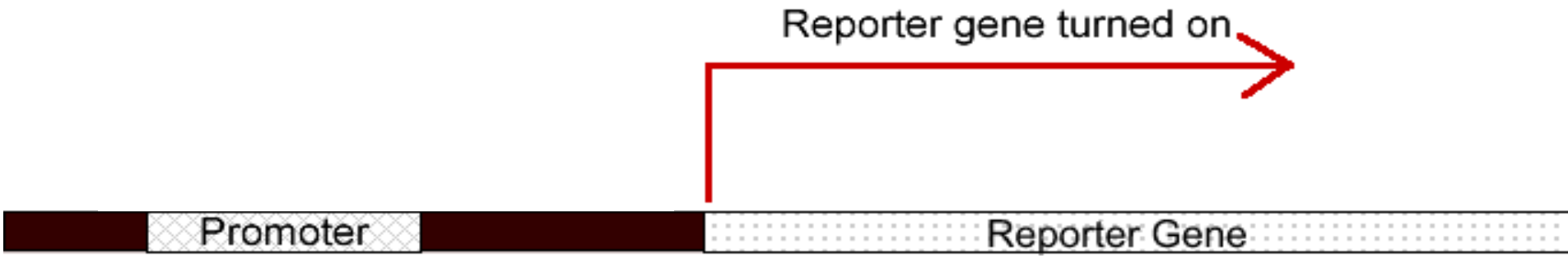
Transcriptional activator

- A special protein that has the ability to cause specific *reporter genes* to be switched on (or transcribed).
- E.g. GAL4
- contains two functional subparts ("domains"): a DNA-binding domain and an activator domain



Yeast-Two-Hybrid (Y2H)

Transcriptional activation



Yeast-Two-Hybrid (Y2H)

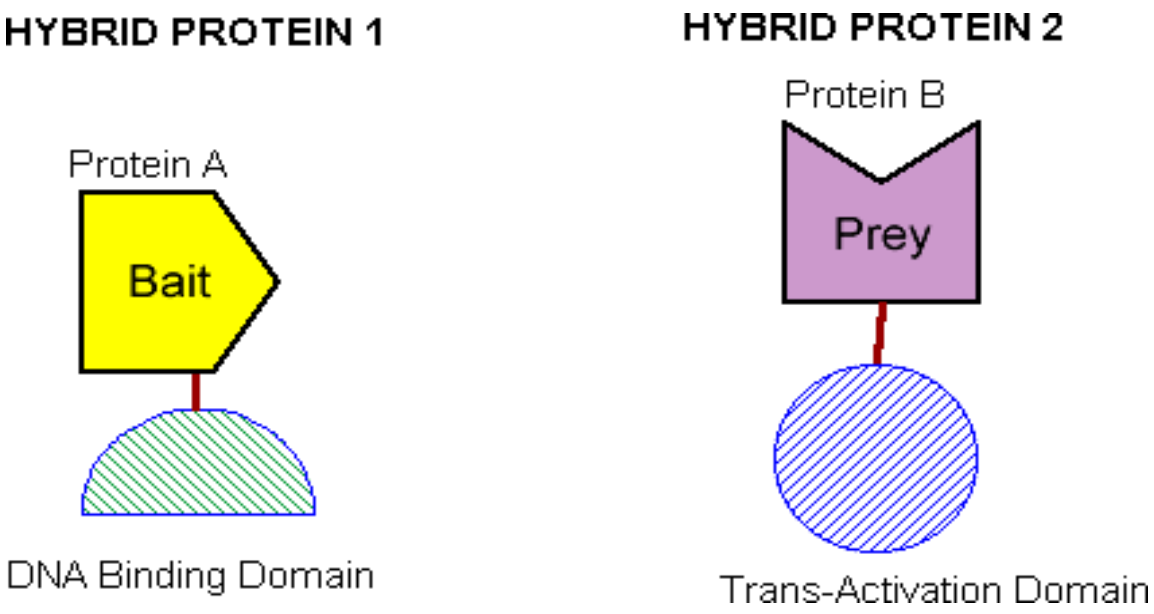
- Want to check whether Protein A physically interacts with Protein B

Method:

  - Break the transcriptional activator molecule into two halves, each containing one of the two functional domains
  - Create two hybrid proteins by attaching Protein A to one halve, and Protein B to the other halve
  - We call the one attached to the DNA-binding domain halve the “Bait” protein, and the one attached to the activation domain halve the “Prey” protein

Yeast-Two-Hybrid (Y2H)

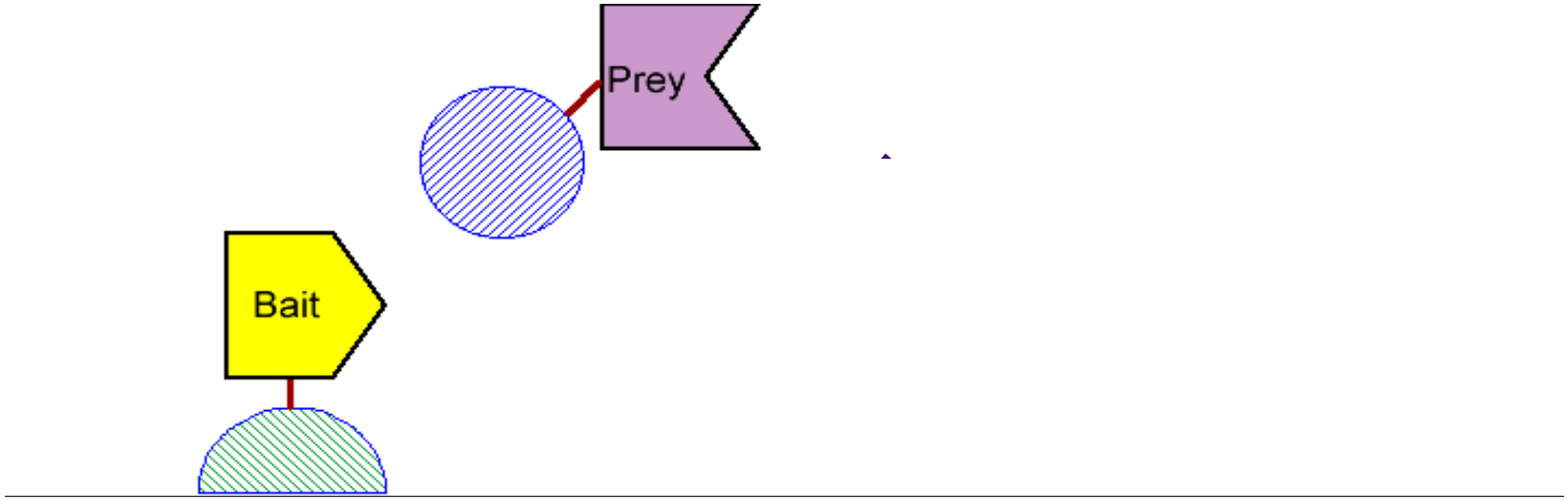
- Results: Two hybrid proteins (hence the name Y2H)



Yeast-Two-Hybrid (Y2H)

CASE 1: Protein A does not interact with Protein B

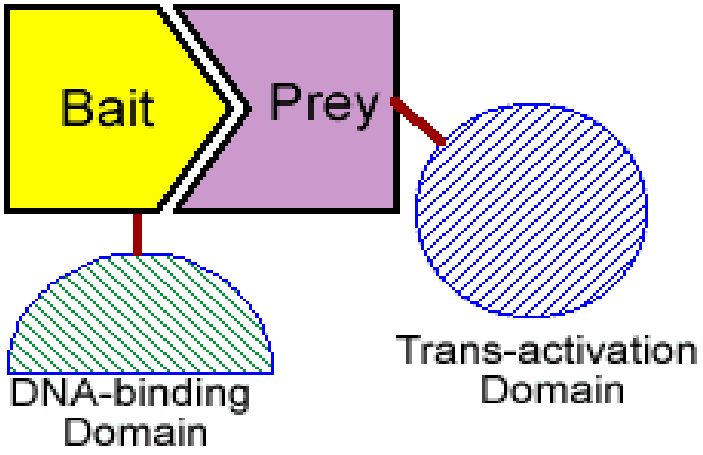
- the DNA binding domain will not be “reunited” with the activation domain



Yeast-Two-Hybrid (Y2H)

CASE II: Protein A physically interacts with Protein B

- then the two functional domains of the transcriptional activator molecule are “reunited” together



- This causes the reporter gene to be turned on as before

Yeast-Two-Hybrid

Advantages

- *in vivo* technique
- Simple and inexpensive
- Amenable to high-throughput methods

Yeast-Two-Hybrid

Limitations

- *In vivo* technique:
  - Must take place in nucleus where transcription takes place
  - Cannot detect interactions between proteins that do not exist in the nucleus
  - E.g. membrane proteins

Yeast-Two-Hybrid

Limitations

- Relies on fusion proteins
  - False positives
    - Hybrid protein may adopt non-native folding – giving rise to spurious interactions
    - Some proteins have transcriptional properties themselves
  - False negatives
    - Non-native folding can also cause non-interactions
    - Many proteins require post-translational modifications in order to interact

Yeast-Two-Hybrid

Limitations

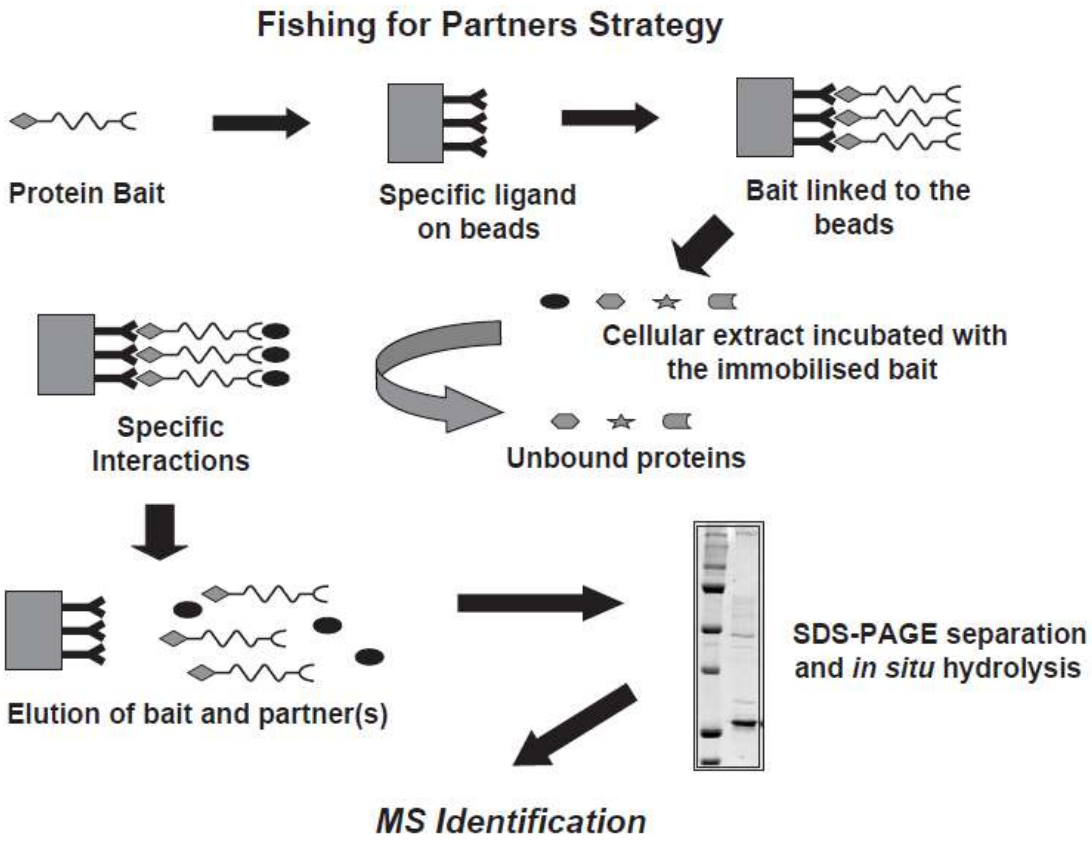
- Can only detect pairwise interactions

Yeast-Two-Hybrid

Popular high-throughput method for proteome-wide interactome dissection

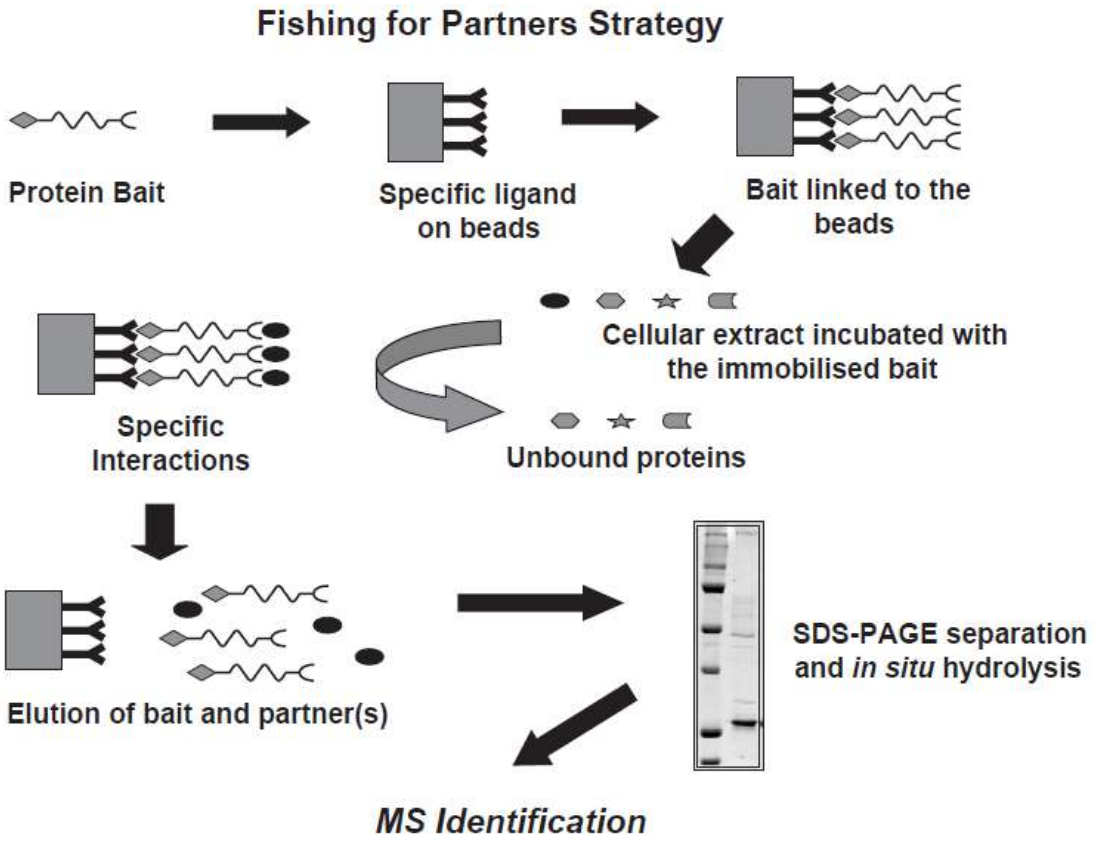
- Yeast
- Worm (C. elegans)
- Bacteria (H. pylori)
- Fly (Drosophila)
- … etc

Fishing partner strategy



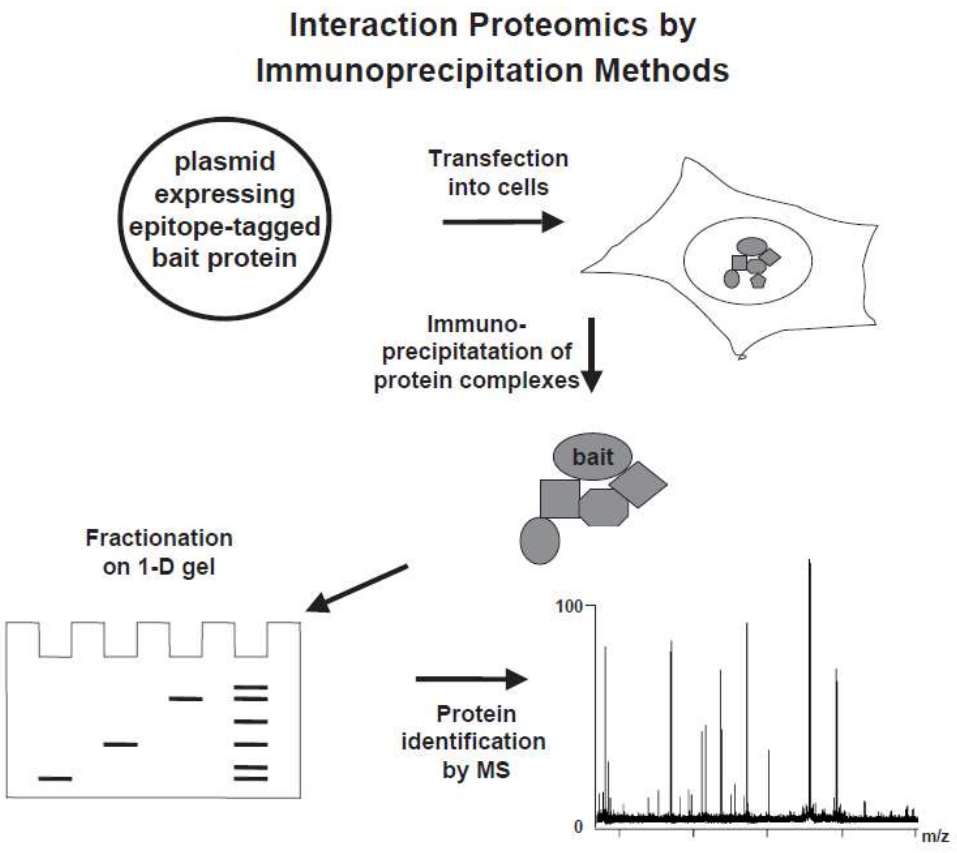
- Using commercially available protein expression systems, the protein bait can be produced as a hybrid protein fused to the glutathione S-transferase (GSTfused protein) or to small peptide epitopes (i.e., FLAG, HA, or c-myc), or containing a poly-His tail, or covalently modified with biotin.
- In all cases, the tagged bait can be immobilized onto agarose beads derivatized with the appropriate anti-tag ligand (glutathione, anti-epitope antibodies, nickel ions, streptavidin etc.).

Fishing partner strategy (Contd..)



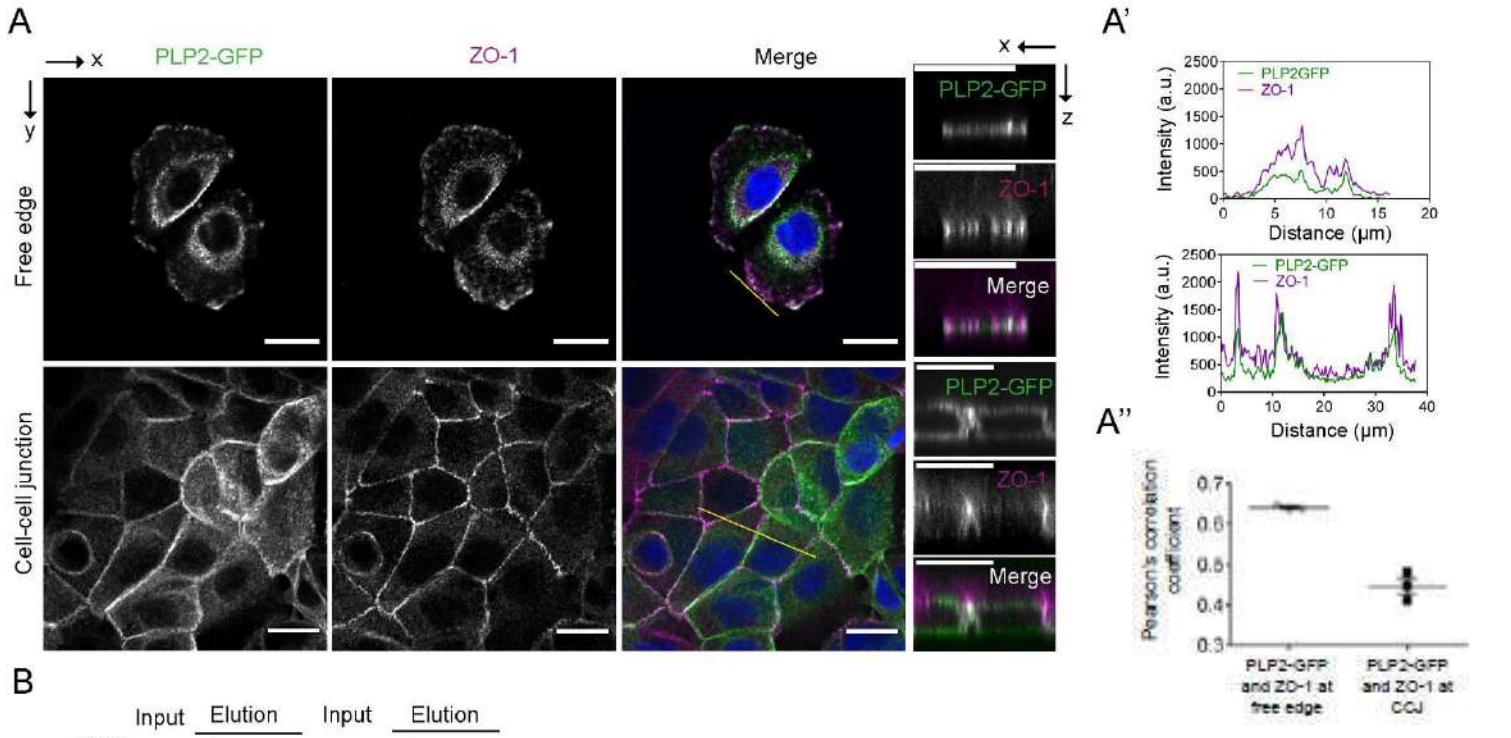
- The entire cellular extract and/or, when appropriate, the extract from specific organelles can then be incubated with the immobilised bait. The immobilised protein forms stable non-covalent interactions with specific partners occurring in the cellular extract, whereas the unbound proteins will be eluted during the washing.
- The protein components specifically recognised by the bait and retained on the agarose beads can then be eluted and fractionated by SDS-PAGE.
- The protein bands detected on the gel are in situ enzymatically digested and the resulting peptide mixtures are analysed by MALDI/MS fingerprinting and/or LC-MS/MS techniques, leading to the identification of the proteins by database search.

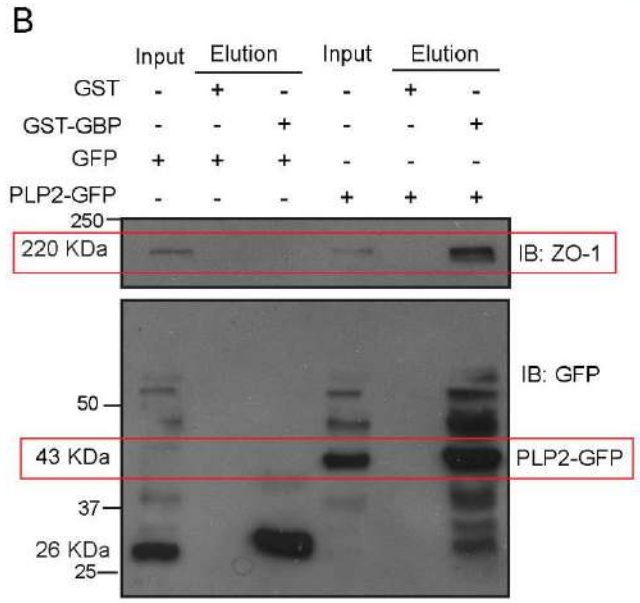
Co-immunoprecipitation

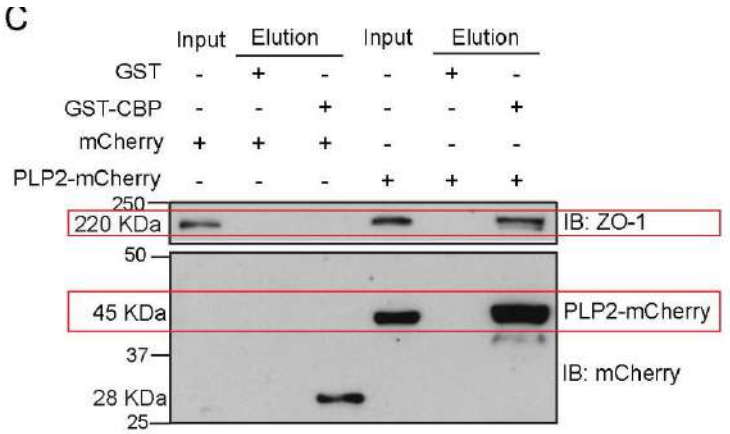


- The gene coding for the bait tagged with an epitope against which good antibodies exist (FLAG, HA, c-myc, etc.) is transfected into the appropriate cell line and expressed in the cognate host.
- Protein complexes are allowed to form in vivo within the cell and the cell extracts are immunoprecipitated
- with anti-tag monoclonal antibodies using suitable experimental conditions to avoid dissociation of the complexes.
- The immunoprecipitated material containing the protein bait and its interacting partners can then be fractionated by SDS-PAGE and the individual protein components identified by different mass spectrometric methodologies.

Example from research: PLP2 associates with ZO-1

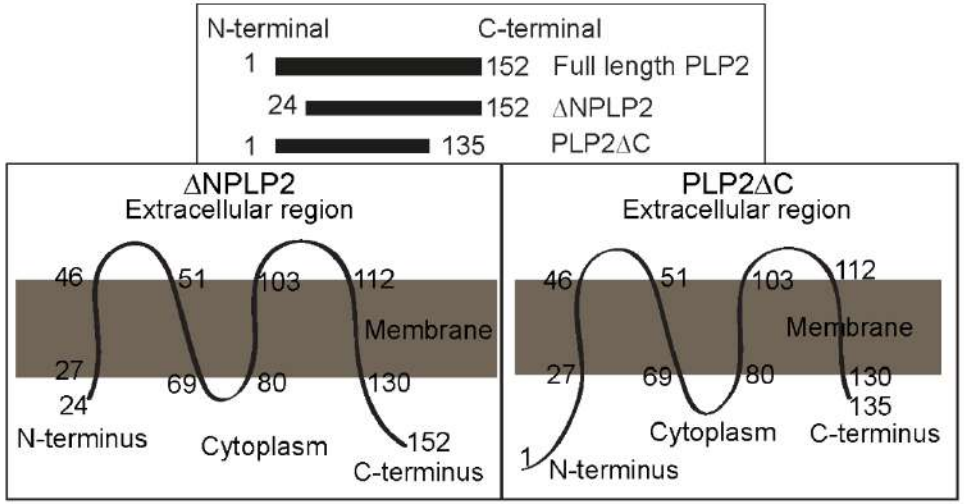


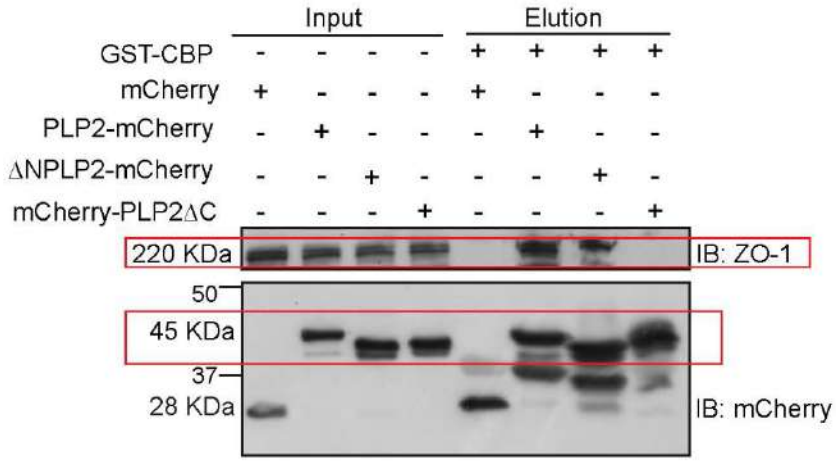




Ghosh et al., JCS, 2021

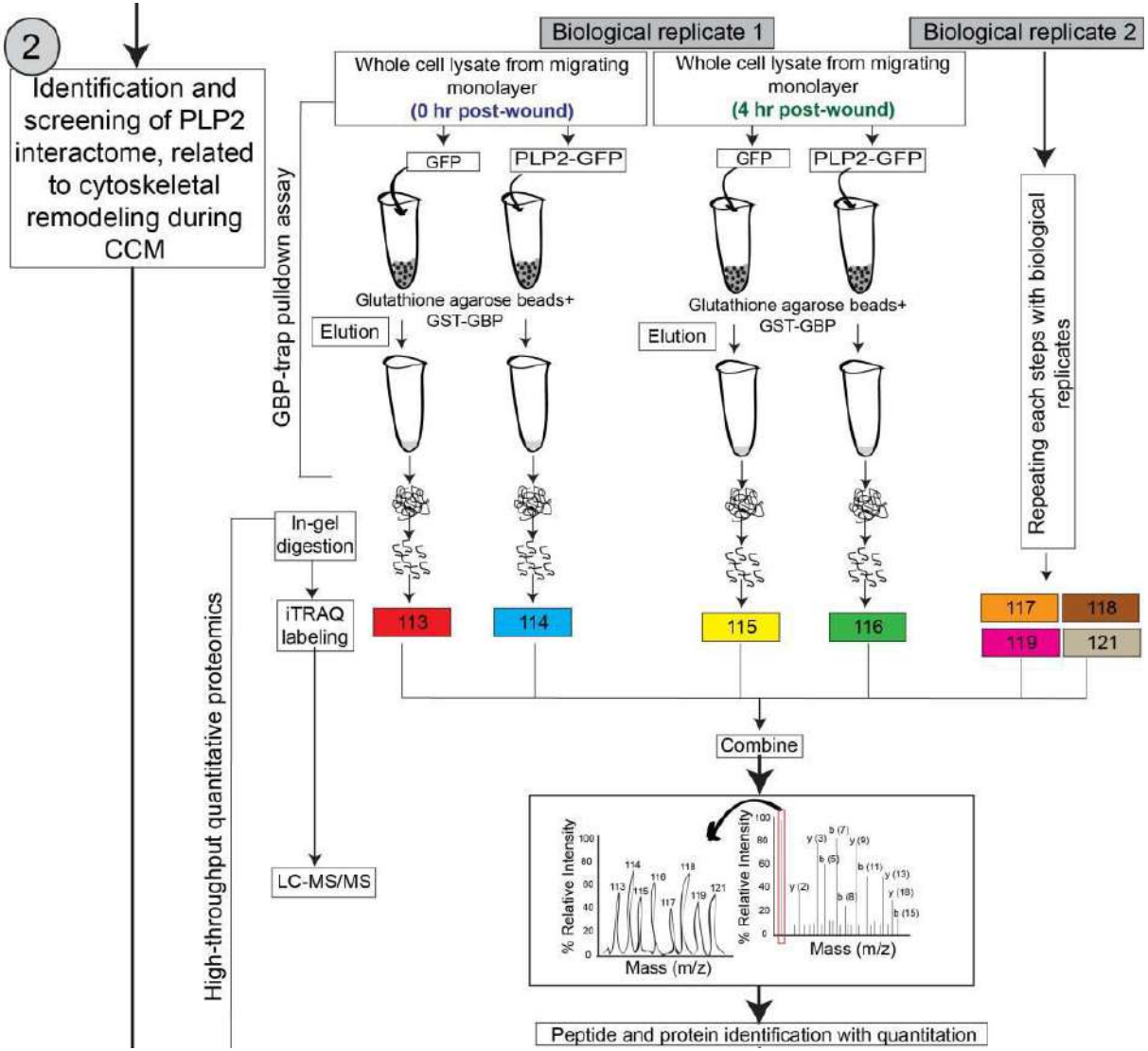
Example from research: C-terminal cytoplasmic tail of PLP2 associates with ZO-1





Ghosh et al., JCS, 2021

Example from research: Plan for identifying interacting partners of PLP2 using co-immunoprecipitation



References

- Partly adopted from study materials by Dr. See-Kiong Ng, NTU
- Clinica Chimica Acta 357 (2005) 140–150

PROJECT PROPOSAL PRESENTATION (MSC-307)

by Dr. Dipanjana Ghosh

Date 06 January 2022

TOPICS TO BE COVERED

- What is a project proposal?
- Components of a project proposal
- How to write a project proposal?
- How to present the project proposal?

  How to deliver a presentation?

What is a project proposal? (Scientific in your case)

- An idea or a concept of an individual or a team, drafted on paper in a systematic manner

What are the usual components of a project proposal?

- Title

- Origin of the Proposal: (Maximum 1/2 page)

(Scientific rationale for doing this work should be elaborated)

- Review of status of Research and Development in the subject

1. International Status: (Maximum 1 page)

(Researchers working in the area worldwide and their contributions must be properly highlighted with recent references and reviews. A correct and faithful description of the international research status must be given)

Components of a project proposal (Contd..)

2. National Status: (Maximum 1/2 page)

(Same as above to cover the contribution of Indian Scientists in the project area)

3. Importance of the proposed project in the context of current status (Maximum 1/2 page)

(Highlight what is the new area or gap which will be solved in the project in relation to what is already known. This is a very important section to project the novelty content of the proposal)

4. If the project is location specific, basis for selection of location be highlighted: (Maximum 1/2 page)

❖ Work Plan:

1. Aim (scientific goal in a broad aspect)
2. Objectives (specific scientific goals, usually 3-4 points)
3. Methodology: (Maximum of 2 pages)

(It should contain all the details of how each of the objectives will be addressed. This section must be detailed and have clear plans, not vague and generalized statements. It should have several schemes, tables, figures, equations etc. in addition to text, explanation and justification of why the project research plan will work)

Components of a project proposal (Contd..)

- **Timeline:**

  Time Schedule of activities giving milestones through BAR diagram.
  (Maximum 1/2 page)

- **Environmental impact assessment and risk analysis.**
  (Maximum ½ page)

- **References:** Combine all the references from each section sequentially here.

How to write a project proposal?

Examples:

- **Title:** Choose your topic

- **Origin of the Proposal:** About the topic…find literature that describes about the topic; Review articles for example on the disease of concern, the protein of concern etc.

- **Review of status of Research and Development in the subject**

Find literature on the scientific work done in the area chosen i.e. the research articles.

For example: You may find 10 articles all experimentally studied the area of your interest. You need to read all of them, understand the summary of their findings and write that in 1 or 2 sentences for each paper in your own language and cite the paper at the end of the sentence.

How to write a project proposal? (Contd..)

❖ Work Plan:

1. Aim (scientific goal in a broad aspect)
2. Objectives (specific scientific goals, usually 3-4 points)
3. Methodology: (Maximum of 2 pages)

Experimental methodology that needs to be performed to fulfill the proposed objectives. Can be adopted from published papers with proper citation of references.

❖ Timeline: (For you 6 months total, divide into each months)

Phases	Research activities/ Milestones of phases	Year 1				Year 2				Year 3			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
SCREENING	*Lab set-up*												
	Sample collection												
	Sample processing												
	Protein extraction, quantification and pooling												
	2D-DIGE												
	Protein visualization and image analysis												
IDENTIFICATION	*Protein identification using MS/MS*												
	Short-listing of candidates using bioinformatics												
VALIDATION	*Validation*												
	Publications, presentations, patent filing etc.												

❖ Environmental impact assessment and risk analysis:

Write about biohazards involves if any, involves animal ethics or human ethics or GMO in the study etc.

❖ References:

Combine all the references from each section sequentially here. It is important to include all references where you are adopting the knowledge from.

❖ Important to remember

Except for the methodology and references, you have to write in your own language. You may adopt the idea but not the exact sentences. This is called plagiarism.

Final ASSESSMENTS

- Write a project proposal on your own chosen topic and submit
  (Format will be provided)

- Present your proposal (30 min power point presentation)

Internal ASSESSMENT
Date: 13/01/2022

Choose your topic for proposal from the list below:

1. Cancer cell biology (CCB) and focal adhesion dynamics
2. Quantifying forces exerted by cancer cells during migration
3. Role of exosomal Arp2/3 in colorectal cancer
4. Role of exosomal Fibronectin in colorectal cancer
5. Involvement of Actin cytoskeleton in CCB
6. Role of different classes of interleukins in CCB
7. Targeting cancer stem cells by natural products for chemoprevention
8. Nano formulation based approaches for chemoprevention.

How will you choose?

- Search the topics in Google/ Pubmed
- Select a latest review article
- Read it
- Refer to the back references in the review article
- Read few (minimum 3) of the back references that you feel are relevant to the given topic
- Choose the most comfortable topic
- Each one of you should have individual topics, should not overlap

Sterilisation andDisinfection

By: Dr. Kamal Uddin Zaidi

Why we need Sterilization?

- Microorganisms capable of causing infection are constantly present in the external environment and on the human body
- Microorganisms are responsible for contamination and infection
- The aim of sterilisation is to remove or destroy them from materials or from surfaces

Definitions:

Sterilisation :

- It is a process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form

Disinfection :

- Destruction of all pathogens or organisms capable of producing infections but not necessarily spores.
- All organisms may not be killed but the number is reduced to a level that is no longer harmful to health.

Antiseptics :

- Chemical disinfectants which can safely applied to living tissues and are used to prevent infection by inhibiting the growth of microorganisms

Asepsis :

- Technique by which the occurrence of infection into an uninfected tissue is prevented.

Uses of Sterilization /Disinfection

- ✓ Sterilization of materials, instruments used in surgical and diagnostic procedures.

- ✓ For media and reagents used in the microbiology laboratory.
- ✓ In food and drug manufacturing to ensure safety from contaminating organisms.

Methods

1. Physical methods
2. Chemical methods

Physical methods:

- Physical methods:
  1. Sunlight
  2. Drying
  3. Heat
     1. Dry heat
     2. Moist heat
  4. Filtration
  5. Radiation

Chemical methods

- **Chemical methods:**
  1. Alcohols
  2. Aldehydes
  3. Phenols
  4. Halogens
  5. Oxidizing agents
  6. Salts
  7. Surface active agents
  8. Dyes
  9. Vapor phase disinfectants

Physical methods

1. Sunlight

- ✓ Sunlight has an active germicidal effect due to its content ultraviolet rays and heat rays
- ✓ It is a natural method of sterilisation of water in tanks, rivers and lakes

2. Heat

- ✓ Heat is the most reliable and commonly employed method of sterilization.
- ✓ Two types of heat are used, dry heat and moist heat.

Principle -

(i) Dry heat kills the organisms by denaturation of bacterial protein, oxidative damage and by the toxic effect of elevated levels of electrolytes.

However, the possibility of DNA damage is also incriminated as one of the mechanisms of inactivation of microbes.

(ii) Moist heat kills the microorganisms by denaturation of their enzymes and coagulation of structural proteins.

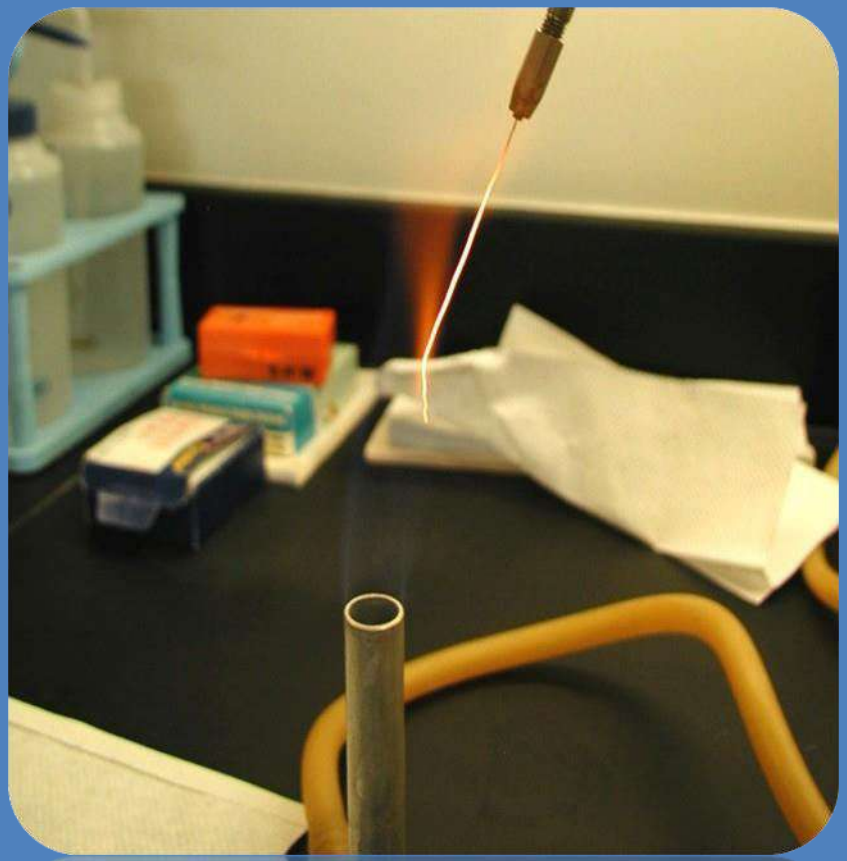
Factors influencing

(i) *Nature of heat: Dry heat or moist heat*
(ii) Temperature and duration: The time required for sterilization is inversely proportional to temperature to which organisms are exposed.

(iii) Characteristic of microorganisms and spores present:
Bacterial spores are killed by moist heat at 1**21 °C** for 15 minutes. Most vegetative bacteria, fungi and viruses are killed in 30 minutes at 65°C by moist heat.

(iv) Type of material:
Materials containing organic substances require more time for sterilization. Proteins, sugars, fats and starch are some of the organic substances.

- Dry heat:
  1. Red heat
  2. Flaming
  3. Incineration
  4. Hot air oven

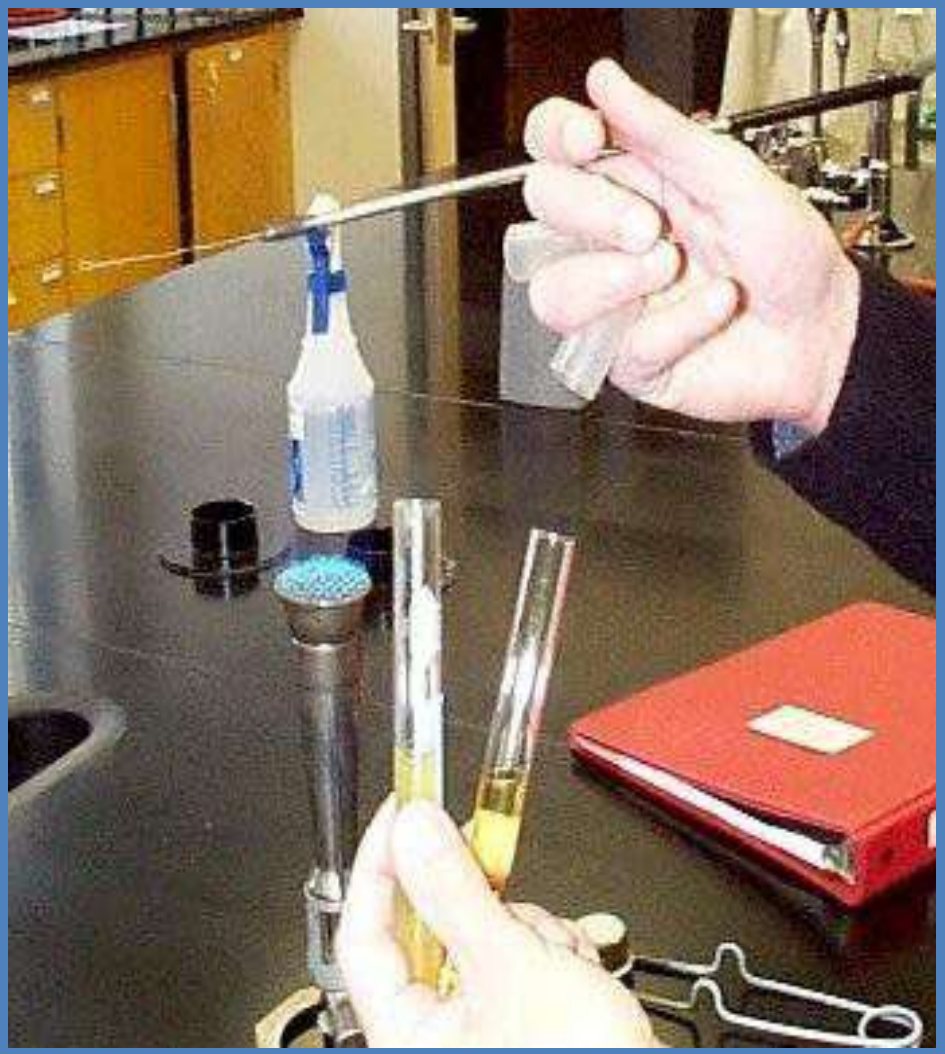


Red heat

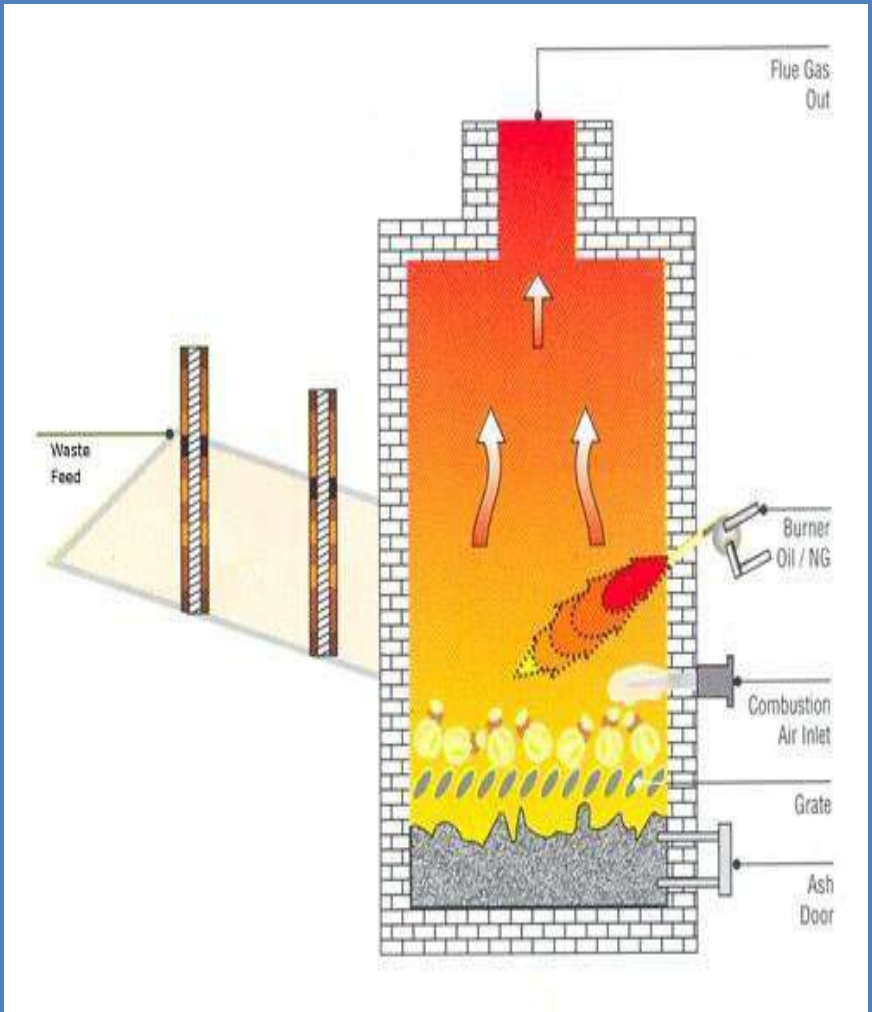
- Materials are held in the flame of a bunsen burner till they become red hot.
  - Inoculating wires or loops
  - Tips of forceps
  - Surface of searing spatulae
  - Needles

Flaming

- Materials are passed through the flame of a bunsen burner without allowing them to become red hot.
  - Glass slides
  - scalpels
  - Mouths of culture tubes and bottles



Incineration:



- Materials are reduced to ashes by burning.
- Instrument used was incinerator.
- Soiled dressings
- Animal carcasses
- Bedding
- Pathological material

Hot air oven

- Most widely used method of sterilisation by dry heat
- It is used to process materials which can withstand high temperatures, but which are likely to be affected by contact with steam
- It is a method of choice for sterilisation of glassware, forceps, scissors, scalpels, swab sticks packed in test tubes
- Materials such as oils, jellies and powders which are impervious to steam are sterilised by hot air oven
- Hot air oven is electrically heated and is fitted with a termostat that maintains the chamber air at a chosen temperature
- Fitted with a fan that distributes hot air in the chamber

Hot air oven


memmert
30 9 10AM

Holding temperature & time

General purpose

Temperature and time:
160^{0}C for 2 hours
170^{0}C for 1 hour
180^{0}C for 30 minutes

Cutting instruments such as those used in ophthalmic surgery,
Should be sterilized at 150^{0}C for 2 hours

Oils, glycerol and dusting powder should be sterilised at
150^{0}C for 1 hour

Precautions

1. Should not be overloaded
2. Arranged in a manner which allows free circulation of air
3. Material to be sterilized should be perfectly dry.
4. Test tubes, flasks etc. should be fitted with cotton plugs.
5. petridishes and pipetts should be wrapped in paper.
6. Rubber materials and inflammable materials should not be kept inside.
7. The oven must be allowed to cool for two hours before opening, since glass ware may crack by sudden cooling.

Uses of Hot Air Oven

Sterilisation of

1. Glassware like glass syringes, petri dishes, pipettes and test tubes.
2. Surgical instruments like scalpels, scissors, forceps etc.
3. Chemicals like liquid paraffin, fats etc.

Sterilisation controls

1. Spores of ***Bacillus subtilis subsp. Niger***

2. Thermocouples

1. Browne's tube

   (Tube containing red colour solution is inserted in each load and a colour change from red to green indicates proper sterilization)

Moist heat

Moist heat is divided into three forms

1.Temperature below 100^0C
2.At a temperature of 100^0C
3.Temperature above 100^0C

Temperatures below 100^0C

1. Pasteurization

2. Vaccine bath

3. Water bath

4. Inspissation

5. Low temperature steam-formaldehyde (LTSF) sterilization

Pasteurization

¬ Milk is sterilised by this method; Two methods

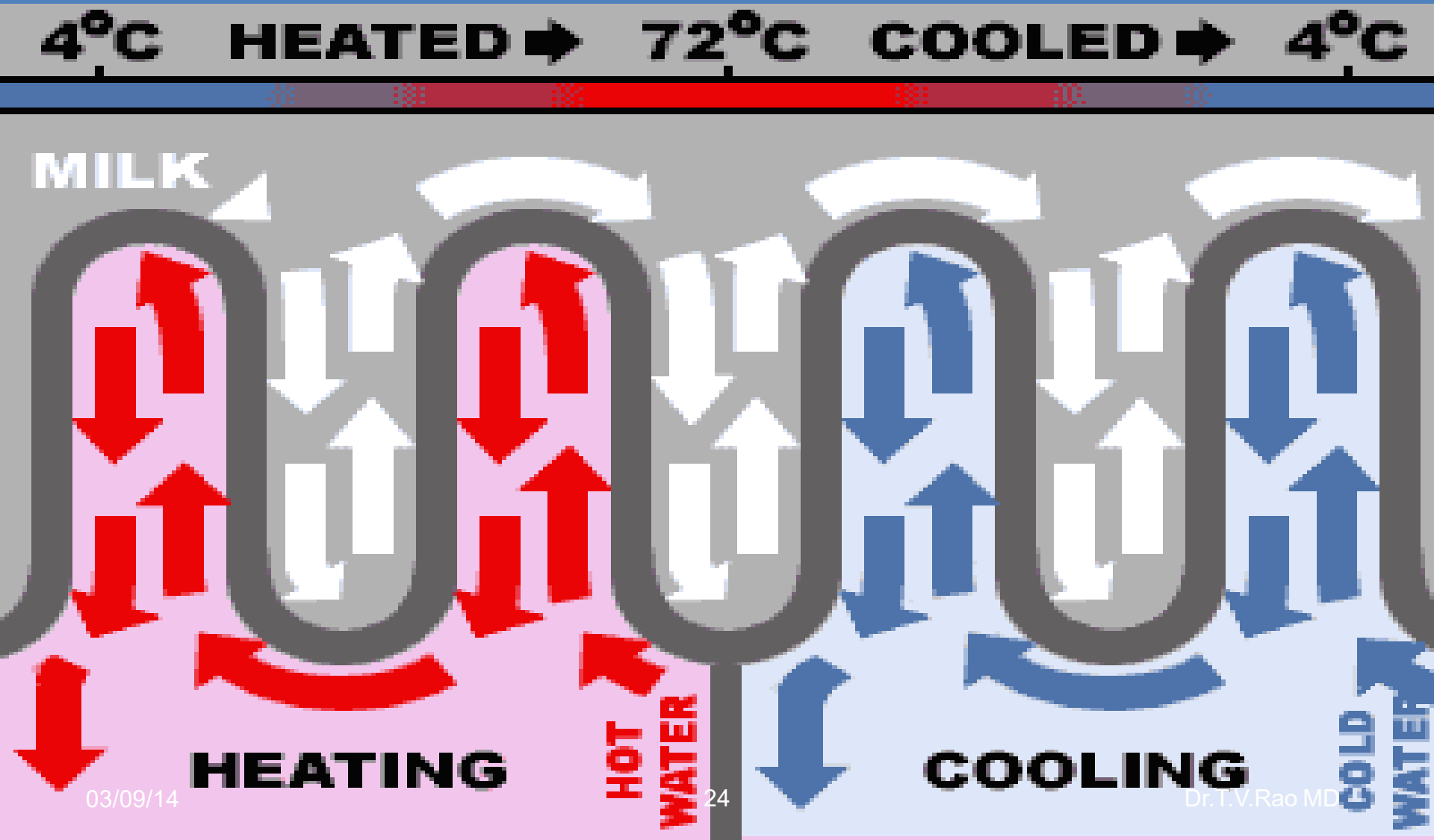
1. Holder method (63^{0}C for 30 min followed by rapid cooling to 13^{0}C or lower)
2. Flash method (72^{0}C for 15-20 seconds followed by rapid cooling to 13^{0}C or lower)

¬The dairy industry sometimes uses ultrahigh temperature (UHT) sterilization (140 to 150^{0}C for 1-3 seconds followed by rapid cooling to 13^{0}C or lower)

¬All nonsporing pathogens such as mycobacteria, brucellae and salmonellae are destroyed by these processes

¬*Coxiella burnetii* is relatively heat resistant and may survive the holder method

Principle of Pasteurization



Vaccine bath

Vaccines prepared from nonsporing bacteria may be inactivated in a water bath at 60^0C for 1 hour

Water bath

Serum or body fluids containing coagulable proteins can be sterilized by heating for 1 hour at 56^0C on several successive days

Inspissation

Media containing egg or serum such as Lowenstein-Jensen and Loeffler's serum slope are rendered sterile by heating at 80-85^{0}C for 30 min on three successive days

This process is called inspissation and instrument used is called inspissator

LTSF sterilization

Used for sterilizing items which cannot withstand the temperatue of 100^{0}C

In this method steam at 75^{0}C with formaldehyde vapor is used

Inspissator

Water bath

Temperature at 100°C

1. Boiling
2. Tyndallisation
3. Steam sterilisation

Boiling at 100^0C

- Boiling at 100^0C for 10-30 min kills all vegetative bacteria and some bacterial spores
- Sporing bacteria required prolonged periods of boiling
- Therefore, it is not recommended for sterilization of surgical instruments
- Addition of 2% sodium bicarbonate may promote sterilization
- For the disinfection of medical and surgical equipment – when sterility is not essential in emergency or under field conditions

steam sterilizer at 100^0C

- Steam at normal atmosheric pressure is at 100^0C
- Used to sterilize heat-labile culture media
- A Koch or Arnold steam sterilizer is used
- It consists of a vertical metal cylinder with a removable conical lid
- Single exposure to steam for 90 min ensures complete sterilization

Tyndallisation

An exposure of steam100^{0}C for 20 min on three consecutive days is known as Tyndallization or intermittent sterilisation

The instrument used is Koch or Arnold steam sterilizer

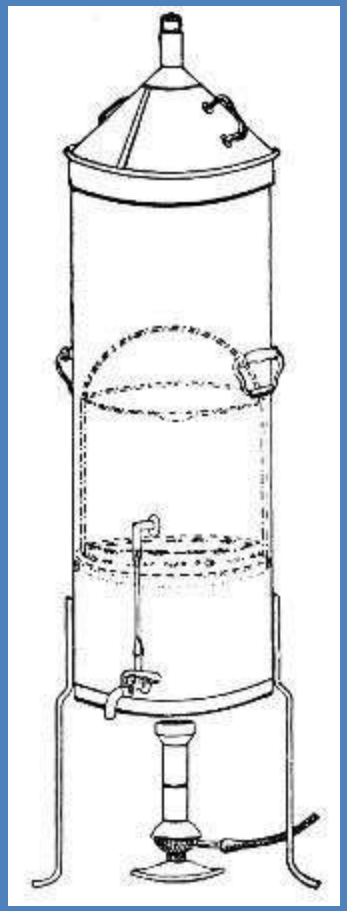
Principle

First exposure kills all the vegetative forms, and in the intervals between the heatings the remaining spores germinate into vegetative forms which are killed on subsequent heating

Uses

Used for sterilisation of egg, serum or sugar containing media

Koch or Arnold steam sterilizer



Temperature above 100^0C

Steam under pressure

Saturated steam is more efficient sterilizing agent than hot air because

1. Bacteria are more susceptible to moist heat as bacterial protein coagulates rapidly.
2. Saturated steam can penetrate porous material easily.

3. When steam comes into contact with a cooler surface it condenses to water and liberates its latent heat to that Surface.
4. The condensed water produces moist conditions for killing the microbes present.

for example, 1600 ml of steam at 100°C and at atmospheric pressure condenses into one ml of water at 100°C and releases 518 calories of heat.

Autoclave

Sterilization conditions

Temperature - 121 °C
Chamber pressure - 15 lb per square inch
Holding time - 15 minutes

These conditions are generally used.

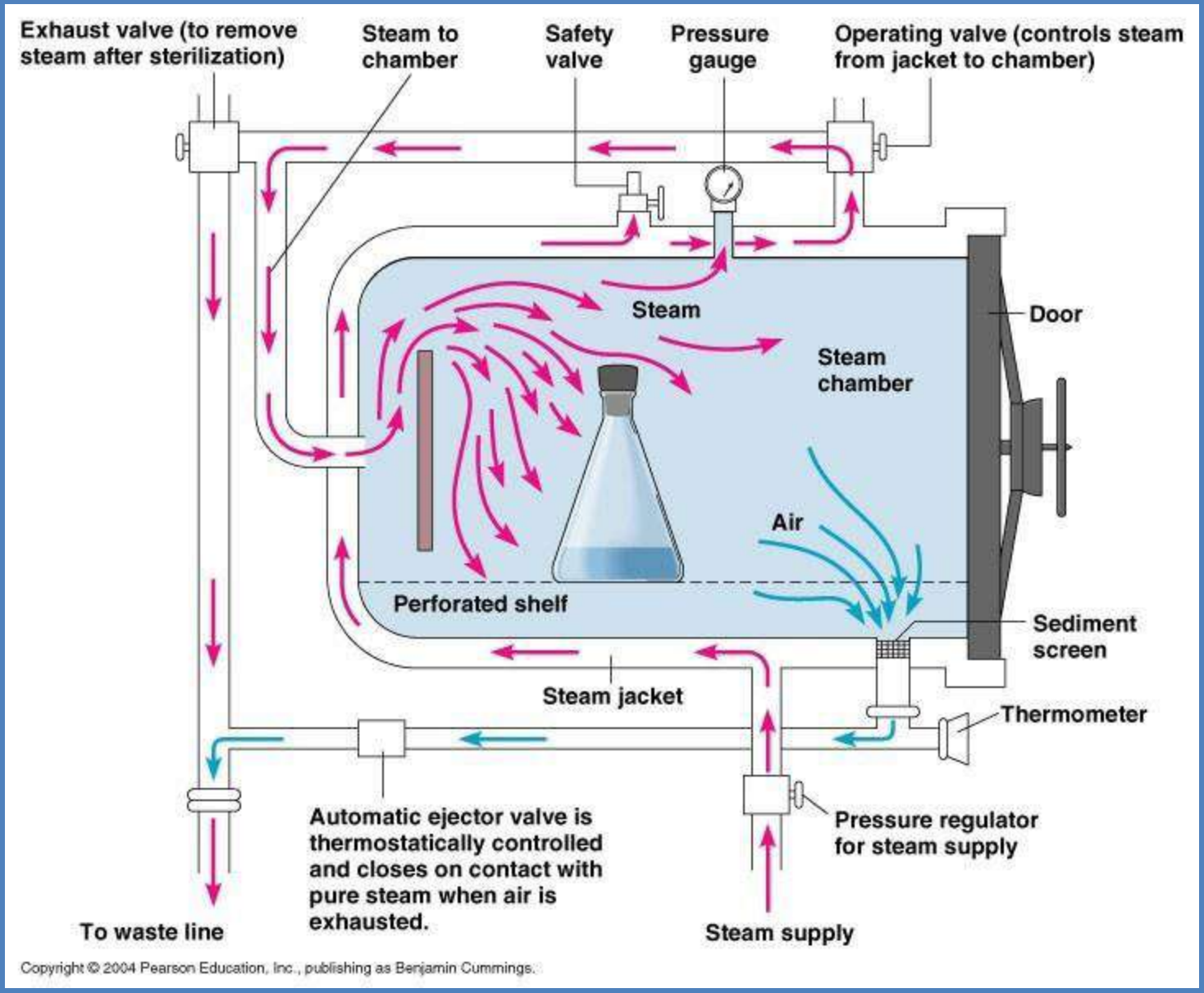
however, sterilization can also be done at higher temperatures, at:

126°C (20 lbs/square inch) for 10 minutes

133°C (30 lbs/square inch) for 3 minutes.

Components of autoclave:

- Consists of vertical or horizontal cylinder of gunmetal or stainless steel.
- Lid is fastened by screw clamps and rendered air tight by an asbestos washer.
- Lid bears a discharge tap for air and steam, a pressure gauge and a safety valve.




TECHMECH
GROUP

Precautions

1. All the air must be removed from the autoclave chamber

2. Materials should be arranged in such a manner which ensures free circulation of steam inside the chamber

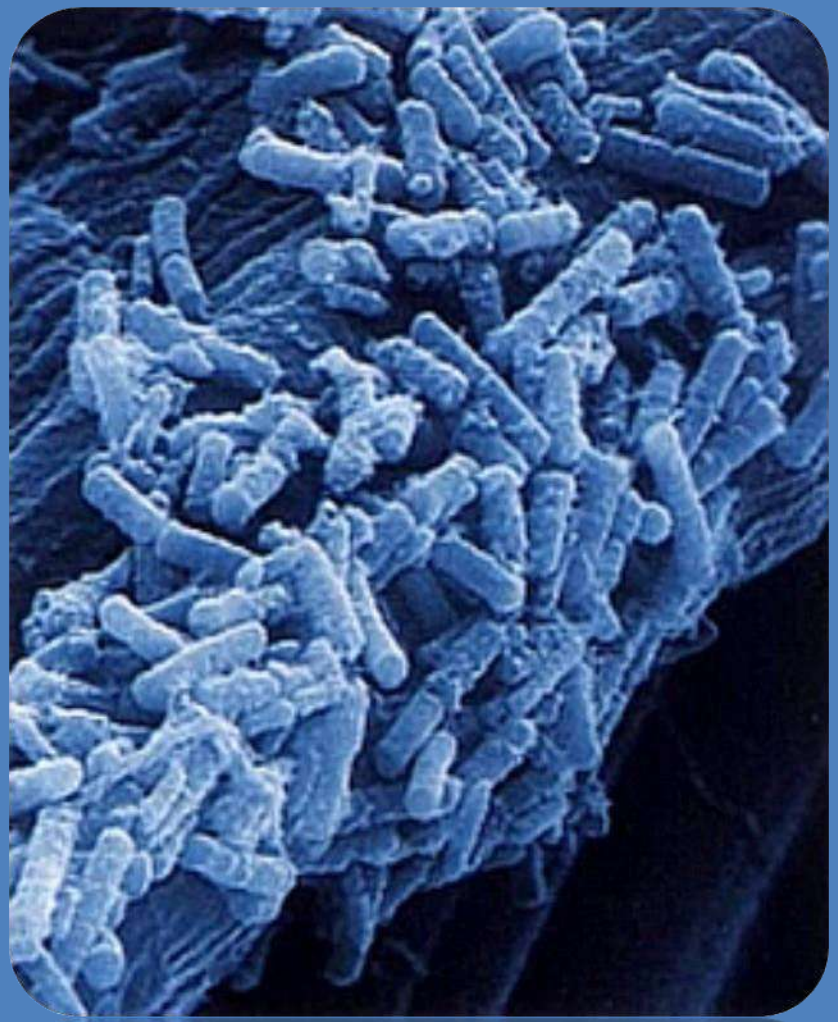
3. Lid should not open until inside pressure reaches to the atmospheric pressure

Uses :

1. To sterilize culture media, rubber material, gowns, dressings, gloves, instruments and pharmaceutical products
2. For all materials that are water containing, permeable or wettable and not liable to be damaged by the process
3. Useful for materials which cannot withstand the high temperature of hot air oven

Sterilisation controls

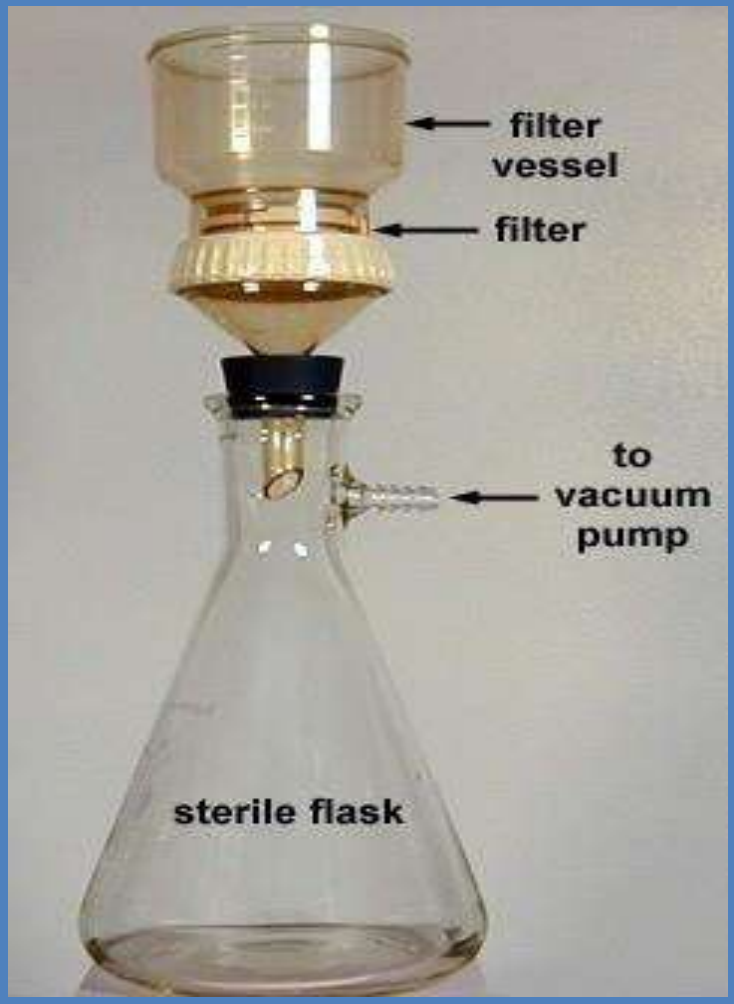
1. Thermocouples
2. Bacterial spores- ***Bacillus stearothermophilus***
3. Browne's tube
4. Autoclave tapes



Filtration

Sterilize solutions that may be damaged or denatured by high temperatures or chemical agents

Used for the sterilization of heat labile materials such as sera, sugar solutions, and antibiotics



Types of Filters

1. Earthenware filters
   (Candle filters)
2. Asbestos disc
   (Seitz) filters
3. Sintered glass filters
4. Membrane filters
5. Syringe filters
6. Air filters

Earthenware filters

- ✓Manufactured in several different grades of porosity
- ✓Used widely for purification of water for industrial and drinking purposes
- ✓They are of two types

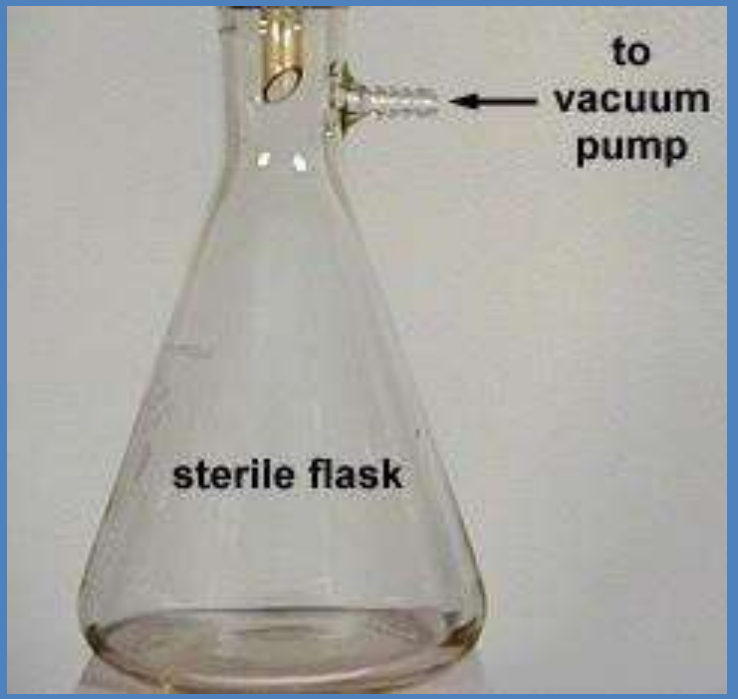
1. Unglazed ceramic filters
   eg: Chamberland and Doulton filters
2. Compressed diatomaceous earth filters eg: Berkefeld and Mandler filters

Earthenware (Candle) filters

Asbestos filters

- ✓ Made up of a disc of asbestos (magnesium trisilicate)
- ✓ Discs are available with different grades of porosity
- ✓ The filter disc is supported on a metal mount.
- ✓ The filter is attached to a vacuum flask through a silicone rubber bung. After use, the filter disc is discarded .
- ✓ Each time a fresh disc is used and the outfit is sterilized by autoclaving .

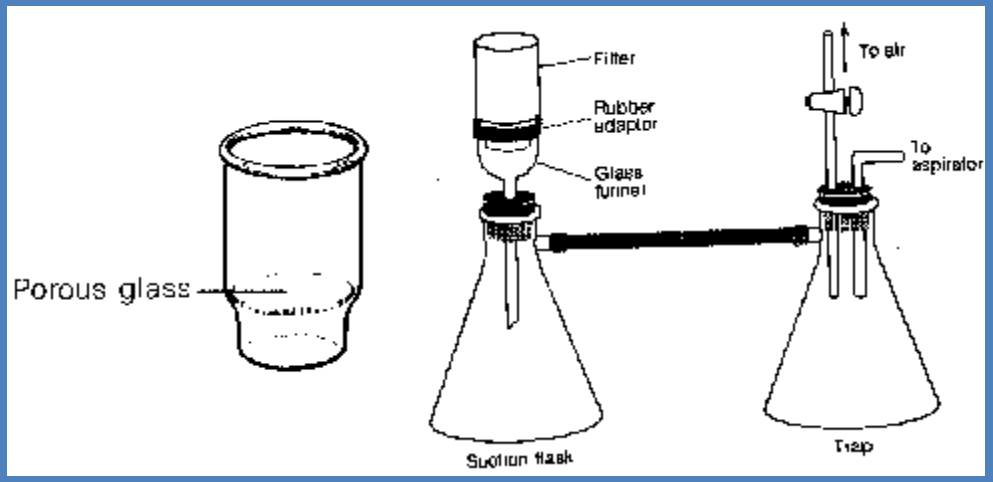
Asbestos Filter holder

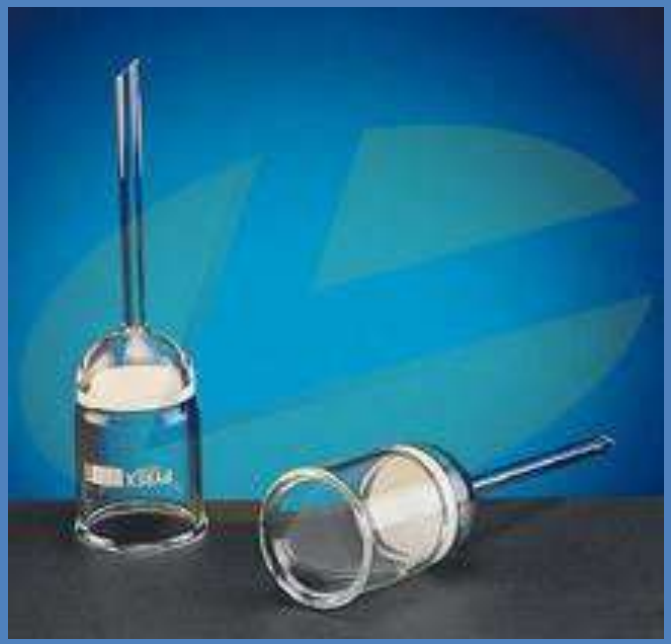


Sintered glass filters

- Prepared by fusing finely powdered glass particles
- Available in different pore sizes
- Pore size can be controlled by the general particle size of the glass powder
- The filters are easily cleaned, have low absorption properties
- But they are fragile and relatively expensive

Sintered glass filter

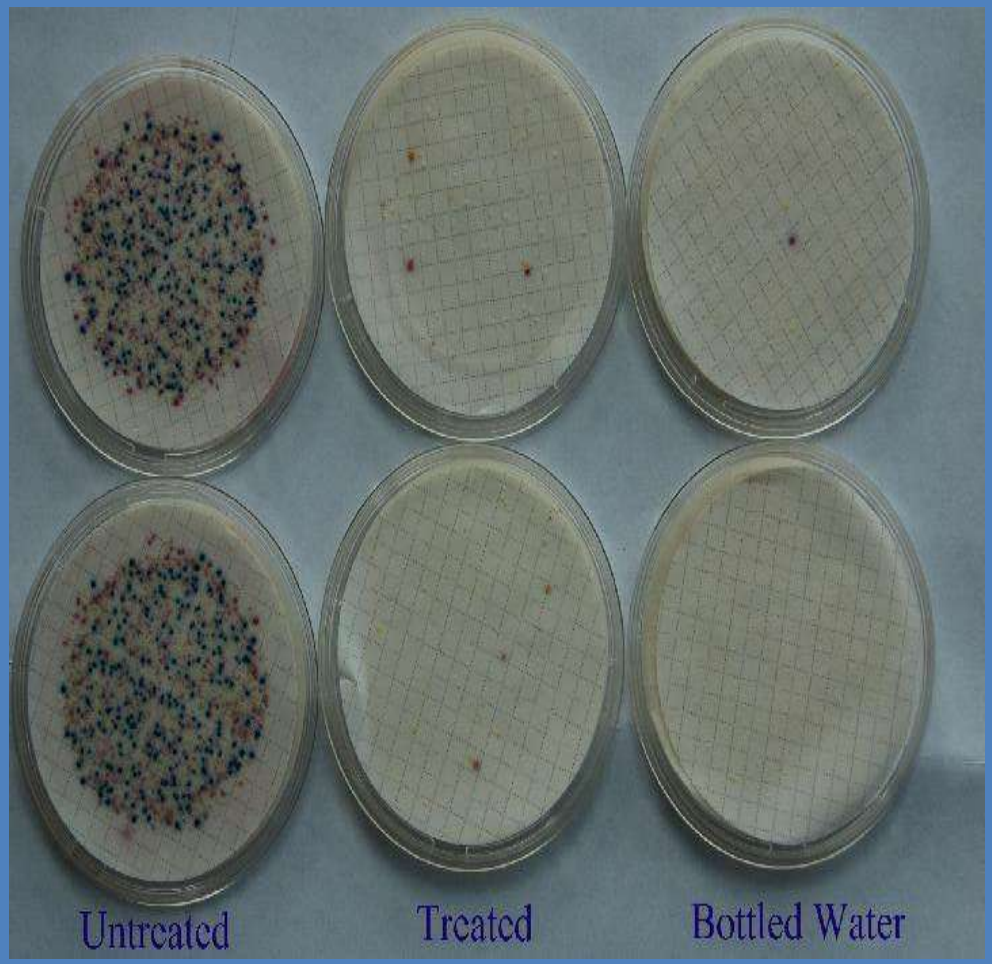




Membrane filters

- ✓ Made of variety of polymeric materials such as cellulose nitrate, cellulose diacetate, polycarbonate and polyester
- ✓ Membrane filters are available in pore sizes of 0.015 to 12 µm
- ✓ The 0.22 µm filter is most commonly used because the pore size is smaller than bacteria
- ✓ These are routinely used in water analysis, bacterial counts of water, sterility testing, and for the preparation of solutions for parenteral use

Membrane filters



Syringe filters

- Syringes fitted with membrane filters of different pore sizes are available
- For sterilization, the fluid is forced through the the disc (membrane) by pressing the piston of the syringe

Air filters

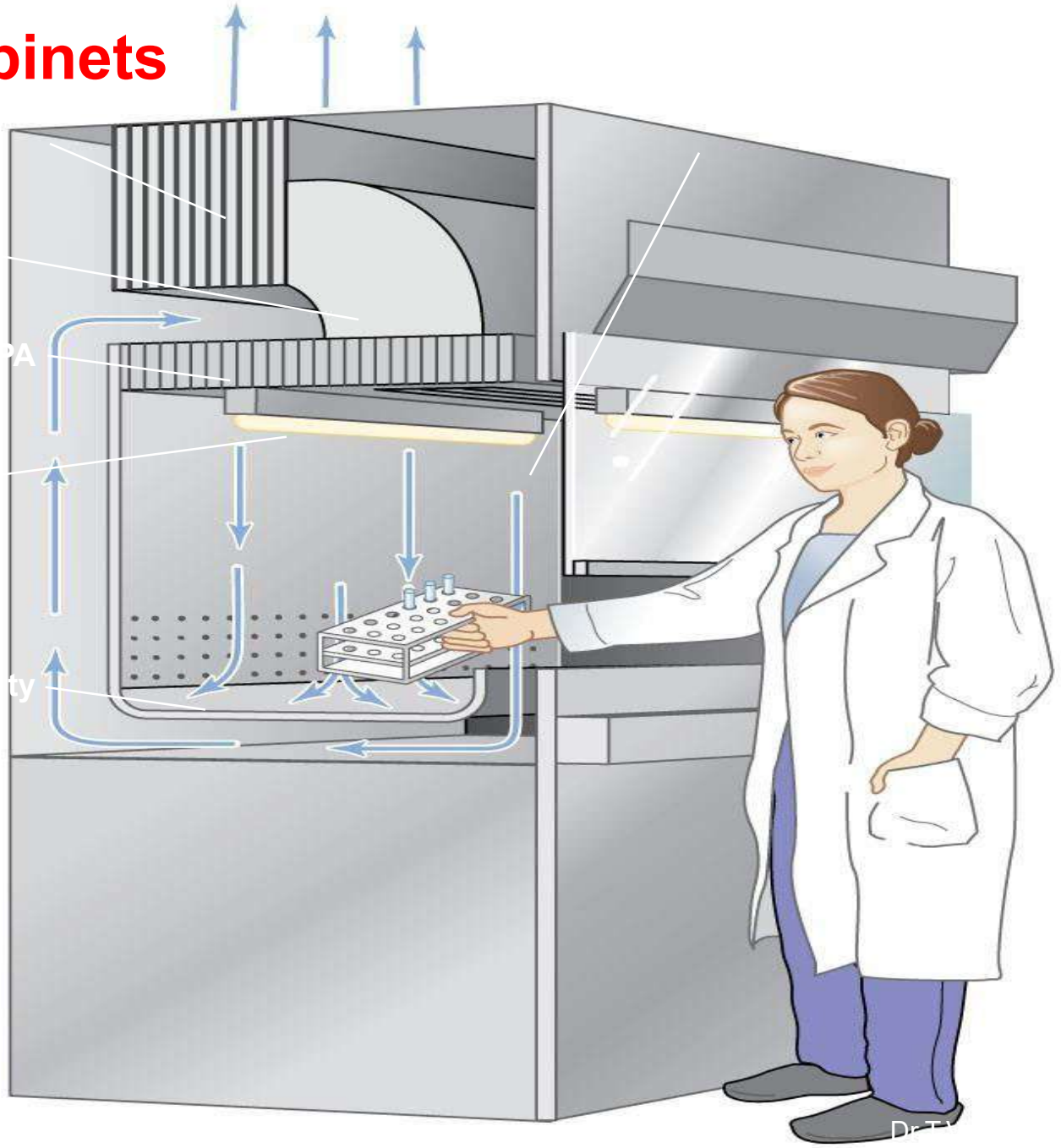
¬Air can also be sterilized by filtration

¬Large volumes of air may be rapidly freed from infection by passage through high efficiency particulate air (HEPA) filters

¬They are used in laminar air flow system in microbiology laboratories

¬HEPA filters can remove particles of 0.3 μm or larger

Bio-safety cabinets



Radiation

Two types of radiations are used for sterilization

1. Non-ionising
2. Ionising

Non-ionising radiations

- These inculde infrared and ultraviolet (UV) radiations

- Infrared is used for rapid mass sterilisation of syringes and catheters

- UV radiation with wavelength of 240-280 nm has marked bactericidal activity

- It acts by denaturation of bacterial protein and interference with DNA replication (produces thymine dimers)
- They can penetrate only a few mm into liquids and not at all into solids
- UV radiation is used for disinfecting enclosed areas such as bacteriological laboratory, inoculation hoods, laminar flow and operation theatres
- Most vegetative bacteria are susceptible but spores are highly resistant
- Susceptibility of viruses is variable
- Source of UV radiations must be shielded otherwise causes damage to skin and eyes.

Ionising radiations

- These include X-rays, Y (gamma) rays and cosmic rays
- Possess high penetrative power and are highly lethal to all cells including bacteria
- They damage DNA by various mechanisms
- Gamma radiations are used for sterilization of disposable items such as plastic syringes, swabs, culture plates, cannulas, catheters etc

- Since theres is no appreciable increase in the temperature, in this method it is known as “cold sterilisation”

- Large commercial plants use gamma radiation emitted from a radioactive element, usually cobalt 60

- The advantage of this method include speed, high penetrating power (it can sterilise materials through outer packages and wrappings)

- *Bacillus pumilis* used to test the efficacy of ionizing radiations

Chemical methods

A variety of chemical agents are used as antiseptics and disinfectants. An ideal antiseptic or disinfectant should

1) Be fast acting in presence of organic substances
2) Be effective against all types of infectious agents without destroying tissue or acting as a poison if ingested
3) Easily penetrate material to be disinfected, without damaging/discoloring it
4) Be easy to prepare, stable when exposed to light, heat or other environ-mental factors
5) Be inexpensive, easy to obtain and use
6) Not have an unpleasant odor

Chemical agents act in various ways. The main modes of action are

1. Protein coagulation
2. Distruption of cell membrane
3. Removal of free sulphydryl groups
4. Substrate competition

Factors that determine the potency of disinfectants are

a) Concentration of the substance
b) Time of action
c) pH of the medium
d) Temperature
e) Nature of organism
f) Presence of organic matter

Disinfectants can be divided into three groups

1. High level disinfectants
   (Glutaraldehyde, hydrogen peroxide, peracetic acid and chlorine compounds)

2. Intermediate level disinfectants
   (Alcohol, iodophores and phenolic compounds)

3. Low level disinfectants
   (Quarternary ammonium compounds)

Alcohols

- Ethanol and isopropanol are the most frequently used
- Used as skin antiseptics and act by denaturing bacterial proteins
- Rapidly kill bacteria including tubercle bacilli but they have no sporicidal or virucidal activity
- 60-70% is most effective
- Isopropyl alcohol is preferred to ethyl alcohol as it is a better fat solvent, more bactericidal and less volatile
- Methyl alcohol is effective against fungal spores

Aldehydes

Two aldehydes (formaldehyde and glutaraldehyde) are currently of considerable importance

Formaldehyde

- Formaldehyde is active against the aminogroup in the protein molecules
- It is lethal to bacteria and their spores, viruses and fungi
- It is employed in the liquid and vapor states
- A 10% aqueous solution is routinely used

Uses

- To sterilise bacterial vaccines
- 10% formalin containing 0.5% sodium tetraborate is used to sterilize clean metal instruments
- Formaldehyde gas is used for sterilizing instruments, heat sensitive catheters and for fumigating wards, sick rooms and laboratories

Glutaraldehyde

- Action similar to formaldehyde
- More active and less toxic than formaldehyde
- It is used as 2% buffered solution
- It is available commercially as ‘cidex’

Uses

- For sterilization of cystoscopes, endoscopes and bronchoscopes
- To sterilize plastic endotracheal tubes, face masks, corrugated rubber anaesthetic tubes and metal instruments

Phenols

- Obtained by distillation of coal tar between temperatures of 170^0C and 270^0C
- Lethal effect is due to cell membrane damage
- Phenol (1%) has bactericidal action
- Phenol derivatives like cresol, chlorhexidine and hexachlorophane are commonly used as antiseptics

Cresols

- Lysol is a solution of cresols in soap
- Most commonly used for sterilization of infected glasswares, cleaning floors, disinfection of excreta

Chlorhexidine

- Savlon (Chlorhexidine and cetrimide) is widely used in wounds, pre-operative disinfection of skin
- More active against Gram positive than Gram negative bacteria
- No action against tubercle bacilli or spores and have very little activity against viruses
- Has a good fungicidal activity

Halogens

- Chlorine and iodine are two commonly used disinfectants
- They are bactericidal and are effective against sporing bacteria and viruses
- Chlorine is used in water supplies, swimming pools, food and dairy industries
- Chlorine is used in the form of bleaching powder, sodium-hypochlorite and chloramine
- Hypochlorites have a bactericidal, fungicidal, virucidal and sporicidal action
- Bleaching powder or hypochlorite solution are the most widely used for HIV infected material
- Chloramines are used as antiseptics for dressing wounds

Iodine

- Iodine in aqueous and alcoholic solution used as skin disinfectant
- Iodine often has been applied as tincture of iodine (2% iodine in a water-ethanol solution of potassium iodide)
- Actively bactericidal, moderate action against spores
- Also active against the tubercle bacteria and viruses
- Compounds of iodine with surface active agents known as iodophores
- Used in hospitals for preoperative skin degerming
- Povidine-iodine (Betadine) for wounds and *Wescodyne* for skin and laboratory disinfection are some examples of iodophores

Dyes

¬Aniline and acridine dyes are used extensively as skin and wound antiseptics

¬Aniline dyes include crystal violet, brilliant green, and malachite green are more active against gram positive organisms

¬No activity against tubercle bacilli

¬They interfere with the synthesis of peptidoglycan of the cell wall

¬Their activity is inhibited by organic material such as pus

¬Acridine dyes also more active against gram positive organisms

¬Acridine dyes affected very little by the presence of organic matter

- More important dyes are proflavine, acriflavine, euflavine and aminacrine
- They interfere with the synthesis of nucleic acids and proteins in bacterial cells

Metallic salts

- Salts of silver, copper and mercury are used as disinfectants
- Protein coagulants and have the capacity to combine with free sulphydryl groups
- The organic compounds thiomersal, phenyl mercury nitrate and mercurochrome are less toxic and are used as mild antisepics
- Copper salts are used as fungicides

Surface active agents

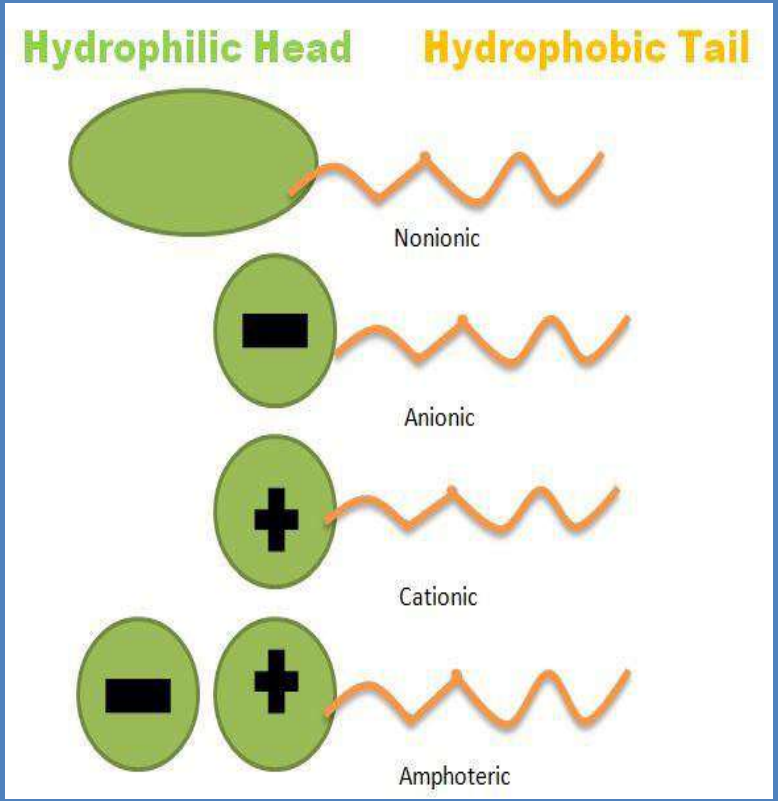
1. Anionic
   Common soaps, have strong detergent but weak antimicrobial properties

2. Cationic
   Act on phosphate group of the cell membrane

   Eg: Quaternary ammonium compounds such as benzalkonium chloride and cetrimide (acetyl trimethyl ammonium bromide)

3. Nonionic

4. Amphoteric (Tego compounds)



Stratia
Lab Notes

SURFACTANTS
(surface active agents)

TYPE	DEFINITION	EXAMPLES
NON-IONIC 	- No charge whatsoever - Non-ionic detergents are super harsh and rarely seen in skincare - More commonly found as emulsifiers	- Polysorbates - Sorbitans - PEGs - Laureth-[*number*]s
ANIONIC 	- Strong negative charge - Extremely effective, but can also be harsh - Higher incidence of irritation - Lathers well and makes a lot of foam	- Soaps - Sodium lauryl sulfate (SLS) - Sodium lauryl sulfate (SLS)
CATIONIC 	- Strong positive charge - Cationic detergents are extremely harsh - Cationic emulsifiers are much more common in beauty products	Detergents - Benzalkonium chloride - Cetrimonium bromide Emulsifiers - Ending in "-quat"
AMPHOTERIC 	- Has both positive and negative charge - Final charge depends on the pH - Milder and less irritating but foam less	- Cocoamidpropyl betaine - Sodium cocoamphoacetate

Vapour Phase Disinfectants

Formaldehyde gas

- Employed for fumigation of heat-sensitive equipment (anaesthetic machine and baby incubators), operation theatres, wards and laboratories etc

- Formaldehyde gas is generated by adding 150 gm of $KMnO_4$ to 280 ml of formalin for 1000 cubic feet of room volume

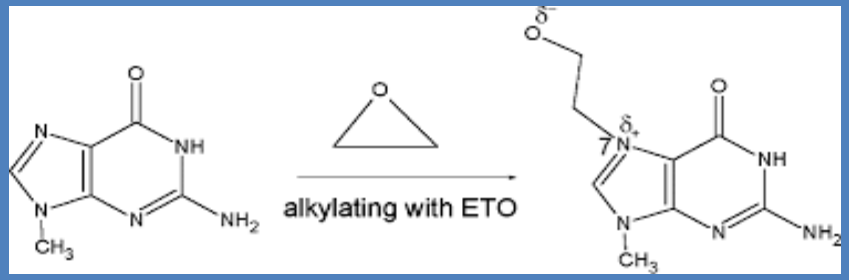
- This reaction produces considerable heat and so heat resistant containers should be used

- Sterilisation is achieved by condensation of gas on exposed surfaces

- After completion of disinfection, the effect of irritant vapours should be nullified by exposure to ammonia vapour

Ethylene oxide (ETO)

- Colourless liquid with a boiling point of 10.7^0C
- Highly lethal to all kinds of microbes including spores
- Action is due to its alkylating the amino, carboxyl, hydroxyl and sulphydryl groups in protein molecules
- In addition it reacts with DNA and RNA
- Highly inflammable and in concentrations (>3%) highly explosive
- By mixing with inert gases such as CO_2, its explosive tendency can be eliminated



Uses

- Used for sterilising plastic and rubber articles, respirators, heart-lung machines, sutures, dental equipments and clothing
- It is commercially used to sterilise disposable plastic syringes, petridishes etc
- It has a high penetrating power and thus can sterilise prepackaged materials
- *Bacillus globigi* (a red pigmented variant of *B. subtilis*) has been used as a biological control fot testing of ETO sterilisers

Betapropiolactone (BPL)

- Condensation product of ketane and formaldehyde
- Boiling point: 163^0C
- Has low penetrating power but has a rapid action
- For sterilization of biological products 0.2% BPL is used
- Capable of killing all microorganisms and is very active against viruses

Uses

In the liquid form it has been used to sterilize vaccines and sera

Recent vapor phase disinfectants

Hydrogen peroxide

- Used to decontaminate biological safety cabinets

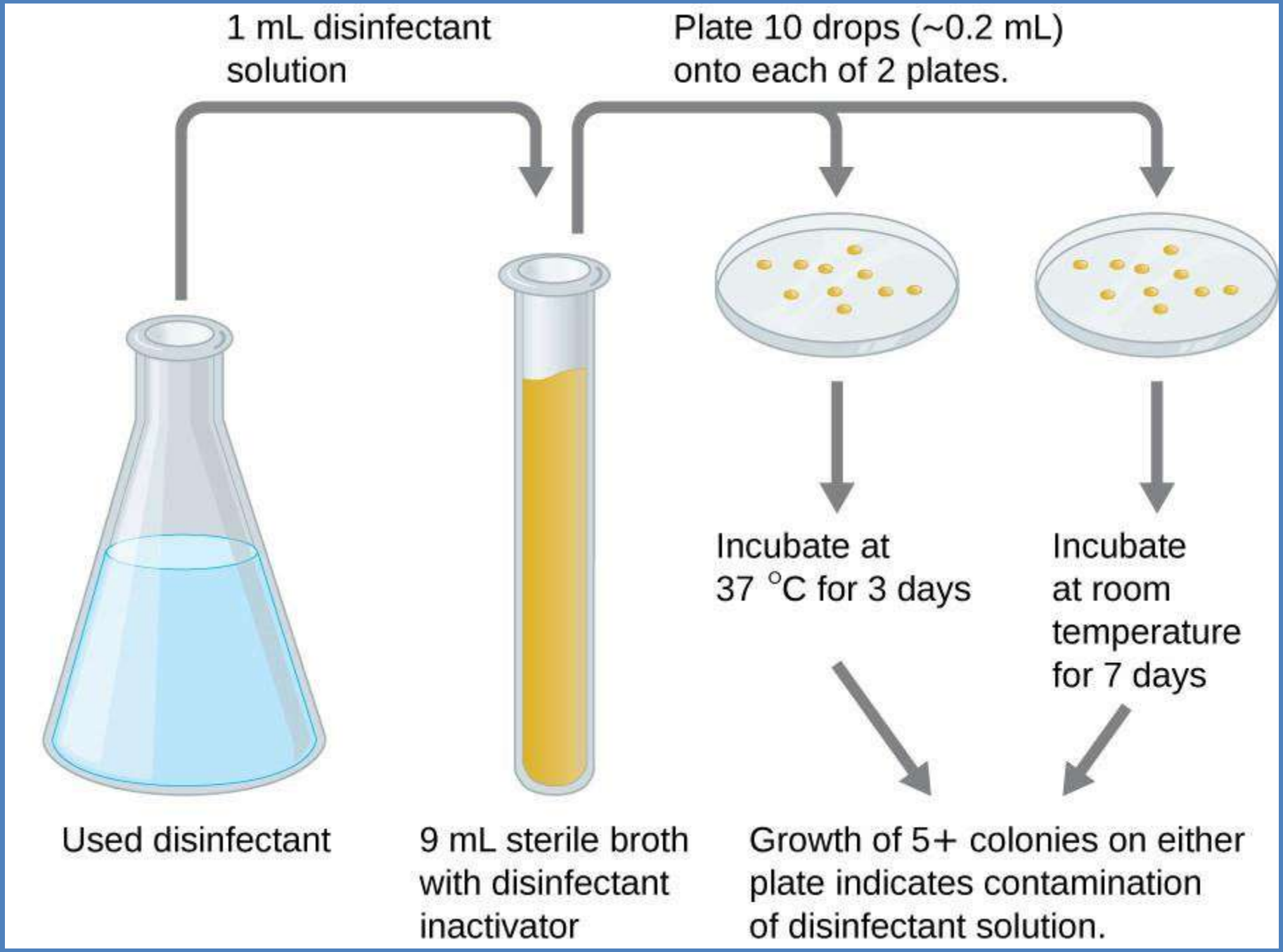
Peracetic acid

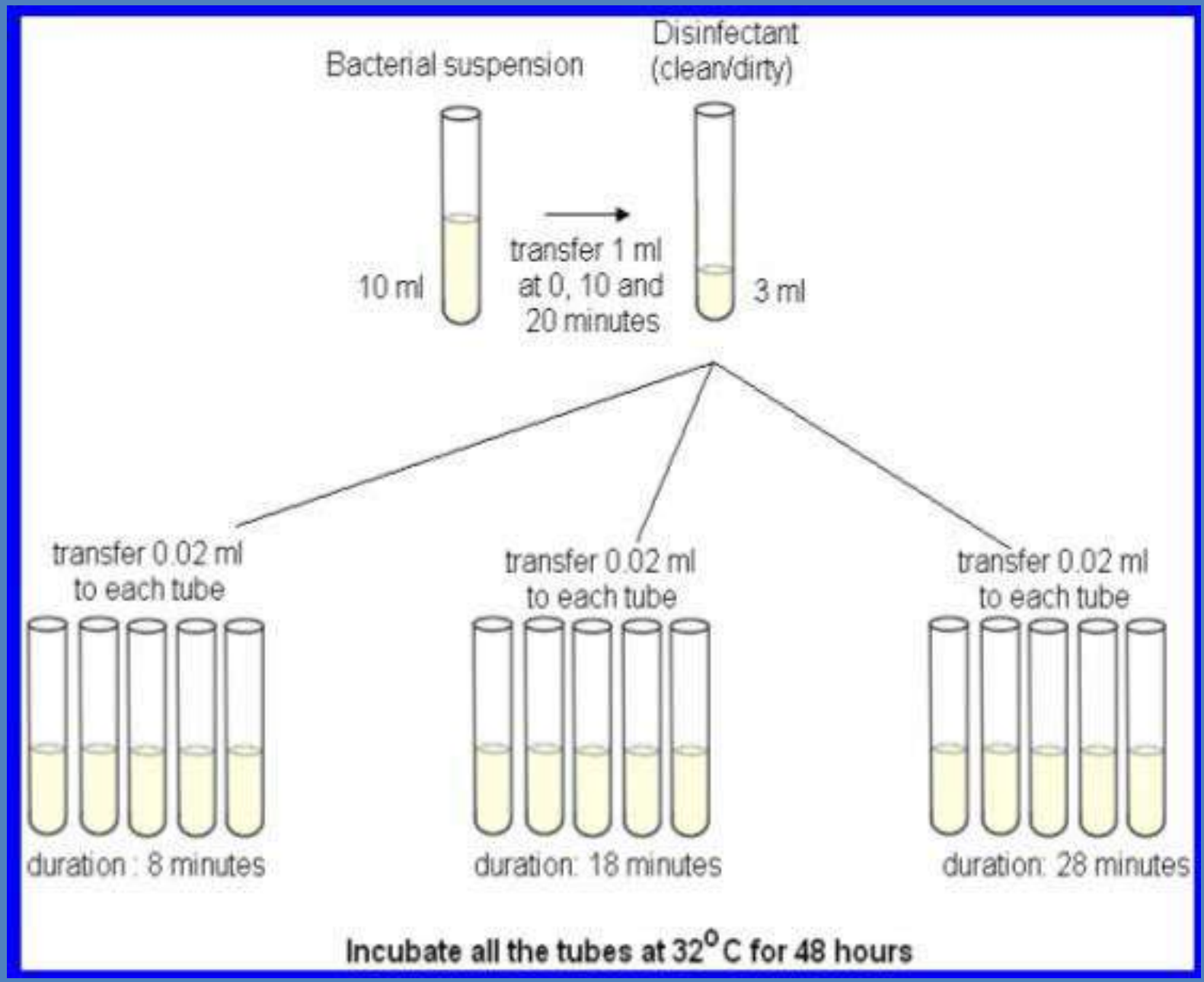
- It ia an oxidising agent
- One of the high level disinfectants
- Used in plasma sterilizers

Testing of disinfectants

1. Minimum inhibitory concentration (MIC)
2. Rideal Walker test
3. Chick Martin test
4. Capacity test (Kelsey and Sykes test)
5. In-use test

	Rideal -Walker	Chick-Martin
Volume medium	5.0 ml	10.0 ml
Diluent for test disinfectant	Distilled water	Water with yeast suspension or feces
Reaction temperature	17.5±0.5°C	30°C
Organism	*Salmonella typhi*	*Salmonella typhi, Staphylococcus aureus*
Sampling times	2.5, 5.0, 7.5, 10.0 min.	30.0 min.
Calculation of coefficient	Dilution test killing in 7.5 mins divided by same for phenol	Mean concentration of phenol showing no growth after 30 min. divided by same for test


1 mL disinfectant solution
Plate 10 drops (~0.2 mL) onto each of 2 plates.
Incubate at 37 °C for 3 days
Incubate at room temperature for 7 days
Used disinfectant
9 mL sterile broth with disinfectant inactivator
Growth of 5+ colonies on either plate indicates contamination of disinfectant solution.


Bacterial suspension
Disinfectant
(clean/dirty)
10 ml
transfer 1 ml
at 0, 10 and
20 minutes
3 ml
transfer 0.02 ml
to each tube
transfer 0.02 ml
to each tube
transfer 0.02 ml
to each tube
duration : 8 minutes
duration: 18 minutes
duration: 28 minutes
Incubate all the tubes at 32°C for 48 hours

Sterilisation of prions

Dry heat

360^{0}C for one hour

Moist heat

134-138^{0}C for 18 min

Chemicals

25% sodium hypochlorite for one hour

Sensitive to household bleach, phenol (90%) and iodine disinfectants