Microbiological investigations for infectious diseases

BCHM 4608

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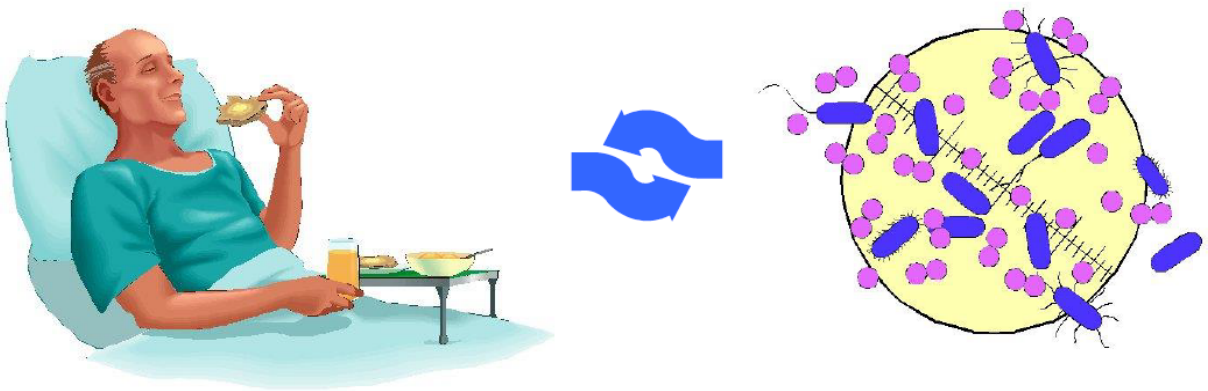
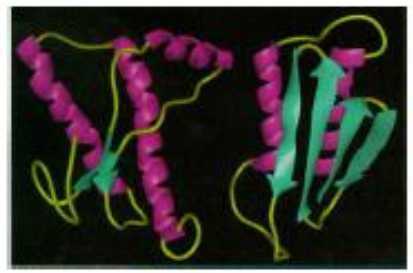


Pathogens for Infectious Diseases

Infectious diseases: 1) diseases caused by microbes; and 2) that spread

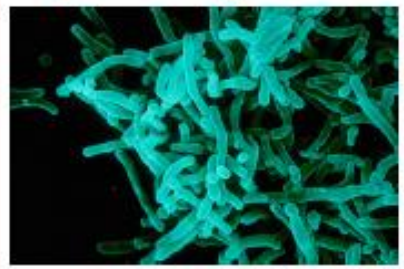
non-cellular

Virus



Prion

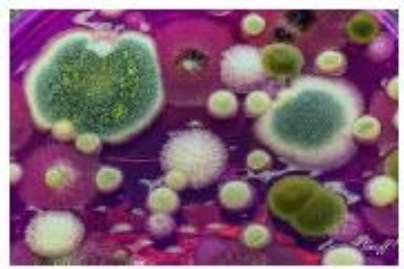
prokaryotic



Bacterium

Protozoan

Helminth



Fungus

eukaryotic

The microbiology laboratory

- Microbiological examination of clinical specimens.
- Consultation on the investigation and management of patients with infectious problems.
- Control of hospital infections.
- Public health surveillance.

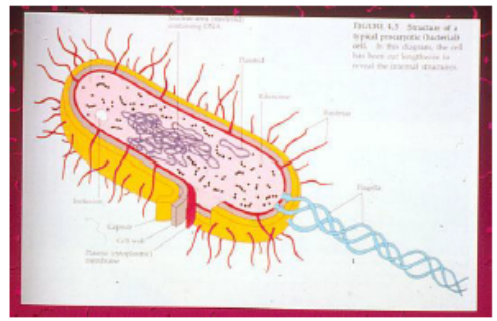
Learning objectives

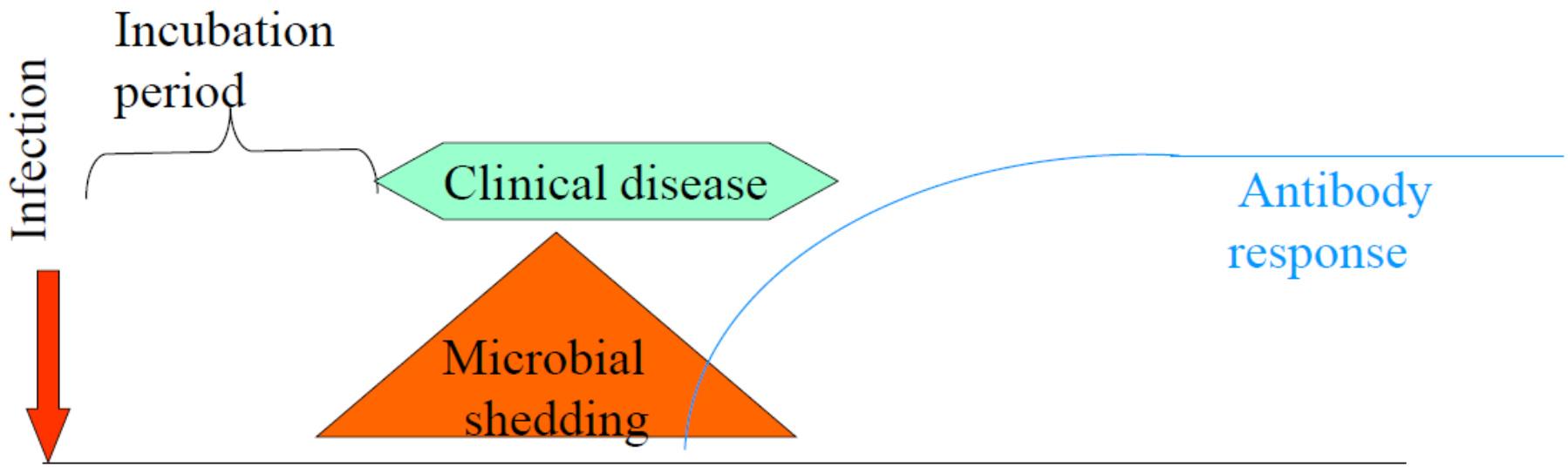
- List the reasons why microbiological laboratory diagnosis is important for good clinical care?
- Describe the factors that need to be considered in collection and transport of specimens for microbiological laboratory diagnosis?

- Describe the available options for diagnosis of viral infections?

Microbial diagnosis: How?

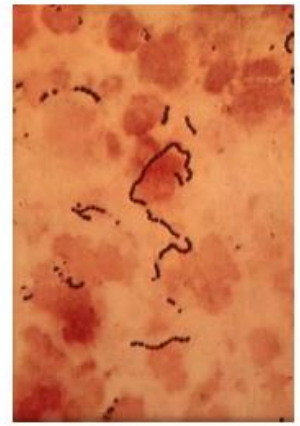
- Detect the microbe
- Detect antibody response - serology





Detect the microbe: How

- see - microscopy, *minutes*



- grow (culture) – *days*
- Microbial antigen detection – *hours*
- Microbial nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

The laboratory services

- Microscopic examinations.
- Culture.
- Antimicrobial susceptibility testing.
- Antigen/antibody detection (serology)
- Nucleic acid detection.
- Therapeutic drug monitoring.
- Epidemiological typing.

Diagnosis of infections

- Clinical diagnosis
- Laboratory diagnosis
  - Confirmation
  - Antibiotic susceptibility
  - Epidemiological relationships

Laboratory diagnosis

- Specimens
- Direct microscopic examination
- Culture
- Antigen/antibody detection
- Nucleic acid amplification, gene sequencing
- Histopathology

Good quality specimens

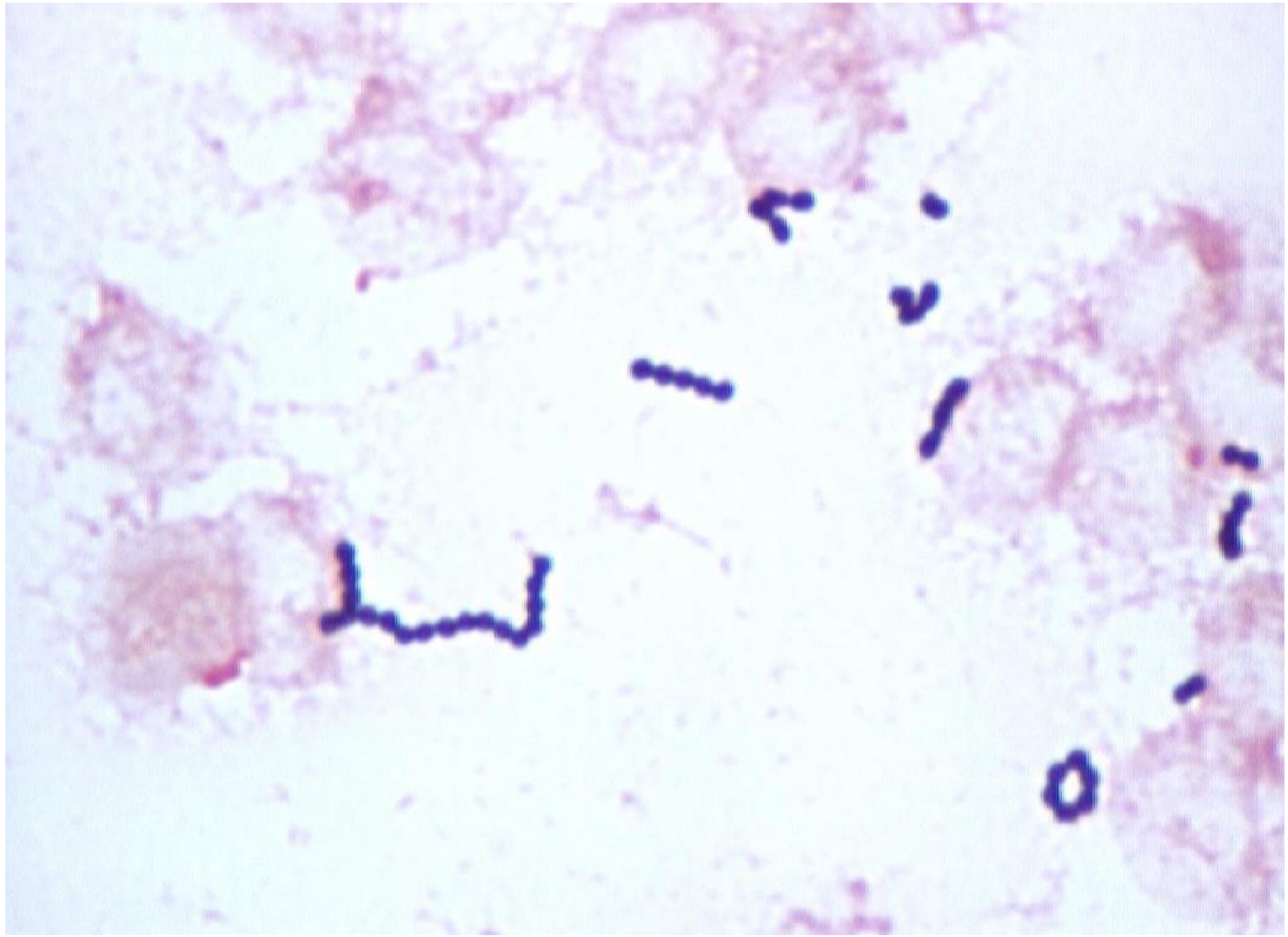
Learning objective !

- Optimal time of collection.
- Correct type.
- Minimum contamination.
- Adequate quantities.
- Appropriate number.
- Correct labelling.
- Safe transport.
- Prompt delivery.

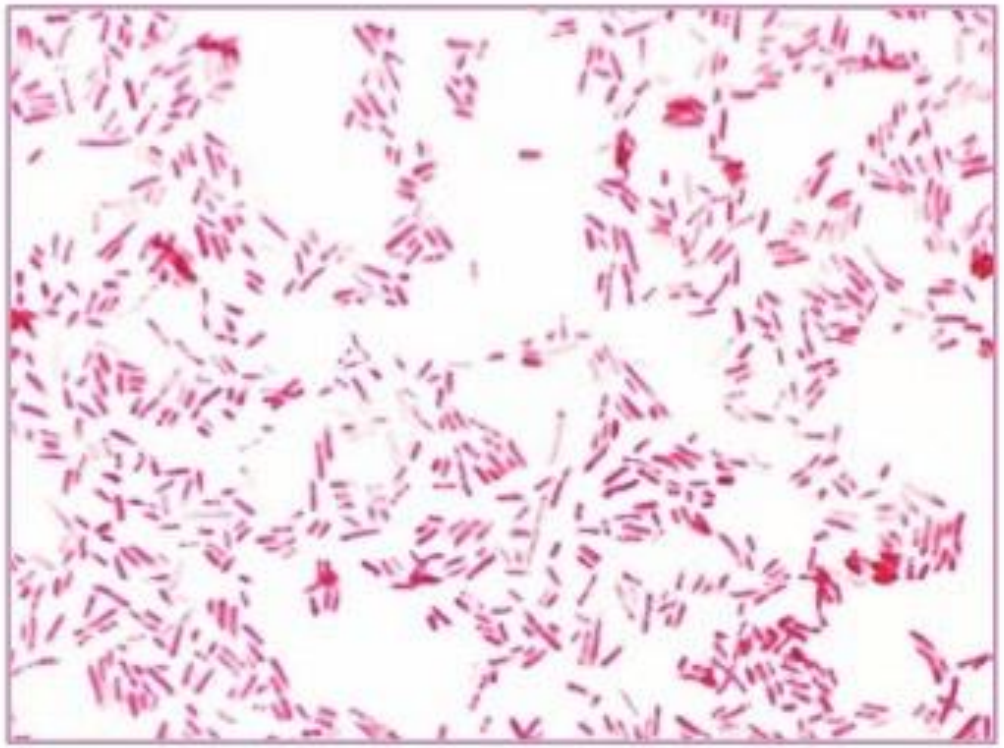

QM8623/10
Nikon
OPTIPHOT
SLIDES

Microscopic examination

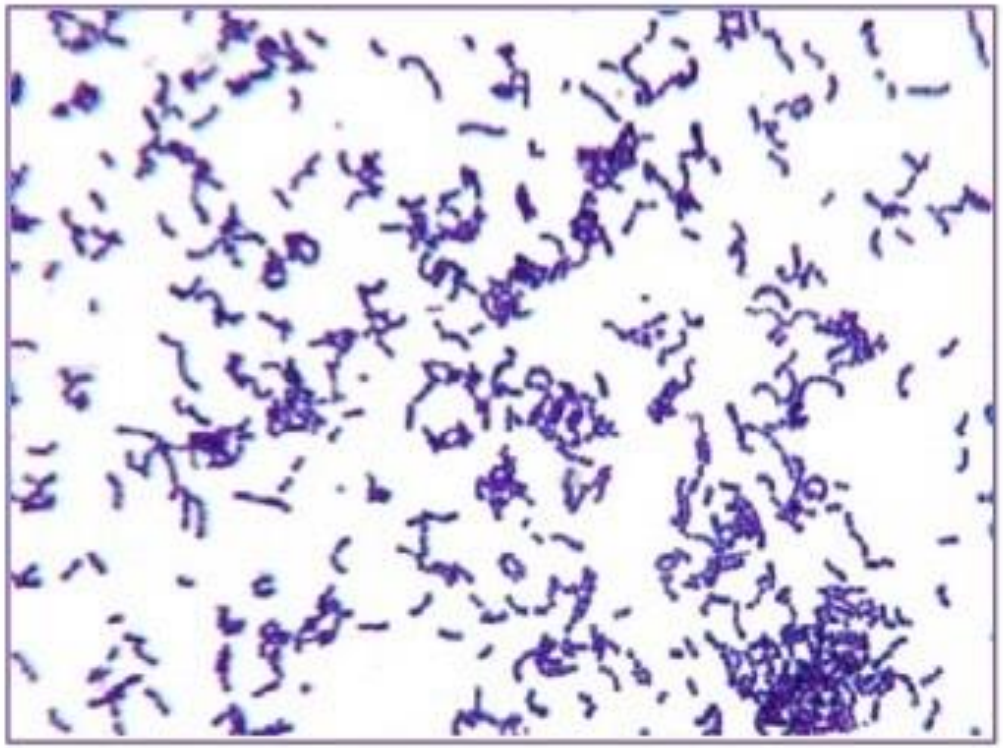
- Direct microscopic examination
- Staining
  - Gram stain
  - Ziehl-Neelsen stain
  - Other special stains
  - Immunofluorescence
- Electron microscopy
- Histopathology



Gram Stain

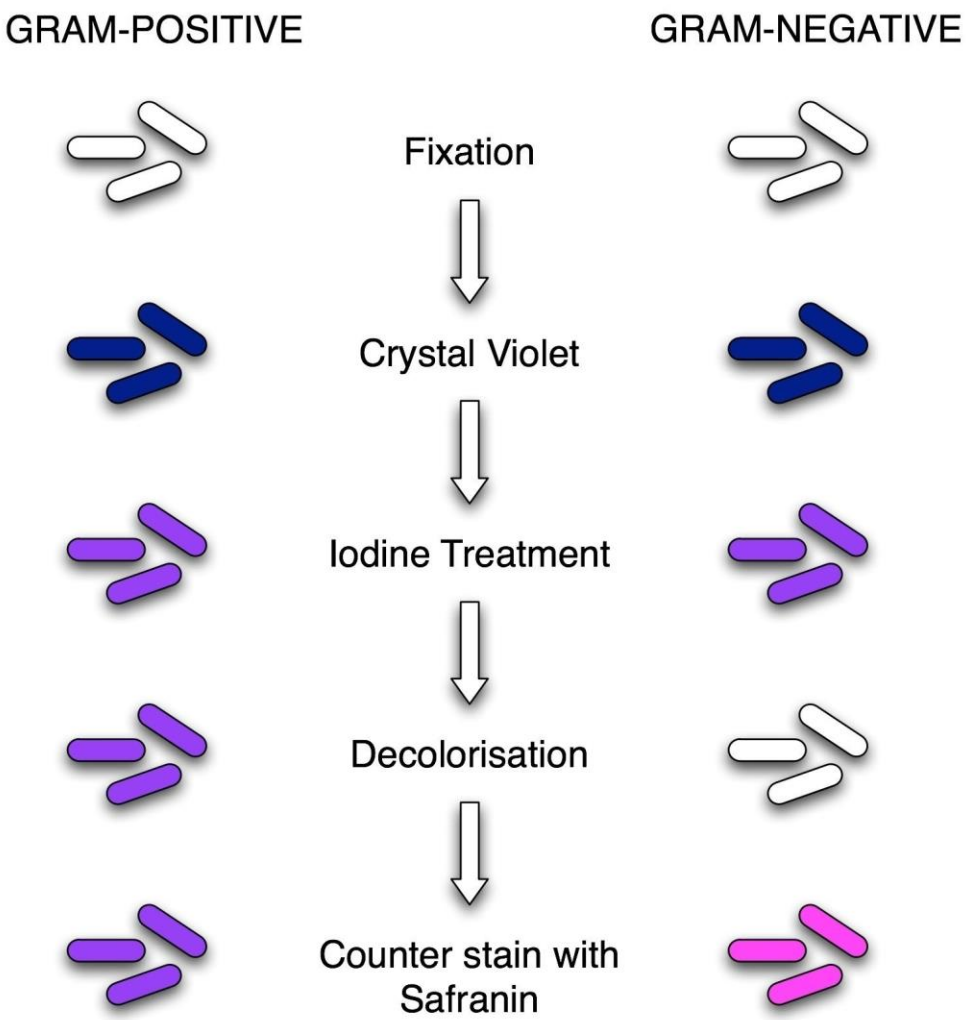


Gram-Negative Bacteria

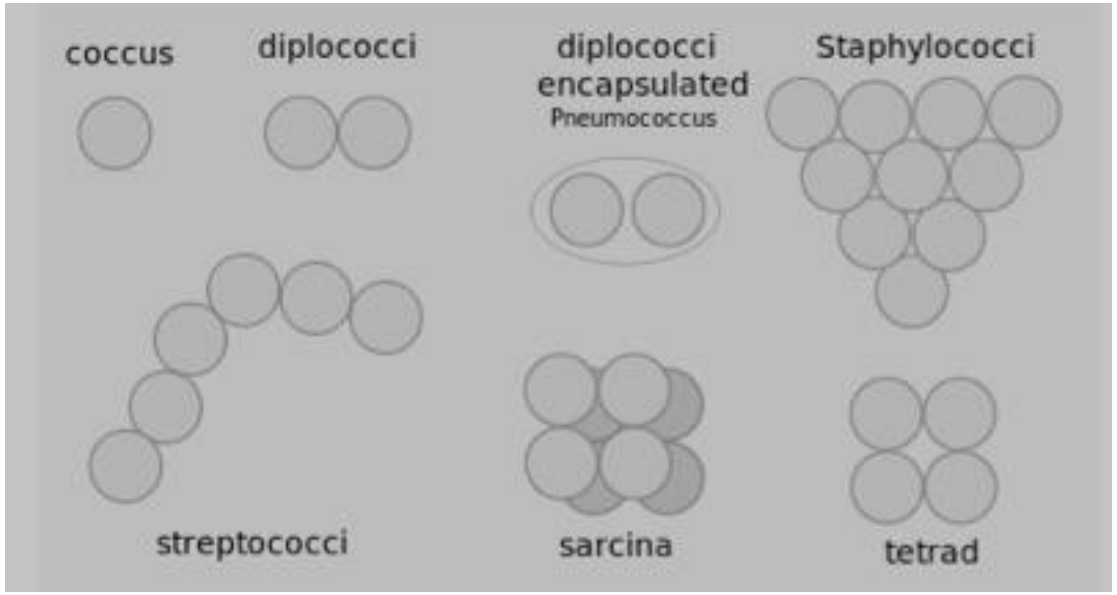


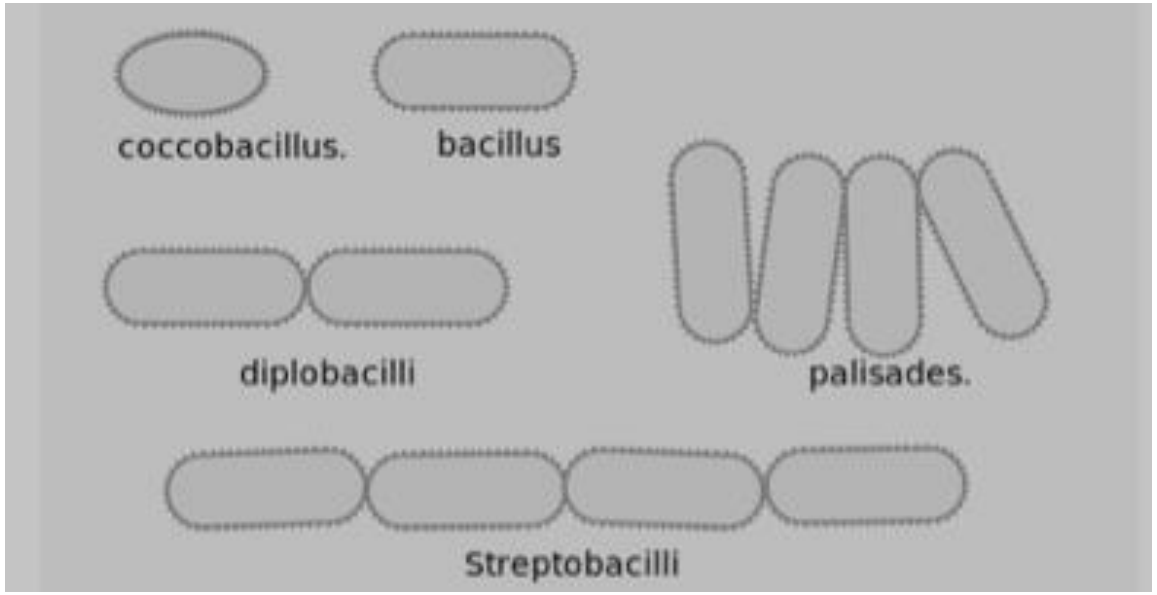
Gram-Positive Bacteria

Gram Stain


GRAM-POSITIVE
GRAM-NEGATIVE
Fixation
Crystal Violet
Iodine Treatment
Decolorisation
Counter stain with Safranin

Cellular morphology and bacteria arrangements





https://milnepublishing.geneseo.edu/suny-microbiology-lab/chapter/differential-staining-technic

Culture

- Solid media
  - Differential media
  - Selective media
- Liquid media
- Cell cultures (mainly for viruses)
- Animal inoculation (rarely used)


Dept. of Microbiology, GMH
Strontium Selenite Broth (SF Broth)
Batch : 01M03711
Prep. Date : 03/10/01
Exp. Date : 02/11/01
Storage : 4eC
QC Pass : Y
Prep. By : 54
Check By : 54
Sign :


Concept Plus

Identification

- Phenotypic
  - Colony morphology
  - Microscopic morphology
  - Biochemical tests
- Genotypic
  - Probe hybridization
  - Polymerase chain reaction
  - Gene sequencing
- Mass spectrometry (MALDI-TOF MS)

Biochemical test

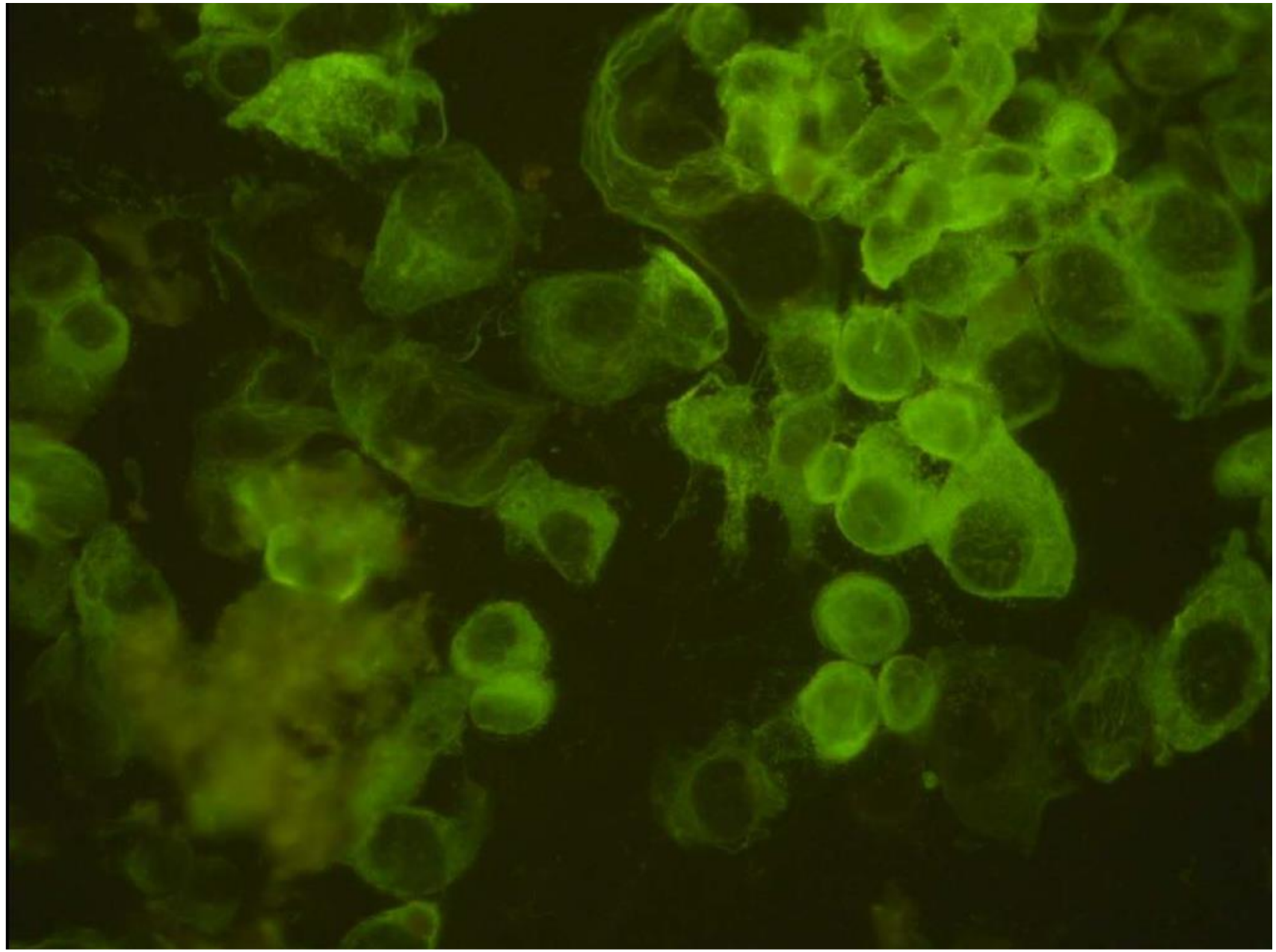
The difference in protein and fat metabolism, carbohydrate metabolism, enzyme production, compound utilization ability, etc. are some factors that aid in bacterial identification.

Serology

- Detection of antibody/antigen.
- Blood.
- Other body fluids, e.g. urine, saliva.

Antibody detection

- Pros
  - Fast.
  - Does not depend on culture.
  - Non-invasive.
- Cons
  - Cross reactions.
  - Past/new infections.
  - Time needed for development of antibody response.
  - Patients with immunosuppression.




Starlet Diagnostics
PENICILLIUM
ELISA
Anti-Penicillium Antibody ELISA Kit
Department of microbiology
Universityof HongKong
96
Tests
gate Reagent
H980328P
IN VITRO TEST
Substrate
IN VITRO TEST
Substrate

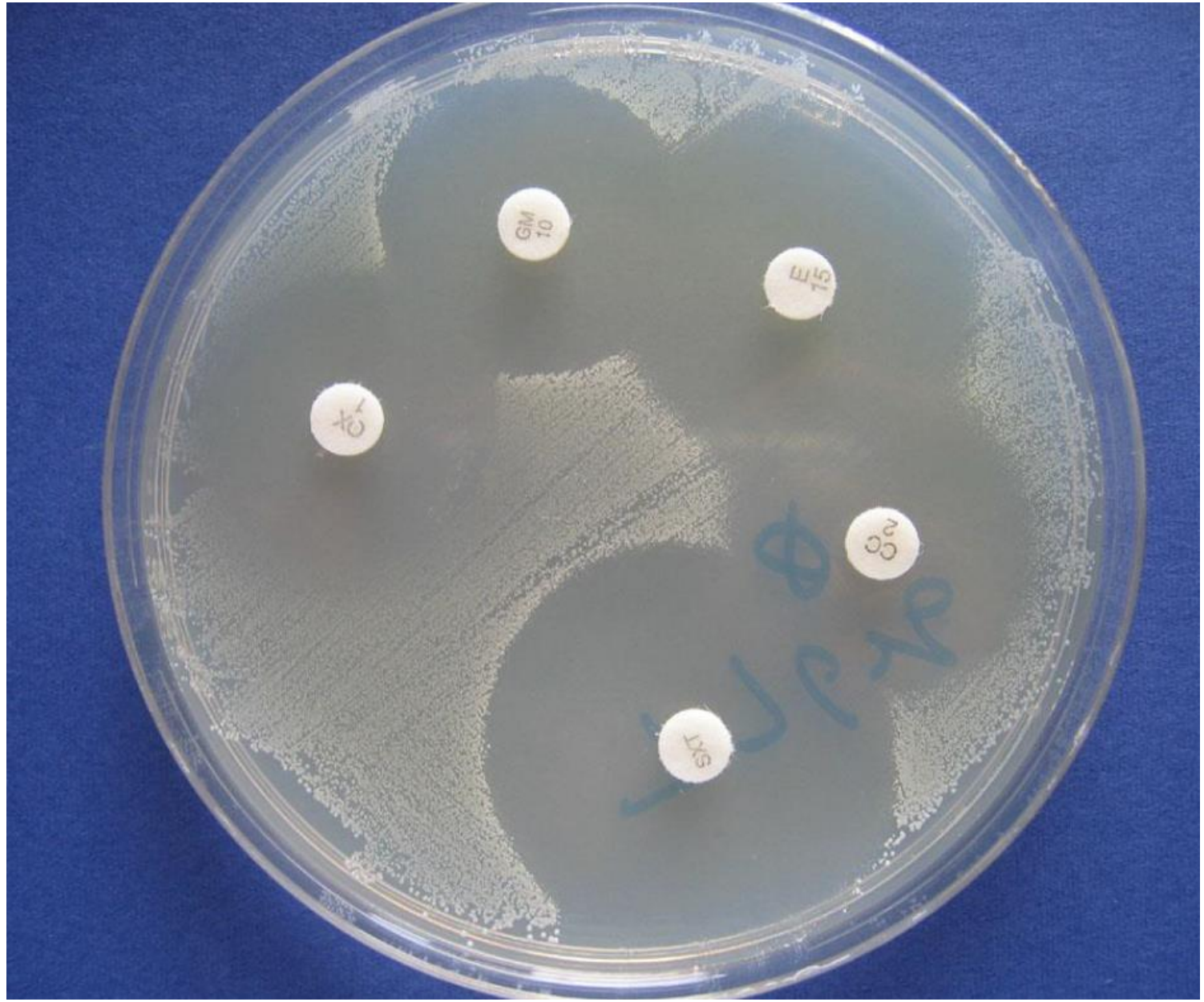
Antigen detection

- Can be performed in various body fluids or secretions.
- Less problem than antibody detection.
- Applicable to relatively few pathogens.


CONTROL
SAMPLE
(+)
(-)
Alere
BinaxNOW®
Streptococcus pneumoniae

Antimicrobial susceptibility testing

- To guide the choice of antimicrobial agents for treatment of infections.
- Sensitive/susceptible.
- Resistant.


GM 10
E 15
CX
C 2
TXT

NAME : XXXXX XXXX XXX
SEX/AGE : F/65
WARD/BED : K8N/24
HOSP.NO : 9456877
REQ. DATE : 1/4/2014

LAB.NO :94156788
SPECIMEN : Blood
INVEST : C&ST

Culture : Pure growth of *E. coli*

Ampicillin	R
Cephalothin	S
Gentamicin	S
Co-trimoxazole	R

Bacterium is Resistant
Cannot use for treatment

Bacterium is Sensitive
to antibiotic.
Can use for treatment

Nucleic acid amplification

- e.g. polymerase chain reaction (PCR).
- Pros
  - Relatively fast.
  - Does not depend on culture.
  - Very sensitive.
- Cons
  - Live/dead organisms.
  - Inhibitors.
  - Cost.

Principles for laboratory diagnosis of infections

Learning objective !

- Specimen
  - Quality.
  - Quantity.
  - Timing.
  - Collection technique.
  - Preservation.
  - Delivery.
- Requests
  - Right test for the right purpose.
  - Clinical information.
  - Prior discussion with the laboratory if necessary.

Blood culture

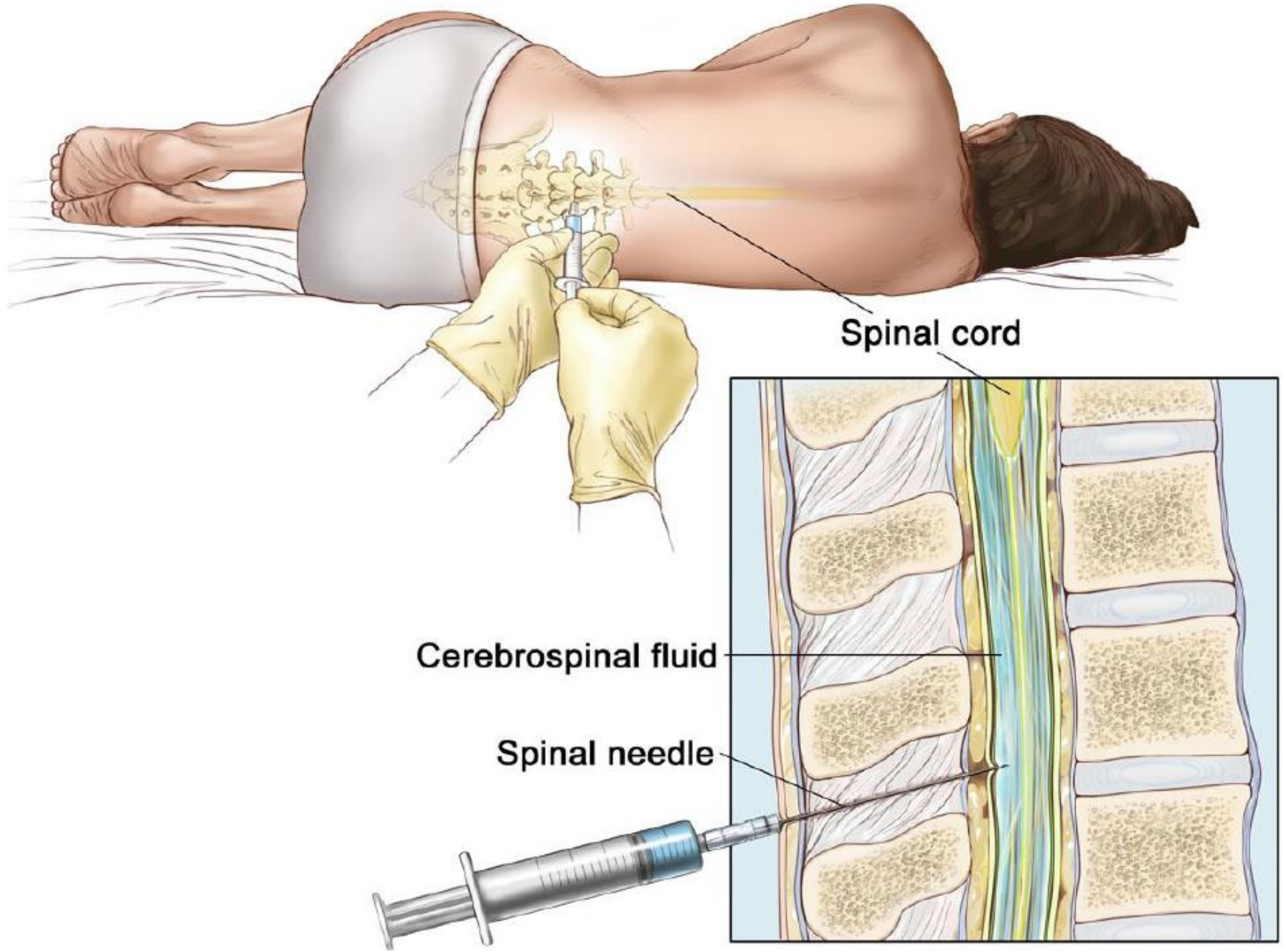
- Must be taken in any patient with suspected sepsis or other severe infections, e.g. meningitis, pyelonephritis, fever of unknown origin.
- Aerobic + anaerobic culture.
- Fungal and mycobacterial cultures as needed.
- At least 2 sets from different sites.
- Strict aseptic techniques.
  - Proper skin disinfection important.
- Adequate volume
  - 5–10 mL per bottle.
  - Number of bacteria in blood usually small (1–10 per mL).

Central nervous system

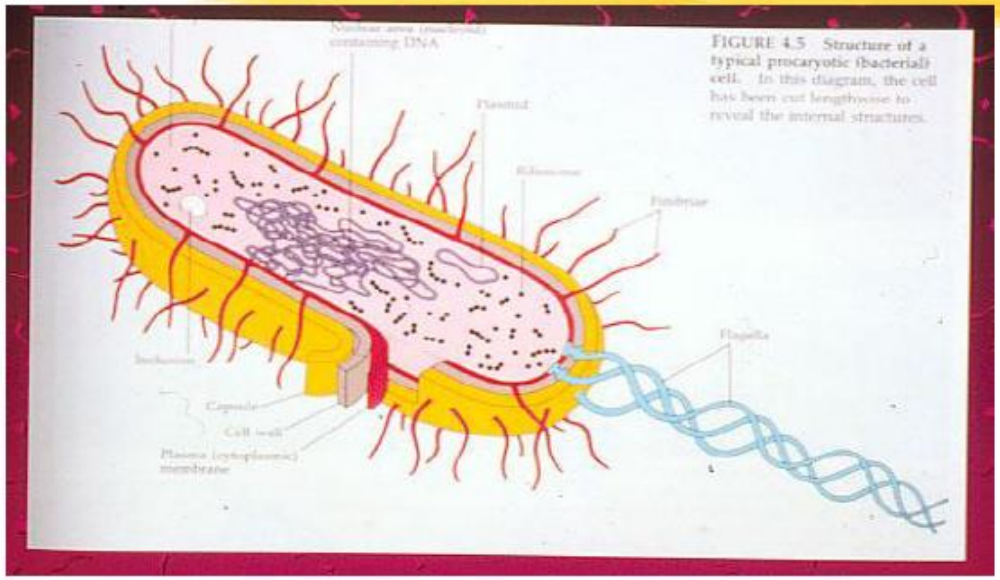
- Common types of infections:
  - Meningitis
  - Encephalitis
  - Brain abscess, epidural/subdural abscess
  - Ventricular shunt infections
- Spine
  - Epidural/subdural abscess

Specimens for CNS infections

- Cerebrospinal fluid
  - Lumbar puncture
  - Ventricular drains
- Brain abscess aspirate/drainage
- Brain biopsy
  - Microbiology, histopathology
- Blood culture (in pyogenic meningitis)
- Serology
  - Virus antibodies (e.g. Japanese encephalitis)
  - Cryptococcal antigen (cryptococcal meningitis)

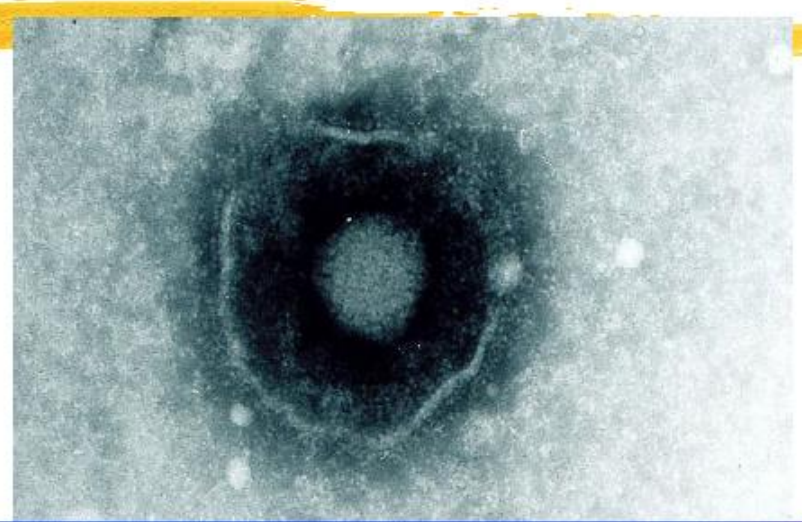

Spinal cord
Cerebrospinal fluid
Spinal needle

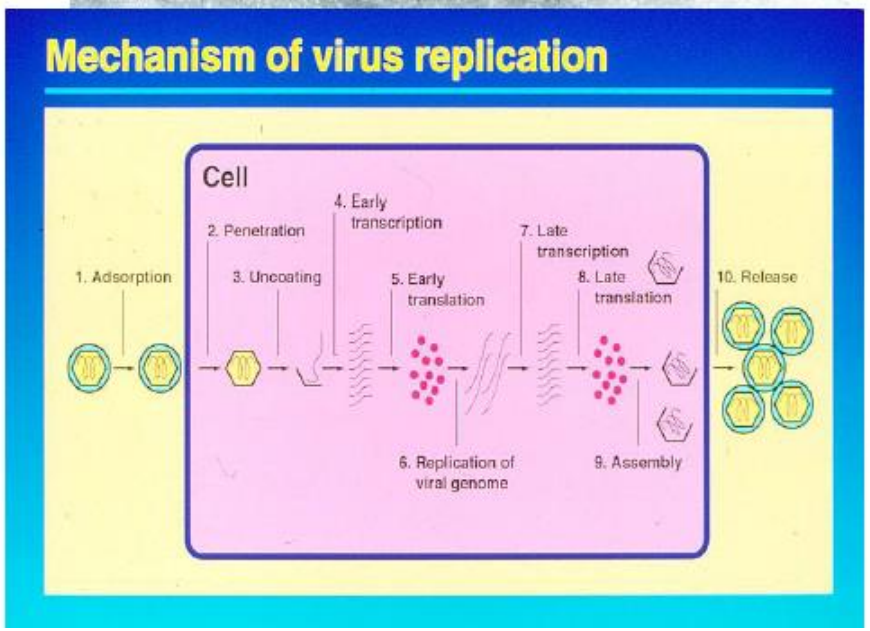
Bacteria



	No of genes
⌘ Human	>20,000
⌘ Bacterium	~ 4000
⌘ Herpesvirus	~ 100
⌘ Hepatitis B v	<10

Virus

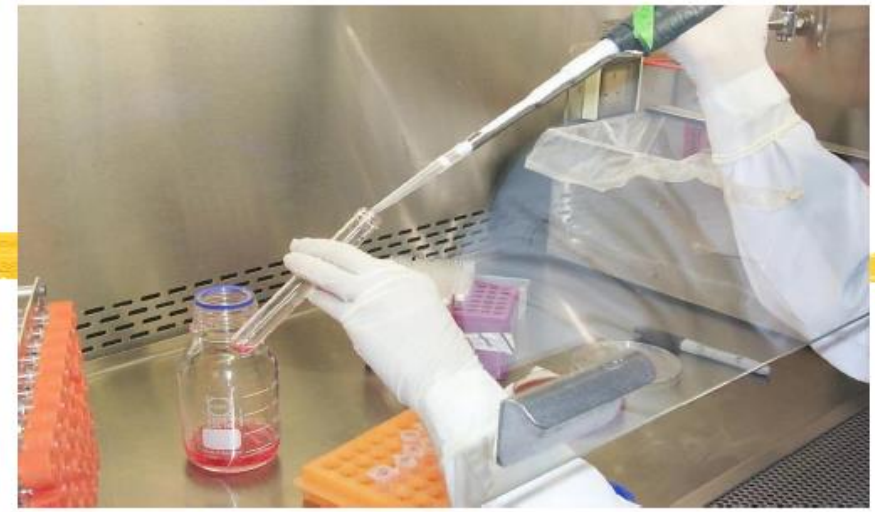


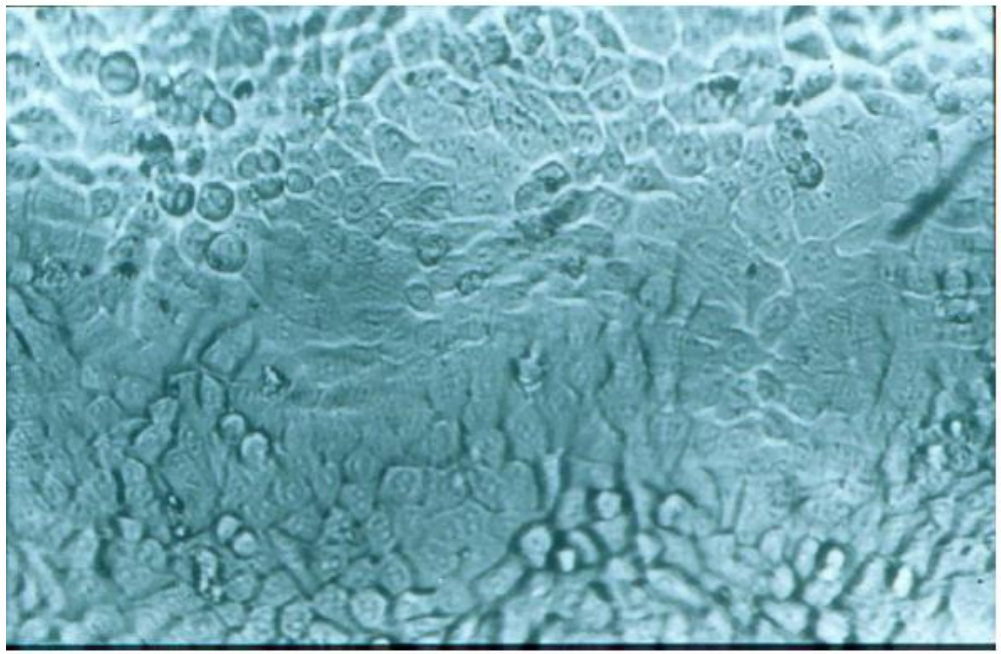


Viral diagnosis: Detect virus

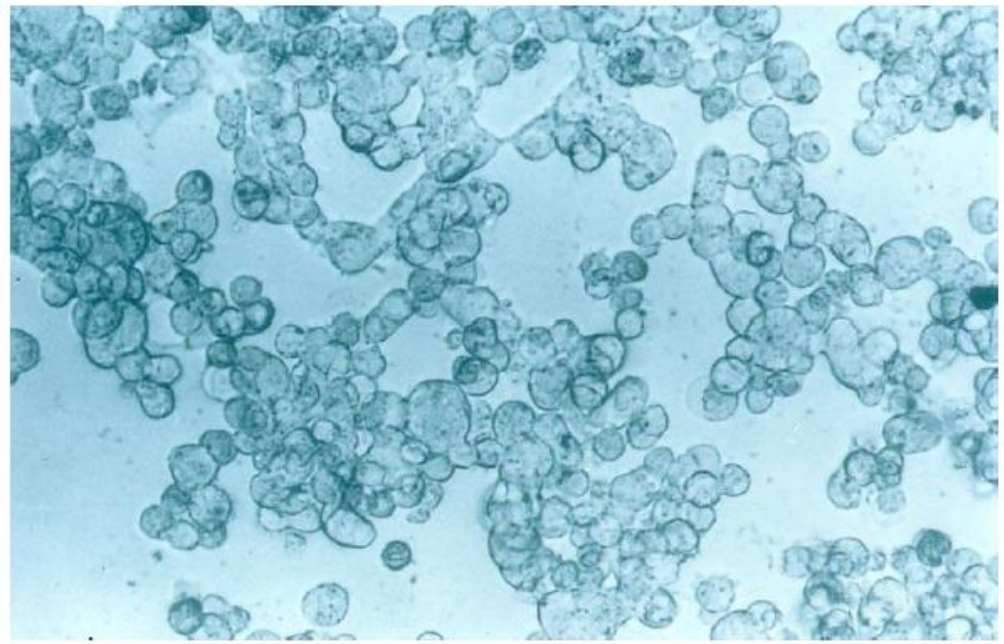
- grow (virus culture in cell lines) - *days / weeks*
- viral antigen detection - *hours*
- viral nucleic acid detection (e.g. Polymerase chain reaction) (PCR) - *hours / days*

Cell culture





Normal cells



Virus cytopathic effect

Virus definition

A virus is

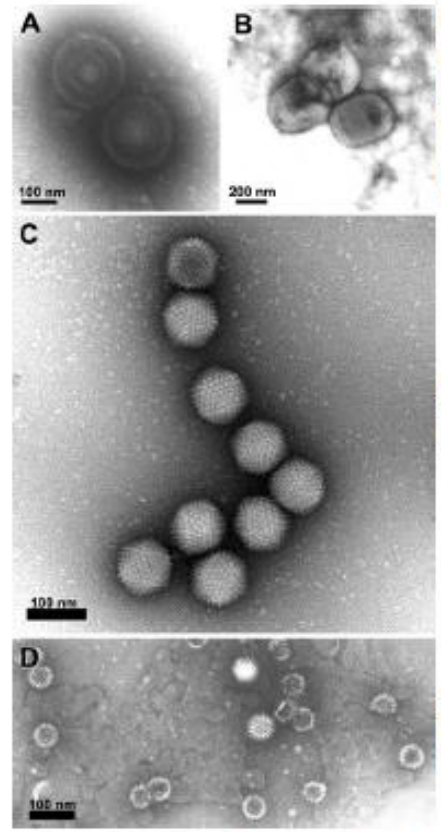
a small, infectious, obligate intracellular parasite, capable of replicating itself in a host cell.

Its genome is

composed of either DNA or RNA, enclosed in a protein coat.

Electron Microscopy

FEI Tecnai TEM



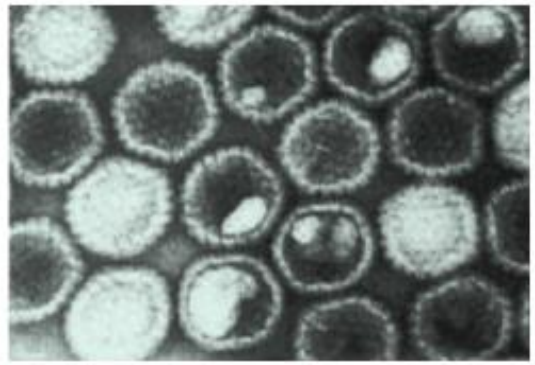
Herpes virus
(Varicella) - A
Parapoxvirus
(Orf virus) - B

Adenovirus
Icosahedral form

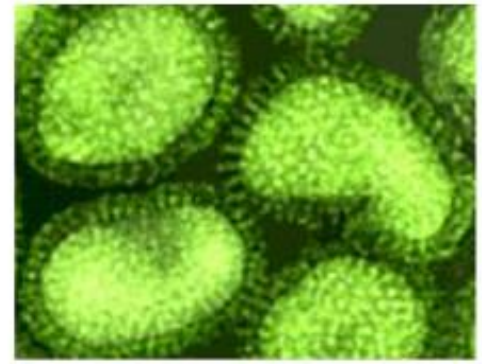
Rotavirus
Wheel like

Roingeard P. 2008 Biology of the Cell 100:491

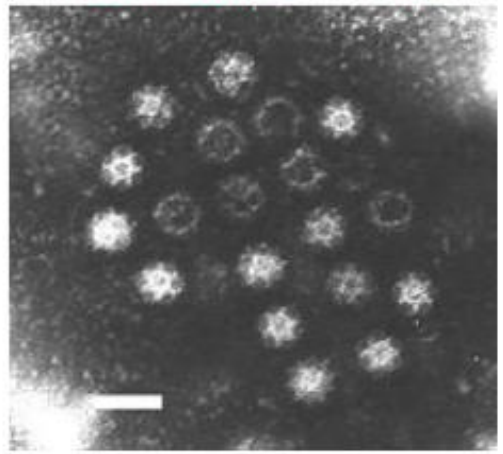
Human Herpes Virus (~100 nm)



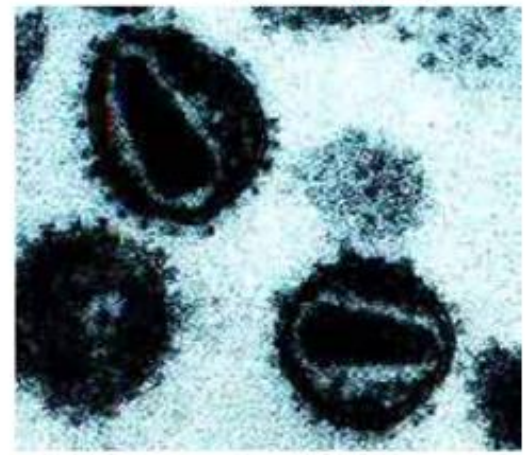
Influenza virus (50-120 nm)



Calicivirus (~35 nm)



HIV (~140 nm)



Genome size

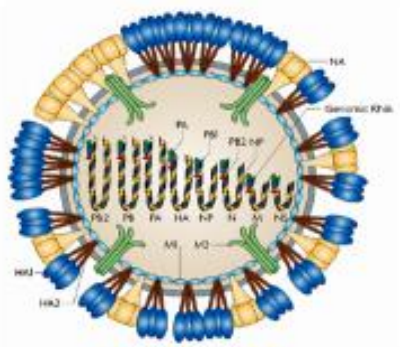
kb (= kbp) = kilo base pairs = 1,000bp
Mb (= Mbp) = mega base pairs = 1,000,000 bp
Gb (=Gbp)= giga base pairs = 1,000,000,000 bp

Organism	Genome size	No. of genes*
Human	3 Gb	~ 21,000
Yeast (*S. cerevisiae*)	12.1 Mb	~ 6,300
E. coli	4.6 Mb	~ 4,000
Pandoraviruses	1.9 - 2.5 Mb (dsDNA)	~1,500 - 2,500
Herpes viruses	120 - 230 kb (dsDNA)	60 - 120
Coronaviruses	27 - 32 kb (+ssRNA)	14 -16
Influenza virus	14 kb (-ssRNA)	14
Hepatitis B virus	3 kb (dsDNA-RT)	4
Hepatitis D virus	~1.7 kb (-ssRNA)	1 (with 2 isoforms)

*Protein coding genes

Structure of a virus

- Nucleic acid (genome)
  - either DNA or RNA
  - segmented or in one piece
  - inside the capsid (often with virus proteins)
- Capsid (protein shell, matrix)
  - made of protein subunit called capsomeres/protomeres
  - accounts for the main structure and major mass of virus
- Envelope (with or without)
  - lipid bilayer lying outside of the capsid
  - providing antigenic structures of lipid, protein and carbohydrate molecules
- Viral Spikes (surface proteins)
  - protruding from the envelope or matrix, usually made of glycoprotein
  - have enzymatic and/or adsorption and/or hemagglutinating activity
  - highly antigenic, targeted by the immune response

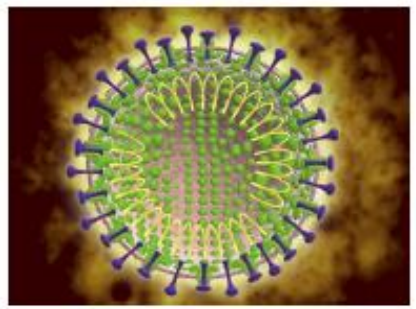


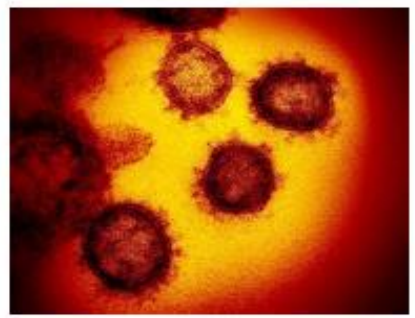
Influenza A virus

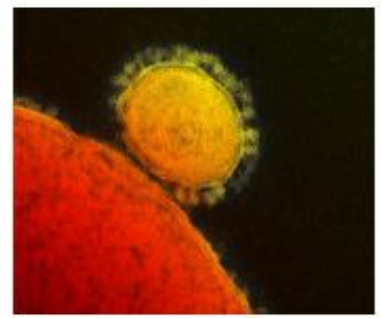
Classifying viruses

- By morphology
  - Helical
  - Icosahedral
  - Complex morphologies
- By envelop
  - enveloped
  - non-enveloped (naked)
- By genome
  - dsDNA, ssDNA, dsRNA, ssRNA
  - linear, circular
- By mechanism of mRNA production
  - Baltimore classification

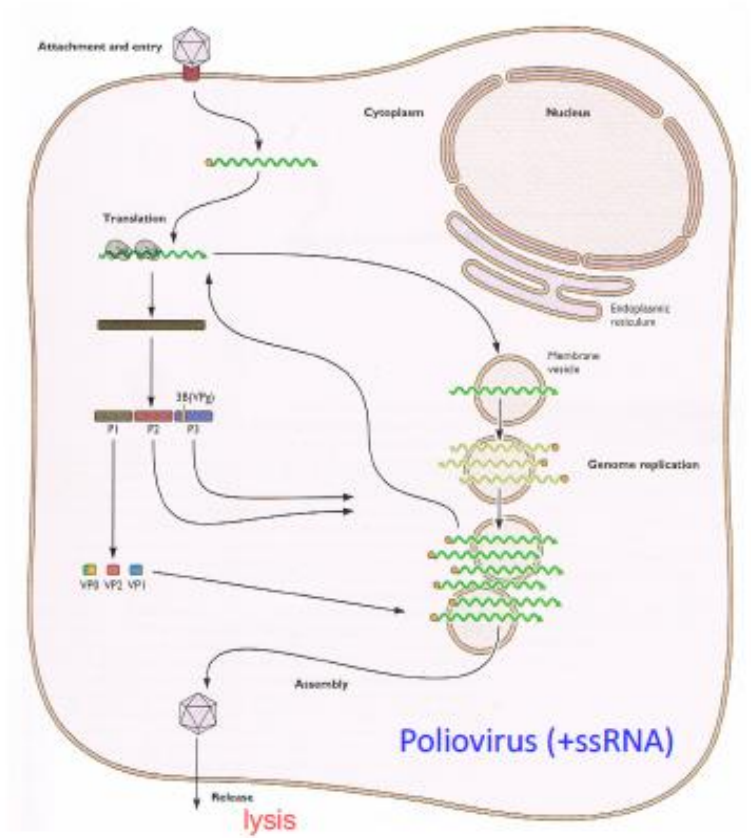
Coronavirus



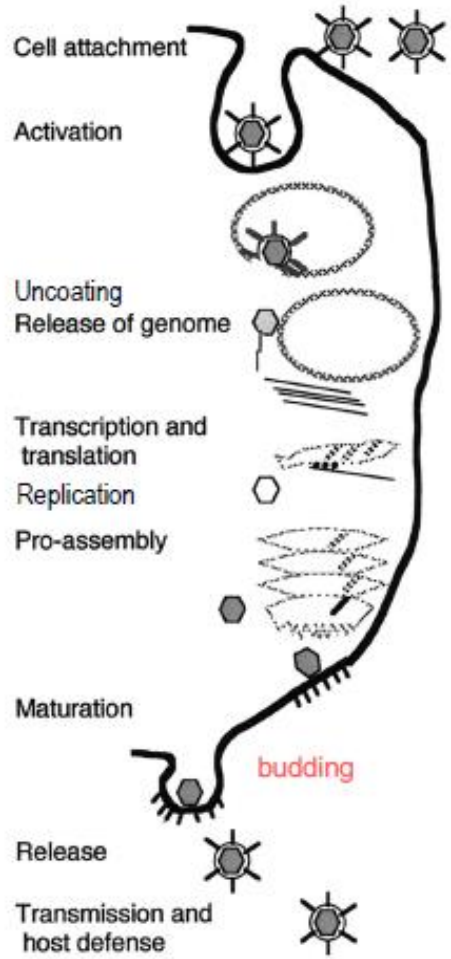




Virus ‘life’ cycle



Flint et al 2009 Principles of Virology, ASM Press



Baker et al 1999 Microbiol Mol Biol Rev 63:862

Overview Of Virus Replication

1. Attachment
2. Entry
3. Transcription
4. Translation
5. Genome replication
6. Assembly
7. Exit

AETTGAE

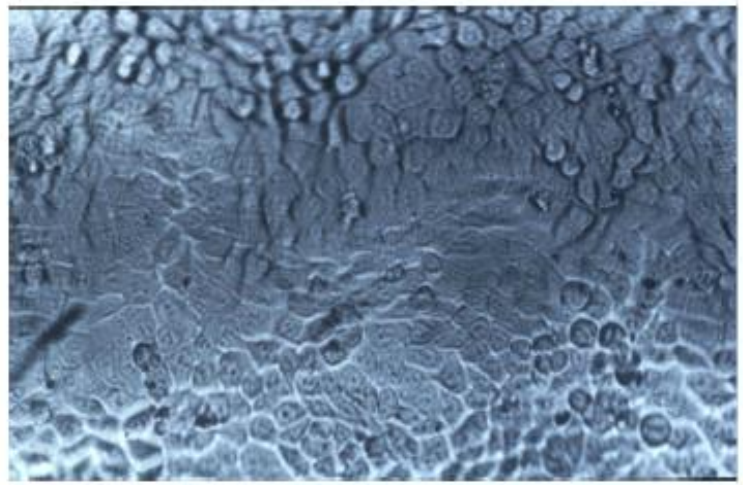
Specimens for virus culture

- Send to the lab within hours of collection.
  Otherwise the viruses will 'die'.
- If delayed, keep cool (at 4°C; do not freeze).
- Put viruses in transport medium (with antibiotics)

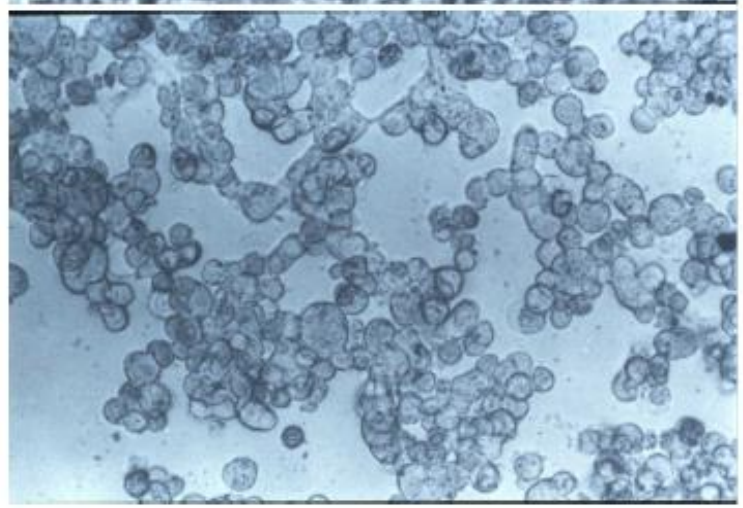
Transport medium

Effect of a virus on a cell culture

Normal cells

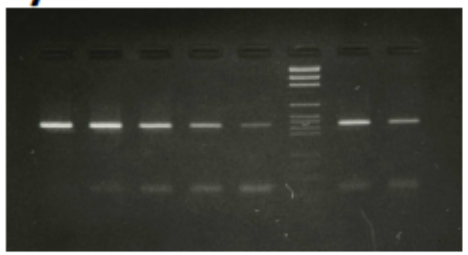


Infected cells showing CPE "cytopathic effect"



Virus culture in embryonated eggs

Detecting virus genes by PCR



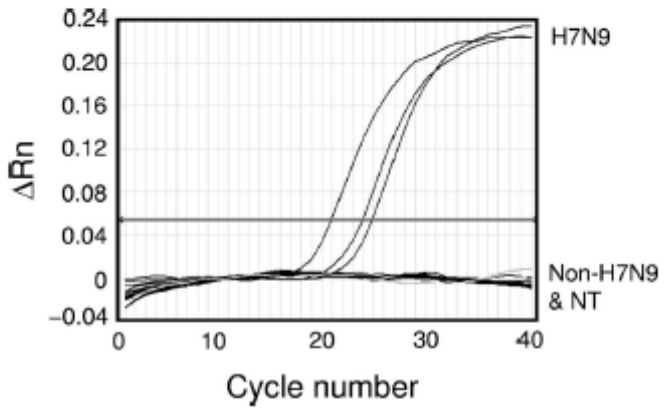
Polymerase Chain Reaction

Requires species-specific “primers”

Use “polymerase” to amplify

(and Reverse Transcriptase [RT] for RNA viruses)

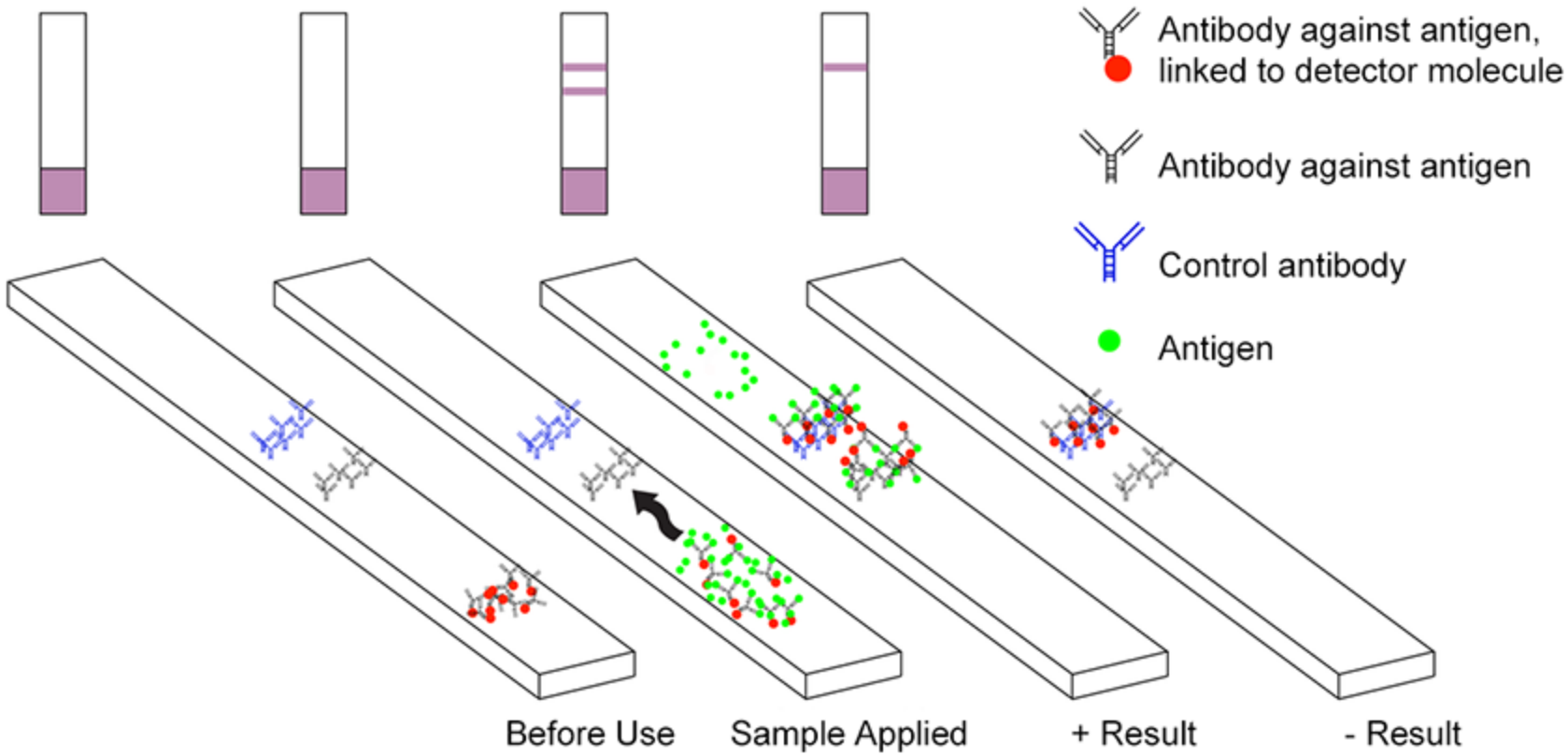
Visualize by hybridization with complimentary DNA probes; gel electrophoresis; “Real-time” PCR.



Real-time (quantitative) RT-PCR to identify and distinguish the recent H7N9 viruses from other H7 viruses.

Wong et al 2013, ClinChem 59:1062

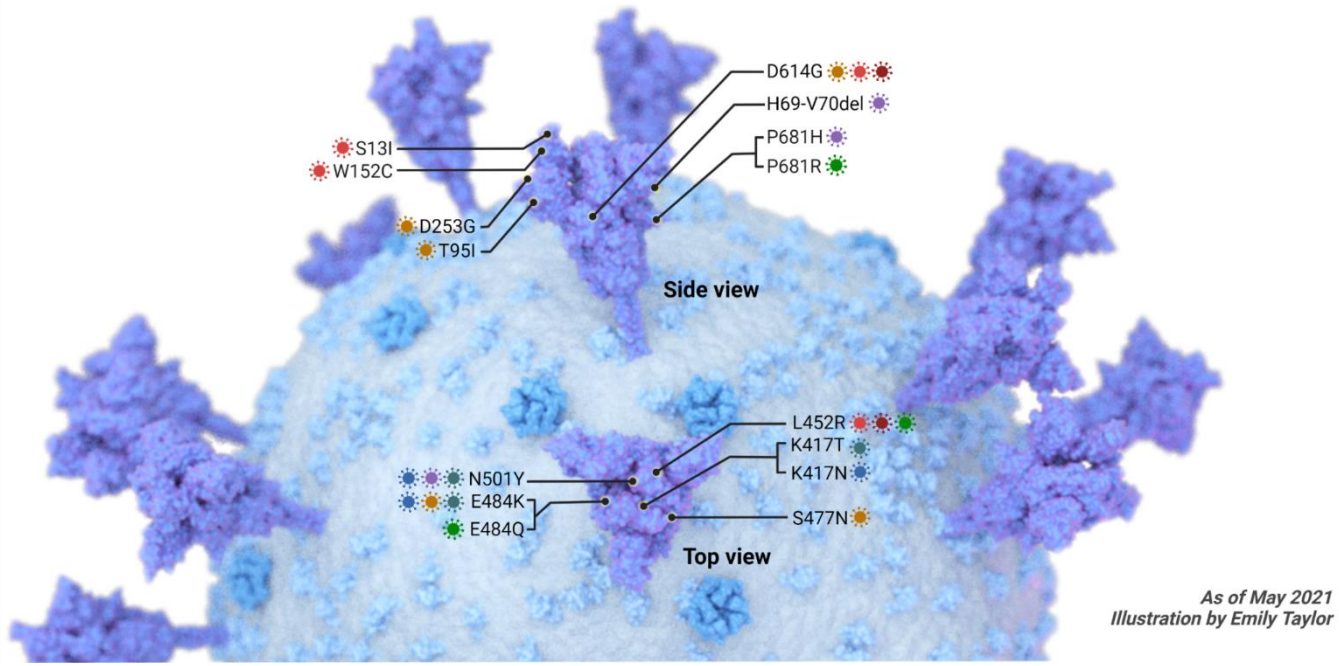
Rapid Antigen Test



The SARS-CoV-2 Variants of Concern

Key mutations in the spike protein are shown, but mutations in other areas of the genome have been identified and are currently under investigation. Mutations occur on all spike protein subunits.

B.1.1.7	B.1.351	P.1	B.1.526	B.1.427	B.1.429	B.1.617
Discovered: Dec. 14 2020	Discovered: Dec. 18 2020	Discovered: Dec. 4 2020	Discovered: Nov. 2020	Discovered: Dec. 2020	Discovered: Nov. 2020	Discovered: Oct. 2020

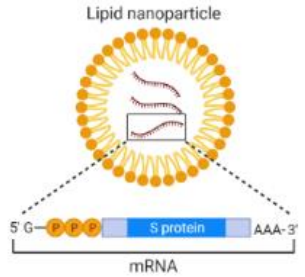


As of May 2021
Illustration by Emily Taylor

Omicron

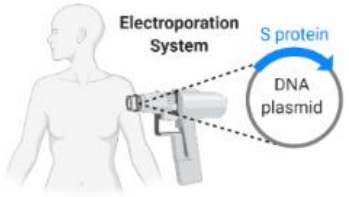
Vaccine

Moderna (mRNA-1273)



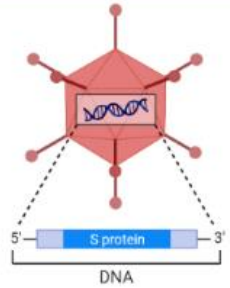
Platform: LNP-encapsulated mRNA encoding S protein.

Inovio Pharma (INO-4800)



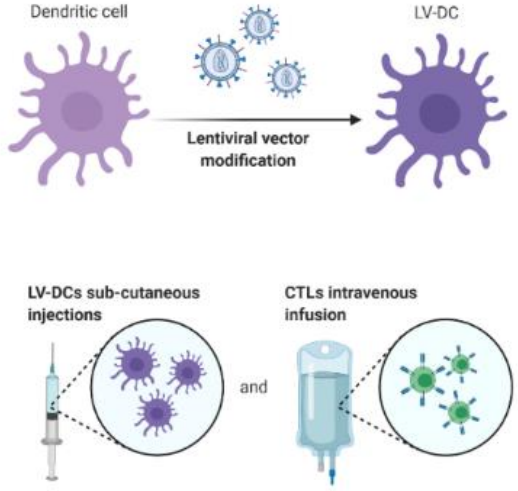
Platform: Electroporation of DNA plasmid encoding S protein.

CanSino Biologics (Ad5-nCoV)



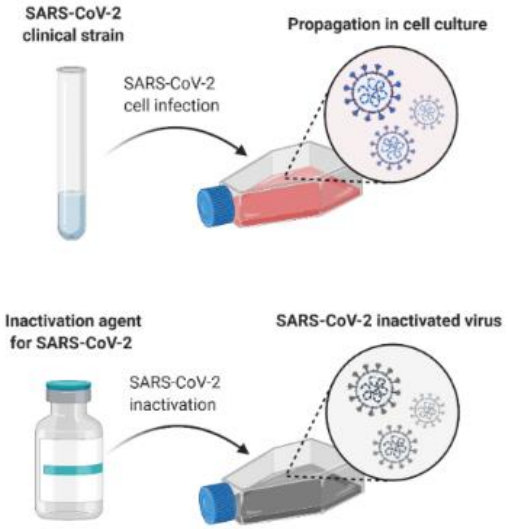
Platform: Adenovirus type 5 vector that expresses S protein.

Shenzhen Medical Institute (LV-SMENP-DC)



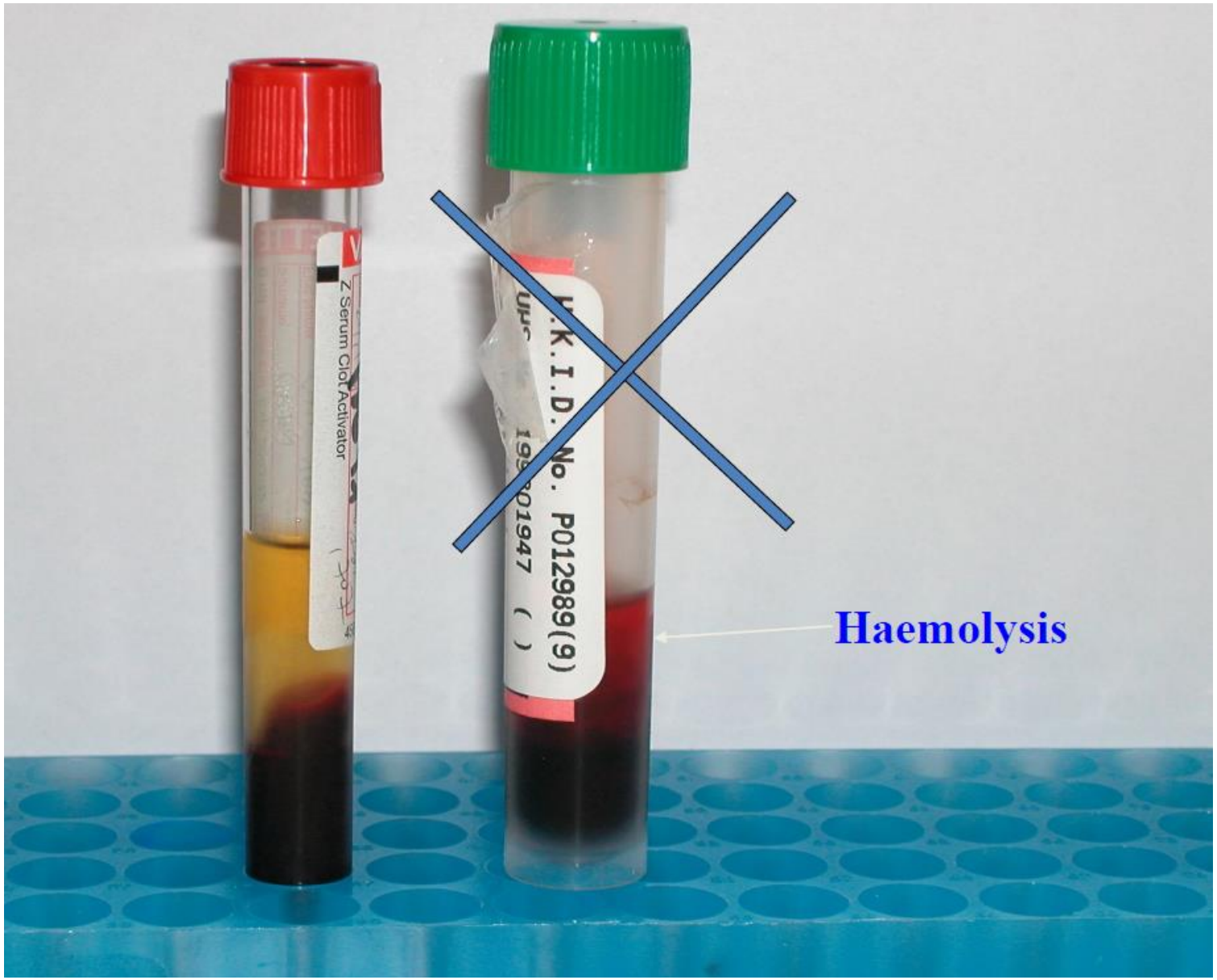
Platform: Lentiviral vector modification of dendritic cells (LV-DCs) and antigen-specific cytotoxic T lymphocytes (CTLs).

Sinovac Biotech (unnamed)



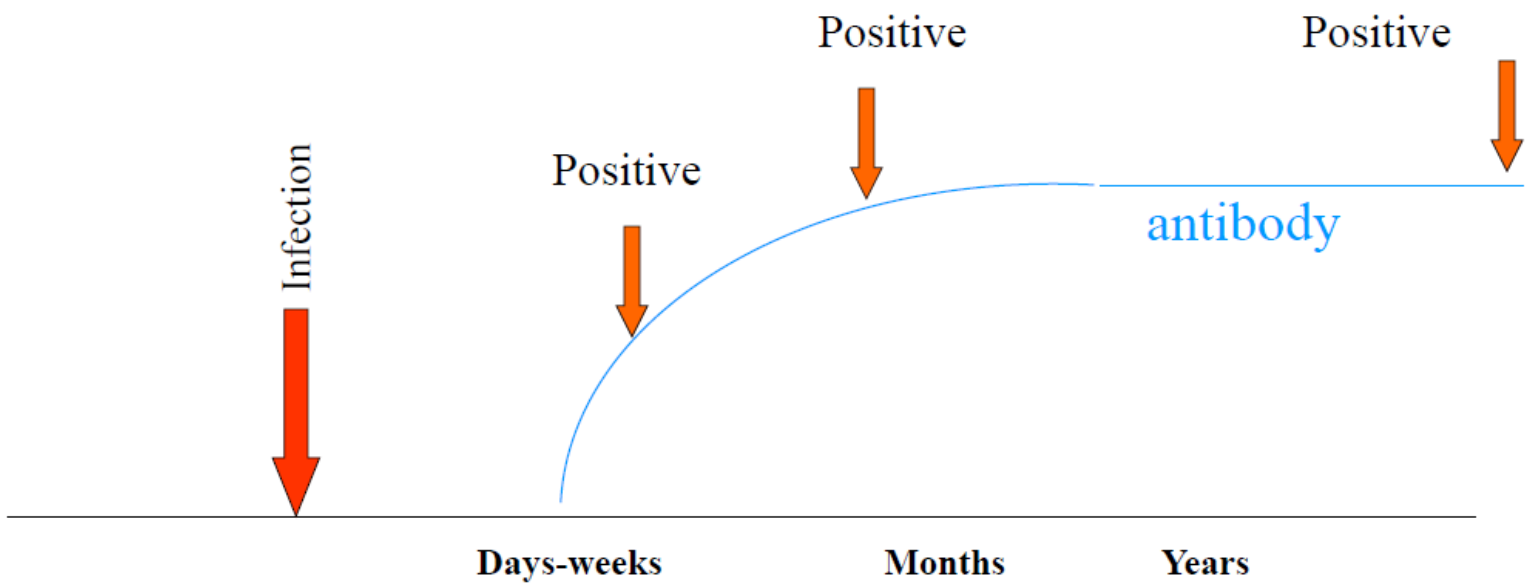
Platform: Inactivated virus vaccine produced from viral propagation in cells infected with a SARS-CoV-2 clinical strain.

*As of April 2020


Z Serum Clot Activator
Haemolysis

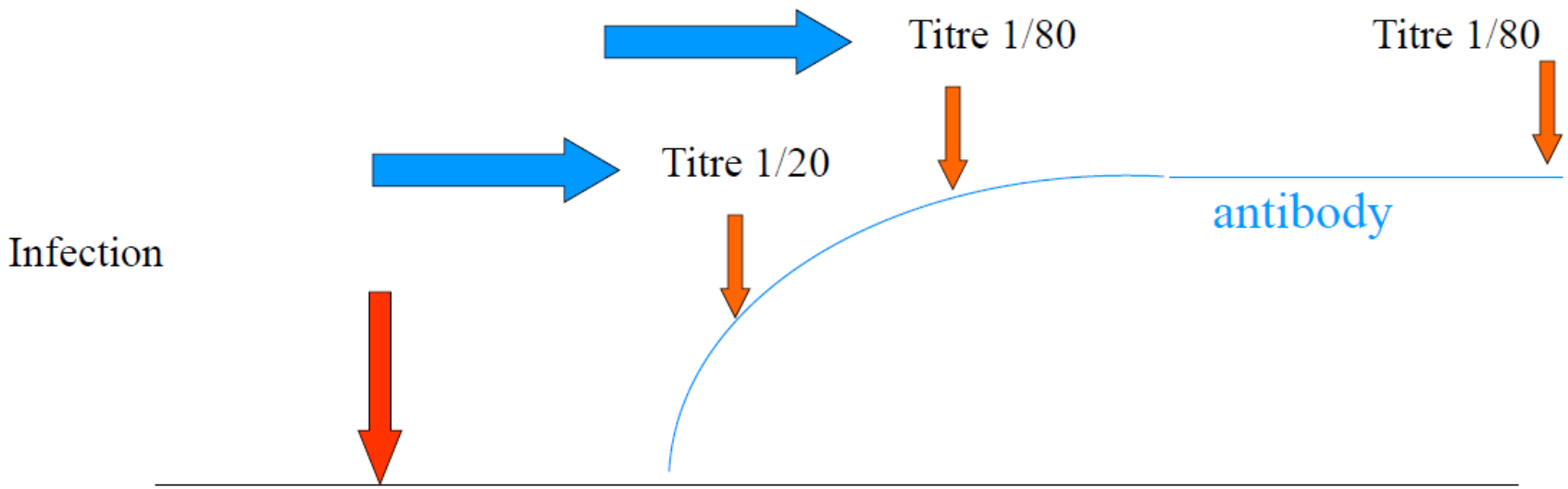
Detect antibody response

- A single antibody test? What does it mean?
- Infection at some time during lifetime.



Detect antibody response

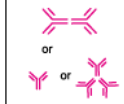
- Paired sera 10-14 days apart, tested for antibody titre (quantitation)
- Rising antibody titre: ≥4 fold increase



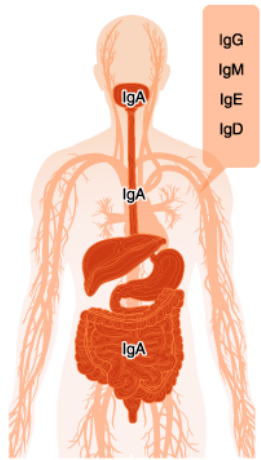
Detect antibody response

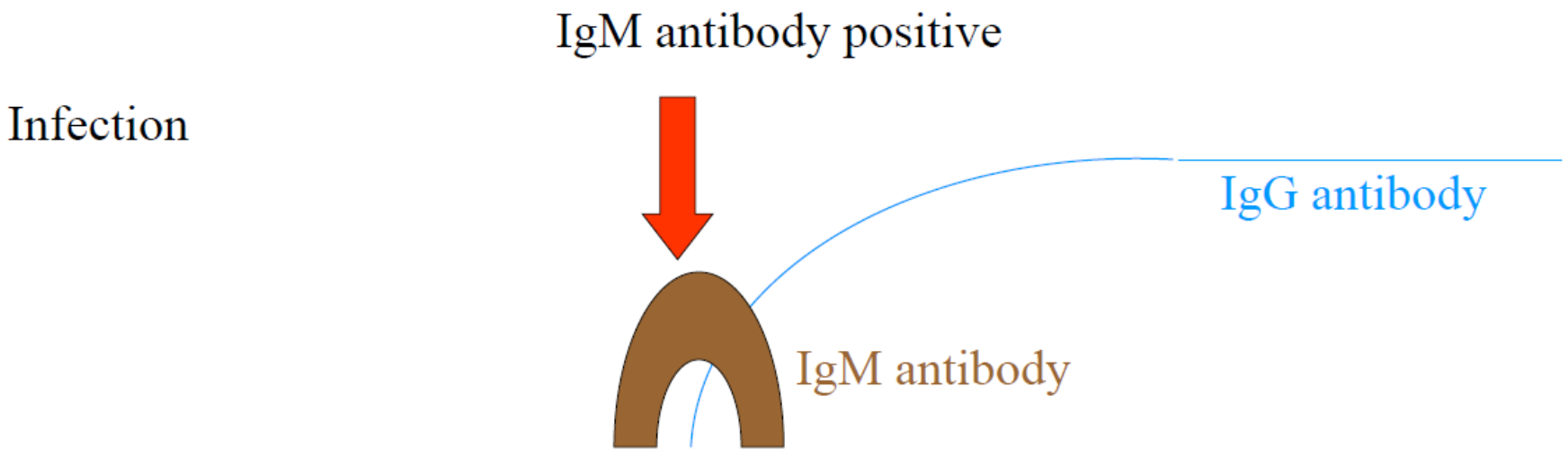
- Antibody classes: IgM antibody / IgG antibody
- Recent infection within last 1-3 months

Types and characteristics of antibodies

IgG		• Highest opsonization and neutralization activities. • Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4).
IgM		• Produced first upon antigen invasion. Increases transiently.
IgA		• Expressed in mucosal tissues. Forms dimers after secretion.
IgD		• Unknown function.
IgE		• Involved in allergy.

Distribution in the body





Learning objectives

- List the reasons why microbiological laboratory diagnosis is important for good clinical care?
- Describe the factors that need to be considered in collection and transport of specimens for microbiological laboratory diagnosis?

- Describe the available options for diagnosis of viral infections?