Diagnosis of infectious diseases

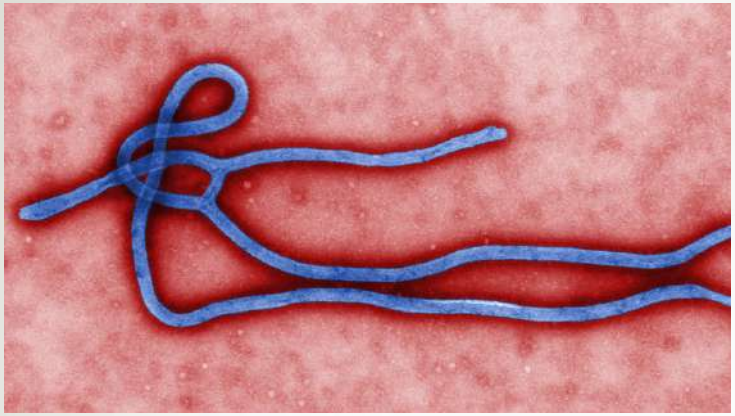
Microscopy

Culture

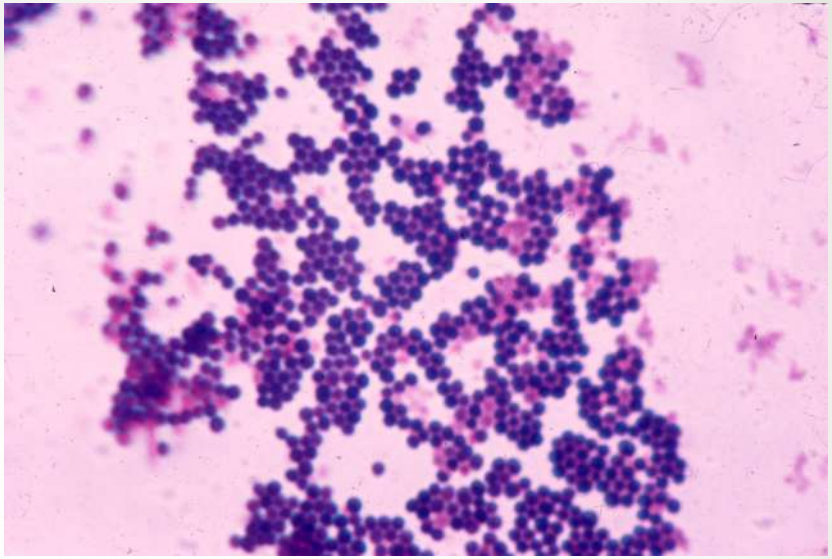
Immunologic tests

Nucleic acid based tests

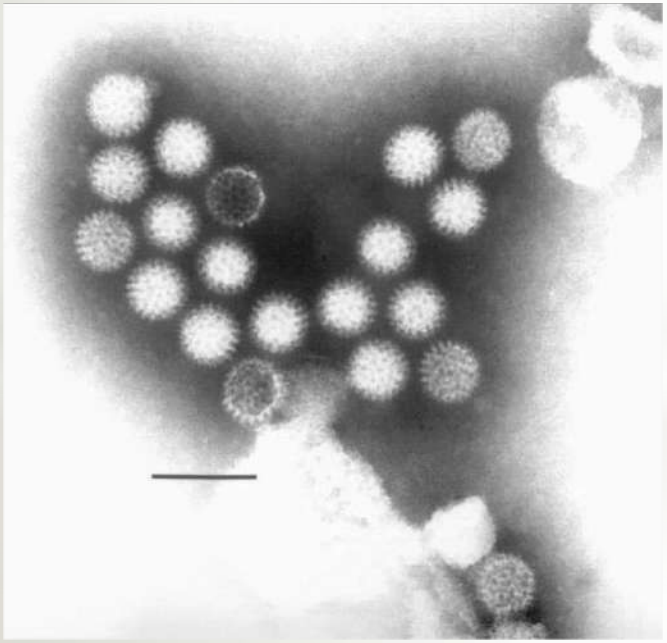
Microscopy in diagnostics



Ebola virus (electron microscopy)



Staphylococcus aureus in gram stain



Roatvirus (electron microscopy)

Sources: npr.org
cmapspublic.ihmc.us
lamedicinasiqueduele.blogspot.com

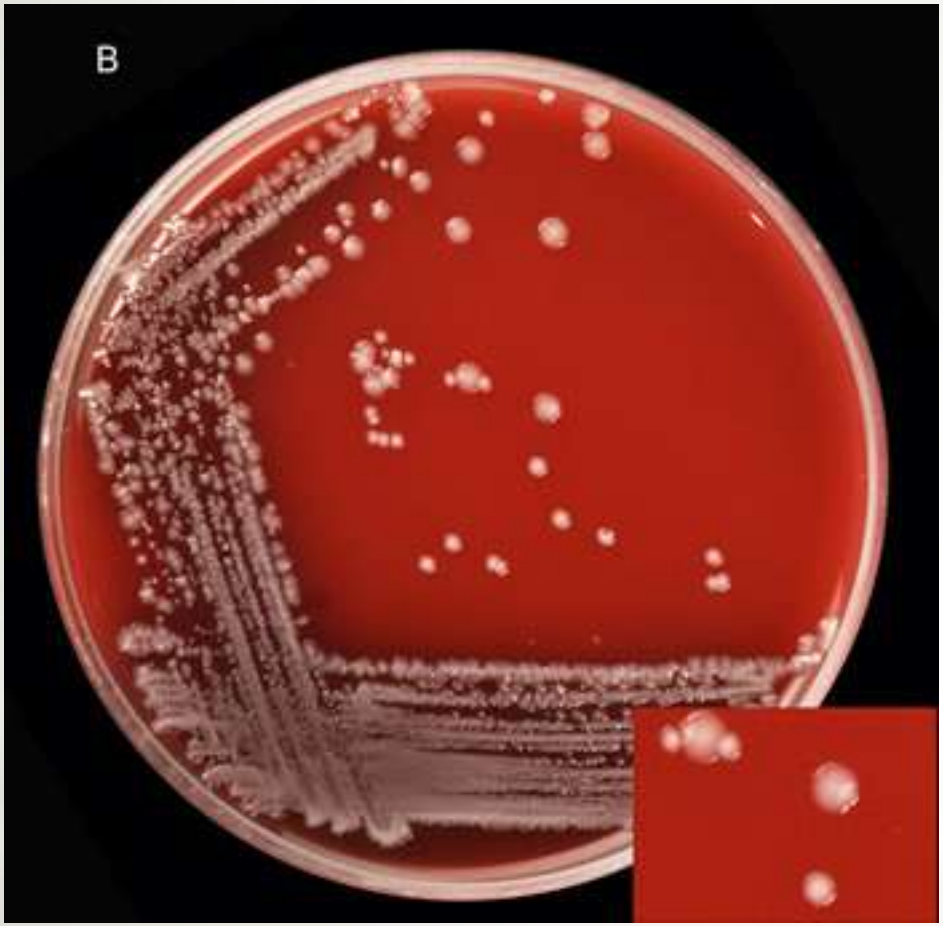
Microscopy in diagnostics

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)
calcoflour 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)

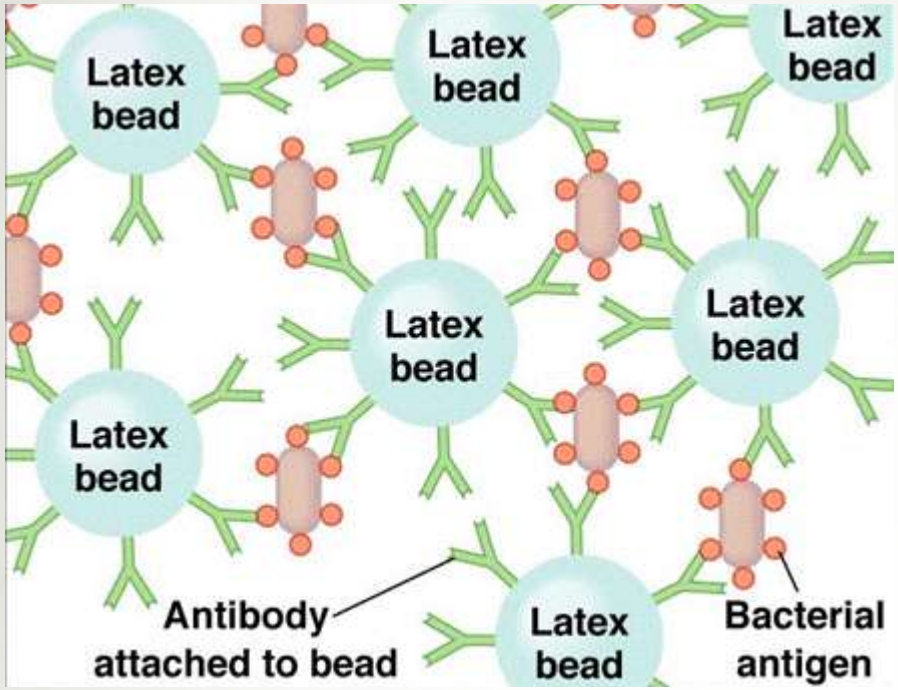
Prevention of contamination very important

Susceptibility tests for antibiotics Possible, helpful in treatment

Specific issues:
Viruses do not grow well in pure culture
Mycobacteria grow slowly

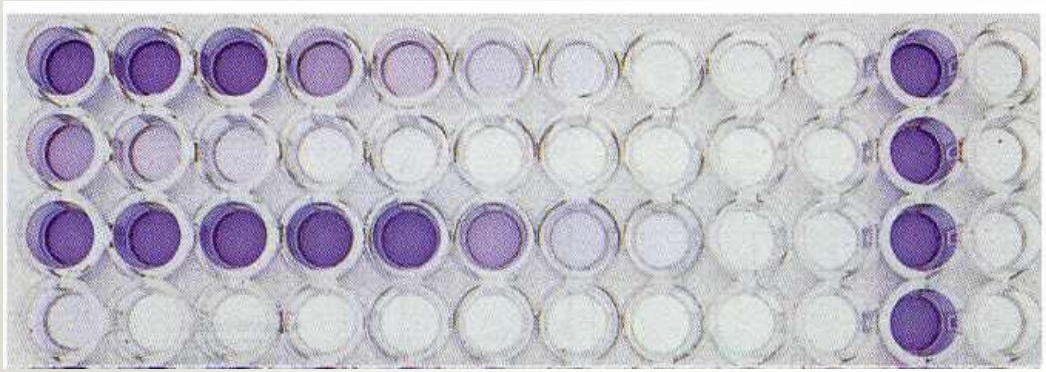
Source : lhsc.on.ca

Immunologic tests



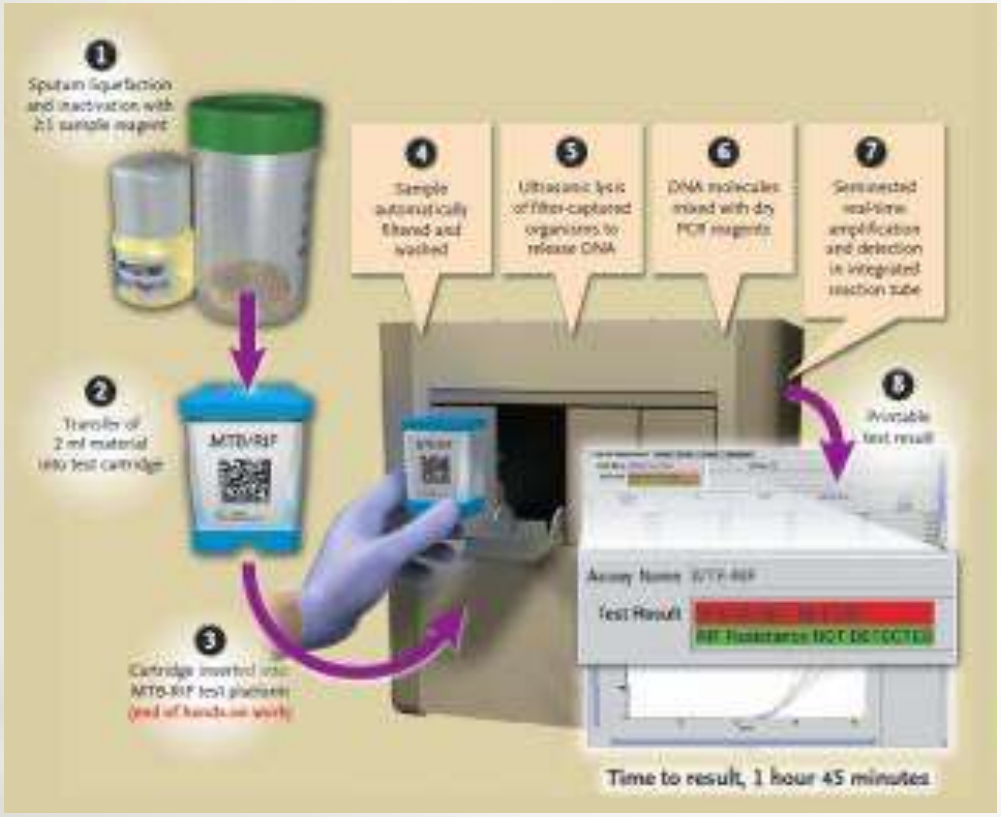
Agglutination tests:
Agglutination of latex beads by antigen-Antibody interaction
Formation of thick precipitate

ELISA
Based on antigen-antibody binding
Colorimetric detection of binding



Sources: lookfordiagnosis.com
Source: lucidlimos.com

Nucleic acid based diagnostic tests



PCR-based kit against *mycobacterium*

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
Dairrhea	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

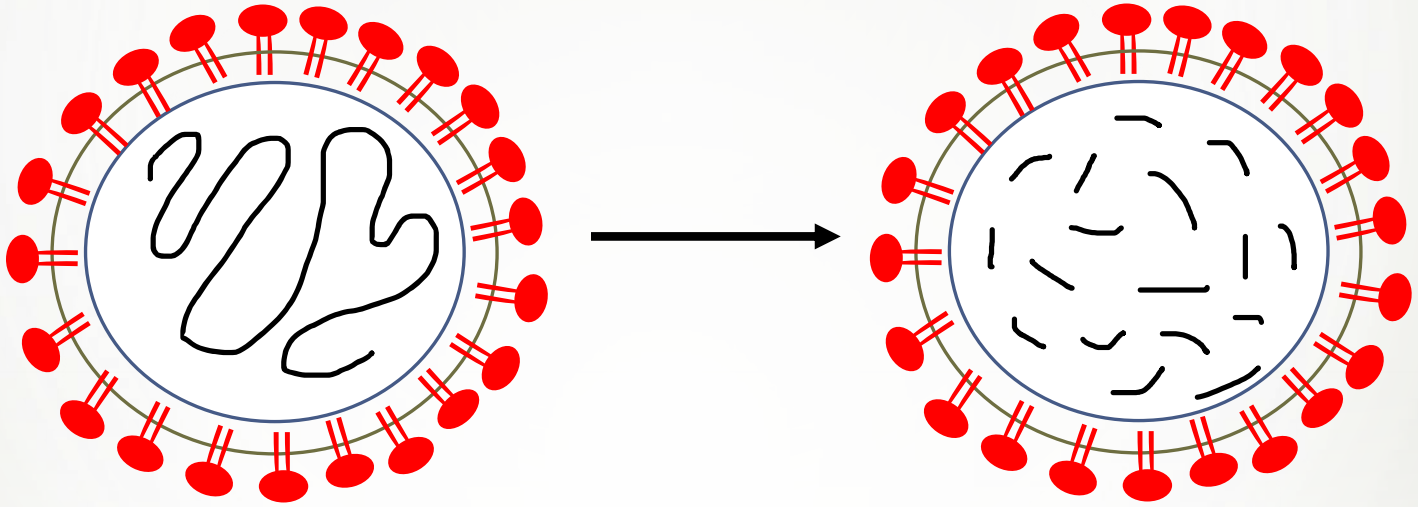
Principles of vaccination

I. Exposure of immune system to “treated pathogen”

II. Stimulation of effective immune response

III. Long lasting immunity – saved in “memory”

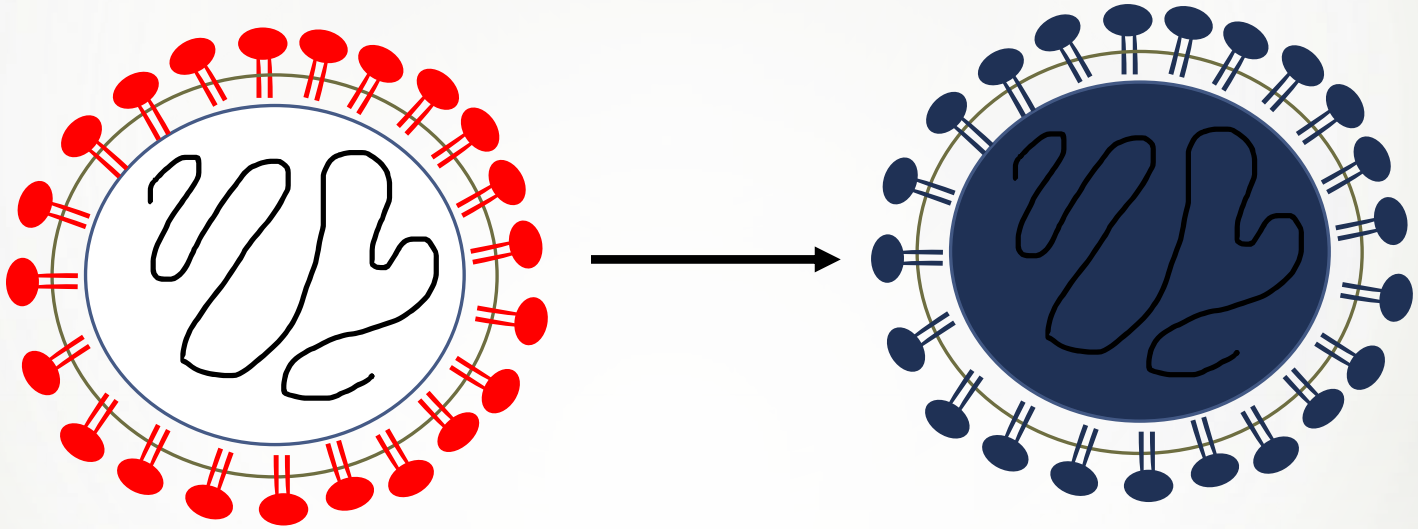
Inactivated vaccine



Heat/chemicals like beta-propiolactone

Salk polio vaccine

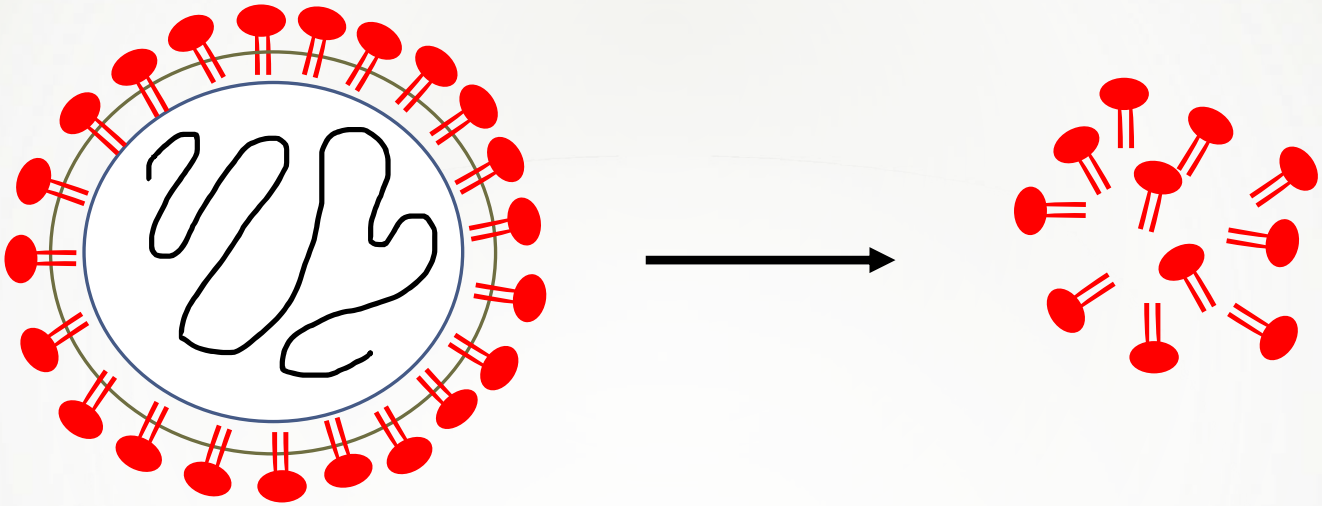
Live attenuated vaccine

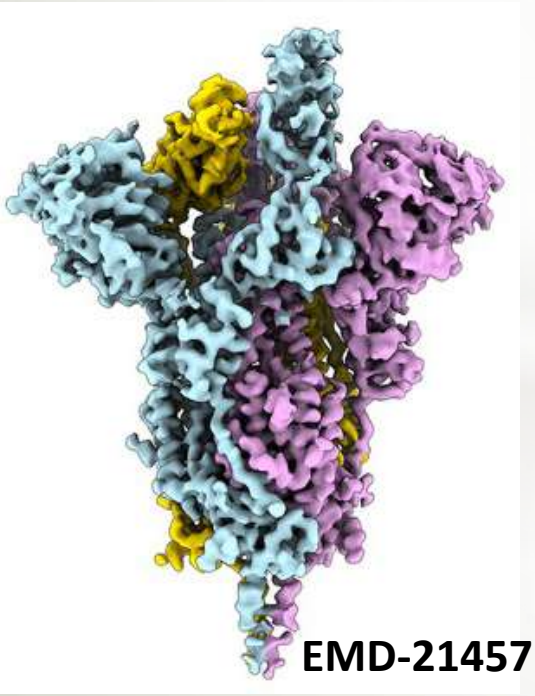


Weaken strain by mutations, growth conditions

Tuberculosis vaccine, MMR

Subunit vaccine



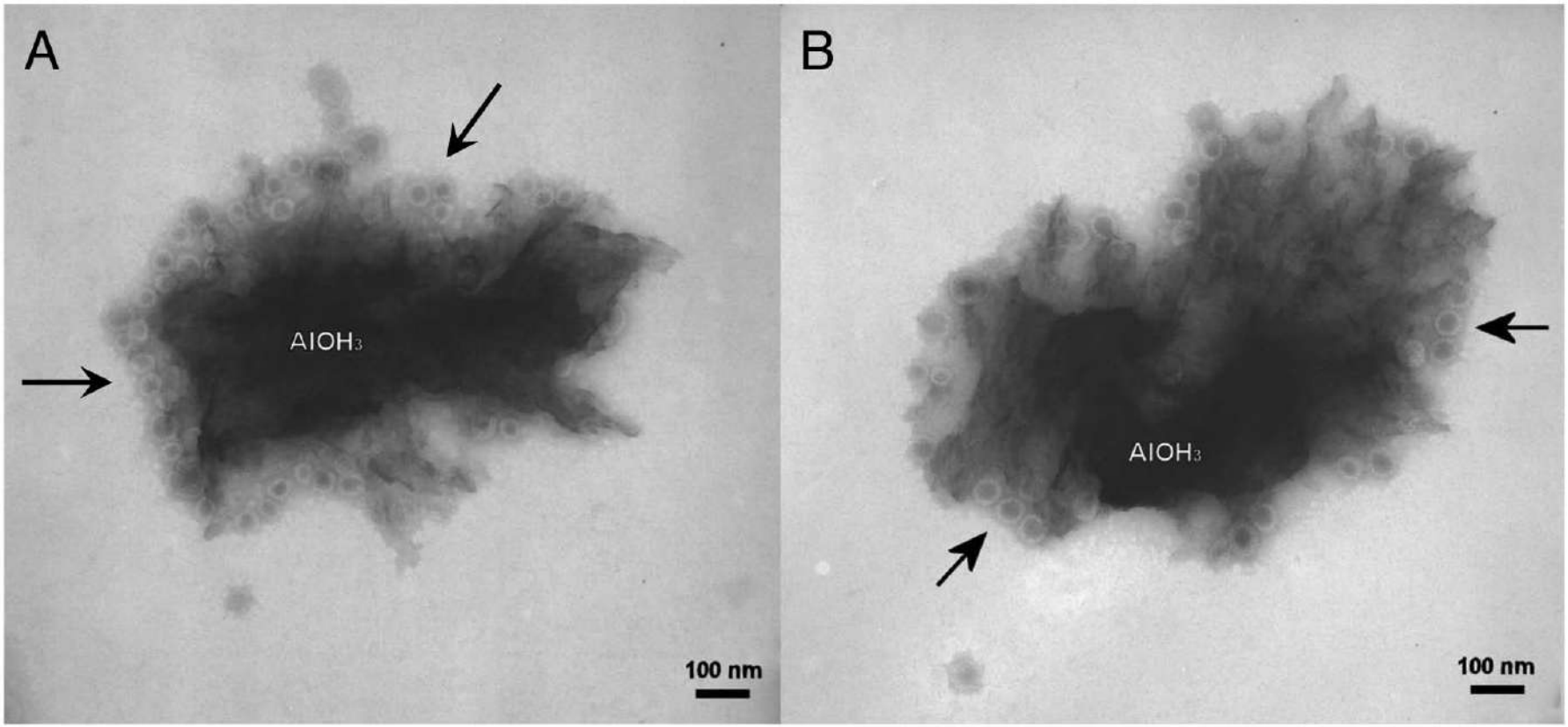


Recombinant production of antigenic proteins

Administration with adjuvant

Tetanus vaccine, HBV vaccine, HPV vaccine

Example of subunit/protein vaccines



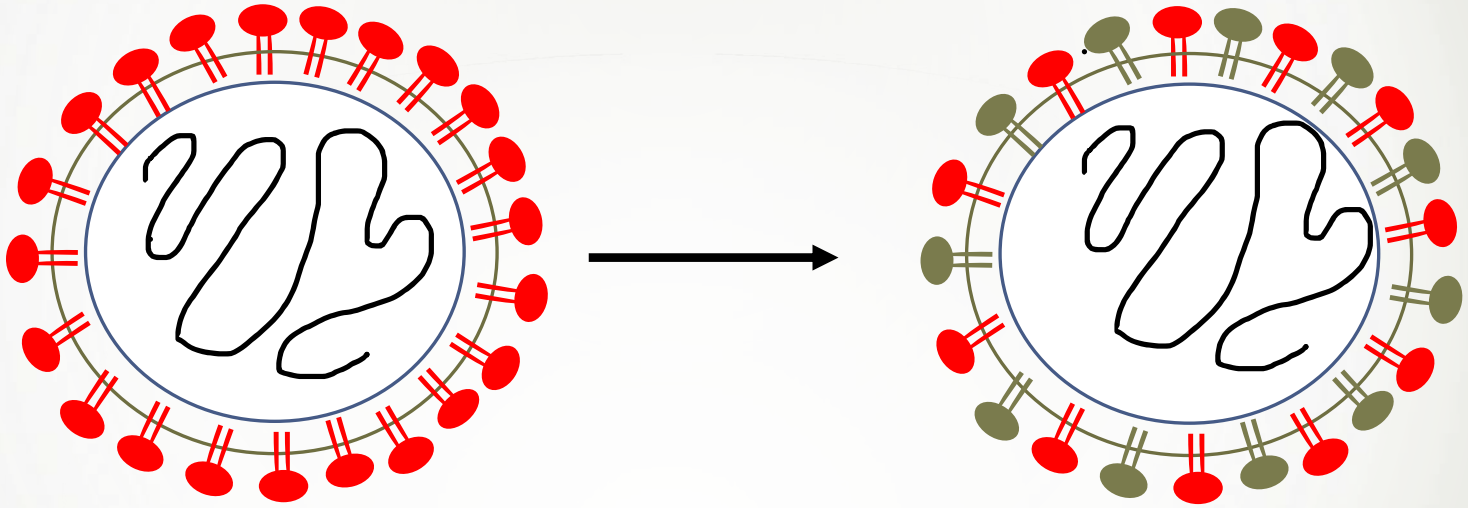
Use of virus-like particles (VLPs)

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

Protection against ~ 70% of all cervical cancers

Source: Deschuyteneer, Hum Vaccin, 2010

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)

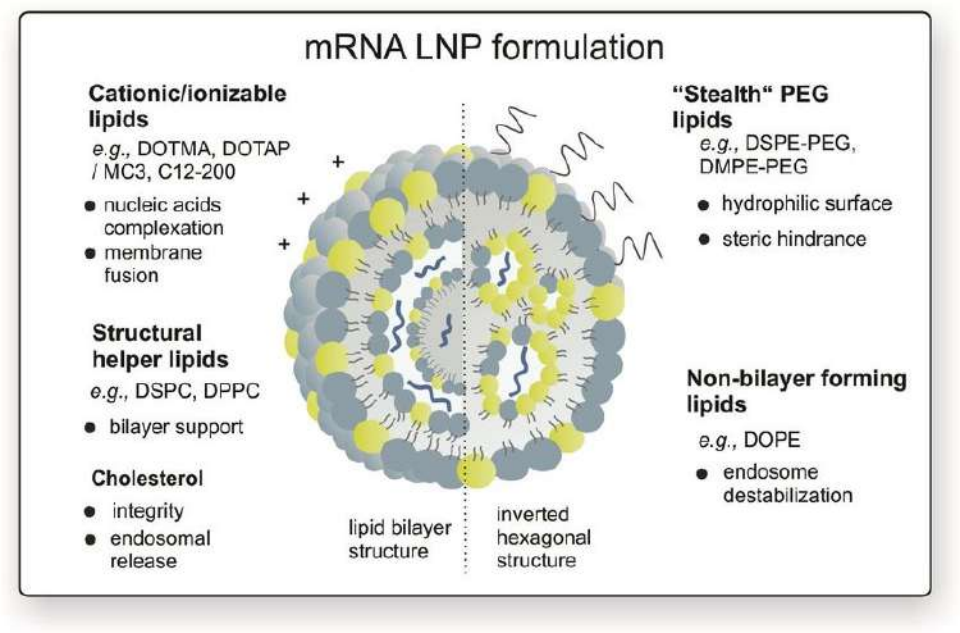
RNA vaccine

mRNA encapsulated in lipid nanoparticles

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

moderna™
messenger therapeutics

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



Verbeke et al, Nanotoday, 2019

Vaccine trial pathways

Pre-clinical evaluation

Production

Characterization

Toxicity

Potency

Immunogenicity

Adjuvants/Additives

Phase I trials

20-100 low risk human volunteers

Safety/ Immunogenicity Profile

Non-randomized

Phase II trials

100s of high risk human Volunteers

Compile immunological data

Define optimal dose/route/schedule

Randomized

Evaluate factors like ethnicity, age, gender specific variation

Phase III trials

Thousands of subjects

Randomized

Safety and efficacy

Duration of protection

Requirement of booster

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

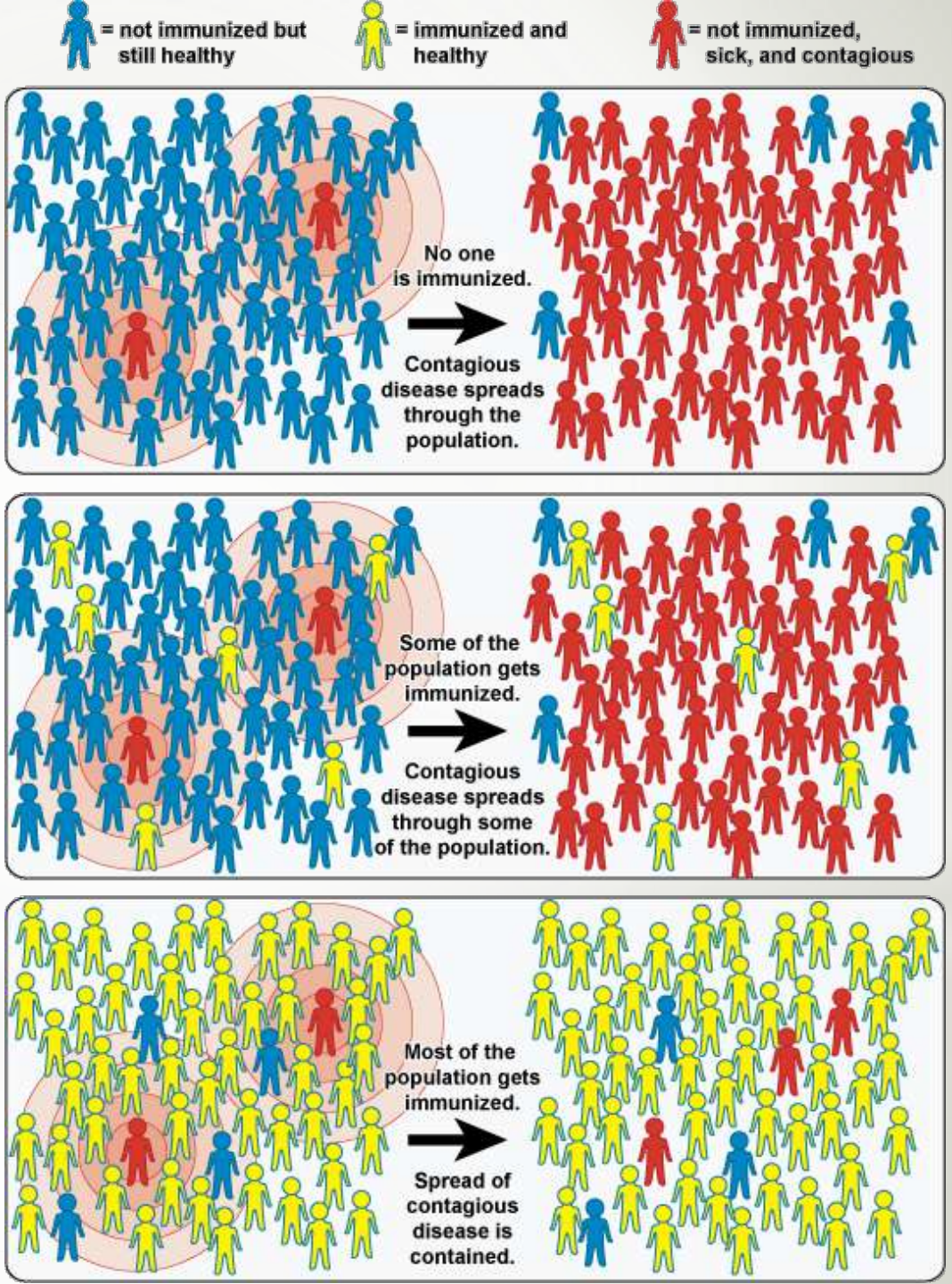
Recent increase in number of infections

III. Poliovirus -

Oral live vaccines

Virtually abolished polio

Herd Immunity



Source: niaid.nih.gov

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

Examples of therapeutics

Compound	Mode of action	Susceptible viruses
Amantadine	Blocks the M2 proton channel	Type A influenza viruses
Alpha, beta interferrons	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