BASIC MICROBIOLOGY

RECOMMENDED TEXTBOOK

MICROBIOLOGY - Prescott, Harley and Klein

Fundamentals of Microbiology – Frobisher

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Course Outline:

Introduction to the Science of Microbiology

Nature and kind of microorganisms

Nutrition and growth of microorganisms

Isolation, characterization and staining

Culture of microorganisms

Bacterial characterization

Bacteriophage and nature of viruses

Microbiology is the study of microorganisms.

Microorganisms are minute living creatures that individually are too small to be seen with the naked eye.

Microorganisms include:

- Bacteria
- Fungi (yeast and molds)
- Protozoa
- Cyanobacteria (Blue-green algae)
- Viruses

Microorganisms are found everywhere in our environment

Air

Soil

Water

Majority of microbes do not cause disease

Instead they are used to promote our well-being

This explains the various branches of microbiology.

Medical Microbiology: deals mainly with diseases

Pathogens are disease causing microbes

Microbes can either produce more of itself to cause the problem or produce chemicals (usually called a toxin) to interfere with the normal processes

VIRAL :

Poliomyelitis, AIDS, Hepatitis, Influenza

BACTERIA

Diphtheria, Cholera, Typhoid, Tuberculosis, Leprosy, Legionella

FUNGI: Athlete's foot, Ringworm

Protozoa: Malaria, Cryptosporidium, Giardiasis

CYANOBACTERIA (Blue-green algae): Swimmers, allergies, nephritis.

Food microbiology

Food- Microbes as food: mushrooms, algae, single cell protein (Marmite)

Microbes in food production: cheese, yoghurt, bread, kenkey, wagashi, sauerkraut, beer, wine, vinegar, soya source.

Food processing: canning, pasteurization, freezing, irradiation, packaging, transport, ripening, spoilage (decay and disease).

Agricultural microbiology

Viral, bacterial and fungal diseases of animals and crop plants

Beneficial symbiosis: bacteria and protozoa in ruminants, bacterial nitrogen fixation in legumes, fungal (mycorrhizal) associations with plants, cyanobacteria in *Azolla*.

Composting, ensilaging, biological control of weeds by fungi and insects by bacteria and genetic engineering of plants with *Agrobacterium*.

Applied microbiology

Sewage disposal, oil spill clean ups (bioremediation),

methanogenesis (rubbish tip explosions)

pesticide breakdown

microbial fertilizers, water supply industry, single cell protein , fine chemicals, antibiotics, vitamins, alcohol, flavourings, enzymes, quality control, basis of biotechnology

Microbial ecology

Recycling of nutrients e.g. carbon, nitrogen (nitrogen fixation, proteolysis, ammonification, nitrification, denitrification), sulfur, phosphorus, primary production in oceans and lakes, grazing.

How did it all Start

Louis Pasteur Grape juice went sour on standing for sometime

Heated & Bottled, tightly closed, it remained the same

However left ajar went sour

Certain microbes in the air responsible for the spoilage.

Breakthroughs in microbiology was the development of the Koch's postulates (1881).

Diseased tissues often yield more than one microbes

Consequently, not always obvious which microbe is the cause of disease

Big problem in medical science in the 19th century

Robert Koch Rules-of-proof of causality

Robert Koch – 1843-1910

Koch's Postulates

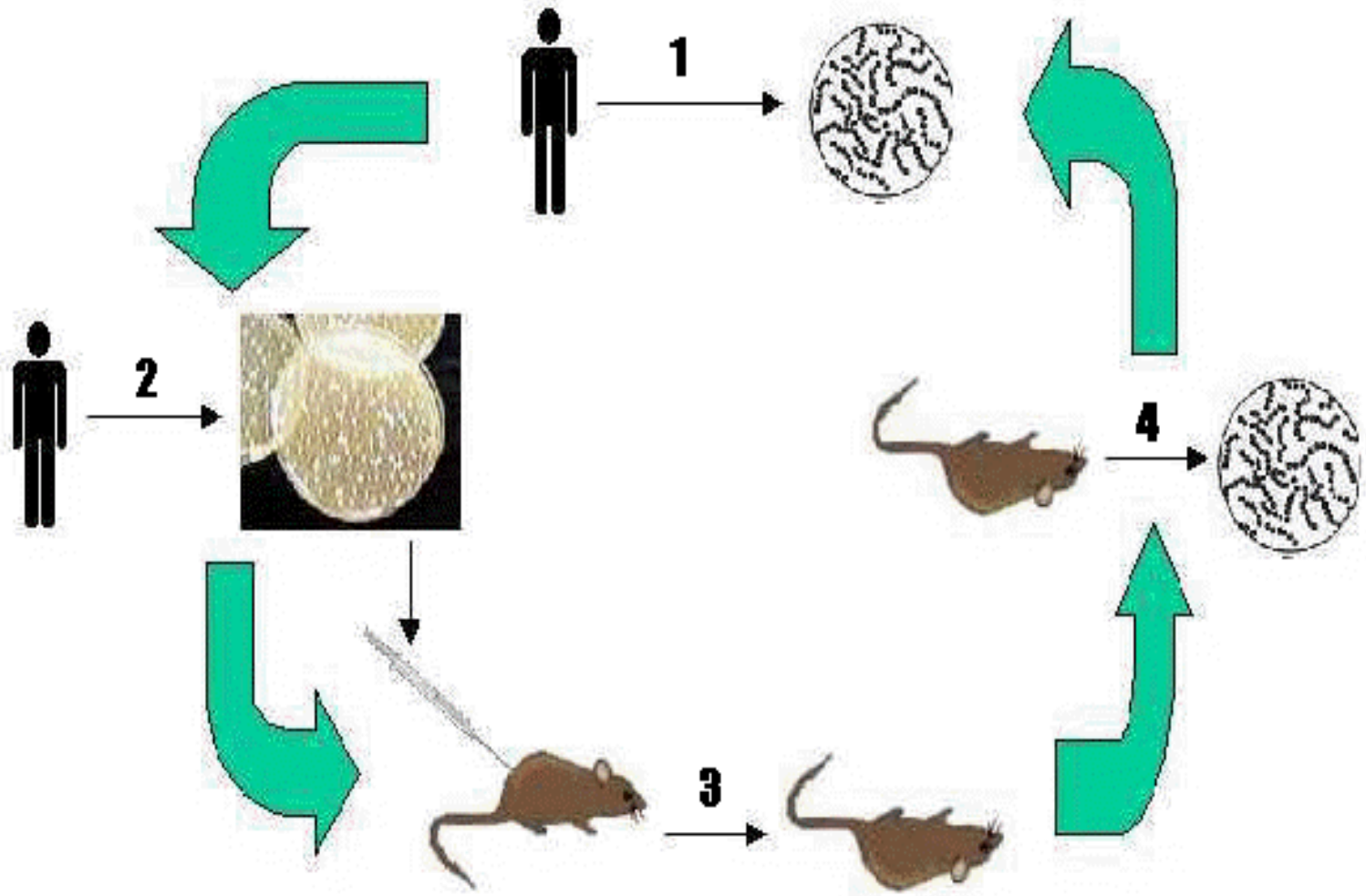
The organism in question must always be found associated with a particular disease

The organism must be isolated and grown in pure culture.

The organism grown in pure culture must be inoculated into a healthy host under favourable conditions and induce a characteristic disease.

The organism must be re-isolated from the second host and compared with the first culture

Both the diseased condition produced by inoculation and the organisms recovered from the inoculated host must correspond to the original diseased condition and to the first organisms isolated, respectively.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NATURE AND KINDS OF MICROBES

Prokaryotic cells (bacteria) differ from eukaryotic cells in several ways

Differences between prokaryotic and eukaryotic cells

Feature	prokaryote	eukaryote
cell size	small $>$ 2 μm	larger than 2 μm
nuclear membrane	-	+
# chromosomes	1	more than 1
mitosis	-	+
organelles	-	chloroplast & mitochondria
endoplasmic reticulum	-	+
cell wall	thin & usually peptidoglycan	thick or absent chemically diverse
cytoplasmic ribosomes	70s	80s
organella ribosomes	-	70s
cilia	-	+
flagella	+	+
	3-threaded helices	9:2 fibril arrangement

NATURE AND KINDS OF MICROBES

Some of these differences are exploited as targets for antibiotics e.g. the cell wall of bacteria.

Cell structures are involved in the cause of disease: flagella, fimbriae and pili, which recognize and stick to cells (adhesions) capsules which prevent phagocytosis

Cell walls which are susceptible to antibiotics and which may act as endotoxins

NATURE AND KINDS OF MICROBES

Bacteria are differentiated

Morphology (Shape)

Chemical composition (staining reactions)

Nutritional requirements

Biochemical activities and some source of energy (sunlight and chemicals)

NATURE AND KINDS OF MICROBES

Three major forms of bacteria

Spherical or round forms

Coccus (Plural- cocci)

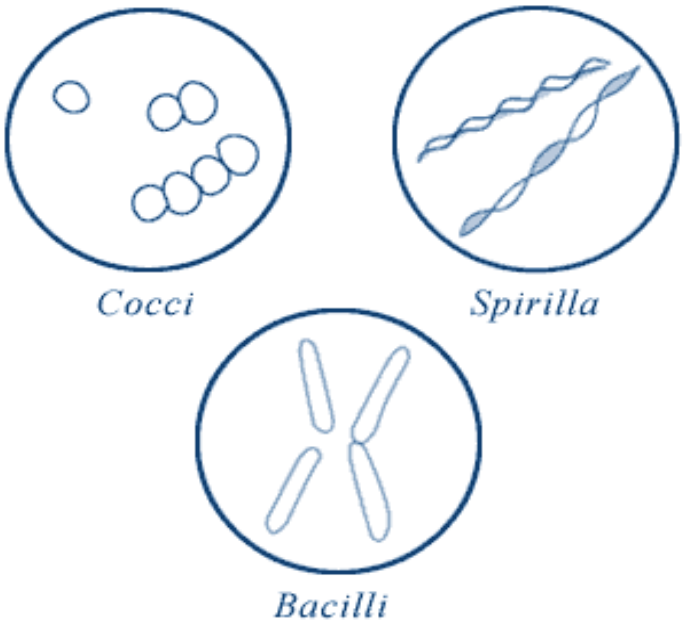
Rod-shaped forms

Bacillus (Plural-Bacilli)

Spiral forms or Spirillum

E.g. *Treponema pallidium*

(Syphilis) like cockscrew



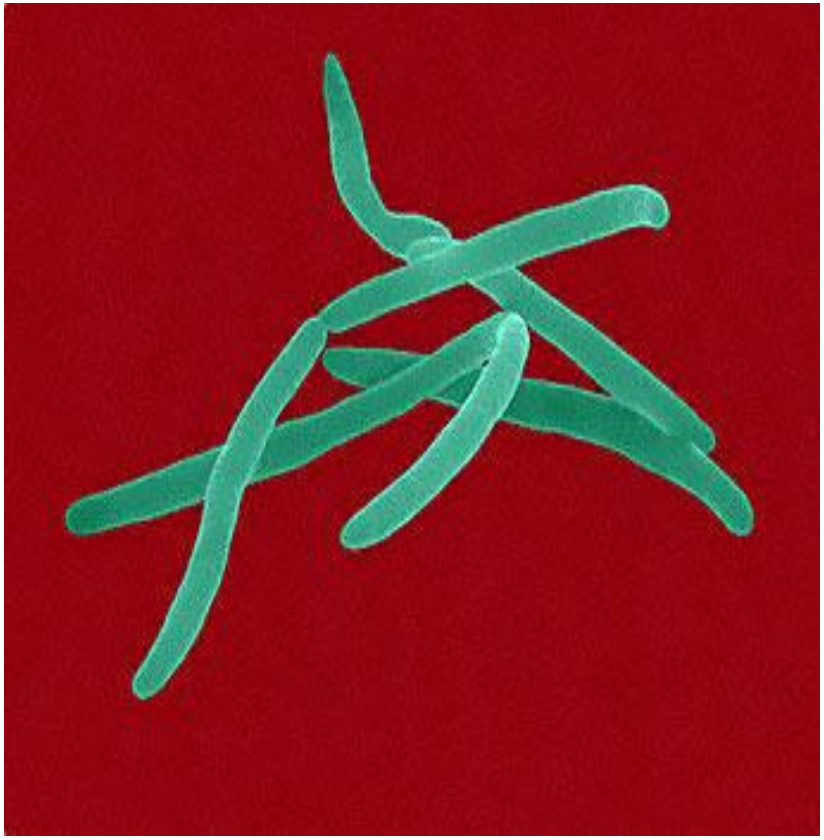
Coccibacillus is neither rod-shaped nor spherical but in between

Fusiform – they are tapered at the ends

Fusobacterium - Filamentous long threads

Vibrios curved at one end like a comma

Fusobacterium cholerae

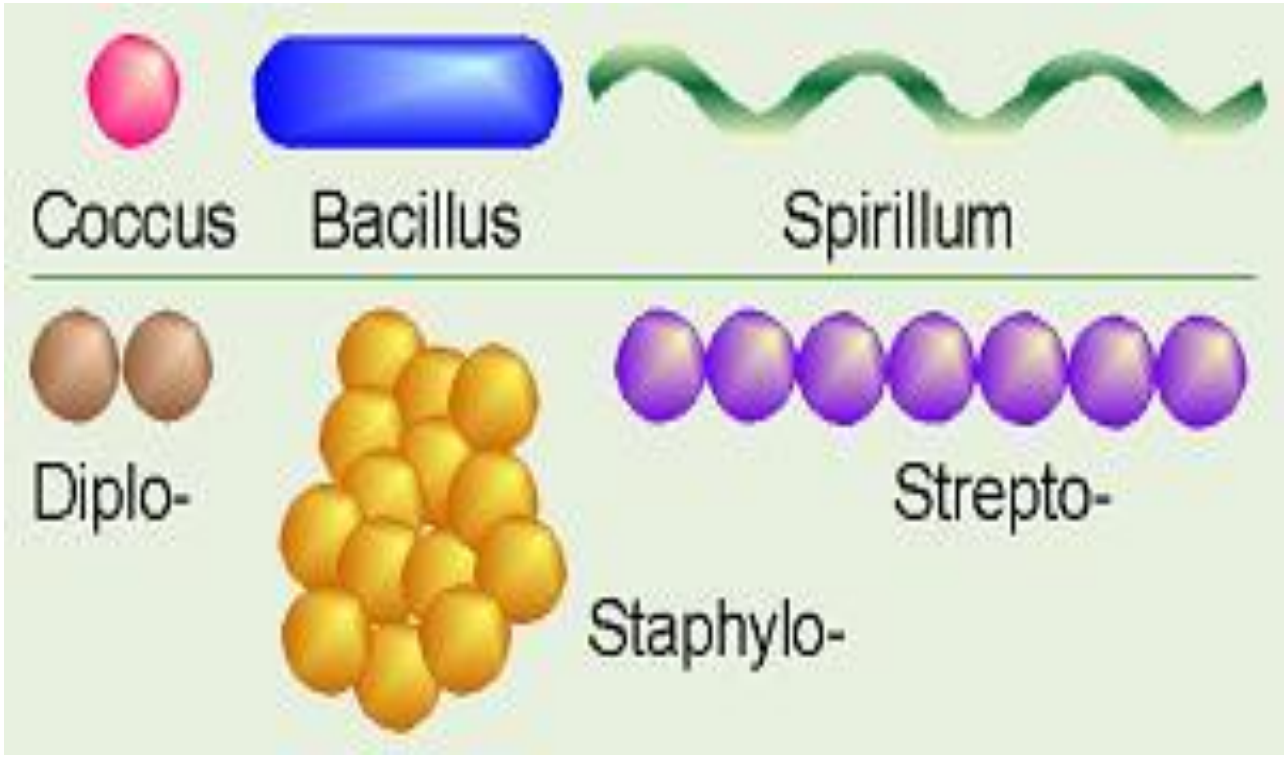


Vibrio

NATURE AND KINDS OF MICROBES

Arrangements:

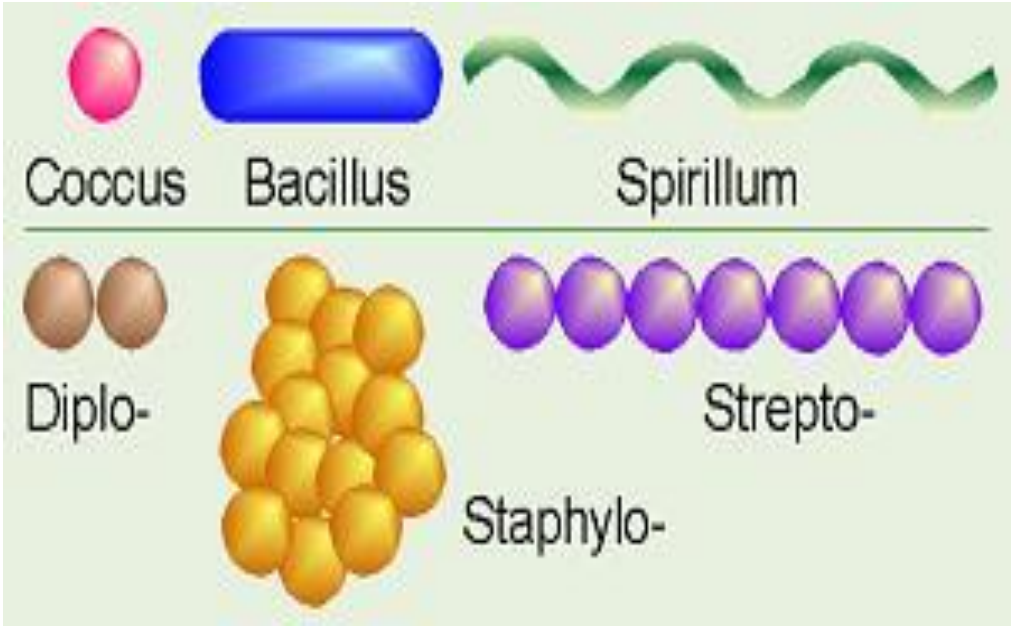
Cocci that divide and remain in pairs after dividing are *Diplococcus*



NATURE AND KINDS OF MICROBES

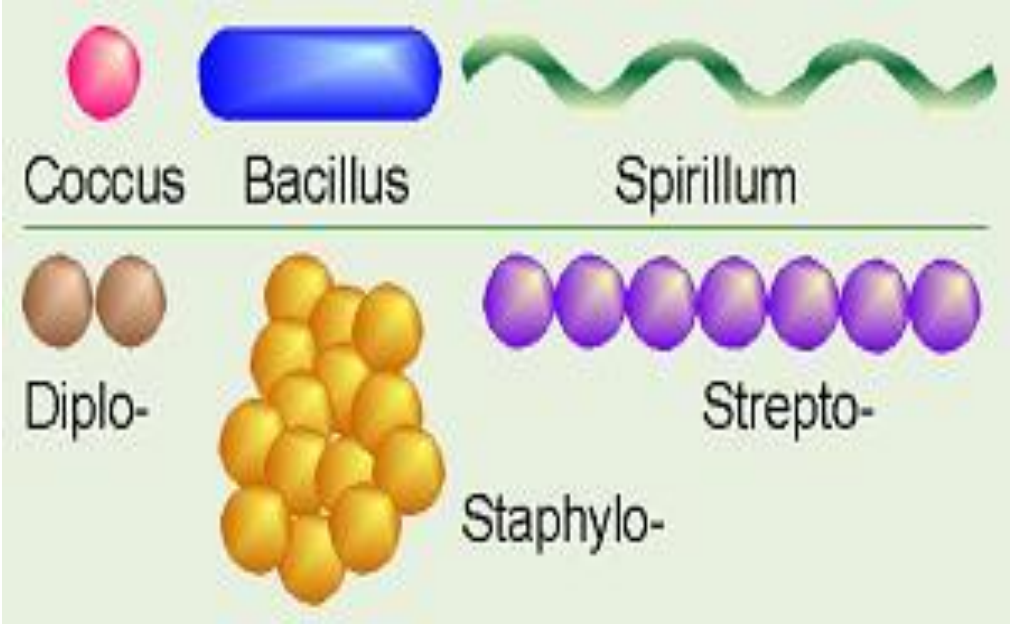
Those that divide and remain attached in chainlike patterns are **Streptococcus**

Those that divide in multiple planes and form a grape-like cluster **Staphylococcus**

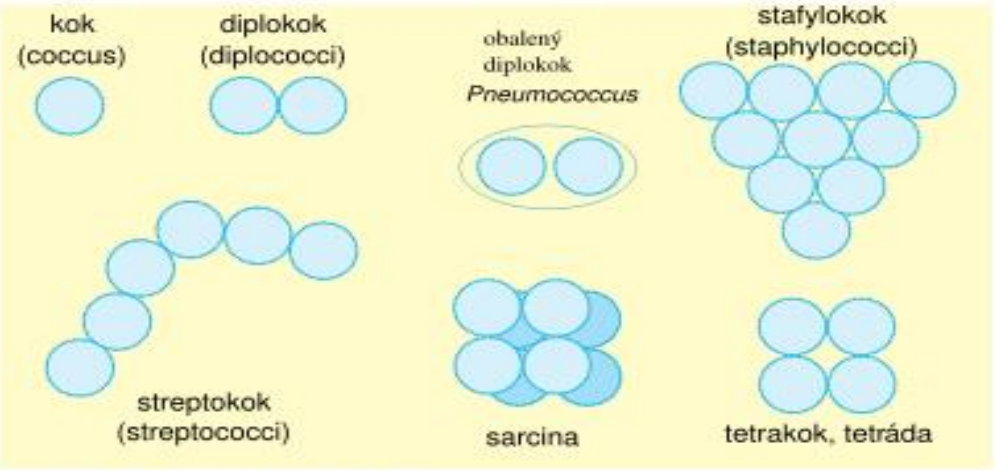


Those that divide in two planes and remain in groups of four are **Tetrads**

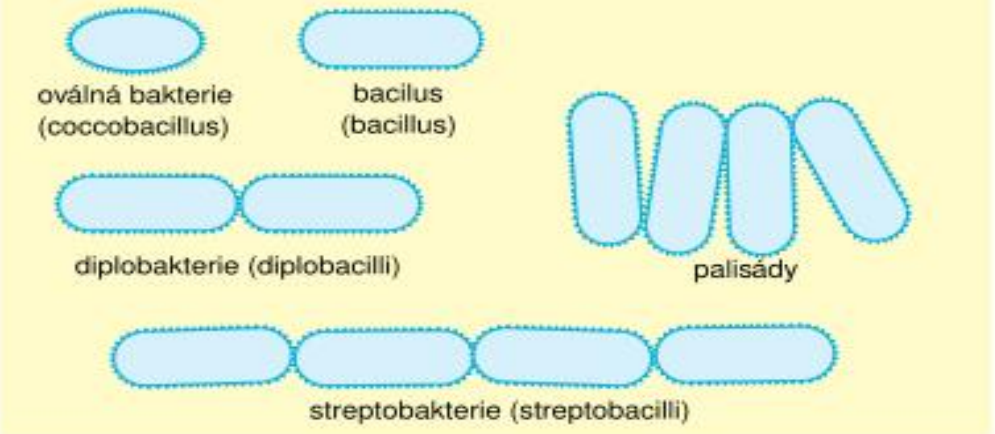
Those that divide in three planes and remain attached in cube-like groups of eight are called **Sarcina**



koky (cocci)

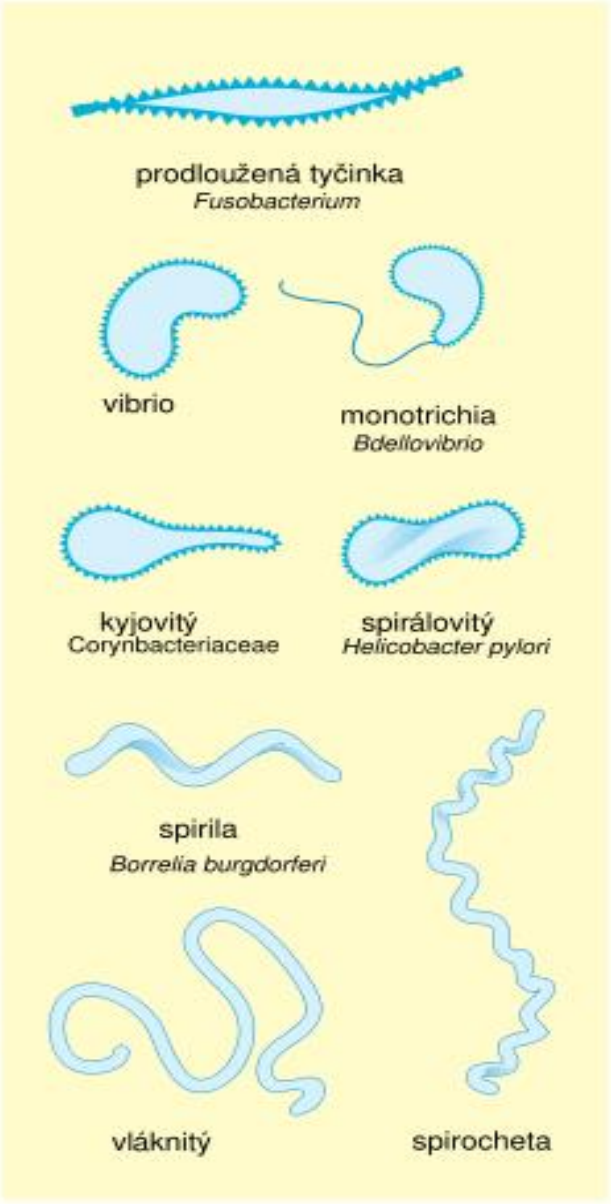


tyčinkovité bakterie (bacilli)



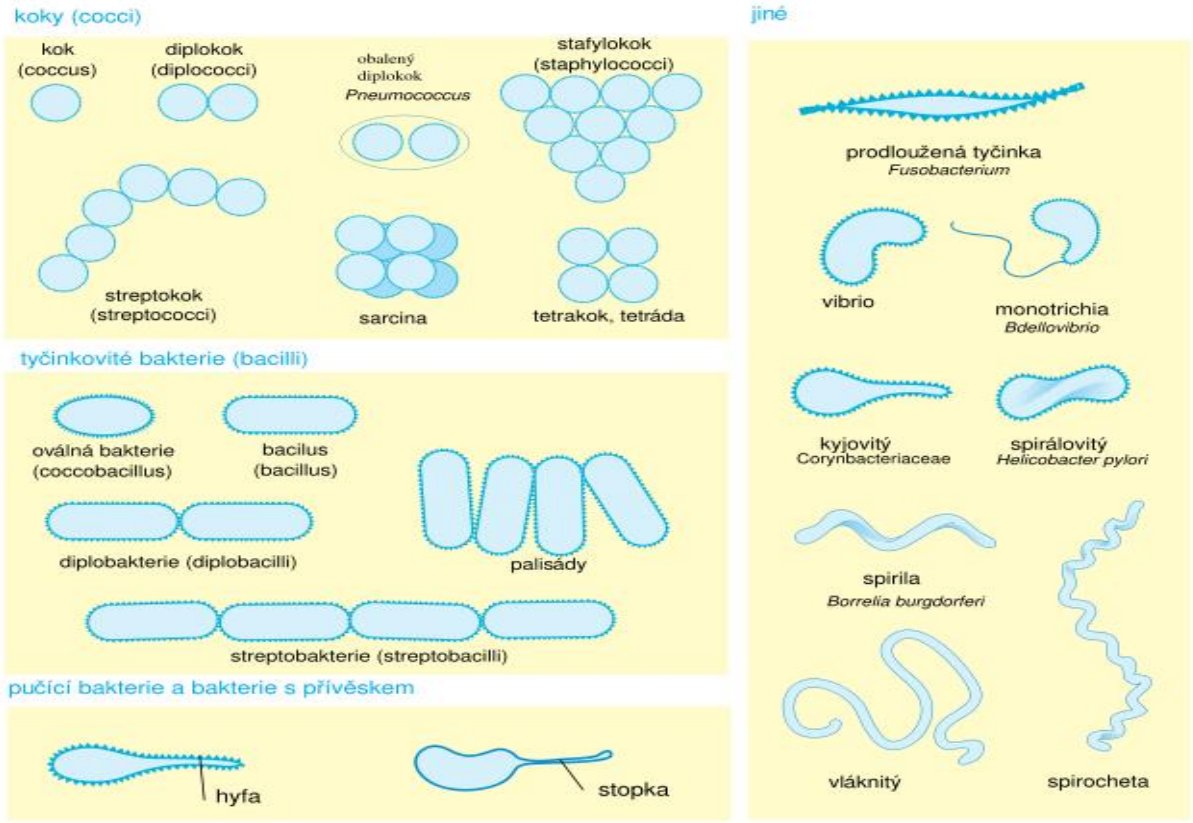
pučící bakterie a bakterie s přívěskem

jiné



NATURE AND KINDS OF MICROBES

Bacilli divide only across their short axis, so there are fewer groups of bacilli than are coccus



NATURE AND KINDS OF MICROBES

spirals are helical and flexible; they are called **Spirochaetes**

spirilla use whip-like external appendages called flagella to move

spirochaetes move by means of an axial filament, which resembles a flagellum but is contained under an external flexible sheath.

NATURE AND KINDS OF MICROBES

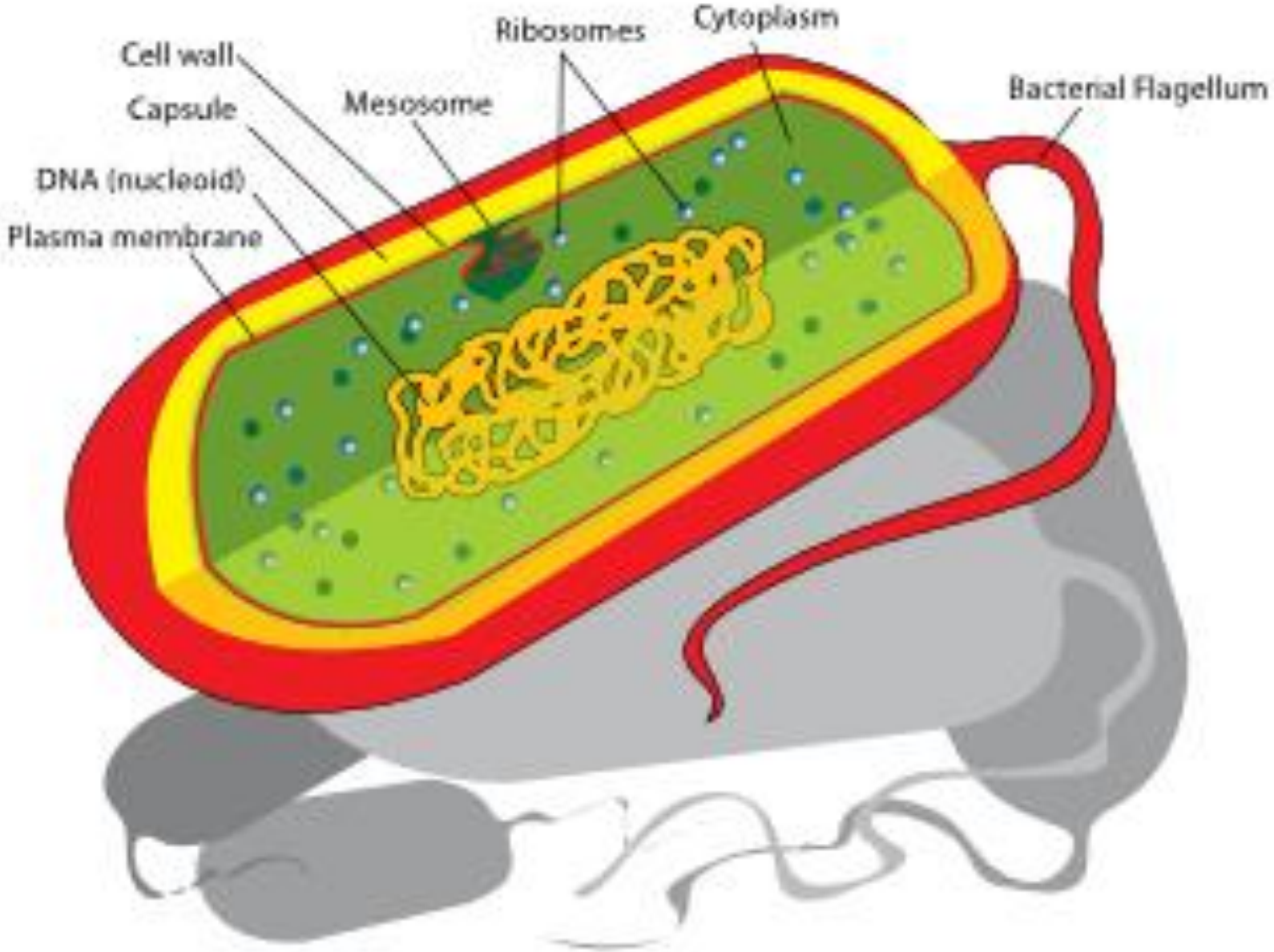
Generally bacterial shape is determined by heredity

Most bacteria are **Monomorphic** that is they maintain a single shape

However, a number of environmental conditions can alter that shape

If the shape is altered, the identification becomes difficult

For example the shape of Rhizobium and Corynebacterium are genetically **Pleomorphic** that is they can have many shapes.


Cell wall
Capsule
DNA (nucleoid)
Plasma membrane
Mesosome
Ribosomes
Cytoplasm
Bacterial Flagellum

Morphology of a Typical Gram positive bacteria

Features or structures outside are

Glycocalyx

Flagella

Axial Filaments

Fimbriae and Pili

GLYCOCALYX:

consists of polysaccharide, polypeptide or both but this varies depending on the species

Viscous (sticky), gelatinous substance and can take the form of either a capsule or a slime layer

Is often produced from within the cell and excreted outside

If Glycocalyx is firmly attached to the cell wall it is called a **CAPSULE**

But if loosely attached it is called a **SLIME LAYER**.

Capsules are important in

Determining bacterial virulence

Degree to which a bacteria can cause a disease

Often protect pathogenic bacteria from phagocytosis by the cells of the host

E.g. *Streptococcus pneumoniae* causes pneumonia when the cells are protected by a capsule

Uncapsulated *S. pneumoniae* cells are readily phagocytized and cannot cause pneumoniae

Same as *B. anthracis* which causes anthrax when capsulated.

Enables organisms attach themselves to various surfaces such as

plant roots, human teeth and tissues

E.g. *S. mutans* on teeth

Klebsiella pneumoniae to the respiratory tract.

FLAGELLA

Structures on bacteria which enable them to move either in a clockwise or counterclockwise direction

Proteus for e.g. can “swarm” on solid culture media.

Movement of a bacterium towards or away from a stimulus is called a **TAXIS**

Stimulus can either be chemical (chemotaxis) or light (Phototaxis)

Through this receptor, microbes can either be attracted or repelled from a stimulus.

FLAGELLA

Flagella is about 12 microns in length

The major protein component is **flagellin**

Can be observed by the hanging drop technique.

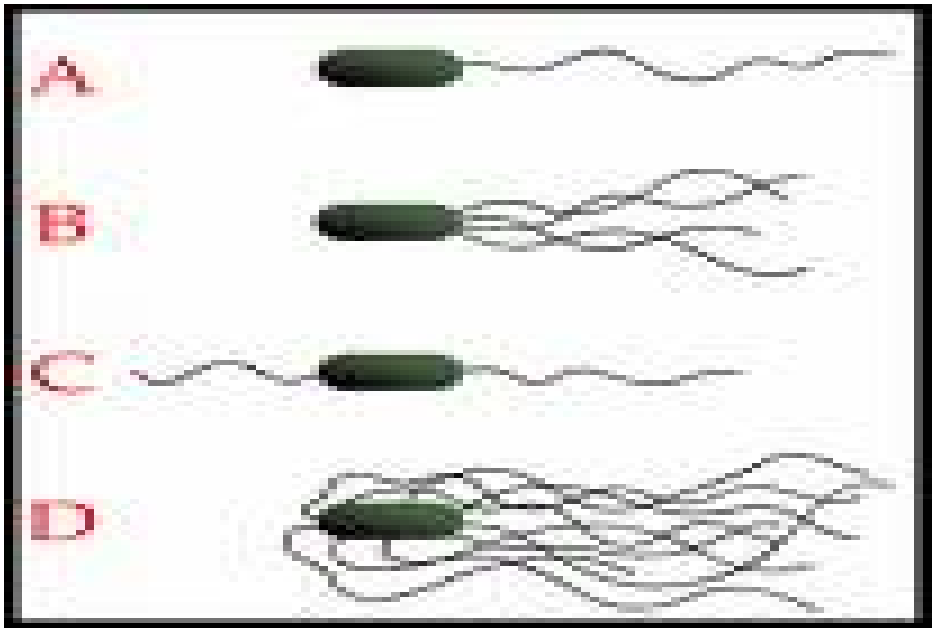
Bacteria have four major arrangements of flagella

(A) Monotrichous – single polar flagellum

(B) Lophotrichous – two or more flagella at one or both poles of the cell

(C) Amphitrichous - single flagellum at each end of the cell

(D) Peritrichous – flagella distributed over the entire cell.



FIMBRIAE AND PILI

Are hair-like appendages that are shorter, straighter and thinner than flagella

Used for attachment rather than for motility.

Originates from the cytoplasm and extend outwards from the surfaces

Mainly found on Gram –ve rods

Chemical composition confers antigenic specificity.

Environmental factors such as temp and oxygen tension tend to affect the presence or absence of the pili.

Pili help in the transfer of DNA from one cell to the other

CELL WALL

Responsible for the maintenance of the shape of the cell.

Protects the plasma or cytoplasmic membrane from the surrounding environment.

Prevents the bacterial cell from rupturing when the osmotic pressure inside the cell is greater than that outside the cell.

Enables some bacterial species to cause diseases and is the site of action of some antibiotics

Its composition is used to differentiate between major bacterial types.

The bacterial cell is composed of

Macromolecular network called PEPTIDOGLYCAN

It is either present singly or in combination with other substances

Peptidoglycan consists of a repeating disaccharide attached by polypeptides to form a lattice that surrounds and protects the entire cell

The disaccharide portion is made up of monosaccharides called N-acetylglucosamine (NAG)

and N-acetylmuramic acid (NAM) which are related to glucose.

GRAM +ve	GRAM –ve
Many layers of peptidoglycan forming a thick, rigid structure	only one or few thin layers
Contain large amounts of teichoic acid, alcohol (glycerol or ribitol and phosphate	Do not contain teichoic acid

NUTRITION AND GROWTH

Every organism must find in its environment all of the substances required for energy generation

These chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients**

In the laboratory, bacteria are grown in **culture media**

which are designed to provide all the essential nutrients in solution for bacterial growth

Bacteria are ubiquitous

They exhibit a wide range of tolerance to the environment

Obtain energy from an amazing variety of substrates

Show the most extreme forms of metabolism for any given environmental factor

For e.g. they can be classified based on their **oxygen requirements**

Aerobic:

Require oxygen to grow

Anaerobic

Do not require oxygen to grow

Microaerophilic

Require very little oxygen to grow

Campylobacter

Obligate aerobes: grow only in the presence of oxygen

Obligate anaerobes:Do not need or use it to grow

In fact, oxygen is toxic to it, as it either kills or inhibits their growth

Obligate anaerobic may live by fermentation, anaerobic respiration

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism

Under anaerobic conditions (no O2) they grow by fermentation or anaerobic respiration

But in the presence of O2 they switch to aerobic respiration For e.g. Yeasts

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism

They are insensitive to the presence of O2

They live by fermentation alone whether or not O2 is present in their environment e.g. lactic acid bacteria

Carbon- can be classified based on their carbon source

When they can fix carbon dioxide using light, they are called **Photoautotrophs** e.g. Cyanobacteria

Or from chemical reactions usually oxidations i.e. reduced inorganic molecules, **chemoautotrophs**

Litotrophs - oxidation of inorganic substances

Organotrophs - oxidation of organic substances

Most growth is heterotrophic and ranges from the use of simple hexoses to utilising complex carbon compounds.

Autotrophs – pure inorganic diets

Nitrogen- there are parts of the nitrogen cycle which can only be carried out by bacteria

Nitrogen fixation,

Nitrification and

Denitrification

Bacterial nitrogen metabolism ranges from the use of molecular nitrogen (N fixation) through to

Proteolysis as found in gas gangrene. (A type of gangrene that arises from dirty, lacerated wounds infected by anaerobes e.g. *Clostridium*).

Temperature-

Psychrotrophic bacteria can grow in refrigerators (*Listeria*, *Proteus*)

Most soil and water bacteria are **mesophilic** with an optimum temperature of 25^{o}C and many pathogens of man grow best at 37^{o}C.

Thermophilic bacteria are found in hot springs and volcanic vents

Thermoplasma

Growth curve

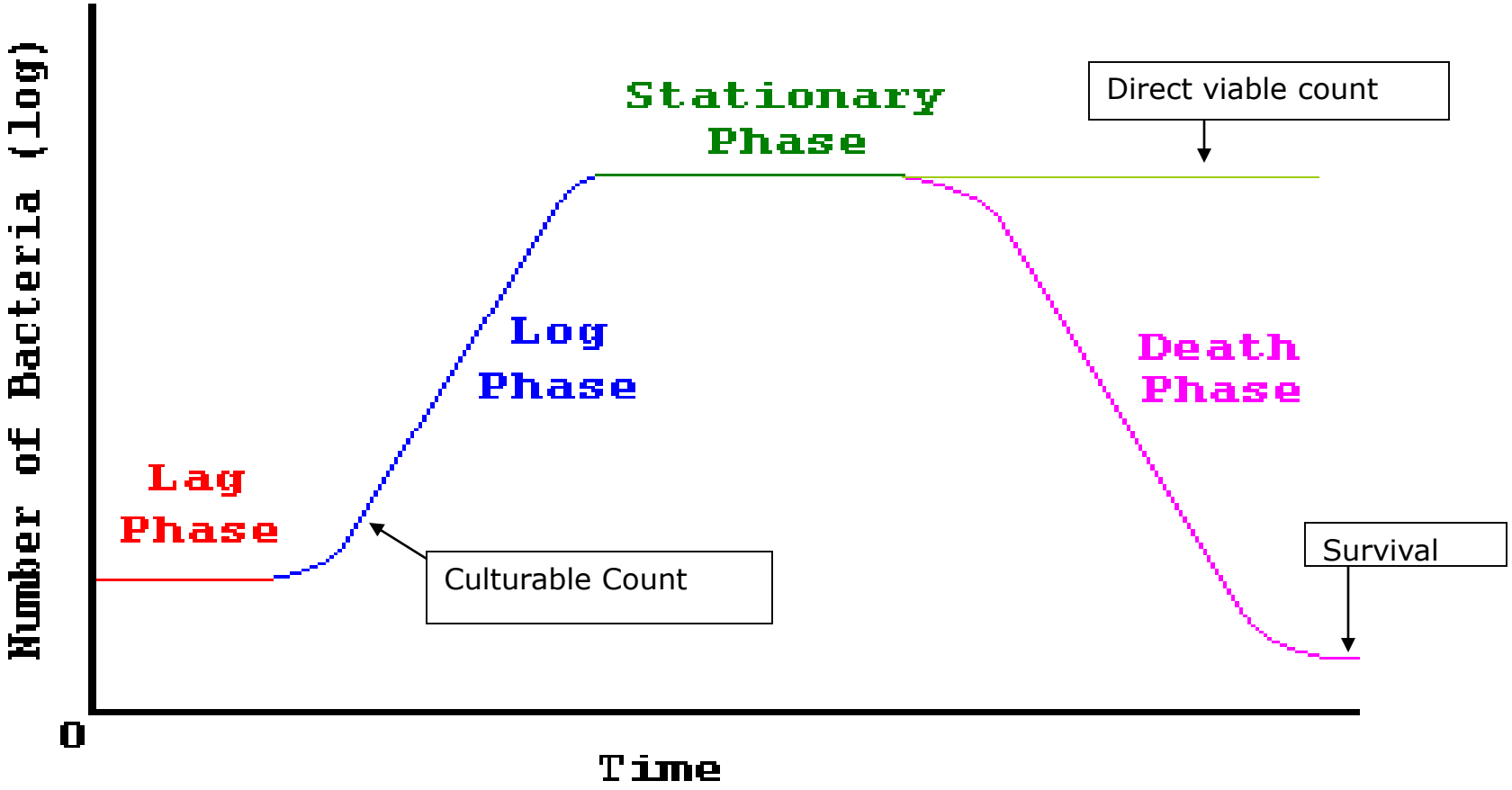


Figure 1 Stages of the bacterial growth curve

There are four main stages in the bacterial growth curve:

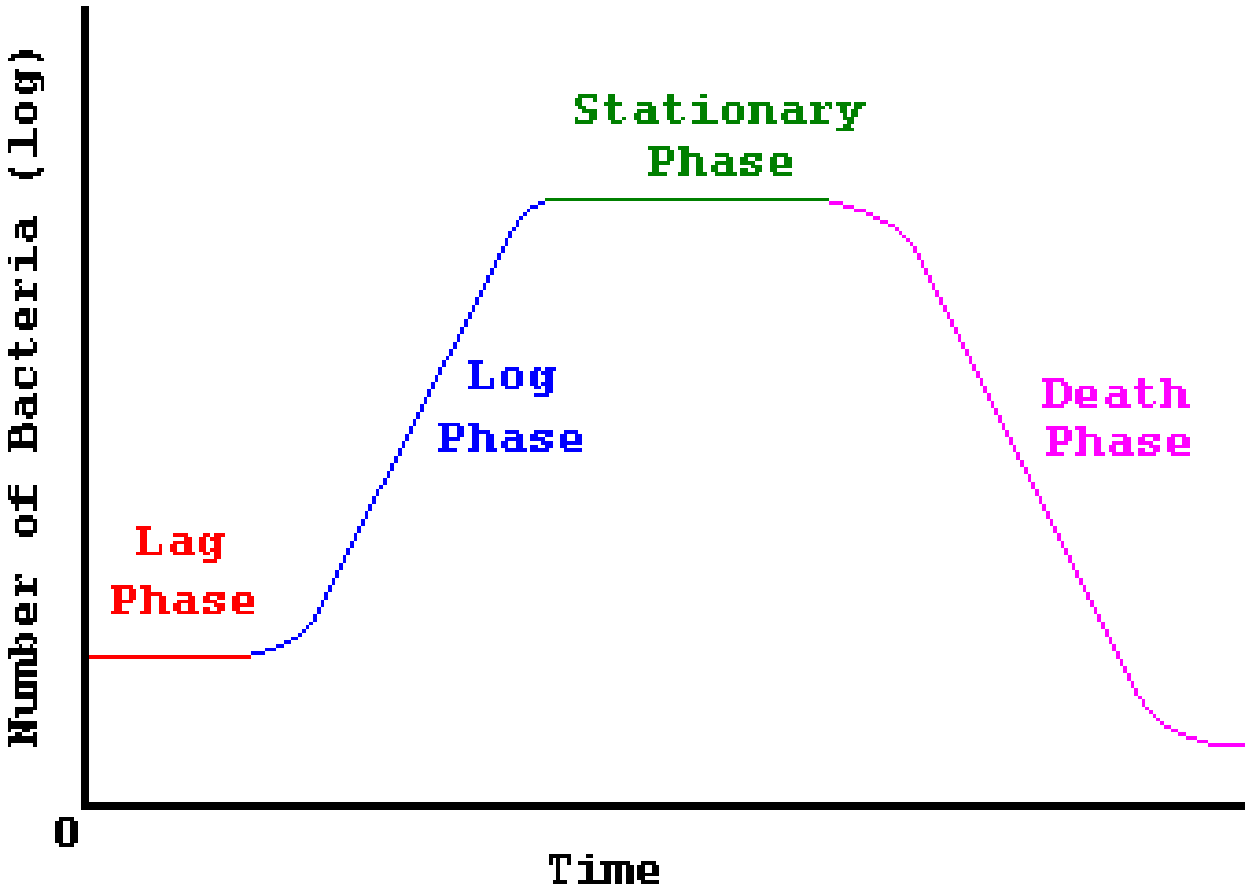
Lag phase: on transfer to a new medium there is a period of adjustment, the length of which depends on

The Bacterium

Closeness of the medium in which the bacteria had been growing to that into which it has been inoculated

Size of the inoculum relative to the volume of medium

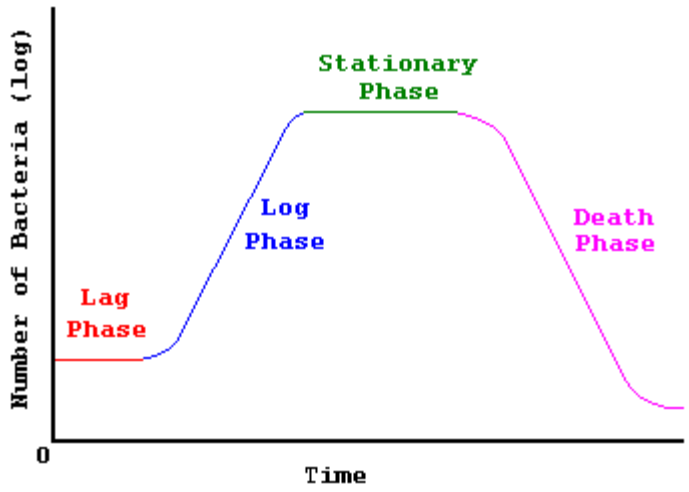
The main cause of the delay is the time taken for appropriate enzymes to be induced.


Number of Bacteria (log)
Stationary Phase
Log Phase
Death Phase
Lag Phase
0
Time

There are two main types of enzyme:

Inducible enzymes - are produced only when the substrate is present

Constitutive- are produced all the time



Log phase:

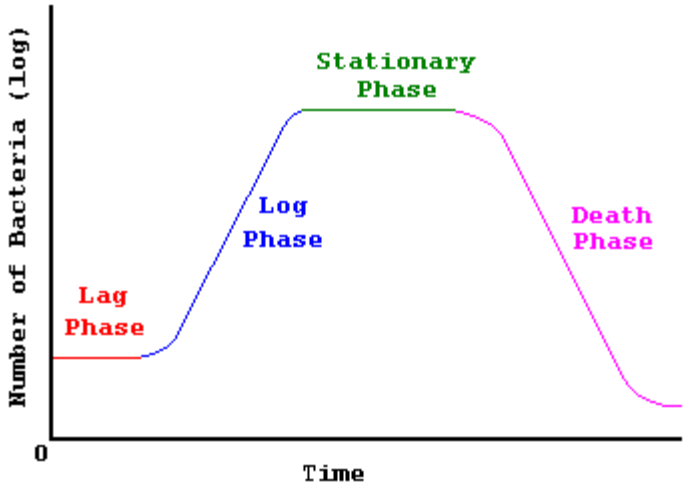
During log phase bacteria grow and divide at a constant rate.

After a while growth will slow down

Due to overcrowding,

build up of toxic products

starvation.



Possible to keep bacteria in a log growth phase using continuous culture and synchronous growth

Stationary phase:

Number of cells dividing and dying is in equilibrium

Nutrient supplies are depleted

Toxic waste products accumulate and a steady state in cell numbers is reached.

Passage through stationary phase prepares bacteria for survival in unfavourable conditions and primes bacteria for growth when environmental constraints are removed.

Death and declining phase:

Net decrease in numbers as more cells die than are replaced by new cells

It is not clear that all of the cells die

For example, *Salmonella typhi*, *Vibrio cholerae* and *Campylobacter jejuni*, form viable but non-culturable forms.

Survival Phase

When conditions become unfavourable bacteria begin to form resistant structures

endospores, cysts

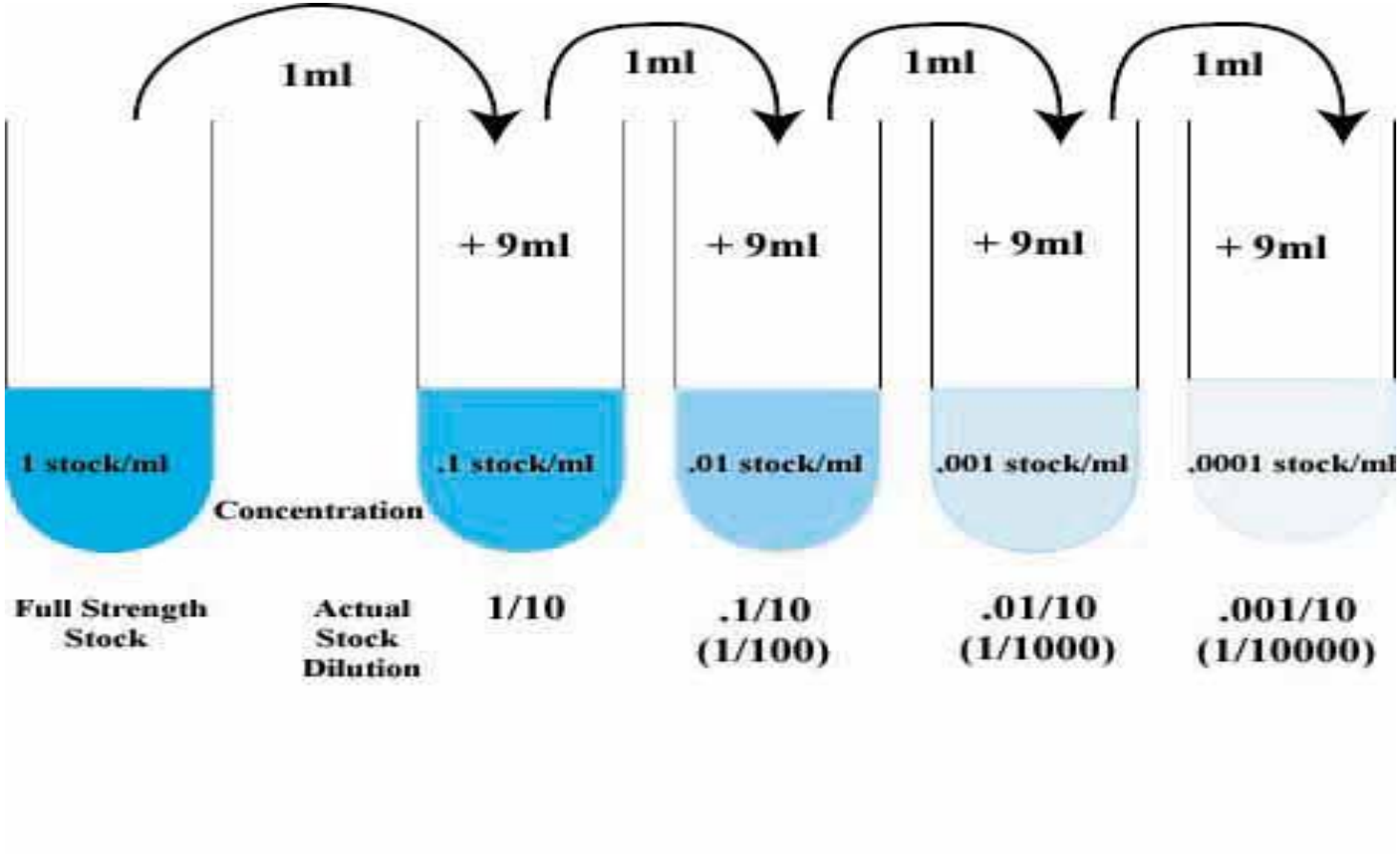
Akinetes – specialised non-motile, dormant, thick walled resting cells formed by some cyanobacteria and fungi

KINETICS OF GROWTH

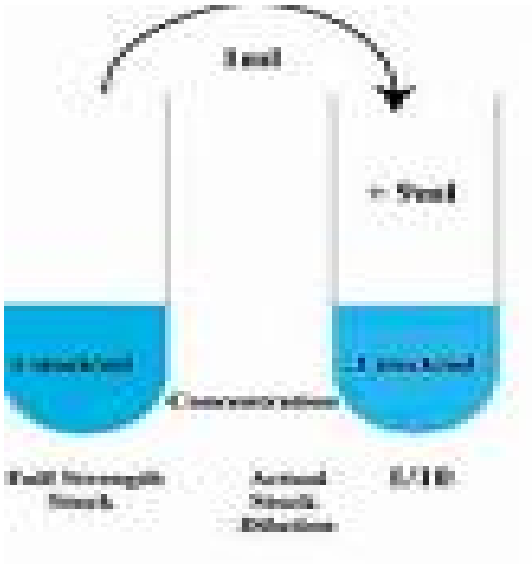
Under ideal conditions, e.g. laboratory conditions, where the microbes experience uniform and optimum chemical and physical conditions, the population change in a perfectly regular and predictable manner

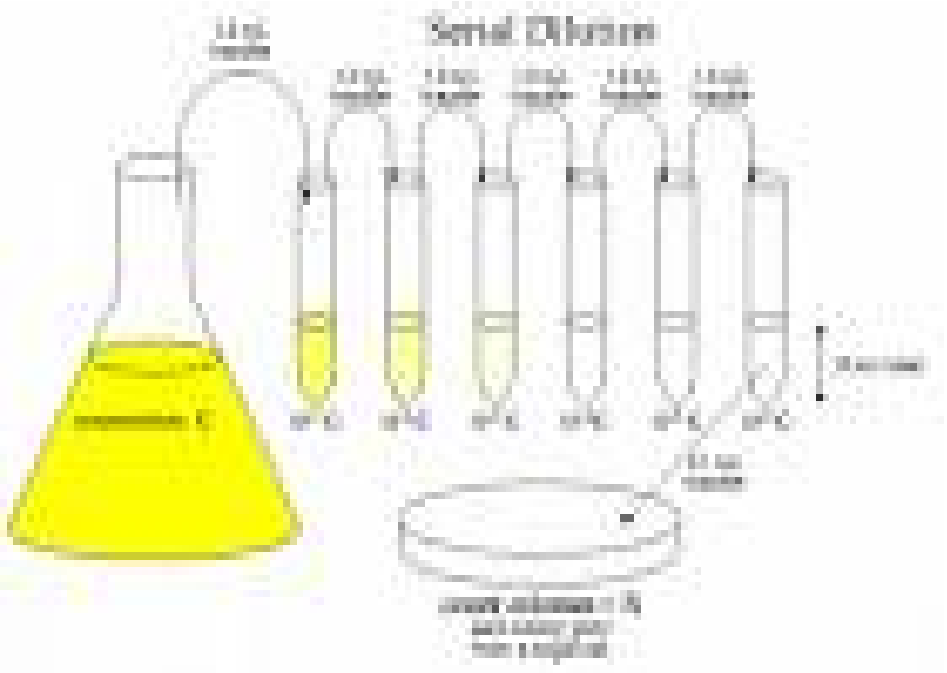
Most bacteria multiply by binary fission.

MEASUREMENT OF MICROBIAL GROWTH

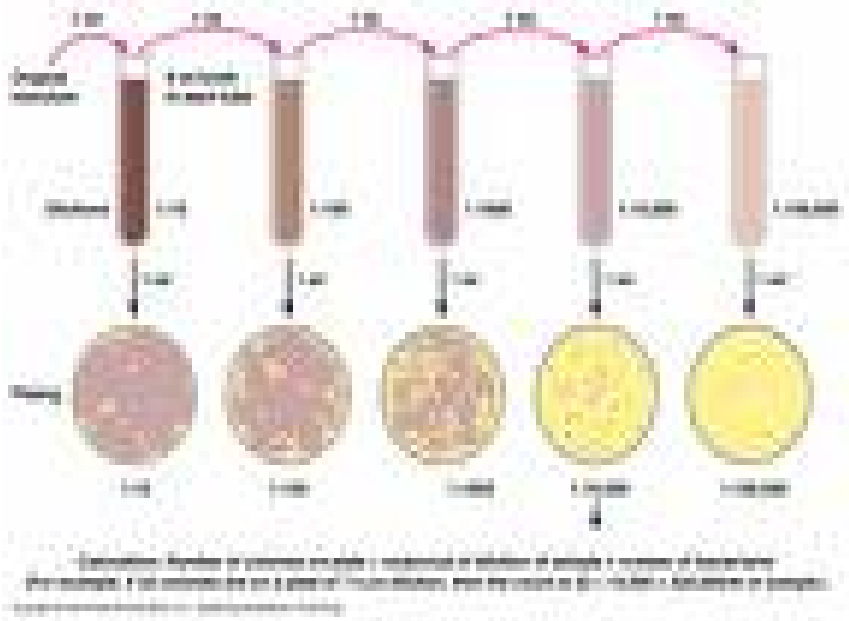


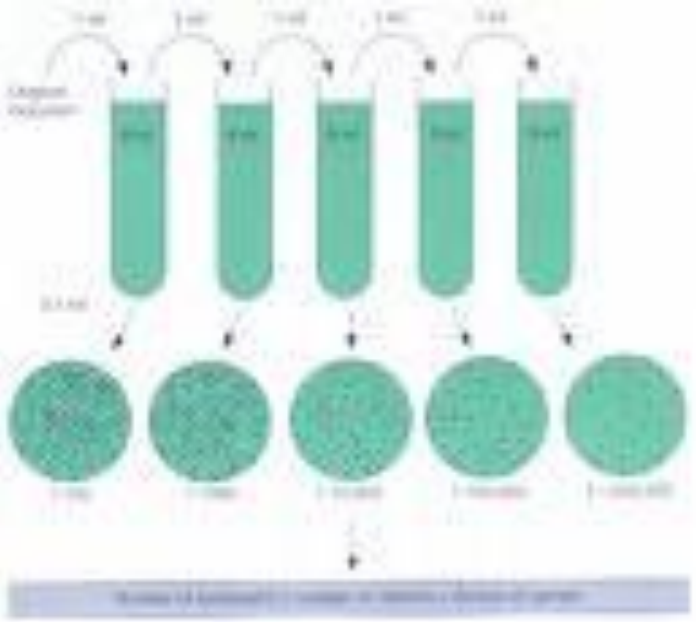
Serial Dilution





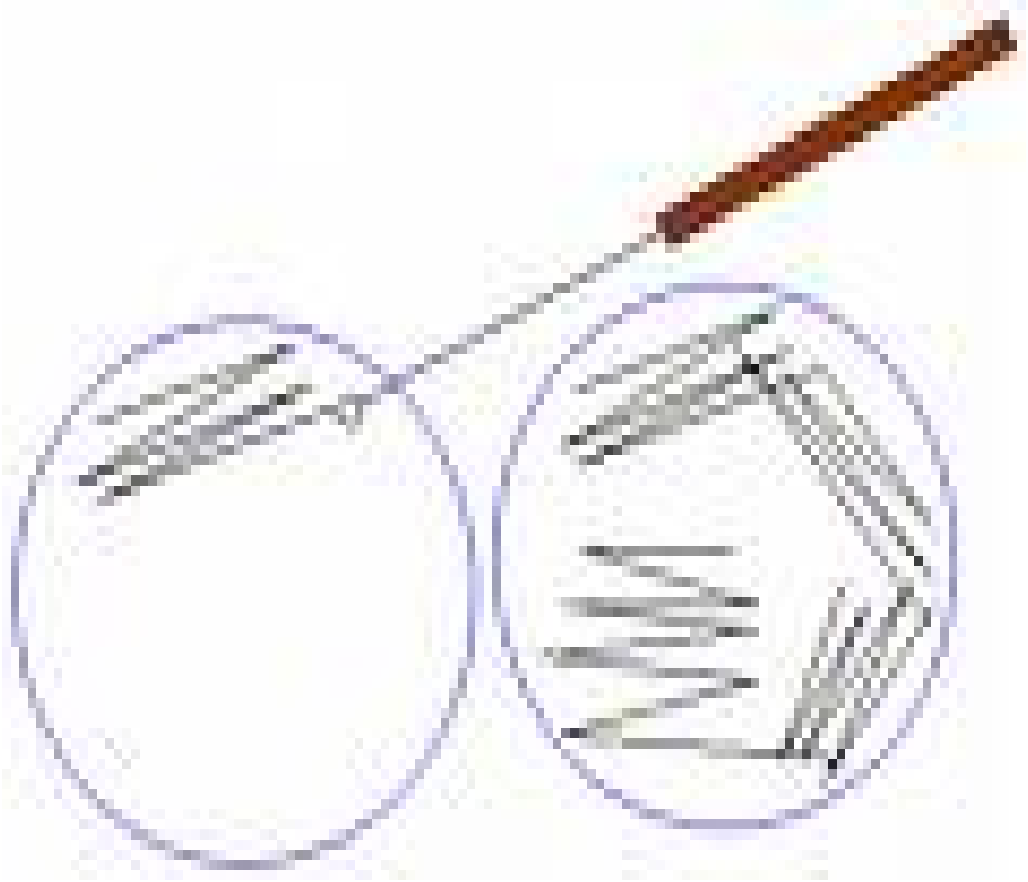
Serial dilution

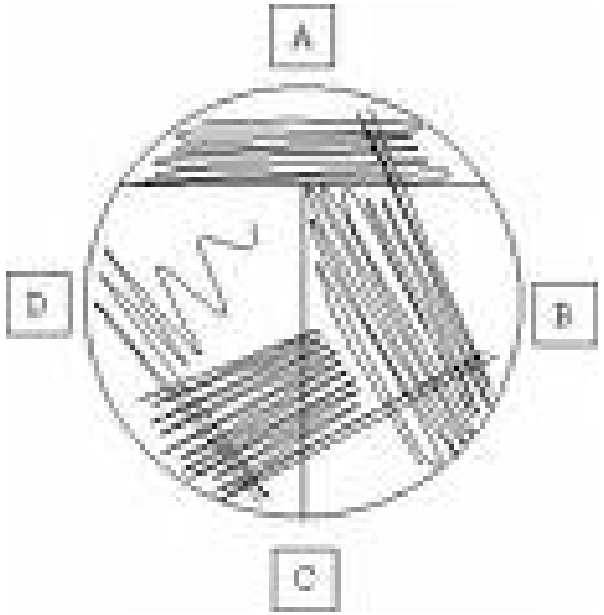




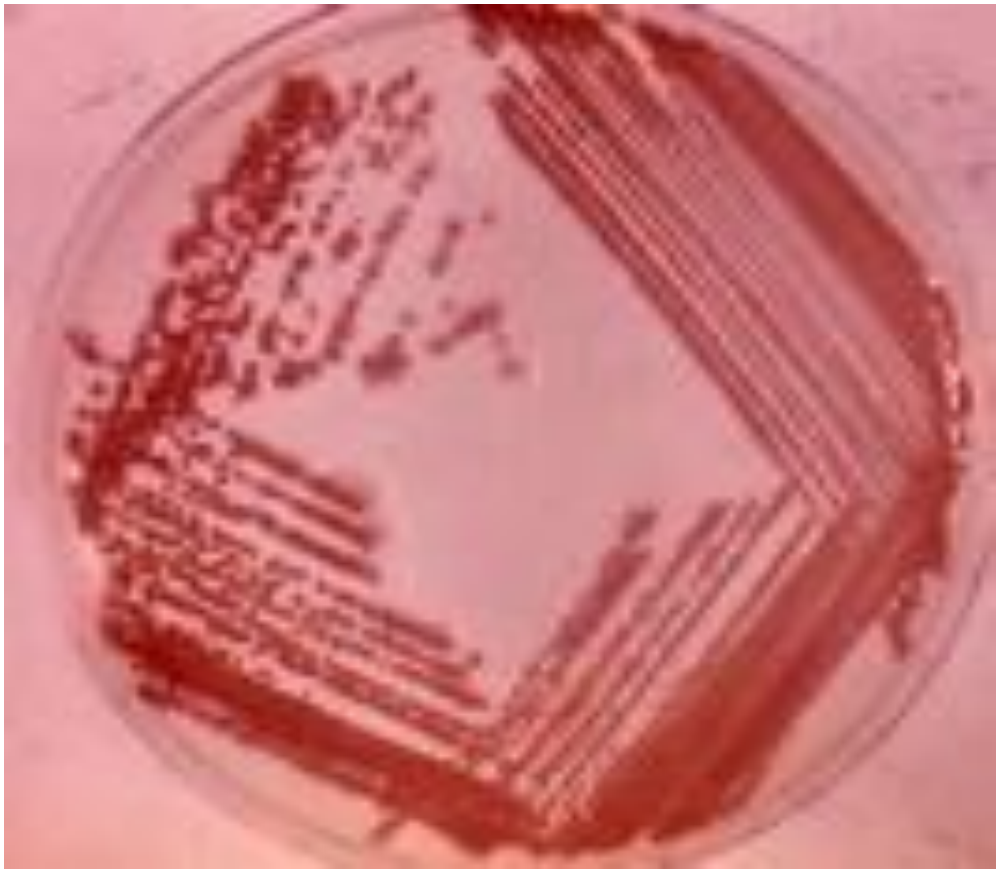
Pour Plate Technique

Streak Plate and Spread Plate





STREAK AND SPREAD PLATE



Streak Isolation on Nutrient Agar - Trypticase Soy Agar (TSA)

Generation Time

The time required for a cell to divide or its population to double.

It varies from one organism to the other. For example that for *E. coli* is 20 minutes

For microbes to grow, they require appropriate media. Depending on the type of microbe, different media are required

Nutrient media for bacteria

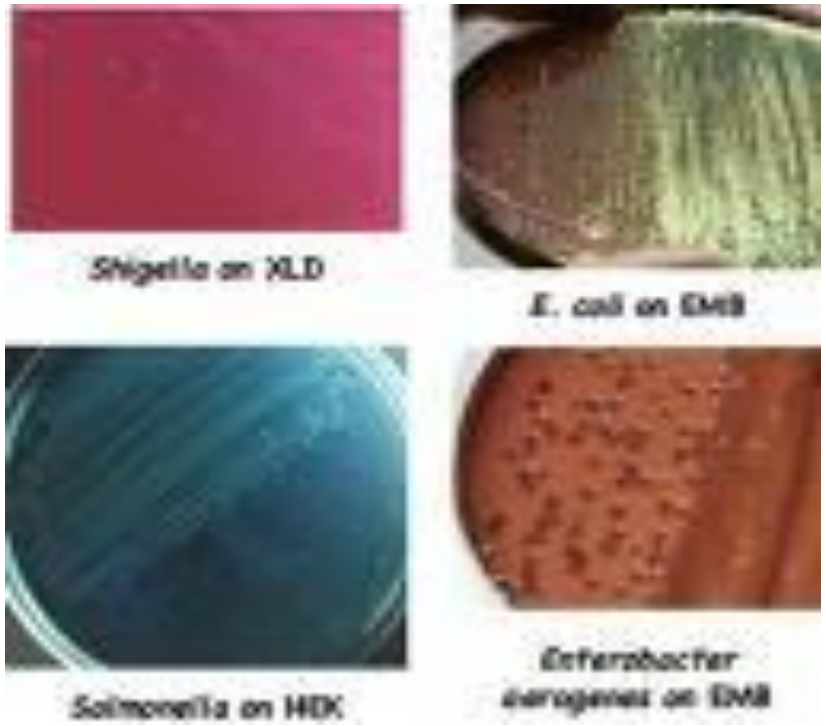
Sabouroud agar, Yeast Extract agar, malt extract agar for yeast

deMann Rogosa Sharpe's medium for lactic acid bacteria

Potato dextrose agar or cassava dextrose agar for molds and fungi etc.

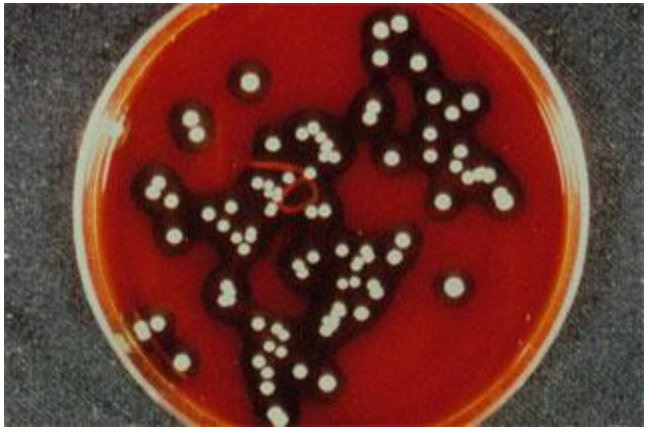
DIFFERENTIAL MEDIA

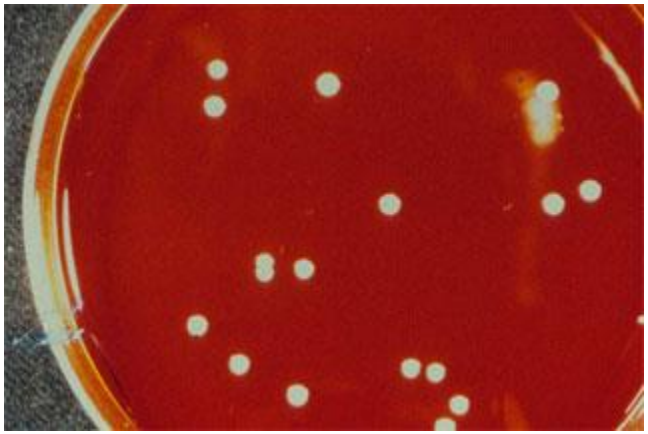
This media makes it easier to distinguish colonies of the desired organisms from other colonies growing on the same plate. Salmonella Shigella media



A: Beta Haemloysis on enriched agar - Blood agar

B: Non-Haemloytic growth on enriched agar - Blood agar





SELECTIVE MEDIA

This media suppresses unwanted organisms and encourage the growth of desired microbes.

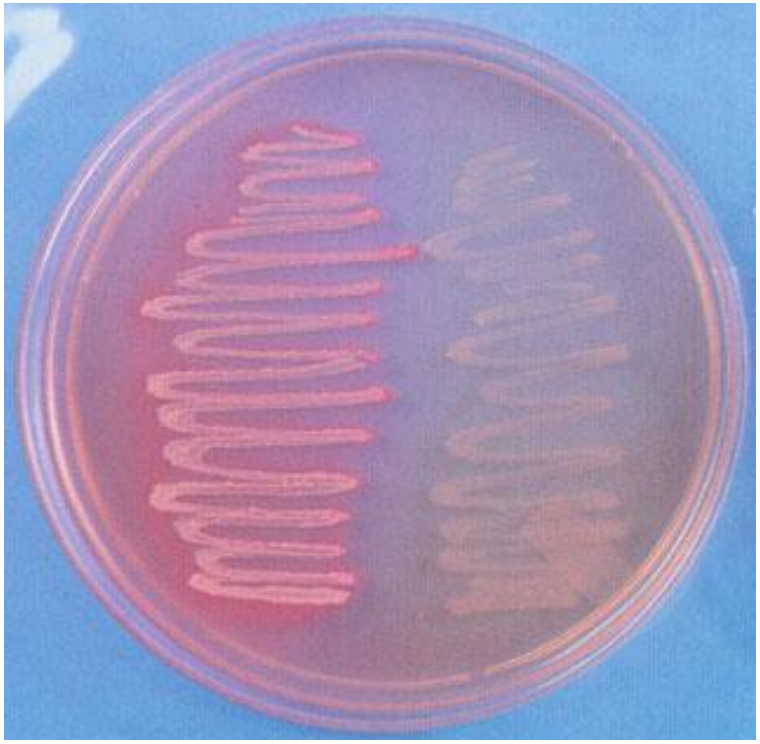
Thiosulphate citrate bile salt sucrose agar (TCBS) for Cholera

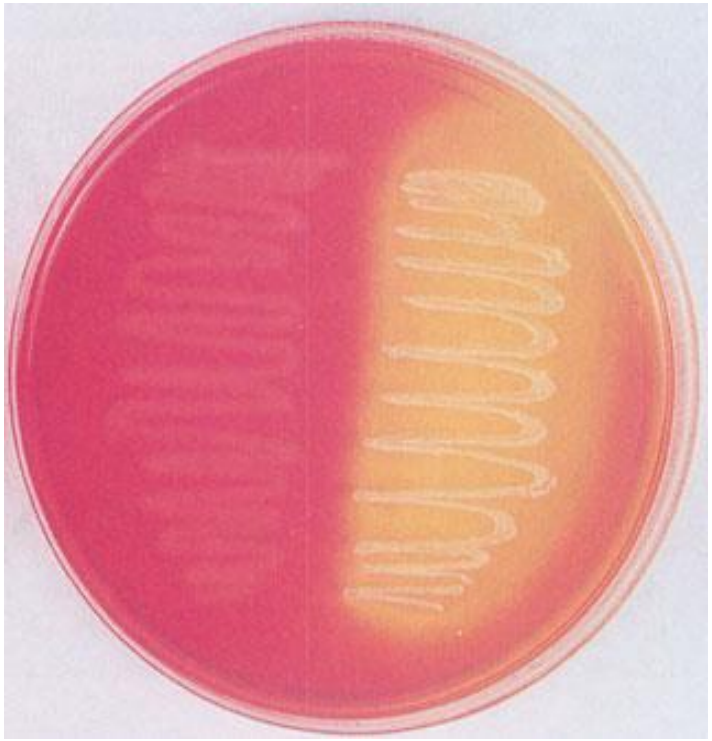
modified campylobacter charcoal deoxycholate agar for campylobacter.

However, some media are both selective and differential i.e. MacConkey agar.

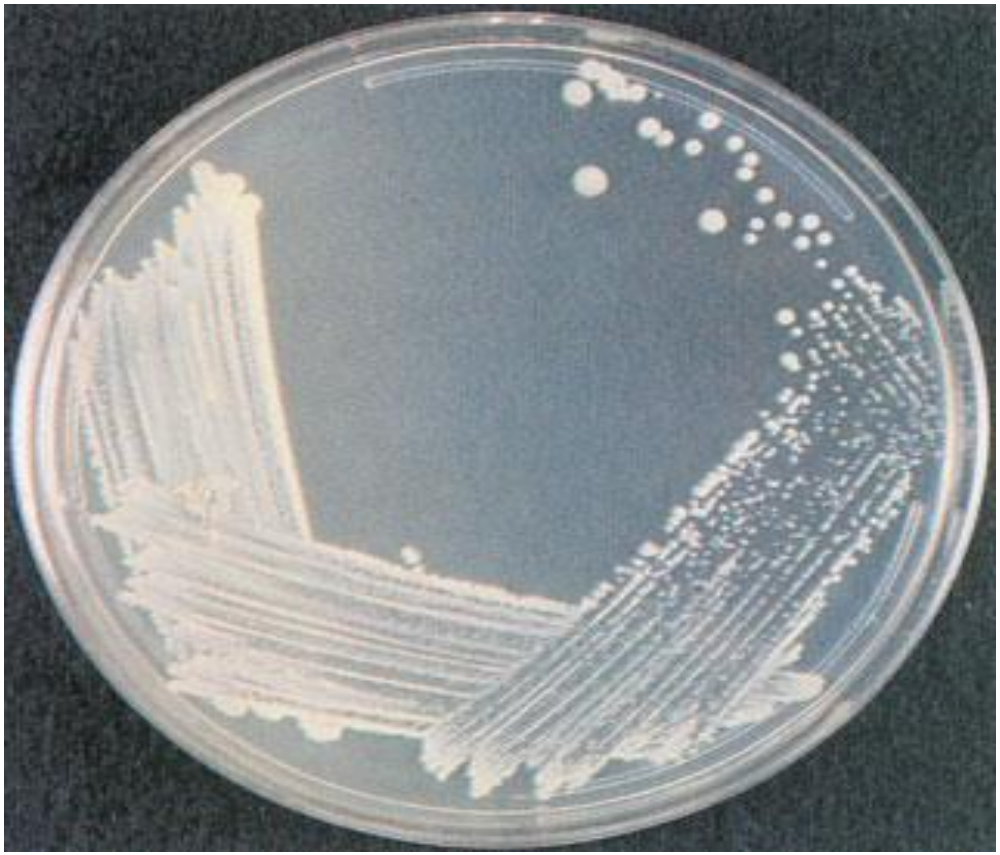
The ability to distinguish between lactose fermenters (red or pink colonies) and non-fermenters (colourless colonies) is useful in distinguishing between the pathogenic Salmonella bacteria and other related bacteria.

E. coli and *Proteus* on selective/differential media – MacConkeys
S. epidermidis and *S. aureus* on selective/differential - Mannitol Salt Agar (MSA)





Gram Positive Organisms on selective agar - Colistan Naldixic Acid Agar (CNA)

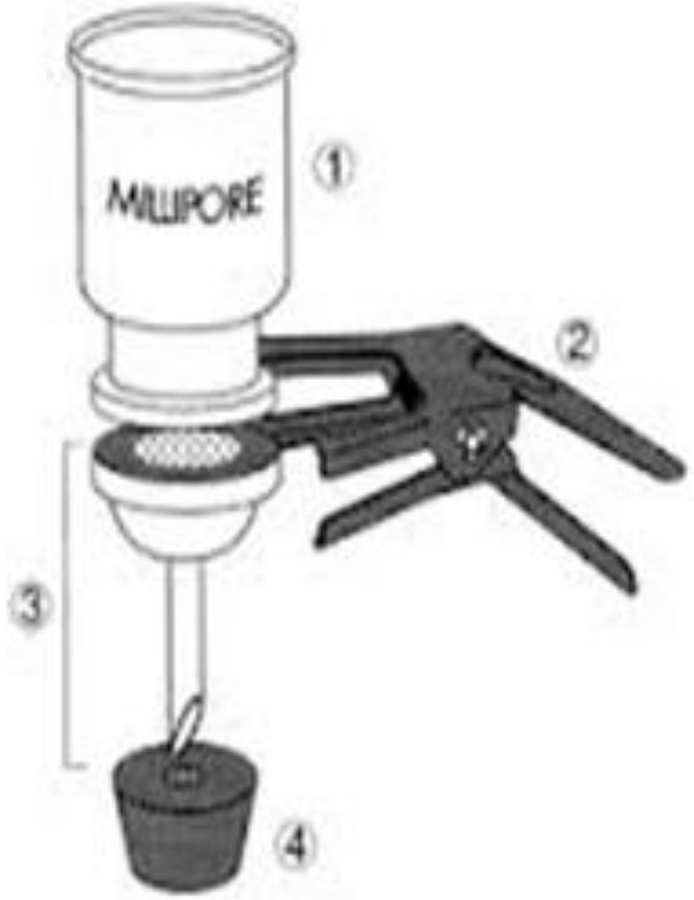


MEMBRANE FILTRATION

Used to enumerate microorganisms found in clear solutions and where they may be in small numbers including pathogens; S*almonella, Campylobacter* and Enterococci

A triple glass filtration unit with funnels and cups used

Water filtered through a white, grid marked, 47 mm diameter Millipore HA-type cellulose filters with a pore size of 0.45 μm


MILLIPORE
1
2
3
4


Sample filtration

Place 100ml of water in each cup in triplicate

Using a vacuum pump at a pressure of 65 kPa (500 mm Hg)

After filtering, using sterile forceps, the filter membrane is aseptically removed and placed grid side upwards onto dried plates

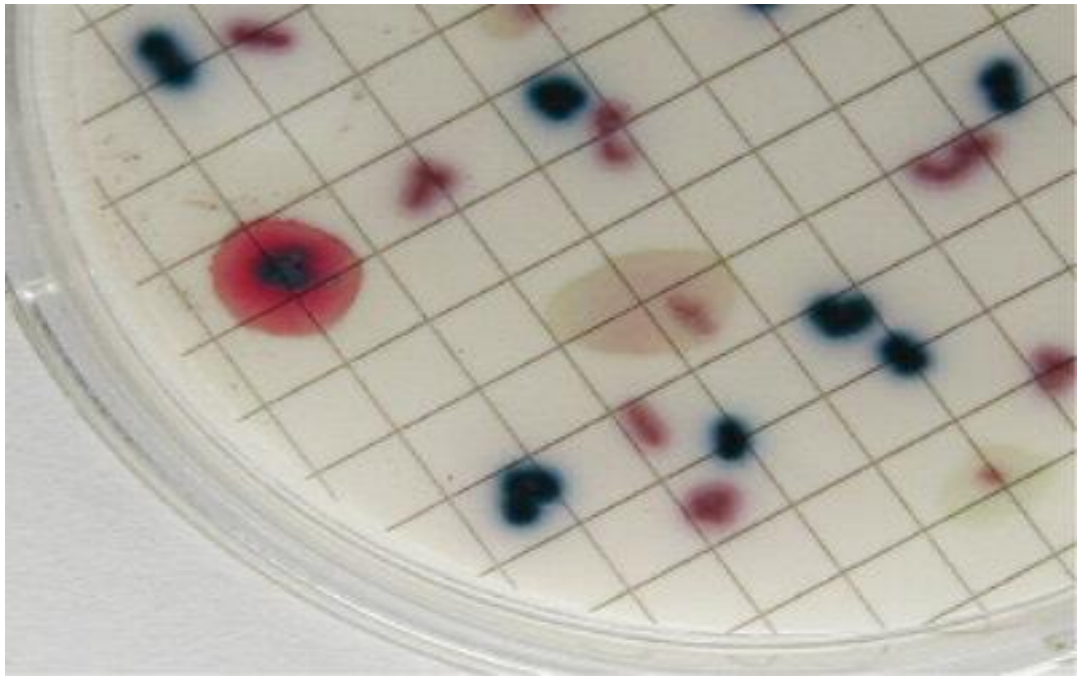
Faecal coliforms grow on lauryl sulphate agar

Enterococci on Slanetz and Bartley

Between samples, the glass funnels is disinfected by immersion in boiling distilled water for at least 2 min.

Golden green sheen colonies are counted as presumptive faecal coliforms and confirmed

Results were expressed as cfu 100 ml-1.



MOST PROBABLE NUMBER

Faecal coliforms can be estimated using a three-tube Most Probable Number method (MPN)

It is often used for dirty water and sediments, leaves, food, generally solid materials

Seawater and river water dilutions of 10^{-1} to 10^{-6} are prepared in 0.1% buffered peptone water or sterile distilled or saline water

1 ml of each dilution inoculated in triplicate into 5 ml Minerals Modified Glutamate medium in test tubes with inverted Durham tubes.

The tubes incubated for 24 h at 44oC.

Temperature prevents growth of majority of coliforms of non-faecal origin, which are unable to survive at this elevated temperature.

Tubes scored positive if both acid and gas is produced.

No. of bacteria 100 ml-1 is deduced from MPN table for each sequence of positive tubes

Loop inoculation is made from each positive tube onto MacConkey No. 3 agar and incubated for 24 h at 42oC

Growth of characteristic dry, pink colonies confirmed the presence of faecal coliforms

DIRECT MICROSCOPIC COUNT OR TOTAL COUNT

Here a measured volume of bacterial suspension is placed inside a defined area on a microscope slide

0.1 ml sample is spread over a marked square cm of slide, stained and looked at under oil immersion.

Bacteria is counted in several fields and average no. of bacteria per viewing field is calculated

Often used in the dairy industry.

A specially designed slide called the Petroff-Hausser counter is also used in direct microscopic counts

A shallow well of known volume is indented into the surface of a microscope slide inscribed with squares of known areas and covered with a thin cover glass.

The well is filled with the microbial suspension

The average number of bacteria in each of a series of these squares is calculated and then multiplied by a factor that produces the count per ml

Advantage is that incubation time is not required.

TURBIDIMETER

Estimating turbidity is a practical way of monitoring bacterial growth.

As bacteria multiply in a liquid medium, the medium becomes turbid or cloudy with cells.

Instrument used is either a spectrophotometer or colorimeter.

In the Spec, a beam of light is transmitted through a bacterial suspension to a photovoltaic cell.

As bacterial numbers increase, less light will reach the photovoltaic cell

This change of light will register on the instrument's scale as the percentage of transmission and this is often expressed logarithmically as absorbance or Optical density.

Absorbance or OD is used in plotting a STANDARD graph

If absorbance readings are matched with plate counts of the same culture, this correlation can be used in future estimations of bacterial numbers obtained by measuring turbidity.

METABOLIC ACTIVITY

Assumes that the amount of a certain metabolic product, such as acid or CO_2 is in direct proportion to the number of bacteria present.

Bacteria numbers can also be estimated by a reduction test; which measures oxygen directly or indirectly.

A dye that changes color in the presence or absence of oxygen, such as methylene blue, is added to a medium such as milk

The bacteria then use the oxygen as they metabolize the milk

Because methylene blue is blue in the presence of oxygen and colorless in its absence

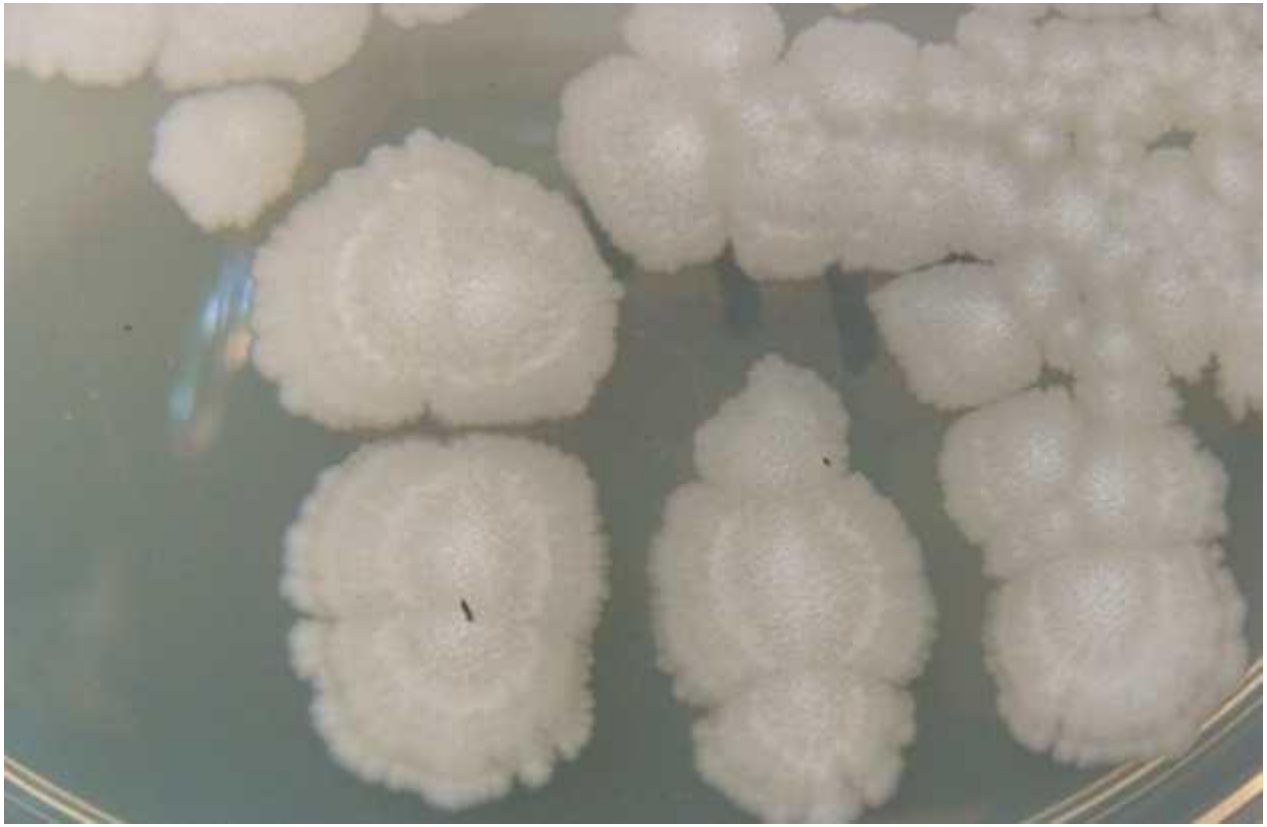
the faster the dye (and thus the milk) loses color, the faster the oxygen is being depleted and the more bacteria are presumed to be present in the milk.

Reduction test are frequently used in microbiology teaching laboratories but they lack accuracy and are seldom used in commercial applications.

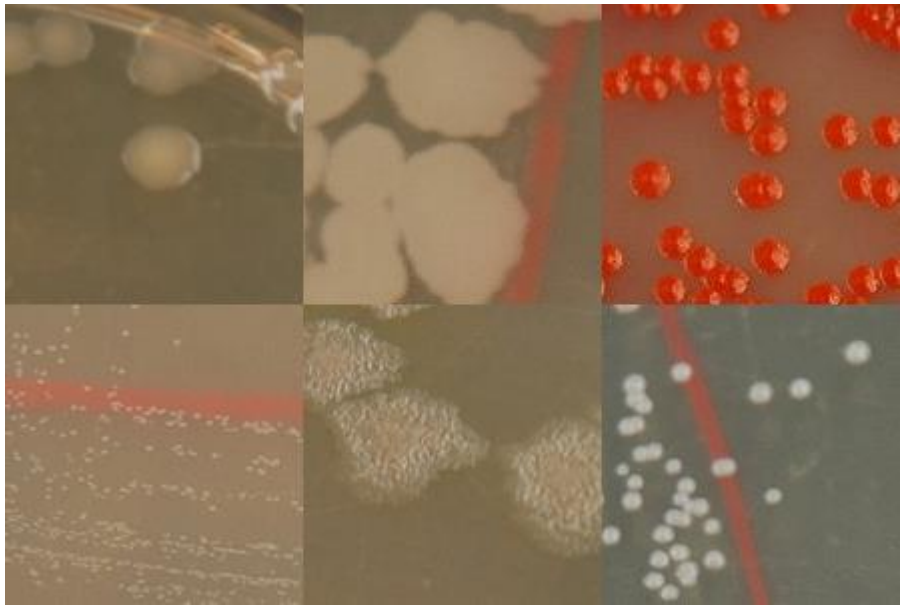
DRY WEIGHT

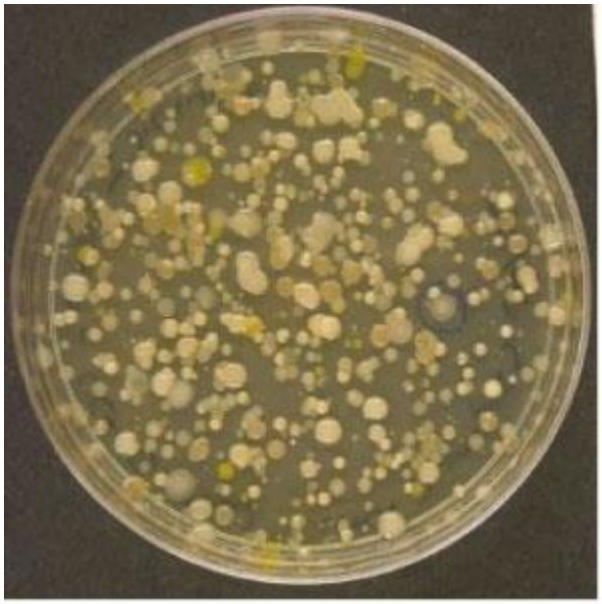
mostly for molds and fungi especially filamentous organisms

CHARACTERISATION OF BACTERIA

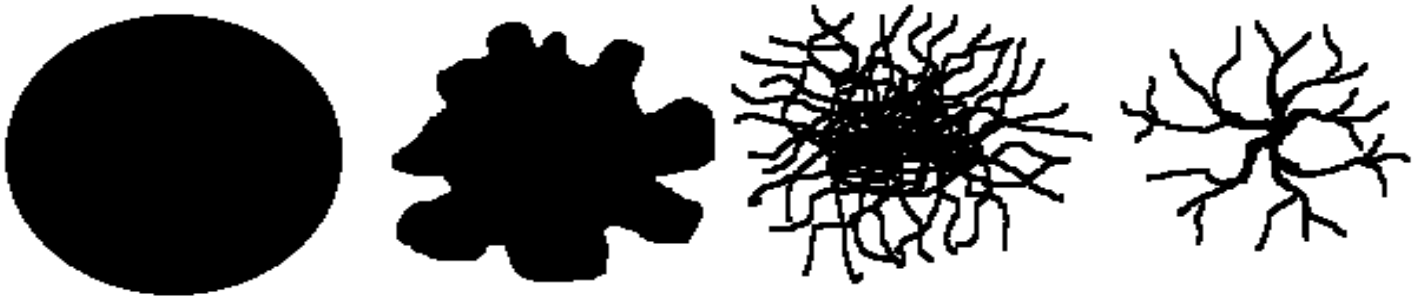


CHARACTERISATION OF BACTERIA





Form



Elevation

Margin

Whole colony:

Punctiform

Circular

Rhizoid

Irregular

Filamentous

Edge:

Entire

Undulate

Lobate

Filamentous

Curled

Surface:

Smooth, glistening

Rough

Wrinkled

Dry, powdery

Elevation:

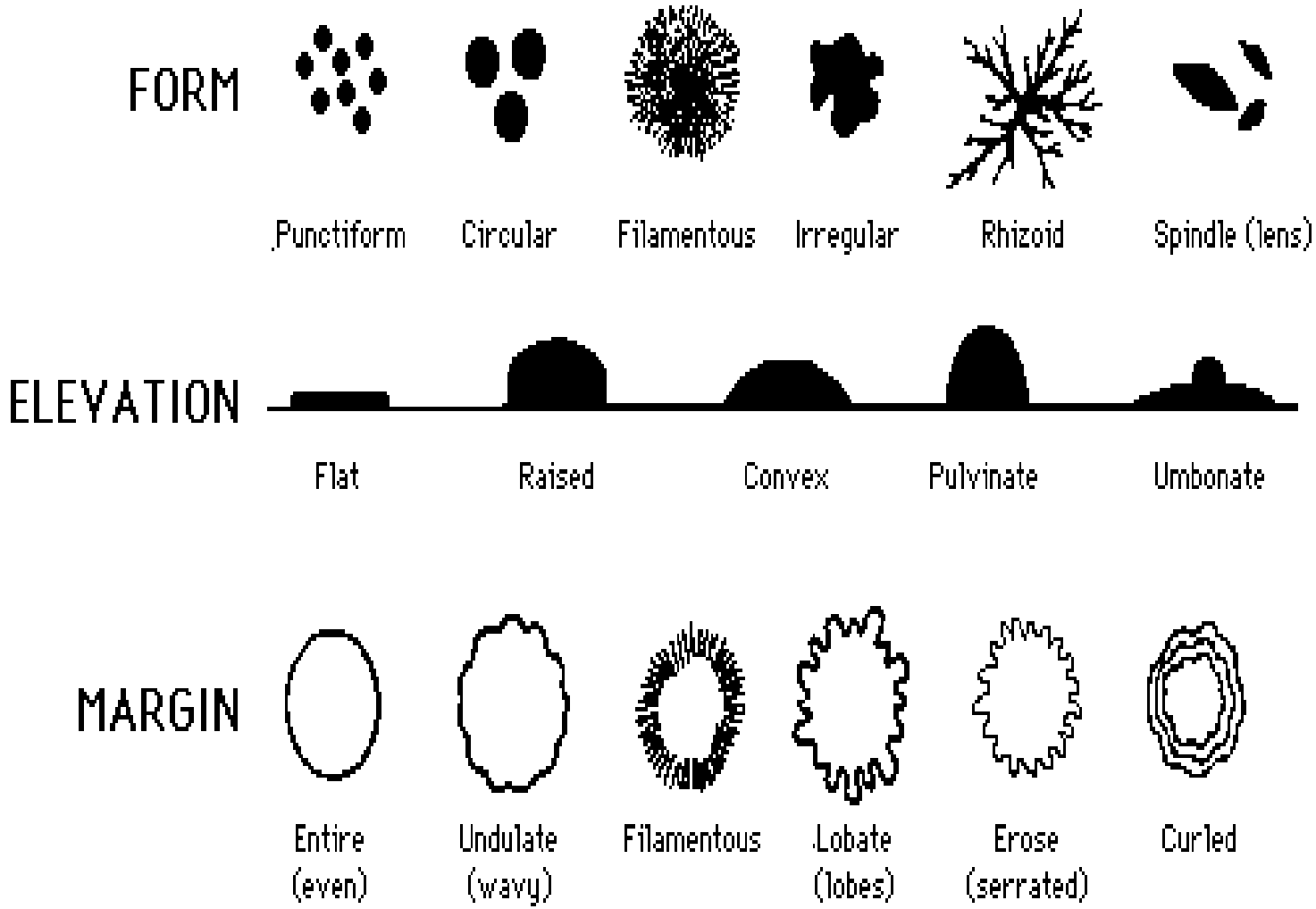
Flat

Raised

Convex

Pulvinate

Umbonate


FORM
Punctiform
Circular
Filamentous
Irregular
Rhizoid
Spindle (lens)
ELEVATION
Flat
Raised
Convex
Pulvinate
Umbonate
MARGIN
Entire
(even)
Undulate
(wavy)
Filamentous
Lobate
(lobes)
Erose
(serrated)
Curled

FORM

Circular

Irregular

Filamentous

Rhizoid

ELEVATION

Raised

Convex

Flat

umbonate

Crateriform

MARGIN

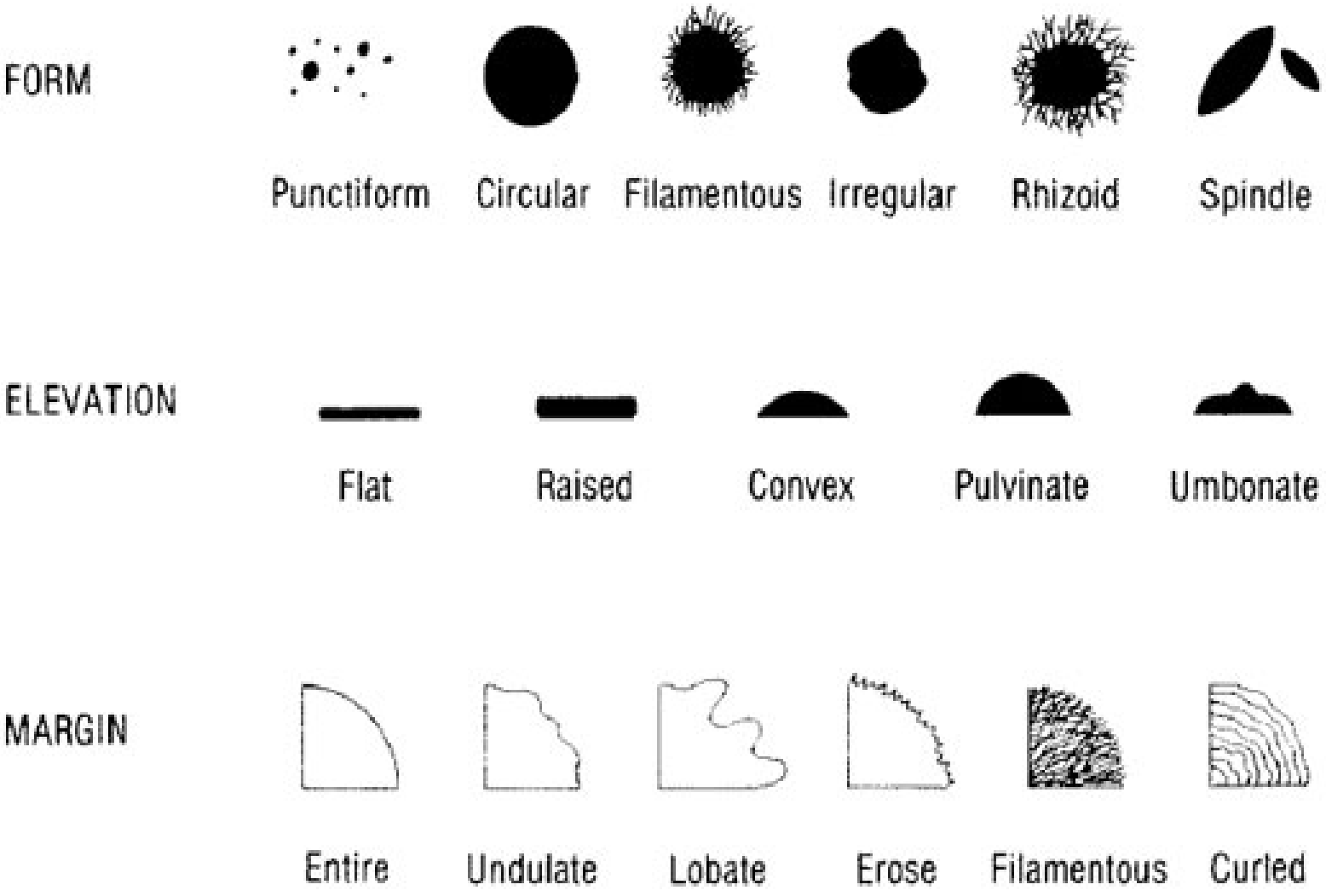
Entire

Undulate

Filiform

Curled

Lobate


FORM
Punctiform
Circular
Filamentous
Irregular
Rhizoid
Spindle
ELEVATION
Flat
Raised
Convex
Pulvinate
Umbonate
MARGIN
Entire
Undulate
Lobate
Erose
Filamentous
Curled

CHARACTERISATION OF BACTERIA

MICROSCOPIC

To be able to examine microbes microscopically, they need to be stained as this enables us to appreciate their shape and arrangement.

Staining means coloring the microbes with a dye that will emphasize certain structures.

Before staining, a culture or SMEAR of the microbe is placed on a microscope slide

FIXED by passing it through a Bunsen flame or heat to dry

Basic dyes include Crystal violet, methylene blue and safranin.

Negative Staining

When bacteria cells are observed to be colourless against a coloured background it is called **Negative Staining**

Helps to appreciate the cell shape, size, arrangement etc

Eg are eosin, nigrosin and Indian ink

Presently this is not being done routinely because of the Phase Contrast Microscope.

Simple Staining is also staining the cells themselves and not the background

Can also be done using methylene blue, carbol fuschin, crystal violet, safranin etc.

Differential Staining

Often used to differentiate between different groups of bacteria

Gram and Acid fast Staining

Gram Stain Developed by Christian Gram, a Danish Bacteriologist in 1884

Useful as it divides bacteria into two broad groups i.e. Gram +ve and Gram –ve

If a liquid culture of bacteria is to be examined, place a drop of culture on a slide

Spread it with a flamed sterile loop

Allow to dry and fix by passing it 2 or 3 times through a Bunsen flame

If an agar culture is to be used, place a **very small** drop of water on the slide and add a little of the bacterial colony to the drop with a sterile loop

Spread the drop and fix the bacteria as with the liquid culture.

Stain with 0.5% crystal violet for 2 minutes

Wash with water and drain off the water.

Stain with dilute iodine for 2 minutes

The crystal violet and iodine form a purple/black complex inside the bacterial cell

Slowly drip 95% alcohol or acetone onto the smear until the colour stops running

Wash off with water

Alcohol dissolves the lipid layer surrounding Gram negative cells and allows the crystal violet/iodine complex to wash out

Counter stain with 1% safranin for 2 min

Wash and drain the slides

Microscopy

After staining, the slides are examined without a coverslip

Find the stained smear using a low power objective, x10

Put a small drop of immersion oil directly onto the smear

Examine using oil immersion (x100) lens

Gram +ve bacteria are stained purple or dark violet and Gram –ve pink.

These colours develop on the bacteria because of either the retention or escape of the combination of crystal violet and iodine complex

Gram +ve bacteria have a thicker peptidoglycan cell wall than Gram-ve ones.

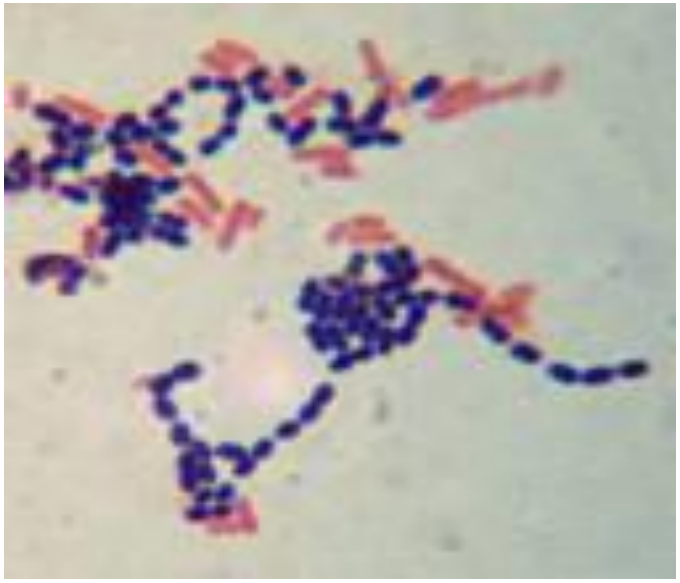
Most essential technique in Medical Microbiology

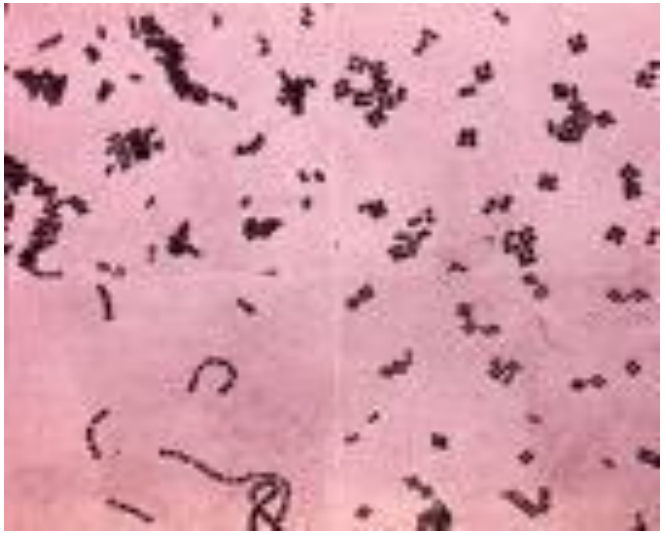
Provides information that helps in the treatment of diseases

Eg. Gram +ve bacteria tend to be killed easily with Penicillin and sulfonamide drugs

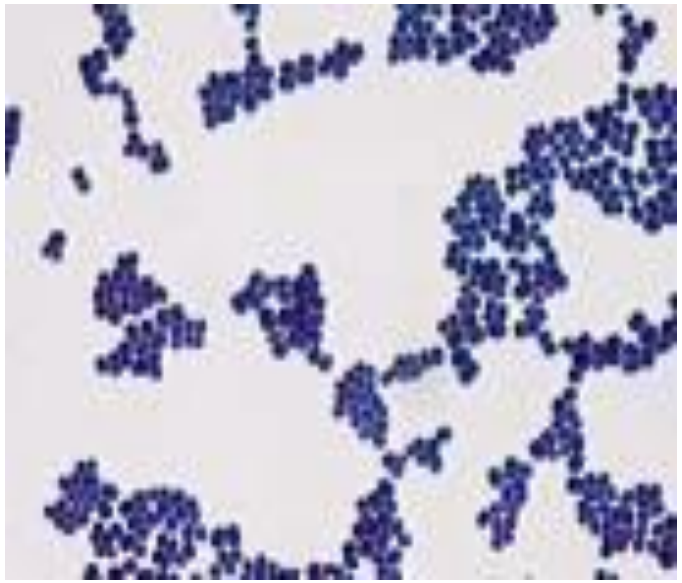
Gram –ve resist these drugs but are much more susceptible to such drugs as streptomycin, chloramphenicol and tetracycline.

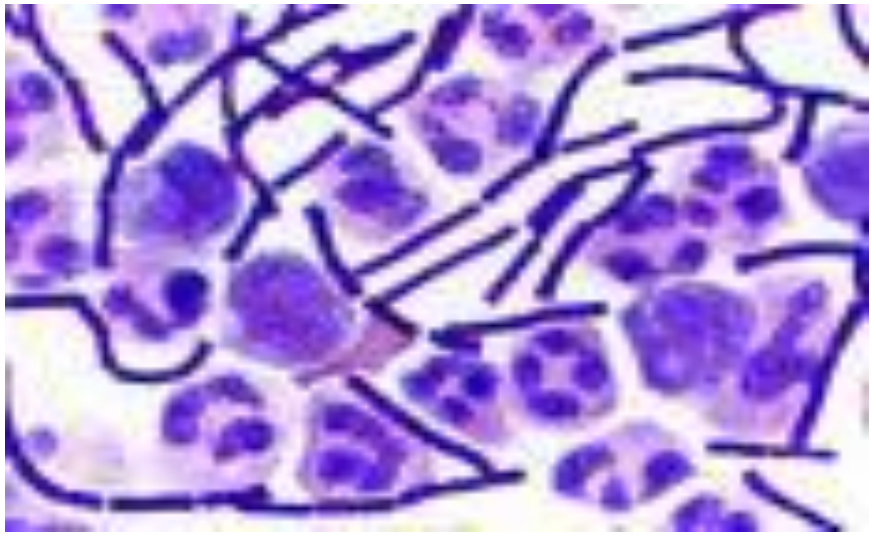
Streptococcus





Staphylococcus
Lactobacillus





Cult. No	*TCBS*	*YDC*	*TZC (glucose)*	*GRAM*	*KOH*	*O/F Test*	*Motility*	*Org. Shape*
12WA	white	white acid yellow	white small	positive	NS	O	not motile	cocci
12Y	white	yellow	orange small	positive	NS	O	not motile	rods
13WA	milky	white acid	trans-parent small	positive	NS	O	not motile	short rods/cocci
13Y	NG	yellow acid	white small	positive	NS	O	not motile	cocci
16	NG	white acid	white	positive	NS	O	not motile	cocci
18	milky	yellow	orange small	positive		O	not motile	rods
20	blue, green, yellow, orange	yellow, flat trans-parent colonies, slightly acid	NG	negative	S	F	motile	small rods
30	light yellow	yellow, raised	peach, opaque	positive	NS	O	not motile	cocci
44	Yellow dry	beige, acid, flat	white pink center	positive	NS	O	not motile	short rods/cocci
53	blue green yellow	flat cream colonies, slight acid	NG	negative	S	F	motile	mixed cocci and rods
70	yellow, raised slightly mucoid	slight acid small flat cream colonies	trans-parent, poor growth	positive	NS	O	not motile	thin short rods
81TCBS	yellowish-white	light yellow colonies	orange, opaque, small	positive	NS	O	not motile	cocci and rods
81YDC	yellow	creamy acid colonies	opaque small	positive	NS	O	not motile	cocci and rods
91	yellow, changed media to yellow	flat acidic undulated borders	white flat	positive	NS	O	not motile	cocci

Table 3. Some characteristics of different isolates enriched from Khewra salt mine samples.

Sample code	Media* used	Isolate code	Colony morphology				Cell shape and size (μm)	Endos-pores	Mean generation time t_d (h)	Specific growth rate μ (h^{-1})
			Colour	Form	Elevation	Margins				
DS	LB	BPT-12	Orange	Irregular	Raised	Undulate	Rods, 1-1.5	+	2.1	0.33
	HP	BPT-15	Cream	Punctiform	Flat	Entire	Rods-coccus, 1-1.5	-	2.3	0.30
	AP	BPT-23	Yellow	Circular	Raised	Entire	Rods, 2-3	-	3.0	0.23
	AP	BPT-25	White	Circular	Raised	Entire	Rods, 1	+	4.3	0.16
P1	LB	BPT-7	Yellow	Irregular	Raised	Entire	Rods, 1-1.5	+	1.2	0.58
P3	LB	BPT-3	Cream	Circular	Flat	Entire	Rods, 1-2	-	3.3	0.21
	LB	BPT-6	Red	Irregular	Umbonate	Undulate	Rods, 1-1.5	+	1.0	0.69
	AP	BPT-18	Orange	Irregular	Umbonate	Undulate	Rods, 1-1.5	-	2.4	0.29
P3 (5)	AP	BPT-20	Yellow	Irregular	Raised	Lobate	Rods, 2-2.5	+	1.5	0.45
S1	LB	BPT-2	Yellow	Circular	Raised	Entire	Rods-Coccus, 0.5-0.8	-	2.0	0.35
	LB	BPT-4	Cream	Circular	Flat	Entire	Rods, 1-2	-	2.7	0.26
	AP	BPT-19	Cream	Circular	Flat	Entire	Rods, 2-3	-	2.9	0.24
SL-1	LB	BPT-5	White	Irregular	Flat	Undulate	Rods, 1-1.5	+	1.5	0.47
WD1	LB	BPT-8	White	Circular	Raised	Entire	Coccus, 0.5-1	-	2.3	0.30
	LB	BPT-11	Red	Circular	Pulvinate	Entire	Ovoid, 0.5-1	-	2.4	0.28

*LB-Luria Bertani, HP-Halophilic Medium, AP-Alkaliphilic Medium.

Acid Fast Stain

Important in identifying bacteria with a waxy material in their cell walls

Use in identifying the *Mycobacterium*

M. leprae and *M. tuberculosis, M. ulcerans*

After fixing a smear of the organism, apply the red dye carbol-fuchsin and gently heat for several minutes

Heating enhances the penetration and retention of the dye.

The slide is cooled and washed with water

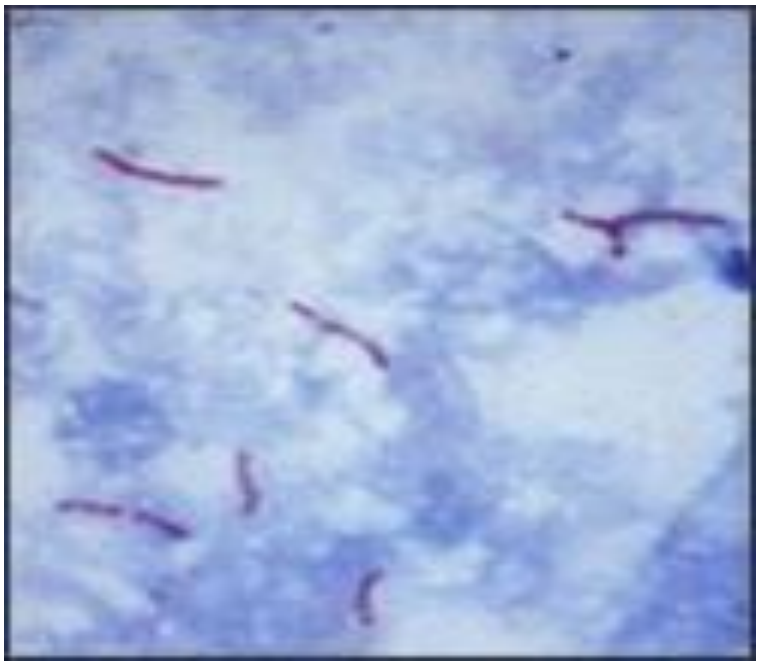
Next treat with alcohol, a decolorizer, which removes the red stain from bacteria that are not acid fast

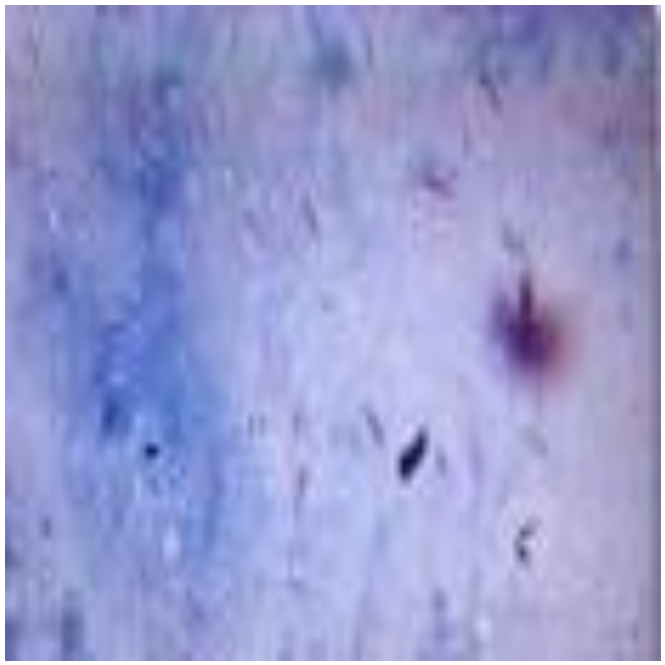
However, acid fast organisms retain the red colour because carbol-fuchsin is more soluble in the cell wall waxes than in the acid alcohol other wise the stain is readily washed off.

Counter stained with methylene blue

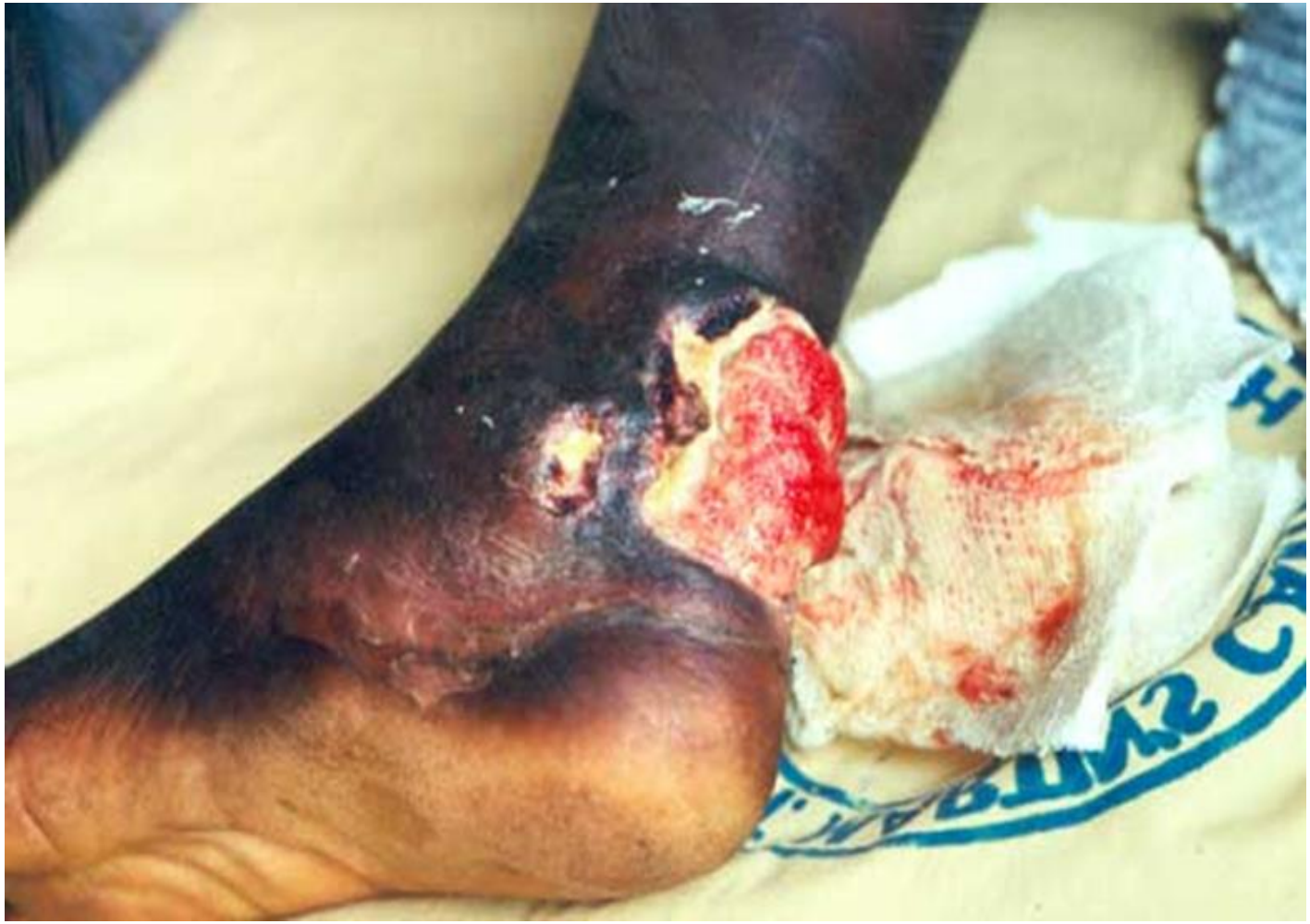
Non acid fast cells appear blue after application of the counter stain.

This photomicrograph reveals Mycobacterium tuberculosis bacteria using acid-fast Ziehl-Neelsen stain; Magnified 1000s

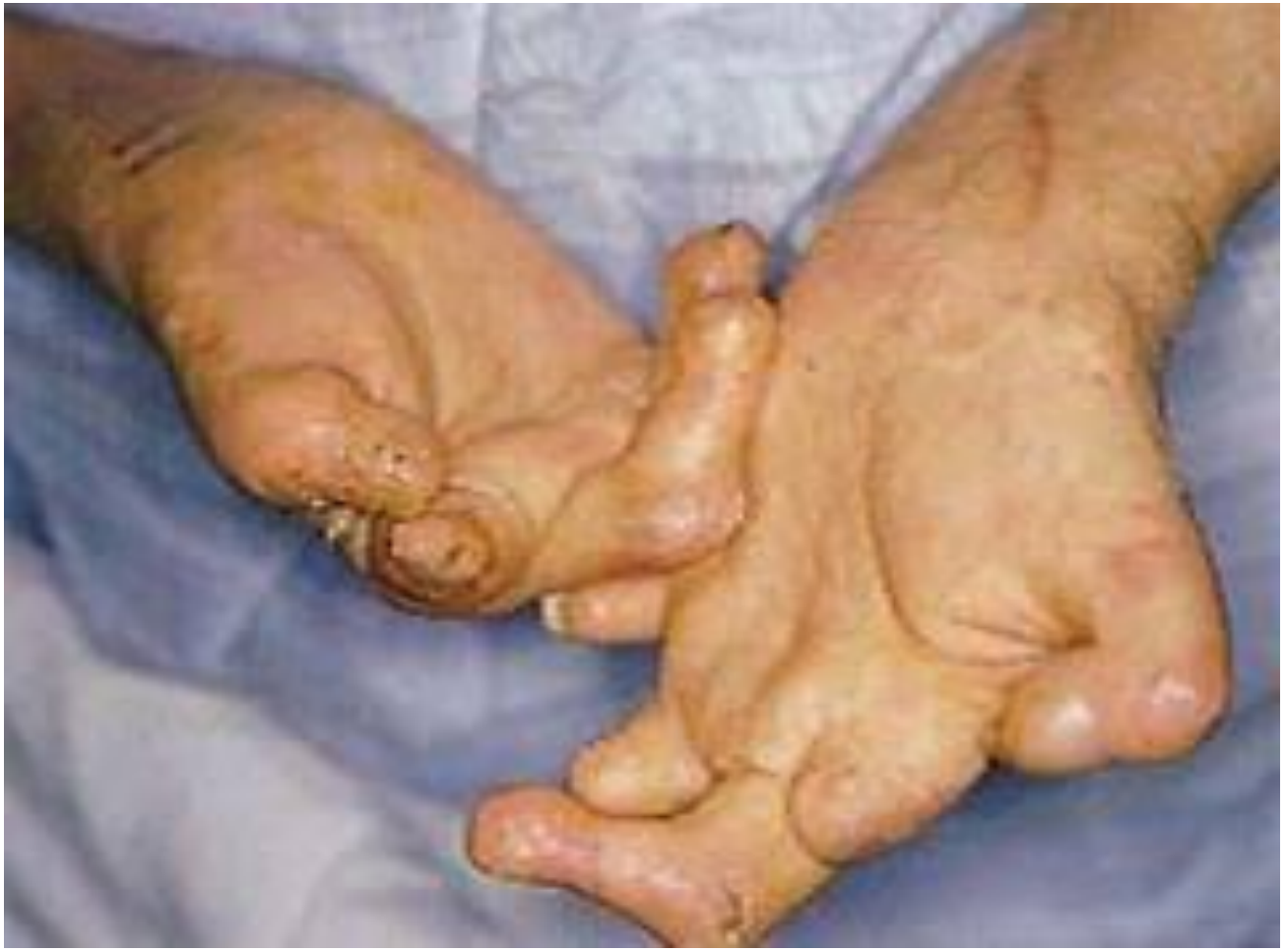


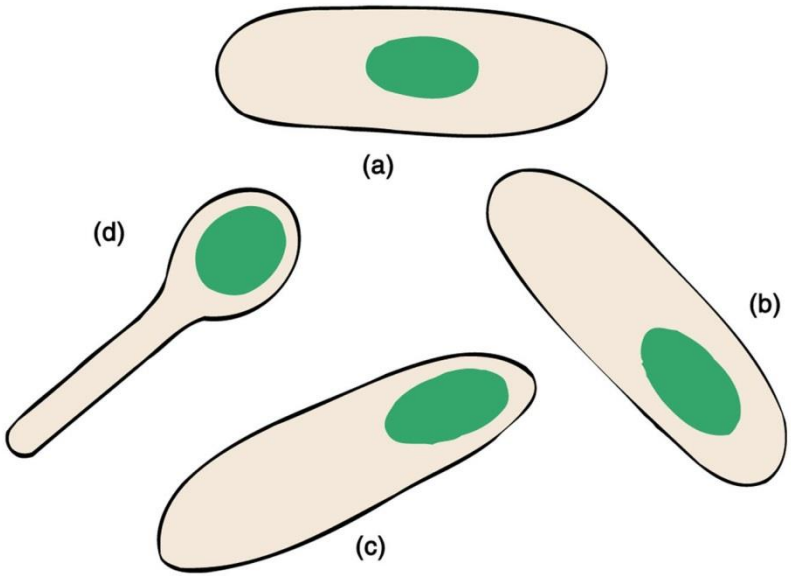


Buruli Ulcer



Leprosy




(a)
(b)
(c)
(d)

Spore staining

A spore is a special resistant, dormant structure formed within a cell that protects microorganisms from harsh environmental conditions

cannot be stained by ordinary methods – simple or Gram stain because the dyes do not penetrate the wall of the endospore

Stain used is Schaeffer-Fulton endospore stain

Malachite green, the primary stain is applied to a heat-fixed smear and heated to steaming for 5-10 mins

Heat helps the stain to penetrate the endospore wall

Washed for 30 secs with water to remove the malachite green and counterstained with safranin.

BIOCHEMICAL CHARACTERISTICS

Fermentation test

Fermentation is an anaerobic enzyme decomposition of organic compounds usually carbohydrates in which part of the substrate is oxidized and part reduced to produce carbon dioxide, acids and alcohol as by-products.

Must contain a vial (Durham tube to collect the gas

Must contain an indicator to detect any acid formation. Indicators include

Bromocresol purple changes from purple to yellow

Methyl red changes from red to yellow

Methyl orange changes from red to yellow

Phenol red from yellow to red.

Phenolphthalein from colourless to pink

Bromothymol Blue from green grass to yellow

Hydrogen Sulphide

Product of sulphur containing organic compounds metabolism

E.g. *Salmonella paratyphi* B causes gastroenteritis but the *S. paratyphi* A doesn't

The two can be differentiated by using the hydrogen sulphide test: *S. paratyphi* B produces H_2S but the *paratyphi* A doesn't.

Two methods can be used

- Moist lead acetate paper turns black in the presence of H_2S gas or the lead acetate can be incorporated into the medium and stab the organisms into the medium.

Catalase Test

Catalase is an enzyme which catalysis the breakdown of hydrogen peroxide to water and free oxygen.

Catalase is produced by many bacteria species

Micrococcus, Bacillus and *Staphylococcus* but not for e.g. in *Lactobacillus*

Use 3% hydrogen peroxide ……

Oxidase Test

A diagnostic test for the presence of cytochrome c using p-amino dimethyl aniline

Cytochromes are proteins that function as electron carriers in respiration and photosynthesis.

Use of oxidase strips…….

Proteolysis

Anything capable of hydrolyzing proteins

Can be demonstrated by the decomposition of gelatin

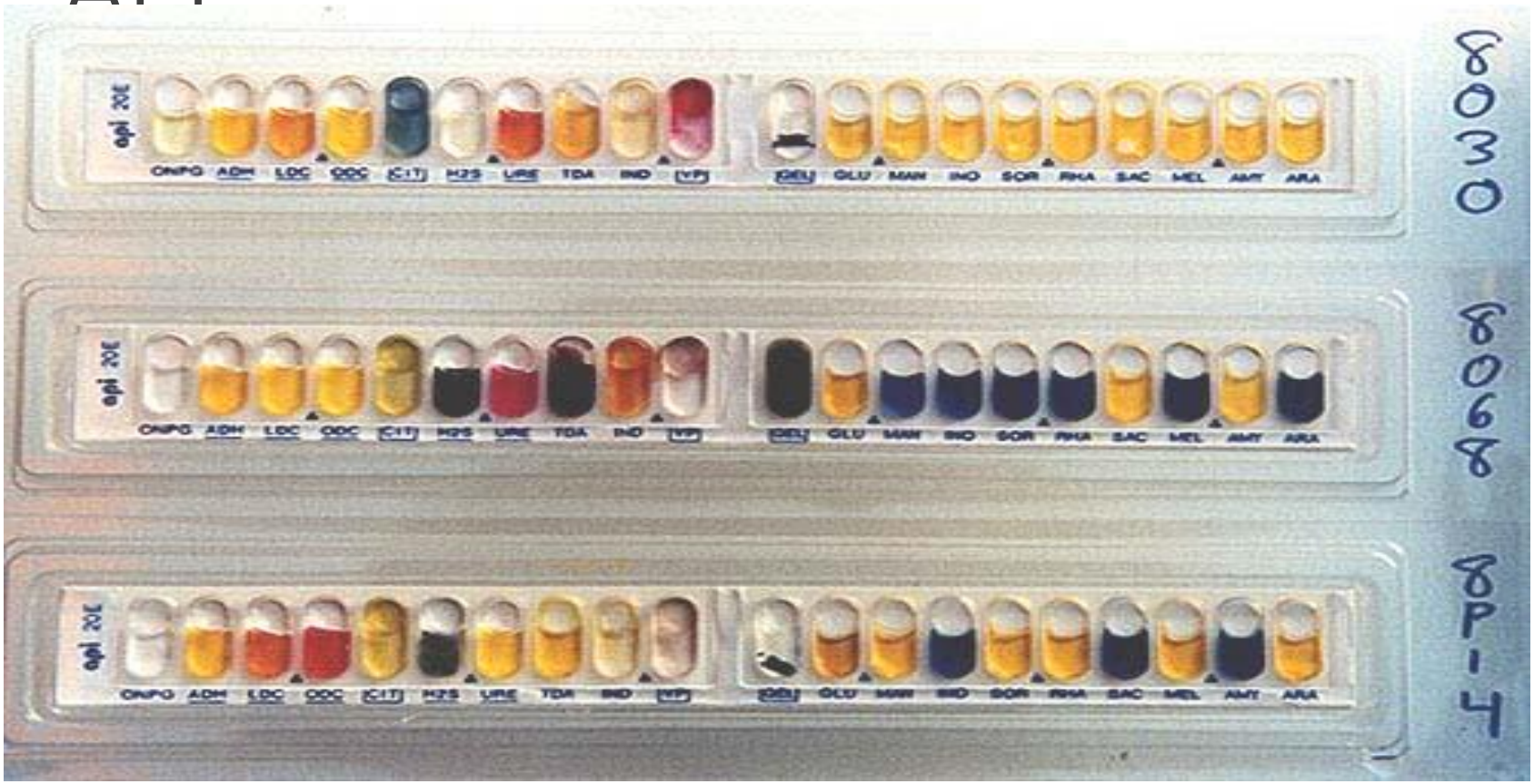
Test microbes are cultured on nutrient agar containing 1% gelatin

These are distributed into about 2 ml portions and stabbed with the test microbe

After incubation (5 hrs), several drops of acidified mercuric chloride is added

Hydrolysis is shown by a clear zone in the upper layer of the gelatin.

API


api 20E
ONPG ADH LDC ODC CIT H2S URE TDA IND VP
GEL GLU MAN INO SOR RHA SAC MEL AMY ARA
6030
6068
API-T


api 20E
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GEL GLU MAN INO SOR RHA SAC MEL AMY ARA
1 2 3 4 5 6 7 8 9 10

api 20 E

07223 A

REF. : _____

Origine / Source / Herkunft / Origen / Prelievo :

bioMérieux

Imprimé en France / Printed in France

Autres tests / Other tests / Weitere Tests / Altri tests / Otros tests :

Ident. :

Escherichia coli

excellent identification

Few thoughts

One of the successes of modern medicine is the control of infectious diseases in the developed world

Typhoid, cholera, diphtheria are now rare and smallpox is eradicated globally

Reasons include

Improved sanitation

Improved nutrition

Good housing

Smaller families

Immunization

antibiotics

Pathogen	non-pathogen
Staph. aureus	Staph albus
Boils, wound infections	harmless,lives on skin Osteomyelitis
pneumonia	
Coryn. diphtheria	Coryn. xerosis
Diphtheria	harmless,throat,skin

Now there can be S. aureus without illness and S. albus damaging heart valves and prosthetic joints

Infection sustained uncontrolled growth of microbes, doing the patient some harm

Colonisation: presence of microbes which is well controlled and only has the potential for harm

Colonising microbes can go on to cause infections when our defences are impaired

Defences?????

General Health: Measles in healthy and malnourished child

Mechanical: intact skin, flushing urine, coughing up phlegm, prevent microbes gaining access

Immunity: protects us against diseases but also make transplant difficult.

Ability of an individual to resist infection

Lowered resistance: Poor nutrition, ill health

Invasion: surgery, catheters, cannulas, intubation, prostheses

Immunosuppression: malignancy, drugs, illness, AIDS

Opportunistic infection: where the occurrence or severity of an infection is determined by the condition of the patient rather than by the microbe. More virulent microbes are a lot worse and harmless microbes become serious

Nosocomial infection: infection occurring in hospital or hospital acquired infection

Infectivity: ease with which a microbe can spread or how easy it is to catch it

Serology

Microbes, should they enter a human system will stimulate it to produce antibodies.

An **antigen** is a molecule that stimulates an immune response or stimulates antibody generation.

Antigens are usually proteins or polysaccharides

This includes cell components such as coats, capsules, cell walls, flagella, fimbrae, and toxins of bacteria, viruses and other microbes

Non-microbial exogenous (non-self) antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells.

Antigens that enter the body from the environment; these would include

- inhaled macromolecules (e.g., proteins on cat hairs that can trigger an attack of asthma in susceptible people)
- ingested macromolecules (e.g., shellfish proteins that trigger an allergic response in susceptible people)
- molecules that are introduced beneath the skin (e.g., on a splinter or in an injected vaccine)

Antibodies are proteins that circulate freely in the blood or other body fluids and often combine in a highly specific way with the microbes that caused their production

and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.

E.g. the immune system of an animal injected with killed cholera bacteria (antigen) responds by producing antibodies against the cholera bacteria

Antisera (antiserum) are commercially available solutions of such antibodies used in the identification of many medically important microbes

If an unknown microbe is isolated from a patient, it can be tested against known antisera and often identified quickly.

Slide agglutination test

Slide Agglutination tests are used to test an unknown organism against known antisera.

Used in the serotyping of *Salmonella* species.

Bacteria, provided they form stable suspensions in saline, can be agglutinated directly by antibody.

Bacterial agglutination tests may be performed on a slide, in microtitre tray wells or in tubes.

Tube agglutination tests are usually more sensitive than slide tests as they require a longer incubation period which allows more antigen and antibody to interact

Here samples of an unknown microbe are placed in a drop of 0.85% saline on each of several slides.

Then a different known antiserum is added to each sample.

The microbe agglutinate (clump) when mixed with antibodies that were produced in response to that species or strain of microbe, a +ve test is indicated by the presence of agglutination

ELISA (Enzyme-linked iummunosorbent assays)

ELISA is a fundamental tool of clinical immunology used as an initial screen for HIV detection

Based on the principle of antibody-antibody interaction

Test allows for easy visualization of results

They are now widely used because they are fast and can be read by a computer scanner

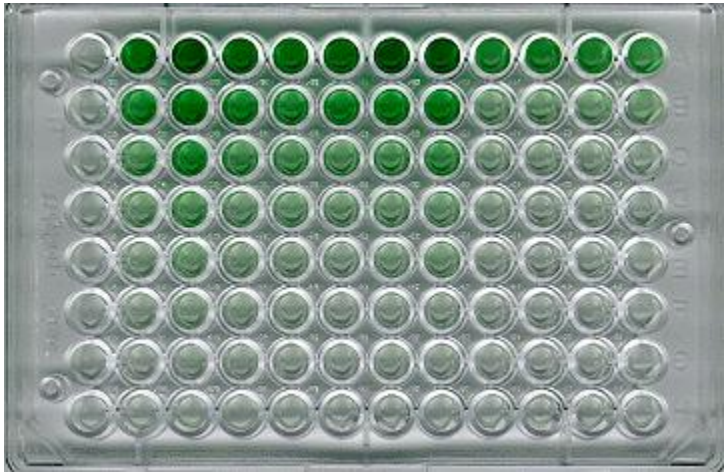
ELISA, known antibodies are placed in the wells of a microplate

and an unknown type of bacteria is added to each well

A reaction between the known antibodies and the microbe provides identification of the microbe ELISA is used in AIDS testing worldwide

ELISA is the first and most basic test to determine if an individual is positive for a selected pathogen, such as HIV.

The test is performed in a 8 cm x 12 cm plastic plate which contains an 8 x 12 matrix of 96 wells, each of which are about 1 cm high and 0.7 cm in diameter.



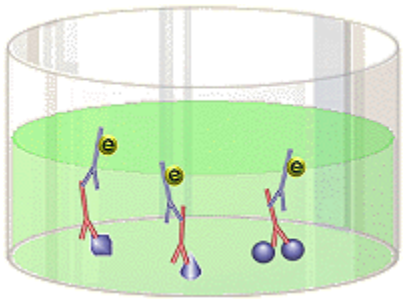
The Elisa Method

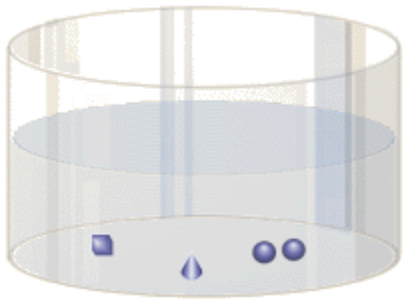
Partially purified, inactivated HIV antigens pre-coated onto an ELISA plate

Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate

Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.

Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody





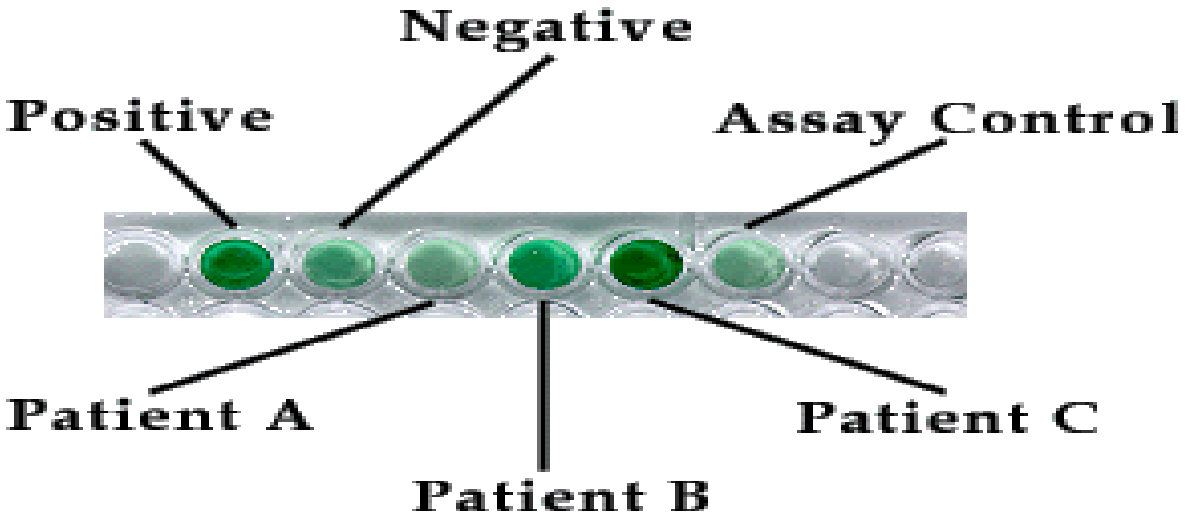
False positives

It is entirely possible that an individual not infected with HIV has antibodies which may give a positive result in the HIV ELISA

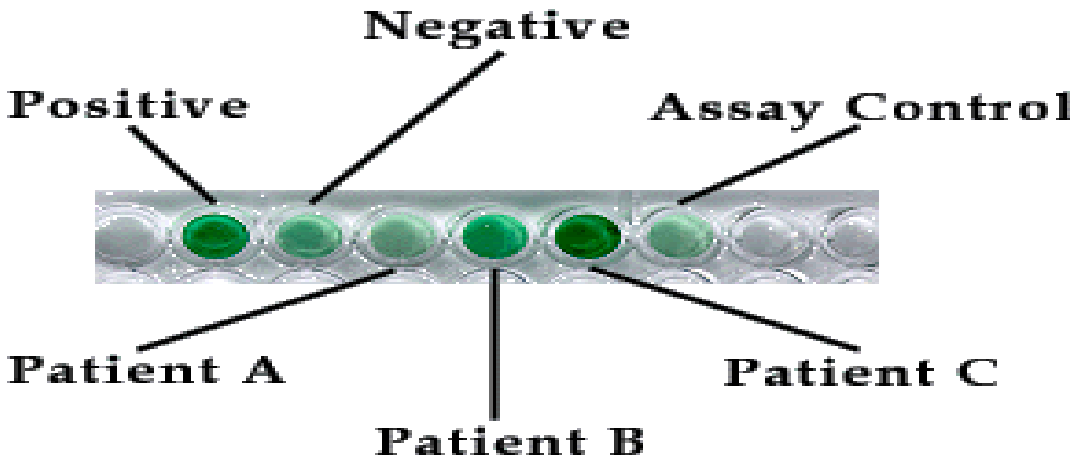
This is called a false positive.

One reason for this is that people (especially women who have had multiple pregnancies) may possess antibodies directed against human leukocyte antigens (HLA) which are present on the host cells used to propagate HIV.

As HIV buds from the surface of the host cell, it incorporates some of the host cell HLA into its envelope. False negatives can occur during the window between infection and an antibody response to the virus (seroconversion)



Positive Control	Negative Control	Patient A	Patient B	Patient C	Assay Control	
1.685	0.153	0.055	0.412	1.999	0.123	



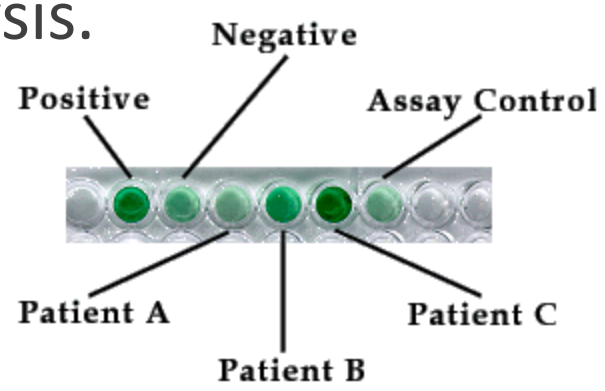
Above is ELISA data from three patients. Numbers are expressed as optical density at 450 nm. The cutoff value indicating a positive result is 0.500

Optical densities of 0.300 to 0.499 are indeterminate and need to be retested

Values below 0.300 are considered to be negative

In most cases, a patient will be retested if the serum gives a positive result.

If the ELISA retests are positive, the patient will then be retested by western blotting analysis.



Western Blot

Western blot is a method used in detecting a specific protein in a given sample of tissue homogenate or extract

It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions)

Gel electrophoresis is a technique used for the separation of DNA, RNA, or protein molecules using an electric current applied to a gel matrix.

The proteins are then transferred to a membrane (typically nitrocellulose where they are probed (detected) using antibodies specific to the target protein

are probed (detected) using antibodies specific to the target protein

Now many companies specialize in providing antibodies (both monoclonal and polyclonal antibodies) against many thousands of different proteins.

Previously large animals (e.g. sheep, goat - lots of serum) had to be immunised with the target protein twice (secondary immune response generates high affinity antibodies)

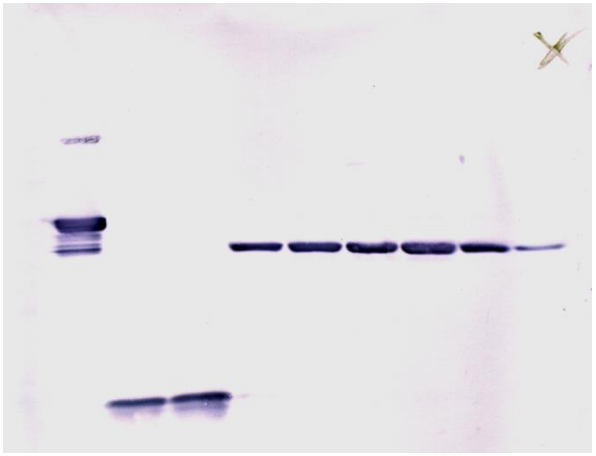
Then either serum could be purified and used (polyclonal antibodies)

Bacterial proteins in the patients serum are separated by a process called electrophoresis

Proteins are then transferred to a filter by blotting.

Antibodies tagged with a dye are washed over the filter

If the specific antigen (in this case the microbe proteins) is present in the serum, the antibodies will combine with it and will be visible as a coloured band on the filter



The method originated from the laboratory of George Stark at Stanford. The name **western blot** was given to the technique by W. Neal Burnette and is a play on the name Southern blot, a technique for DNA detection developed earlier by Edwin Southern. Detection of RNA is termed northern blotting

Phage typing

Bacteriophages are bacterial viruses that usually cause the breakdown or lysis of bacteria cells they infect.

They are used in looking for similarities amongst organisms and is therefore useful in tracing disease outbreak sources.

Phage typing is highly specialized as they usually infect only members of a particular species, or even particular strains within a species.

Flood a agar plate with the bacterial culture or suspension.

Drops of each different phage type to be used in the test is then placed on the bacteria.

Whenever the phage are able to infect and lyse the bacteria cells, clearings in the bacteria growth called plagues appear.

Such a test might show that the bacteria isolated from a surgical wound have the same pattern of phage sensitivity as those isolated from the operating surgeon or surgical nurses. This establishes that the surgeon or nurse might be the source of infection

BACTERIA CLASSIFICATION

Systematic arrangement of organisms into some system of reference

i.e. groups or categories - taxa based on their distinguishing characteristics or features or based on their similarities

There are several difficulties with the classification of bacteria for example because

There are no natural species boundaries because multiplication is asexual

- We can only depend on theory to learn about evolutionary and phylogenetic history of a species.
- Bacteria left no fossil evidence and therefore their evolution is a mere speculation
- many characteristics of microbes are subject to change

This explains why many bacterial classification systems have depended on descriptive keys for identification of an organism.

When new organisms are encountered references are made to sample cultures kept at collection centres

e.g. American Type Culture Collection (ATCC),

International Collection of Phyto-pathogenic bacteria

Again some microbes may have the distinctive features of others

Research ‘s manipulation of genetic constitution of bacteria for research activities and other commercial ventures

What is the way out?

To rely on qualitative characteristics which has produced a more stable classification systems

e.g. form, motility, oxygen requirement

These are less subject to change unlike the quantitative characteristics like size, rate of growth, pigment intensity etc.

Microbiologists have cooperated in the use of standardized procedures, knowledge of characteristics that are variable, knowledge and appreciation of the general principles and problems of taxonomy.

Nomenclature is the application of names both to the microbes themselves and also to the groups in which they are placed.

The name should be descriptive and binomial.

The first name refers to the genus starting with an uppercase letter and the second name is the epithet of the first name

It may refer to the colour, origin or the disease. A name is either descriptive, Geographical or commemorative.

Identification is the recognition of microbes and involves marching microbes with those already known.

DIFFERENT CLASSIFICATION SCHEMES

A species is a group of cells that show a high degree of overall phenotypic similarity and that differ from related cell groups.

The species concept in microbiology is often doubtful in bacteria.

Theoretically it is a kind of bacteria with identical or near so individual cells. In reality however, mutant forms always exist in a given population even though they may sometimes be temporary.

NATURE OF VIRUSES

1. Viruses are very small microorganisms that can only be seen with the electron microscope.

2. They are acellular.

3. Contain only a single type of nucleic acid, either DNA or RNA

Normal living cells all have DNA and RNA and therefore viruses can only reproduce by using the cellular material of other organisms

4. contain a protein coat (sometimes itself enclosed by an envelope of lipids, proteins and carbohydrates) that surrounds the nucleic acid

This protein coat structure is called CAPSID

Capsid is made up of many structural units called CAPSOMERESS

The composition and numbers and forms of which vary with different kinds of viruses.

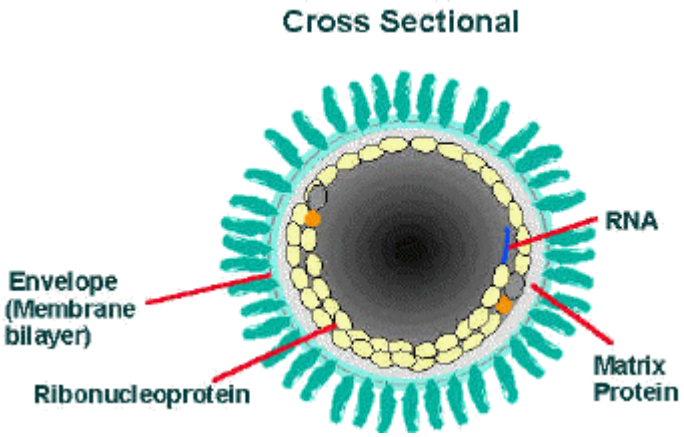
capsid is physiologically inert.

5. Multiply inside living cells by using the synthesizing machinery of the cell

6. They have a few or no enzymes for their own metabolism

For example they lack the enzymes for protein synthesis and ATP generation.

Rabies virus



7. Viruses are most difficult to handle in medical science, WHY?

because most drugs that would interfere with viral multiplication would also interfere with the functioning of the host cell and hence are too toxic for clinical use

However, the presence of lipids in the coverings of some viruses makes these viruses sensitive to disinfection (outside the host cell)

For example, a 1:10 dilution of household chlorine bleach can inactivate HIV (the AIDS virus, which is in the genus Lentivirus) outside the body.

8. Viruses are about 20 – 14000 nm in length.

9. Animal viruses are enveloped

Plant and Bacteria viruses are non-enveloped

The viral particle exhibit a great variety of shapes and sizes

Some appear as rids which on careful examination prove to be helical e.g. influenza virus

Spherical forms characteristic of polyhedral herpes virus

A combination of helical and spherical forms i.e. a polyhedral head attached to a spherical tail, is common among phages

but is never found in animal or plant viruses. Usually all the nucleic acid material is in the head and the tail is

10. Viruses can infect a host of microorganism including inverts, verts, plants, protists, fungi and bacteria

Viruses that infect bacteria are called Bacteriophages or phages

Viruses are all parasites.

Many diseases of humans are known to be caused by viruses and are responsible for thousands of deaths

Viruses were previously endemic (low levels of infection in localised areas but have now crossed the species barrier

Polio was endemic but now a Pandemic (high levels of infection worldwide)

Polio spread has been due to urban populations that have developed as a result of the industrial revolution providing an ideal environment.

Polio vaccine in 1950 has halted the spread but still pockets of infection. WHO said it will eradicate it by **2006**

Measles population and travel

Reservoir (healthy individuals harbouring an infectious agent that is available to infect another host)

Vector (carriers) for a virus

Yellow fever – carried by *Aedes aegypti* but now also by Haemagogus

Stayed amongst monkeys until people involved in timber cut the trees to enable the mosquito come to the forest floor and infect humans

Many virologist believe that similar events have occurred in the case of the Human immunodeficiency virus (HIV)

Retrovirus which are similar to HIV exist in domesticated cats called the FIV

Feline Immunodeficiency virus in 1987

Simian AIDS, immune deficiency disease in green monkeys reported in 1986

It is possible that a mutated form of SIV crossed over to humans from contact with an infected monkey

Antibodies to SIV have been found in humans

CATS AND DISEASE

Chimp version of HIV finally linked to human epidemic

HIV originated in a subspecies of chimpanzee in west Central Africa, an international research team has concluded.

AIDS researchers have long suspected that HIV-1 evolved from one of the simian immunodeficiency viruses (SIVs) that infect other primates. But virologists had never identified a SIV strain similar enough to HIV-1 to be its forerunner.

Now Feng Gao of the University of Alabama in Birmingham and his colleagues have found a match between the three major groups of HIV and SIV sequences from the chimp subspecies *Pan troglodytes troglodytes* (*Nature*, vol 397, p 436). "But these animals don't seem to get AIDS," says Gao, who hopes this may help in designing a vaccine.

In spite of the many theories, e.g., polio vaccine or life-style it seems that the original theory that human mmuno-deficiency virus evolved from simian viruses (SIVs) is correct

In USA is an emerging virus called Hantavirus

It is transmitted by Rodents faeces and urine to humans

It causes Hantavirus Pulmonary Syndrome

Also causes haemorrhagic fever

It is an acute often fatal respiratory disease

Bats - Nipah virus

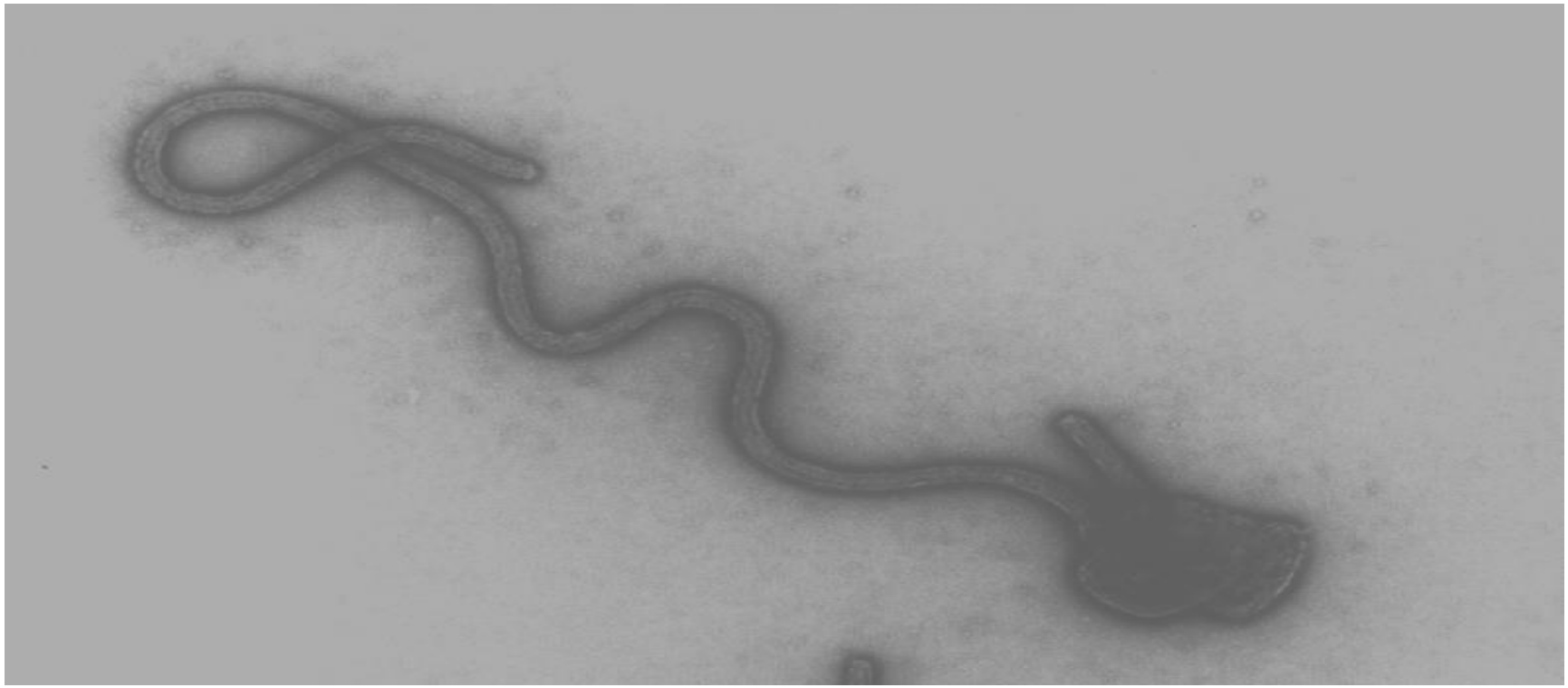
birds - West Nile virus

Dengue fever

Influenza virus

Ebola virus

Unknown route of transmission (highly infectious through contact with body fluids); Zoonotic virus



Why they are appearing now

Travel to increasingly exotic locations (esp. last minute with no time for vaccinations)

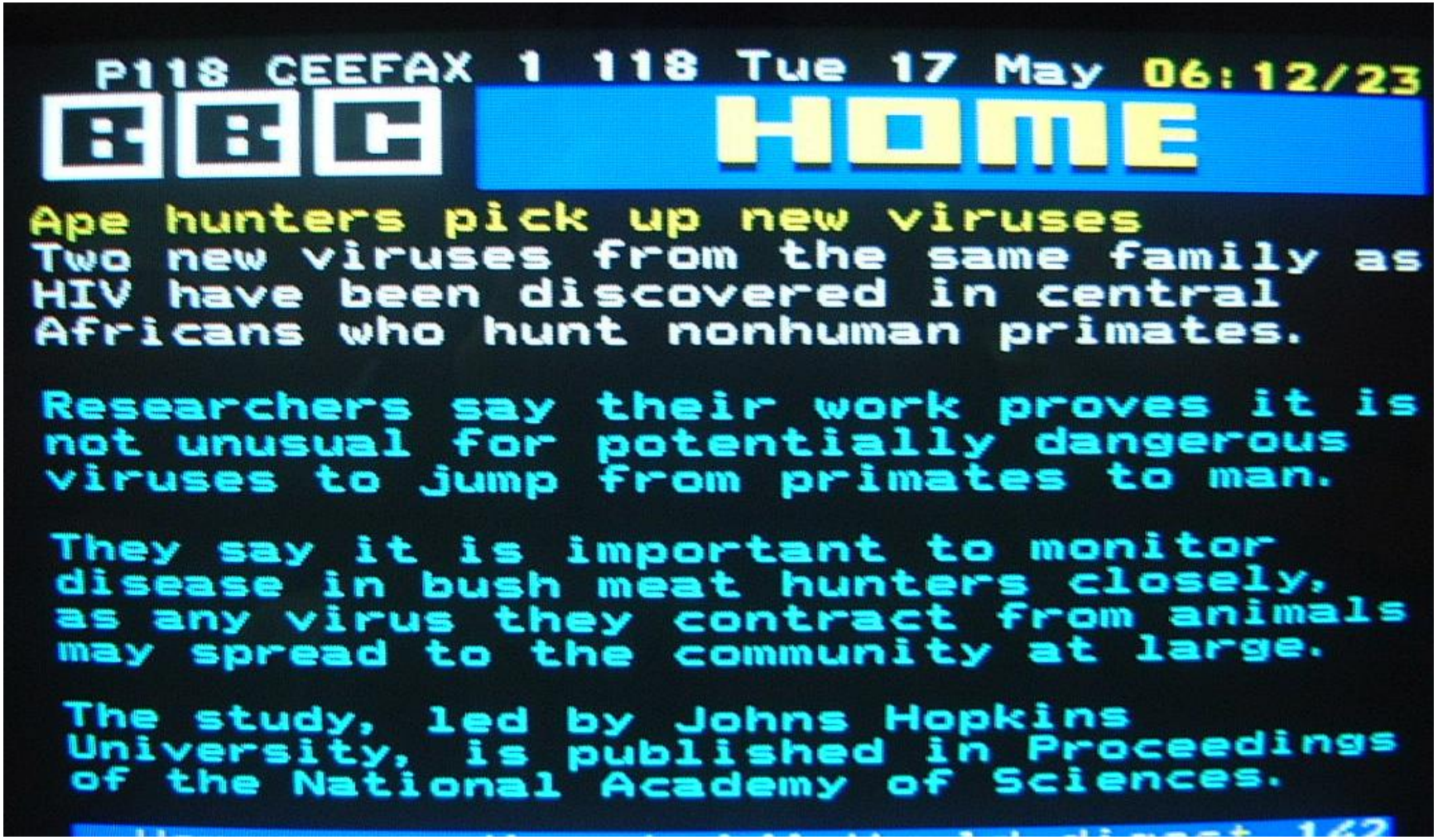
Pilgrimages (Haj in 2000 - meningitis)

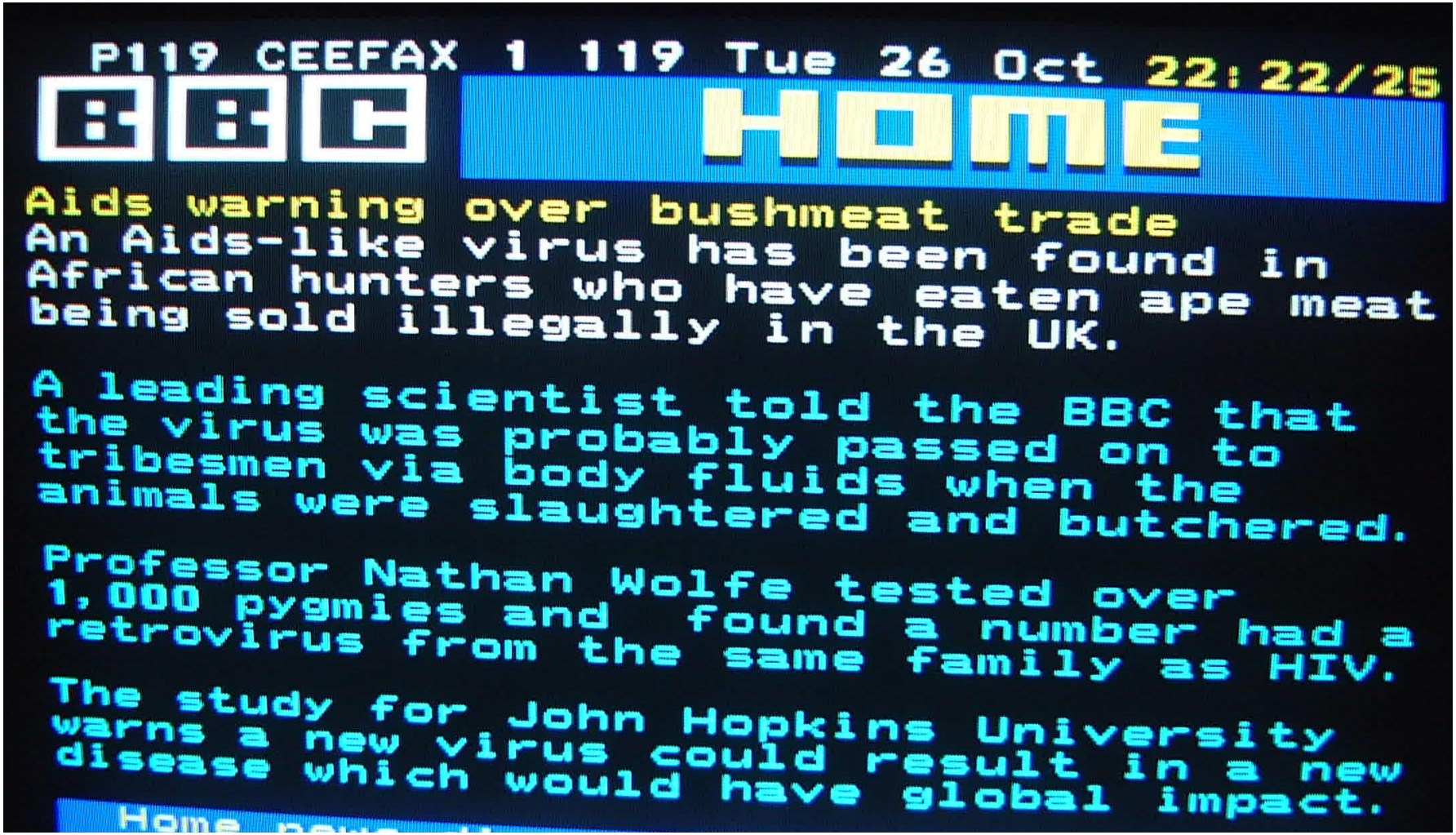
Cruise ships – winter vomiting disease

Travel getting closer to nature

Forest clearance and eating (and/or importation) of bush meat:

This leads to increased contact with animals, their bodily excretions and their faeces, frequently in an aerosolised dust


P118 CEEFAX 1 118 Tue 17 May 06:12/23
BBC
HOME
Ape hunters pick up new viruses
Two new viruses from the same family as HIV have been discovered in central Africans who hunt nonhuman primates.
Researchers say their work proves it is not unusual for potentially dangerous viruses to jump from primates to man.
They say it is important to monitor disease in bush meat hunters closely, as any virus they contract from animals may spread to the community at large.
The study, led by Johns Hopkins University, is published in Proceedings of the National Academy of Sciences.


P119 CEEFAX 1 119 Tue 26 Oct 22:22/25
BBC
HOME
Aids warning over bushmeat trade
An Aids-like virus has been found in African hunters who have eaten ape meat being sold illegally in the UK.
A leading scientist told the BBC that the virus was probably passed on to tribesmen via body fluids when the animals were slaughtered and butchered.
Professor Nathan Wolfe tested over 1,000 pygmies and found a number had a retrovirus from the same family as HIV.
The study for John Hopkins University warns a new virus could result in a new disease which would have global impact.

P312 Teletext 312 Mar23 05:54:44

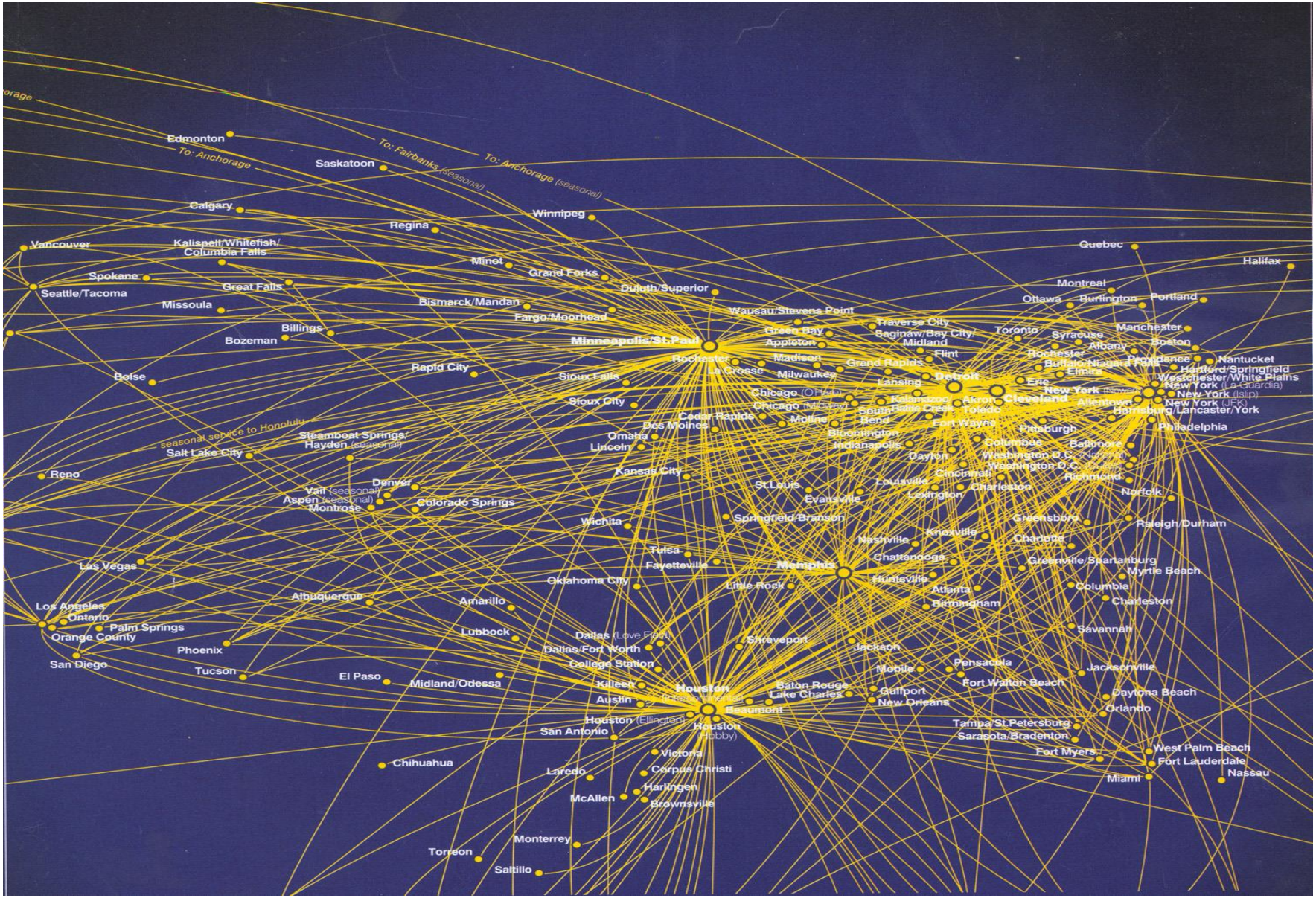
News

WARNING OVER MEAT IMPORTS

Illegal meat imports threaten a repeat of the foot-and-mouth outbreak, the National Audit Office has warned.

The 2001 epidemic, thought to have been caused by pig swill contaminated by meat unlawfully brought into Britain, cost the country an estimated £8bn.

However, around 12,000 tonnes of meat and meat products are still thought to come in illegally each year. Tighter controls are needed, the NAO said.



World wide travel

Emerging and re-emerging viral diseases

Most emerging diseases are zoonoses and viruses:

DISEASE	ANIMAL RESERVOIR
Hanta, lassa fevers	rodents
Rift valley virus	cattle and sheep
HIV	monkeys
Nipah virus	bats
West Nile virus	birds
Avian flu	birds
Ebola /Marburg	?
SARS*	Horse shoe bats, civet cats

*severe acute respiratory syndrome

DETECTION AND ENUMERATION OF VIRUSES

Plague method where bacteriophage is used

Growth of animal viruses in the laboratory. Involves using living animals like mice rabbits and guinea pigs. Following Koch’s postulate.

However, some human viruses cannot be grown in animals or can be grown but do not cause disease

This has been the problem with the slow progress made in HIV research because there are no animal models for the disease, HIV virus

For example Chimpanzees can be infected with the HIV 1 virus i.e. Lentivirus but because they do not show symptoms of the disease, they cannot be used to study effects of viral growth and disease treatment

This explains why AIDS vaccines or drugs are presently being tested in humans in Kenya and South Africa, but the disease progresses so slowly in humans that it can take years to determine the effectiveness of these vaccines

A breakthrough in this area of worry has been resolved. Presently, genetically engineered human T cells and human gamma globulin is being produced by mice and therefore it is now possible to infect them with human AIDS.