

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

dr.ssa Manuela Riccardi

Venezia-Mestre, 21 febbraio 2019



**S.I.T.R.A.C.**  
SOCIETÀ ITALIANA TRAPIANTO DI CORNEA  
E SUPERFICIE OCULARE

**VENEZIA, 21-22-23 febbraio 2019**

Sede congressuale: NH LAGUNA PALACE - Mestre

**ORGANIZZATORI:**  
Antonella Franch  
Diego Ponzin  
Emilio Rapizzi

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A. Franch, L. Mastropasqua,  
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# AZIENDA ULSS 3 SERENISSIMA

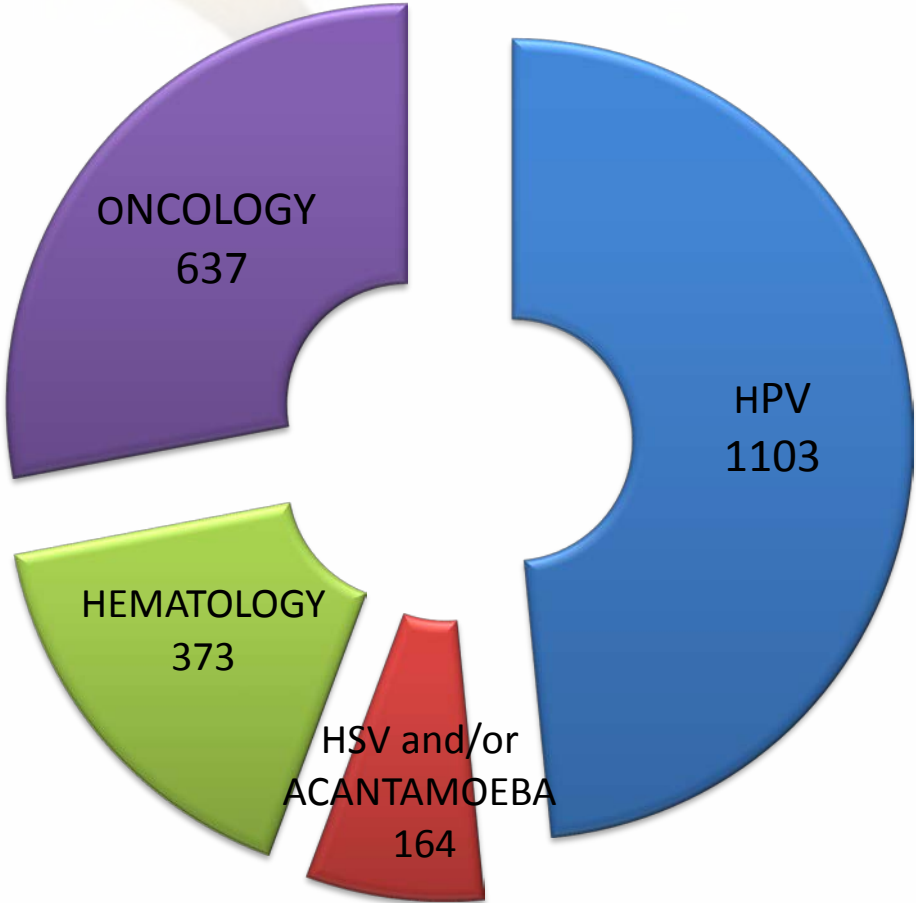
S.S.Giovanni e Paolo Hospital



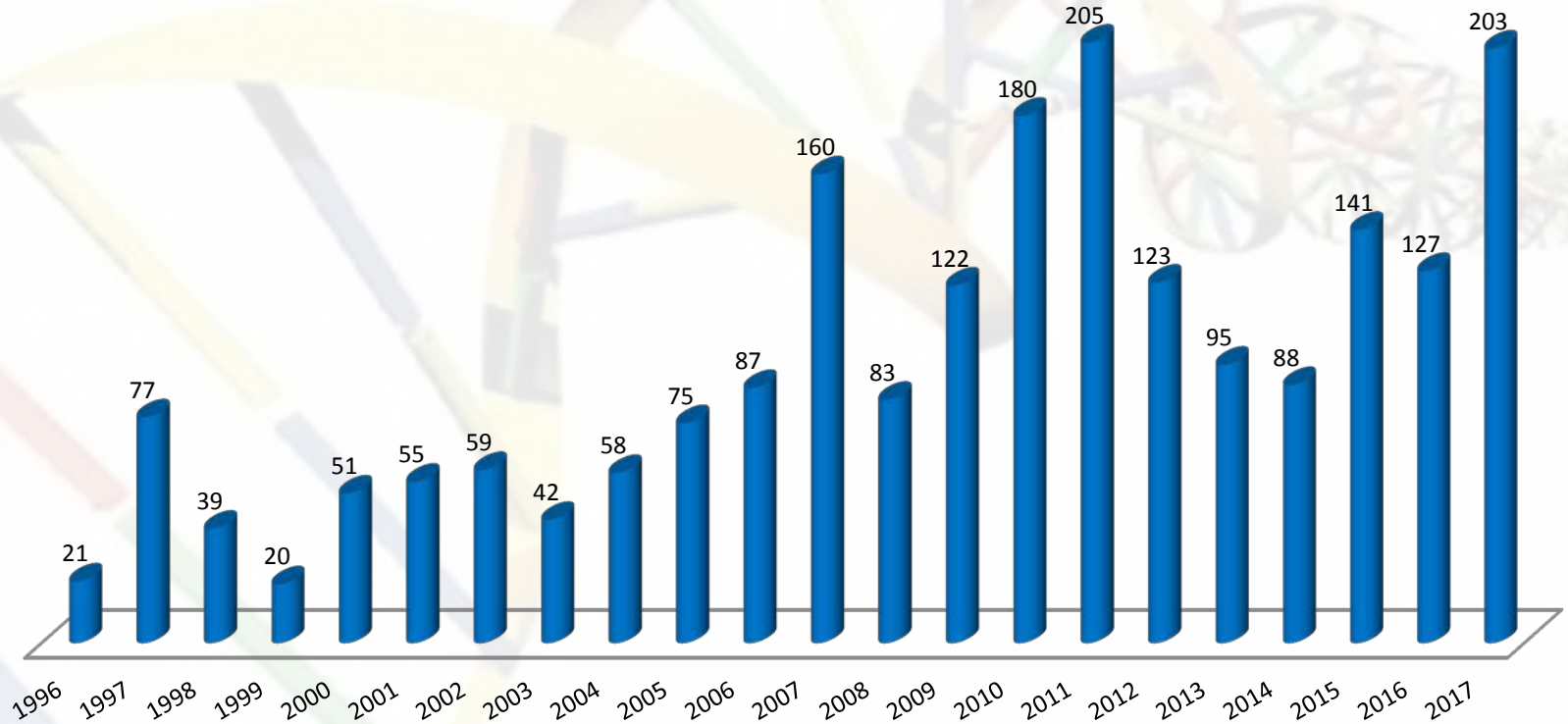
U.O.S.D. Cyto-Hystology  
and Molecular Pathology



# 2018 MOLECULAR PATHOLOGY ACTIVITY



## N° of molecular diagnosis for ophthalmologist



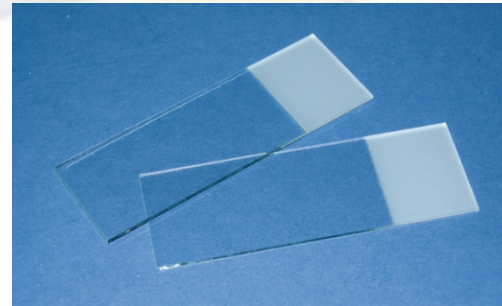
### Different sample analyzed/processed:

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

# Corneal scraping and HSV diagnosis



Microsurgery knife for ophthalmic surgery



*Cytologic  
diagnosis*

smear and staining



*Molecular  
diagnosis*

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

Commercial system available

# SPECIMEN COLLECTION & PRESERVATION

OPTIMIZED FOR MOLECULAR  
APPLICATIONS



**DNA/RNA  
STABILIZATION**

**MICROBIAL  
VIABILITY  
INACTIVATION**

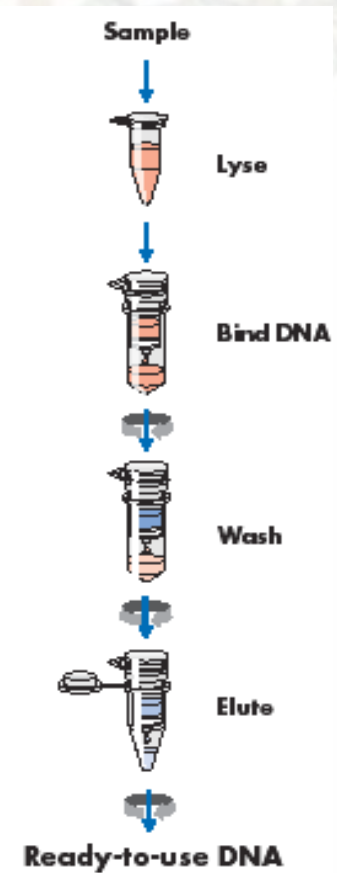
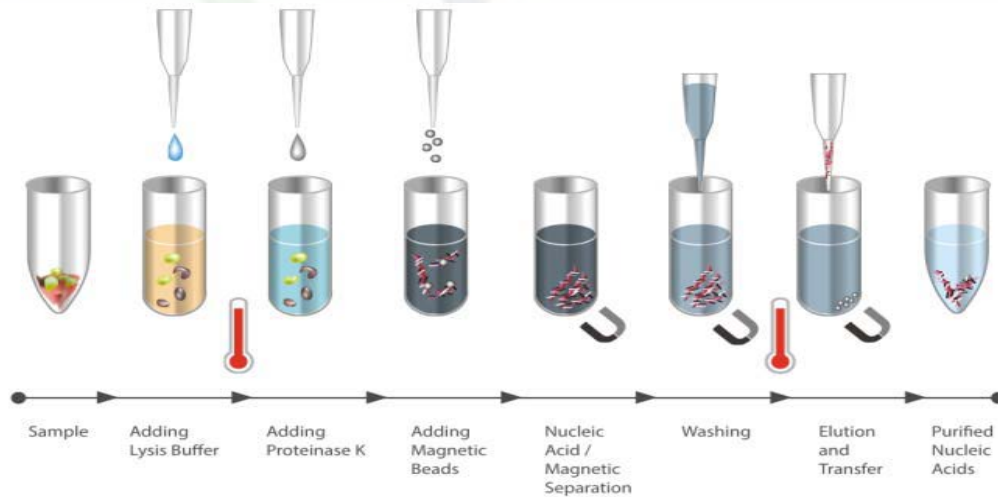
**NUCLEASE  
INACTIVATION**

**COMPATIBILITY  
WITH  
MOLECULAR  
SYSTEMS**

# Molecular diagnostic of HSV

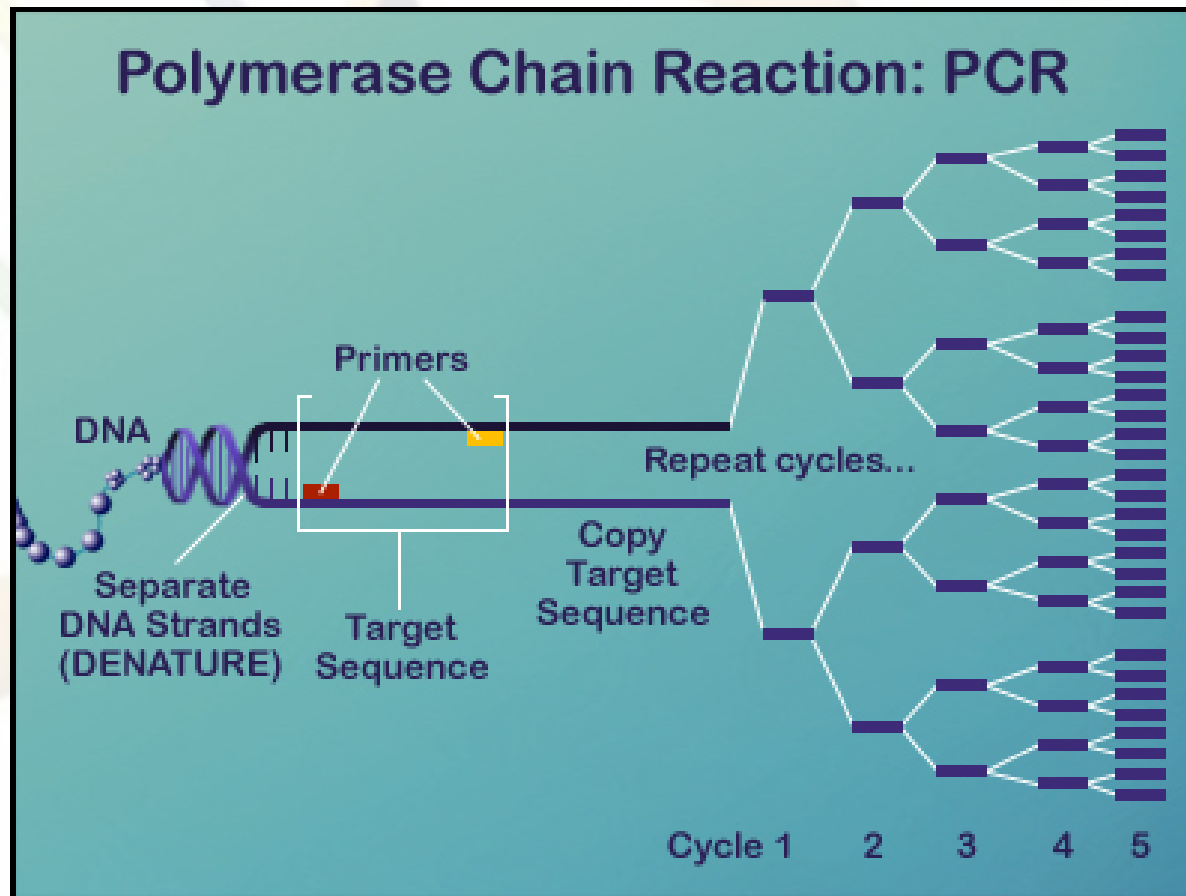
*first step: DNA extraction*

- Spin columns with silica resin →
- Magnetic beads ↓



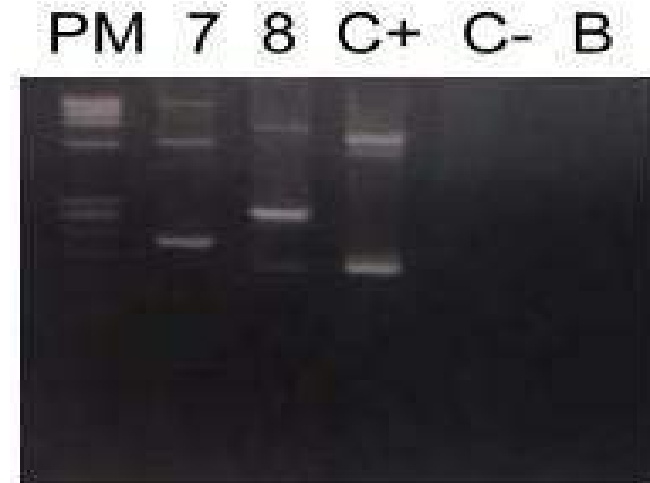
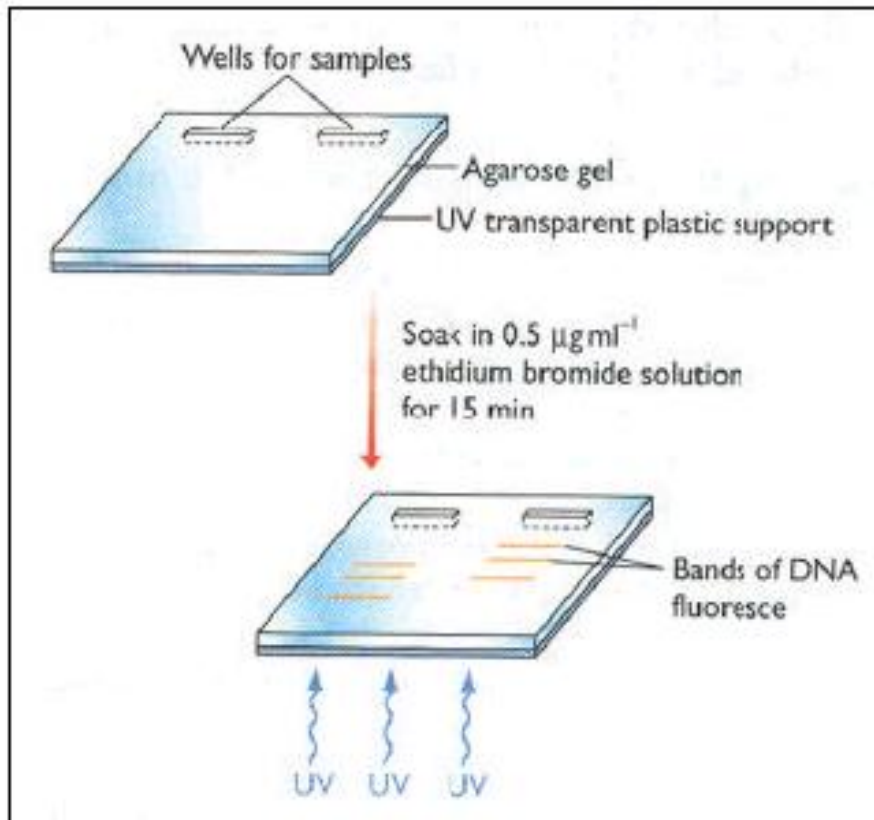
# Molecular diagnostic of HSV

*second step: DNA amplification*





# Agarose gel electrophoresis



# Molecular diagnostic of HSV

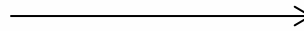
## *our experience: home-made PCR*

### 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.

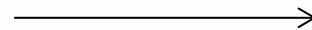
Amplicon size: 92 bp

### HSV I

Primers (UL42 region):

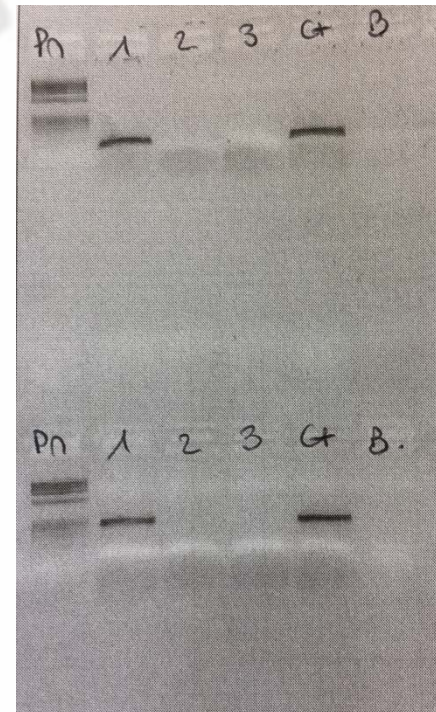
HS13 ACg ACg ACg TCC gAC ggC gA

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  $\beta$ -globin gene as control gene

# Molecular diagnostic of HSV

## *second step: DNA amplification*

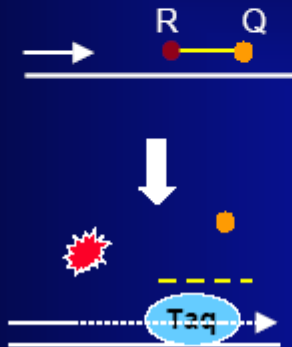
### Real Time PCR

#### Methods of fluorescence detection

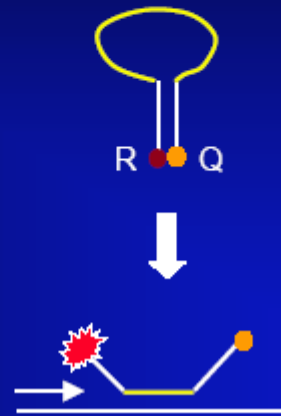
SYBR Green



Taqman



Molecular  
Beacons



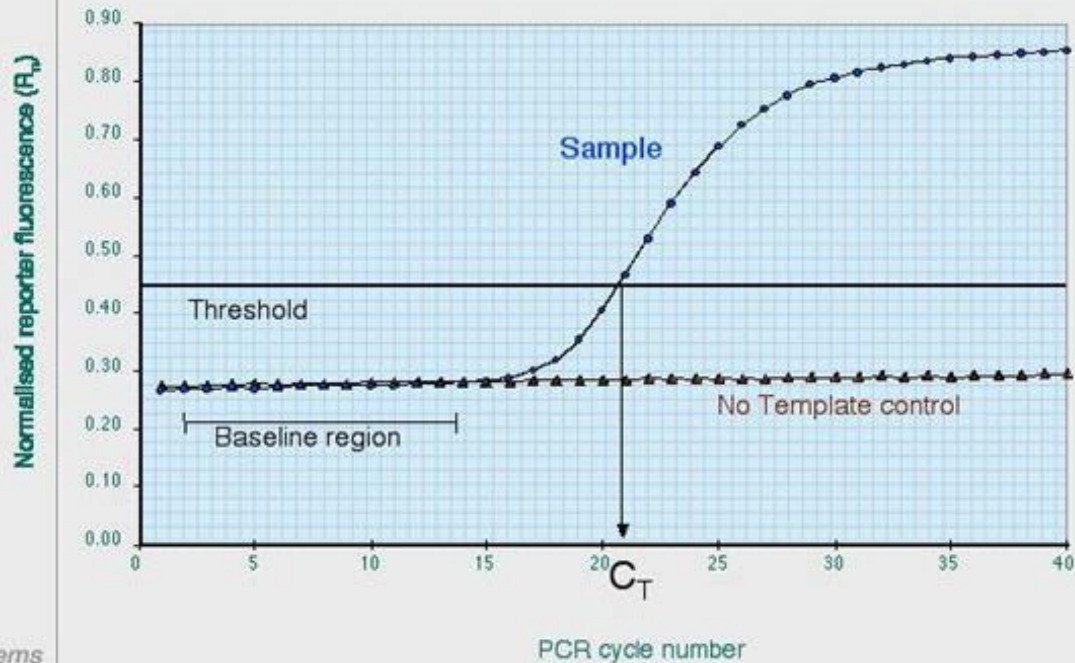
Light  
Cycler



# Real Time PCR

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

## Amplification plot features

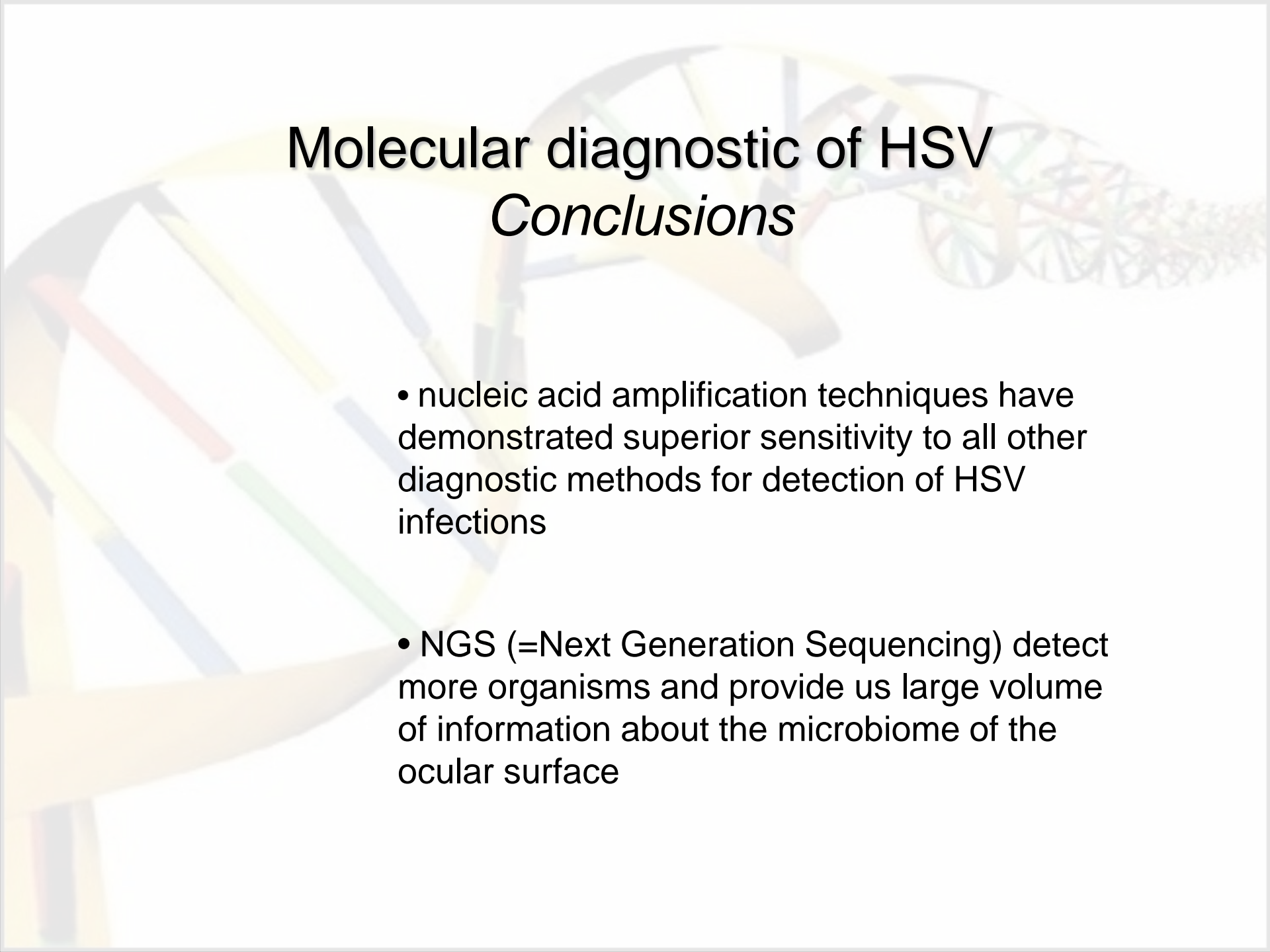




# Molecular diagnostic of HSV

## *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

