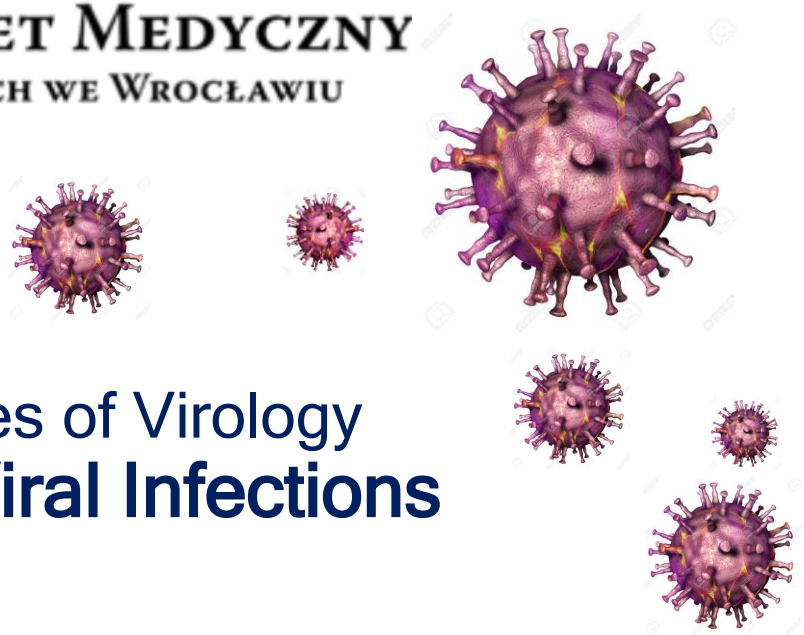
UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU



Subject: Faculty Lectures of Virology
Topic: Diagnosis of Viral Infections

Academic Year 2024/2025

Faculty: Medicine
Field of study: Virology
Level of study (uniform MA)
Form of study (full time)
Year of study: III

Academic title/professional title: professor
Name, last name of the lecturer: Beata Sobieszczańska
Position of person conducting classes: teacher
Wroclaw Medical University

The appropriate management of the patient depends on the knowledge that follows from the diagnosis

General techniques used in the diagnostics of viral diseases:

1) Microscopy
2) Culture
3) Serology
4) Molecular techniques

METHOD	ADNATAGES	DISADAVTAGES
Virus isolation Culture	Produces further material for study of agent	Slow, time-consuming, expensive, difficult
	Usually highly sensitive	Critical cell type or animal selection
		Useless for non-viable virus or non-cultivable agents
Direct observation (electron microscope)	Rapid	Relatively insensitive
	Detects uncultivable viruses	Cumbersome for large number of samples
	Detects non-viable viruses	Limited to a few infections
Detection of viral genomes (NAATs)	Rapid, sensitive	Too high sensitivity (detection of non-related infections)
	Potentially applicable to all viruses (including uncultivable)	Risk of DNA contamination
	Reagents for new viruses easily made	Needs good quality control
	Good quantitation of load	Targeted to a specific agent

METHOD	ADNATAGES	DISADAVTAGES
Serology	Rapid and sensitive	Not applicable to all viruses
	Readily available (diagnostic kits)	Not as sensitive as NAATs
		Targeted at the a specific virus
Antibody seroconversion (acute & convalescent sera)	Useful if appropriate samples for direct detection cannot be obtained or to exclude a particular infection retrospectively	Slow, late (retrospective) Interpretation can be difficult Targeted to a specific agent
IgM serology	Rapid	False positives may occur Targeted at the specific agent

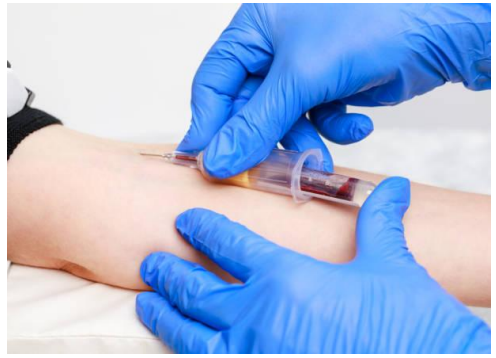
Specimen selection & collection:

The right samples, at the right time, and proper transport to the lab.

The selection of an appropriate sample from the patient depends on:

- Specific syndrome or disease
- Viral etiology suspected

Confusing & complicated in some cases



Sampling and specimen collection are always essential factors

Specimens should be collected as early as possible following the onset of the disease

DISEASE / SYNDROME	SPECIMEN
RESPIRATORY	Nasal or throat swab; nasopharyngeal aspirate; sputum tracheal aspirates, bronchoalveolar lavage fluid, and serum
ENTERIC	Feces
GENITAL	Genital swab, urine
EYE	Conjunctival (and/or) corneal swab
SKIN	Vesicle fluid/scrapings, biopsy, solid lesion
CNS	CSF, feces (enteroviruses)
GENERALIZED	Throat swab, feces, blood, and leukocytes
AUTOPSY	Relevant organ
ANY	Blood for serology

Specimen transport & storage

Specimens:

- should not be allowed to sit at a room or higher temperature
- should be placed in ice & transported to the lab immediately
- in case of delay – they should be refrigerated but not frozen

Storage and transport:

- specimens which need to be held for days before processing:
  - storage up to 5 days: 4^0C
  - storage for 6 or more days – 20^0C or preferably – 70^0C

Before freezing – dilution or emulsification in a particular transport medium

Specimen transport & storage

Special transport media include:

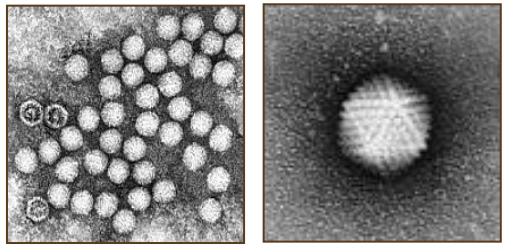
- protein (serum, albumin, gelatin) to stabilize the virus
- antimicrobials (penicillin, streptomycin) to prevent overgrowth of bacteria & fungi

Diagnostic tests

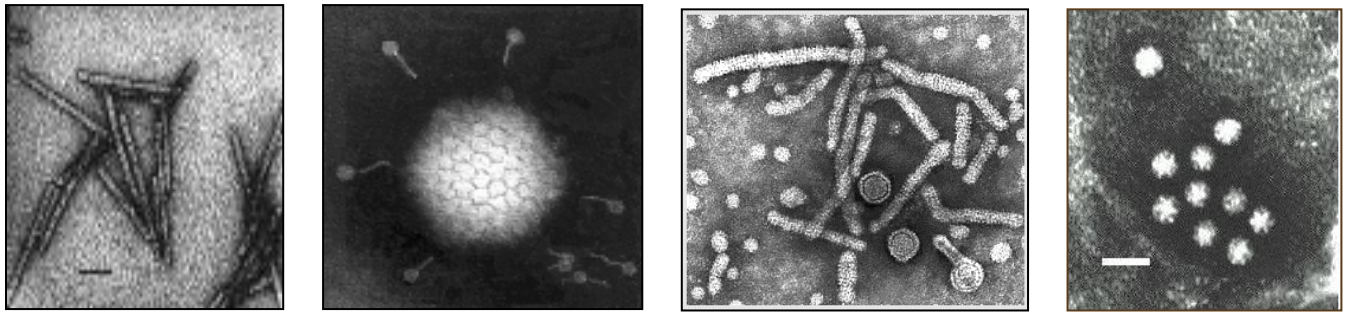
Direct detection

Indirect detection

Direct detection of the virus



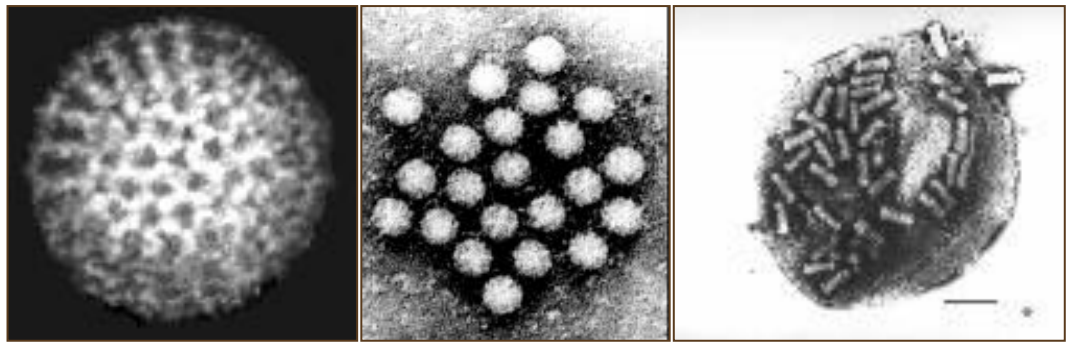
Electron Microscopy – virus visualization



Electron Microscopy (EM)

- Labor, expensive, and insensitive
- Most helpful for the detection of unculturable viruses
- Viruses are detected & identified based on morphology
- Magnification ca. 50,000 – 100,000 times

EM depends on there being a large number of virus particles (at least 10^5-10^6 per gram) present in the sample

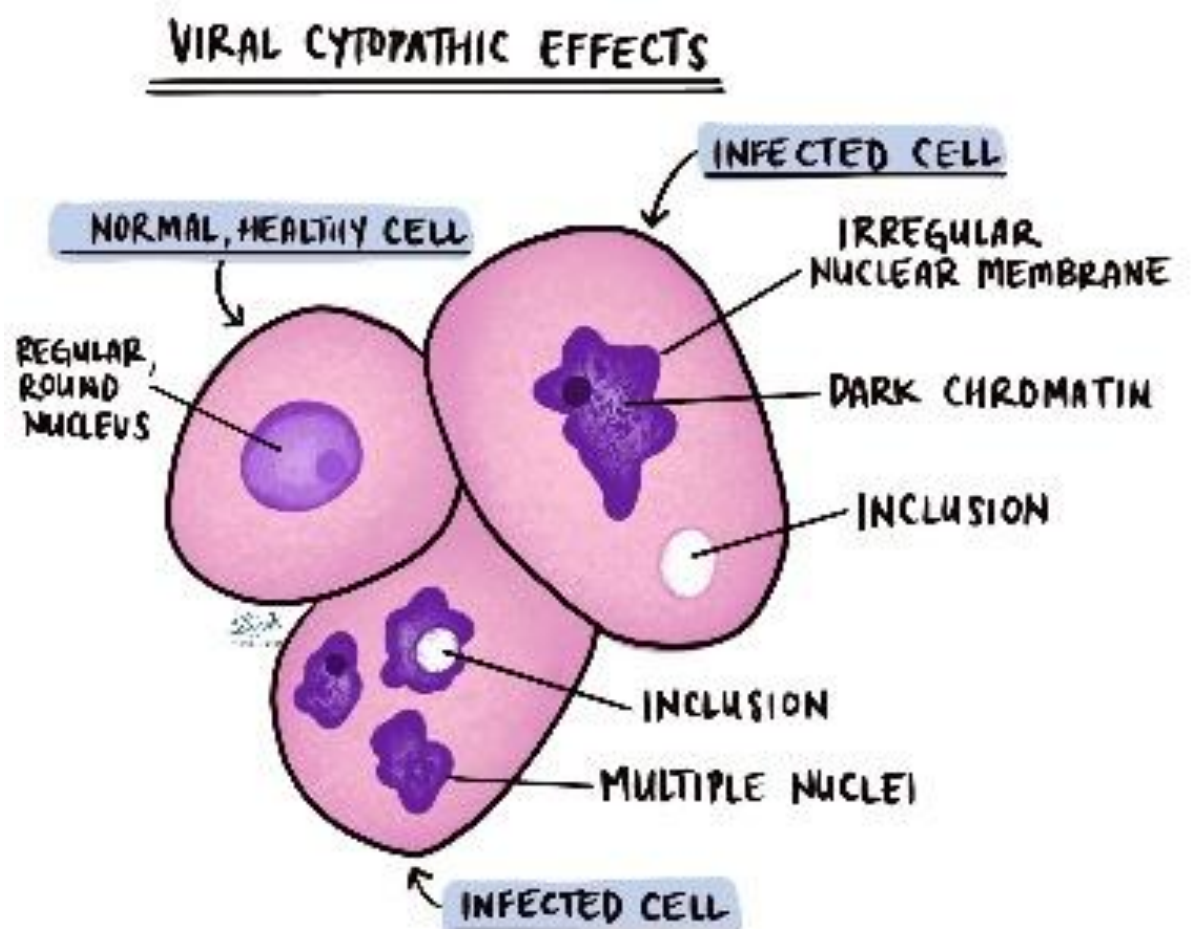


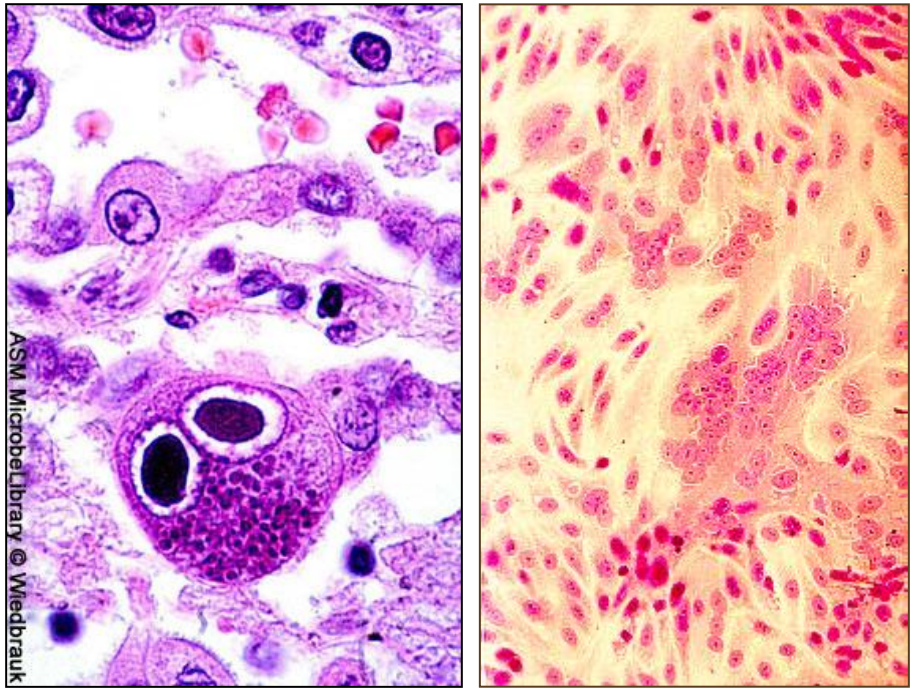
Indirect detection of the virus

Light microscopy:

cytology & histology

The most readily available technique for the detection of a virus





Virus inclusions (inclusion bodies) = histological changes in infected cells = collections of replicating virus particles either in the nucleus or the cytoplasm

CPE = cytopathic effect

Culture of the virus in vitro on cells

Types of cell cultures

- Primary cells - essentially normal cells obtained from freshly killed adult animals
- Semi-continuous cells - cells taken from embryonic tissue
- Continuous cells - immortalized cells, i.e., tumor cell lines that can be cultured indefinitely

More convenient and cost-effective than embryos and animals

The gold standard for virus isolation and identification

Advantages:

Virus propagation (for research)

Virus identification

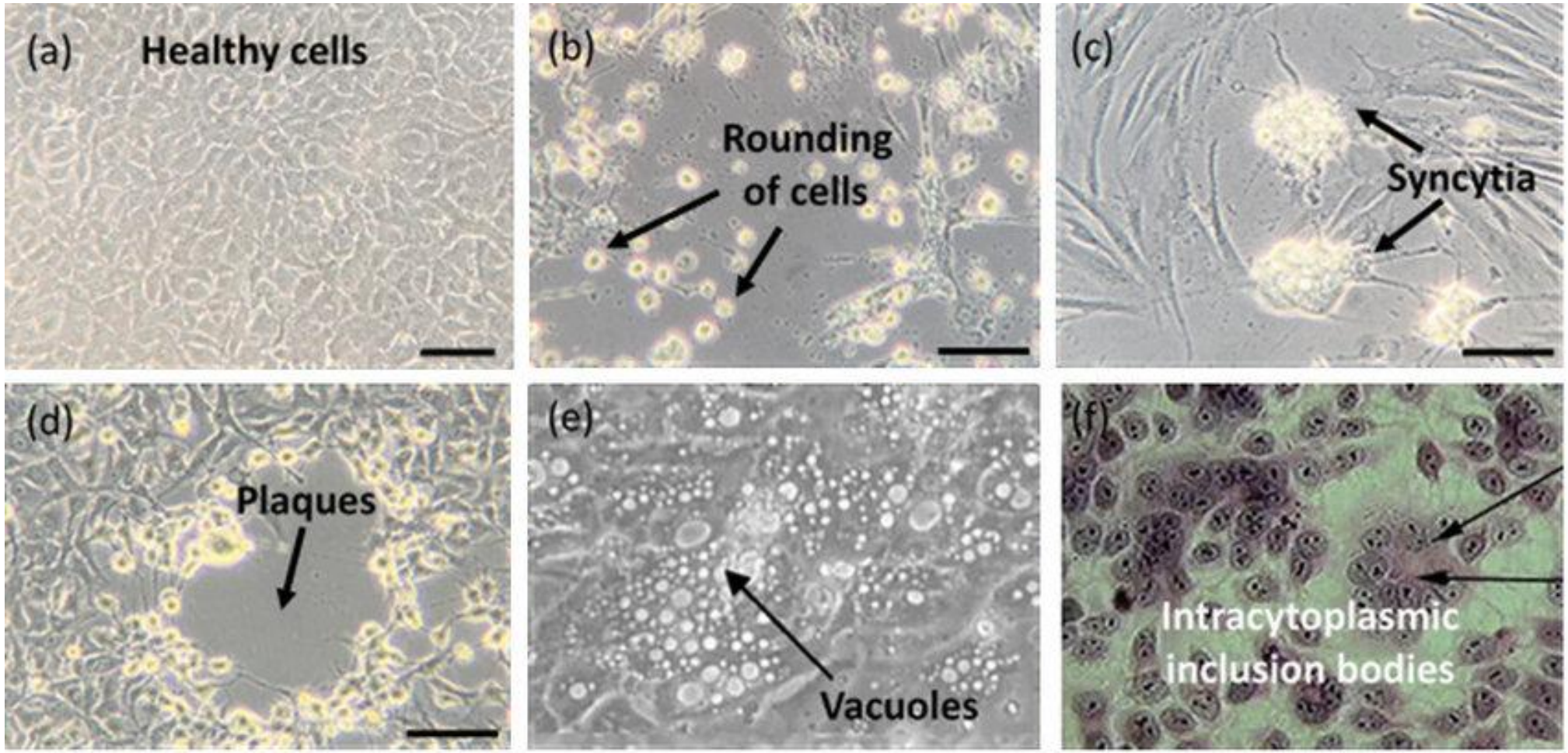
Culture of the virus in vitro on cells

Disadvantages:

Not all viruses produce a cytopathic effect

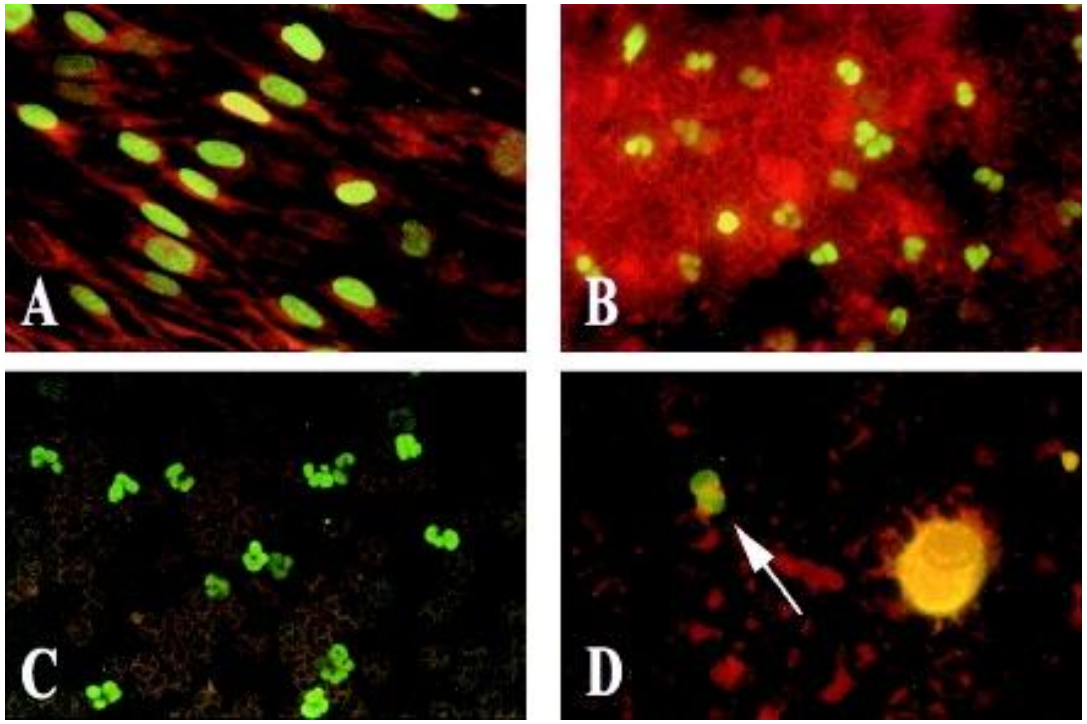
Often time-consuming

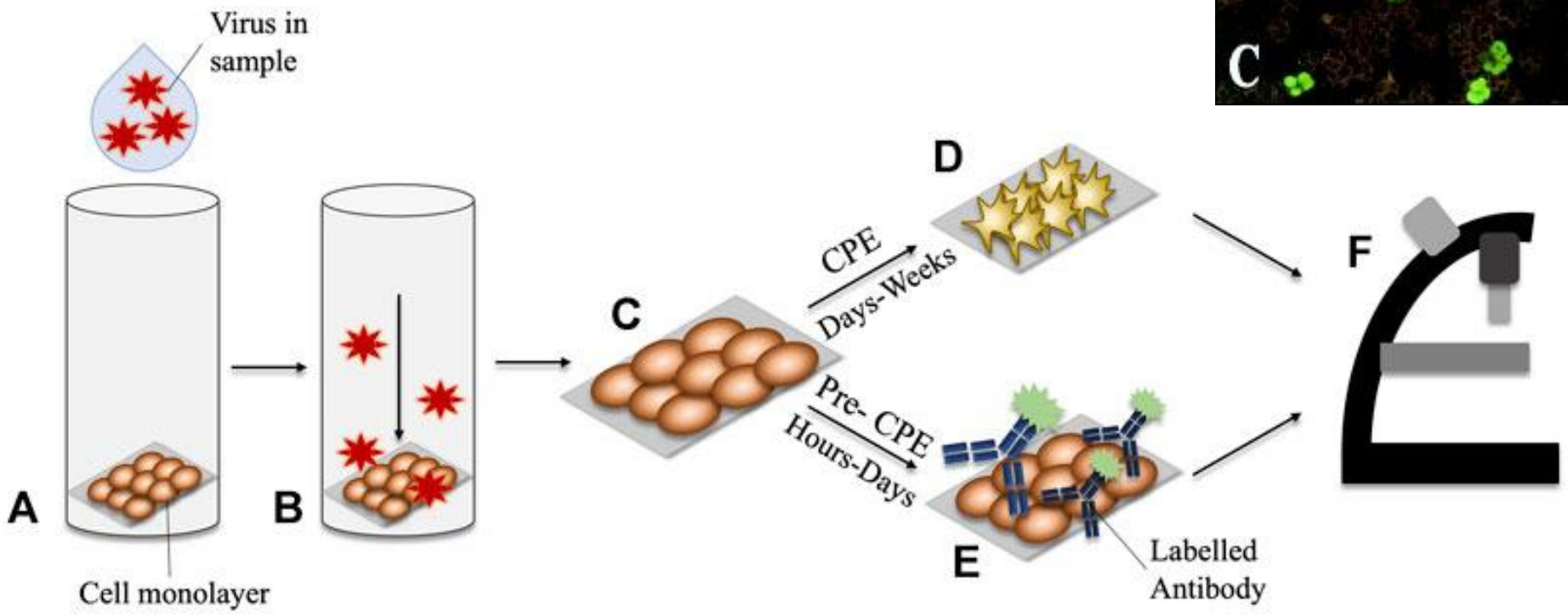
Require highly trained staff in specialised laboratories

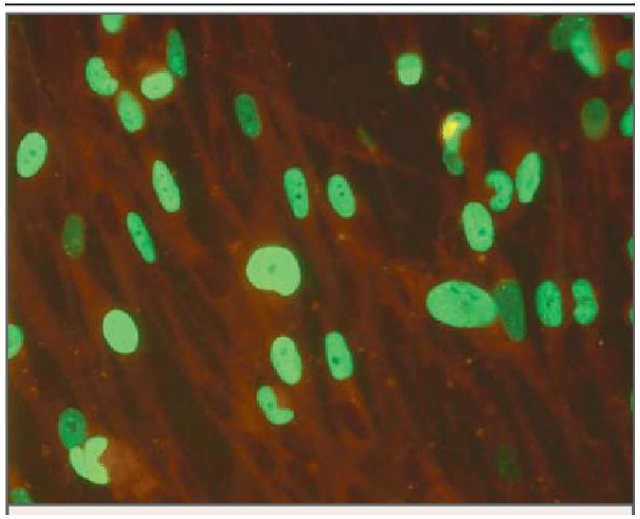


Shell vial culture = modified culture

Allows for more rapid isolation of viruses







Shell vial (task 4)-rapid cultivation – in life threating

+ sample (CSF)

centrifugation

1 d cultivation
t 37 °C

Remove of a cover glass, stick to under glass

TC on small glass in glass test-tube

Indirect IF (CMV)

Direct IF (Herpes 1,2)

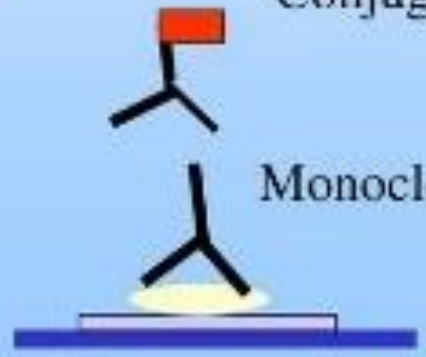
Sample is shining

a.b. marked with an fluorescence colour - conjugate

cover glass + Ag under glass

Conjugate

Monoclonal a.b.



Culture of the virus

The gold standard for virus isolation and identification

- Laboratory animals
- Chicken embryos

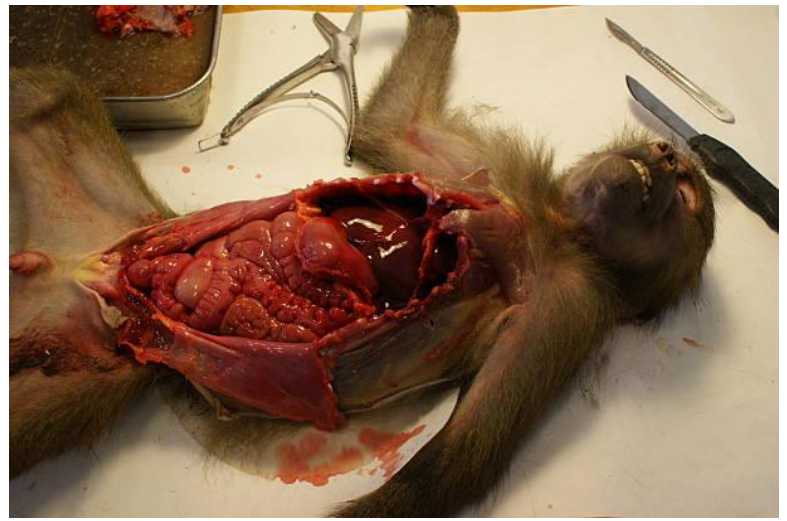
Time-consuming, cumbersome,

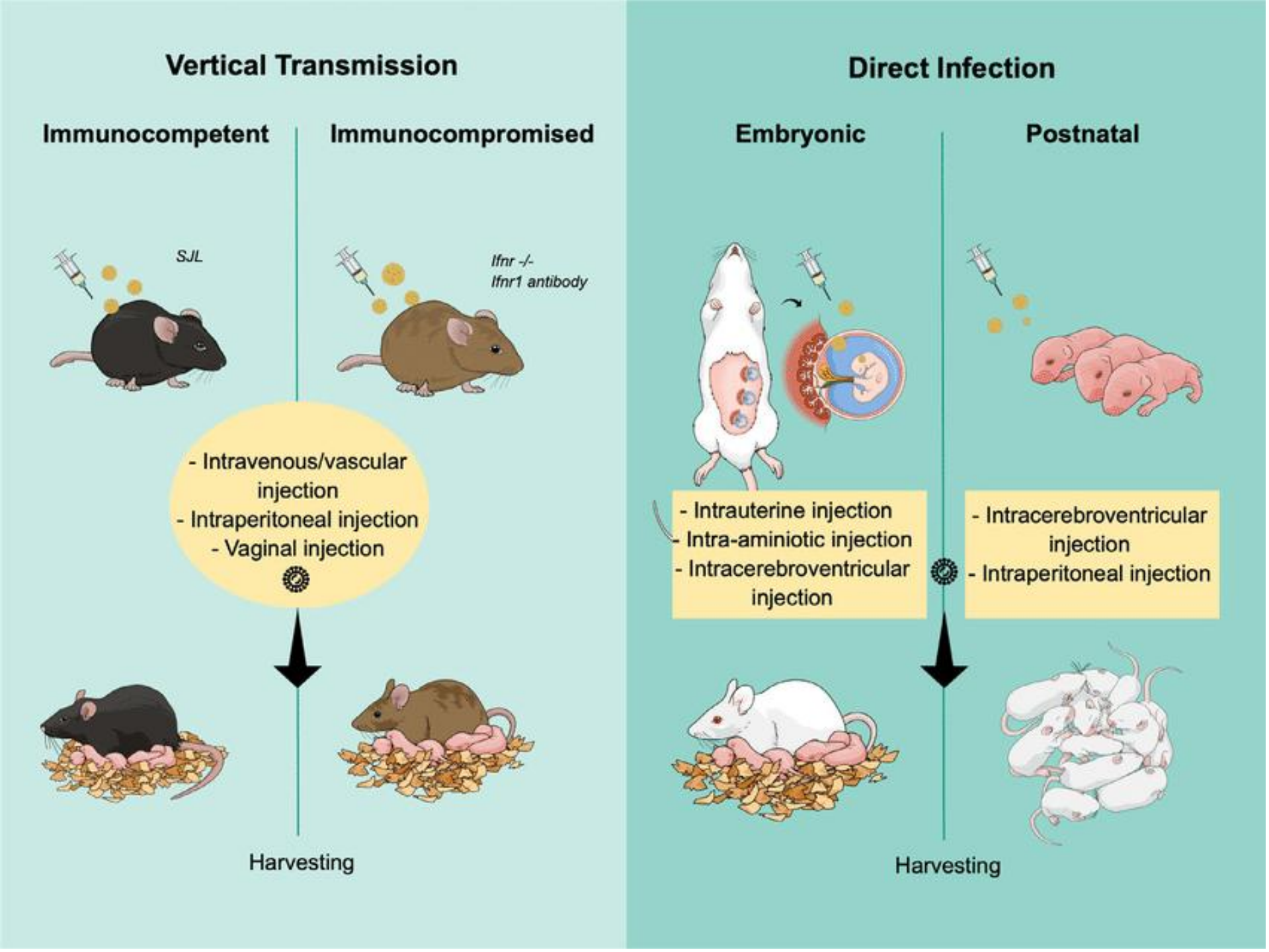
Expensive (special conditions, laboratories)

Moral problems

Animals – host organism

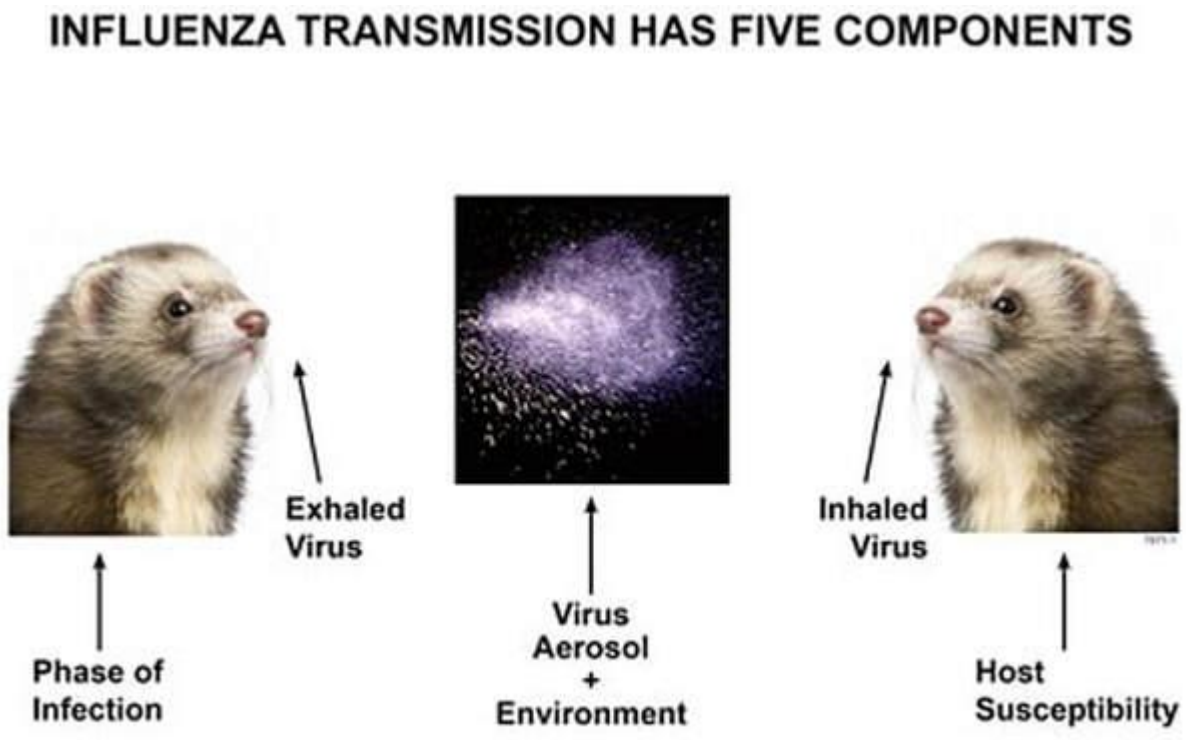
Pathogenesis, clinical symptoms, antibody production, specimens for diagnosis, treatment = all our knowledge about viruses





ZIKA virus pathomechanism study

The selection of the animal species for the type of virus is important



Ferrets are the optimal animal model for studying flu viruses:

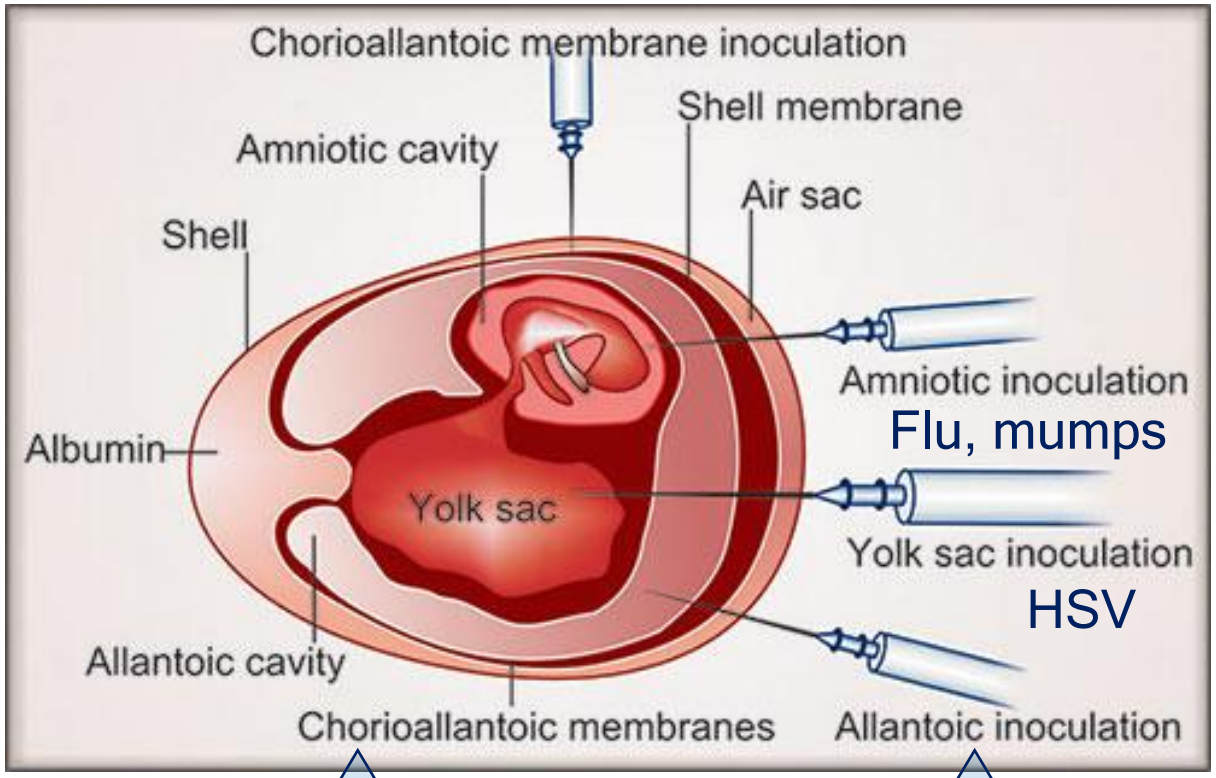
- Highly susceptible to human flu viruses
- Exhibit many of the clinical symptoms observed in humans (fever, listlessness, and nasal discharge)
- They transmit the virus efficiently among themselves

Animals often need to be genetically adapted to be infected with a given virus, e.g., via inserting specific genes

Culture of the virus on embryonated eggs

Economical, convenient as chick embryos contain several different types of cells in which various viruses will undergo replication

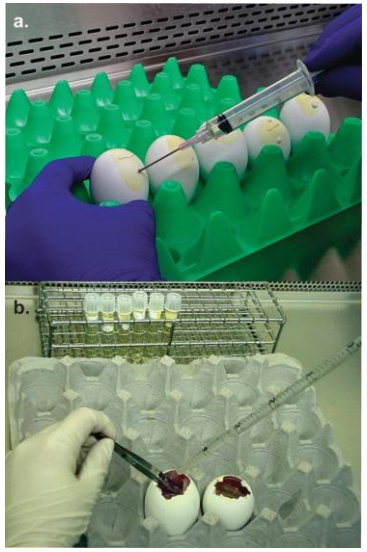
The yolk sac is generally an ideal medium for the growth of viruses

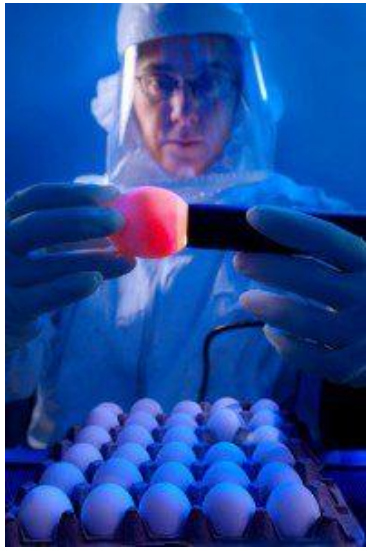


Influenza, HSV poxviruses

Influenza, mumps, adenoviruses

Fertile chicken eggs incubated for 5 to 12 days are aseptically inoculated with virus particles through the shell, and the opening is sealed with paraffin wax



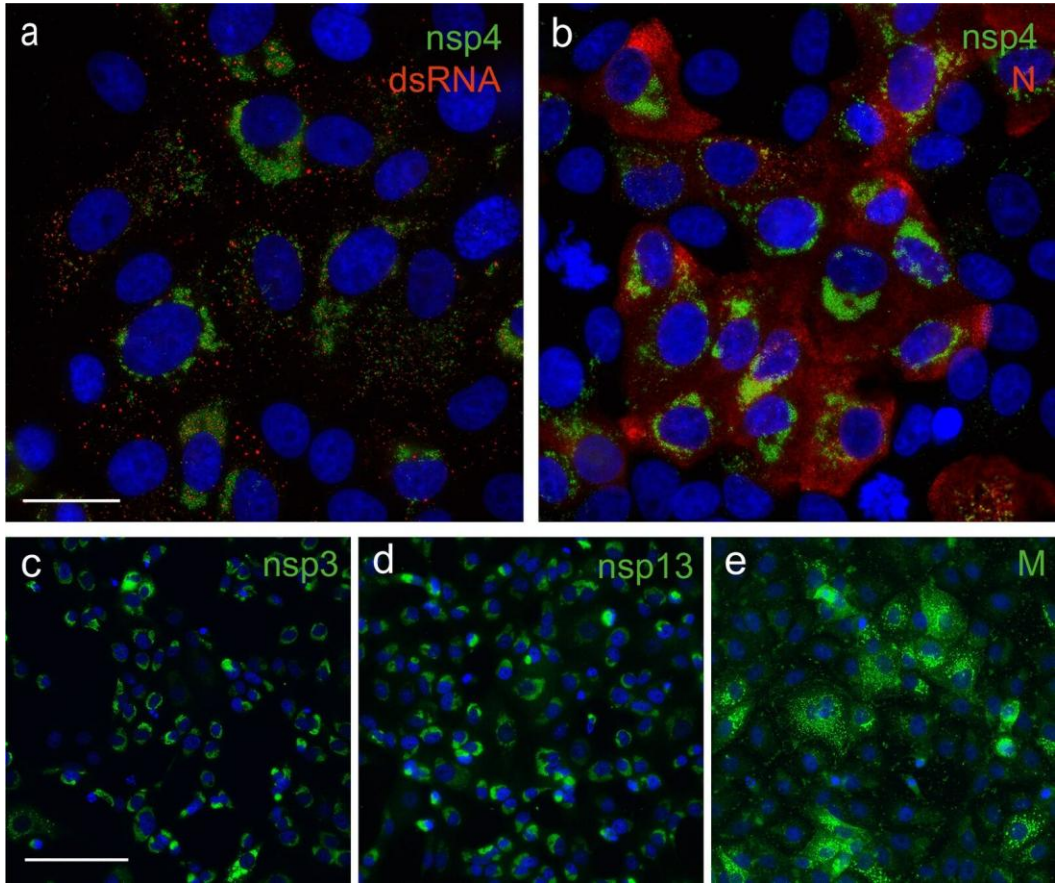


Direct virus detection

Antigen detection

Examples: immunofluorescence testing of Cov2 infection

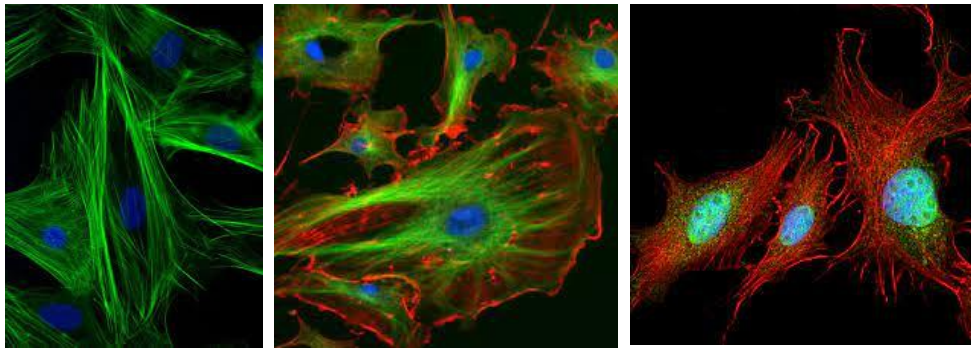
Specific fluorochrome dye-labeled antibodies react with virus antigens present in cells



Requires immunofluorescence (confocal) microscope

Advantages: rapid – results in a few hours (scan)

Disadvantages: tedious, time-consuming, difficult to interpret, poor sensitivity & specificity



Direct detection of the virus

Rapid methods for detecting viruses are based on immunological or molecular techniques

Viral genome detection

Molecular techniques

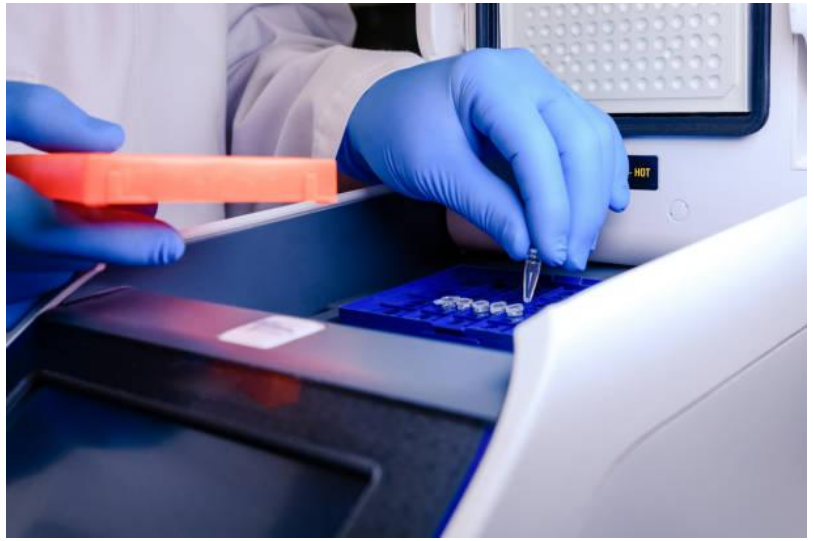
- PCR, RT-PCR, LCR
- Nucleic acid-based amplification
- Genotyping
- Quantification

Advantages

Rapid

High sensitivity and reproducibility

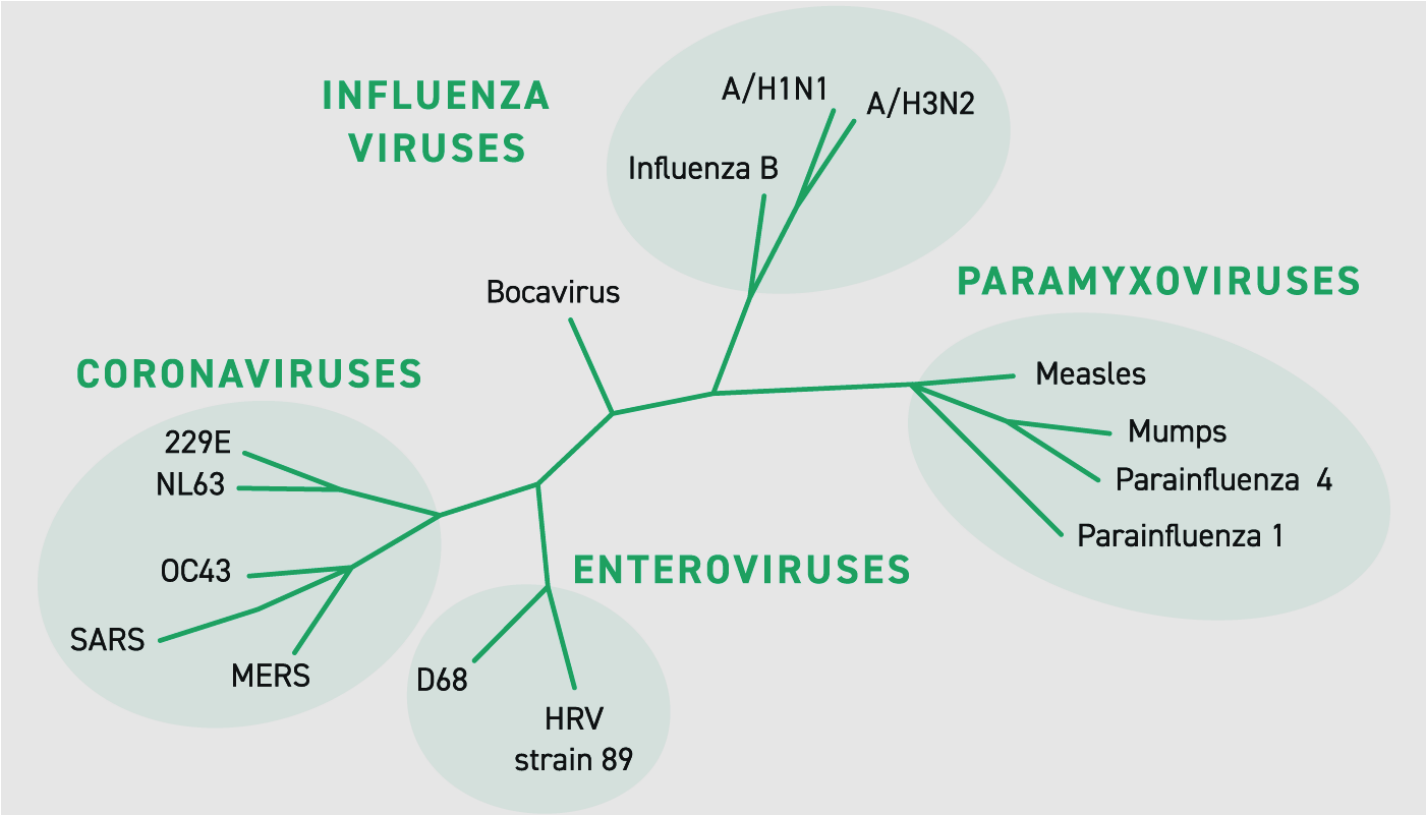
Detection of unculturable viruses



Requires specific equipment

Molecular methods also enable the search for many different viruses at the same time in one sample

Virus Research Panel



Comprehensive Detection of Respiratory Pathogens

Designed to target multiple viruses in a single capture

Targeted against the reference sequences for 29 common human respiratory viruses

Point-of-care testing (POCT) = medical testing done near the patient (outside a traditional laboratory setting) to provide **rapid results for immediate clinical decision-making**

Advantages:

- Faster diagnosis and treatment – superior in terms of both sensitivity and specificity to other tests
- Improved patient management (particularly in settings with limited access to advanced diagnostic facilities)
- Potentially reduced costs

Disadvantages:

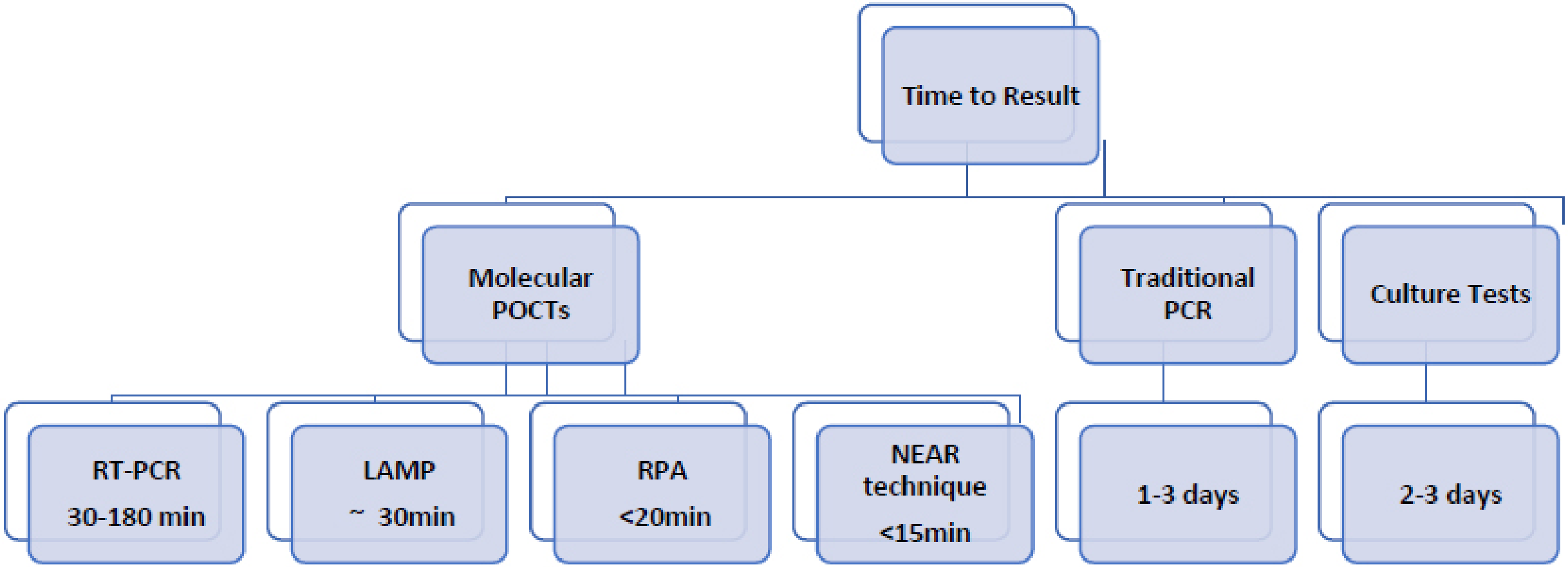
Correct procedures and quality control are crucial to ensure accurate and reliable results

Molecular POCTs are based on:

Nucleic acid amplification technique (NAATs),
Recombinase polymerase amplification (RPA),
Loop-mediated isothermal amplification (LAMP),
Iso-thermal Molecular Nicking Enzyme Amplification Reaction (NEAR)

Benefits of POC Testing: Rapid results enabling timely clinical decisions

a reduction in antibiotic prescriptions and ancillary investigations



User-friendly, cost-effective, and can be performed at the bedside, yielding results within minutes

(a)

Test basis

Overdrachts-cassette

Monsteront-vanger

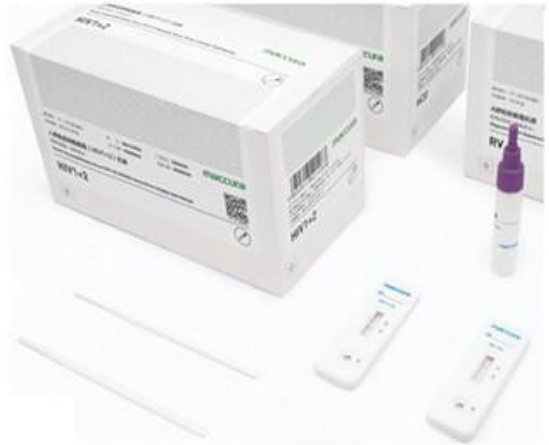
Wattenstaafje voor neus

(b)

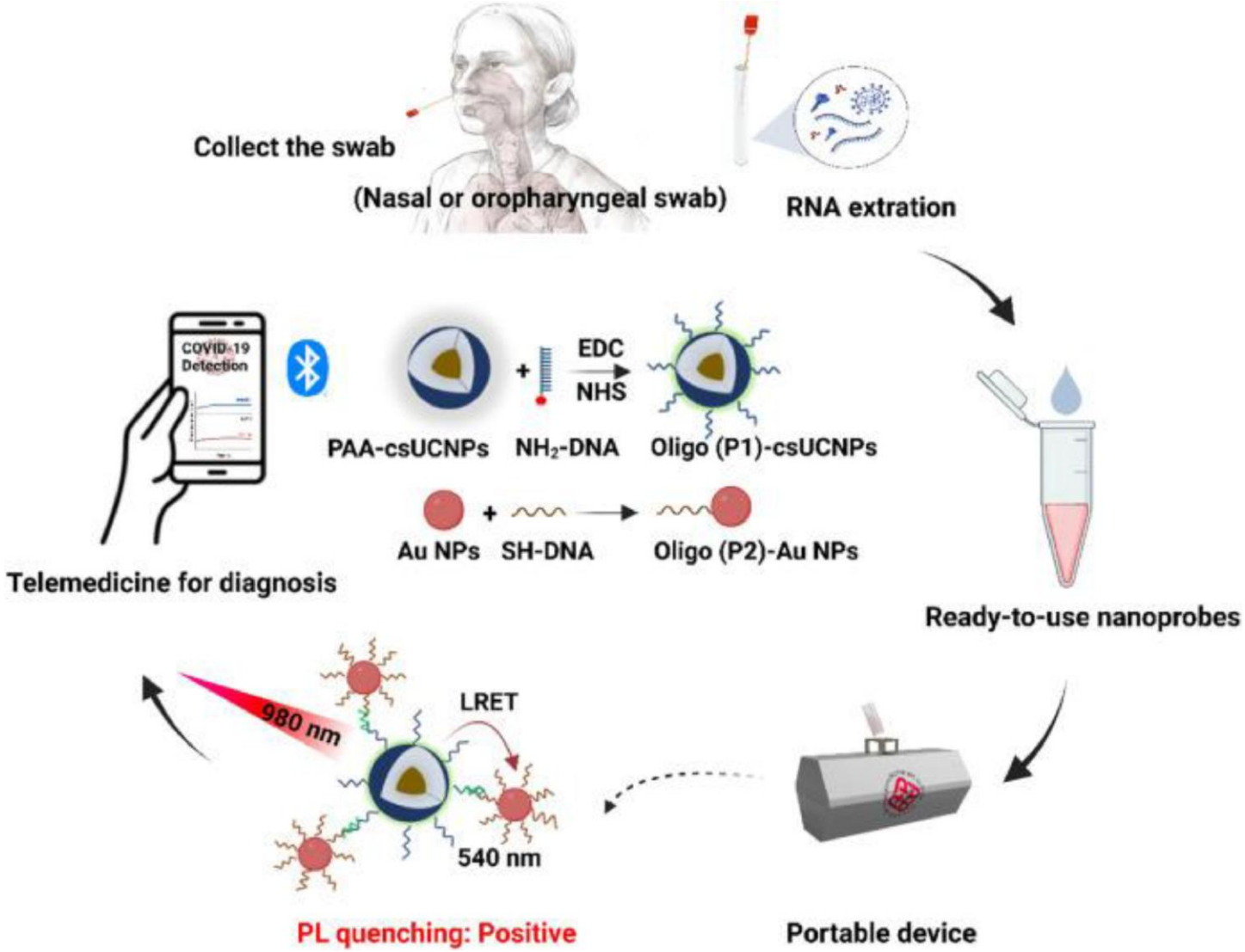
① Sample Capillary ② Air channel ③ Reactor Chamber
④ Buffer Reservoir ⑤ Control Window ⑥ Cartridge Cap

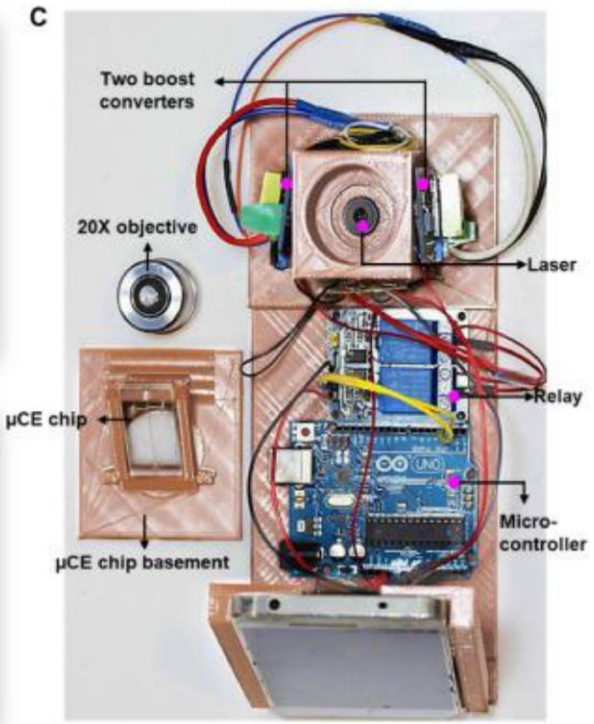
(c)

(d)



Smartphone-based point-of-care testing of the SARS-CoV-2

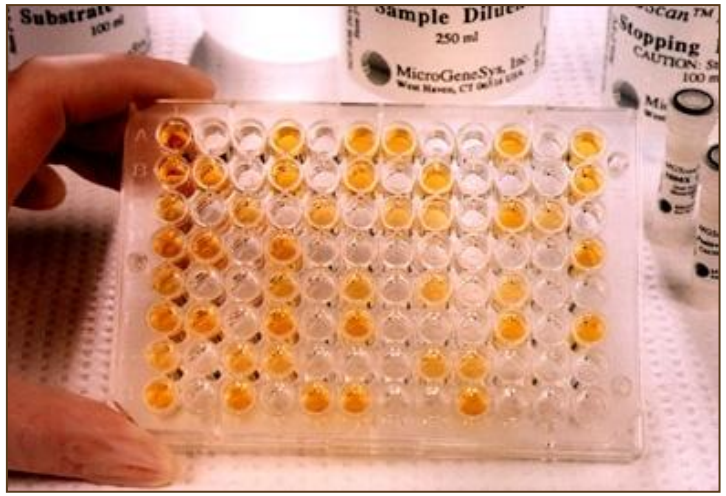


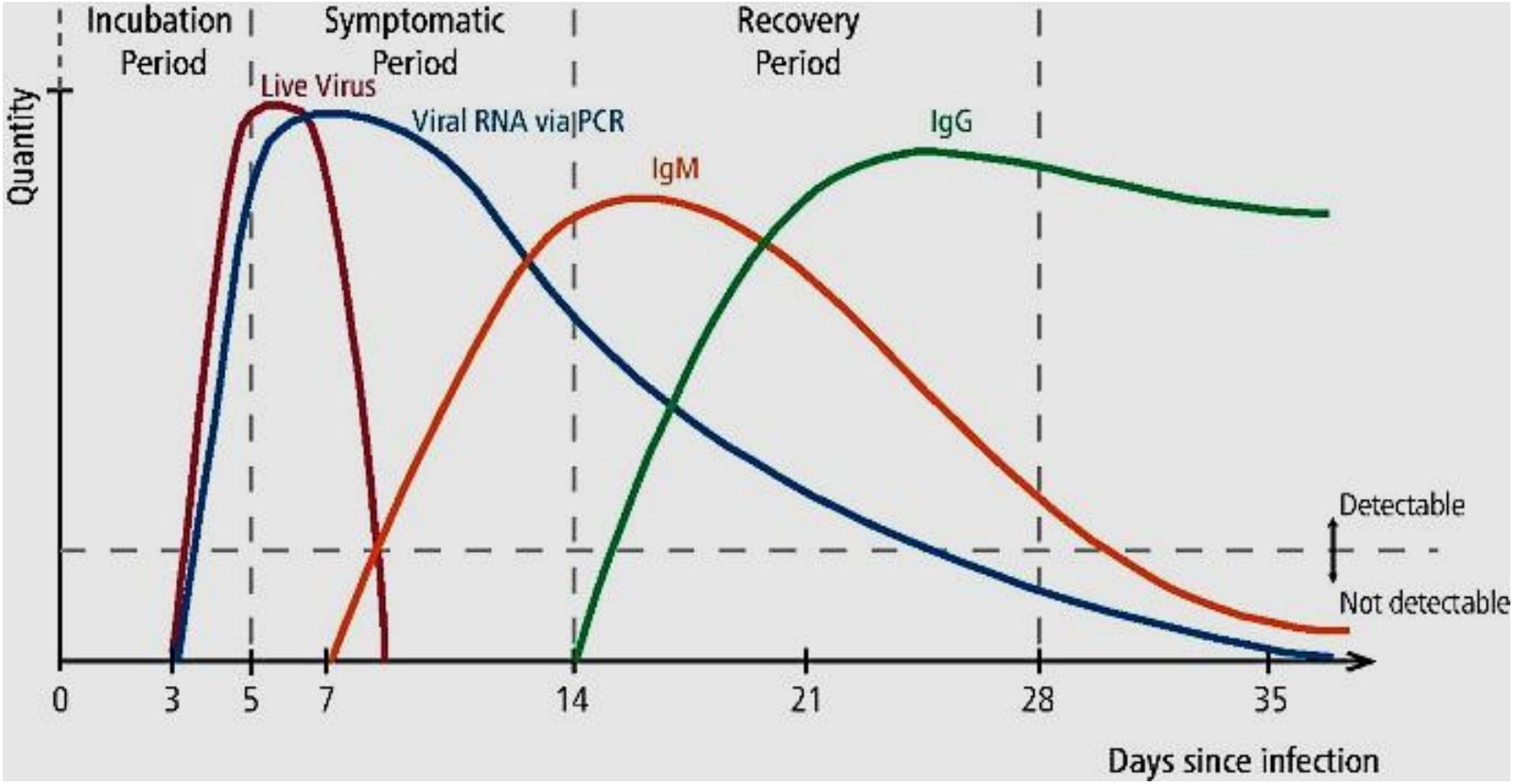


Indirect detection of the virus

Serology (host antibody detection)

Detection of rising titers of antibodies between acute and convalescent stages of infection, or the detection of IgM in primary infection





Questions

1) Which laboratory techniques allow the etiology of a viral infection to be determined as quickly as possible?
2) What techniques are POCTs based on?
3) List three different techniques that enable direct detection of the virus in patient samples.
4) When does serology clearly indicate infection with a given virus?
5) How can a light microscope be used to diagnose viral infections?
6) What is the best model to study the pathomechanisms of viral infections?
7) What is the use of EM in virological diagnostics?

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