Lab 4:
Papillomaviruses (HPV)

450 MIC

PRACTICAL PART

SECTION (30397)

Learning Outcomes

- 2nd Example of Human Specific Disease:
  - Human Papillomaviruses (HPV)
- Classification.
- The disease caused by the virus and the sites of infection.
- The Phylogenetic tree representing the sequences of 118 papillomaviruses
- HPV morphology and structure.
- Pathogenecity and immunity (skin warts as an example).
- The laboratory diagnosis:
  - Molecular tests mainly:
    - In situ hybridization
    - Dat-blot hybridization
    - Southern blot hybridization

2nd: Papillomaviruses

Classification:

Family: Papillomaviruses

Until, recently, newly discovered viruses were described as “types” and named after the host species from which the viruses were isolated (“human papillomavirus,” “bovine papillomavirus”) followed by a number indicating the order of discovery, for example,HPV16, BPV1.

A new system divides the family into 16 different *genera*, each designated by a letter of the Greek Alphabet.

Therefore, papillomaviruses infecting humans fall into five of the recognized genera: alpha, beta, gamma, mu, and nu..

Furthermore, human papillomaviruses can also be grouped biologically according to the usual site of infection, for example, cutaneous (β-clade) or mucosal (α-clade), and the type of pathology.

Diseases Caused by Human Papillomaviruses

And the Sites of Infection

TABLE 19.1 Diseases Caused by Human Papillomaviruses

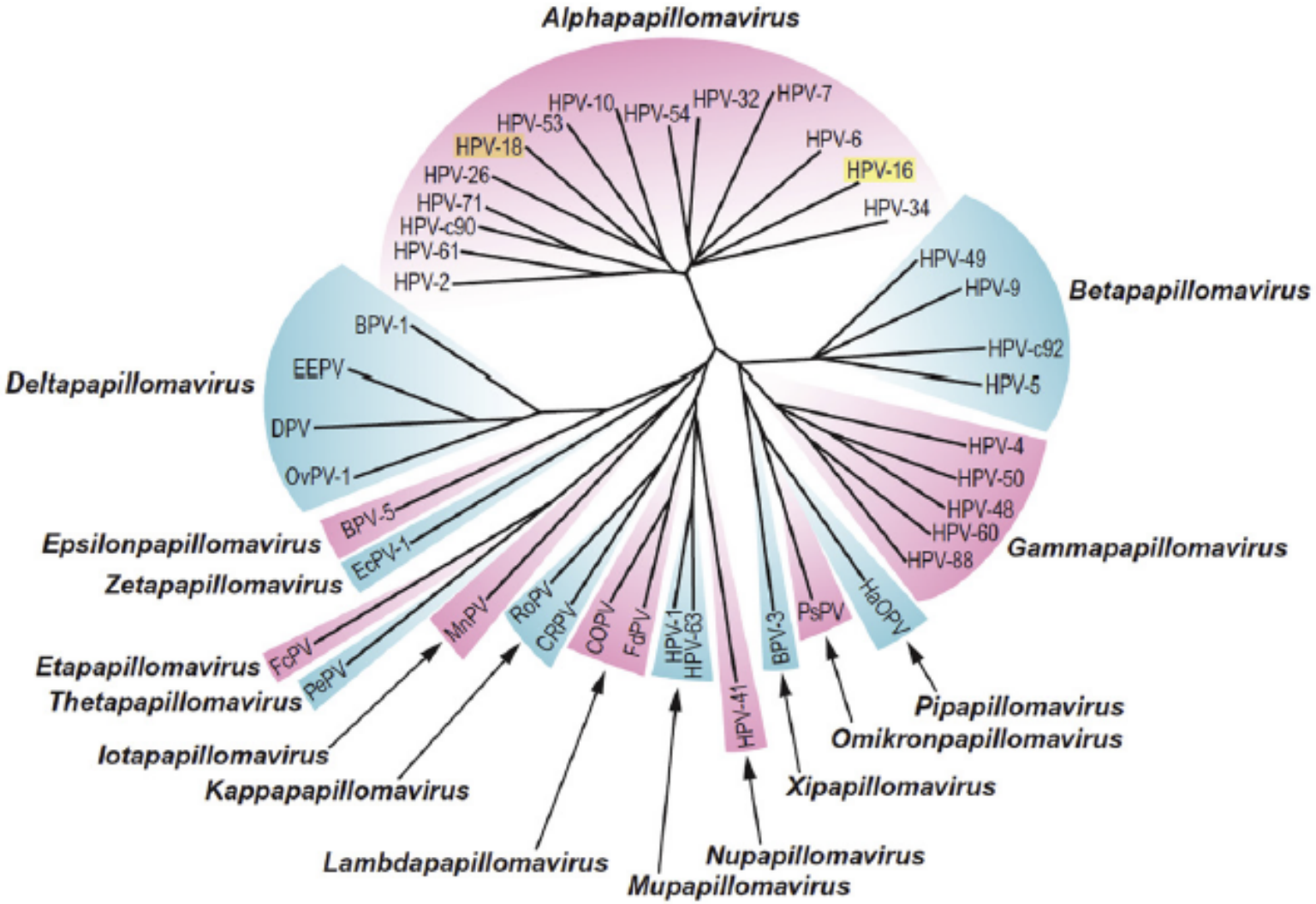
Site	Clinical Presentation	Types[a]
Genital tract	Subclinical infection	All genital types
	Condyloma acuminatum, anogenital warts	**6**, **11**, 42, 43, 44, 55, and others
	Cervical cancer	**16**, **18**, 31, 33, 35, 39, 45, 51, 52, 56
	Vulvar, vaginal, penile, anal cancers	16
Respiratory tract	Recurrent respiratory papillomas	**6**, **11**
Eye	Conjunctival papillomas	**6**, **11**
Mouth	Focal epithelial hyperplasia	**13**, **32**
	Oral papillomas	**2**, **6**, 7, **11**, 16, 32
	Oropharyngeal cancer	16
Skin	Plantar wart	**1**, 2, 4
	Common wart	**2**, **4**, and others
	Flat wart	**3**, **10**, 28, 41
	Butchers' warts	**7**
	Epidermodysplasia verruciformis[b]	**5**, **8**, 9, 12, 14, 15, 17, 19–25, 36, 46, 47

[a]*Common types in bold type.*
[b]*Types 5, 8, and less commonly 17, 20, and 47 are the principal types so far associated with malignant change in epidermodysplasia verruciformis.*

Phylogenetic tree representing the sequences of 118 papillomaviruses.

The circular, dsDNA, viral genome is approximately 8-kb in length.

The genome encodes for 6 early proteins responsible for virus replication and 2 late proteins, L1 and L2, which are the viral structural proteins.



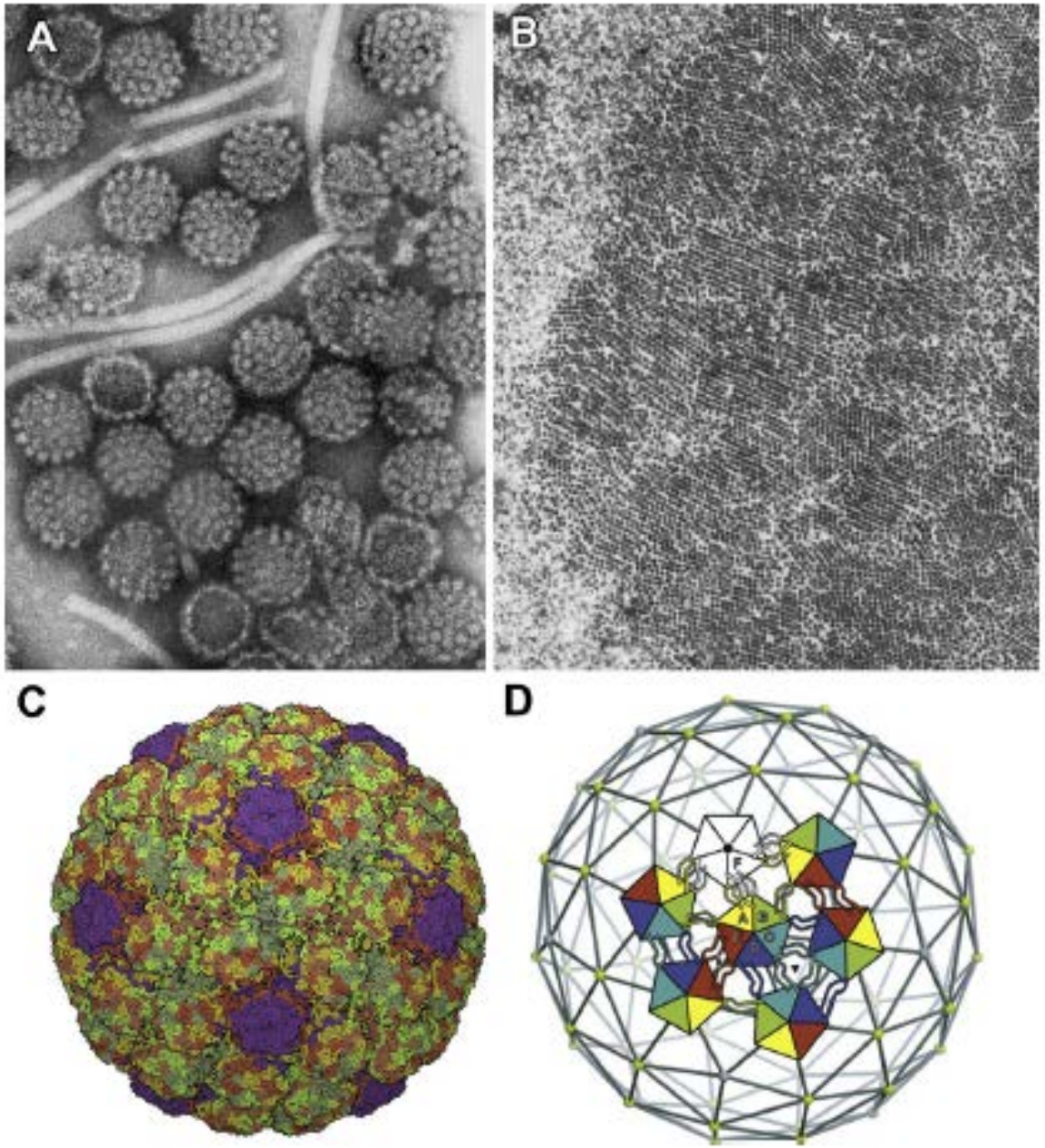
Papillomavirus morphology and structure.

(A) Negative contrast electron microscopy.

(B) Thin-section electron microscopy of the nucleus of a squamous epithelial cell containing a massive number of virions.

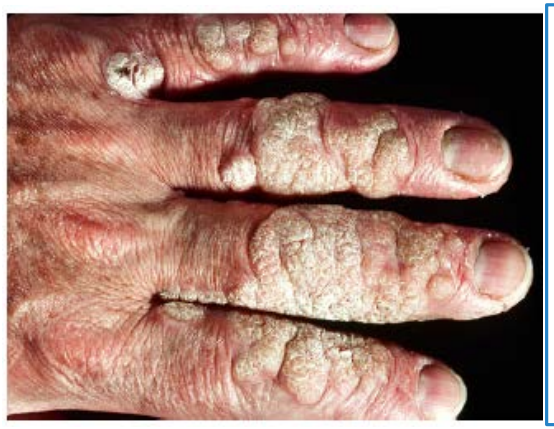
(C) Model of capsid structure

(D) Diagram of packing of structural units.



Wolf, M., et al., 2010. Subunit interactions in bovine papillomavirus. Proc. Natl. Acad. Sci. U.S.A. 107, 6298–6303, with permission

Pathogenicity & Immunity



Multiple warts on the fingers of an immunocompromised patient.

Reproduced from Cooke, R.A. Infectious Diseases, McGraw Hill, with permission.

- Papillomaviruses are highly species and tissue specific
- Virus enters basal cells, leading to expression of early genes, limited DNA replication, viral DNA persists as an episome.
- Early proteins stimulate basal cell proliferation
- Incubation period of warts 6 to 20 weeks
- Significant viral DNA replication, production of capsid proteins, and virions, only occur in outer differentiated keratinocytes
- Lesions usually disappear, possibly after 9 to 18 months after the development of cell-mediated immunity

Laboratory Diagnosis of Human Papillomaviruses (HPV)

1- Biopsy

It is required when malignancy is suspected.

By **Colposcopy**, but the lesions are often flat and can't be distinguished from cervical intraepithelial neoplasia.

Abnormal patches of mucosa are more visible after applying dilute acetic acid ("aceto-white" areas).

Positive VIA

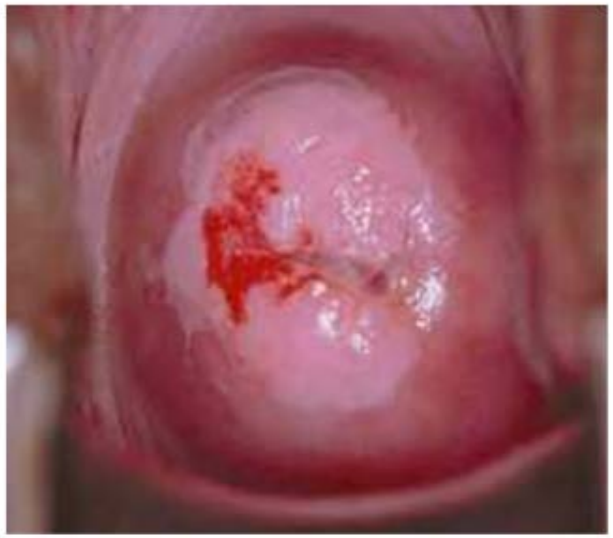
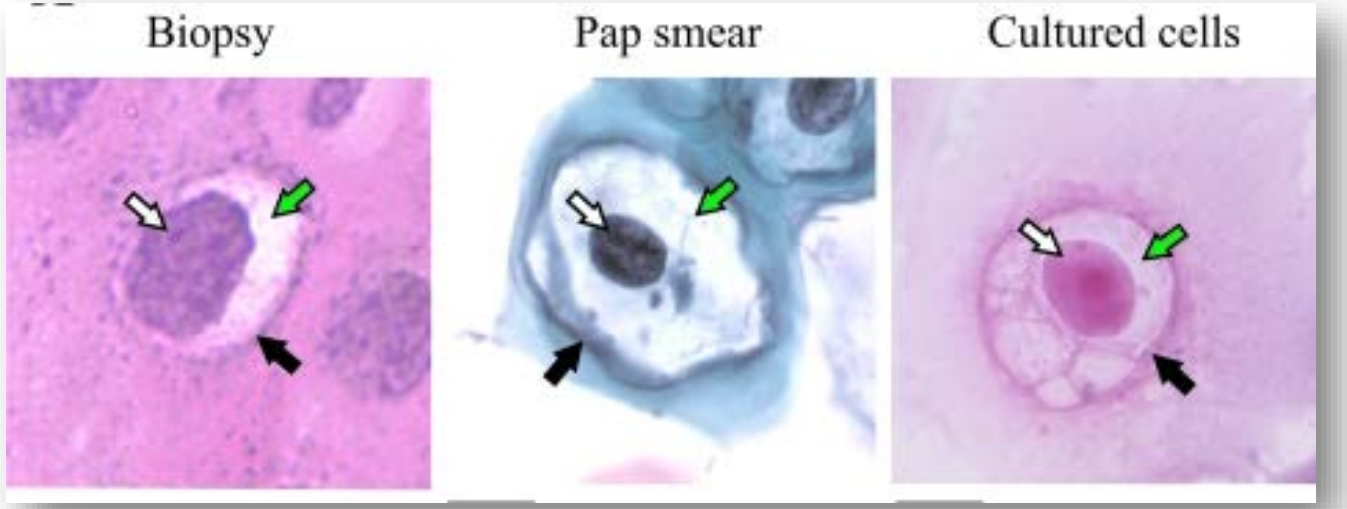


Fig. 15. Photo source: JHPIEGO

2- The Papanicolaou “Pap” smear

A routine screening test of women for early diagnosis of premalignant and malignant cell in the cervix

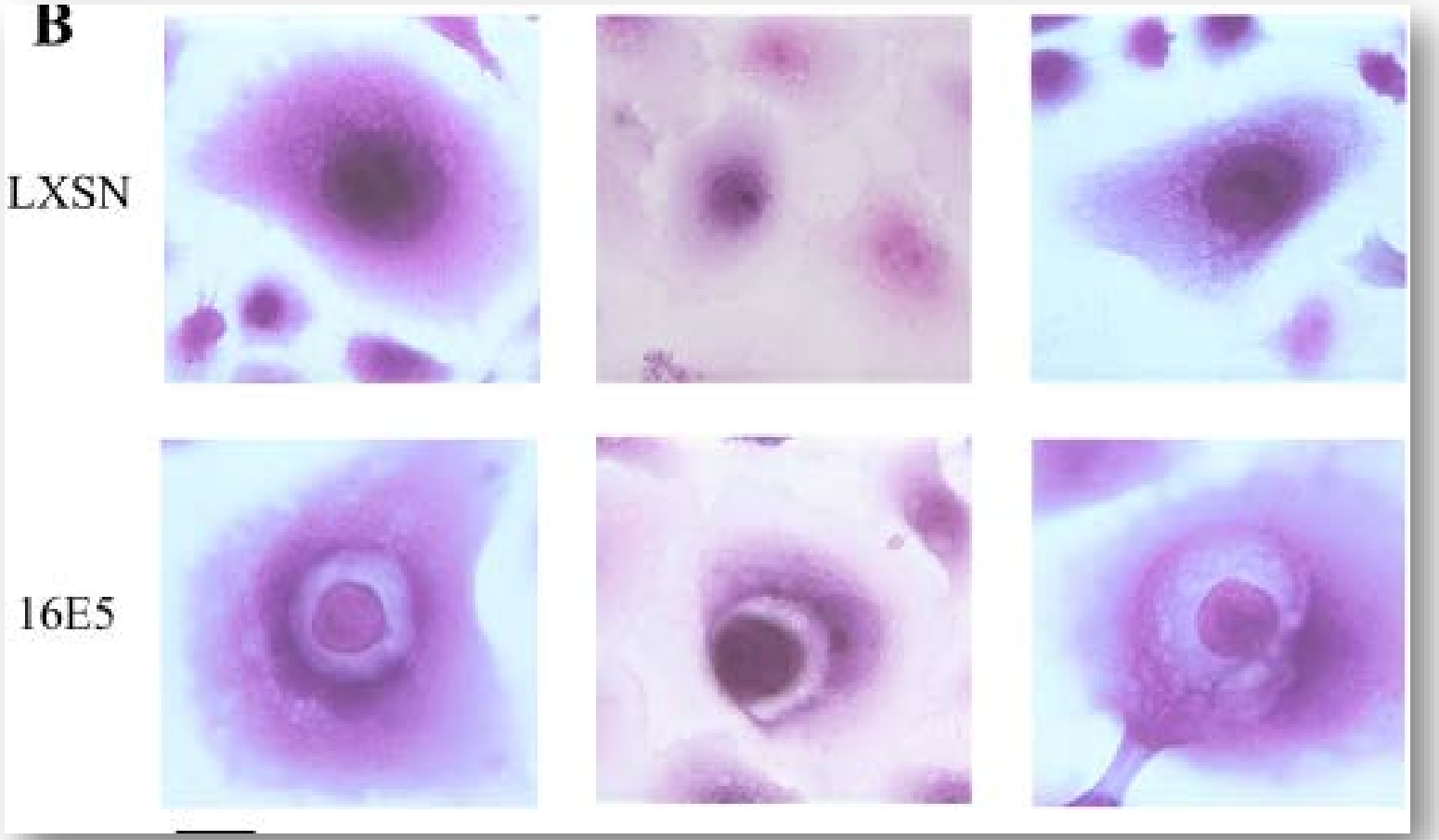
Allows exfoliated cells to be examined for HPV-related changes (koilocytes) and malignant cells (Koilocytotic cells).



The HPV cytopathic effect (koilocytosis) is shown in : a cervical biopsy specimen (**left**, H&E staining), a cervical cytology preparation (**middle**, Pap stain), and a cultured monolayer of 16E6/16E7-immortalized HECs expressing 16E5 (**right**, H&E staining).

Arrows show typical koilocyte features: an acentric, hyperchromatic, moderately enlarged nucleus (**white arrow**) displaced by a large perinuclear vacuole (**green arrow**), surrounded by a thickened cytoplasm (**black arrow**).

2- The Papanicolaou “Pap” smear



H&E staining of 16E6/16E7-immortalized HEC lines demonstrates koilocytes in 16E5-expressing cells, but not in HECs containing the empty expression vector (LXSN). Scale bars = 10 μm

3- Antibody and Antigen Detection:

A- Detection of antibodies to the L1 proteins.

- To study cumulative past or present infection rates.
- The findings are sometimes difficult to interpret and are not reliable for diagnosing current infection.

B- Antigen detection in exfoliated cells.

- Unreliable because viral genomes can persist for years with very limited viral protein expression in basal cells, as episomal DNA in benign papillomas or premalignant cervical dysplasia, or integrated in cancer cells.

4- Detection of viral DNA

The only generally applicable diagnostic approach.

The PCR techniques has been valuable:

- To add information in the case of doubtful or atypical Pap smears.
- In the identification of higher-risk patients needing more frequent testing, for example, those patients infected with the oncogenic types such as HPV16 and 18.

More reliable molecular tests that largely replaced by PCR techniques :

1. Dot-blot hybridization
2. Southern blot
3. *in situ* hybridization.

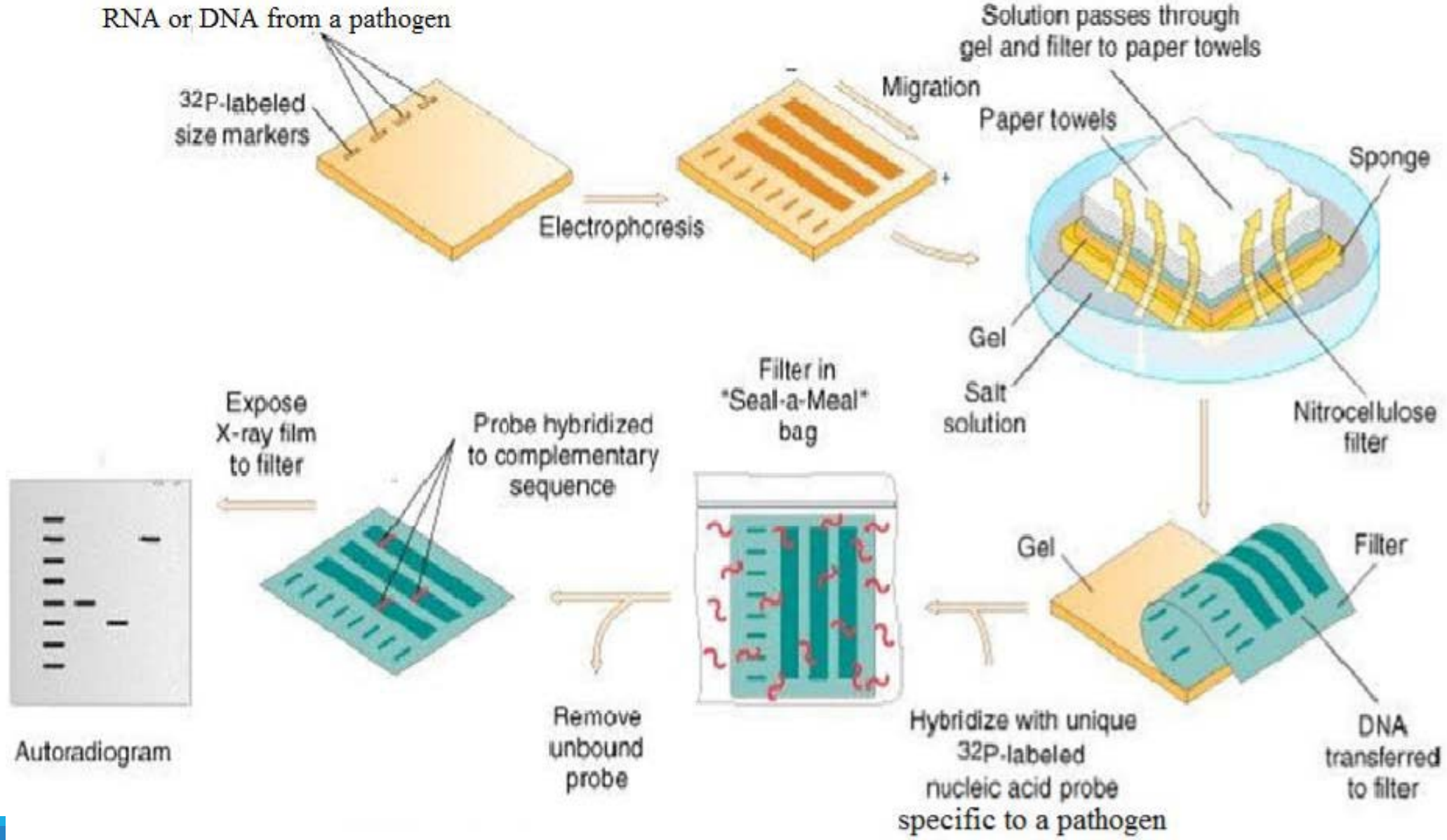
Nucleic Acid Hybridization?

It involves a hybridization reaction between an immobilized nucleic acid target and a labelled probe, washing away unbound probe, followed by subsequent detection of bound probe.

These include:

- **Dot-blot assays** using nucleic acid-containing samples immobilized onto filters.
- **Southern blot hybridization**, where viral nucleic acids are separated by electrophoresis according to molecular weight, blotted to a filter and detected by hybridization.
- ***In situ* hybridization** applied to infected tissue sections or exfoliated cells.

Nucleic Acid Southern Blot Hybridization?

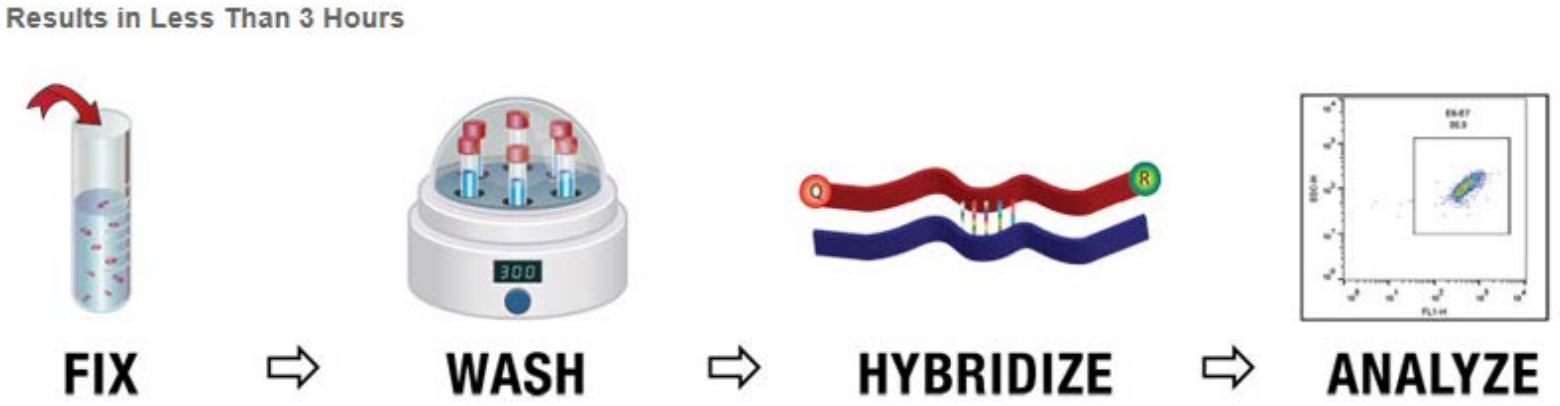


In Situ Hybridization (ISH)

Thin-section microtome sections were prepared from biopsy tissue.

Serial sections adjacent to those used for histologic diagnosis were tested for HPV DNA.

ISH was performed using the GenPoint Catalyzed Signal Amplification System for high-risk (HR)-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, code Y1443) and low-risk (LR)-HPV (types 6 and 11) according to manufacturer protocols.



Kelesidis, T., Aish, L., Steller, M.A., Aish, I.S., Shen, J., Foukas, P., Panayiotides, J., Petrikkos, G., Karakitsos, P., Tsiodras, S. **Human papillomavirus (HPV) detection using in situ hybridization in histologic samples: correlations with cytologic changes and polymerase chain reaction HPV detection.** *Am J Clin Pathol*. 2011;136:119–127.

Benefits and weaknesses of molecular methods of HPV Detection

Method	Benefits	Weaknesses
Nucleic acids hybridization assays	Southern blot is gold standard for HPV genomic analysis Presence of HPV in association with morphology	Low sensitivity , time consuming, relatively large amounts of purified DNA
		Southern blot and hybridization cannot use degraded DNA
Signal amplification assays	Quantitative	Licensed and patented technologies
	FDA-approved test (hc2)	Wasn't designed to genotyping individual
	Lower false-positive rate	
	High sensitivity to genotyping	
Nucleic acids amplification assays	Flexible technology (viral load and genotype)	Lower amplification signals of some HPV genotypes
	Very high sensitivity	Contamination with previously amplified material can lead to false positives
	Multiplex analysis	

HPV = Human *Papillomavirus*; FDA = Food and Drug Administration; hc2 = Hybrid Capture® 2; PCR = Polymerase Chain Reaction.

Treatment

- Cesarean section for infants carried by HPV-infected mothers has been considered for prevention of respiratory papillomatosis
- Interferon α or β injected intramuscularly and/or into the lesion itself has been reported to cause genital papillomas to regress in a majority of cases, but its use for recurrent respiratory papillomatosis has not been so successful.
- Most cases of early dysplasias (CIN1, LSIL) will spontaneously regress and can be managed by close observation, whereas more advanced dysplasias need prompt and adequate surgery.

Prevention

1- Screening asymptomatic women by regular Papanicolaou (Pap) smears allows cytologists to identify HPV affected cells (koilocytes) and also premalignant cells.

Moreover, the coexistence of a highrisk HPV type worsens the prognosis for any dysplasia and is an indication for more frequent screening.

2- the introduction of prophylactic immunization using non-replicating virus-like particles (VLPs) is beginning to revolutionize the impact of this disease.

- The problem of producing HPV antigens in sufficient quantity and in an immunogenic, non-infectious form was overcome by Jian Zhou and Ian Frazer in Brisbane, Australia, who demonstrated that L1 proteins can self-assemble into highly immunogenic VLPs.

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