HERPES SIMPLEX VIRUSES 1 AND 2

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OBJECTIVES

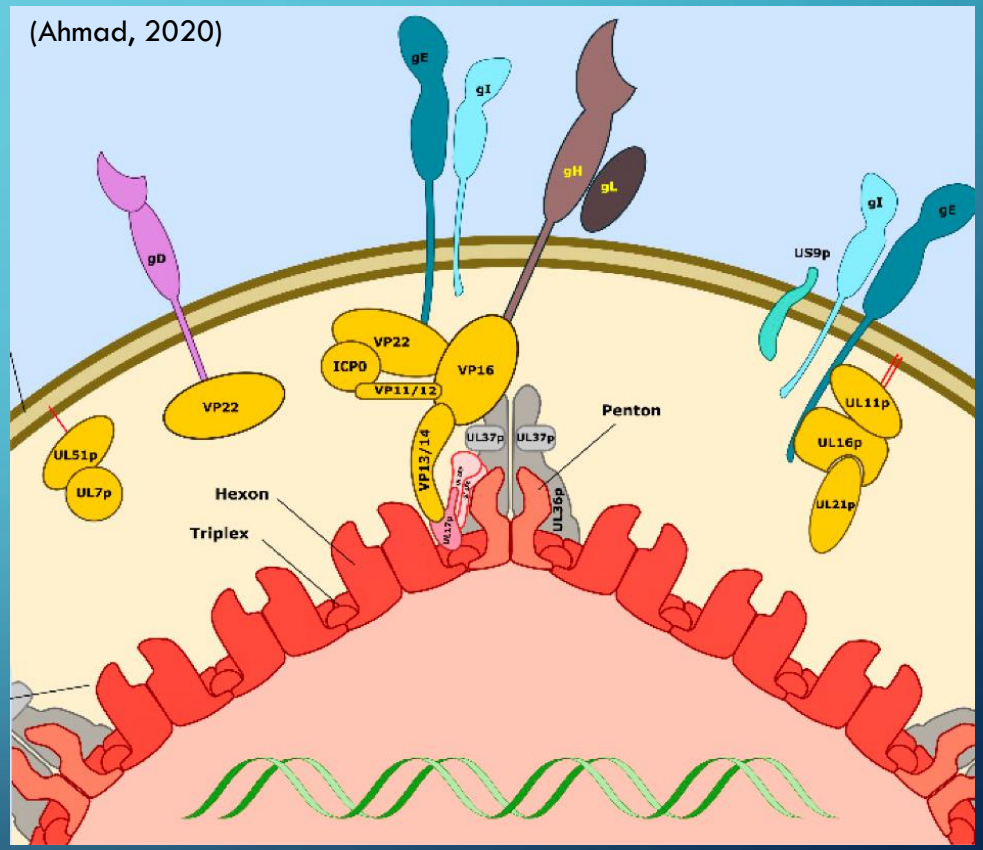
- Examine the evolution and classification of Herpes Simplex Viruses 1 and 2 (HSV-1 and HSV-2)
- Explain the symptoms, transmission, management, and prevention of infection
- Describe the testing modalities for Herpes Simplex Viruses 1 and 2 along with their indications and limitations

HERPESVIRIDAE FAMILY

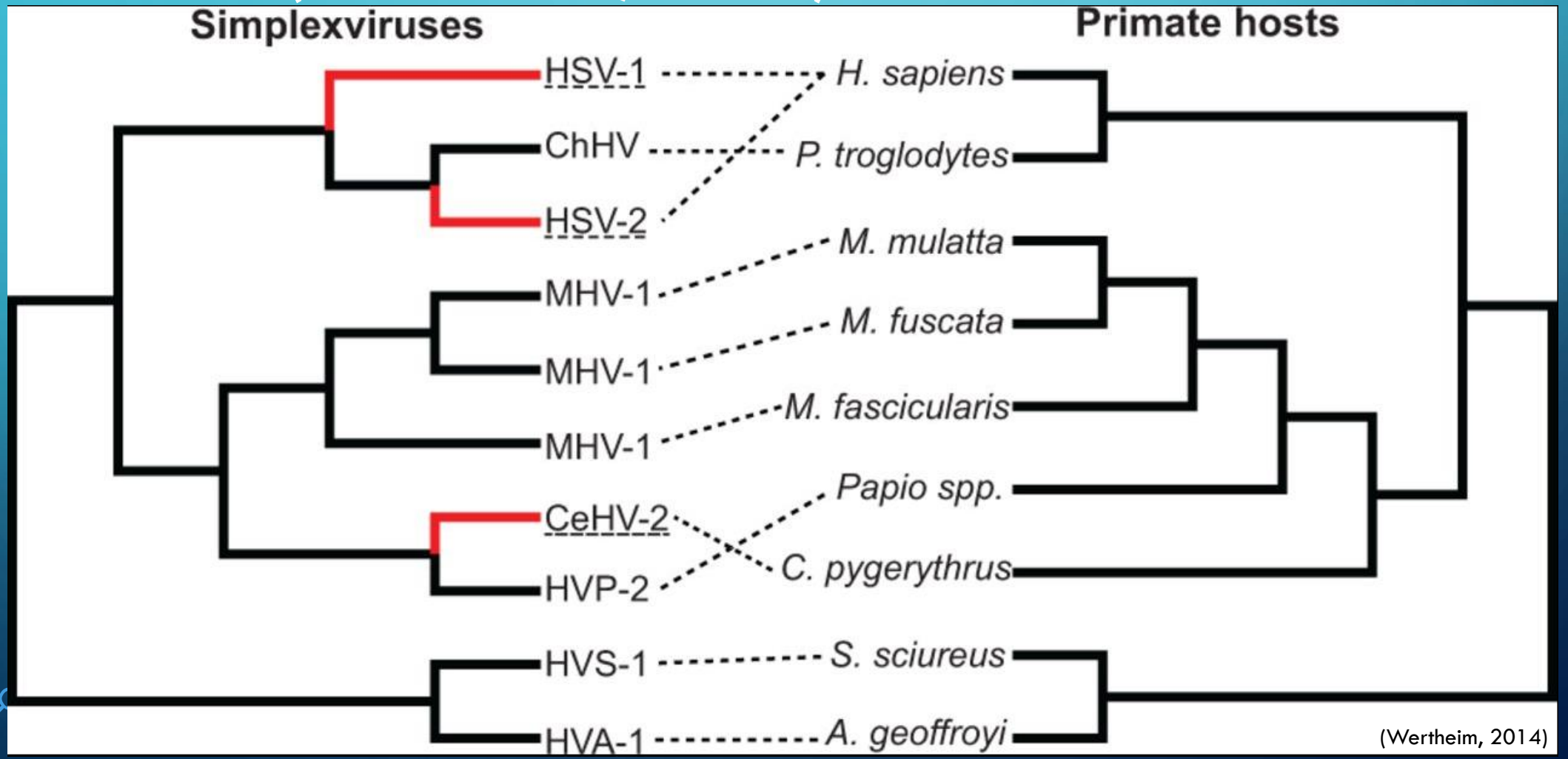
- Enveloped virus with linear, double stranded DNA (Straus, 1990)
- Includes Herpes Simplex viruses 1 and 2, Varicella-zoster virus, Epstein-Barr virus, Cytomegalovirus, and Human herpes virus 6, 7, Kaposi sarcoma-associated herpesvirus (HHV-8), Herpes B virus (Bennett, 2019)
- Divided further into subfamilies:
  - **Alpha herpes viruses: Rapid growth in many tissues, destroy host tissues**
  - Beta – slow growing in limited cell types
  - Gamma – slow growing in lymphoid cells
- Only primates infected by two herpes simplex viruses (Wertheim, 2014)

HERPES SIMPLEX VIRUS STRUCTURE

- DNA: Linear (Ahmad, 2020)
- Capsid: ~125 nm diameter icosahedral
- Envelope: Derived from host organelle with viral membrane proteins
  - Glycoproteins B and D help virus bind and enter host cells (Straus, 1990)
- Tegument: Complex multi-subunit protein layer between the capsid and envelope (Ahmad, 2020)

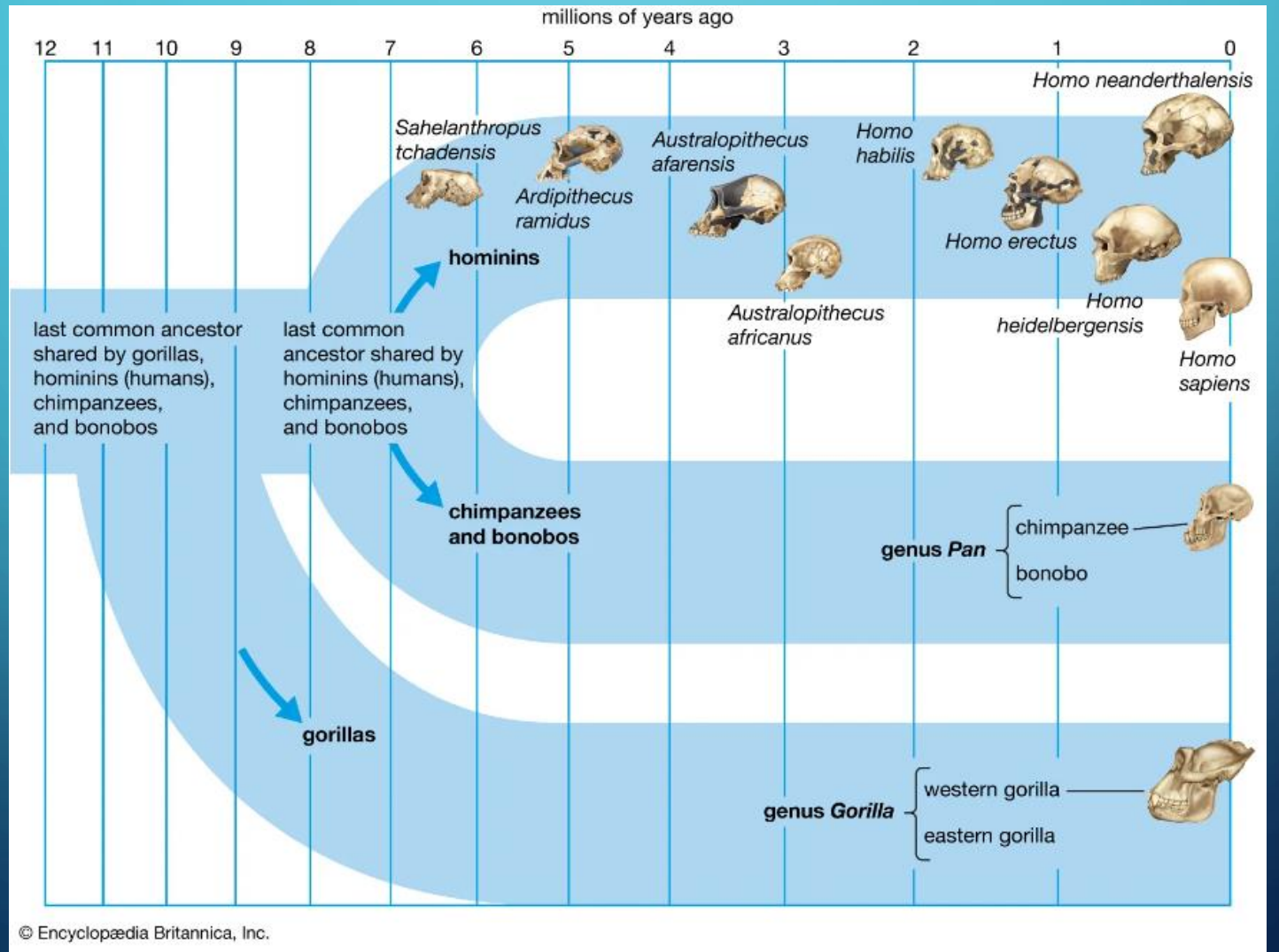


THE EVOLUTION OF THE HUMAN HERPES SIMPLEX VIRUSES (WERTHEIM, 2014)



(Wertheim, 2014)

THE EVOLUTION OF THE HUMAN HERPES SIMPLEX VIRUSES (WERTHEIM, 2014)



HERPES SIMPLEX VIRUS 1

- Primarily oral transmission (World Health Organization, 2022)
  - HSV-1 infections with genital lesions increasing in frequency, especially in young women and men who have sex with men (CDC, 2021)
- Mostly childhood infections (World Health Organization, 2022)
- Estimated 3.7 billion people <50 years old
  - Highest in low and middle income countries (Johnston, 2021)
  - Serologies >90% in sub-Saharan Africa and Latin America
- 50-70% of healthy adults in United States have positive serologies (Mandell, 2020)

HERPES SIMPLEX VIRUS 2

- Primarily sexually transmitted (World Health Organization, 2022)
- Estimated 491 million people ages 15-49
  - 11.9 % of people ages 14-49 have been infected in the United States (CDC, 2021)
- Almost 2 times more women than men (World Health Organization, 2022)
- More frequent recurrence and subclinical shedding (CDC, 2021)
- 2 to 3 fold increased risk in acquiring HIV

COMPARISON OF HSV1 AND HSV2

	HSV-1	HSV-2
Site of lesions	Primarily oral, increasingly genital	Primarily genital
Prevalence (Worldwide)	3,700,000,000	491,000,000
Prevalence (United States)	50-70%	11.9%
Associated risks		HIV, women 2x more than men
Clinical considerations	If infected by HSV-2, 3x more likely to be subclinical	More subclinical shedding, more frequent recurrence

(World Health Organization, 2022; CDC 2021, Mandell, 2020)

SYMPTOMS

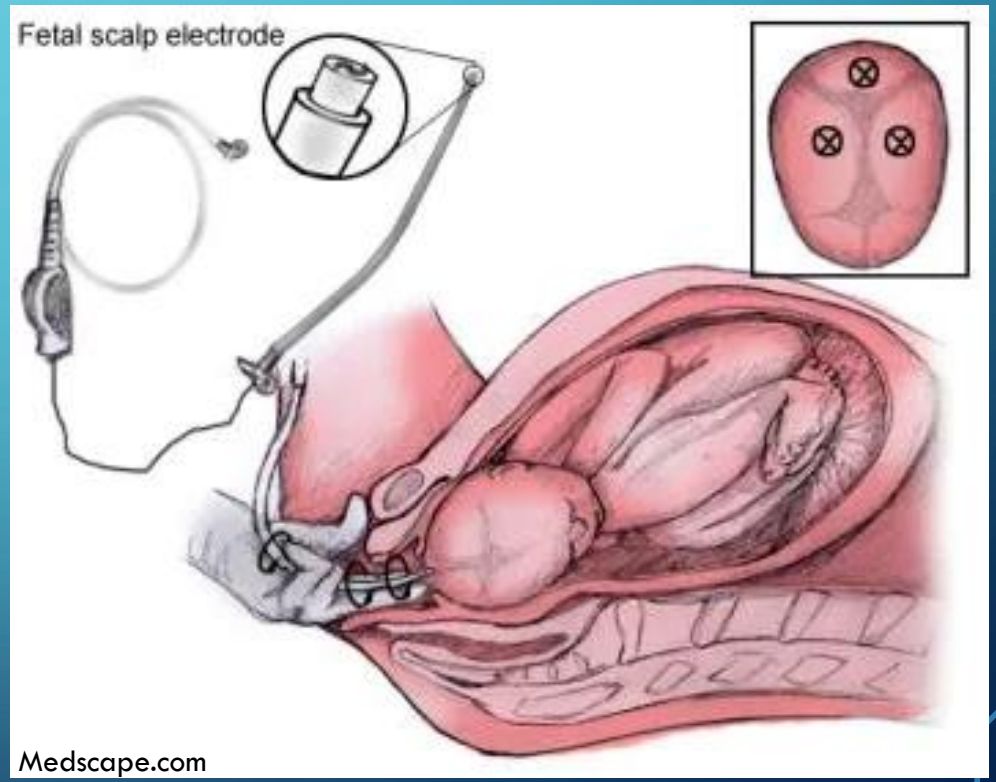
- Initial infection: Fever, body aches, swollen lymph nodes, sores (Johnston, 2021)
- Virus dormant in sensory nerve ganglions (Straus, 1990)
- Subsequent outbreaks: burning or tingling prior to sores appearing (Johnston, 2021)
  - “Cold sores”
- Encephalitis: Altered mental status, headache, seizures (Leonard, 2022)

TRANSMISSION

- Contact with the virus (World Health Organization, 2022)
  - Open sores, saliva, body fluid, mucus membranes
  - Greatest risk with active sores, but can also transmit while dormant
  - Rarely from mother to baby
    - 10 in 100,000 births worldwide

NEONATAL HERPES

- Transmission (Demmeer-Harrison (1), 2022):
  - Intrauterine: Rare, 1 in 250,000 deliveries
    - Ascending infections with prolonged rupture of membranes
  - Perinatal: 85%
  - Postnatal: 10%
- Risk (Demmeer-Harrison (2), 2022):
  - 2% if active lesions
  - 25 to 60% if first time infection
  - <37 weeks gestation, use of scalp electrodes, skin lacerations



(Demmeer-Harrison (1), 2022)

https://www.pratisandhi.com/the-aftermath-of-pregnancy/

Fetal/Neonatal demise

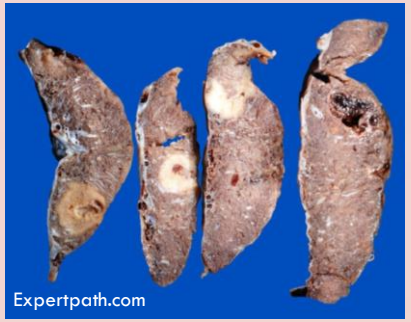
Hydrops fetalis

Viral cytopathic change

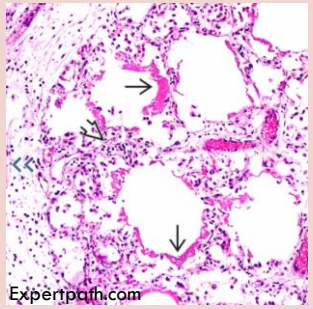
Microcephaly, hydranencephaly

Eye watering, pain, conjunctival erythema

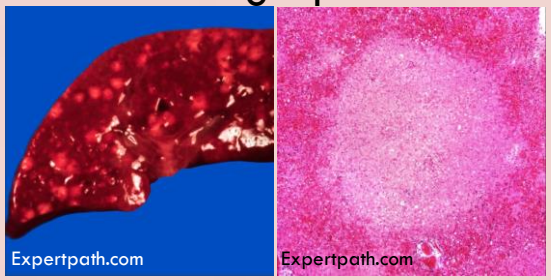
Placenta Infarcts



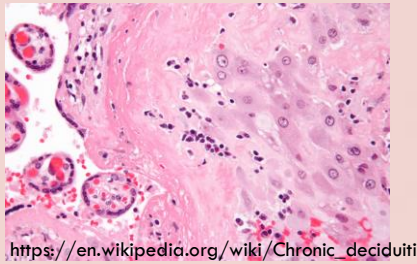
Necrotizing Pneumonia



Liver: Geographic necrosis

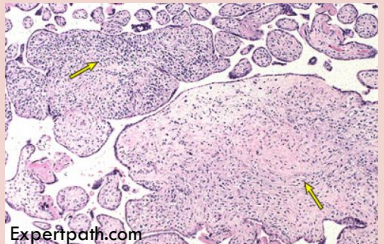


Plasma cell deciduitis



Skin sores and scars

Lymphocytic villitis



Necrotizing, calcifying funisitis

MANAGEMENT OF MATERNAL INFECTIONS

- Not recommended to screen pregnant women for HSV infections, but should collect a thorough history (CDC, 2021)
- If partner is infected by HSV and mother not previously infected, refrain from sex in the third trimester
- Cesarean delivery for active genital lesion (Preboth, 2000)
- No active lesion or prodromal symptoms may proceed with vaginal birth
- Consider antiviral therapy

TESTING, SYMPTOM MANAGEMENT, AND PREVENTION

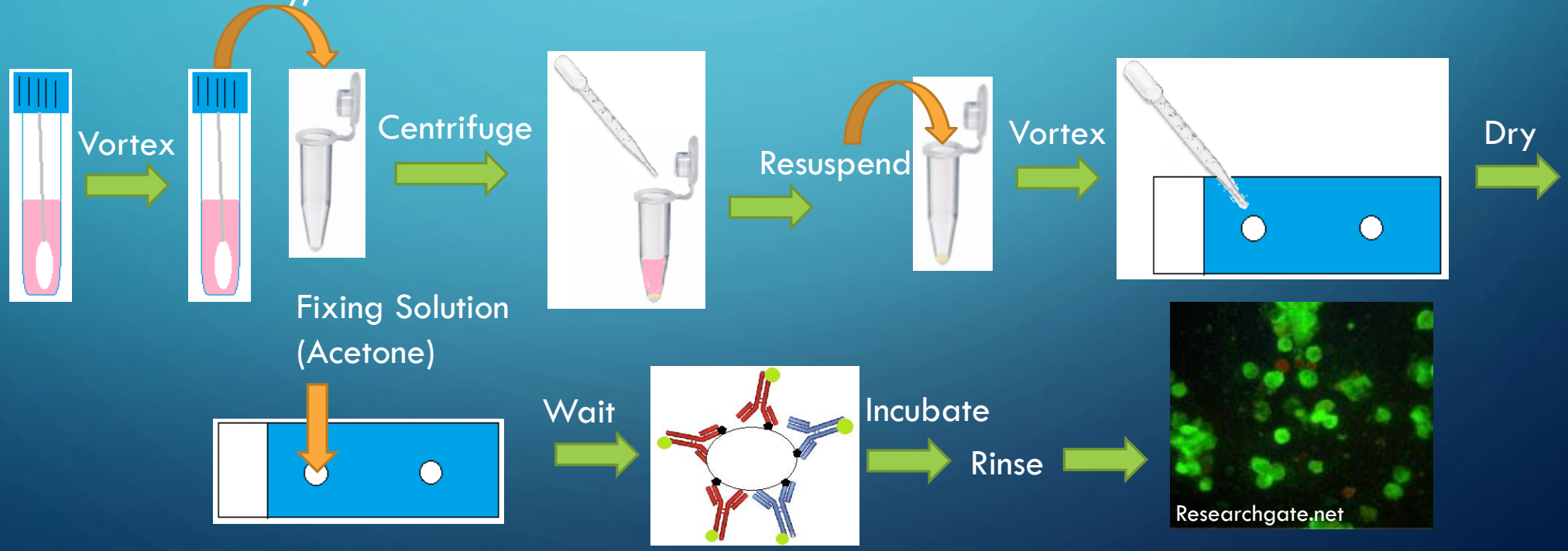
- Testing: (CDC, 2021)
  - Symptomatic, high risk populations including ≥10 sexual partners, HIV+, positive for their sexually transmitted diseases
- Symptom Management:
  - Antiviral medications: **Acyclovir**, famciclovir, and valacyclovir
  - Reduces viral shedding and allows sores to heal quicker (Pethboth, 2000)
- Prevention (World Health Organization, 2022):
  - Avoid oral or sexual contact especially with those with open sores
    - Condoms offer some protection (CDC, 2021)
  - Don't share food, beverages, or cutlery

AVAILABLE TESTING

- Current lesions (Leonard, 2022):
  - Viral culture – traditional gold-standard
    - Direct Fluorescent Antibody Stain
  - Nucleic Acid Amplification Test (NAAT)/Polymerase Chain Reaction (PCR) testing
- History of lesions in the past:
  - Serologic testing
- Immunohistochemical staining

VIRAL CULTURES

- Direct Fluorescent Antibody Stain
  - Rapid results
  - Lower sensitivity, must confirm with viral culture



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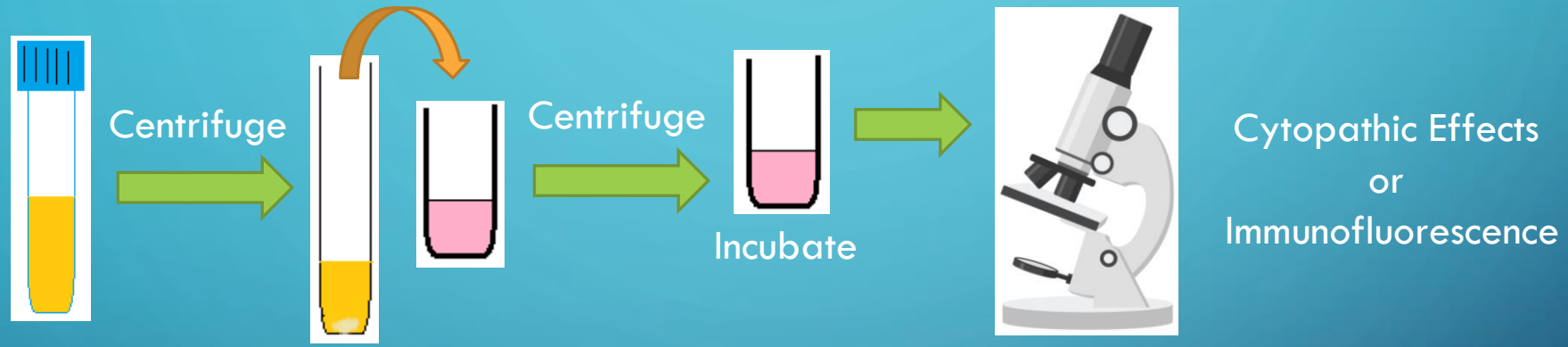
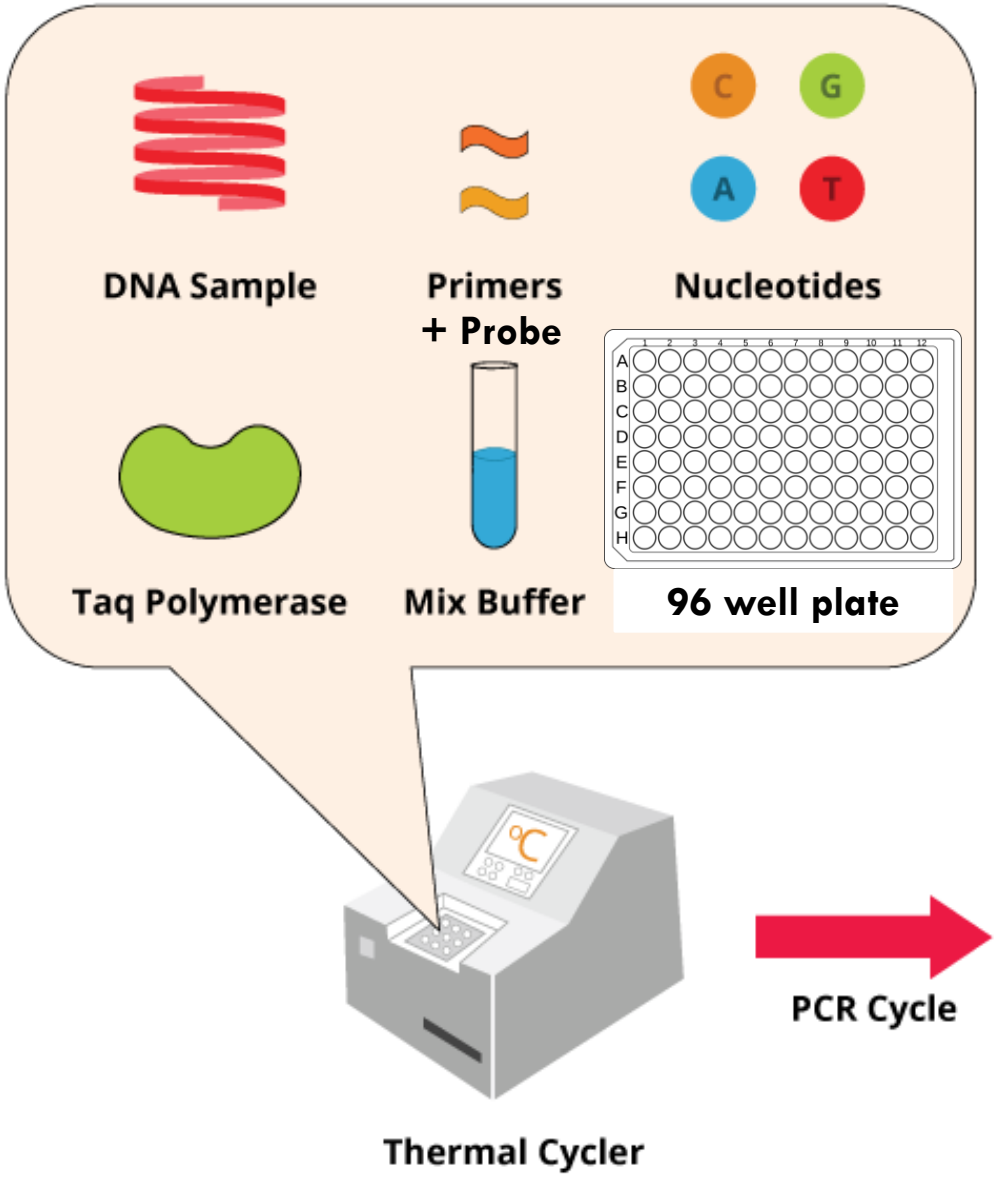
https://www.uline.com/Product/Detail/S-24320/Labware/Transfer-Pipette-7-mL?pricode=WC1579&gadtype=pla&id=S-24320&gclid=EAIaIQobChMIyOrwze77-gIVUeCGCh1abA9vEAQYBCABEgIkZ_D_BwE

VIRAL CULTURES



PCR Components



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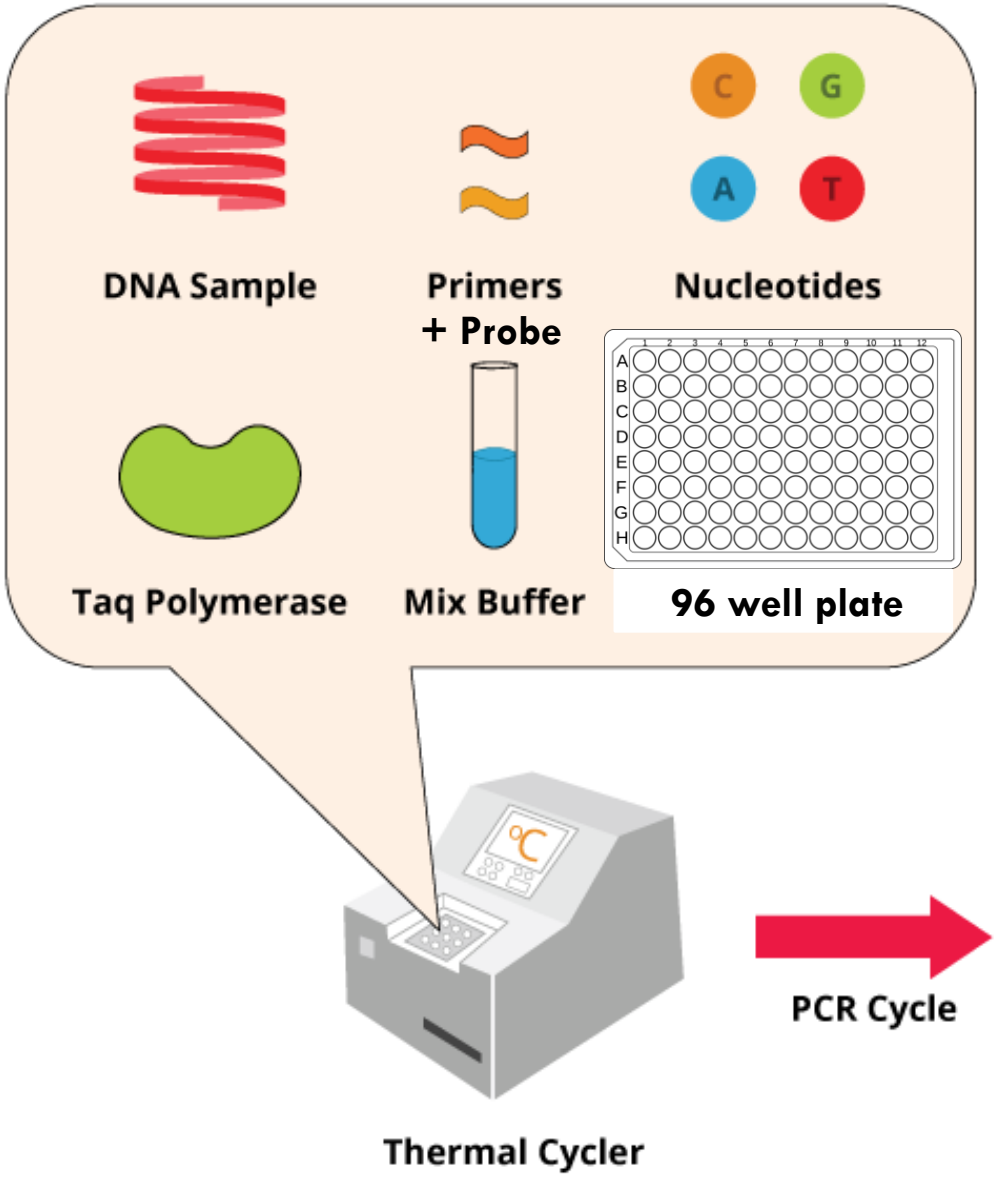
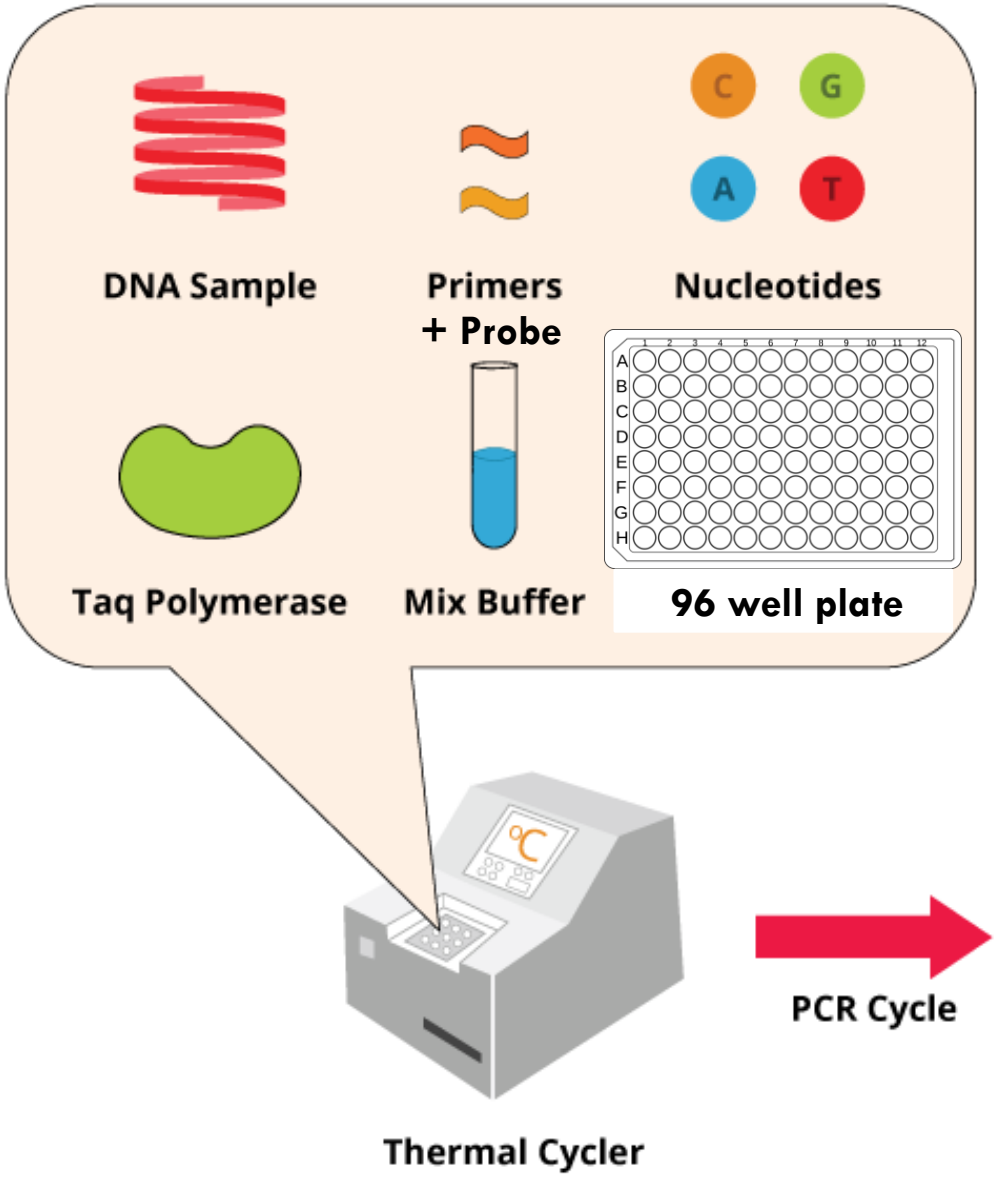


PCR Process (One Cycle)

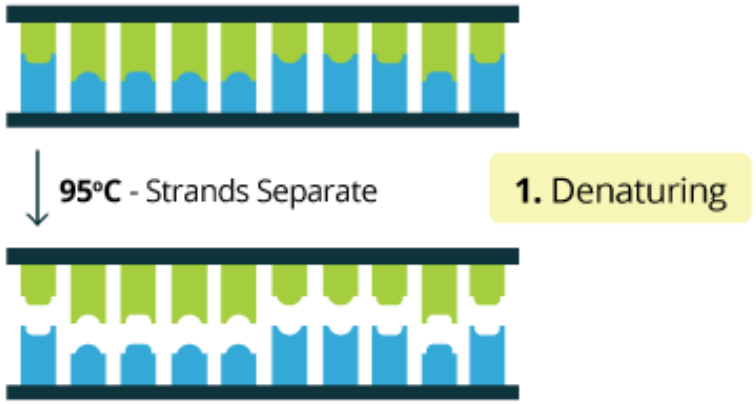
95°C - Strands Separate

1. Denaturing

PCR Components



PCR Process (One Cycle)



PCR Components

DNA Sample

Primers + Probe

Nucleotides

Taq Polymerase

Mix Buffer

96 well plate

Thermal Cycler

PCR Cycle

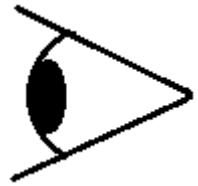
PCR Process (One Cycle)

95°C - Strands Separate

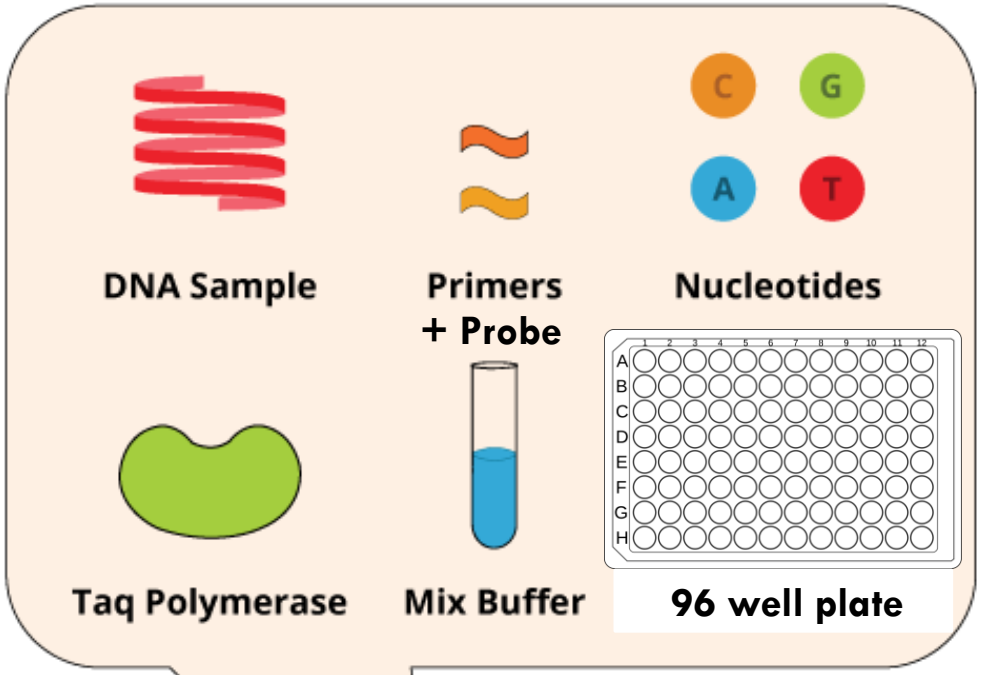
1. Denaturing

56°C - Primers Bind Template

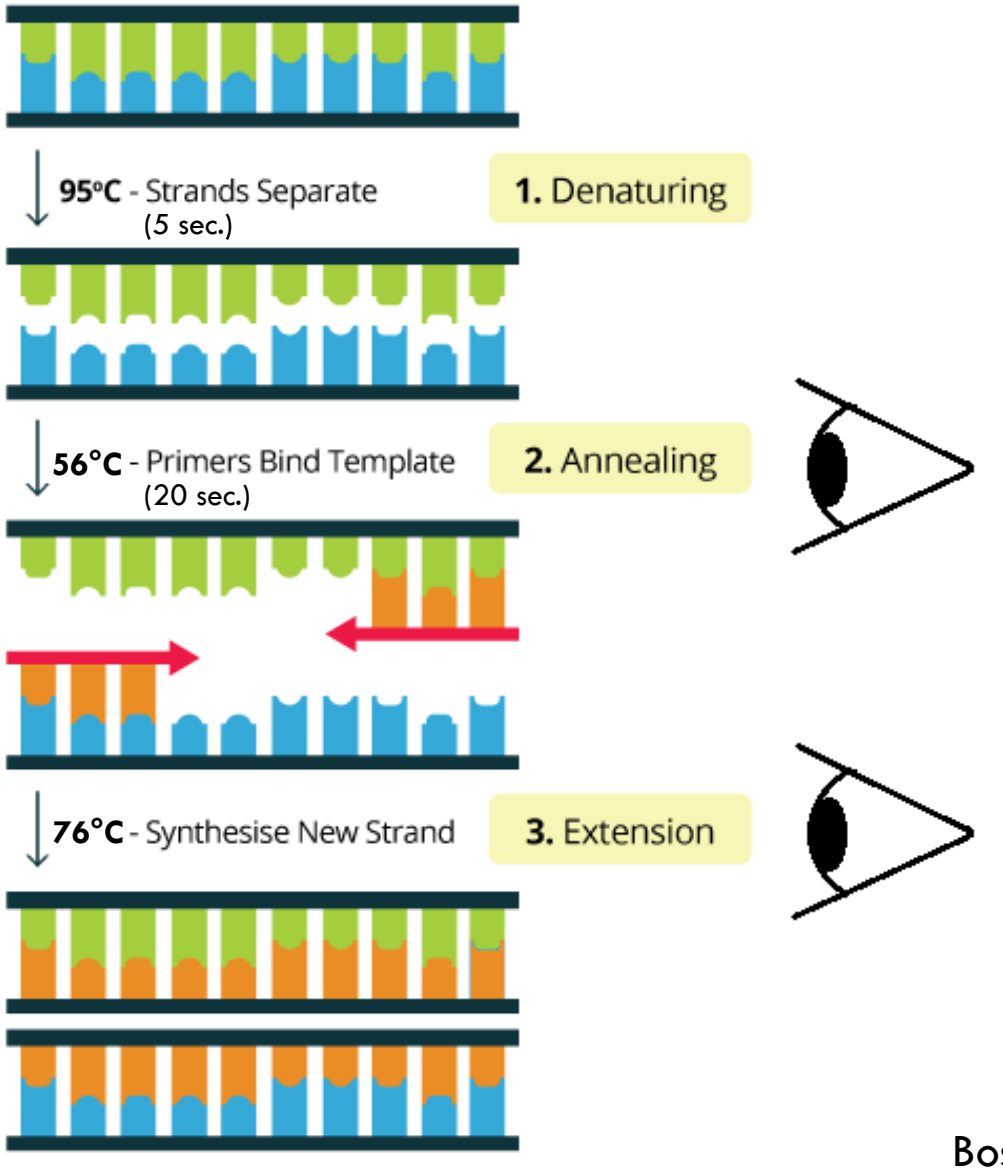
2. Annealing



PCR Components



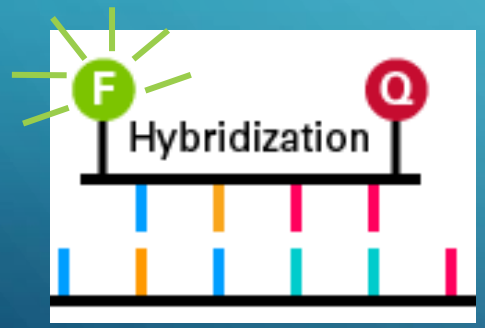
PCR Process (One Cycle)



PCR Cycle

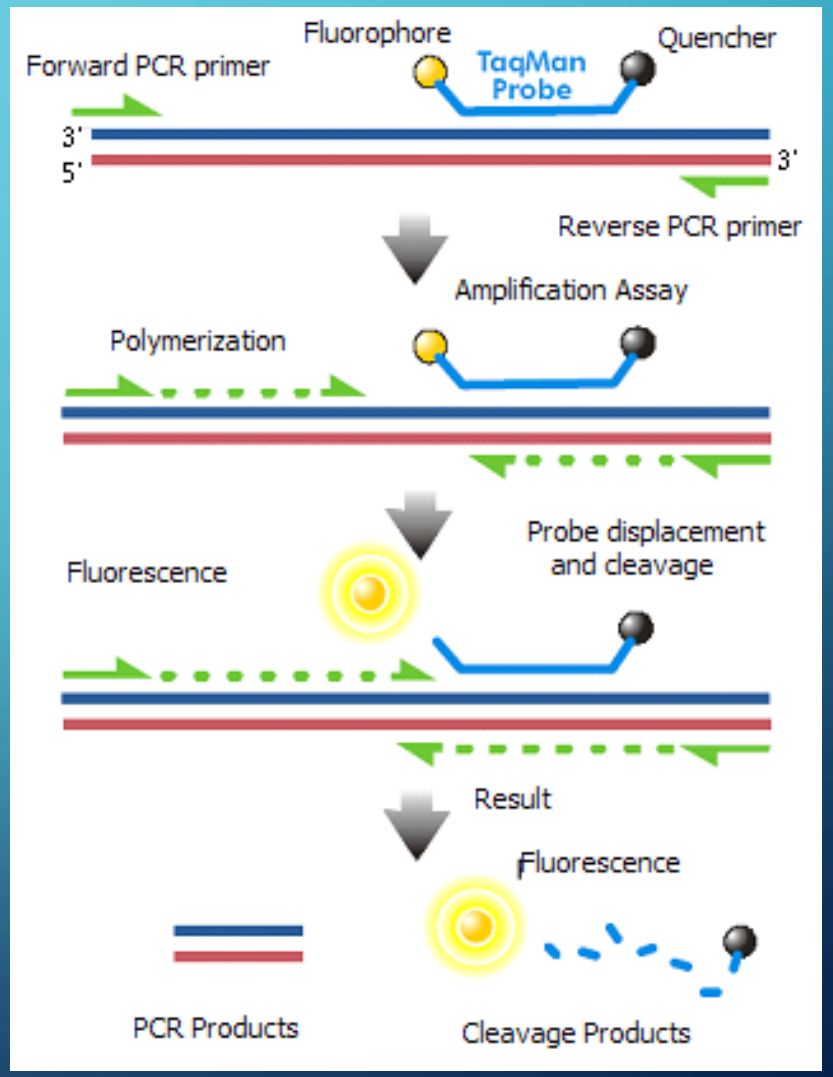
PCR TESTING PROBES

Hybridization Probe



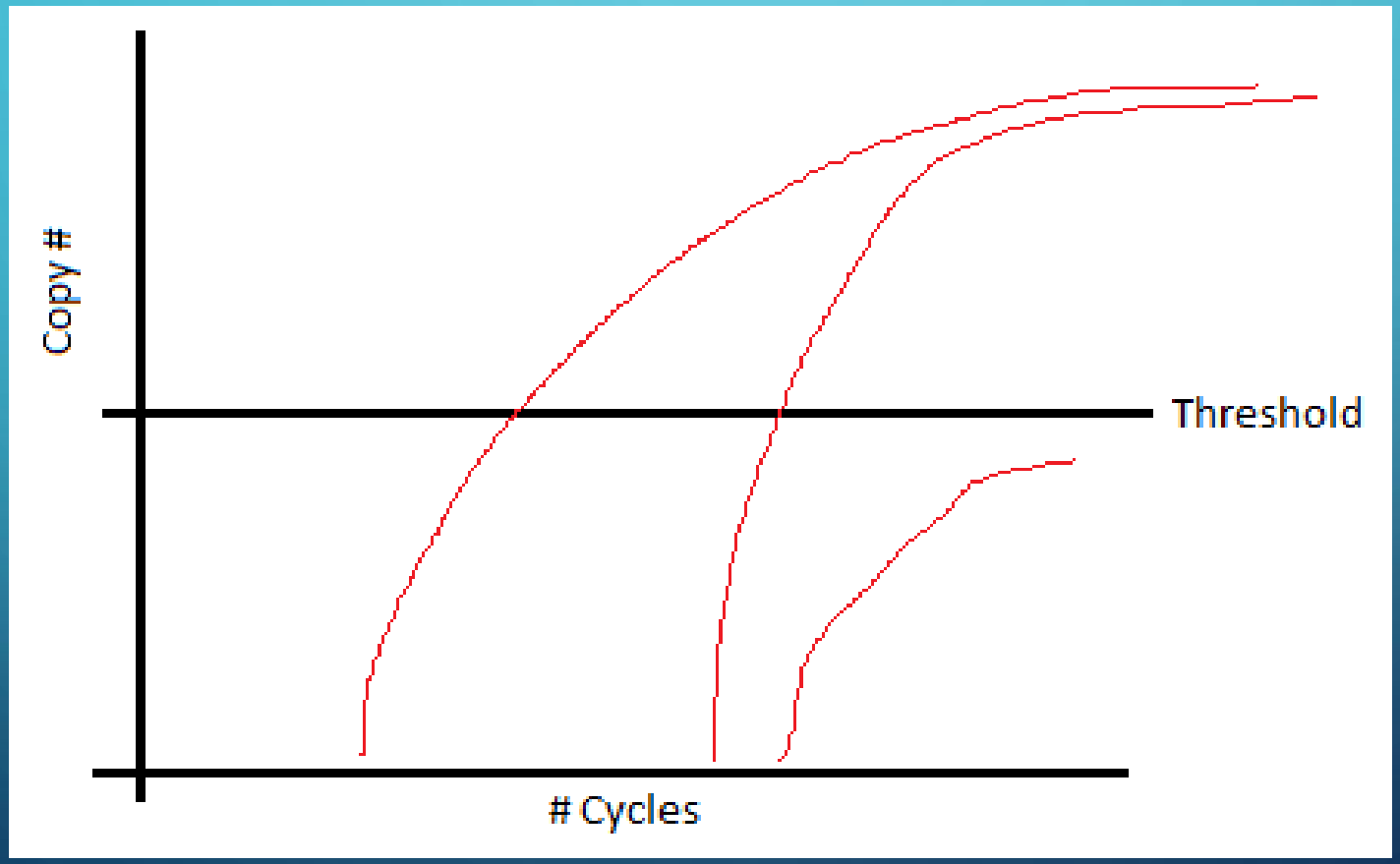
www.takarabio.com

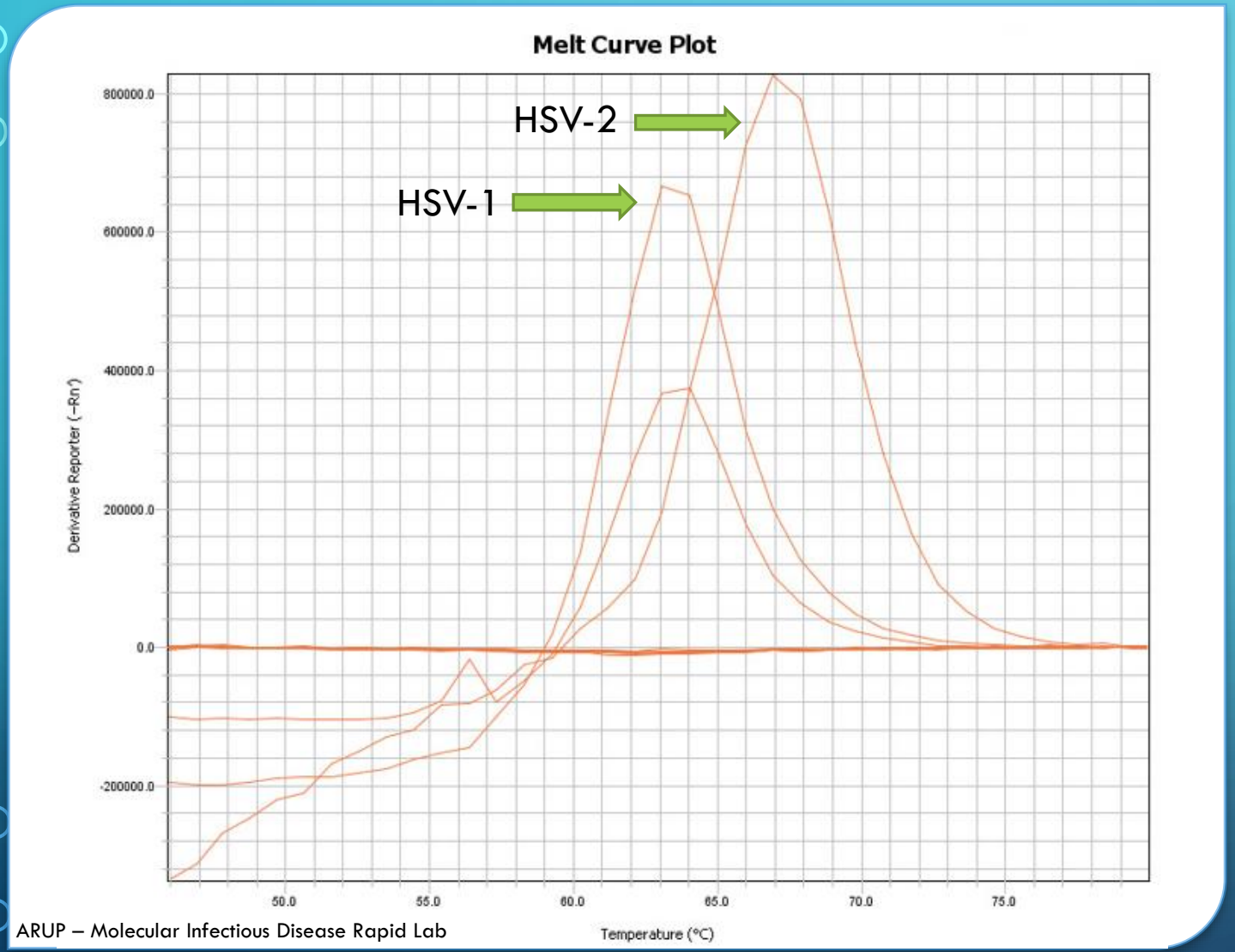
TaqMan Probe



https://en.wikipedia.org/wiki/TaqMan

PCR DATA

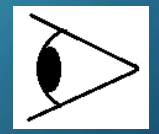




ARUP – Molecular Infectious Disease Rapid Lab

PCR TESTING - TYPING

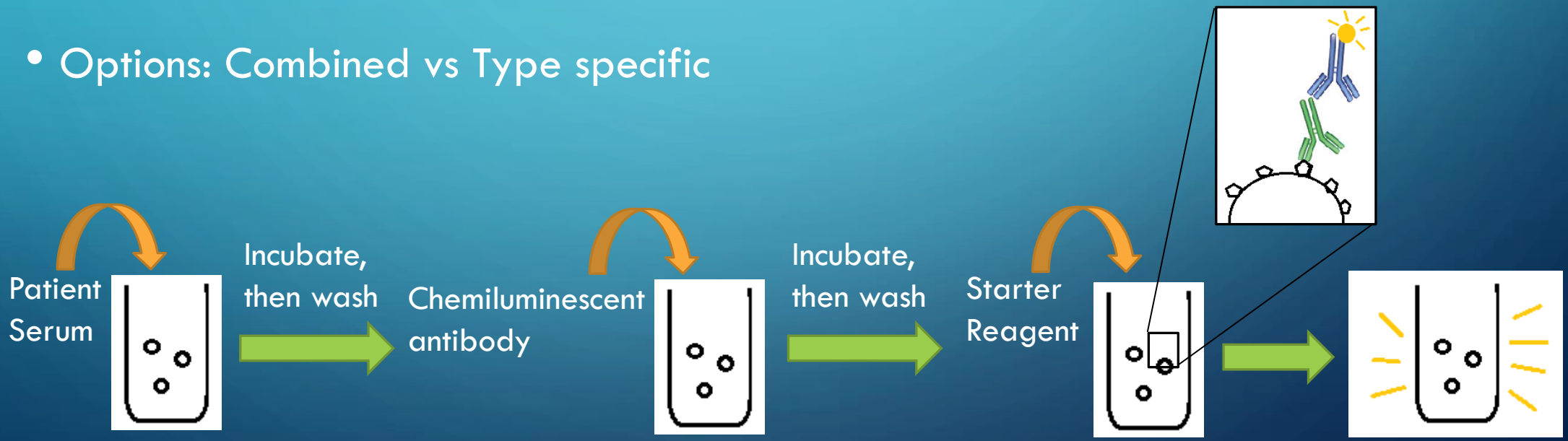
- Melting Curve Stage
  - Denature
  - Anneal
  - Slowly heat until denatured



- Better matching of probe = higher melting point

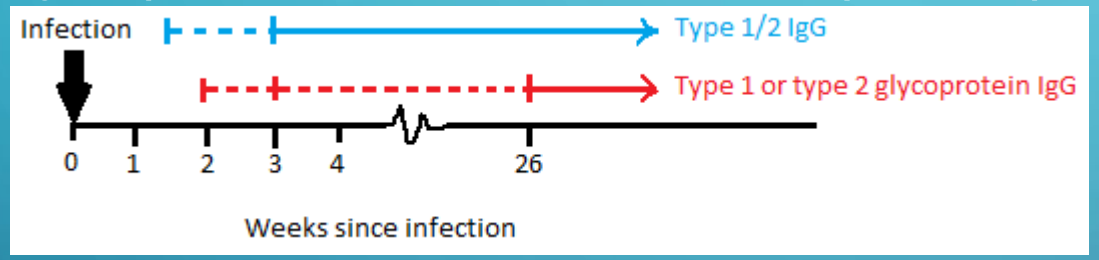
SEROLOGIC TESTING

- Chemiluminescent Immunoassay
- Do not order in neonates (Leonard, 2022)
- Options: Combined vs Type specific



SEROLOGIC TEST INTERPRETATION

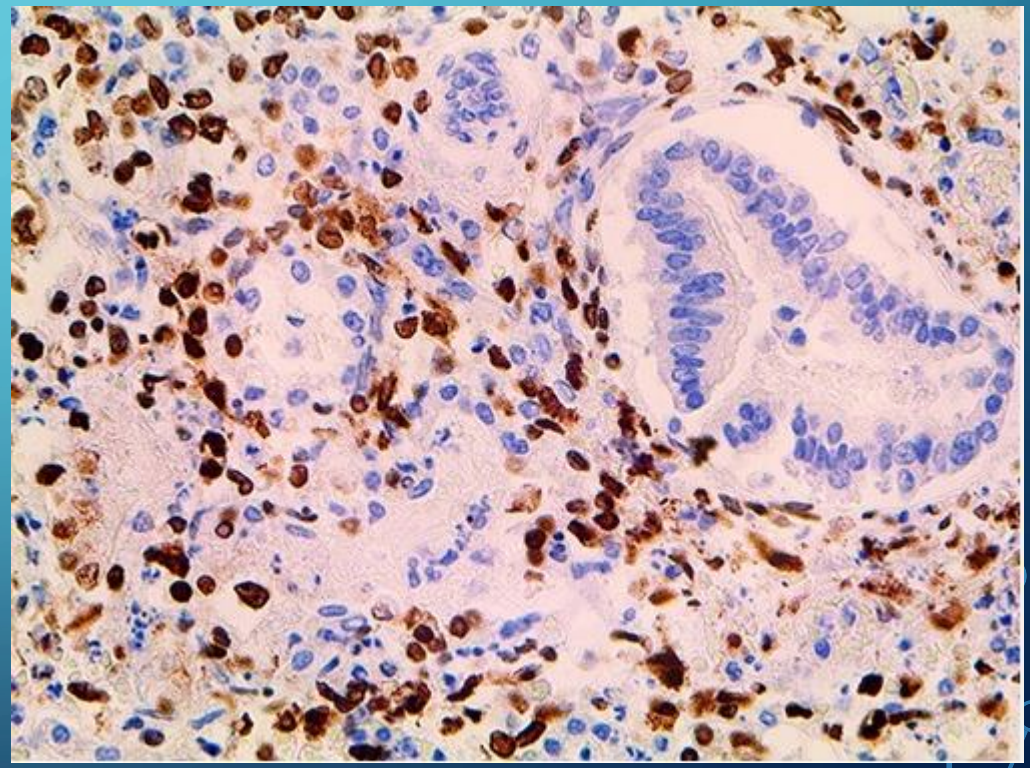
- Combined IgG develops in days to weeks (Leonard, 2022)
- IgG specific to HSV-1 or HSV-2 may take up to 6 months to form



- But why do we even need to know the type?
  - HSV-2 more subclinical shedding and recurrence
  - Epidemiologic information
- Repeat testing in one month

IMMUNOHISTOCHEMICAL STAINING

- Stain thin sections on glass microscope slides (ThermoFisher Scientific, 2022)
- Specific antigens targeted by antibodies
- Coupled to fluorophore or pick up stain to visualize
- Dark brown nuclear staining (Solomon, 2022)



https://www.lsbio.com/antibodies/hsv-antibody-herpes-simplex-virus-antibody-ihc-ls-c743555/769001

COMPARISON OF TESTING MODALITIES (Leonard, 2022)

Test Modality	Indications	Benefits	Limitations	Cost (aruplab.com)
Herpes Virus Culture	Acute infection with active lesions, especially in neonates	Specific	Time May not differentiate type False negatives late in disease	$$
DFA	Acute infections with active lesions, generally not used alone	Quick	Lower Sensitivity, must confirm negatives with cultures	$$
PCR	CSF, blood	Quick, sensitive and specific, able to determine type	False-negatives early in the disease, little data in use in neonates	$$$
Herpes Simplex Virus Combined, IgG and IgM	Blood	No active lesions needed	IgM is not clinically relevant, cross reactive Does not differentiate type False negative early on	$$
Herpes Simplex Virus type specific glycoproteins, IgG	Differentiates type to aid with treatment and counseling, blood	No active lesions needed	False negative early on, some patients never develop type specific	$
Immunohistochemistry	Paraffin imbedded tissue, body fluid		Invasive sample collection, cannot distinguish HSV1/2	$$$

SUMMARY

- Herpes Viridae family with an envelope and double stranded, linear DNA
- Symptoms: Flu-like symptoms, burning/tingling, sores involving mouth, nose, eyes, or genitals
- Transmission: Contact with infected person, more likely if symptomatic
- Symptom Management: Acyclovir
- Testing: DFA, viral culture, PCR, serology, immunohistochemical stain

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