

Diagnosis of infectious diseases

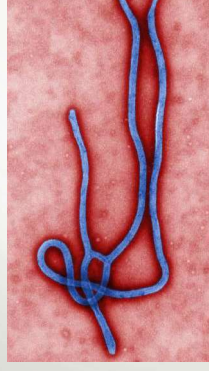
Microscopy

Culture

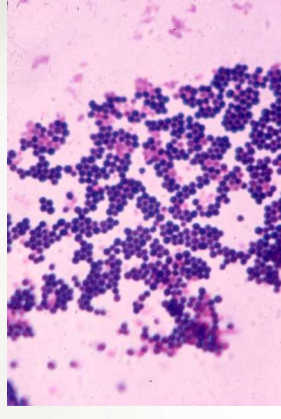
Immunologic tests

Nucleic acid based tests

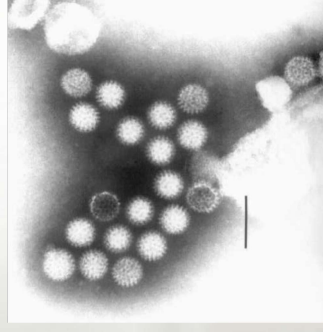
Microscopy in diagnostics



Ebola virus (electron microscopy)



Staphylococcus aureus in gram stain



Rotavirus (electron microscopy)

Sources: npr.org
cmapspublic.lhmc.us
lamedicinasiquehuele.blogspot.com

Microscopy in diagnostics

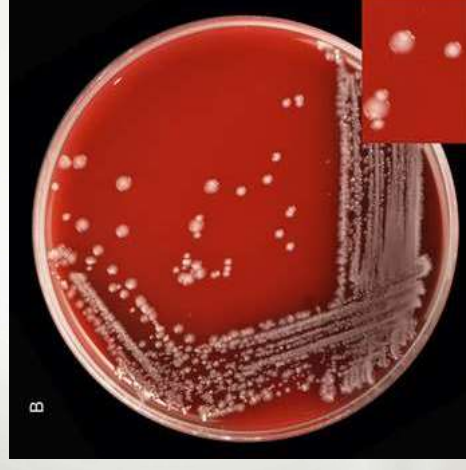
Electron microscopy

Light microscopy with stains: Gram stain, acid-fast stain (mycobacterium),
Giemsa stain (parasites, intracellular pathogens)

Fluorescent microscopy: Detection at lower concentrations

Acridine-orange (bacteria-fungi)
auramine O (mycobacterium)
calcofluor white (fungi)

Diagnosis through cultures



Microbial growth in solid/liquid media

Use of selective inhibitors/nutrients
(Blood agar, chocolate-cystine agar,
use of antibiotics for fungal specimens)

Prevention of contamination very
important

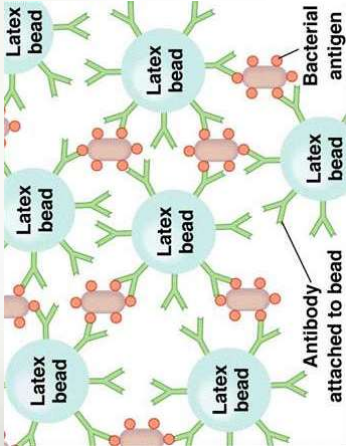
Susceptibility tests for antibiotics
Possible, helpful in treatment

Specific issues:

Viruses do not grow well in pure culture

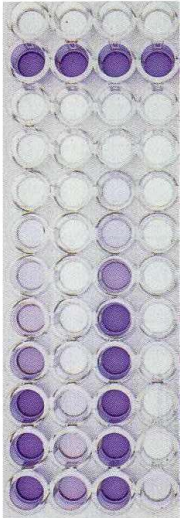
Mycobacteria grow slowly

Immunologic tests



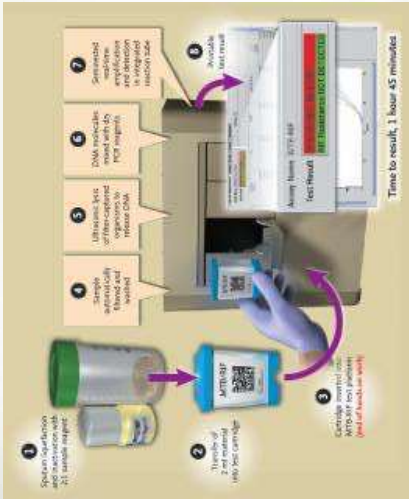
Agglutination tests:
Agglutination of latex beads by antigen-
Antibody interaction
Formation of thick precipitate

ELISA
Based on antigen-antibody binding
Colorimetric detection of binding



Sources: lookfordiagnosis.com
Source: luddlimos.com

Nucleic acid based diagnostic tests



Based on amplification of genomic nucleic acid from pathogens
Very specific, highly sensitive
Can identify more than one pathogen
False positives and false negatives both possible

Source: www.biotechniques.com

Diagnosis of common diseases

Disease	Organism	Common diagnostic tools
Dairrea	Rotavirus, norovirus, astrovirus	Electron microscopy
Hepatitis	Hepatitis A, B, E	Nucleic acid based methods
AIDS	HIV	Nucleic acid based methods, EIA
Smallpox, vaccinia	poxviruses	Nucleic acid based methods, EM
Typhoid fever	Salmonella Typhi	Culture, detection of antibodies in serum (Widal test)
Tuberculosis	Mycobacterium tuberculosis	Microscopy, PCR, cultures, Mantoux test
Cholera	Vibrio cholerae	Cultures

Prevention and cure of infectious diseases

Prevention:
Vaccination
Isolation

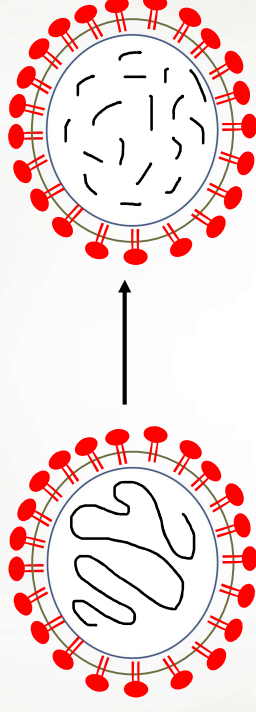
Cure:
Antibiotics
Antifungals
Antivirals

Principles of vaccination

- I. Exposure of immune system to "treated pathogen"
- II. Stimulation of effective immune response
- III. Long lasting immunity - saved in "memory"



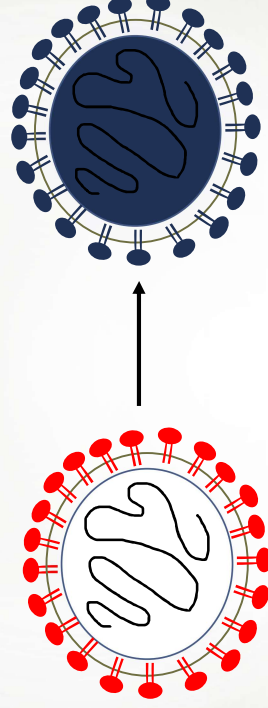
Inactivated vaccine



Heat/chemicals like beta-propiolactone

Salk polio vaccine

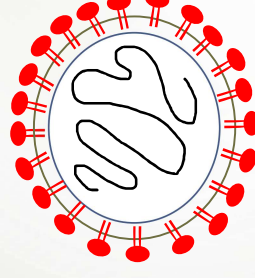
Live attenuated vaccine



Weaken strain by mutations, growth conditions

Tuberculosis vaccine, MMR

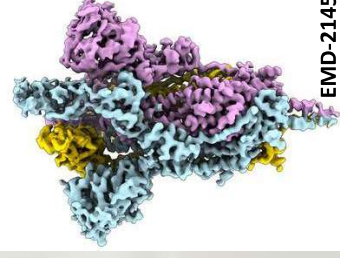
Subunit vaccine



Recombinant production of antigenic proteins

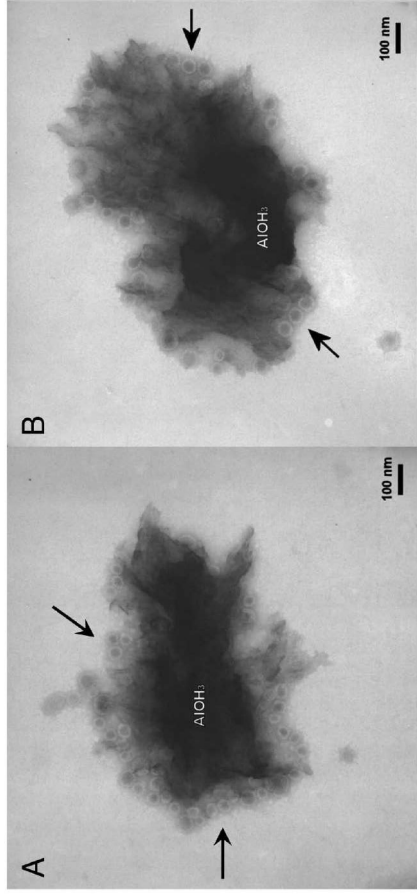
Administration with adjuvant

Tetanus vaccine, HBV vaccine, HPV vaccine



EMD-21457

Example of subunit/protein vaccines



Use of virus-like particles (VLPs)

Vaccine against Human Papilloma Virus (HPV) - Gardasil, Cervarix

Protection against ~ 70% of all cervical cancers

Source: Deschuyteneer, Hum Vaccin, 2010

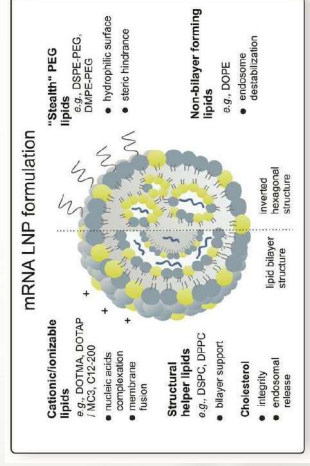
RNA vaccine

mRNA encapsulated in lipid nanoparticles

moderna[™]
messenger therapeutics

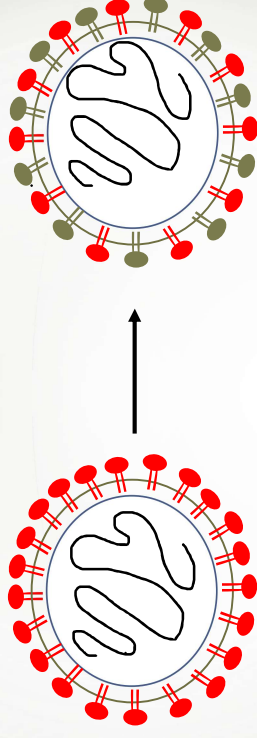
50:10:38.5:1.5 (ionizable lipid:DSPC:cholesterol:PEG-lipid)

Spike protein stabilized in pre-fusion conformation, 2 Proline substitutions



Verbeke et al, Nanotoday, 2019

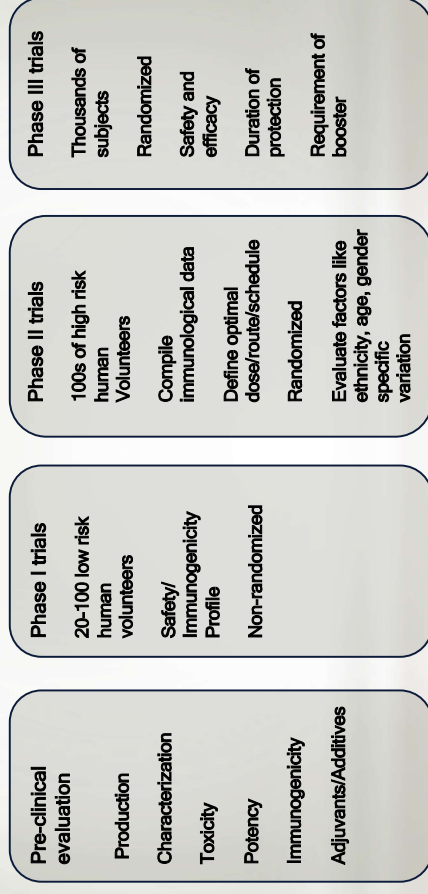
Chimeric viral vaccine



Gene from one virus introduced in another, harmless virus, general platform

AstraZeneca Vaccine - based on Chimpanzee Adenovirus Oxford 1 and 2 (ChAdOx1 and ChAdOx-2)

Vaccine trial pathways



Phase IV: Population study, safety/efficacy profile, rare events, lot-to-lot variation

Vaccination success stories

I. Smallpox -

Mortality was 25% of all children born

II. Measles -

130 million cases annually and 3 million deaths before vaccination

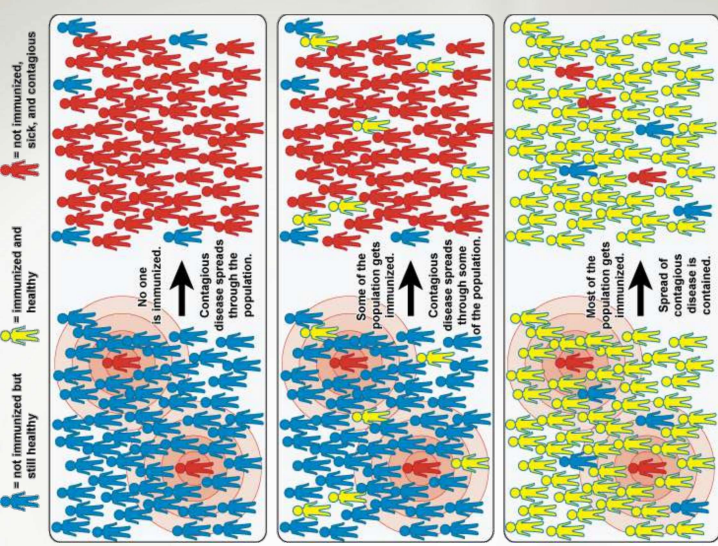
Recent increase in number of infections

III. Poliovirus -

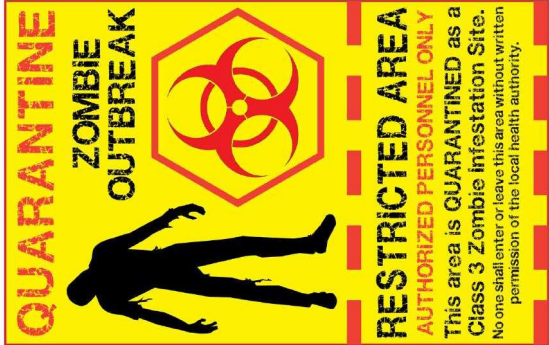
Oral live vaccines

Virtually abolished polio

Herd Immunity



Prevention of disease spread



Isolation

Quarantine

Public safety measures - bioethical considerations

Sources: freerepublic.com

Prevention of disease spread

Isolation - Separation of people infected with contagious diseases from the general population

Quarantine - Separation of people exposed to contagious diseases for monitoring

Travel restrictions

Intervention activities

Treatment of infectious diseases - antifungals

- 1) Polyenes: Bind ergosterol in the fungal membrane
Mammalian cells contain cholesterol, so not affected
Examples: Natamycin, Amphotericin B
- 2) Azoles: Inhibit lanosterol 14 alpha demethylase
(converts lanosterol to ergosterol)
Examples: Fluconazole, Abafungin
- 3) Allylamines: Inhibit squalene epoxidase

Treatment of infectious diseases - antivirals

Viral multiplication is tied too intimately to cellular processes

Therapeutic agents should block attachment, entry, replication, assembly, release of progeny

Compound	Mode of action	Susceptible viruses
Amantadine	Blocks the M2 proton channel	Type A influenza viruses
Alpha, beta interferons	Upregulates MHC class I, antiviral state	Chronic HBV and HCV, papillomavirus
Soluble CD4	Blocks attachment to CD4+ T cells	HIV-1
WIN52084	Prevents disassembly	Rhinoviruses
Oseltamivir	Prevents new virus release (Neuraminidase inhibitor)	Influenza
Remdesivir	Inhibits RNA polymerase activity	SARS-CoV-2
Paxlovid	Inhibits protease activity	SARS-CoV-2