XIII CORSO NAZIONALE SIBO
Società Italiana Banche degli Occhi

HSV LABORATORY DIAGNOSTICS IN TRANSPLANT CORNEAS

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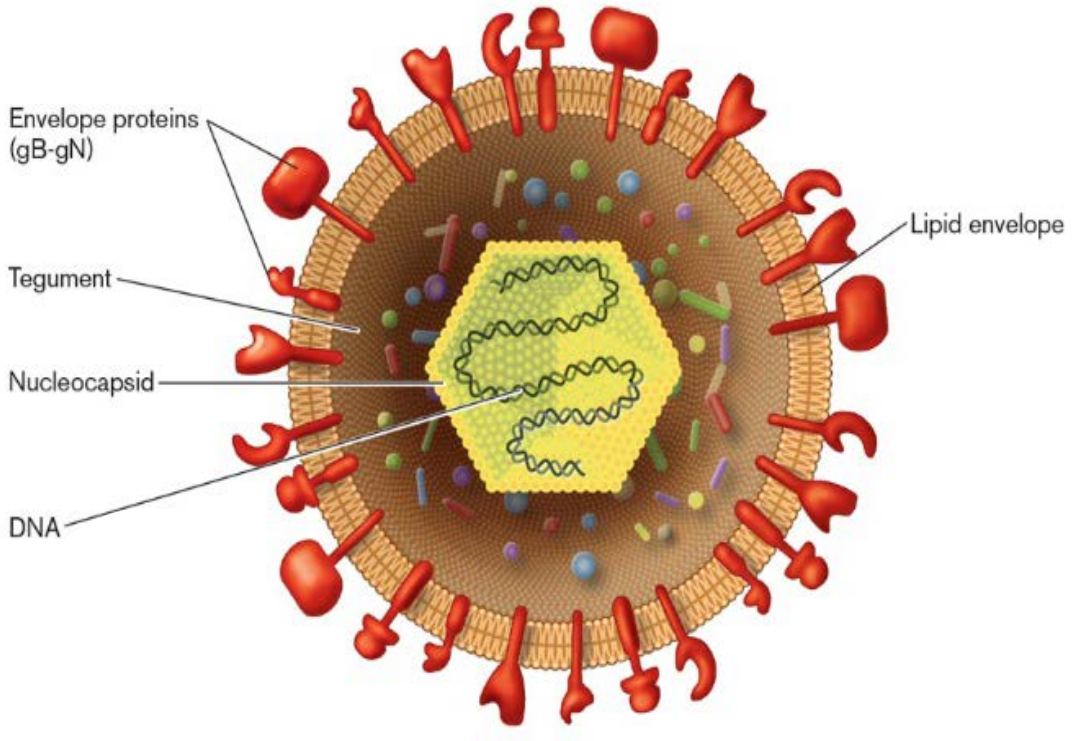
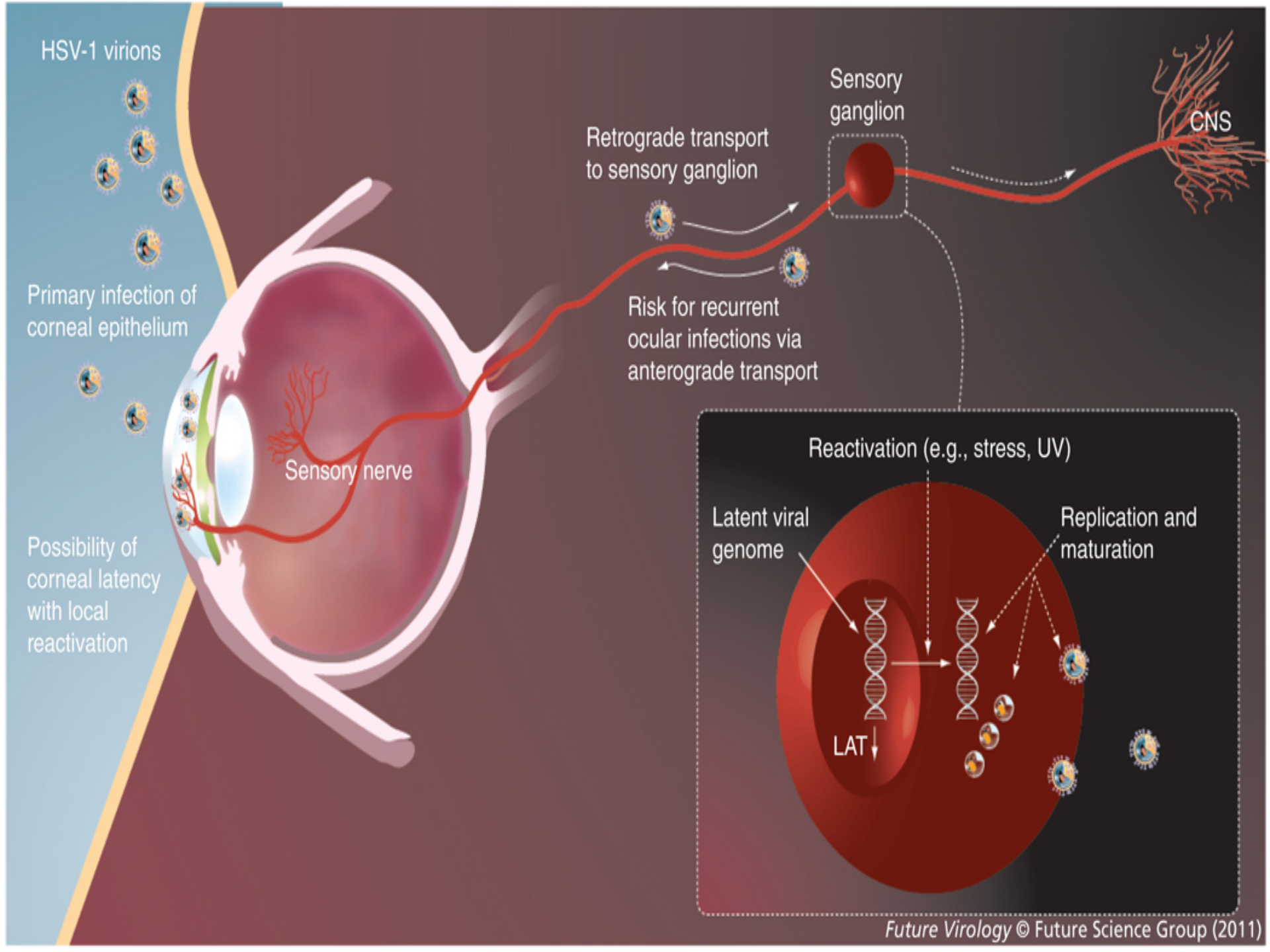
Bologna, 1 Giugno 2019

HERPESVIRUS

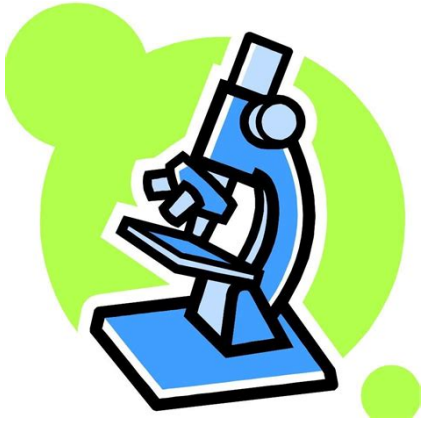


- DNA: Double strand, 120-250 Kbp, 30 nm diameter, cylindrical structure
- Capsid: 162 capsomers, 150 exons, 12 pentons, cable capsomers with 4 nm diameter channel
- Envelope: sensitive to lipid solvents, innermost layer tegument, virion diameter 150-200 nm



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

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)

VIRAL ISOLATION (CLASSICAL METHOD)

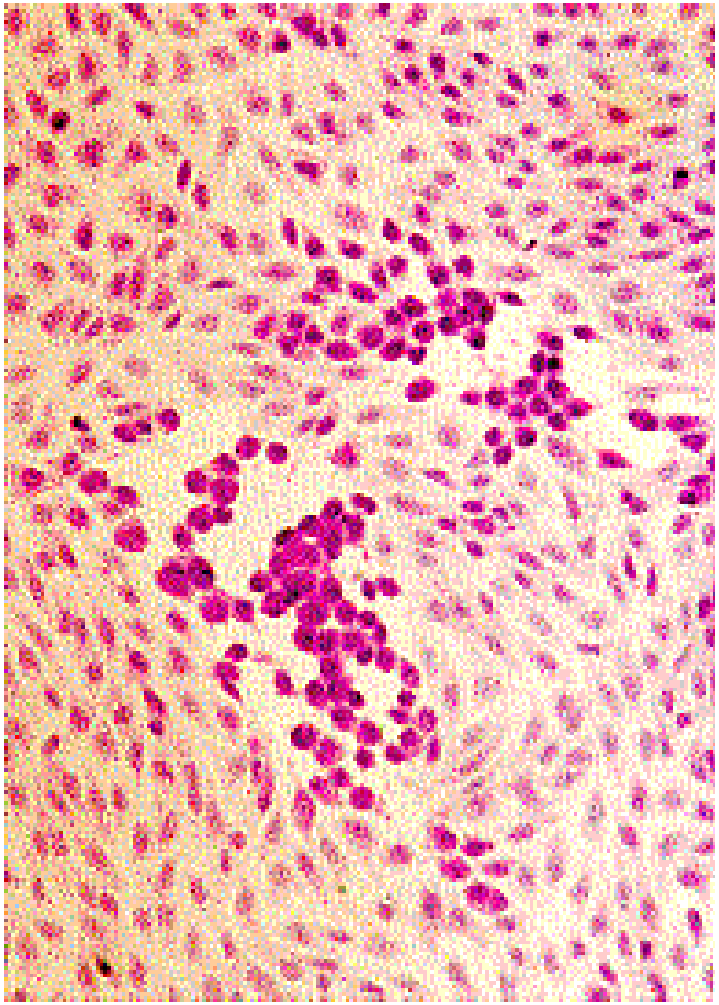
A virus can be revealed by observing the CYTOPATHIC EFFECT in culture.

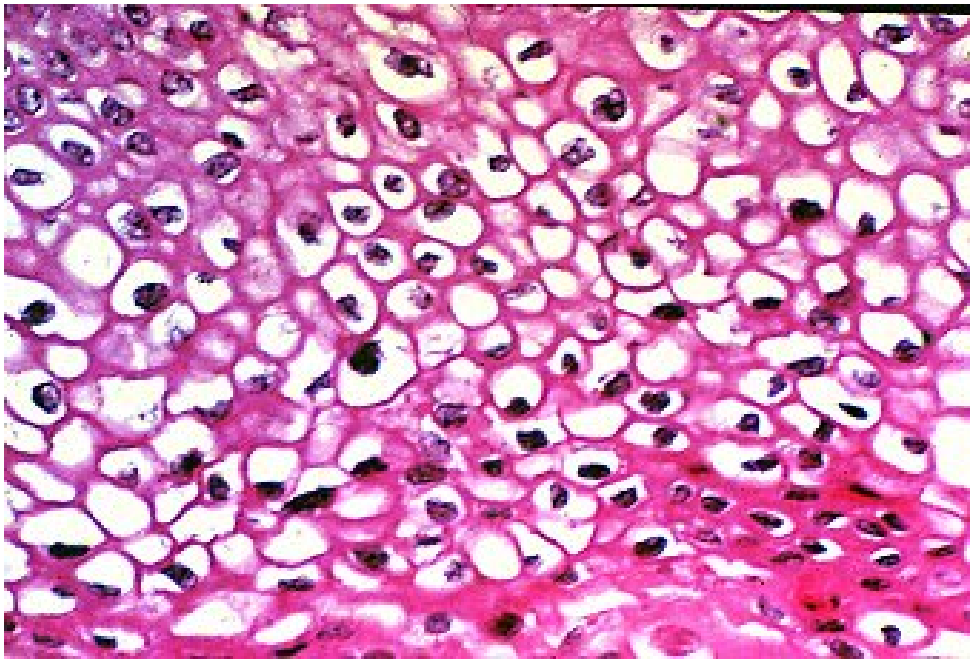
Cytopathic effect:

- 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



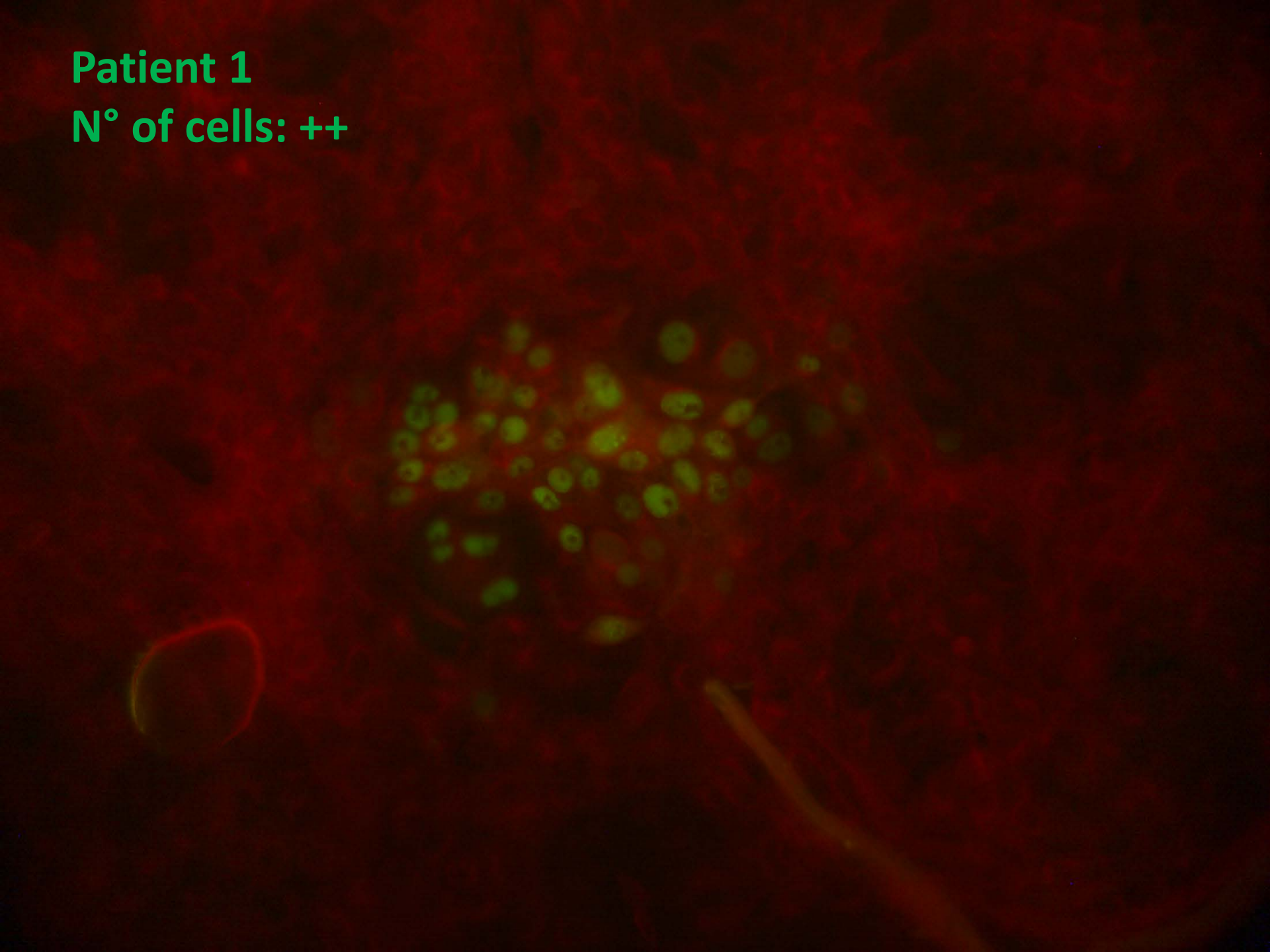


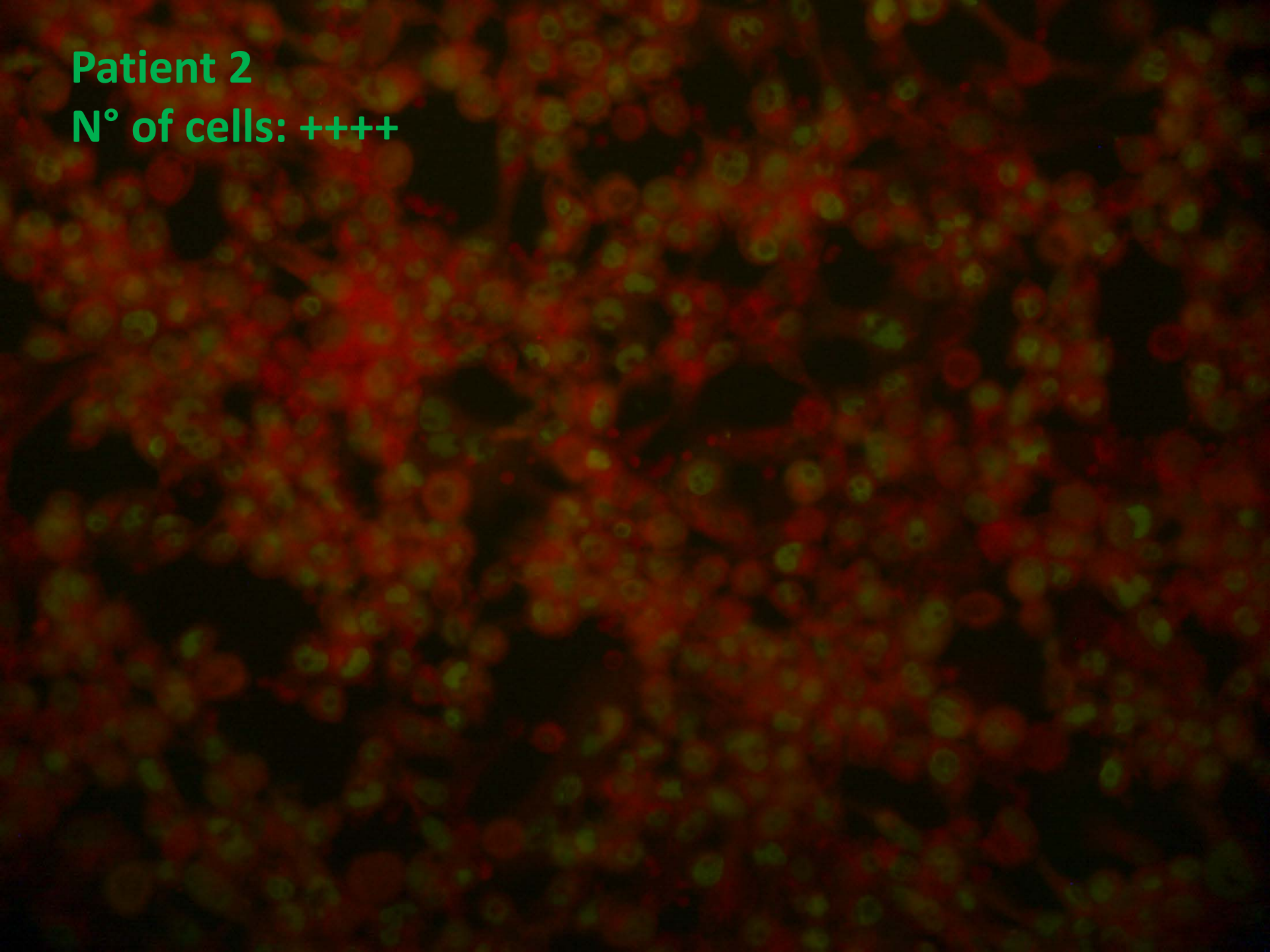
QUICK VIRAL ISOLATION

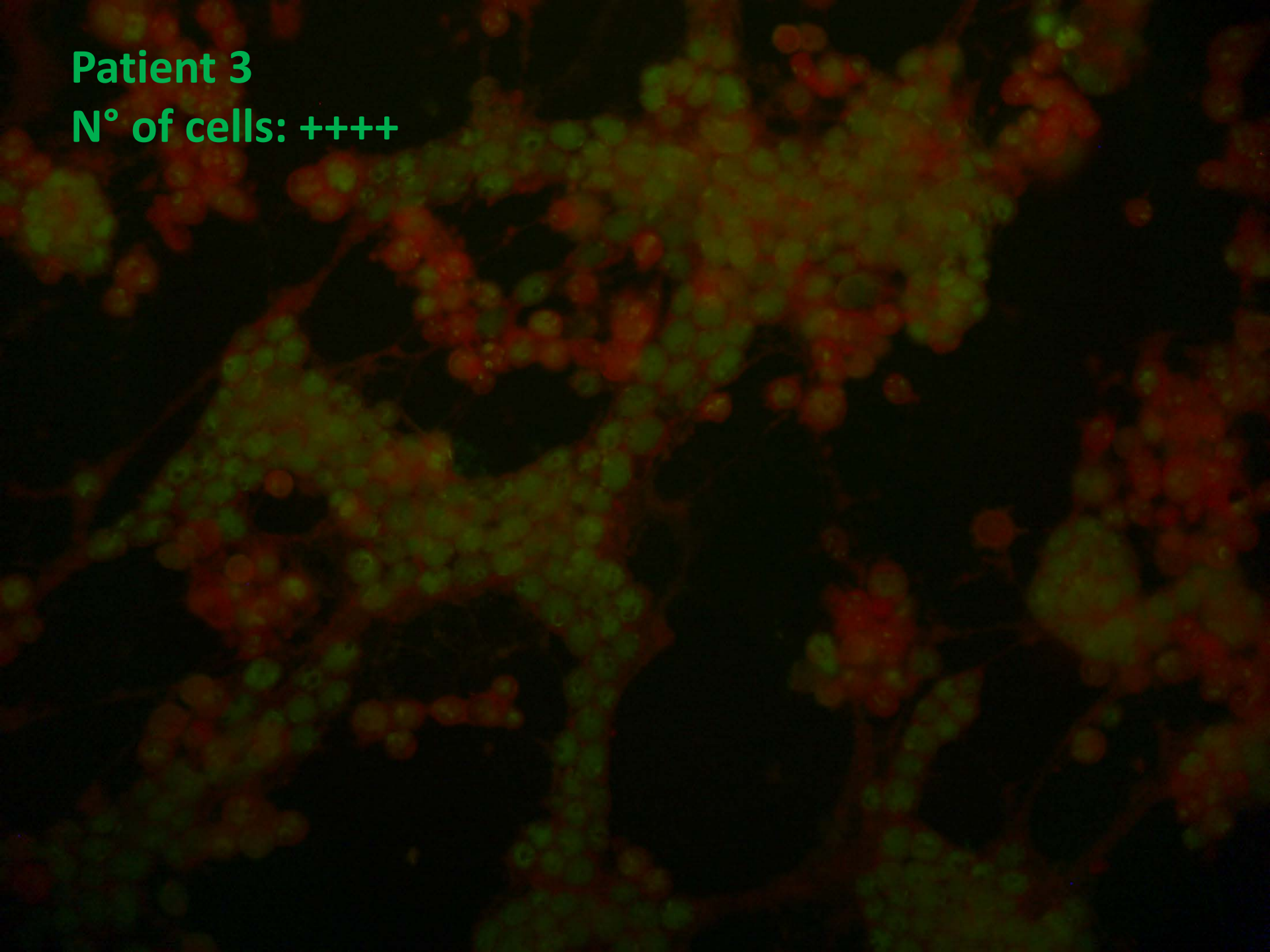
Great contribution to rapid diagnosis.

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: ++++

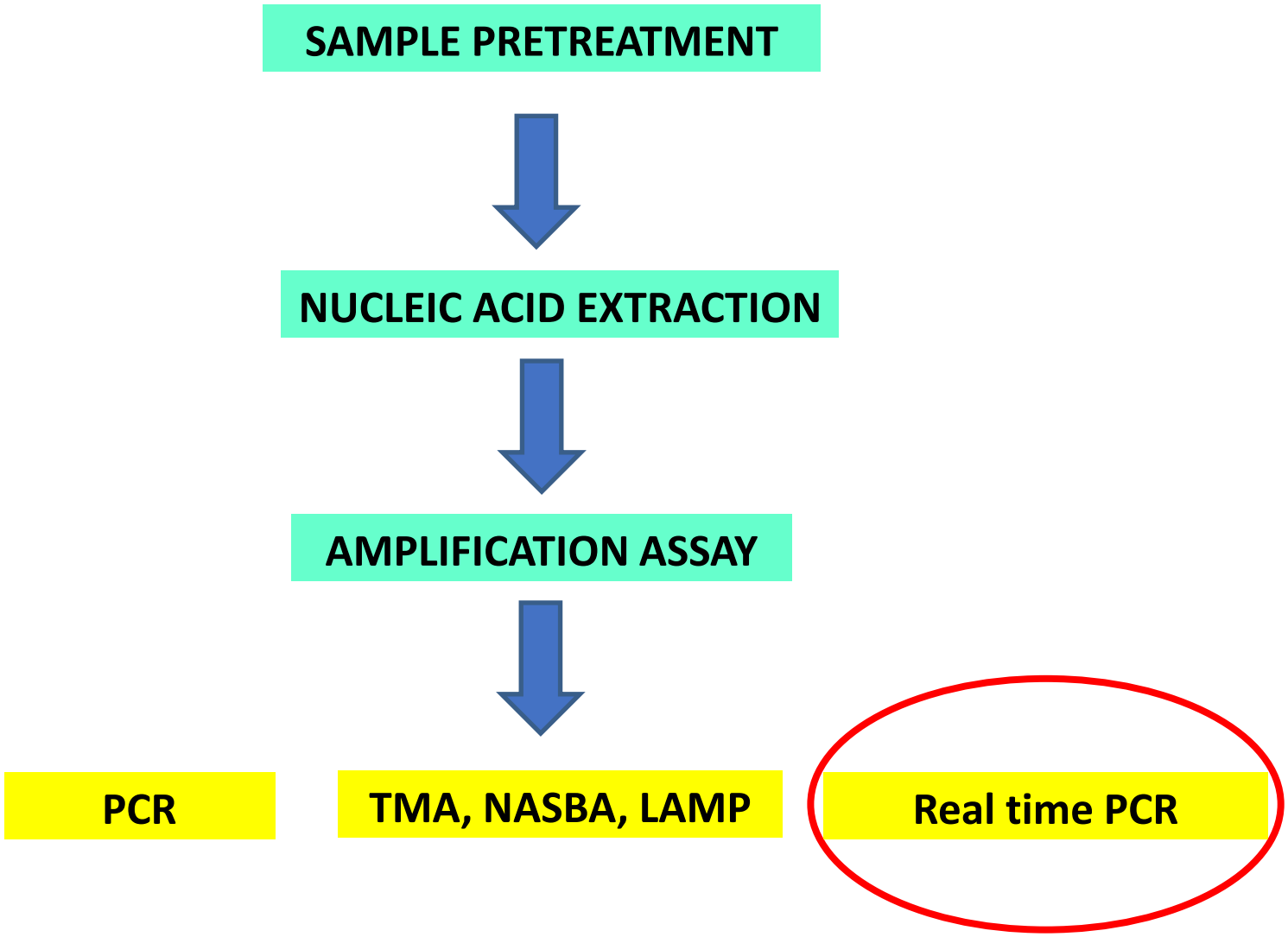

Patient 3
N° of cells: ++++

- 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

MOLECULAR BIOLOGY

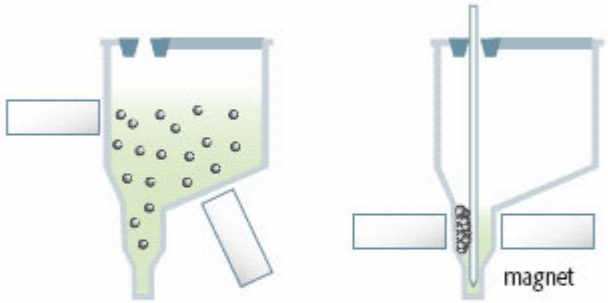
FLOWCHART IN MOLECULAR DIAGNOSTICS



NUCLEIC ACID EXTRACTION

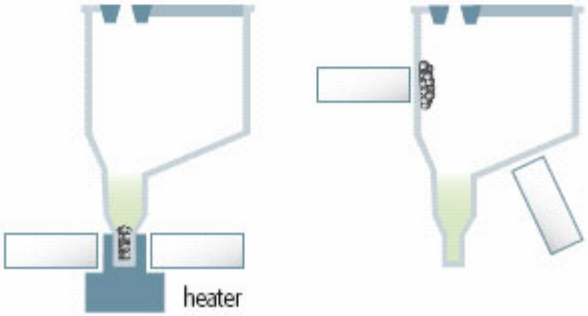
Extraction Principle

A. During incubation of the lysed samples, all the target nucleic acid is captured by magnetic silica particles.
B. The NucliSENS easyMAG magnetic device attracts all the magnetic silica, enabling the system to purify the nucleic acids through several washing steps.
C. The heating step releases the nucleic acids from the silica.
D. At the final step, the magnetic silica particles are separated from the eluate by the magnetic device.



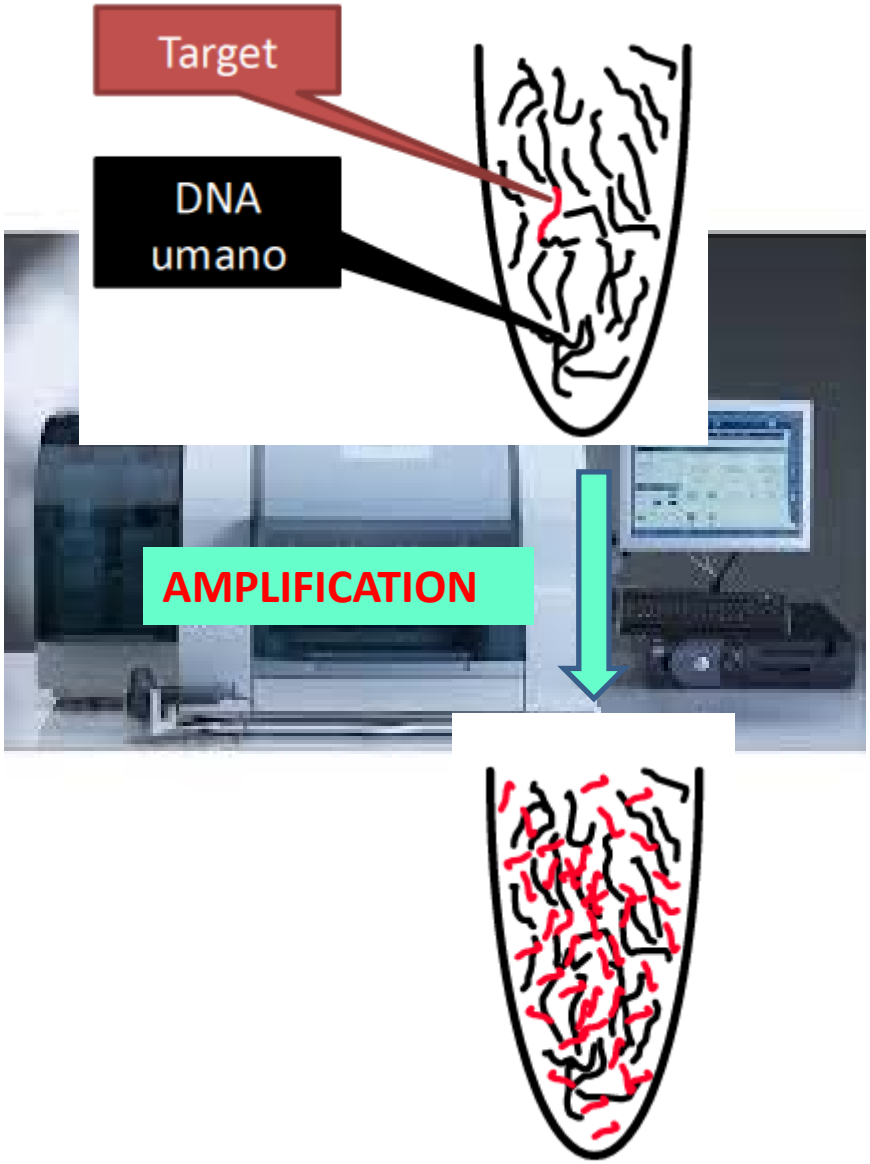
A. Incubation (NA capture by silica)

B. Washing



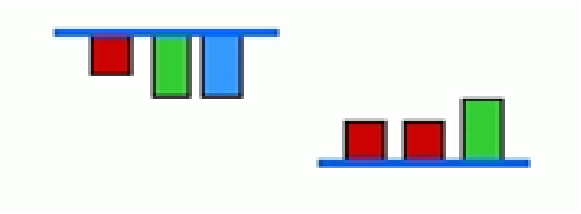
C. Elution

D. Final purification

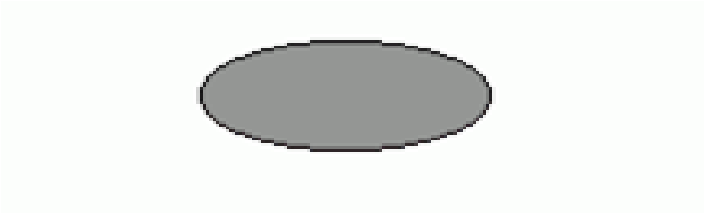


SEVERAL THINGS ARE NEEDED FOR AMPLIFICATION

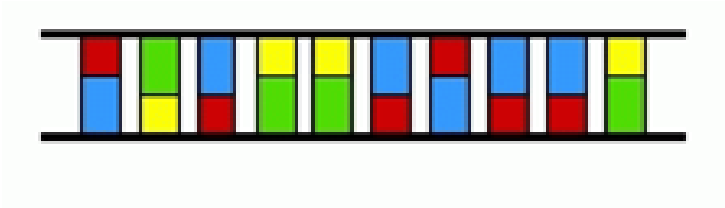
- PRIMERS



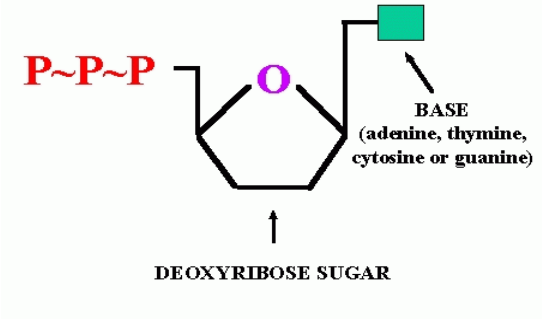
- DNA POLYMERASE



- DNA TO BE AMPLIFIED

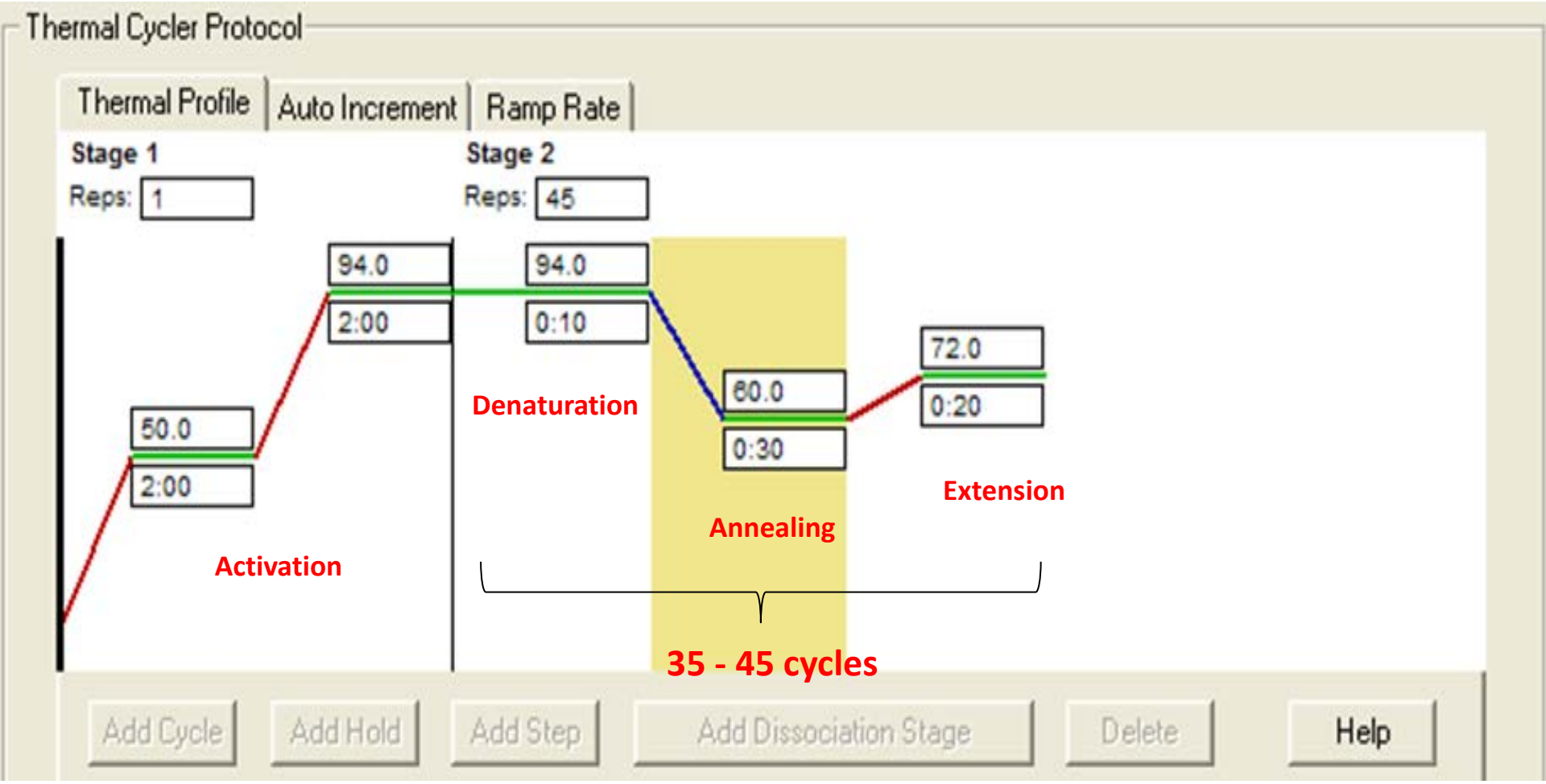


- NUCLEOTIDES

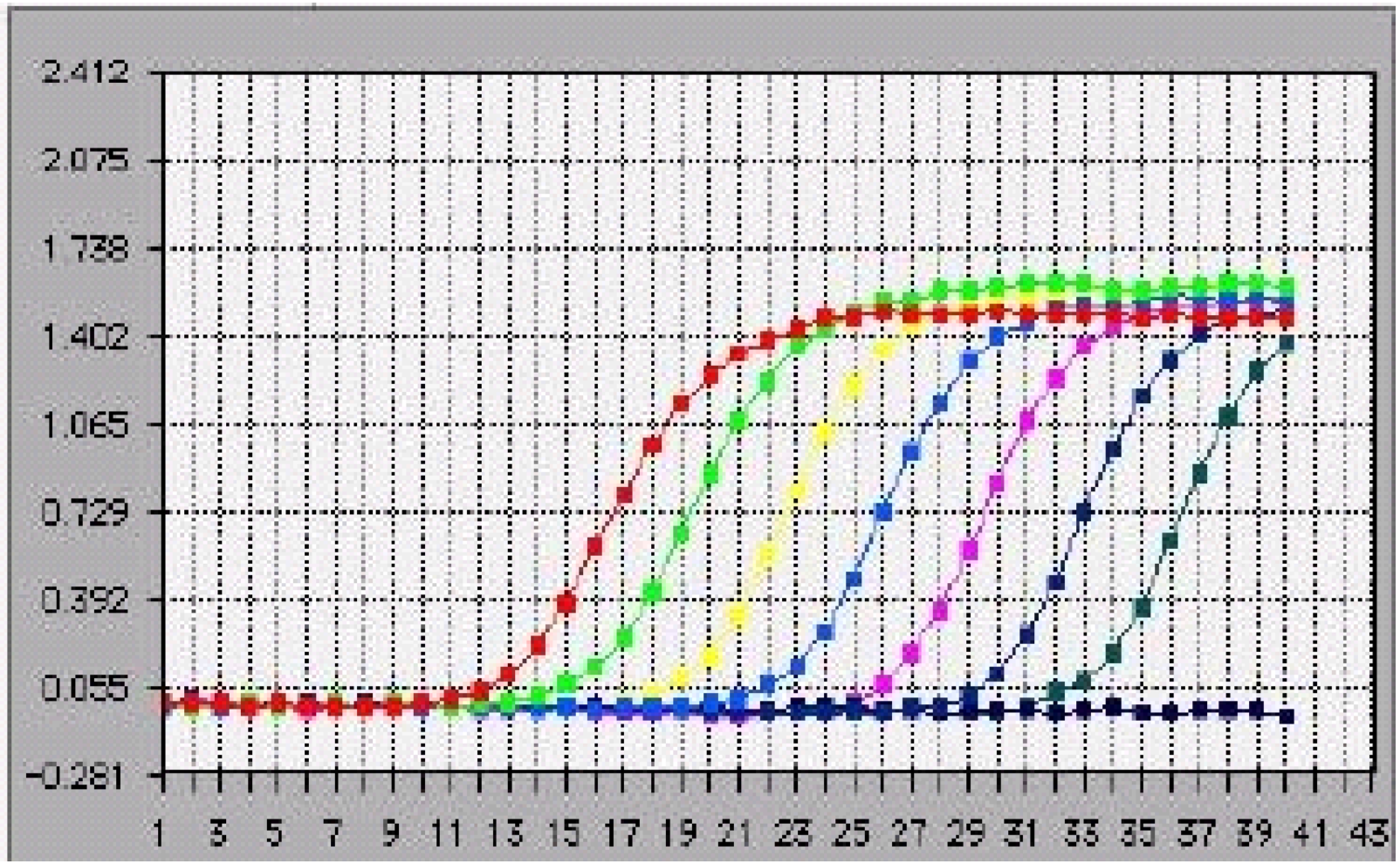


- FLUORESCENT PROBE

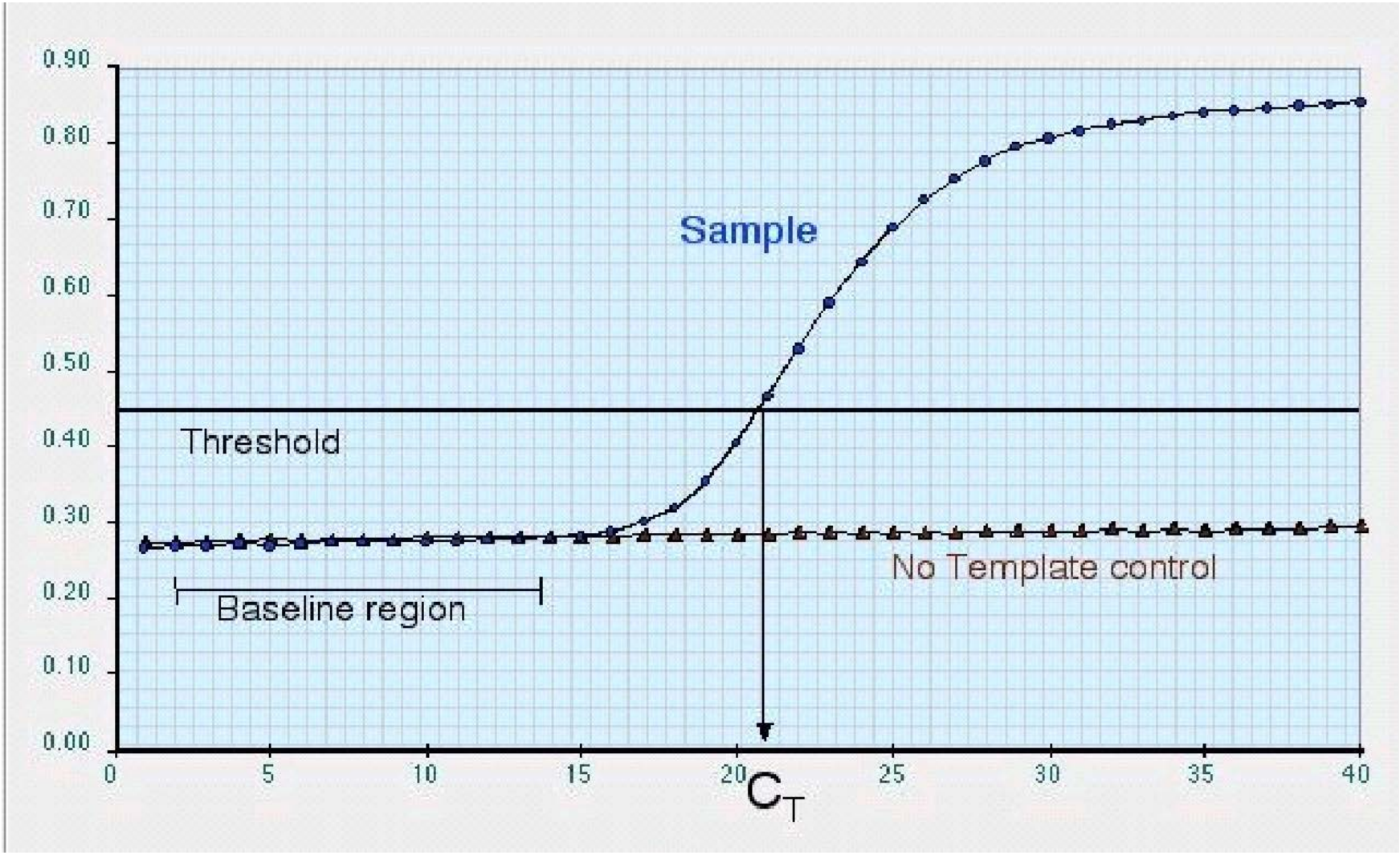
Run Real-Time thermal cycle



Amplification curves



Amplification plot



7500 Fast System SDS Software - [01-04-2014a.sds (Standard Curve)]

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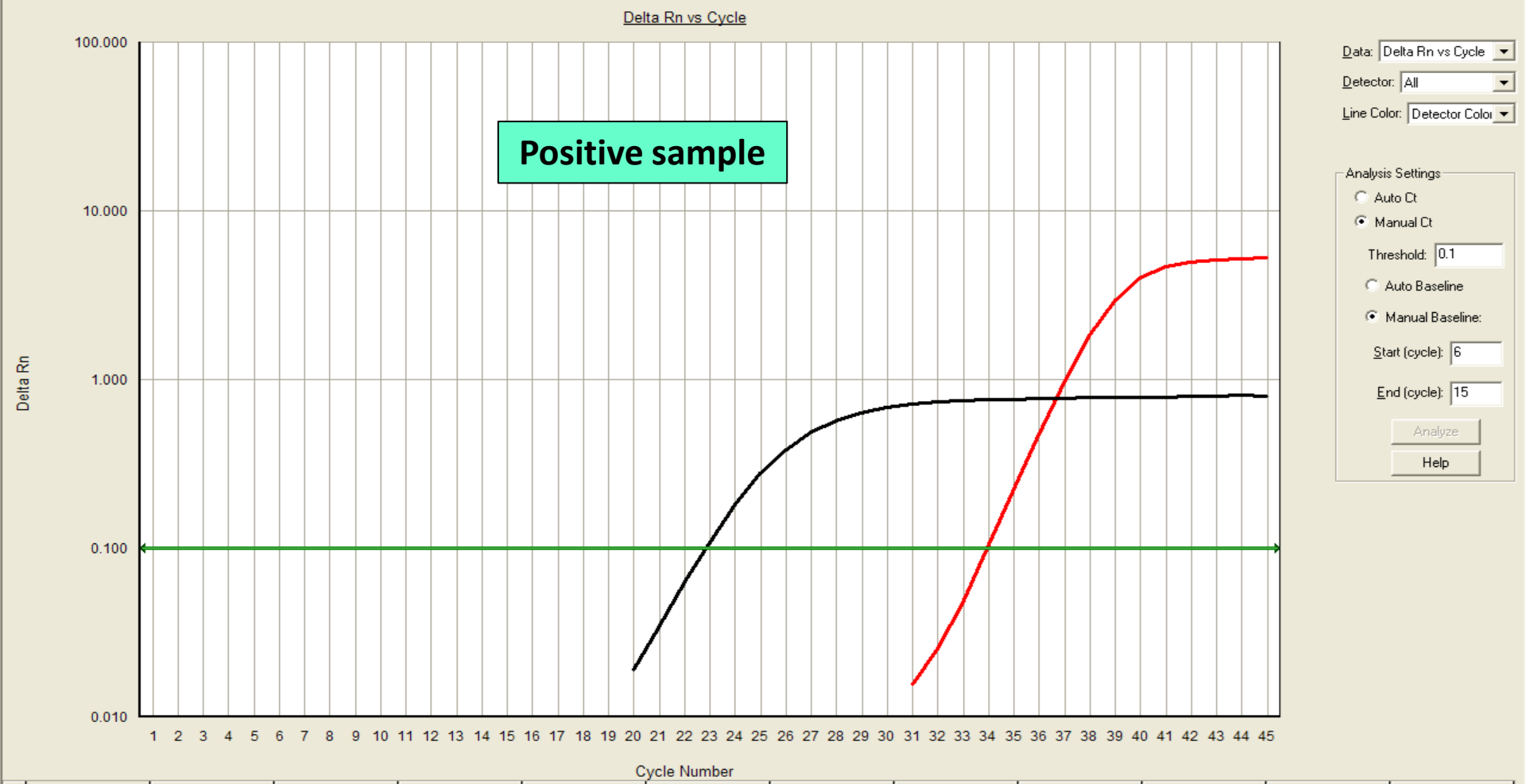
Setup | Instrument | **Results** | Audit Trail | E-Signatures

Plate | Spectra | Component | **Amplification Plot** | Standard Curve | Dissociation | Report



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B	U U	U U	U U	U U	U U	U U	U U	U U	S U			U U
C	U U	U U	U U	U U	U U	U U	U U	U U	N U			
D	U U	U U	U U	U U	U U	U U	U U	U U	U U			
E	U U	U U	U U	U U	U U	U U	U U	U U				
F	U U	U U	U U	U U	U U	U U	U U	U U				
G	U U	U U	U U	U U	U U	U U	U U	S U				
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Ready | Disconnected | User: rt-user | NUM

start | Back-up 7500 Fas... | 7500 Fast System... | untitled - Paint | 12:48 PM

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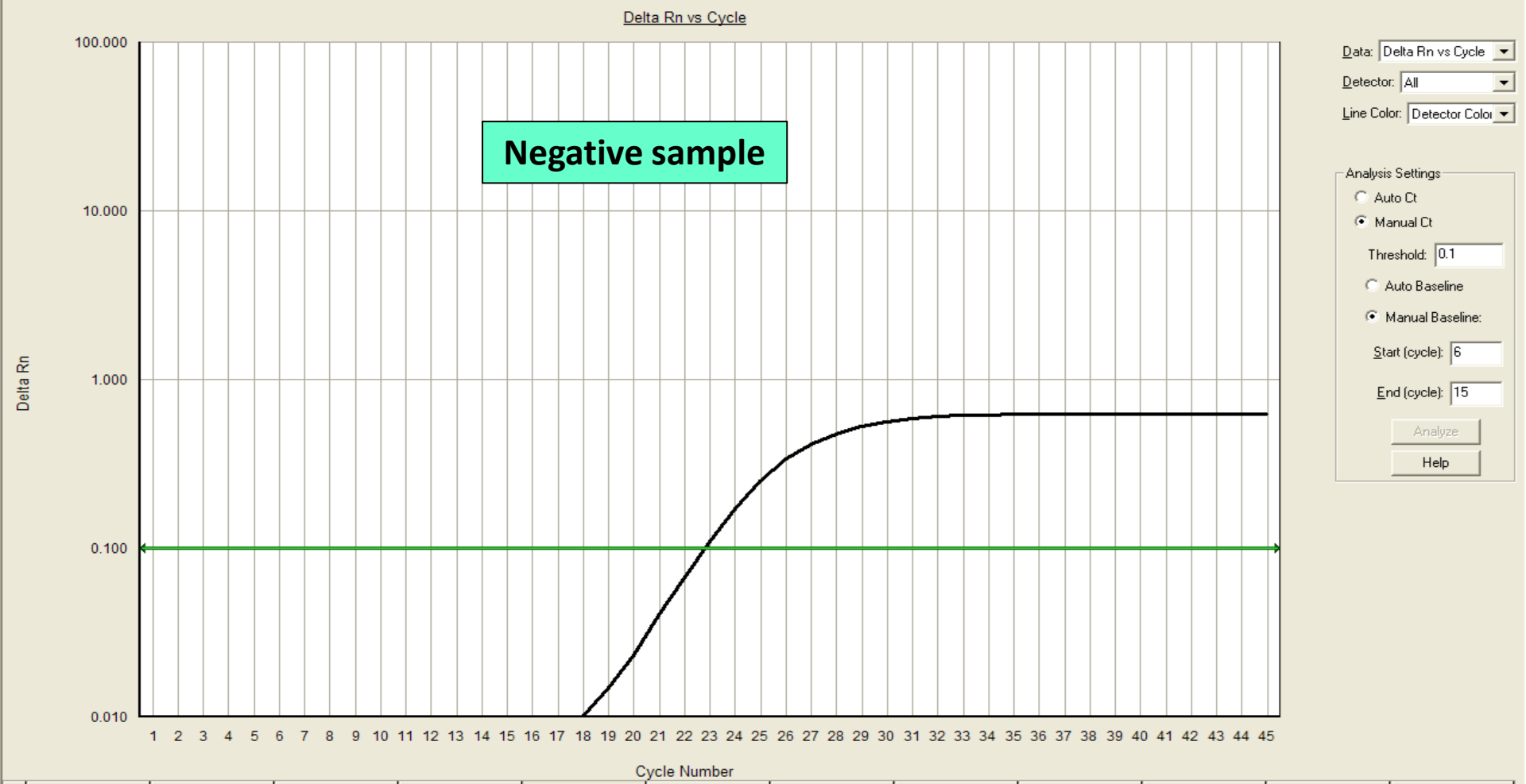

	1	2	3	4	5	6	7	8	9	10	11	12
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C	U U	U U	U U	U U	U U	U U	U U	U U	N U			
D	U U	U U	U U	U U	U U	U U	U U	U U	U U			
E	U U	U U	U U	U U	U U	U U	U U	U U				
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G	U U	U U	U U	U U	U U	U U	U U	S U				
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Ready | Disconnected | User: rt-user | NUM

start | Back-up 7500 Fas... | 7500 Fast System... | 12:48 PM

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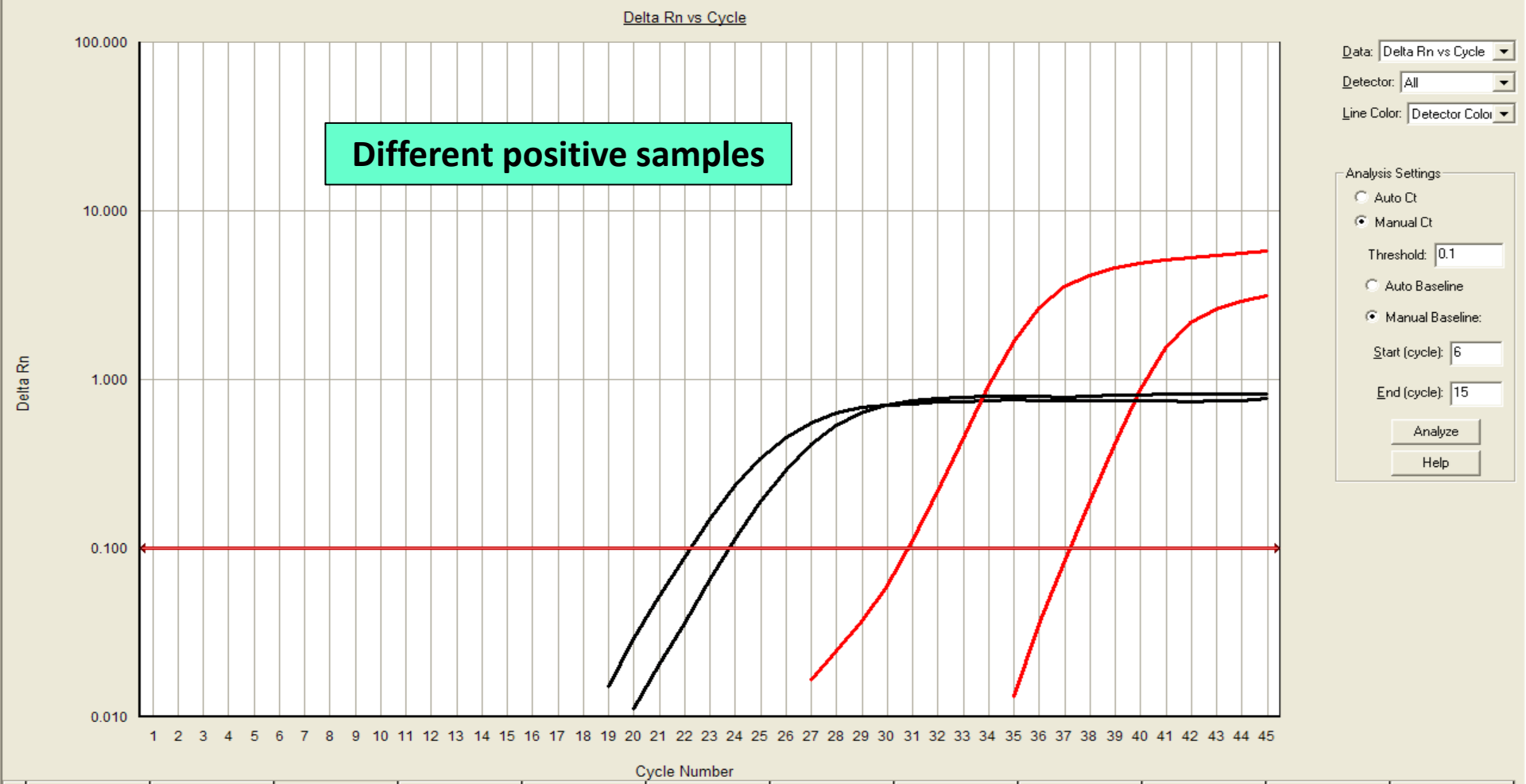

	1	2	3	4	5	6	7	8	9	10	11	12
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B	U U	U U	U U	U U	U U	U U	U U	S U				
C	U U	U U	U U	U U	U U	U U	U U	S U				
D	U U	U U	U U	U U	U U	U U	U U	S U				
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Ready | Disconnected | User: rt-user | NUM

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Molecular diagnostics in virology: advantages

- Very sensitive and specific
- Fast
- Applicable to all clinical materials
- Solves some shortcomings of indirect diagnosis
- Solves some shortcomings of cultural diagnosis

Molecular diagnostics in virology: disadvantages

- Costs
- Viral variants
- Meaning of viral latency

Thank you

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