La diagnostica molecolare di laboratorio dell'HSV nelle cornee per trapianto

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AZIENDA ULSS 3 SERENISSIMA

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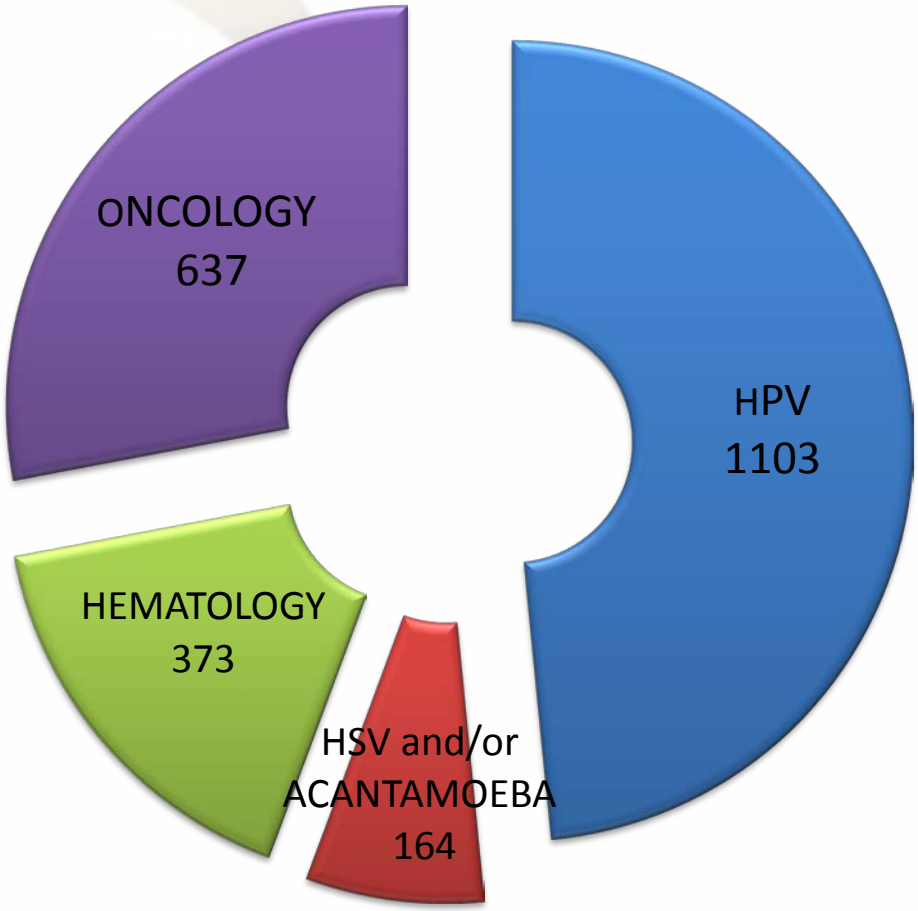
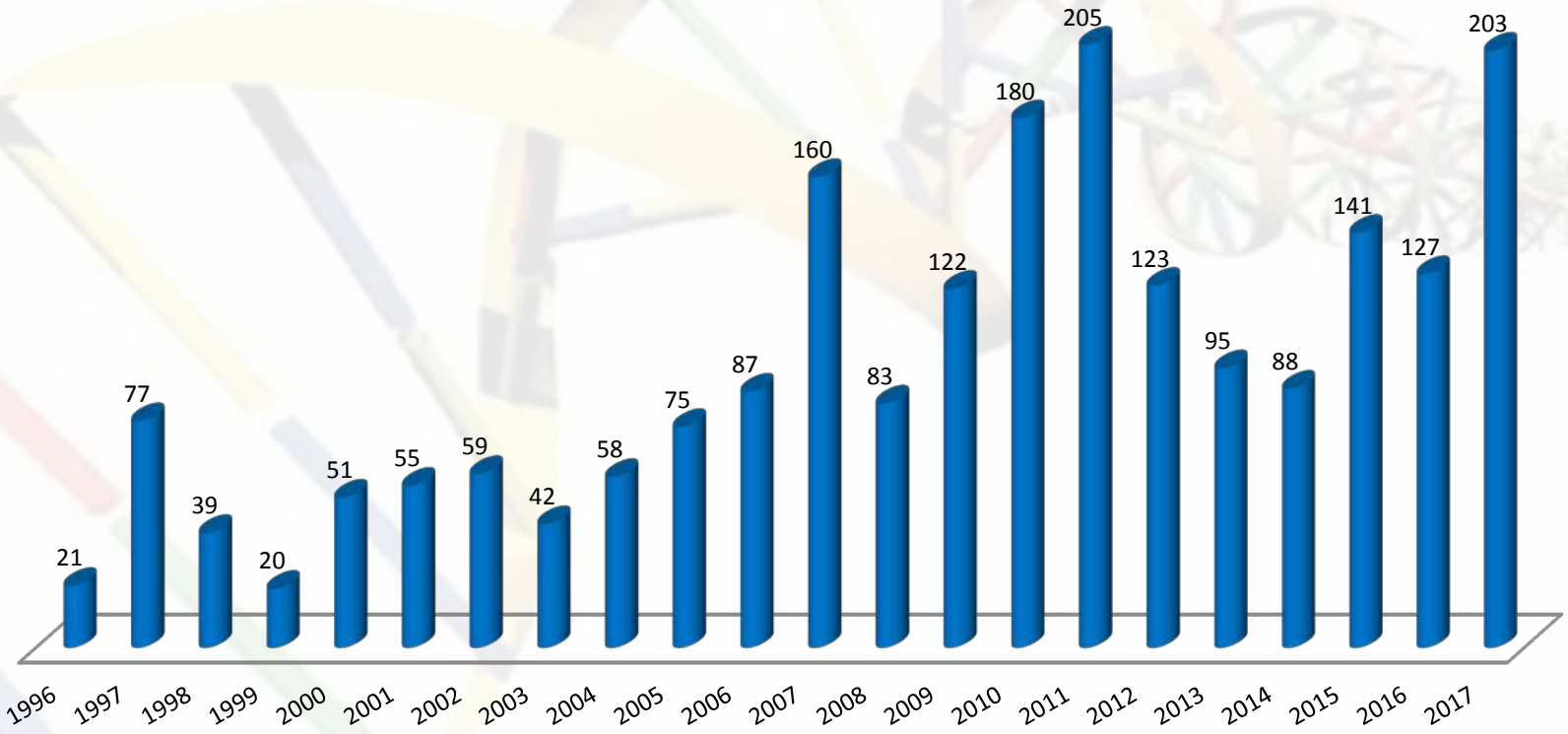
U.O.S.D. Cyto-Hystology
and Molecular Pathology

2018 MOLECULAR PATHOLOGY ACTIVITY



N° of molecular diagnosis for ophtalmologist

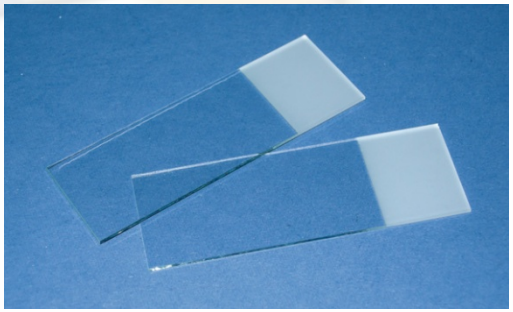


Different sample analyzed/processed:

- corneal tissue
- corneal scraping
- tears
- aqueous humor
- contact-lens solution
- cornea storage medium

Corneal scraping and HSV diagnosis

Microsurgery knife for ophtalmic surgery



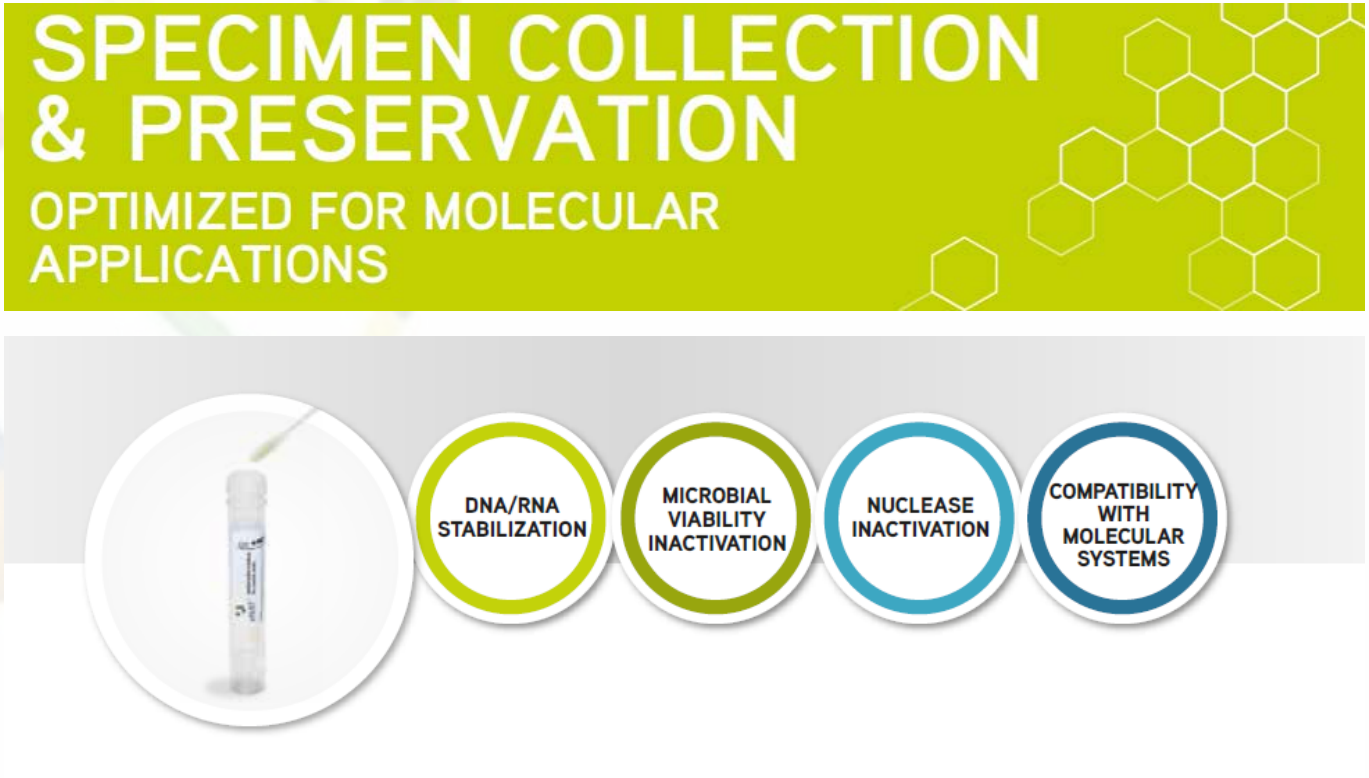
smear and staining

Cytologic diagnosis

DNAase and RNAase free tube with 1,5 ml preservation solution (from liquid-based pap test)

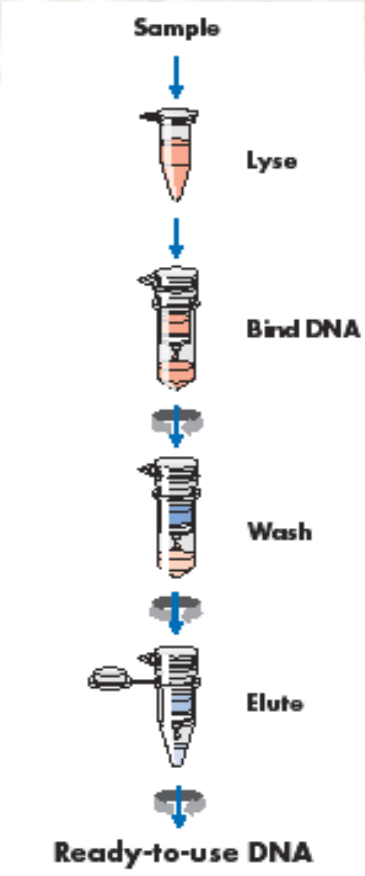
Molecular diagnosis

Commercial system available

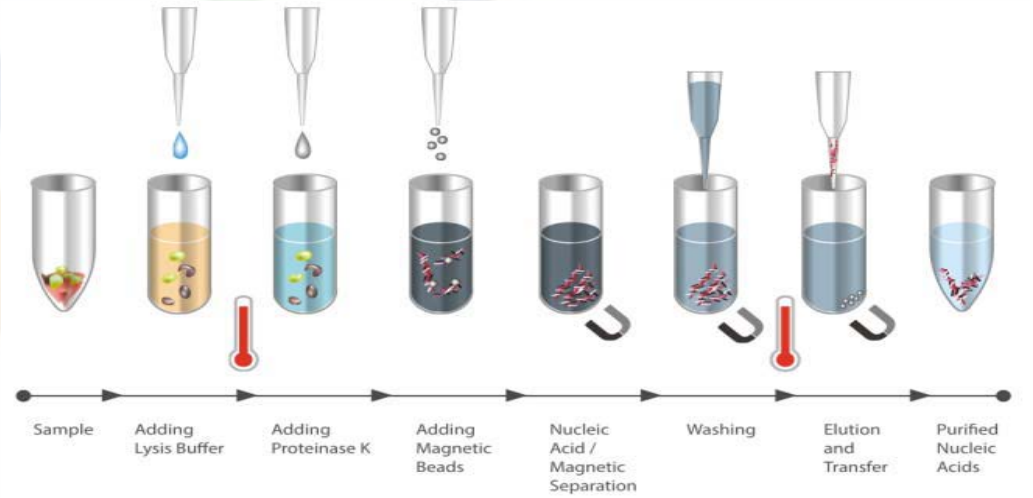


Molecular diagnostic of HSV
first step: DNA extraction

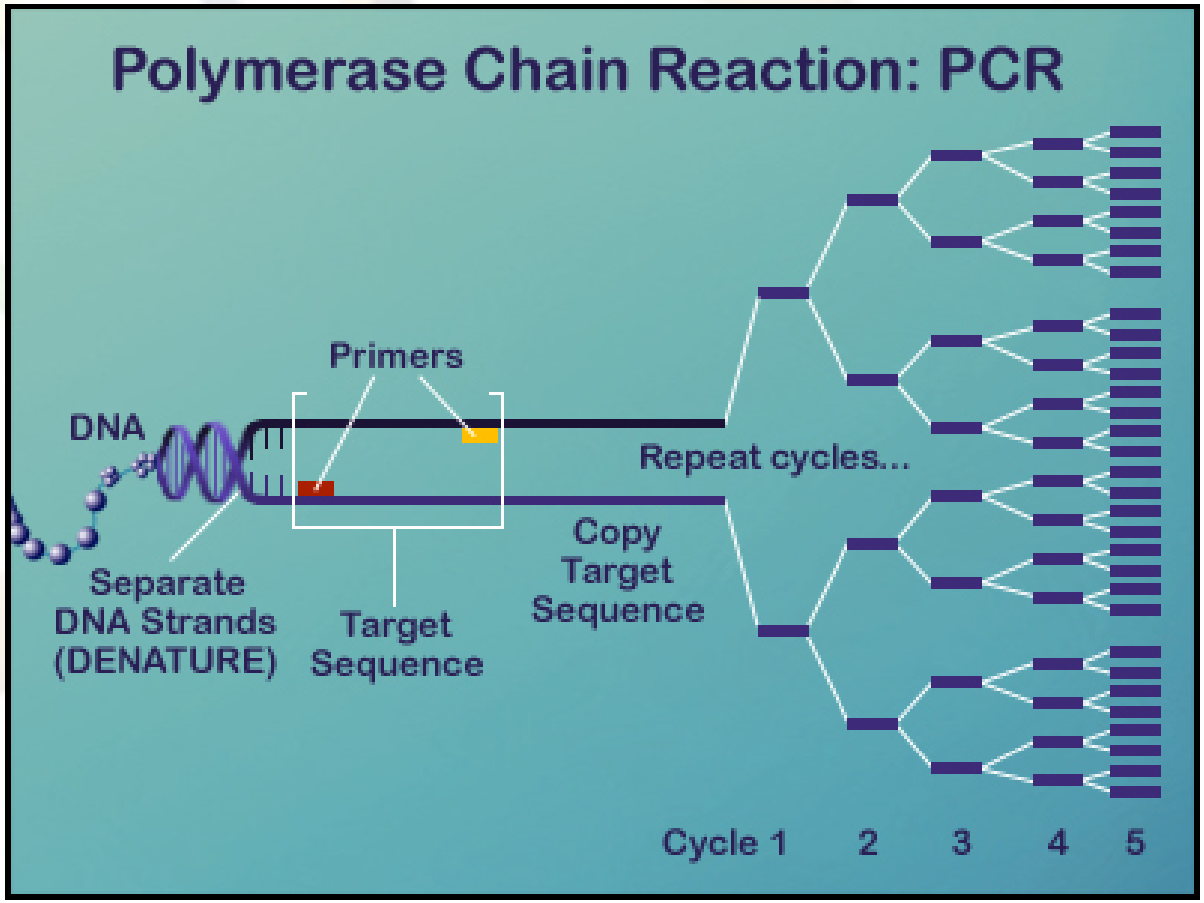
- Spin columns with silica resin



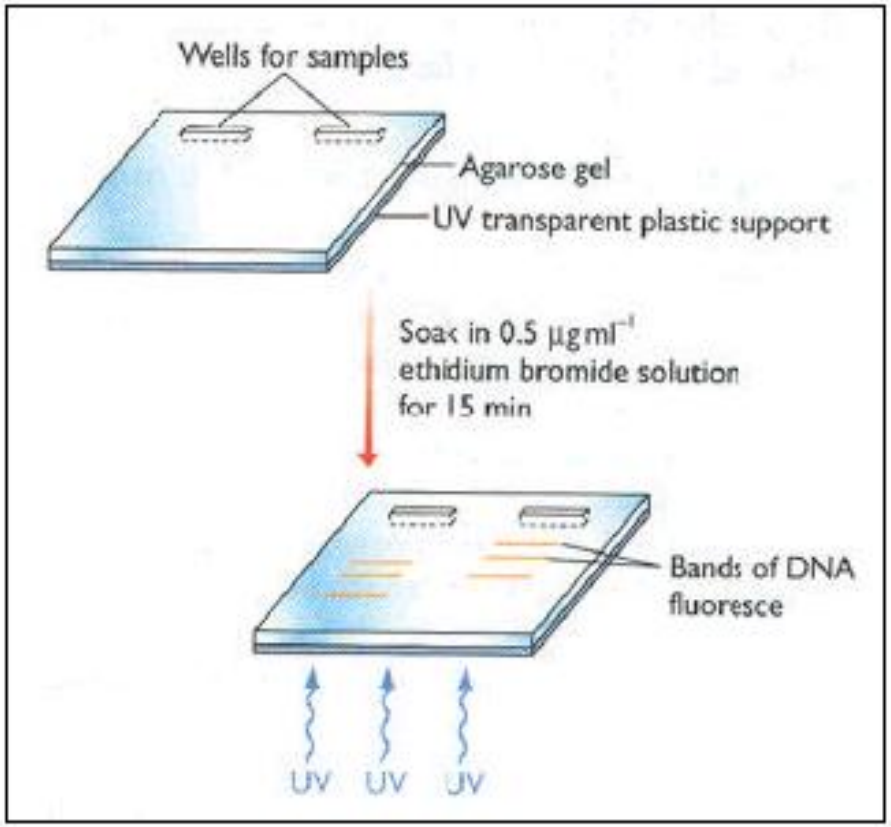
- Magnetic beads



Molecular diagnostic of HSV
second step: DNA amplification



Agarose gel electrophoresis



Molecular diagnostic of HSV
our experience: home-made PCR

HSV I/II

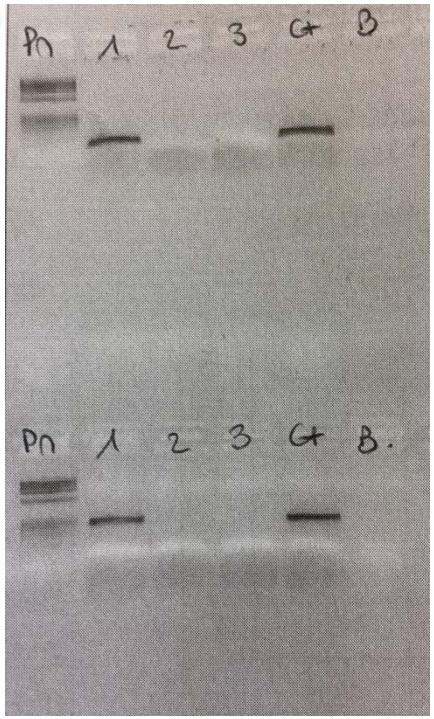
Primers (DNA polymerase gene):
HSV 1012 CAT CAC CgA CCC ggA gAg ggA C
HSV 1013 ggg CCA ggC gCT TgT Tgg TgT A

Cao M. et al. *J Invest Dermatol* (1989) 82, 391-2.
Amplicon size: **92 bp**

HSV I

Primers (UL42 region):
HS13 ACg ACg ACg TCC gAC ggC gA
HS14 gTg CTg gTg CTg gAC gAC AC

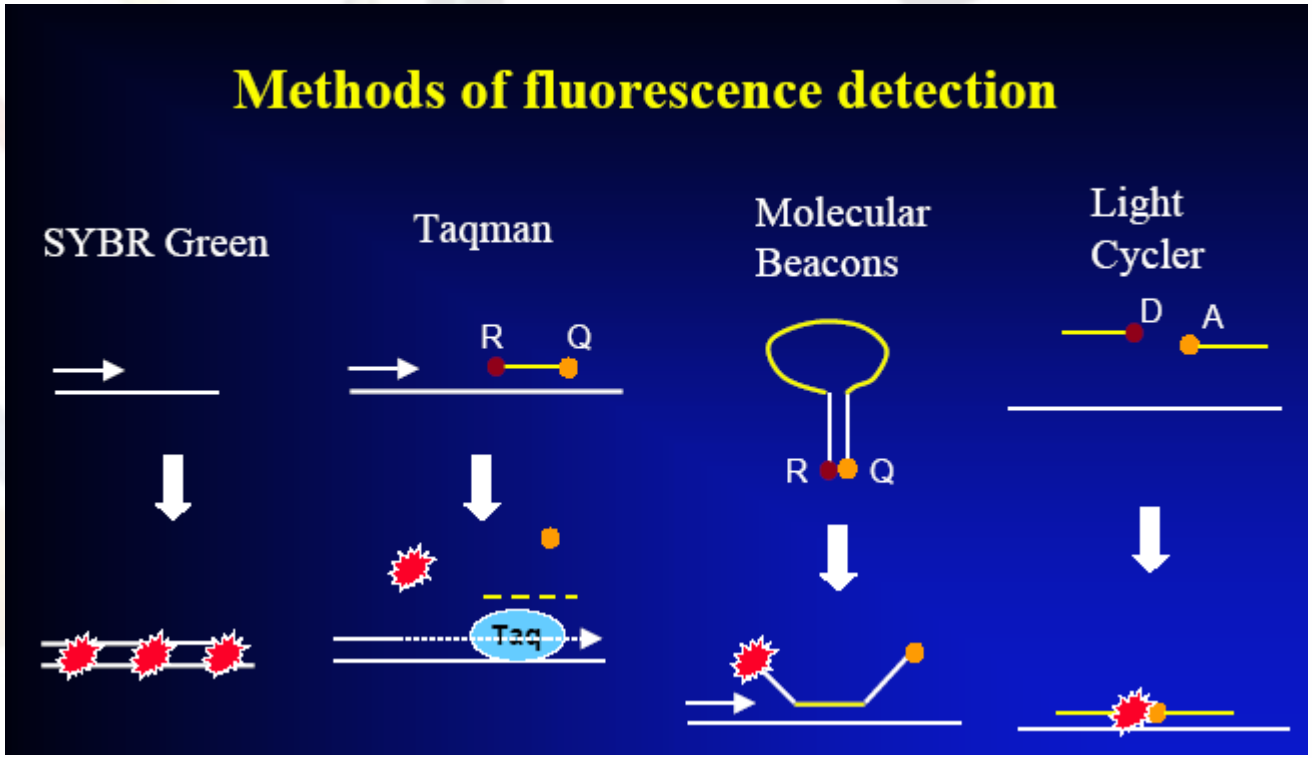
Puchhammer-Stockl E. et al. *J Med Virol* (1990) 32: 77-82
Amplicon size: **278 bp**



Human β-globin gene as control gene

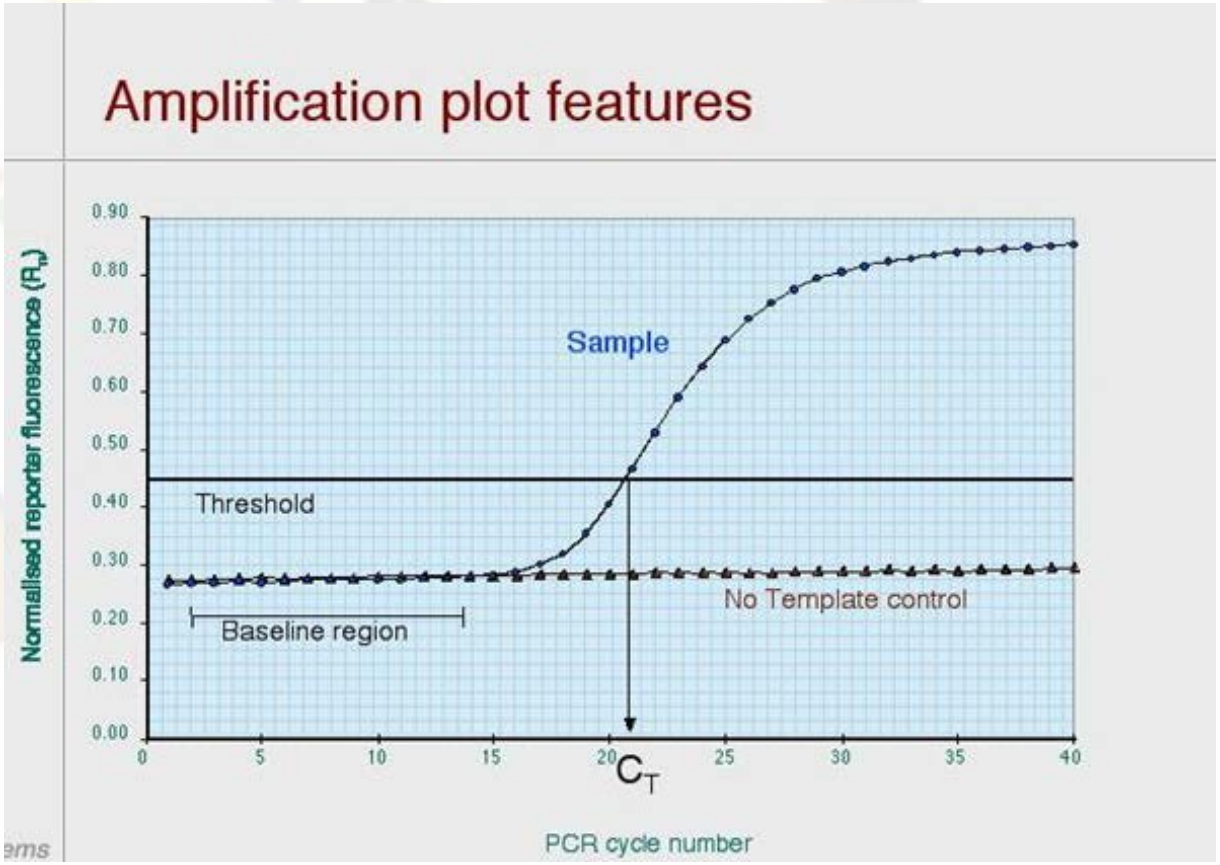
Molecular diagnostic of HSV
second step: DNA amplification

Real Time PCR



Real Time PCR

- Short processing time
- More sensitive
- Quantification (viral load)
- Not require post amplification sample manipulation (reduce risk for carry-over contamination)



Molecular diagnostic of HSV
Limits and pitfalls

- Sample collection
- Nucleic acid extraction (PCR inhibitors)
- Choice of HSV gene target
- Amplification methods

Molecular diagnostic of HSV
Conclusions

• nucleic acid amplification techniques have demonstrated superior sensitivity to all other diagnostic methods for detection of HSV infections

• NGS (=Next Generation Sequencing) detect more organisms and provide us large volume of information about the microbiome of the ocular surface


Thank You