S.I.TRA.C.
SOCIETÀ ITALIANA TRAPIANTO DI CORNEA
E SUPERFICIE OCULARE

LA DIAGNOSTICA COLTURALE DI LABORATORIO DELL'HSV NELLE CORNEE PER TRAPIANTO

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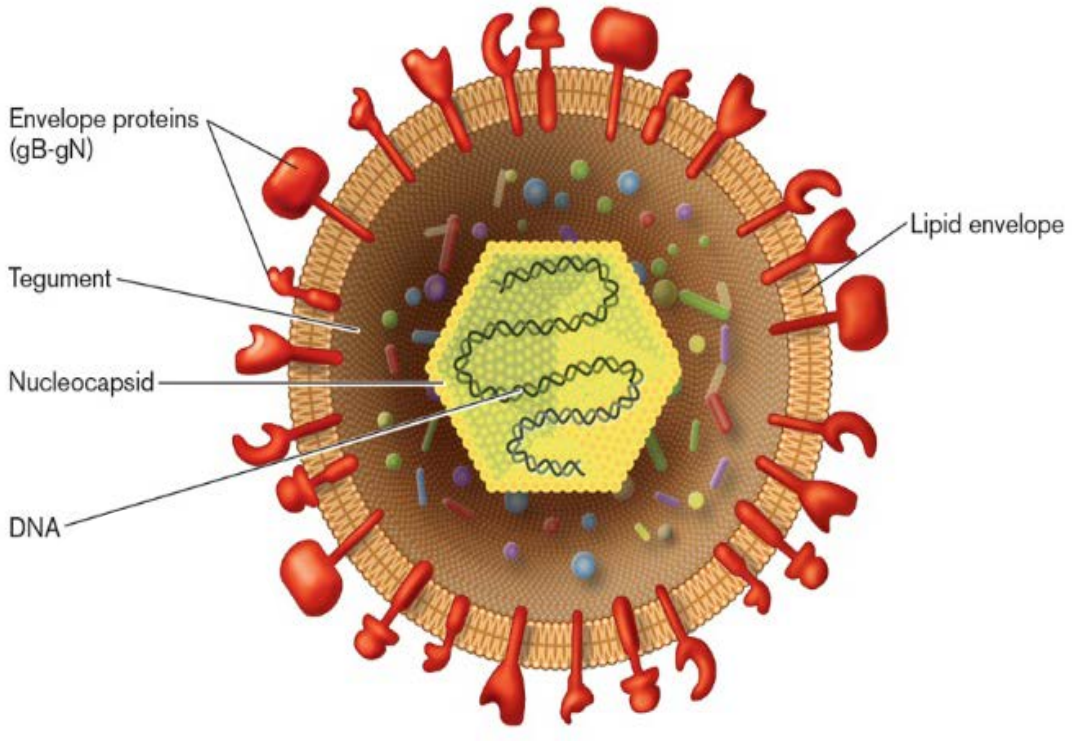
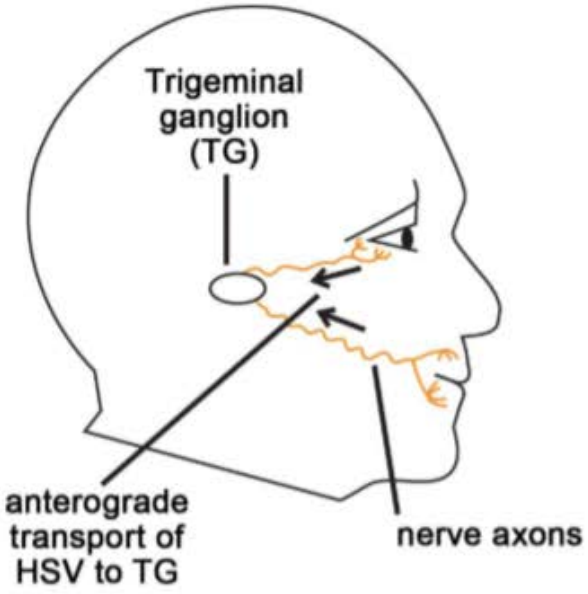
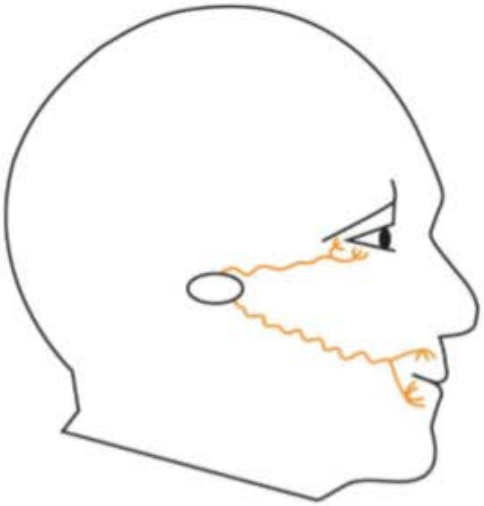
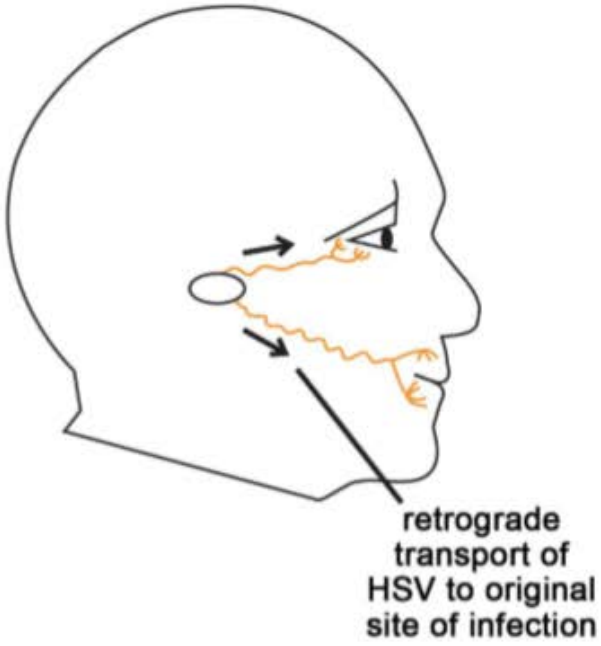
HERPESVIRUS



- DNA: Double strand, 120-250 Kbp, 30 nm diameter, cylindrical structure
- Capsid: 162 capsomers, 150 exons, 12 pentons, cable capsomers with 4 nm diameter channel
- Envelope: sensitive to lipid solvents, innermost layer tegument, virion diameter 150-200 nm







Acute

- primary infection at either lip or eye
- virus replicates locally in epithelium
- virus travels to TG and establishes a latent infection

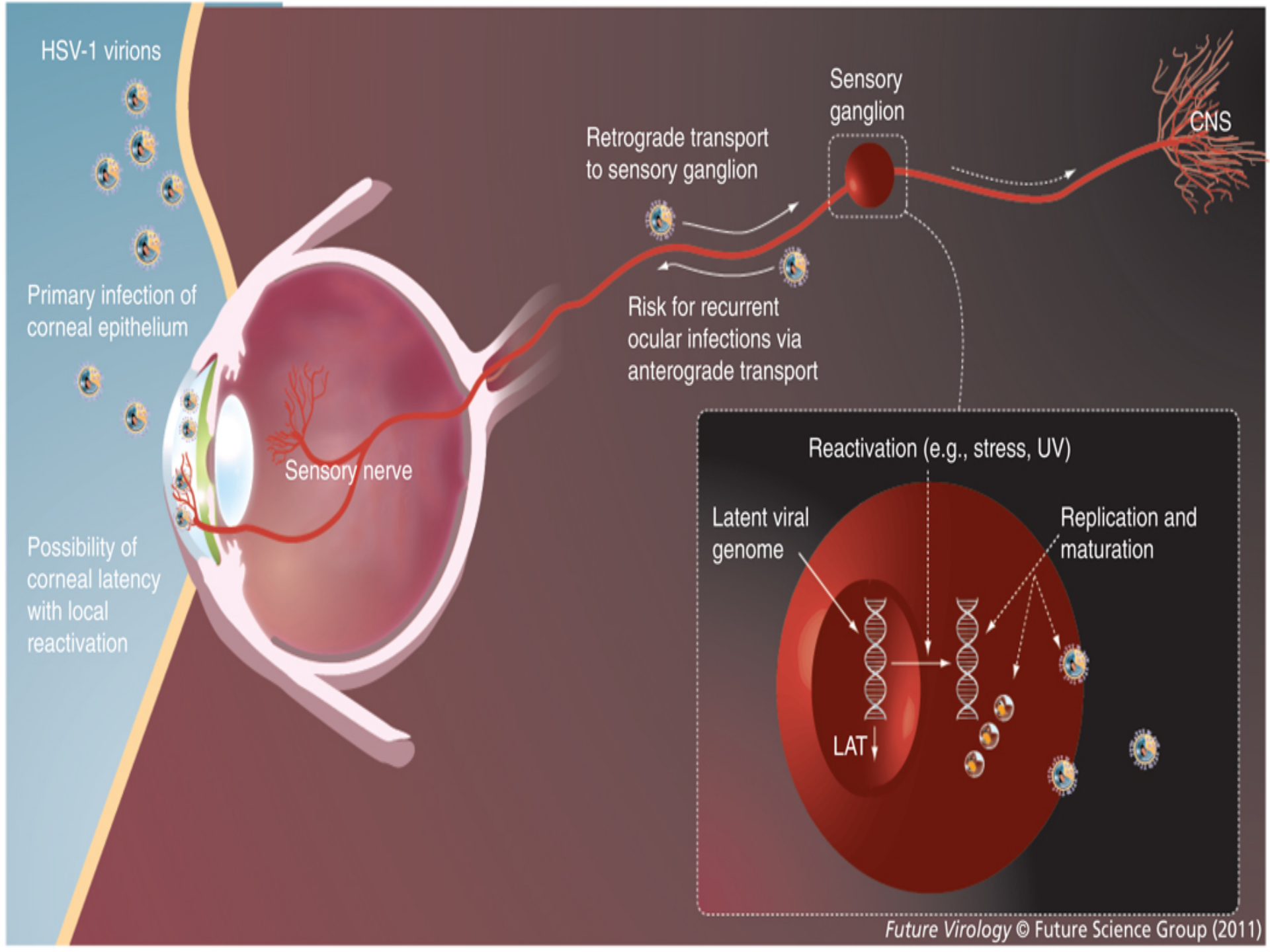
Latent

- no infectious virus detected in TG or at the primary site of infection
- viral gene expression suppressed

Reactivation

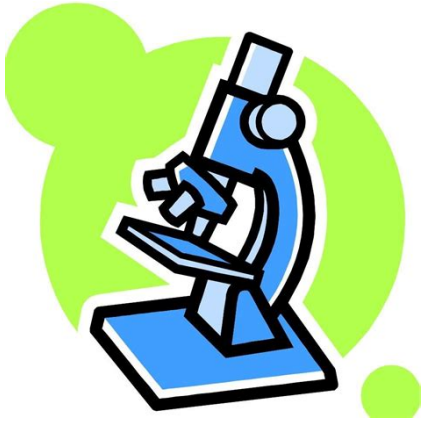
- virus production in infected neurons
- retrograde transport to site of infection
- presence of clinical lesions and/or virus at original site of infection

- Ocular infection is related to latency in the trigeminal ganglion, which provides sensory innervation to the cornea.
- New scientific data suggest that the cornea itself is a site of HSV-1 latency.



HERPES SIMPLEX: LABORATORY DIAGNOSIS

- Viral isolation
- Viral DNA Research (PCR)
- Antibody research in serology



VIRAL CULTURE

Virus = obligate endocellular parasites

↓

growth on living cells

↓

LABORATORY ANIMALS

EMBRYANED EGG

CELL CULTURES

- Embryo egg and laboratory animals are used only in special cases

Cell culture: the most used system in laboratories

- Samples must be collected early in the acute phase of infection
- Transport in a short time to the laboratory (the sample must arrive in ice)

For the growth of viruses different types of cell cultures are used:

- PRIMARY CELL CULTURES: directly from animal organs / tissues for shredding and enzymatic digestion. (1st -2nd passage). The most similar to the cells of origin

- DIPLOID SECONDARY CELL CULTURES: cells able to be kept in vitro for a high number of steps but with a finite life (max 50 steps)

- CELLULAR LINES: transformed cells (derived from tumors or transformed by viruses or chemical agents), heteroploids or aneuploids. Immortalized

- **Primary cultures of monkey kidney:**
  excellent for myxovirus, enterovirus, some adenovirus

- **Diploid cultures of human fetal fibroblasts (PEU, MRC-5):**
  for a broad spectrum of viruses (CMV, HSV, VZV, adenovirus, picornavirus)

- **HEp-2 cell line, human tumor epithelial cells:**
  excellent for RSV, adenovirus, HSV

VIRAL ISOLATION (CLASSICAL METHOD)

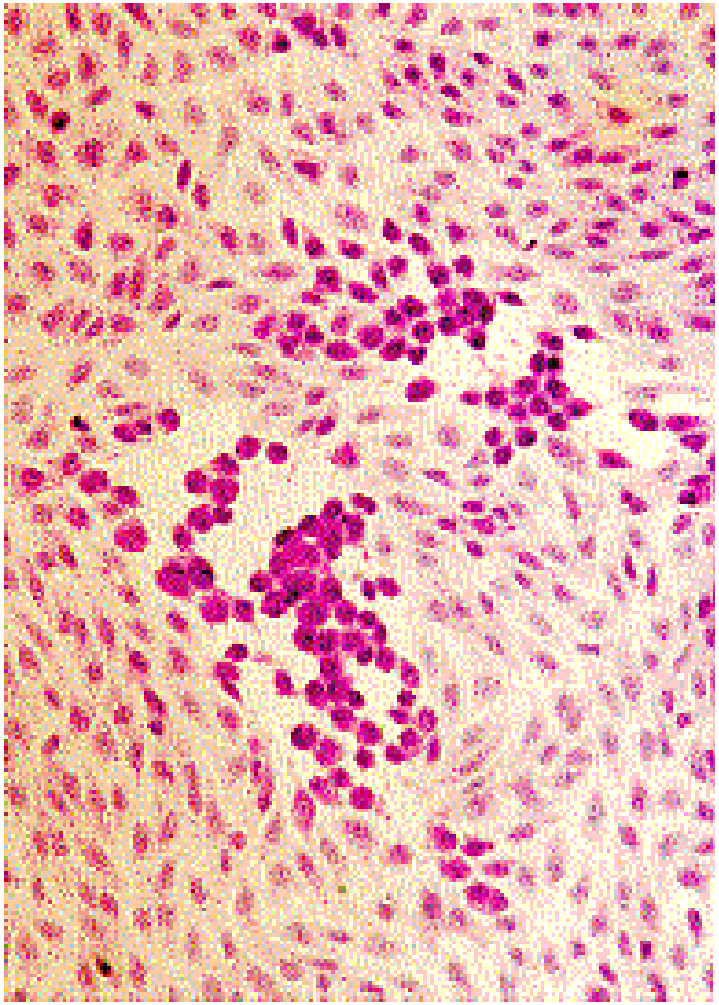
A virus can be revealed by observing the CYTOPATHIC EFFECT in culture.

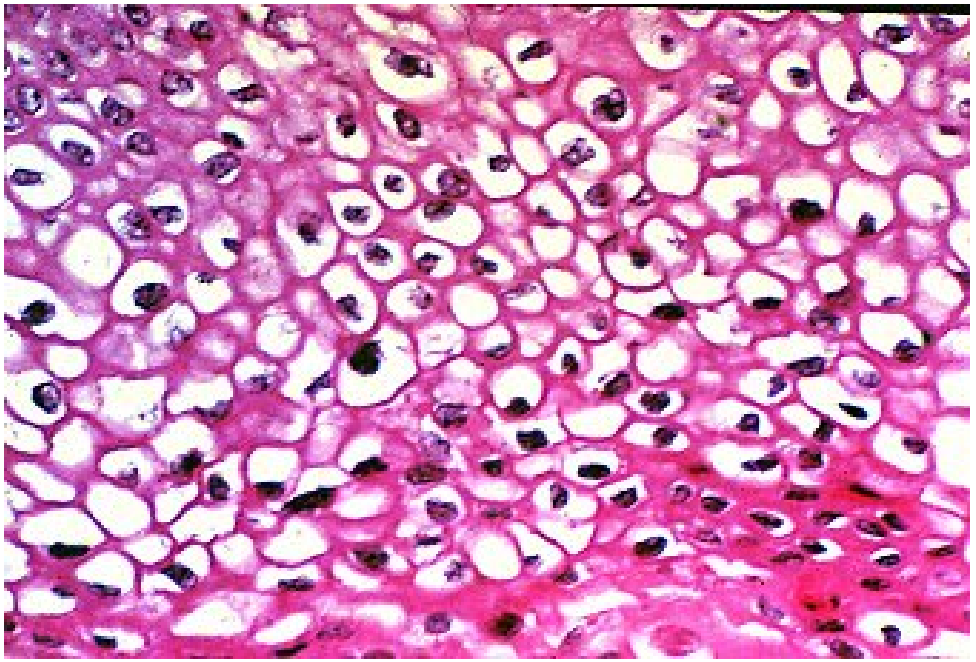
Cytopathic effect:

- Cell death (rounding, degeneration, aggregation, detachment)
- Characteristic histological changes (including nuclear or cytoplasmic bodies, chromatin thickening)
- Multinucleated giant cells (by fusion)
- Changes on the cell surface (viral Ag expression)

To appreciate ECP you have to wait 1-2 weeks (> for CMV)

HSV1: Cytopathic effect and included bodies



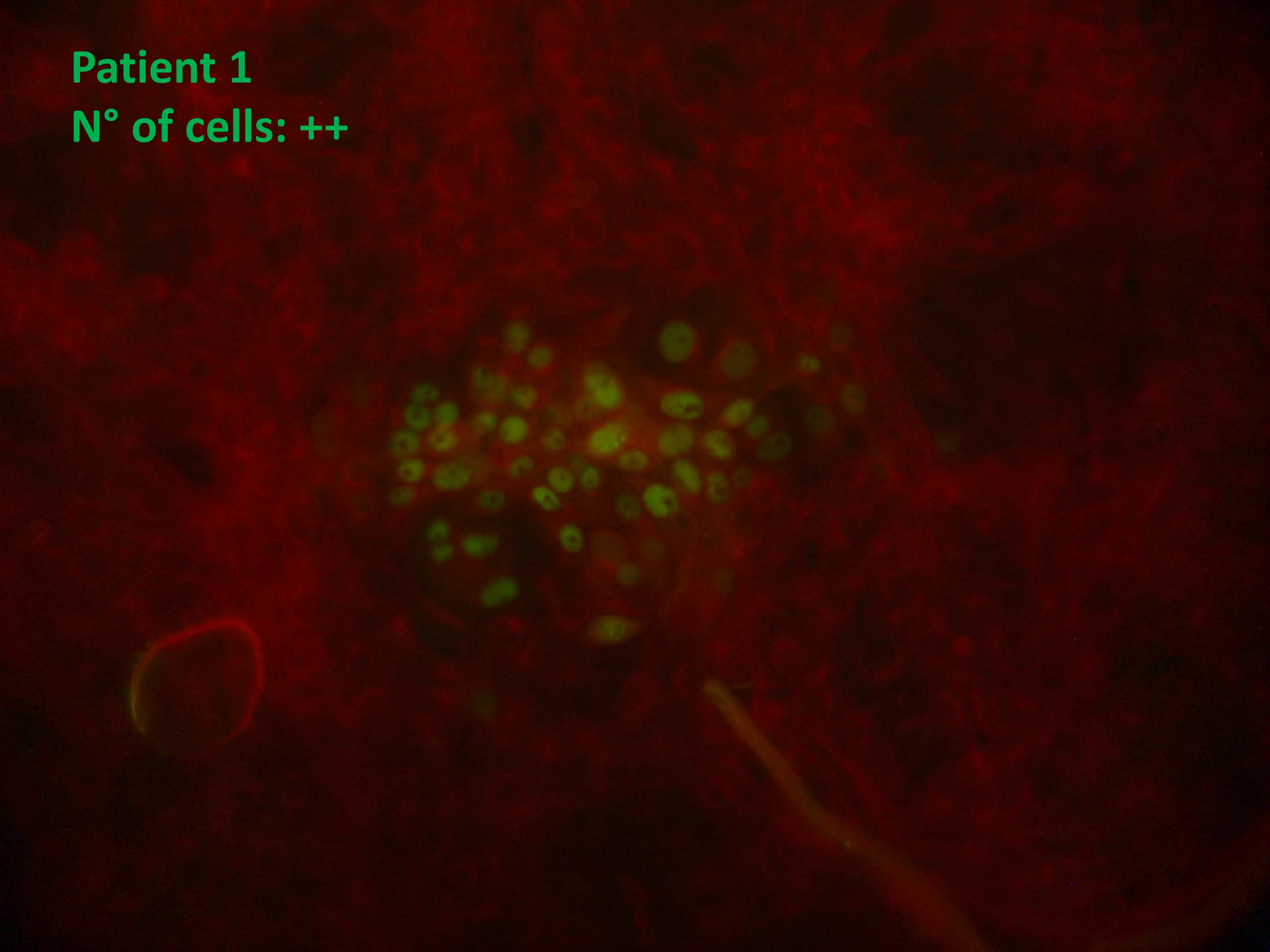


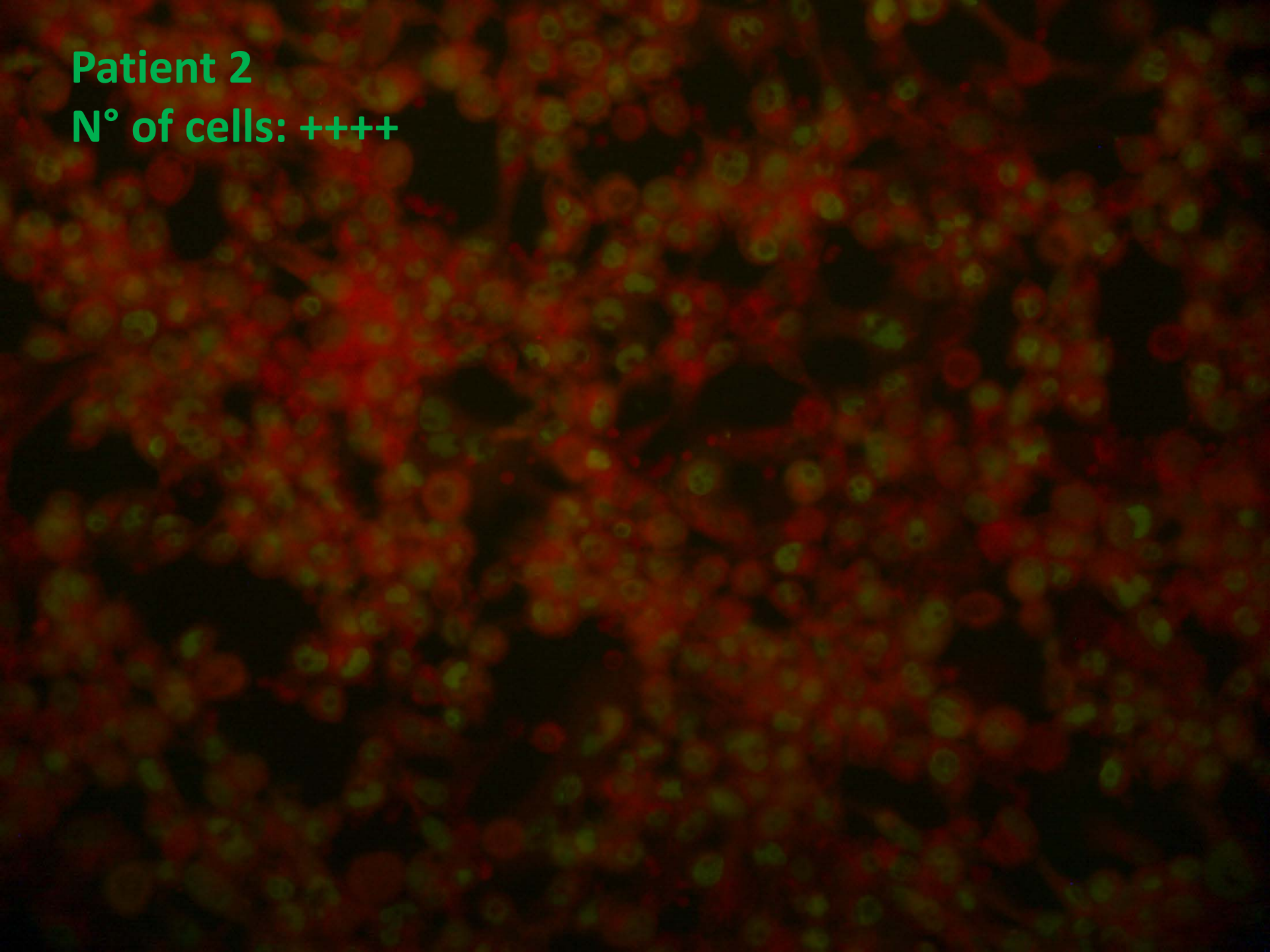
QUICK VIRAL ISOLATION

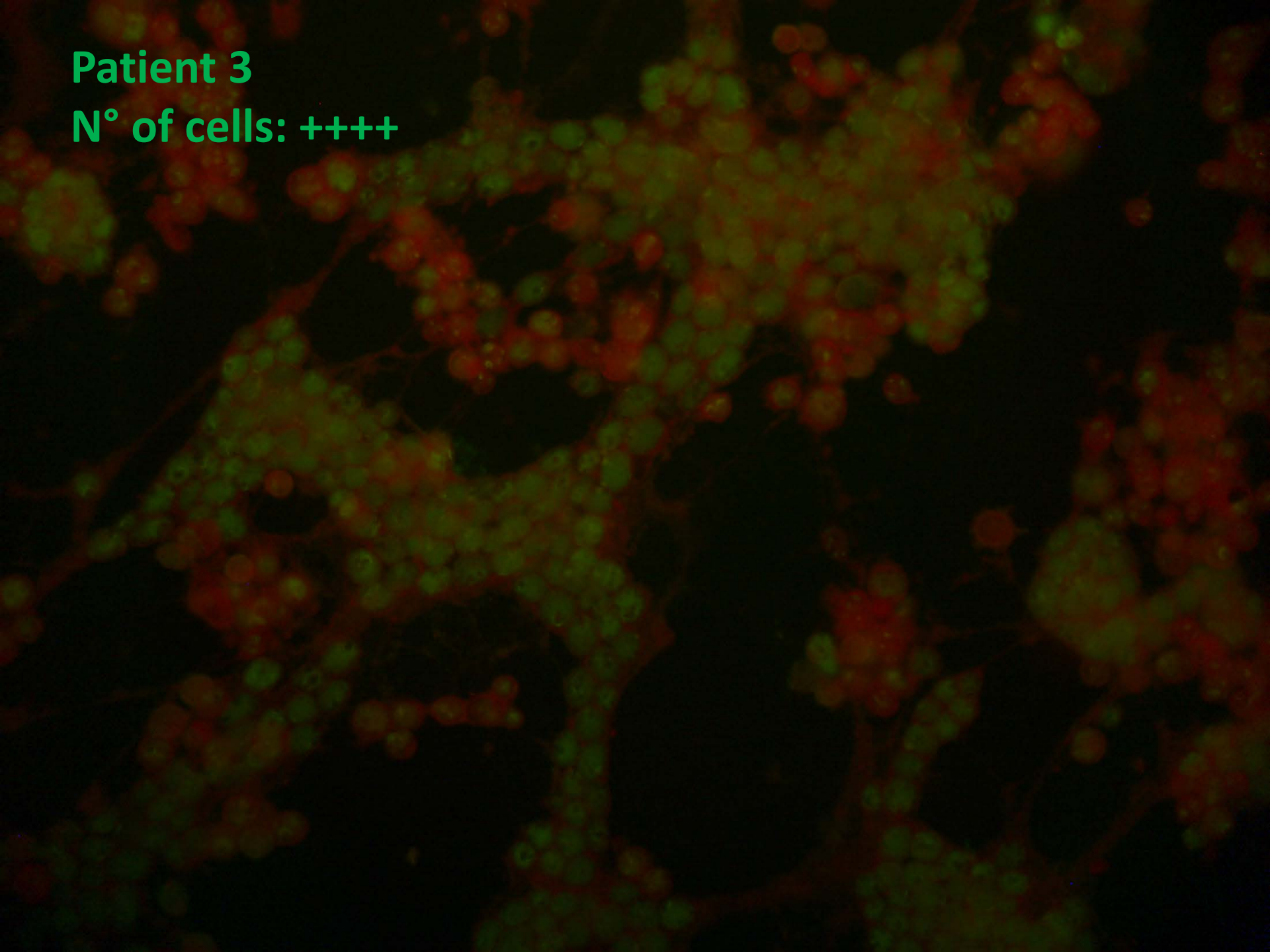
Great contribution to rapid diagnosis.

It includes:

- Increased infectivity by low-speed centrifugation of the sample on monolayer (shell vials)
- Sampling of the slide with the infected culture, fixation, and detection of specific viral antigens by IF with monoclonal Ab 24-48 h after infection


Patient 1
N° of cells: ++


Patient 2
N° of cells: ++++


Patient 3
N° of cells: ++++

- Cell cultures are the "gold-standard" for the detection of the presence of infectious viruses but have a very low sensitivity to detect HSV-1 compared to PCR tests.
- One of the reasons for the low sensitivity of cell cultures is the fragility of HSV-1.
- The lipid envelope is easily degraded making the virus non-infectious and unable to replicate in cell cultures.

The preanalytic is the most critical and important phase

THANKS FOR THE ATTENTION

SALUS SCIENTIA

AZIENDA OSPEDALIERO - UNIVERSITARIA
Città della Salute e della Scienza di Torino