YAKIN DOĞU ÜNİVERSİTESİ
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Infectious Agents and Diagnostic Methods

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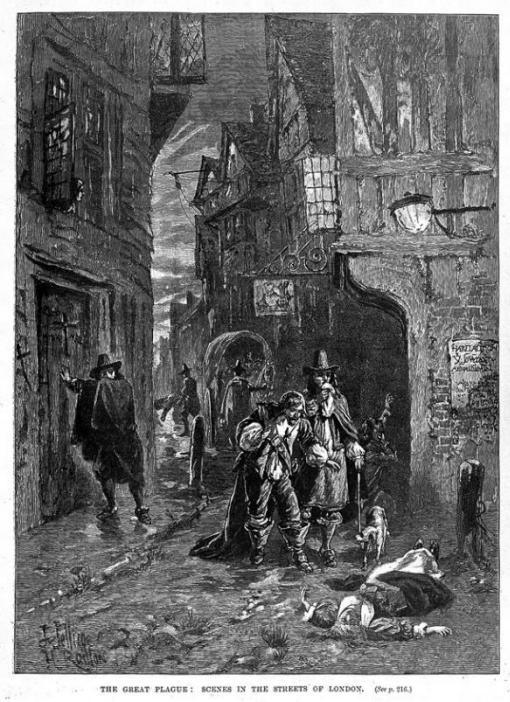
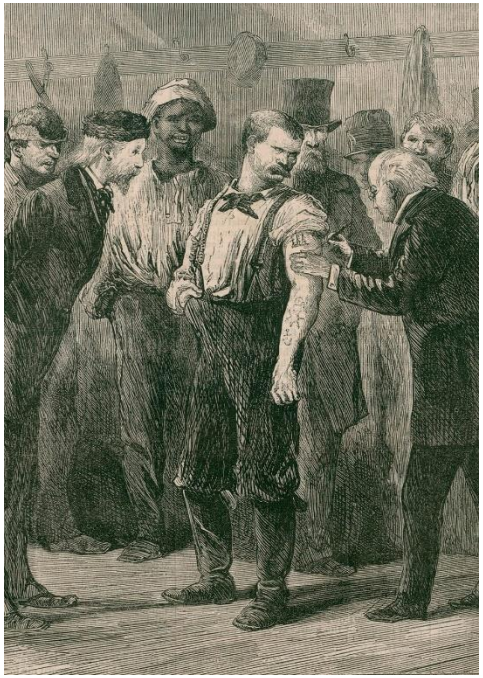
Infectious Diseases...

Throughout history, no other factor has affected human life and development as much as infectious diseases and the microorganisms that cause them



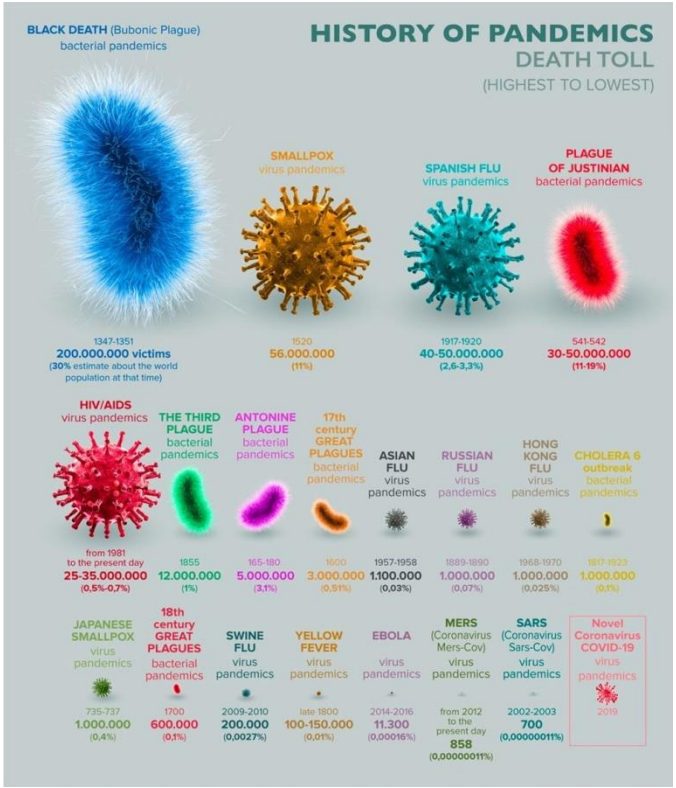


Infectious Diseases...

PAN → ‘All’

DEMOS → ‘people or crowd’

PANDEMIC



Black plaque (1347-1351)...

Yersinia pestis ⟶ 200 million deaths

*Destruction of natural habitats, poor environmental and human hygiene, famine

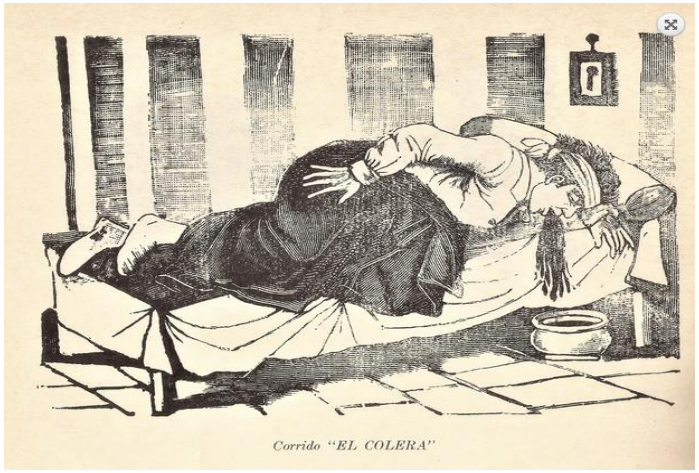
**Rodents, fleas and human-human transmission

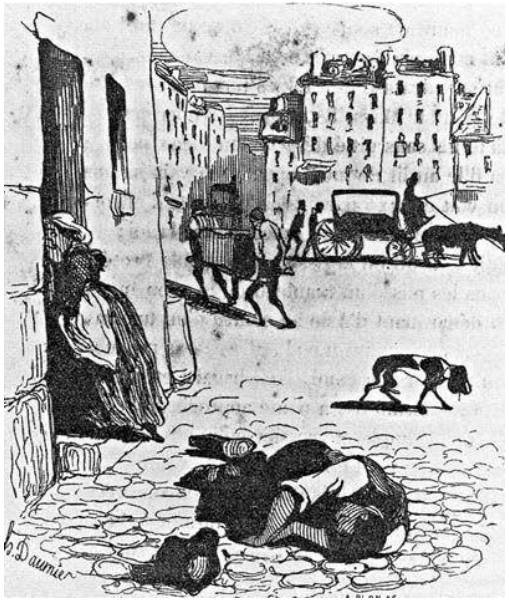
Cholera (1817-1923)...

Vibrio cholerae ⟶ 11 million deaths by 1912 (6 different pandemics)

*From the Ganges river in India to the whole world

**Population growth, difficulties in settlements, trade routes to the west and British colonization



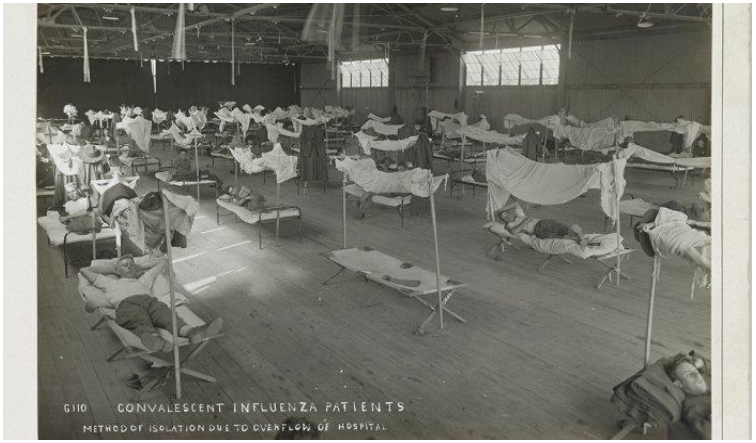


Spanish flue (1918-1920)...

H1N1 (have avian genes) ⟶ 500 million peopel affected (50-100 million deaths)

*The first case appeared in the Spanish press

*The impact of the First World War was great in this pandemic



HIV/AIDS (1981-present)...

According to WHO 2021 data;

- 38.4 millon people living with HIV at the end of 2021
- 1.5 million new cases
- 650.000 deaths
- Approximately 35 million deaths to date

COVID-19 (2019-present)...

Caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

According to WHO data (28.07.2022);

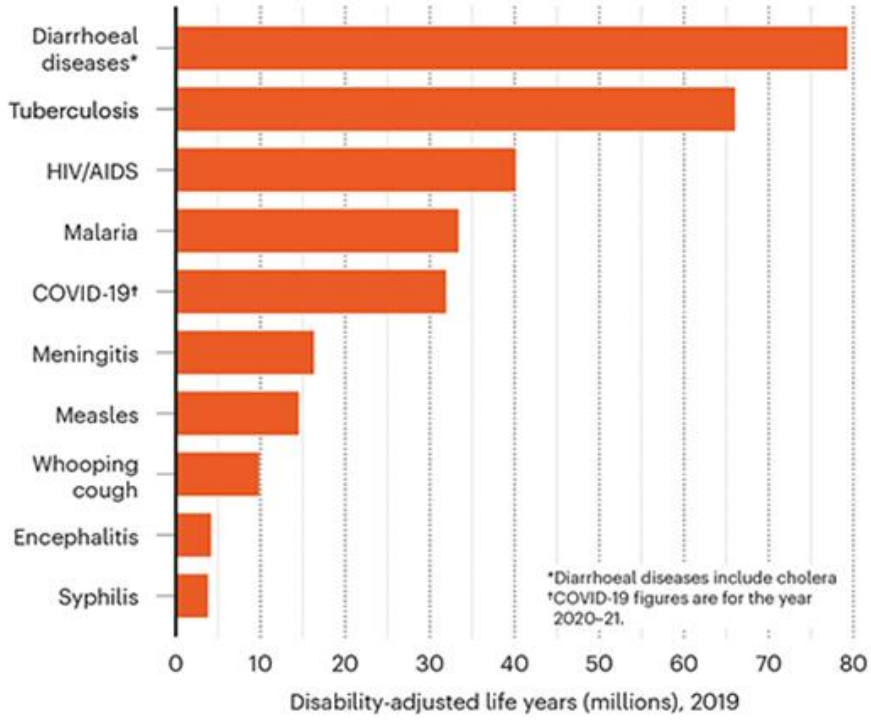
- **571.198.904 confirmed cases** of COVID-19
- **6.387.863 deaths**

Important infectious diseases…

WHICH DISEASES EXACT THE GREATEST TOLL?

One disability-adjusted life years (DALY) represents the loss of the equivalent of one year of full health. COVID-19 has quickly assumed a place among the world's deadliest diseases.

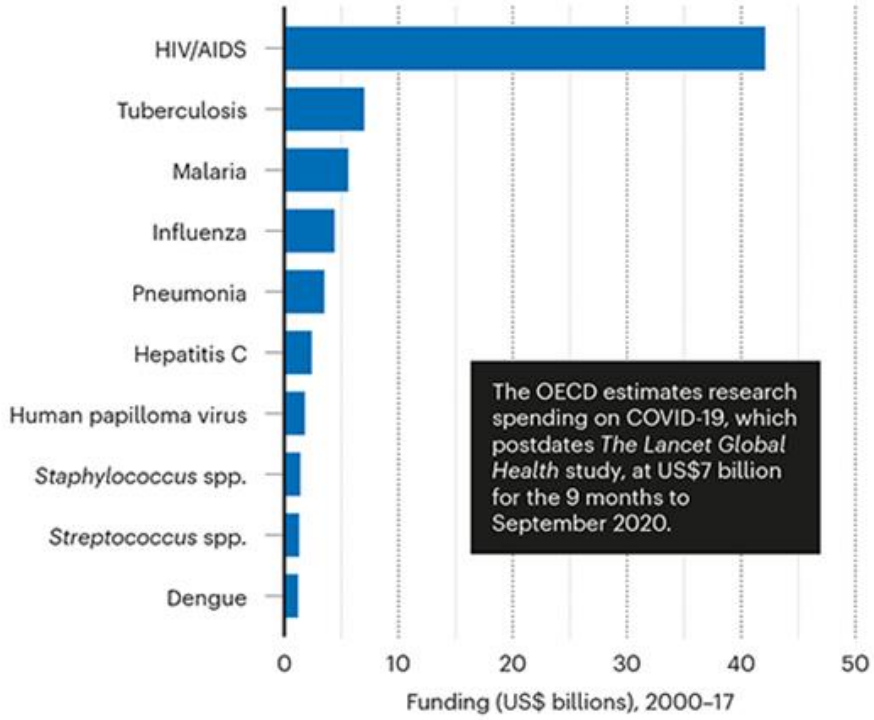
Top 10 infectious diseases tracked by disability-adjusted life years (DALY)



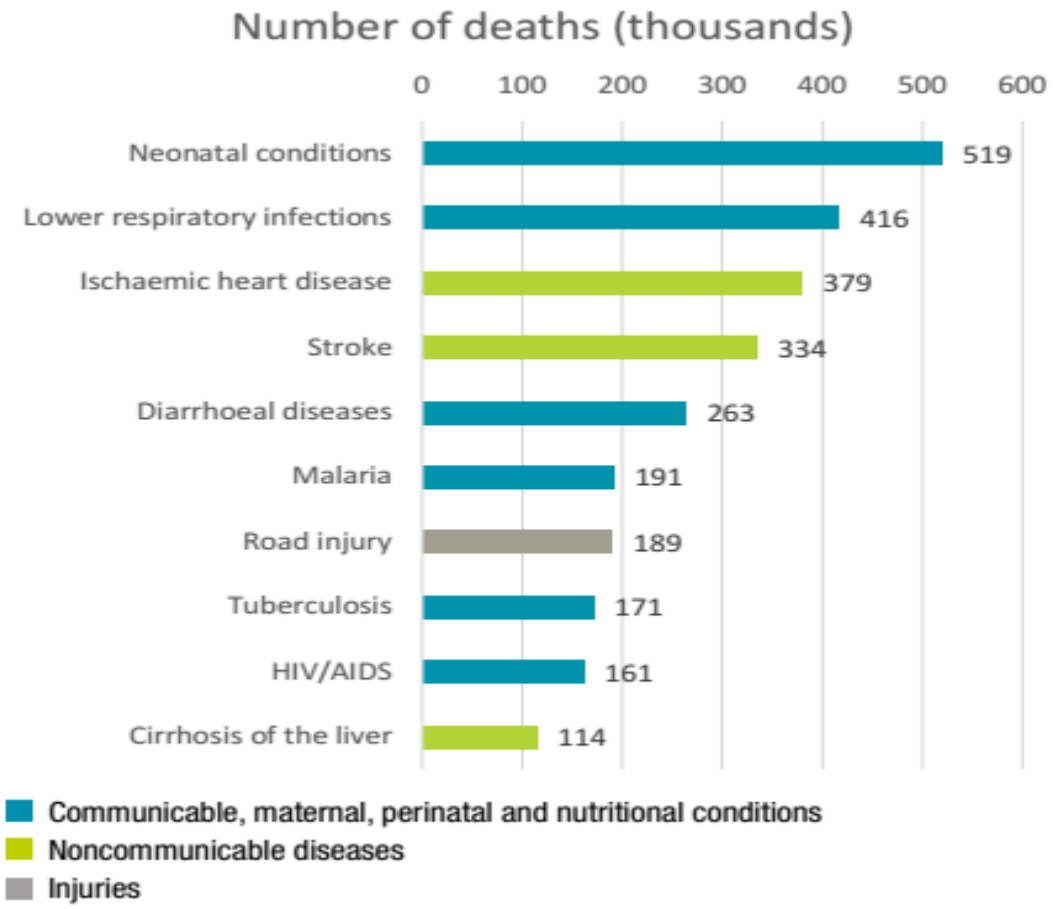
WHICH DISEASES GET THE MOST RESEARCH FUNDING?

In 2000–17, HIV/AIDS received six times more funding than tuberculosis, according to a 2020 study in *The Lancet Global Health*. The study examined grants made for infectious-disease research from public and philanthropic funders in G20 countries.

Top 10 infectious diseases tracked by funding



Mortality rates…

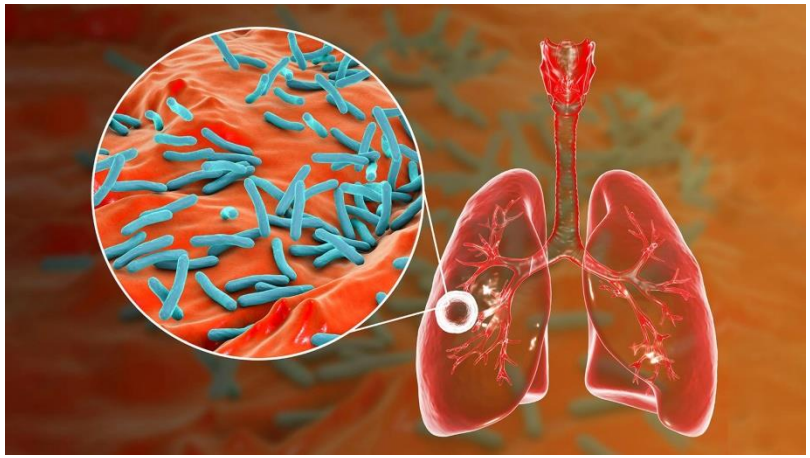


Source: WHO Global Health Estimates, 2019 *(1)*.

Tuberculosis (TB)…

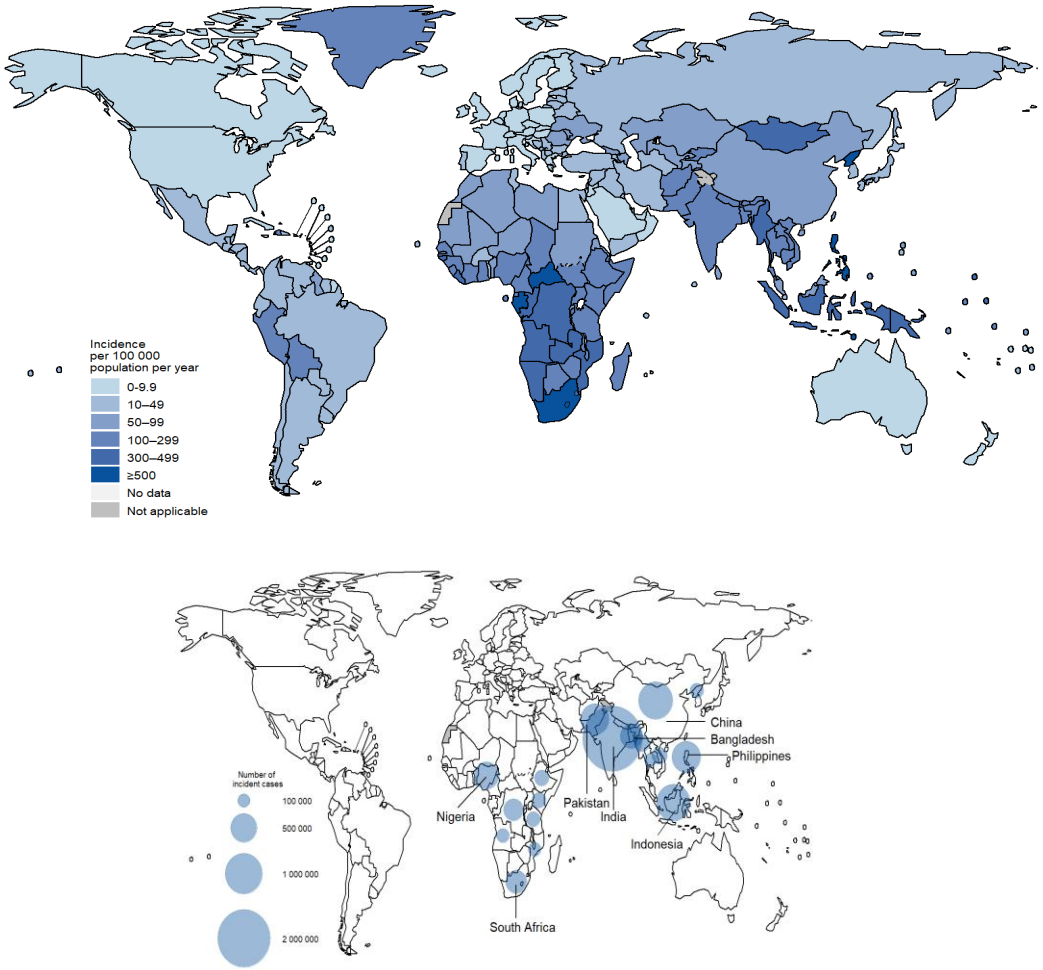
World's leading cause of death from an infectious agent

One of the world's most contagious causes of death



Mycobacterium tuberculosis

Tuberculosis (TB)…



TB incidence worldwide in 2020

Tuberculosis (TB)…

- About **10 million** people infected each year
  - 5.6 million male
  - 3.3 million female
  - 1.1 million children

- **1.5 million** deaths in 2020 (214.000 HIV/TB)

- Between 2000-2020, **66 million** TB cases recovered with treatment

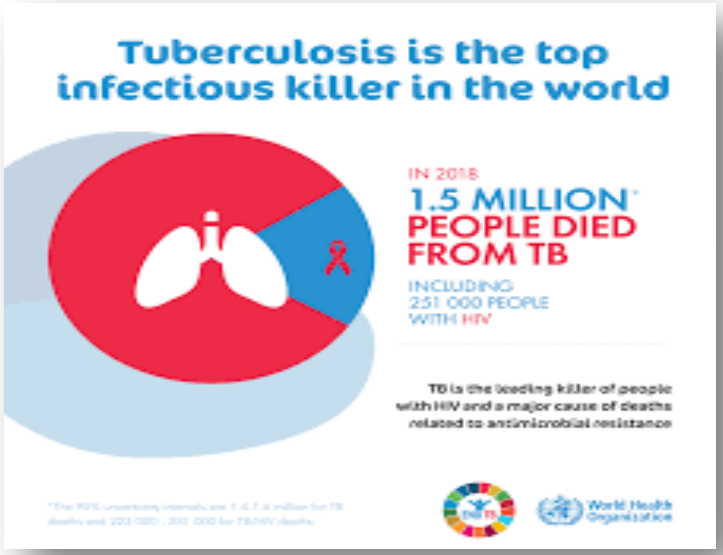
- **13 billion** dollars for prevention, diagnosis, and treatment in 2022

Tuberculosis (TB)…

All of this data trumps TB worldwide;

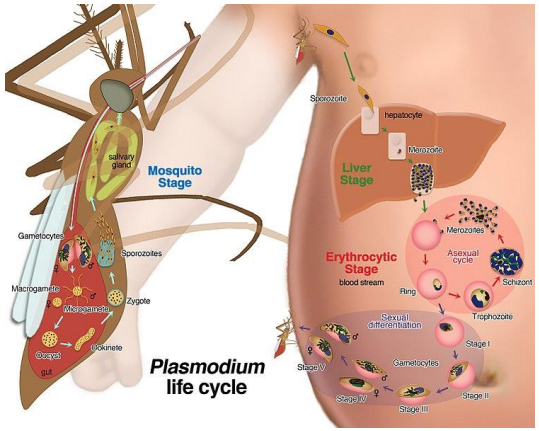
- **13th** leading cause of death
- **2nd** leading infectious agent

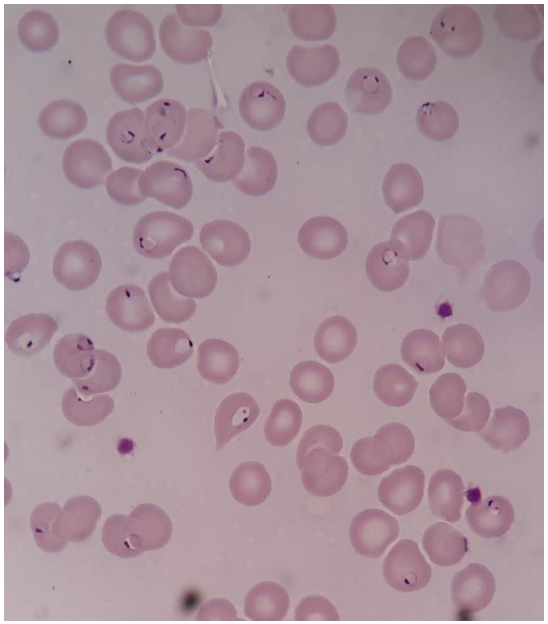
1. COVID-19
2. **Tüberküloz**
3. HIV/AIDS



Malaria…

Plasmodium spp. ----- Female *Anopheles* spp. mosquito

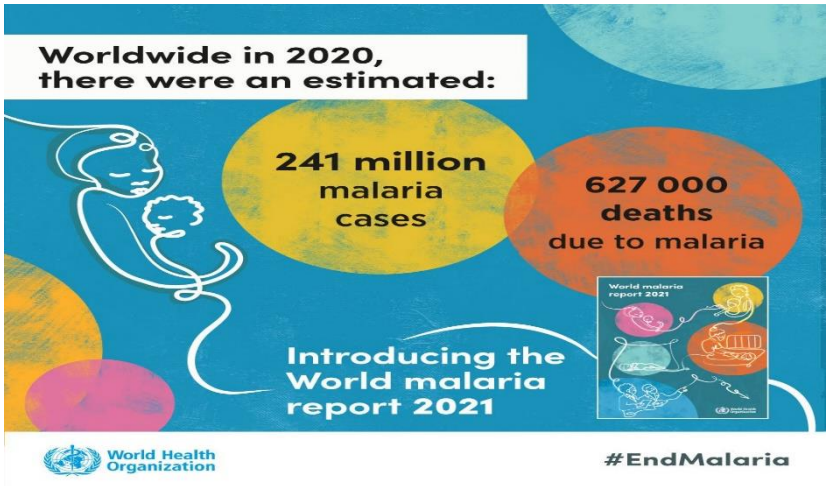


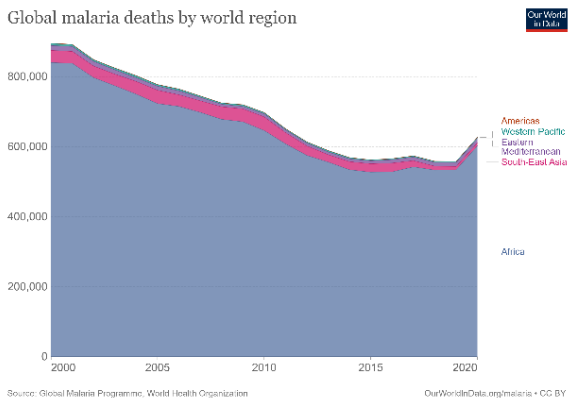


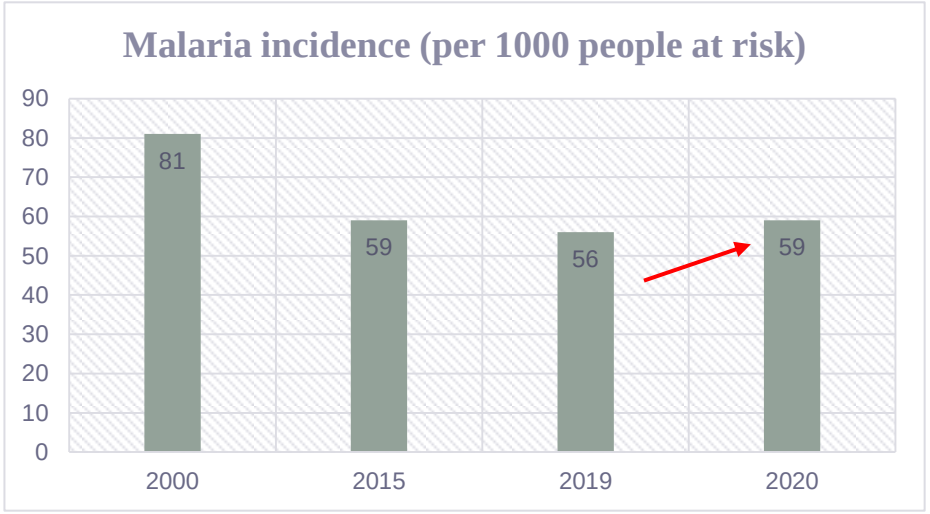
Malaria…

5% increase in case incidence between 2019-2020

12% increase in death rates between 2019-2020





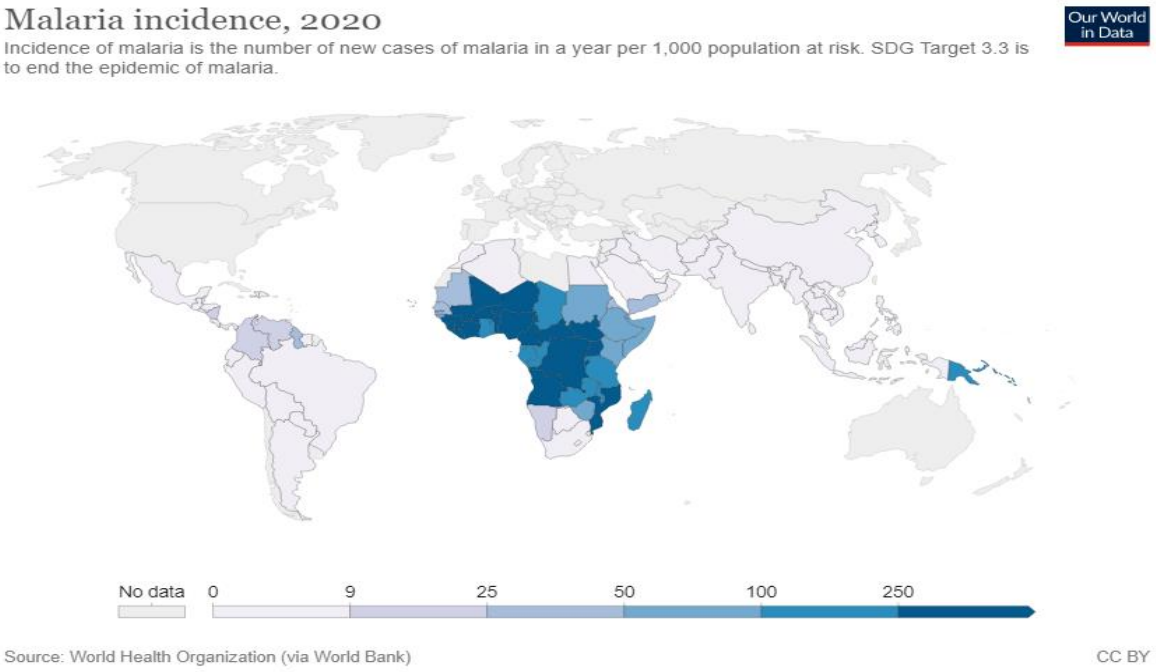


Malaria…

No domestic cases in Cyprus since 1949 and in Turkey since 2010???

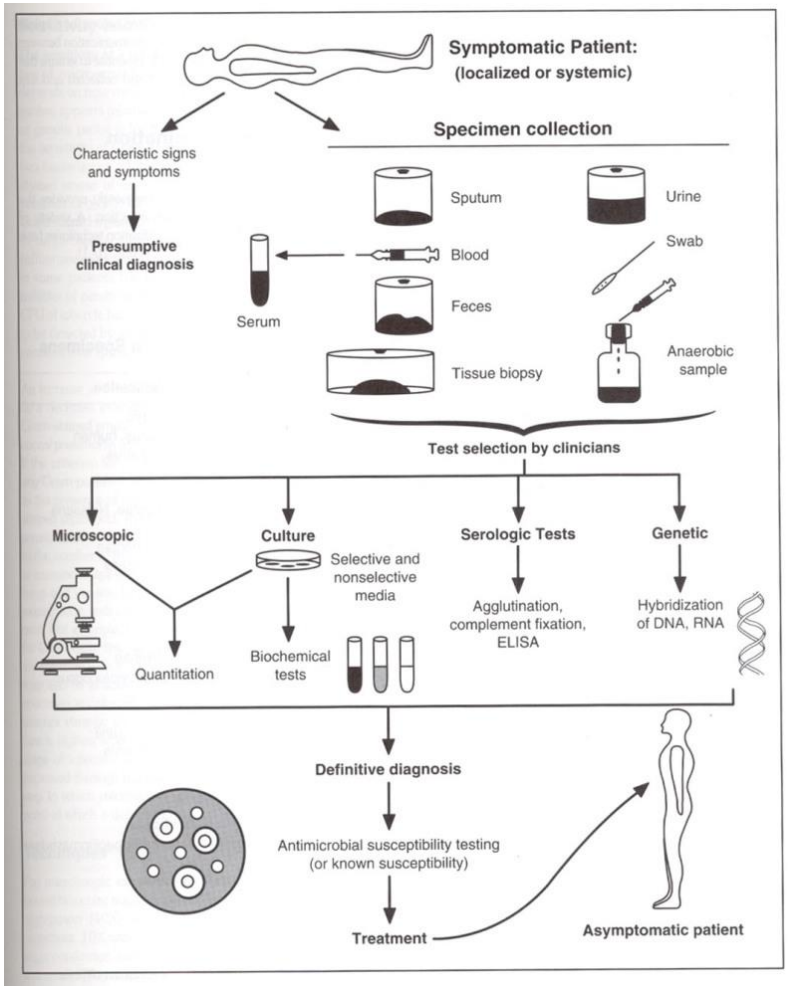
Increase in imported cases

Vector???



Diagnostic Methods in Microbiology…

- **Symptoms and story of patient**
- **Appropriate sample**
- **Correct test selection and diagnosis**
- **Fast and accurate treatment**



Diagnostic Methods in Microbiology…

A microorganism from a patient sample can generally be detected and identified in any of 4 (four) ways:

(1) Direct microscopic examination

(2) Cultivation of microorganisms using artificial media or living hosts

(3) Measurement of microorganism-specific immune responses

(4) Detection of microorganism-specific macromolecules, especially nucleic acids

Diagnostic Methods in Microbiology…

Table: Methods used for microorganisms diagnosis

Test	*Ease of performance*	*Turnaround time*	*Result interpretation*	*Advantages*	*Disadvantages*
Direct examination	Could be performed in routine clinical laboratory and in nurse station	1–3 h	Direct if correlated with symptoms	Rapid	Poor sensitivity and specificity; special skills are needed for interpretation
Culture	Could be performed in sophisticated clinical laboratory and in research laboratory	2–14 days	Definite	For phenotypic drug susceptibility testing	Time-consuming; poor sensitivity; limited microorganisms are culturable
Serology	Could be performed in larger and sophisticated clinical laboratory	4–6 h	Indirect	Automation	Results are generally retrospective; immunosuppressed host may be unable to mount a response
Molecular diagnostics	Could be performed in only a few very sophisticated research and clinical laboratory	2.5 h to 2 days	Direct without knowing microbial viability	High sensitivity and specificity	Facility requirement; false positive due to carryover contamination and false negative due to inhibitors in specimen

(1) Microscopic Examination…

- **Wet mount microscopy**

E.g. *Trichomonas vaginalis* in vaginal discharges or urine

- **Microscopy of stained material**

- Many staining methods are used in routine microbiology laboratory
- Microorganisms can be visualized using special staining methods and classified morphologically and functionally

E.g. Gram staining, Giemsa staining, Acid-fast staining, Iodine staining

Bacteria	Protozoa (malaria)	Mycobacteria	Intestinal parasites

Microscopic Examination…

Stain method	*Organisms detected*	*Advantages*	*Disadvantages*
Gram stain	Bacteria, yeast	Rapid; direct differentiation; assess specimen for culture	Cell wall-deficient bacteria stain unpredictably; pink background often masks Gram-negative organisms
Acridine orange stain	Bacteria, mycoplasma	Good for organisms with damaged cell walls; background stain is relatively weak	Specific light source is needed; cannot differentiate bacterial Gram reaction
Acid-fasting stain (Kinyoun or Ziehl-Neelsen)	Mycobacteria	Direct diagnosis of infection in untreated host	Cannot speciate mycobacteria; high background in tissue slide
Auramine-rhodamine stain	Mycobacteria	Lower power can be used for examining the slide	Fluorescence microscope is needed; artifact staining in tissue slide
Modified acid-fast stain	Nocardia, cryptosporidia, isospora, cyclospora	Rapid and specific diagnosis	Tissue homogenates often mask presence of the organism
Calcofluor white stain with potassium hydroxide	Pneumocystis, fungi	Rapid stain for fungi detection	Fluorescence microscope with specific filter is needed; species differentiation requires skills
India ink stain	*Cryptococcus neoformans*	Diagnosis of meningitis when positive in spinal fluid	Low sensitivity; a messy technique with false positives from lymphocytes in hypotonic solution
Giemsa stain	Plasmodia, trypanosomes, leishmania, toxoplasma, histoplasma, pneumocystis	Detection of multiple organisms; shows the relationship between organisms and host cells	Not specific for viral inclusions; cannot determine bacterial Gram reaction

(2) Culture…

- The production and isolation of a microorganism in an artificial media and/or in a living host is conclusive proof of the existence of that microorganism

- In many cases, the culture method remains the '**gold standart**' despite long incubation time

(2) Culture…

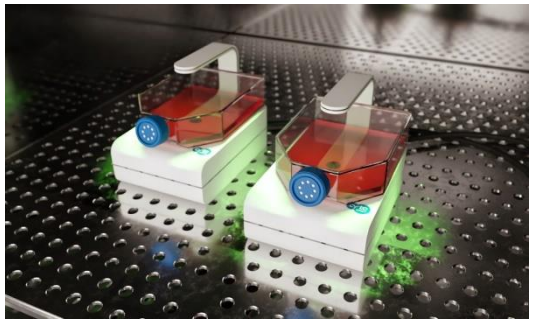
- **Culture Using Artificial Media**

Bacteria, mycobacteria, mycoplasma, and fungi are cultured either in liquid or on a solid artificial media

Culture media can be made selective
(E.g. CHROMagar MRSA)

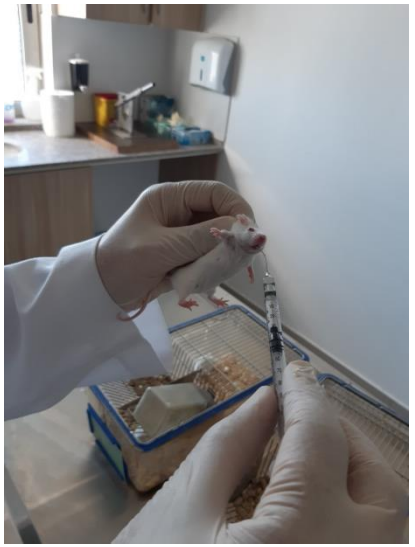
(2) Culture…

- **Culture Using Living Cells**



Many viruses can be recovered and identified in a eukaryotic cell culture system

- **Culture Using Other Living Hosts**



Microorganism isolation, especially for some viruses (e.g. arboviruses) and parasites (e.g. *Plasmodium berghei*, *Leishmania* spp.), occasionally requires the inoculation of test specimens into animals and embryonated eggs (e.g. influenza viruses).

(3) Immunological tecniques…

Serology

- **Agglutination**
- **Immunodiffusion/precipitation reactions**
- **Immunoassays**
  - ELISA
  - Lateral flow immunochromatographic assay

Immunohistochemistry

Flow cytometri

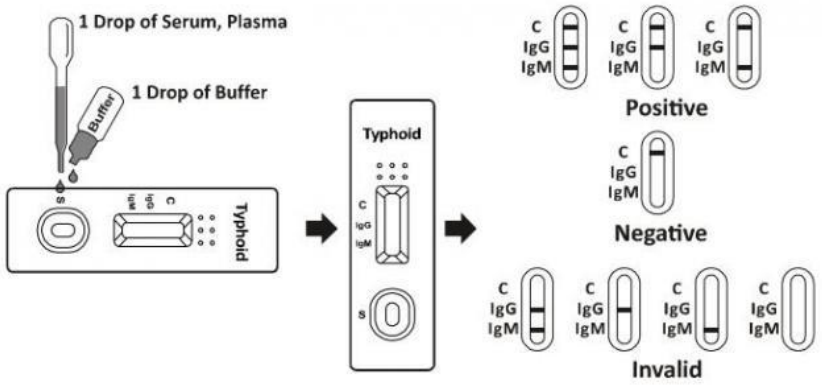
Immunoblotting

- **Western blot**

(3) Immunological tecniques…

Lateral flow immunochromatographic assay

Lateral flow assays (LFAs), also known as **immunochromatographic assays** or **rapid tests**, are **fast**, **easy-to use** and **portable** devices that can detect the presence of a target substance in a liquid sample without the need for specialised staff and expensive equipment.



(3) Immunological tecniques…

Lateral flow immunochromatographic assay

- ***Parts in the structure of immunochromatographic tests***:

1-Plastic base and cassette cover

2-Cellulose-made '**sample pad**' on which the sample is dripped

3-Fiber glass "**reaction pad**" with colored molecules and antibodies where reaction take place

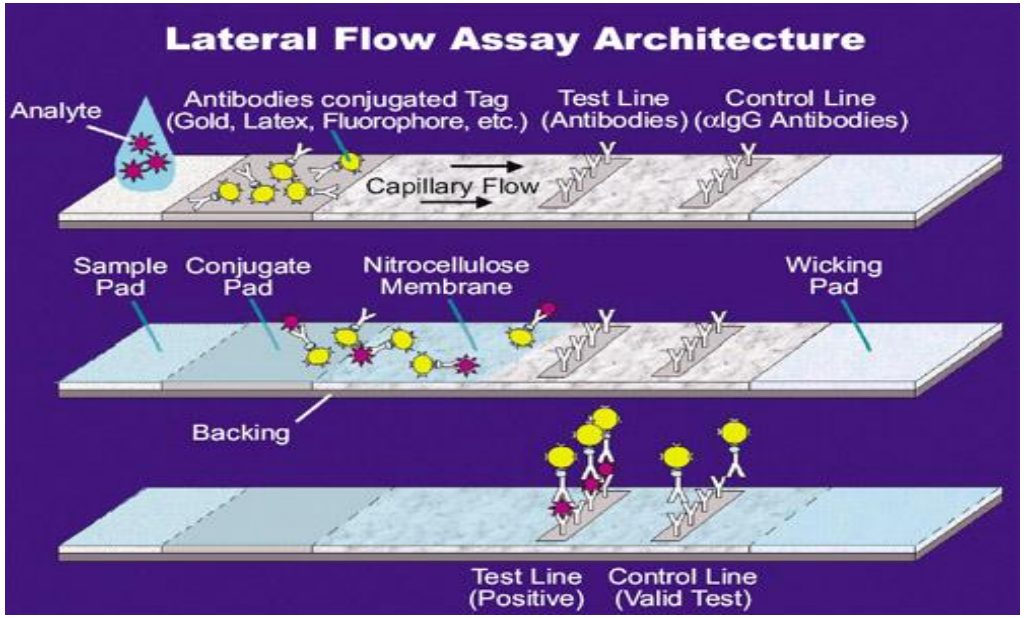
4**-Nitrocellulose membra**ne where capillary flow occurs and the result is monitored

5-Test line(s) and control line

6-A cellulose base '**waste pad**' on which the residual sample is deposited

(3) Immunological tecniques…

Lateral flow immunochromatographic assay



The concept behind Lateral flow assay is simple: a liquid sample (or extract) comprising the analyte of interest moves across many zones of polymeric strips, without the help of external forces (capillary action), where the molecules that interact with the analyte are attached to the polymeric strips.

(3) Immunological tecniques…

Lateral flow immunochromatographic assay

- The sample is applied to the absorbent sample pad at one end of the strip
- The sample pad ensures that the analyte present in the sample will be capable of binding to the capture reagents of conjugates and on the membrane.
- The treated sample migrates through the conjugate release pad, which contains antibodies that are specific to the target analyte and are conjugated to coloured or fluorescent particles – most commonly colloidal gold and latex microspheres
- The sample, together with the conjugated antibody bound to the target analyte, migrates along the strip into the detection zone
- This is a porous membrane (usually composed of nitrocellulose) with specific biological components (mostly antibodies or antigens) immobilized in lines.

(3) Immunological tecniques…

Lateral flow immunochromatographic assay

- Recognition of the sample analyte results in an appropriate response on the test line, while a response on the control line indicates the proper liquid flow through the strip.

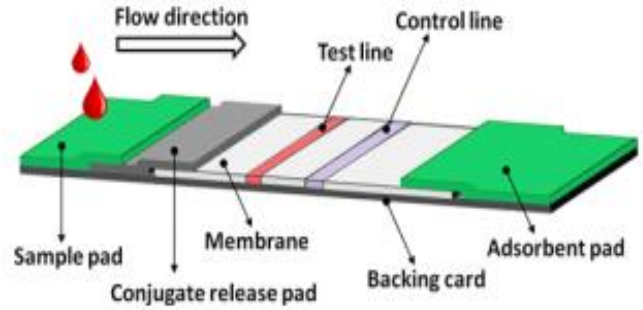
- The read-out, represented by the lines appearing with different intensities, can be assessed by eye or using a dedicated reader

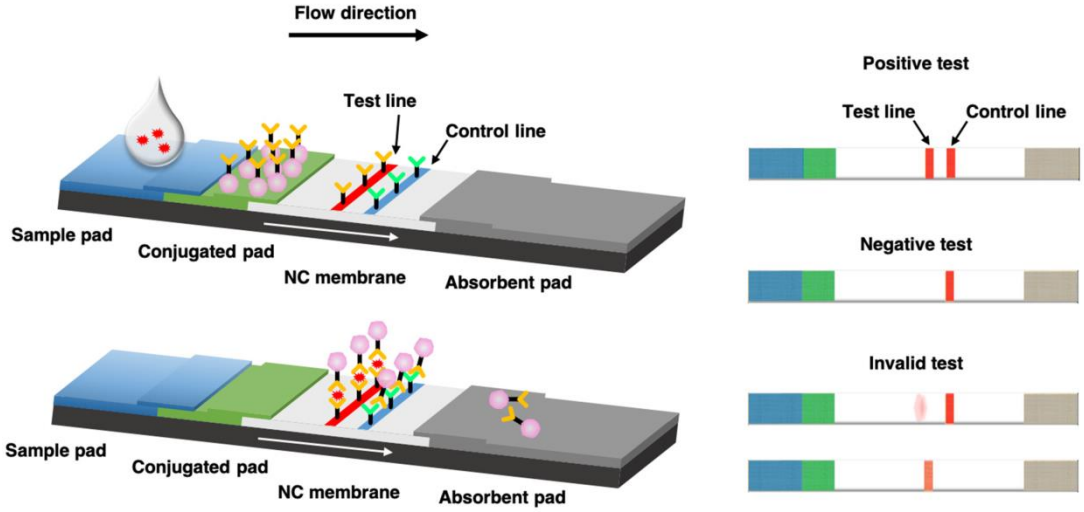
- The principle of this ‘ladder bars’ assay is based on the stepwise capture of colorimetric conjugate–antigen complexes by the immobilized antibody on each successive line, where the number of lines appearing on the strip is directly proportional to the concentration of the analyte

- The liquid flows across the device because of the capillary force of the strip material and, to maintain this movement, an absorbent pad is attached at the end of the strip. The role of the absorbent pad is to wick the excess reagents and prevent backflow of the liquid.

(3) Immunological tecniques…

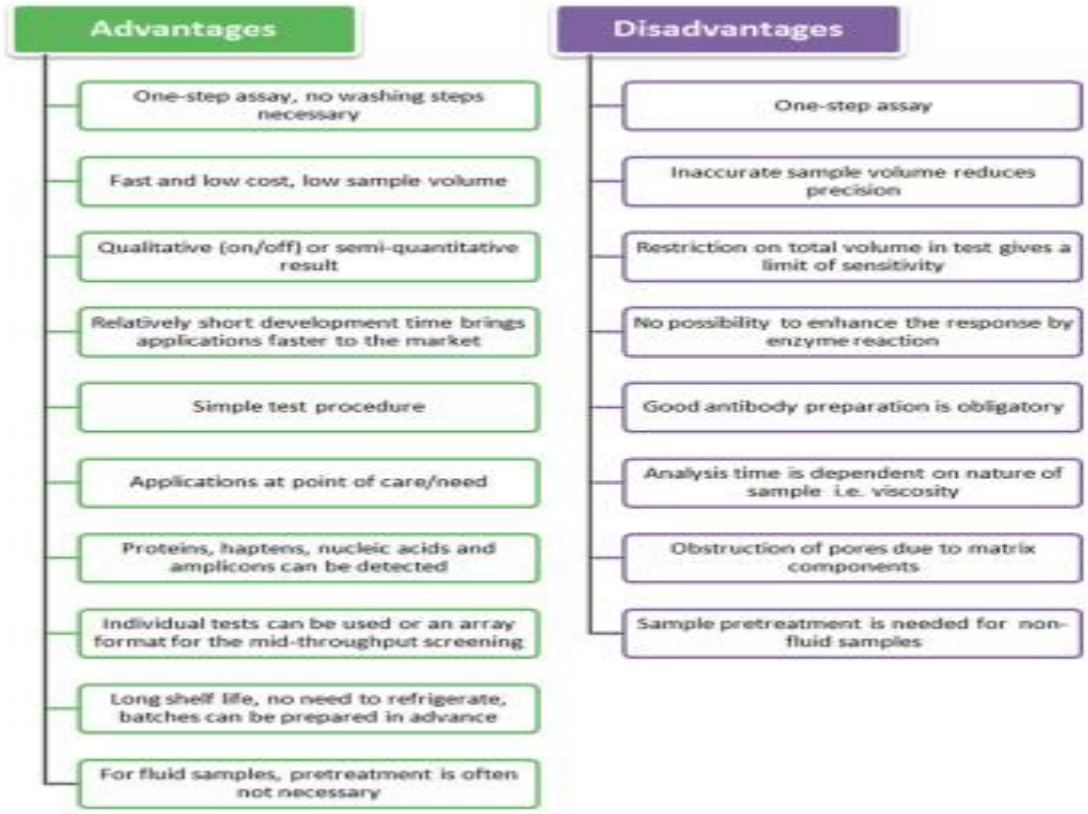
Lateral flow immunochromatographic assay





(3) Immunological tecniques…

Lateral flow immunochromatographic assay

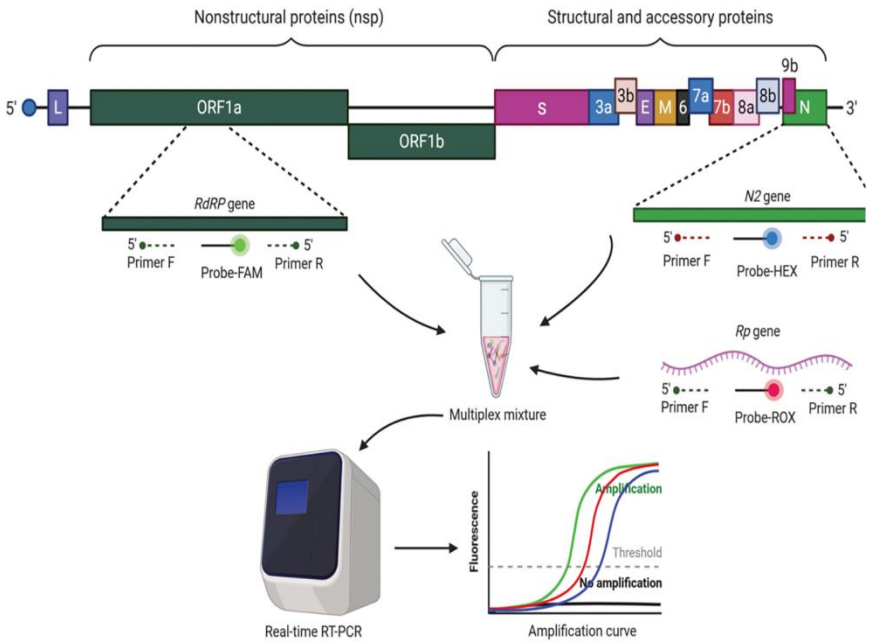


(4) Molecular Methods in Diagnostic Microbiology…

- Diagnosis
- Type-level (species) identification
- Drug resistance analyzes
- Genotype determination

(4) Molecular Methods in Diagnostic Microbiology…

- **Nucleic acid amplification methods**
  - Signal amplification
  - Target amplification
- **Postamplification analyses**
  - Sequencing
  - Reverse hybridization
  - Luminex analysis
- **Large-scale nucleic acid analysis**
  - Whole genome sequencing
  - Nucleic acid arrays
  - Mass spectrometry



(4) Molecular Methods in Diagnostic Microbiology…

- **Nucleic acid amplification methods**
  - Signal amplification
  - Target amplification

- Polymerase chain reaction (PCR)

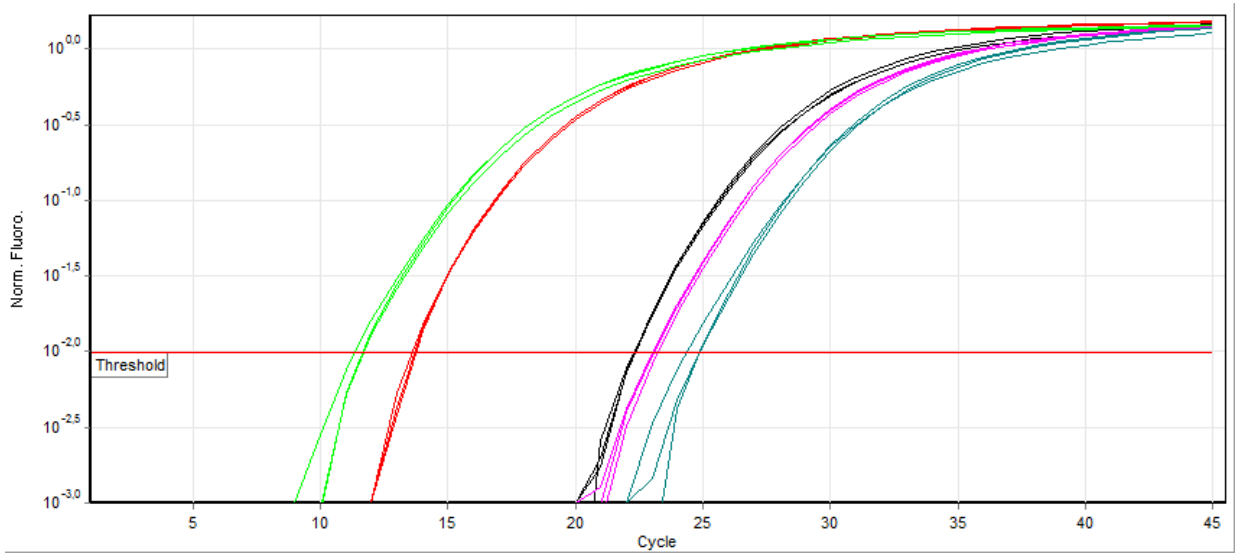
PCR is a method of amplifying target DNA or cDNA sequences using specific primers and a thermophilic DNA polymerase enzyme.

In this method designed primers which amplify a unique region of microorganisms are used. Thus, this method is target specific.

(4) Molecular Methods in Diagnostic Microbiology…

PCR

- Multiplex PCR
- Nested PCR
- **Real time PCR (RT-PCR)**

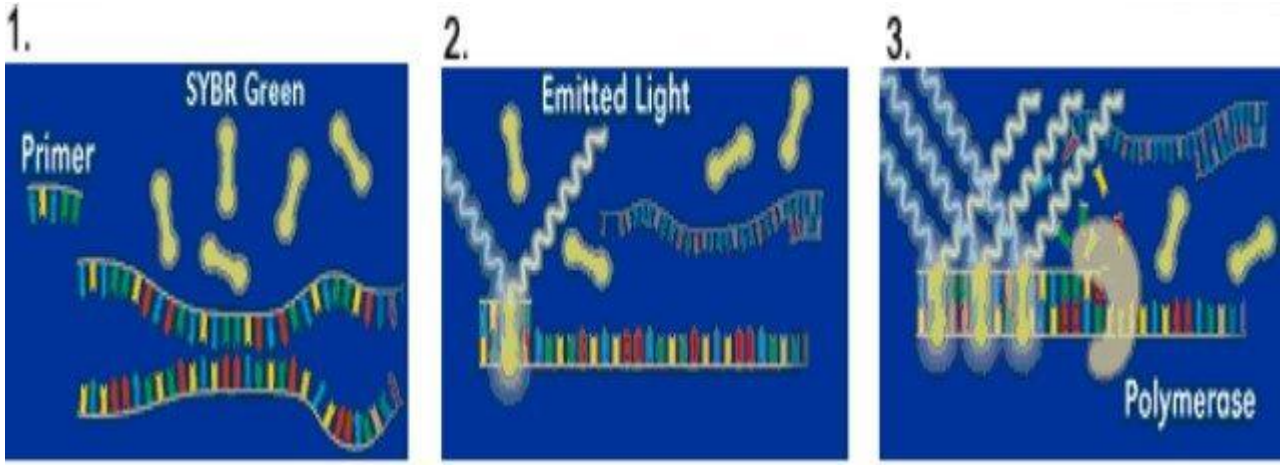


(4) Molecular Methods in Diagnostic Microbiology…

Real time PCR

It allows viewing the increase in the amount of DNA as it is amplified.

All real time PCR systems rely upon the detection and quantitation of the fluorescent reporter, the signal of which increases in direct proportion of the amount of PCR product in a reaction.



(4) Molecular Methods in Diagnostic Microbiology…

In-house PCR

Primers and probes targetin specific gene regions designed in laboratory

Source	Primer/Probe Name	Target Gene	Sequence	Lenght	Genomic Region *
China CDC, China	Forward (F)	ORF1ab	CCCTGTGGGTTTTACACTTAA	21	13,342–13,362
China CDC, China	Reverse (R)	ORF1ab	ACGATTGTGCATCAGCTGA	19	13,442–13,460
China CDC, China	Fluorescence probe (P)	ORF1ab	CCGTCTGCGGTATGTGGAAAGGTTATGG	28	13,377–13,404
China CDC, China	Forward (F)	N	GGGGAACTTCTCCTGCTAGAAT	22	28,881–28,902
China CDC, China	Reverse (R)	N	CAGACATTTTGCTCTCAAGCTG	22	28,958–28,979
China CDC, China	Fluorescence probe (P)	N	TTGCTGCTGCTTGACAGATT	20	28,934–28,953
Institut Pasteur, France	nCoV_IP2-12669Fw	RdRp	ATGAGCTTAGTCCTGTTG	18	12,690–12,707
Institut Pasteur, France	nCoV_IP2-12759Rv	RdRp	CTCCCTTTGTTGTGTTGT	18	12,780–12,797
Institut Pasteur, France	nCoV_IP2-12696bProbe(+)	RdRp	ATGTCTTGTGCTGCCGGTA	19	12,719–12,737
Institut Pasteur, France	nCoV_IP4-14059Fw	RdRp	GGTAACTGGTATGATTTCG	19	14,080–14,098
Institut Pasteur, France	nCoV_IP4-14146Rv	RdRp	CTGGTCAAGGTTAATATAGG	20	14,167–14,186
Institut Pasteur, France	nCoV_IP4-14084Probe(+)	RdRp	TCATACAAACCACGCCAGG	19	14,105–14,123
Institut Pasteur, France	E_Sarbeco_F1	E	ACAGGTACGTTAATAGTTAATAGCGT	26	26,269–26,294
Institut Pasteur, France	E_Sar beco_R2	E	ATATTGCAGCAGTACGCACACA	22	26,360–26,381
Institut Pasteur, France	E_Sarbeco_P1	E	ACACTAGCCATCCTTACTGCGCTTCG	26	26,332–26,357
US CDC, USA	2019-nCoV_N1-F	ORF9b	GACCCCAAAATCAGCGAAAT	20	28,287–28,306
US CDC, USA	2019-nCoV_N1-R	ORF9b	TCTGGTTACTGCCAGTTGAATCTG	24	28,335–28,358
US CDC, USA	2019-nCoV_N1-P	ORF9b	ACCCCGCATTACGTTTGGTGGACC	24	28,309–28,332
US CDC, USA	2019-nCoV_N2-F	ORF9b	TTACAAACATTGGCCGCAAA	20	29,164–29,183
US CDC, USA	2019-nCoV_N2-R	ORF9b	GCGCGACATTCCGAAGAA	18	29,213–29,230
US CDC, USA	2019-nCoV_N2-P	ORF9b	ACAATTTGCCCCCAGCGCTTCAG	23	29,188–29,210
US CDC, USA	2019-nCoV_N3-F	ORF9b	GGGAGCCTTGAATACACCAAAA	22	28,681–28,702
US CDC, USA	2019-nCoV_N3-R	ORF9b	TGTAGCACGATTGCAGCATTG	21	28,732–28,752
US CDC, USA	2019-nCoV_N3-P	ORF9b	ATCACATTGGCACCCGCAATCCTG	24	28,704–28,727
National Institute of Infectious Diseases, Japan	NIID_2019-nCOV_N_F2	N	AAATTTTGGGGACCAGGAAC	20	29,142–29,161

Thank you for listening…