بسم الله الرحمن الرحيم

Laboratory Diagnosis of Viral Infections affect the urinary and Genital Tract

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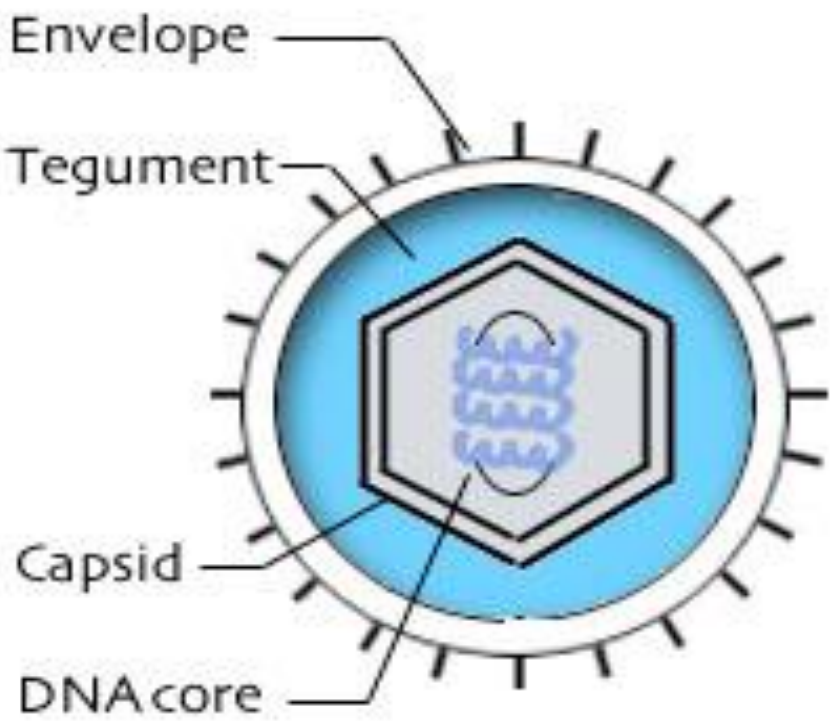
HERPES SIMPLEX VIRUS (HSV)

- **HSV 1 infect the upper part of the body**
  - **mouth and the face**
- **HSV 2 infect the lower part of the body**
  - **genital infections**
- **There is little cross protection**

VIRAL STRUCTURE



Core: Consists of a single linear molecule of dsDNa in the form of a torus

Capsid: Surrounding the core w/ a 100 nm diameter and 162 capsomeres

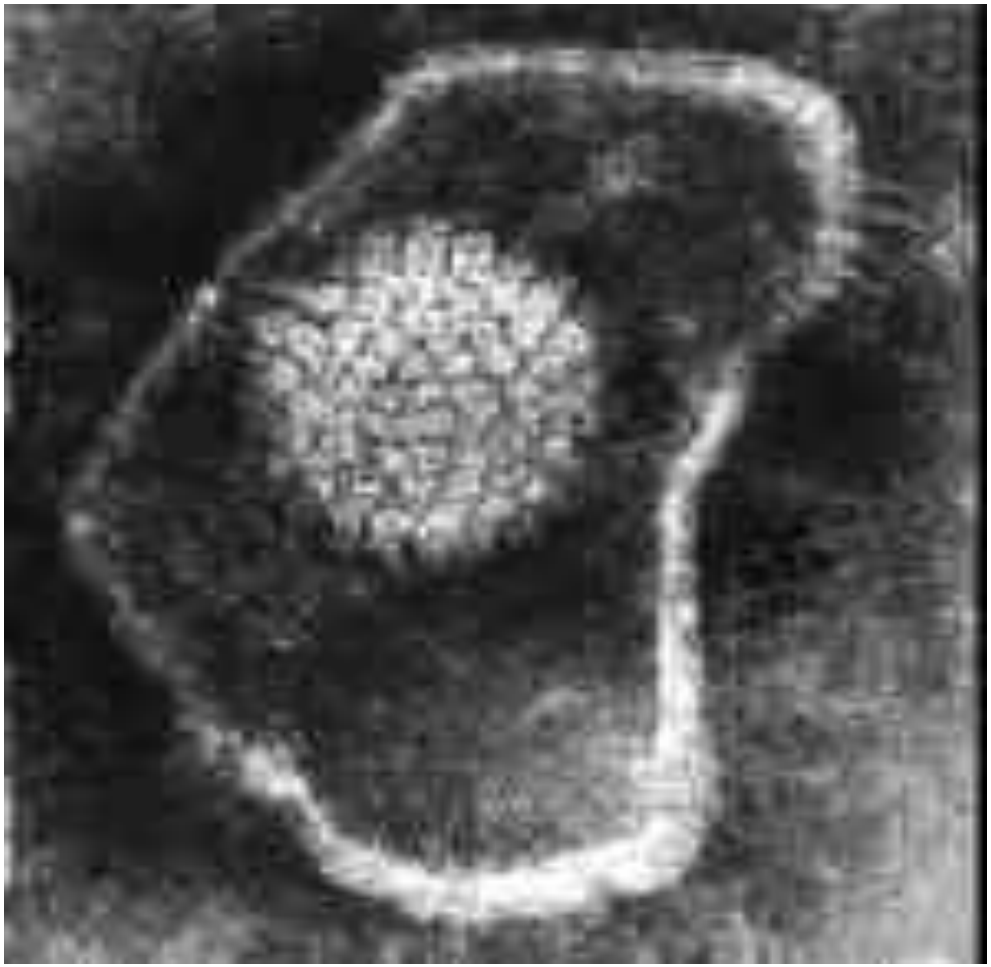
Tegument: Consists of viral enzymes

Envelope: Outer layer composed of altered host membrane and a dozen of viral glycoproteins

Virus Structure

Enveloped, slightly pleomorphic
Spherical
120 – 200 nm in diameter

Capsid
Envelope
Tegument
Genome
double stranded DNA per virion



Herpesviridae

- **Characteristics**
  - **large enveloped, double stranded DNA viruses**
  - **genome encodes for proteins which regulate viral m-RNA synthesis by the cells DNA dependent RNA polymerase**

  DNA replication and assembly occur in the nucleus
  - **virus buds through the nuclear membrane, and is released from the cell by exocytosis or by lysis**
  - **herpesviruses infections can result in lysis, latent persistence, and oncogenesis(immortalization)**
    - **as a group they have a significant tendency toward latent persistence in semi-permissive cells**
  - **viruses in this group are very common**

Herpesvirus Cycle

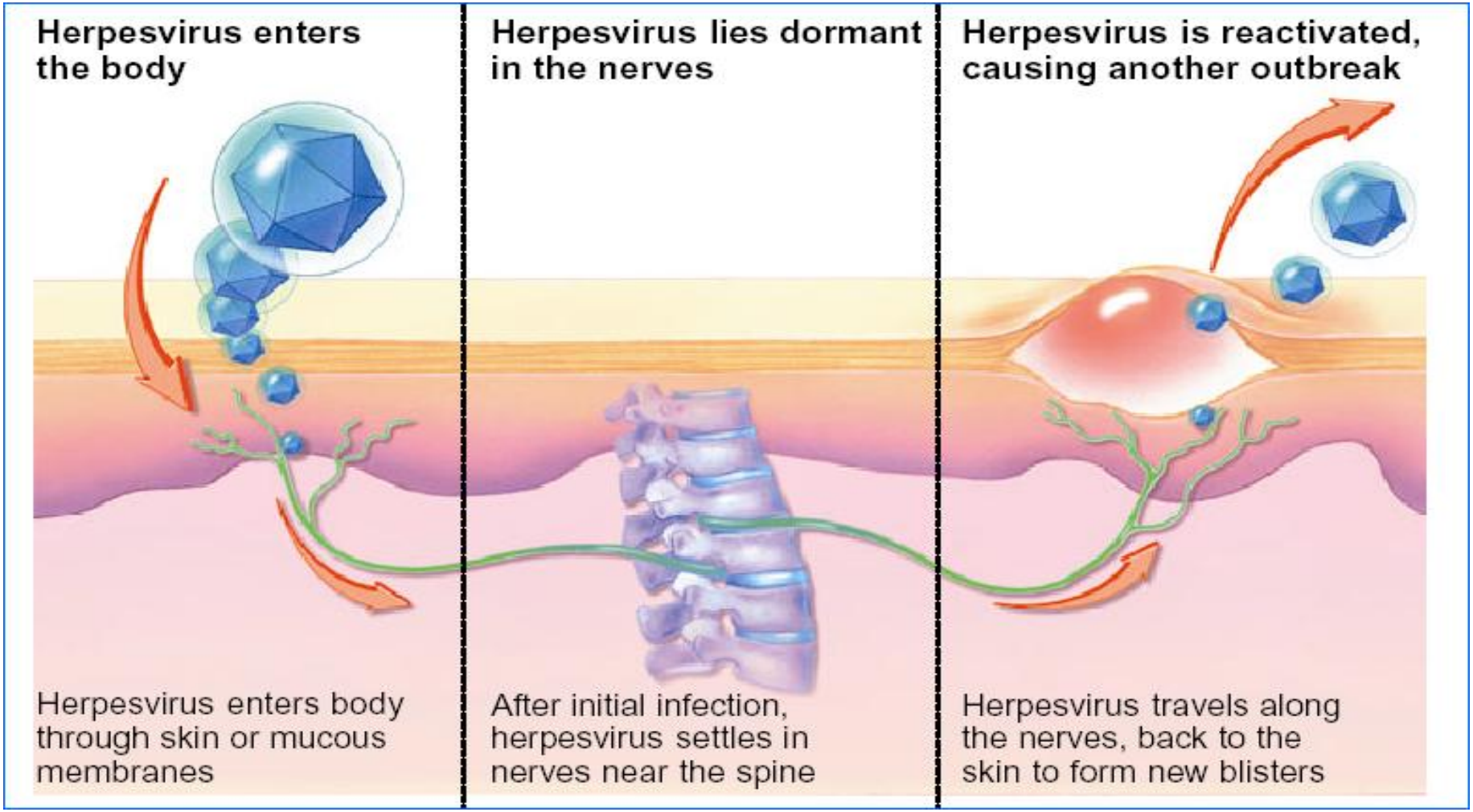
- **Virus Cycle**
  - viral glycoproteins(VAP's) bind virus to host cell receptors
  - virus fuses with the host cell membrane; this removes the envelope and releases then nucleocapsid into the cytoplasm
  - nucleocapsid binds to the nuclear membrane and releases the genome into the nucleus of the host cell
  - early proteins facilitate transcription of viral genome and include the DNA dependent DNA polymerase; viral genome is transcribed by the cellular DNA dependent RNA polymerase
  - late proteins are structural and are synthesized after DNA is replicated
    - viral genome replication requires viral DNA dependent DNA polymerase
  - cells that promote latency restrict viral transcription of early and late proteins
  - cells that complete early and late protein synthesis will die
  - viruses are assembled in the nucleus and bud through the nuclear membrane
  - viruses exit the cell via exocytosis or via cell lysis

Subfamily	Genus	Common Name
Alphaherpesvirinae	Simplexvirus	Herpes simplex virus - 1 (HSV-1)
		Herpes simplex virus - 2 (HSV-2)
	Varicellovirus	Varicella-zoster virus (VZV)
Betaherpesvirinae	Cytomegalovirus	Cytomegalovirus (CMV)
	Roseolovirus	Human herpesvirus 6 (HHV-6)
		Human herpesvirus 7 (HHV-7)
Gammaherpesvirinae	Lymphocryptovirus	Epstein-Barr virus (EBV)
	Rhadinovirus	Human herpesvirus 8 (HHV-8)

	Biologic Properties	
Subfamily ("-herpesvirinae")	Growth Cycle and Cytopathology	Latent Infections
Alpha	Short, cytolytic	Neurons
Beta	Long, cytomegalic	Glands, kidneys
	Long, lymphoproliferative	Lymphoid tissue
Gamma	Variable, lymphoproliferative	Lymphoid tissue

- **Primary infection** -first contact with HSV
- **Latent infection** -persistent virus in root ganglia
- **Reactivation** -production of infective virus by latently infected cell
- **Recurrence** -clinically apparent disease produced by reactivation

Virus Latency



HSV-2 infections

- **Not synonymous with genital herpes however:**
  - **These are primarily infections of the genital tract**
  - **Neonates can be infected at the time of delivery**
  - **Extra genital lesions do occur**
  -
- **Sexual transmission occurs during vaginal, anal and oral sex (although the latter are more often HSV1 viruses)**

- **The prevalence of HSV-2 antibodies in the population depends on the experience of the patient population.**

Laboratory diagnosis of HSV

Direct staining

Tzanck test

Immunostaining

HSV isolation

Serology

PCR

Tzanck test

Cell scrape from base of the lesion

smear on slide

↓

Staining

Wright-Giemsa, Giemsa

↓

Ballooning cell with intranuclear inclusion

multinucleated cell

Tzanck test

Multinucleated cell

Immunofluorescent staining

Cell scrape, smear
fix in cold acetone

↓

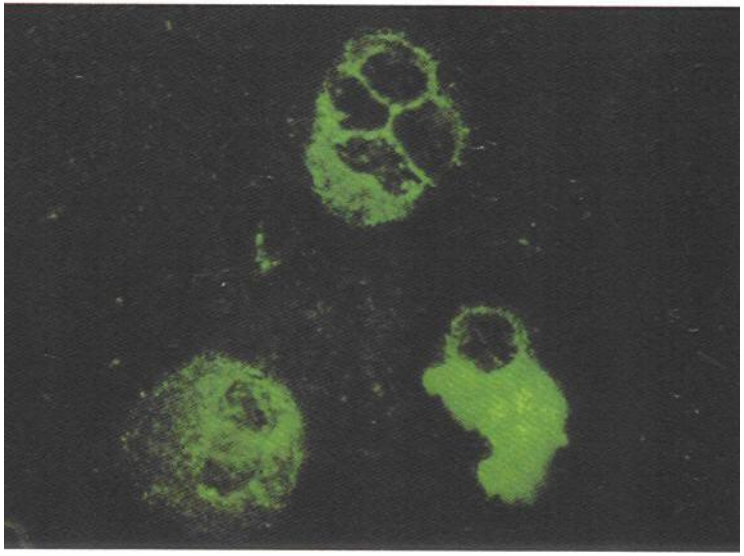
rabbit anti-HSV Ig

↓

goat anti-RaIg conjugated
with fluorescein dye

↓

mount with glycerine buffer

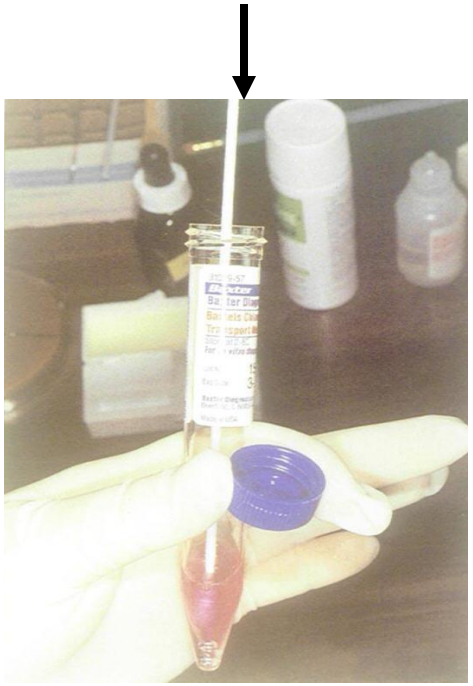


Specimen collection

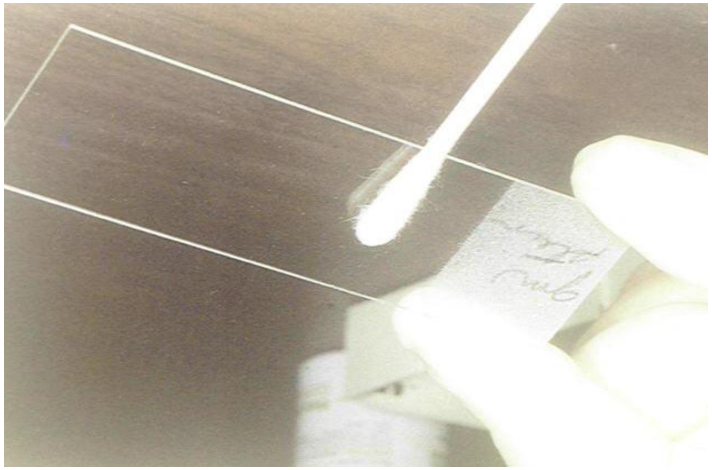
Samples :

vesicle fluid,

lesion swab



Transport media



Smear on slide

Transport media

Isotonic solution or culture media

Protein — **bovine serum albumin**, **bovine serum**

Antibiotics

streptomycin **penicillin** **gentamycin**

Anti-fungus — **amphotericin B**

Viral isolation

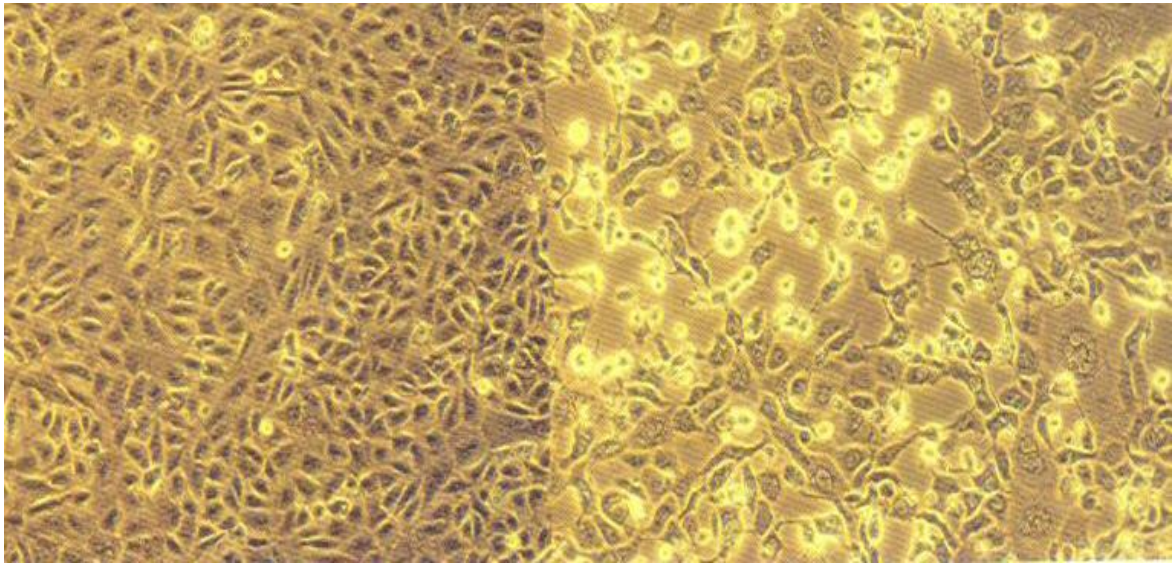
Specimens → **Cell culture (human diploid cells, Vero cells, Hela cells)**

Cytopathic effect
(rounded, enlarged and multinucleated cell)

Identification or typing

***Immunofluorescent staining**

HSV Cytopathic effect



Normal cells **CPE**

Serological test for HSV infection

Immunofluorescent staining

Complement fixation test

ELISA : **IgM capture test**

IgG test

HSV serology

Primary infection

Pair serum: **acute & convalescent serum**

IgG assay ***rising titer** → **≥ ٤ times**

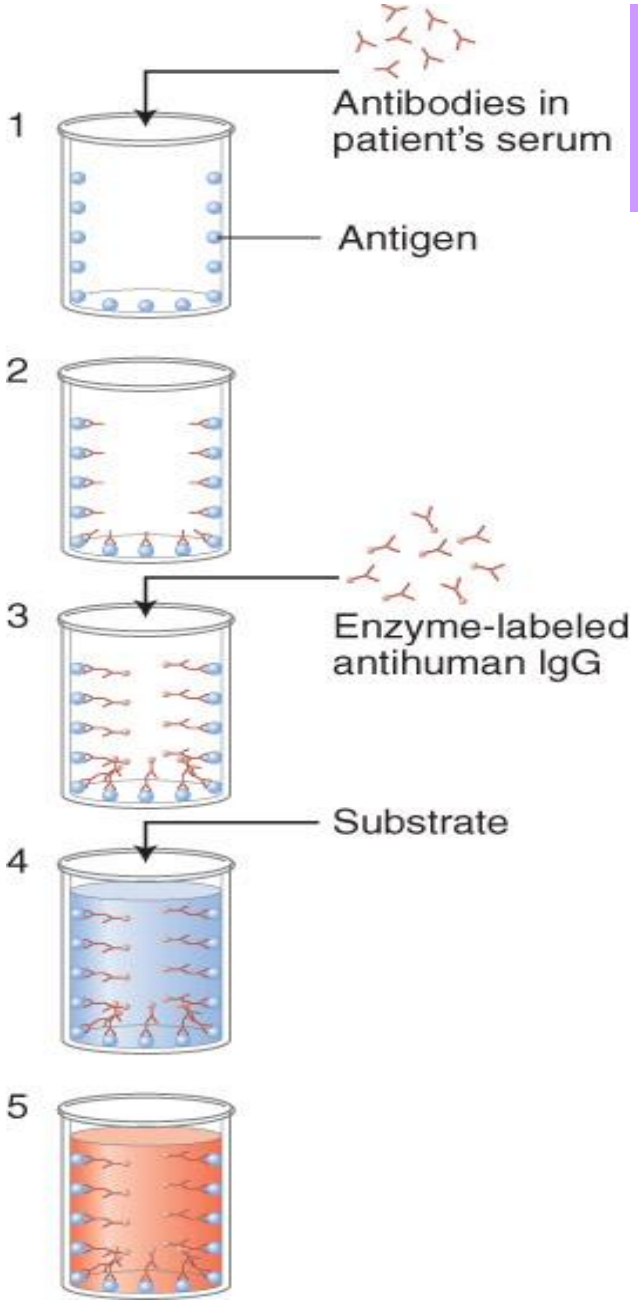
***seroconversion**

Single serum: **IgM assay**

Recurrent infection

not useful; **multiple reactivation**

IgM capture ELISA



Substrate+chromogen

Enzyme labeled anti-viral antibody

HSV antigens

Tested sera (IgM)

Anti-m chain capture Ab

Polymerase chain reaction

Samples

infected cell, vesicle fluid,CSF

↓

DNA extraction

↓

PCR solution

(buffer, dNTP,Taq DNA pol, primres)

↓

Amplify ๒๐-๓๐ cycles

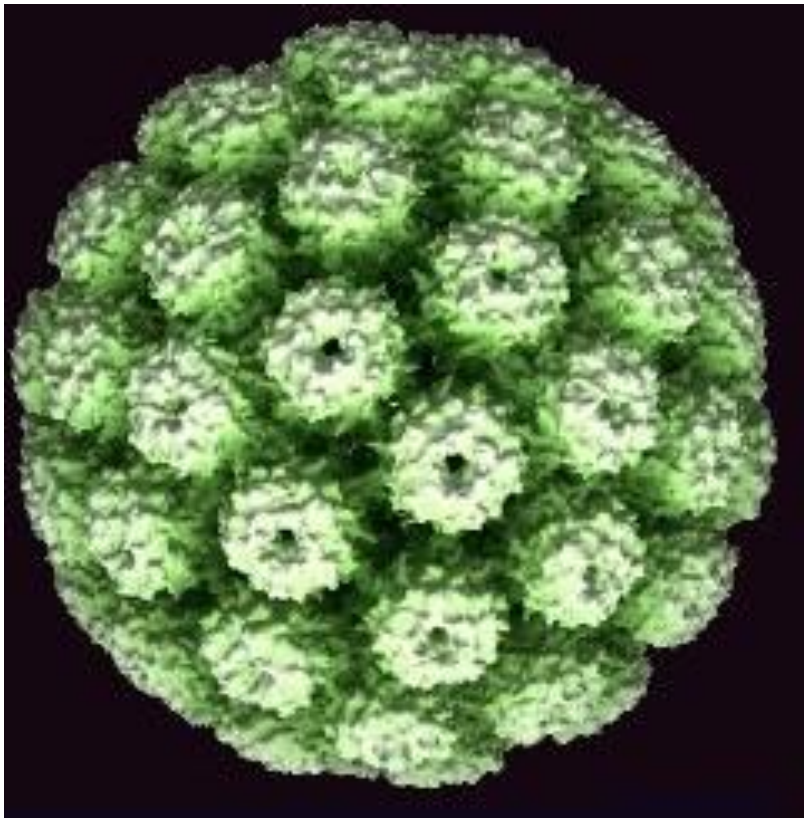
Multiplex primers;

- **cutaneous group; HSV, VZV**
- **lymphotropic group; CMV,**

Detection:

- **gel electrophoresis**
- **dot blot hybridization**
- ***restriction fragment length polymorphism**

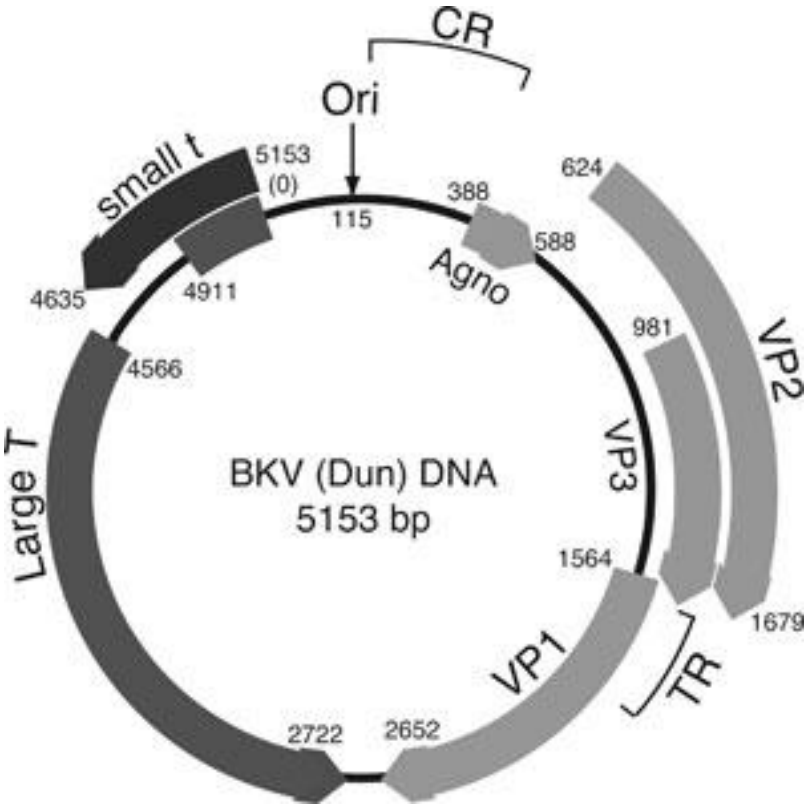
BK Virus - virology



SV40 (www.steve.gb.com/images/science/sv40.jpg)

- **Polyomavirus (related to JC, SV40)**
- **small (30 to 45 nm), icosahedral, nonenveloped**
- **double-stranded, circular DNA, around 5000bp**
- **6 viral genes**

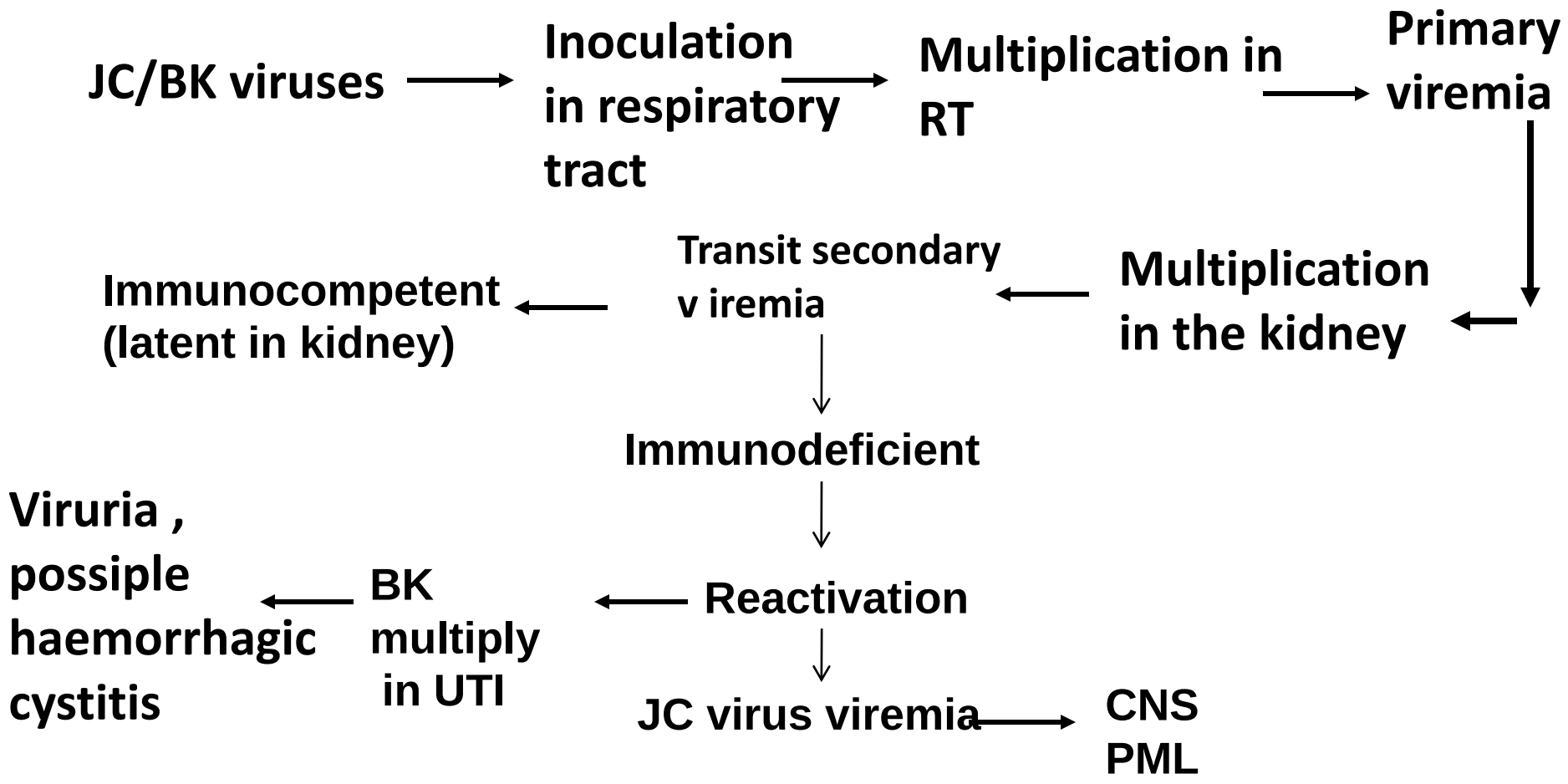
BKV genomic organization



- **Structural genes (capsid proteins)**
  - **VP1, VP2, VP3**
  - **VP1 contains a hypervariable region (TR) useful for genotyping**
- **regulatory proteins**
  - **Early: large T, small t**
  - **Late: Agno**

J. Mol. Evol. 2006, 63:341. PMID 16897259

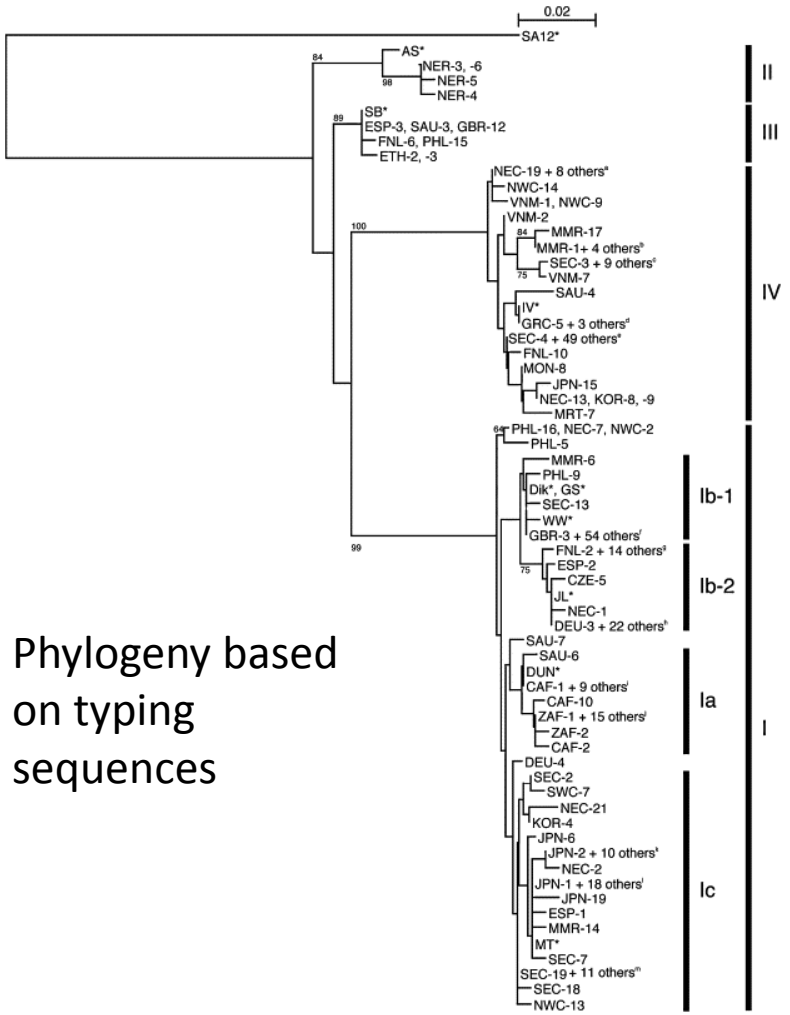
Pathogenesis



Epidemiology

- Overall seroprevalence in humans of 60-90%
- BK primary infection usually occurs in childhood with chronic latent infection in kidney and urinary tract
- Clinical manifestations rare if immunocompetent - primary infection may manifest as childhood viral URI
- Clinically significant in the immunocompromised host

BK subtypes defined by VP1 typing region (TR)

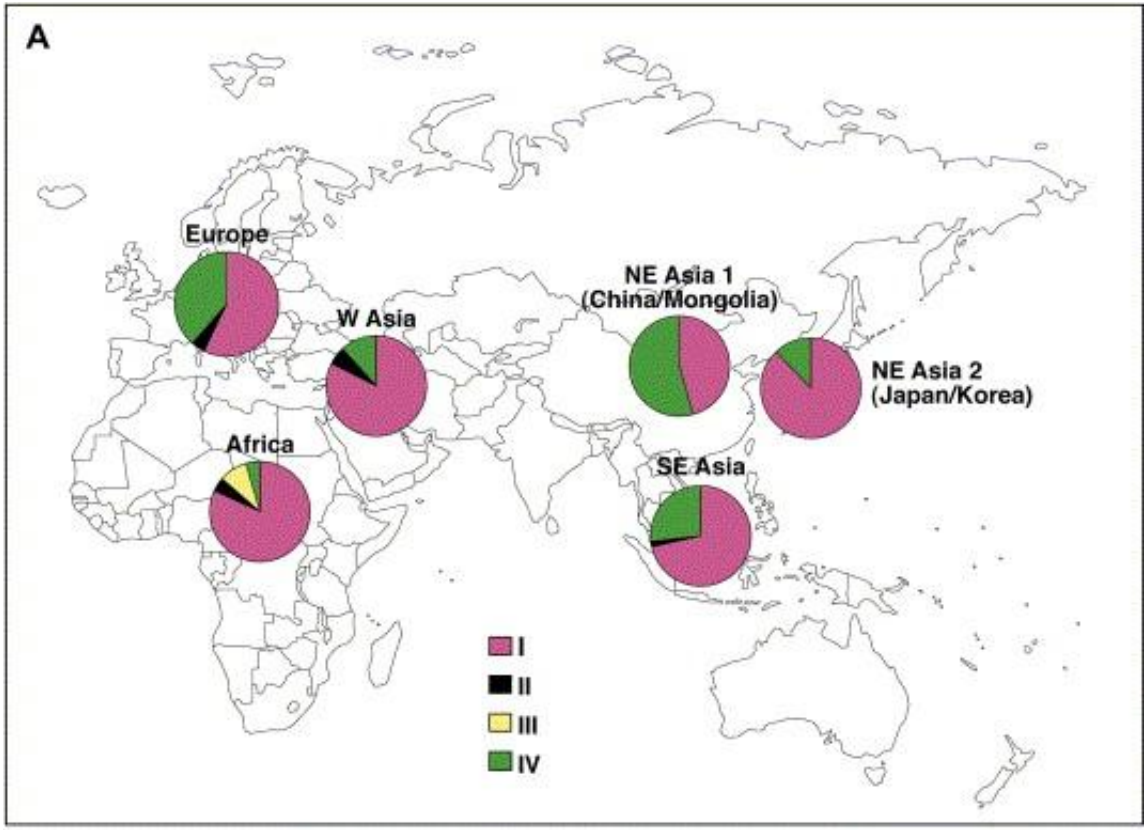


Phylogeny based on typing sequences

- 4 major serologic/genotypic subtypes
- "phylogenetic analyses based on complete DNA sequences supported not only the subtype classification of BKV isolates [by TR sequence] but also the subclassification of subtype-I isolates."

Zheng HY, et al. 2006 (in press) PMID 17208484

Geographic distribution of BK subtypes



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Polyomavirus-associated nephropathy (PVAN)

- Affects 1-10% of renal transplant patients
- Incidence has increased in the last decade
- Most cases in first year after transplantation
- Risk of PVAN rises with intensity of immunosuppression
- Clinically resembles acute rejection
  - Rise in serum creatinine

Why measure BKV?

- BK virus nephropathy (BKVN) in renal transplant recipients
  - screening
  - adjunct to diagnosis
  - monitor success of intervention

- Hemorrhagic cystitis (Hematopoietic Stem Cell Transplant recipients)
  - may play a diagnostic and/or prognostic role

BK detection methods

- Culture - rarely used clinically
- Urine cytology
  - "decoy cells" = enlarged nucleus with a single large basophilic intranuclear inclusion
  - good sensitivity, poor specificity
- Serology
  - high seroprevalence in adults; not diagnostically useful
  - may play a role in pediatric transplantation
- Renal biopsy
  - Indicated in setting of suspected BK-nephropathy versus graft rejection
  - Histology/IHC/in situ hybridization/tissue PCR/EM
- PCR
  - Urine
  - Serum

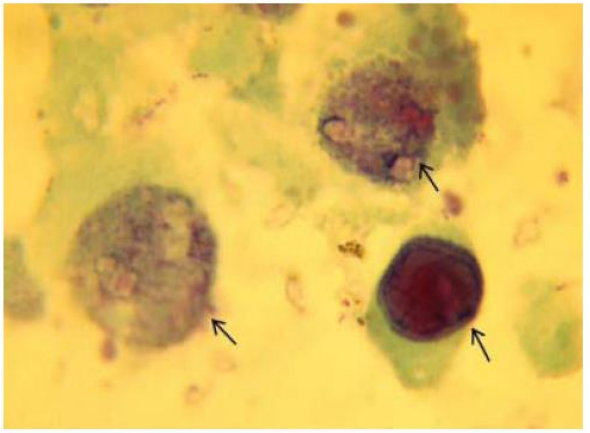
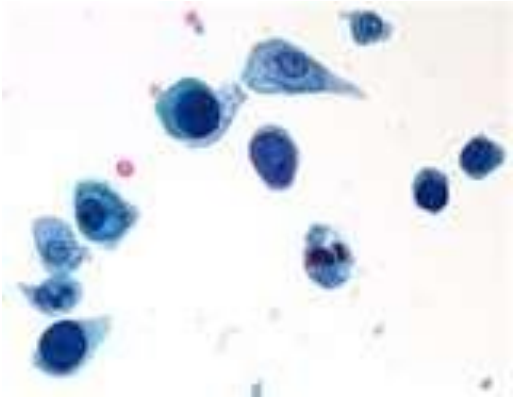
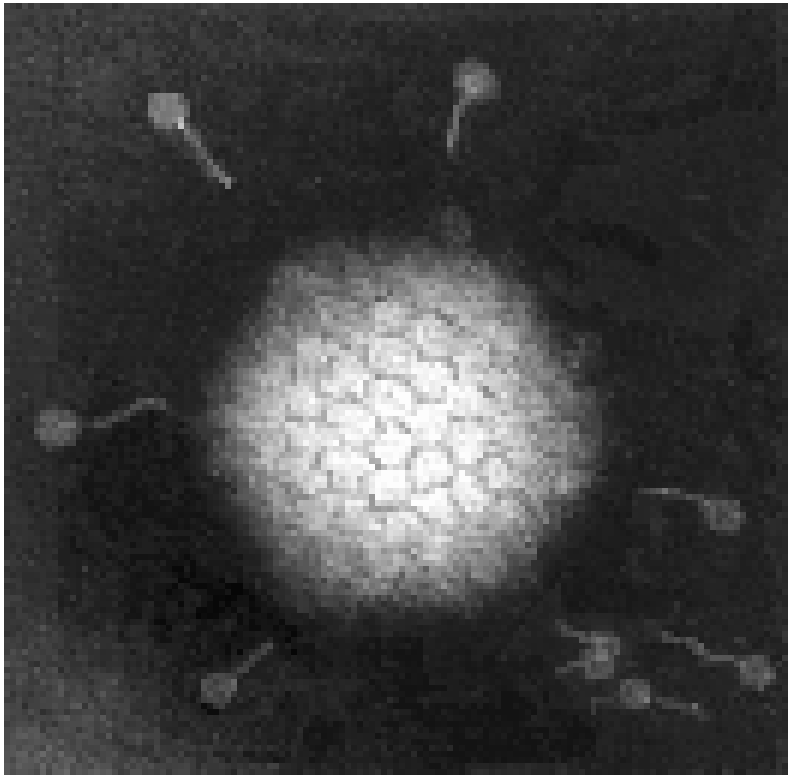
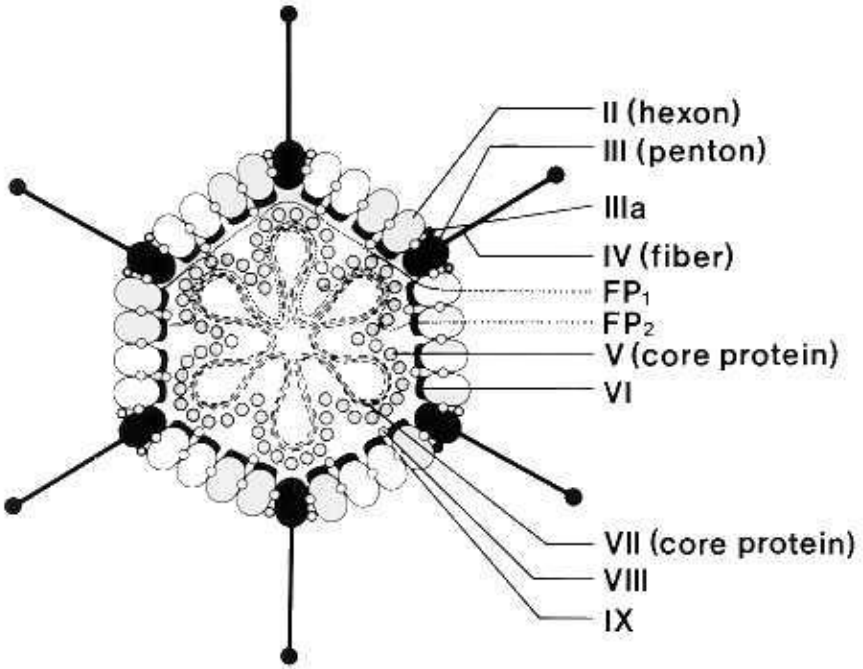


Figura N°2. Papanicolao de orina, muestra *Decoy cells* (Flechas).



Adenovirus

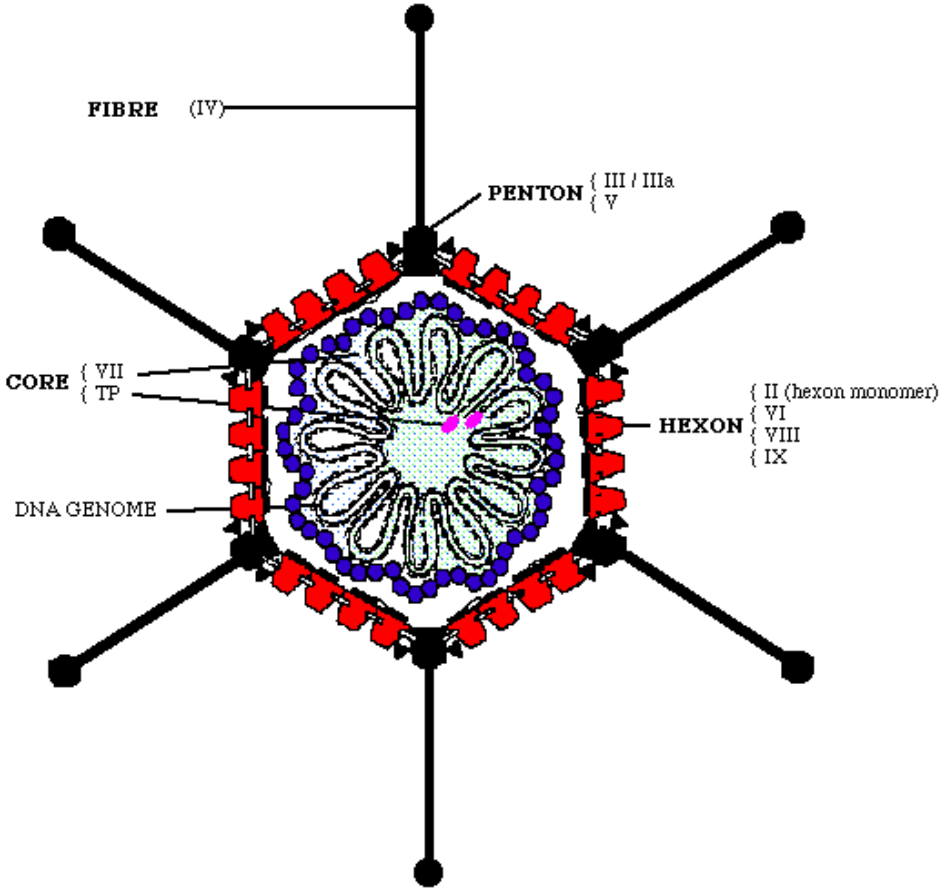




First isolated from adenoidal tissues in 1953

PROPERTIES

- Naked double stranded DNA virus
- Icosahedral symmetry
- 80 nm in diameter
- Capsid consist of 252 capsomeres (240 hexons, 12 pentons)
  Pentons make up the apices and possess projecting fibers.
- The fibers possess hemagglutinating activity and mediate the attachment of the virus to cellular receptors.



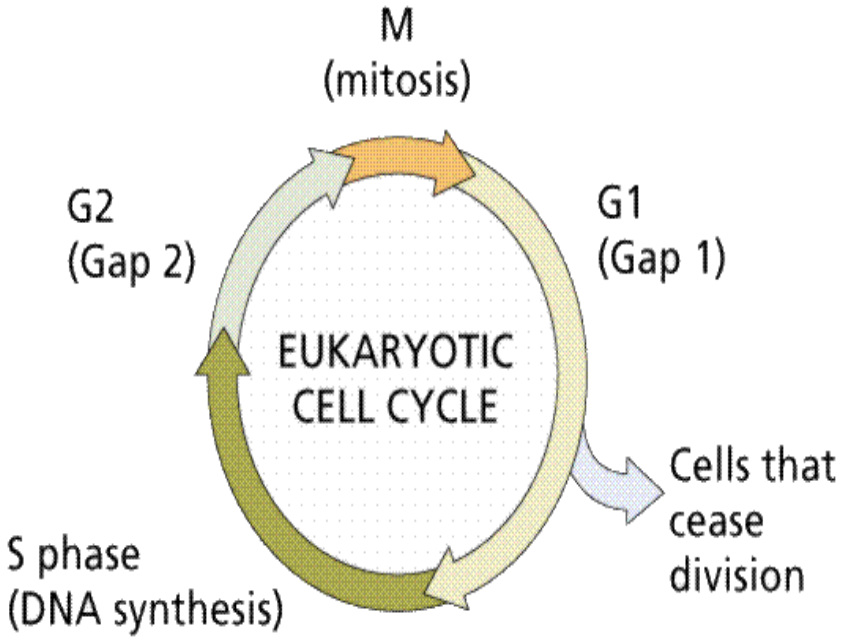
51 human adenoviruses in groups A - F

based upon DNA homology, disease tropism, and fiber antigens

- **Mastadenovirus**
- **Aviadaenovirus**

viral genome encodes many viral proteins

- early proteins promote the growth the infected cell
  - E1A/E1B viral proteins bind and inactivate
  - cellular p53 and pRB, thus stimulating cells growth
- virus also provides its own DNA dependent DNA polymerase
- some viral proteins suppress the host immune response including inflammation
- late proteins provide structural proteins and those carried in mature virion



virus enters the cell by endocytosis, lyses the endosomal vesicle, and capsid is removed as it delivers the DNA to the nucleus

PATHOGENESIS

- Adenoviruses cause diseases that involve ***respiratory***, ***urinary***, ***gastrointestinal tracts*** and ***eye***.
  - **Permissive cells will ultimately exhibit lysis & death**
  - **Non-permissive cells – exhibit latency**
    - **Mostly in lymphoid tissue – tonsils, adenoids, Peyer's Patches**
  - **Replication via general model for DNA viruses**
    - **Attachment to host cell receptor via knobs on tips of the viral fibers**
    - **Entry into cell by receptor-mediated endocytosis**
    - **Uncoating of viral genome, transported to nucleus of host cell**
    - **Transcription of viral genes, genome replication, & assembly all occur w/in Nucleus**

- Virus may be introduced through contact, respiratory droplets or ingestion.

- ***The association of particular types with specific disease syndromes is striking***.

Diseases caused by Adenoviruses

Group Affected	Syndromes	Serotypes
Neonates	Fatal disseminated infection	3, 7, 21, 30
Infants	Coryza, pharyngitis	1, 2, 5 (C)
Children	Upper respiratory disease Pharyngoconjunctival fever Hemorrhagic cystitis Diarrhea Intussusception Meningoencephalitis	1, 2, 4-6 3, 7 (B) 7, 11, 21 (B) 2, 3, 5, 40, 41 (F) 1, 2, 4, 5 2, 6, 7, 12
Young adults	Acute respiratory disease and PNA	3, 4, 7
Adults	Epidemic keratoconjunctivitis	8, 19, 37 (D)
Immunocompromised patients	PNA with dissemination Liver infection Urinary Tract Infection Intestinal Infection CNS disease including encephalitis	5, 31, 34 1, 2, 5 (C) 35, 39 42-51 (D) 7, 12, 32

Diseases caused by Adenoviruses

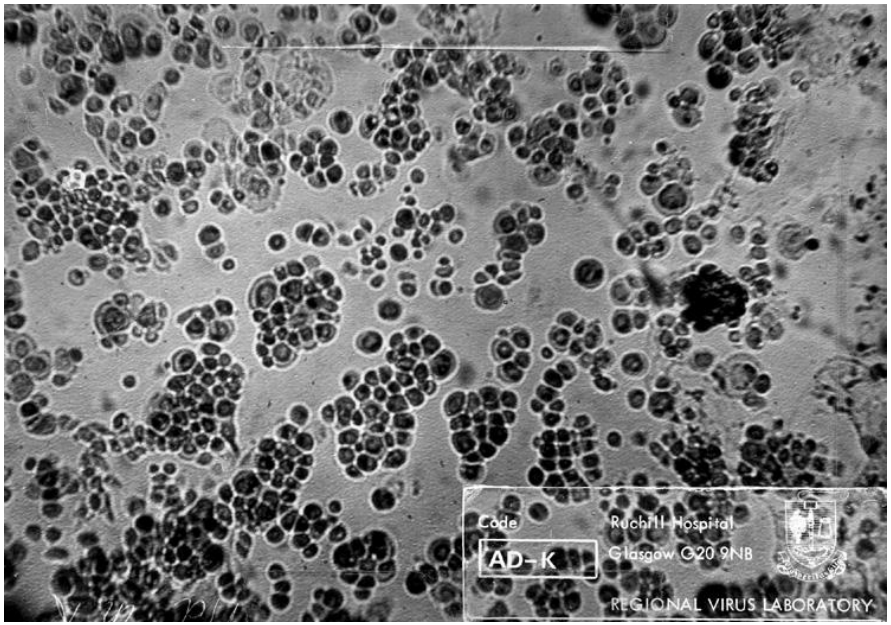
Groups Affected	Diseases	Serotypes
hematopoietic stem cell transplant (HSCT)	Gastroenteritis, pneumonitis, hepatitis, hemorrhagic cystitis, encephalitis, disseminated disease	1-3, 5, 7, 11, 31
Liver transplant	Gastroenteritis, hepatitis pneumonitis, disseminated disease	1, 2, 5, 7, 31
Renal transplant	Hemorrhagic cystitis, pneumonitis, GI disease	11, 34, 35
Lung transplant	Pneumonitis, disseminated diseases	2, 5

- **Acute Hemorrhagic Cystitis**
  - Occurs predominantly in boys (6 – 15 years)
  - Acute dysuria
  - Haematuria
  - Adenovirus serotype 11

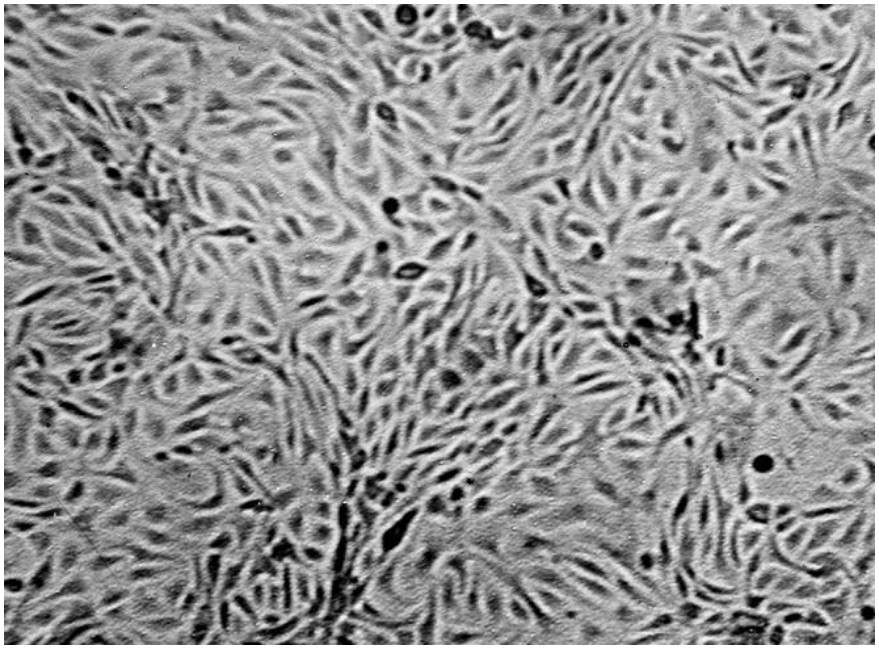
LABORATORY DIAGNOSIS

- **Virus Isolation**
  - Adenovirus may be isolated from most body fluids and secretions; eye swabs, NPA, throat swabs, urine, faeces, and CSF.
  - Human embryonic kidney cells Hep-2 cells
  - Primary monkey kidney cells 293 cells
  - CPE includes rounding, clustering of cells with refractile intranuclear inclusion bodies
- **Detection of antigen by Immunoflurescence (IF)**
- **Serology**
  - Infection of humans with any adenovirus type stimulates a rise in complement-fixing antibodies to adenovirus group antigens shared by all types. *A four-fold or greater rise* in these antibodies between acute phase and convalescent phase sera indicates recent infection.
  - The fastidious (no growth on cell cultures) enteric adenoviruses can be detected by direct examination of fecal samples by ELISA or latex agglutination tests.
- **Polymerase Chain Reaction (PCR)**

Adenovirus CPE in RMK



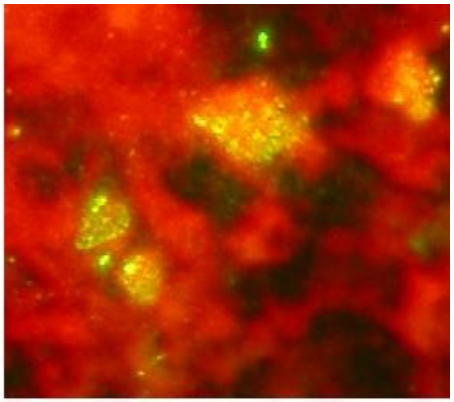
infected RMK



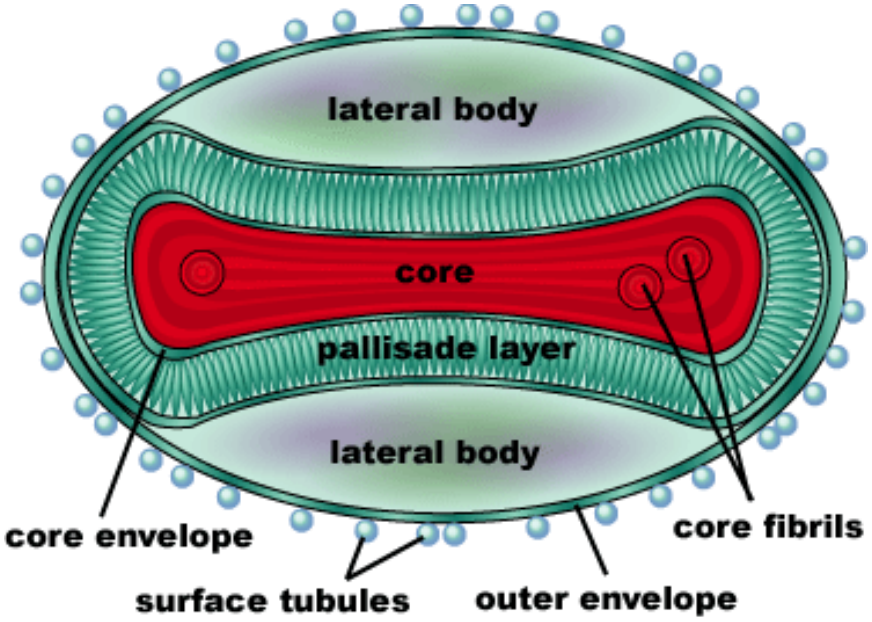
Un-infected RMK

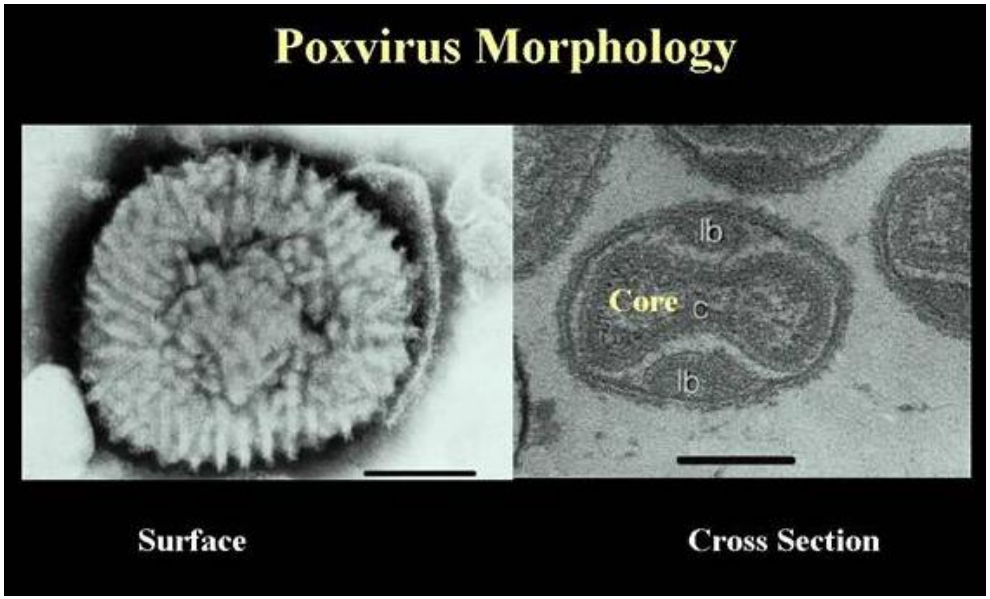
Antigen detection by Immunofluorescence

- **Rapid**
- **Relatively insensitive**
- **Not suitable for all speciemn types**



MOLLUSCUM CONTAGIOSUM VIRUS





- **Poxviridae**

  - Brick-shaped or ovoid
  - Size: 220-450nm long x
    140-260nm wide x
    140-260nm thick
  - Enveloped
  - ds DNA
  - Genome size:130-375kbs (large!)
  - Produce skin lesions eg. Small pox and vaccina virus

POXVIRIDAE

GENERA	Characteristic Members
Orthopoxvirus	Variola Major (Smallpox virus) man Variola Minor (Alastrim virus) Monkeypox Vaccinia virus man Cowpox virus cattle,cats
Parapoxvirus	Pseudocowpox virus Orf virus (milker's nodules)
Leporipoxvirus	Not important to man
Avipoxvirus	
Capripoxvirus	
Suipoxvirus	
Molluscipoxvirus	Molluscum contagiosum virus
Yatapoxvirus	Yaba monkey tumor virus

Molluscum contagiosum virus

- **Molluscum contagiosum is a specifically human disease of worldwide distribution.**

- **The incubation period varies from 1 week to 6 months. The lesion begins as a small papule and gradually grows into a discrete, waxy, smooth, dome-shaped, pearly or flesh-coloured nodule.**

- **Usually 1-20 lesions but occasionally they may be present in hundreds.**

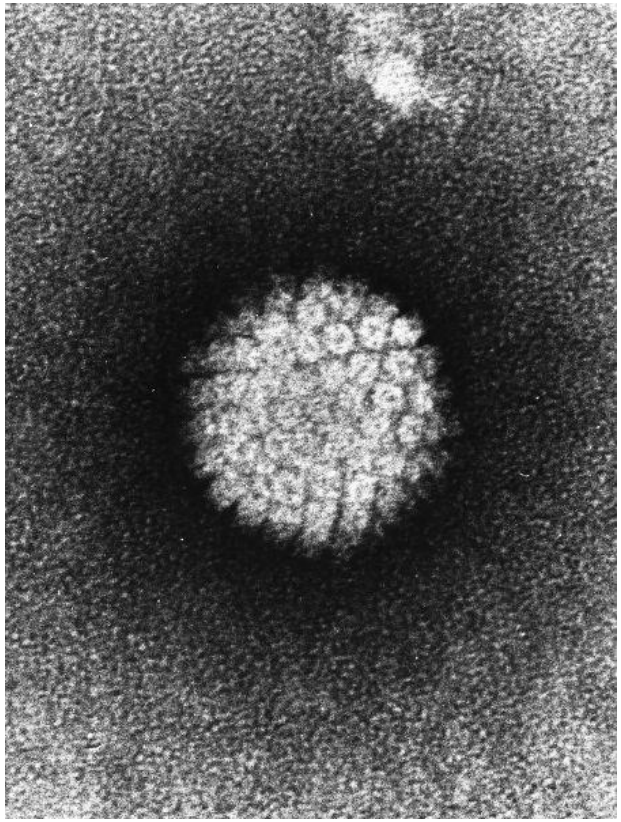
Molluscum contagiosum virus

- **The disease occurs world-wide and is spread by direct contact.**
- **In general it tends to occur in children.**
- **MC is transmitted by close personal contact including sexual contact.**

Diagnosis

- Diagnosis is usually done on clinical grounds alone by the typical appearance of the lesions.
- Expression of materials stained with Giemsa, Wright or Gram stain reveals molluscum bodies.
- Biopsy, which shows characteristic features of epidermal hyperplasia.
- Polymerase Chain Reaction
- The diagnosis can be supported by EM.
- Unlike other poxviruses, molluscum have not been demonstrated to grow in cell culture.

- **Papillomaviridae**
  - Similar to polyomaviruses
  - Diameter: 55nm
  - Genome size: 6.8 - 8.4kbs (larger than polyomaviruses)
  - In humans: May cause warts and genital cancers.
  - Eg. Human Papaillomavirus (HPV)



Papovaviridae

Vaculating agent

Papilloma viruses

100 genotype
Oncogenic

Envelop Protein E6&E7----------cancer by oncogenic HPV genotypes

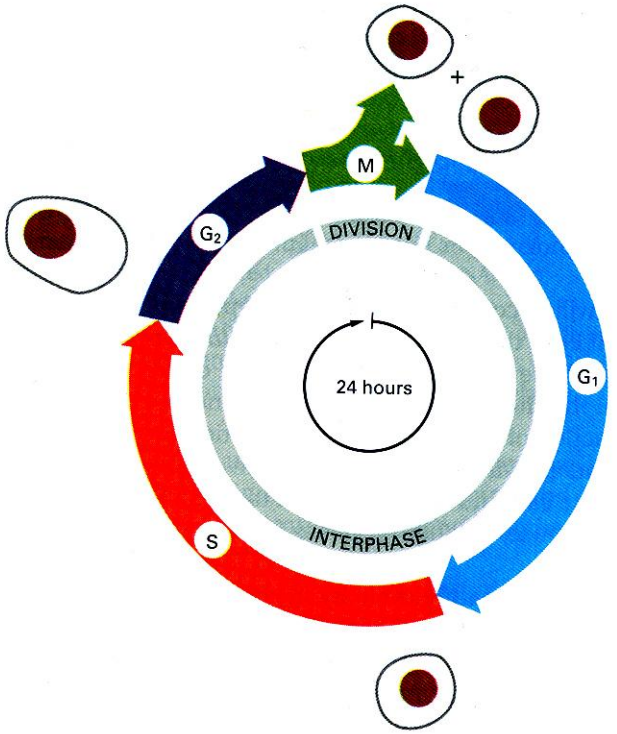
Polyoma viruses

Polyoma virus homonis 1&2 (BK&JC) asymptomatic infection but are associated with renal disease and **progressive multifocal**

cause **warts,** and several genotypes are associated with human cancer (e.g., cervical carcinoma).

Human Papilloma Virus Infections

- **HPV 16/18 cause cervical papillomas and dysplasia in which the virus DNA is integrated into the genome rathe than acting as a plasmid**
  - **E6/E7 genes are oncogenes which produce proteins that bind to and inactivate cellular growth-suppressor proteins, p53 and pRb**
    - **unsuppressed cells are more pron mutations and transformation**
- **infected cells exhibit nuclear chang with large perinuclear vacuoles**
  - **kiliocytosis**
  - **cause both benign and malignan lesions**



HPV Infections/ Lesions

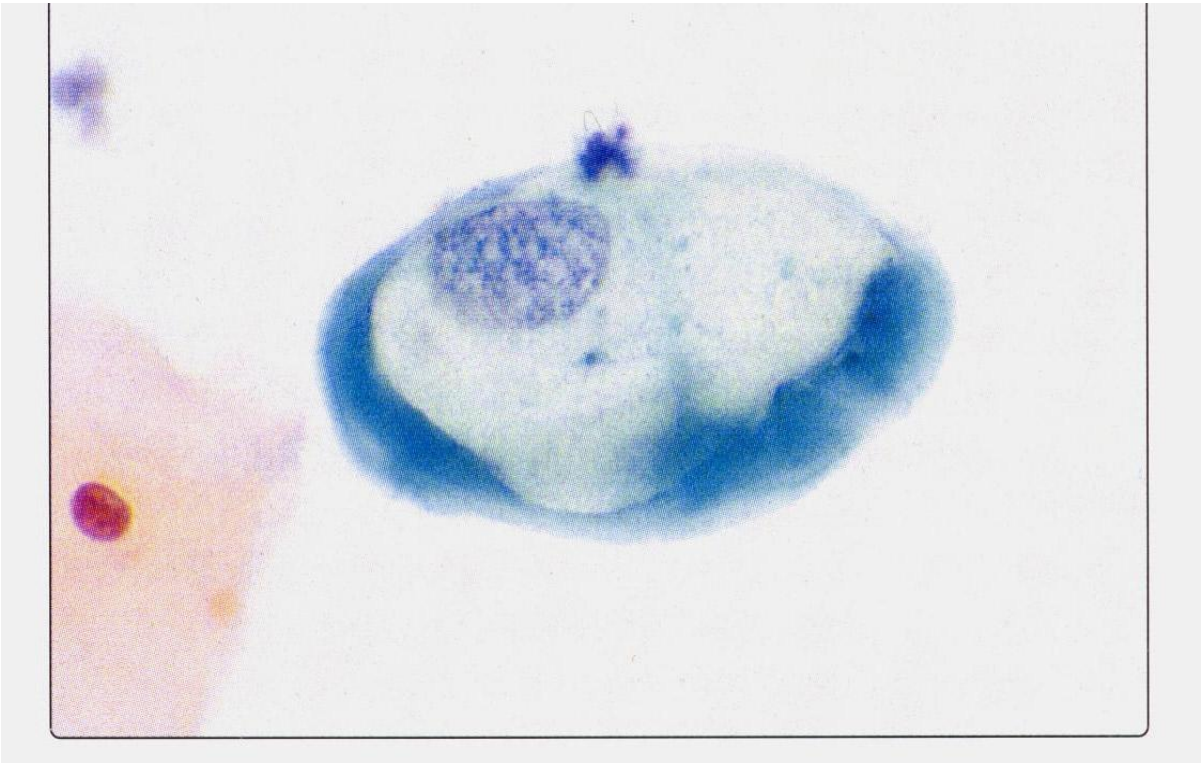
- **Anogenitial Warts**
  - **genital warts HPV 6/11 exclusively on the squamous epithelium of the external genitalia and perianal areas**
    rarely malignant

- **Cervical dysplasia and neoplasia**
  - **malignant changes caused by HPV 16/18 is an intraepithelial cervical dysplasia**
  - **koilocytotic cells observed in Papanicolaou-stained cervical smears**
    - **perinuclear cytoplasmic vacuolization**

	lesion	Associated HPV genotype
Non-malignant lesions	Common warts	1,2,4
	Flate warts	3
	Genital warts	6,11
	Laryngeal papilloma	6.11
Premalignant lesion	Epidermodysplasia verrcuiformis	2,3 and other
Malignant leision	Cervical cancer	16,18,31,33,35, and others

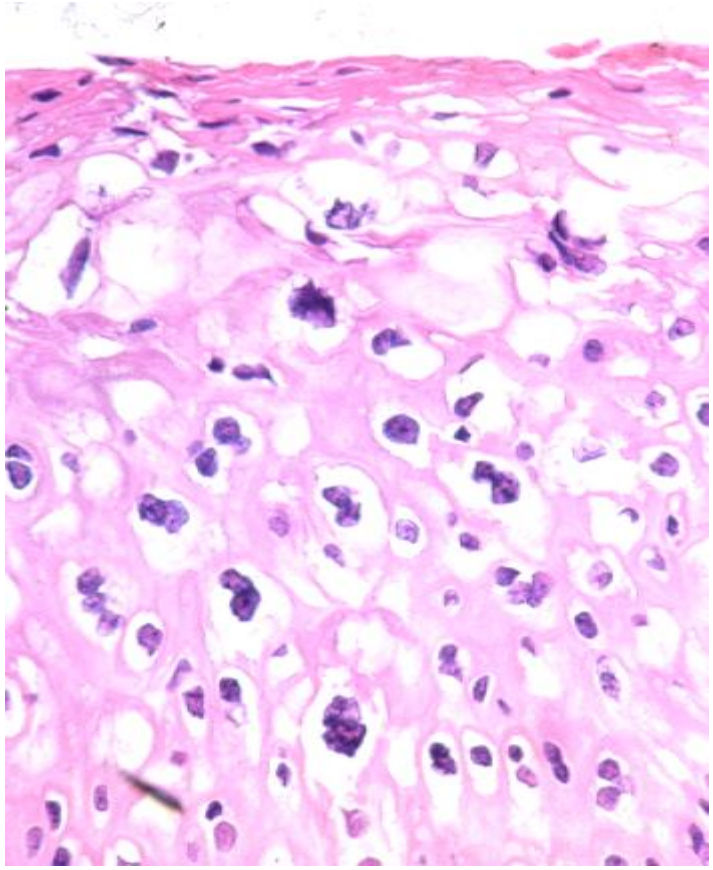
Laboratory Diagnosis of HPV Infections

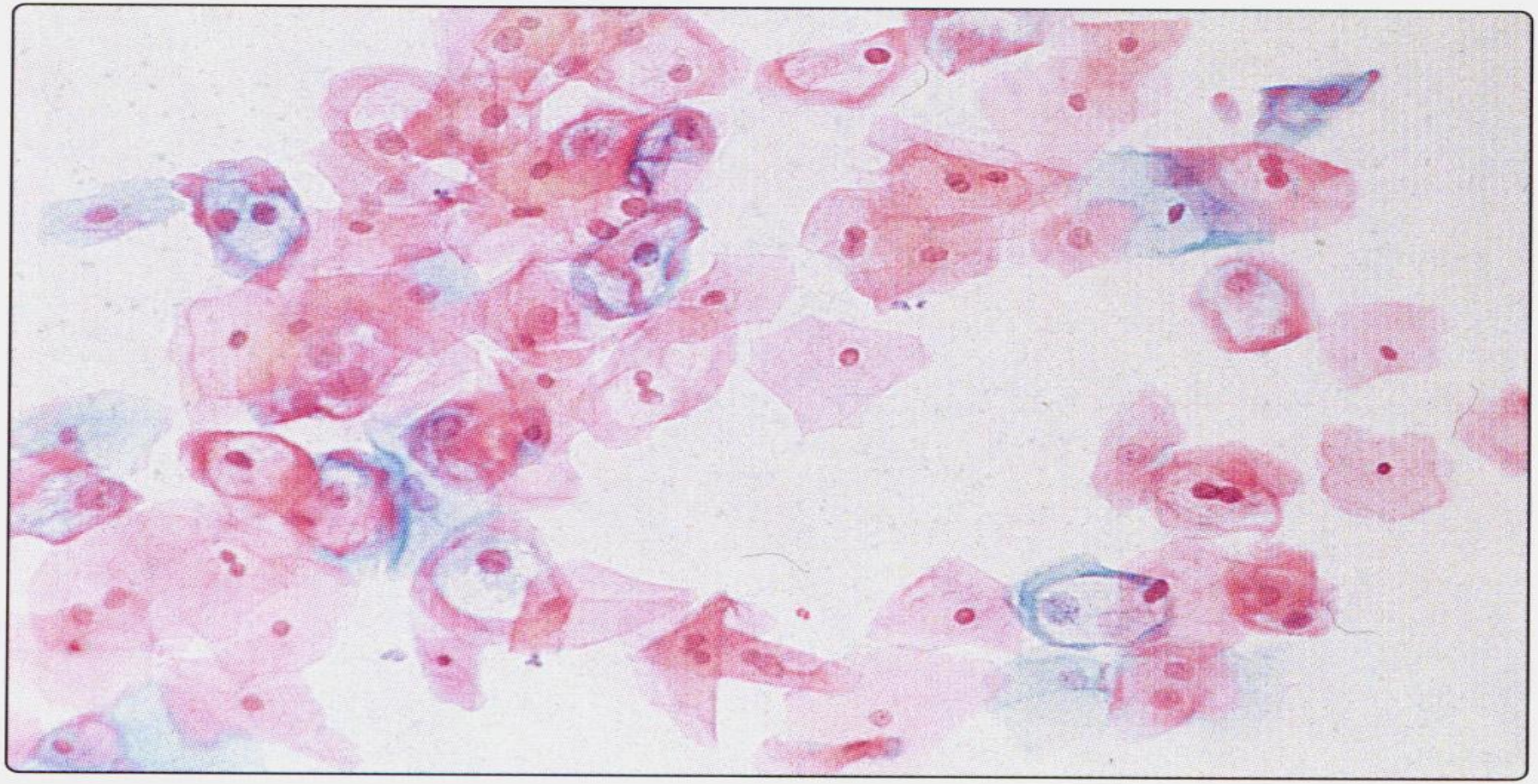
- **Cytology** **detects koilocytotic cells**
  - **warts are characterized by hyperplasia of the prickle cells and increased keratin production known as hyperkeratosis**
  - **koilocytosis of squamous epithelia cells which are rounded and clumped**
    - **as observed in a Papanicolaou smear**



KOILOCYTE

- Hallmark of HPV infection in an epithelium
- "Raisinoid" nucleus (enlarged, hyperchromatic, irregular), perinuclear halo and cytoplasmic thickening
- Upper epithelial layer





Kiolocytosis .this screening power magnification shows scattered mature squamus with well-defined clearing of cytoplasm around the nuclei and slight nuclear enlargement (MP)

Laboratory Diagnosis of HPV Infections

- **In situ DNA probe analysisdetects viral nucleic acid**

  method of choice

- **Polymerase chain reaction detects viral nucleic acid**

  method of choice

- **Southern Blot Hybridization detects viral nucleic acid**
- **Immunofluorescence detects structural viral antigens**
- **Electron Microscopy detects intact virus**
- **Culture: not useful**

Prevention and Control

- **A new HPV recently vaccine Gardasil vaccine which offer protection aganist HPV6, 11, 16 and 18, recommended for girls ages 9-26 y, given as a series of 3 injections over a 6-month period. The second dose is given two months after the first dose, followed four months later by the third dose.**