Human Papillomavirus (HPV); A Comprehensive Overview

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Outline:

- Introduction
- Genome structure & function
- Subtype & clinical significance
- Transmission & risk factors
- Sample collection
- Tests classification
- Troubleshooting

Introduction

Human papillomavirus (HPV) is a ubiquitous viral infection that affects millions of people worldwide. HPV can cause various epithelial lesions and cancers, particularly on skin and mucosal surfaces. This presentation will explore the diverse aspects of HPV, focusing on its subtypes, classification, transmission, and association with various cancers.

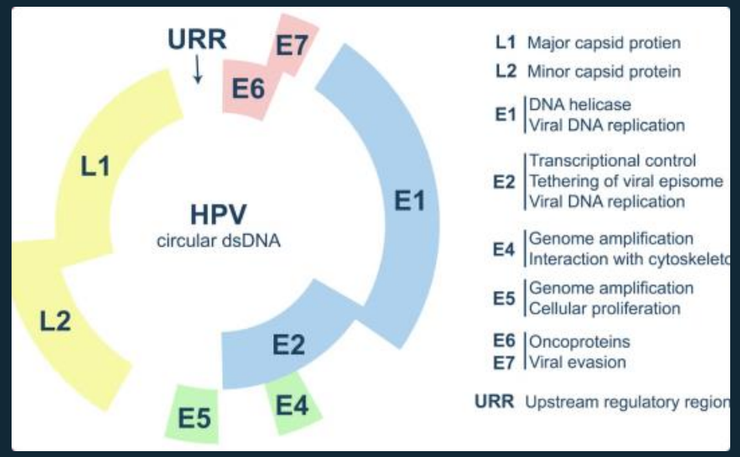
The Human Papillomavirus (HPV) is the initiating force behind multiple epithelial lesions and cancers, predominantly cutaneous and mucosal surfaces.

The current classification of HPV infection is as follows:

- Non-genital (Cutaneous)
- Mucosal or anogenital
- Epidermodysplasia verruciformis (EV)

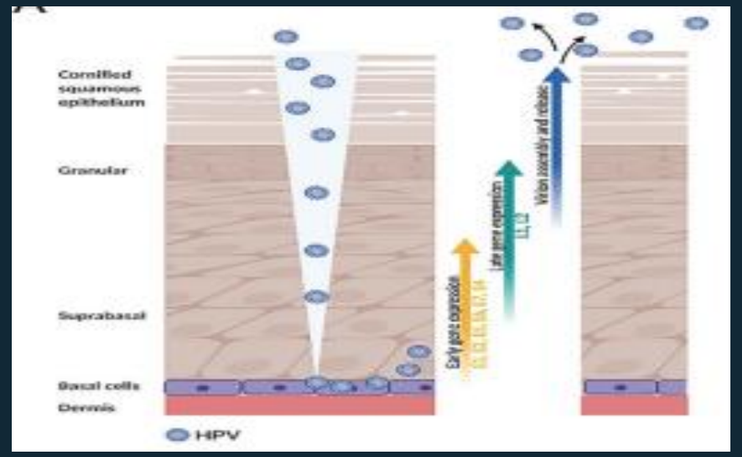
The clinical lesions may be visibly obvious, but in some cases (latent lesions) may require testing for viral DNA. The majority of HPV infections are latent, and most clinical lesions present as warts rather than a malignancy.

HPV Genome Structure and Function



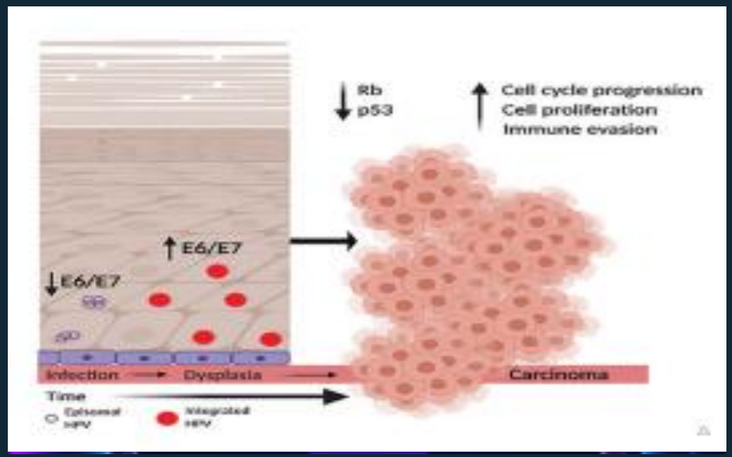
Viral Structure

The HPV genome consists of a circular, double-stranded DNA molecule enclosed within a protein capsid.



Life Cycle

HPV infects cells in the basal layer of the epithelium, where it replicates and integrates into the host genome.



Genome Organization

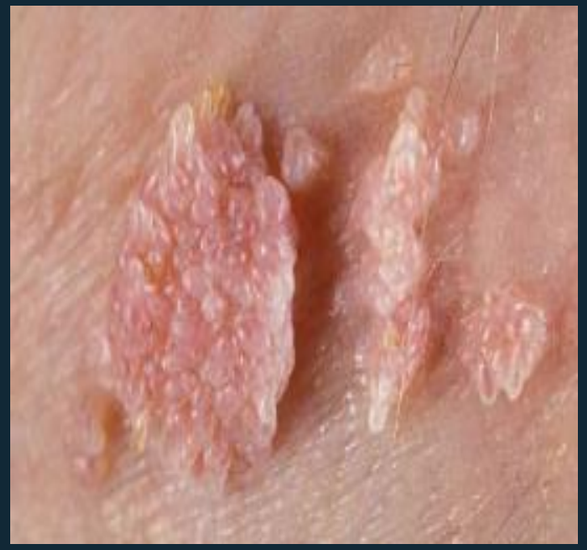
The genome is divided into three regions: early, late, and long control region (LCR).The E6 and E7 oncoproteins are key drivers of cancer progression by disrupting cell cycle regulation.

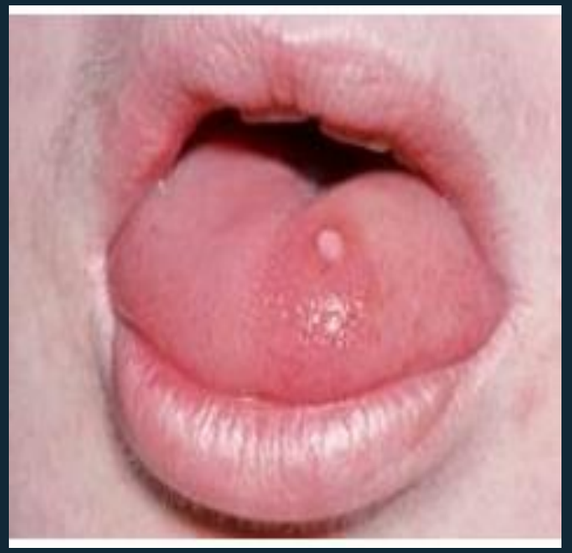
HPV Subtypes and Their Clinical Significance

As of December 2020, 222 types of human papillomavirus have been identified, categorized into five main groups associated with various diseases. These include 65 alpha papillomaviruses, 54 beta papillomaviruses, 99 gamma papillomaviruses, 3 mu papillomaviruses, and 1 neo papillomavirus, each with distinct strains. The gamma, beta, mu, and nu groups exhibit cutaneous tropism, typically resulting in warts on the hands or feet. In contrast, members of the alpha species demonstrate mucosal tropism, which can lead to more severe and potentially life-threatening conditions such as cancer.

Low-Risk HPV Types (6 ,11)

These subtypes are typically associated with benign lesions, such as genital warts. These warts are usually visible and treatable with topical medications or surgical removal.

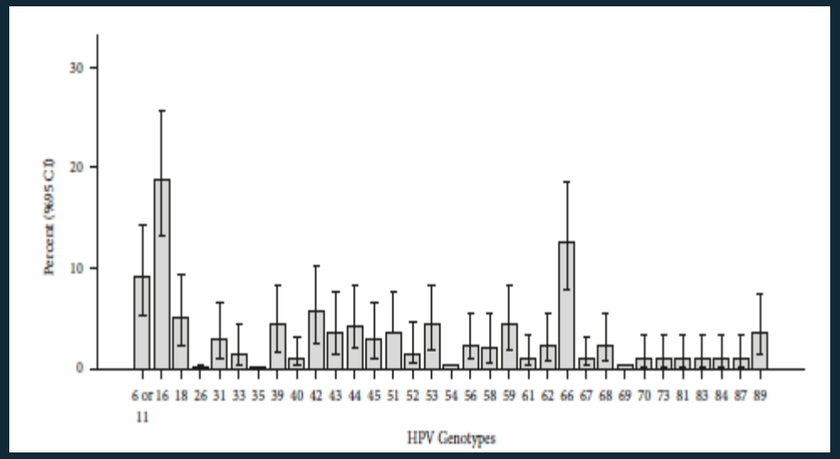




High-Risk HPV Types (16 , 18)

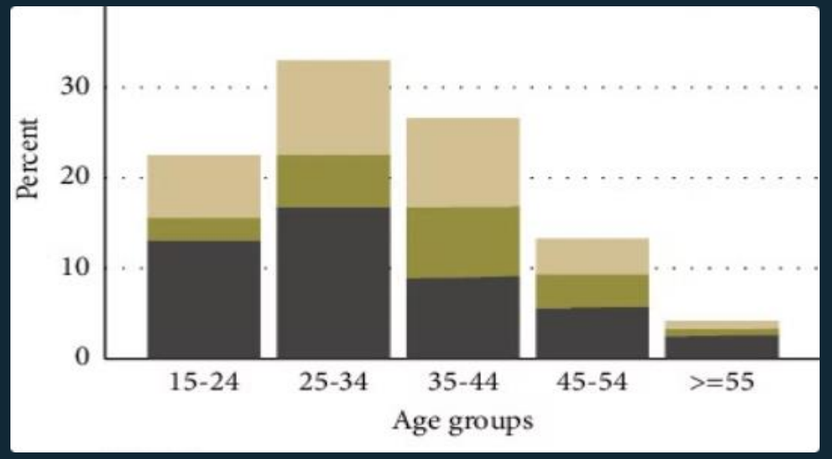
These subtypes are strongly linked to the development of cervical cancer and other cancers. Persistent infections can lead to precancerous lesions, which, if left untreated, may progress to invasive cancer.

HPV Prevalence and Distribution in Iran



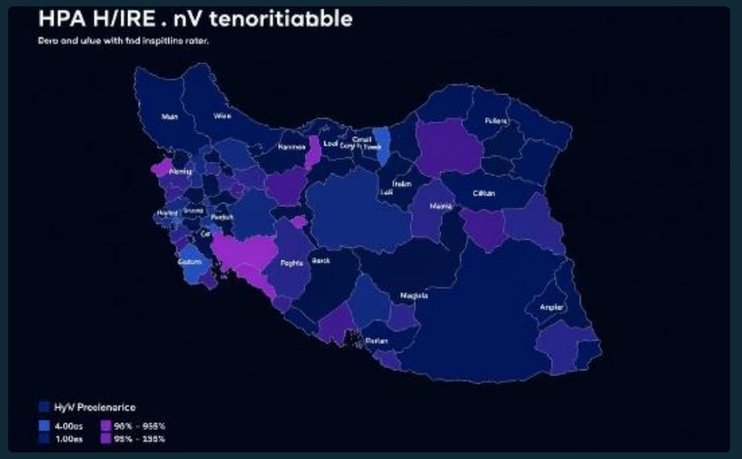
Genotype Distribution

Genotype 16 was most prevalent, followed by 66, 6/11, 42, and 18; varying across provinces.



Age Group Prevalence

Prevalence was highest in the 25-34 age group (4.5%) and lowest in those 55 years and older (3.4%).



Geographical Distribution

Prevalence varied geographically, with Hormozgan highest(9.5%) and Isfahan lowest(2.2%), highlighting cultural and social influences.

Ref ; Evaluation Frequency of Human Papillomavirus and Its Related Genotypes in Women of the General Population Living in 11 Provinces of Iran.2022

HPV Transmission Modes and Risk Factors for Cervical Susceptibility

Sexual Transmission

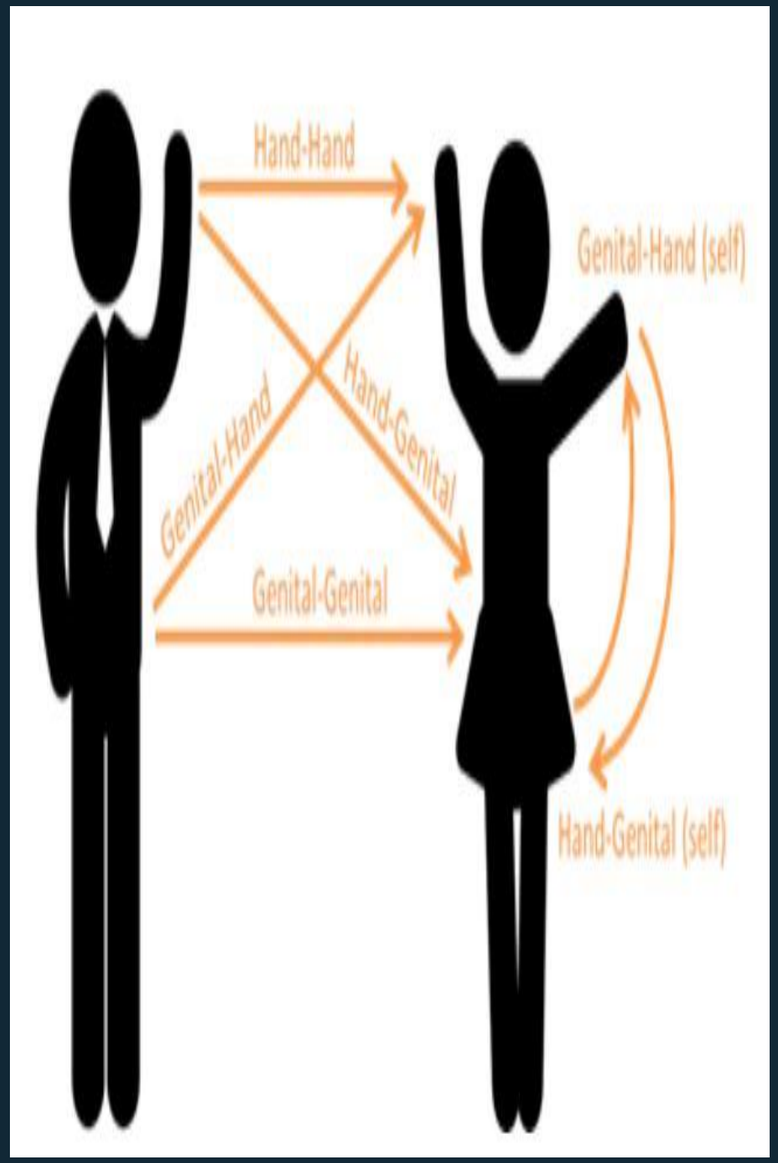
Direct contact during sexual intercourse with an infected individual is the most common mode of transmission.

Close Contact Transmission

Close contact with an infected individual can lead to HPV transmission through skin-to-skin contact.

Medical Procedure Transmission

HPV transmission can occur during medical procedures due to inadequate protective measures.



Vertical transmission

HPV can be transmitted from mother to child during childbirth through contact with the birth canal.

Indirect Contact Transmission

Indirect contact with contaminated surfaces, like clothing or daily necessities, can potentially transmit HPV.

Waterborne Transmission

While not definitively proven, HPV DNA has been found in water habitats, raising concerns about a potential waterborne transmission route. Further research is needed to confirm this mode of transmission.

Sample Collection, Storage and Transport

Cervical Swab Collection

A sterile cervical swab is used to collect cells from the cervix, which is the lower part of the uterus.

Tissue Specimen

Tissue samples are homogenized with a mechanical homogenizer and dissolved in sterile PBS. This allows for DNA extraction from the sample.

Liquid-Based Cytology Samples

Liquid-based cytology samples, like Cytoscreen or PreservCyt, are processed for DNA extraction using a centrifuge, resulting in a highly concentrated sample.

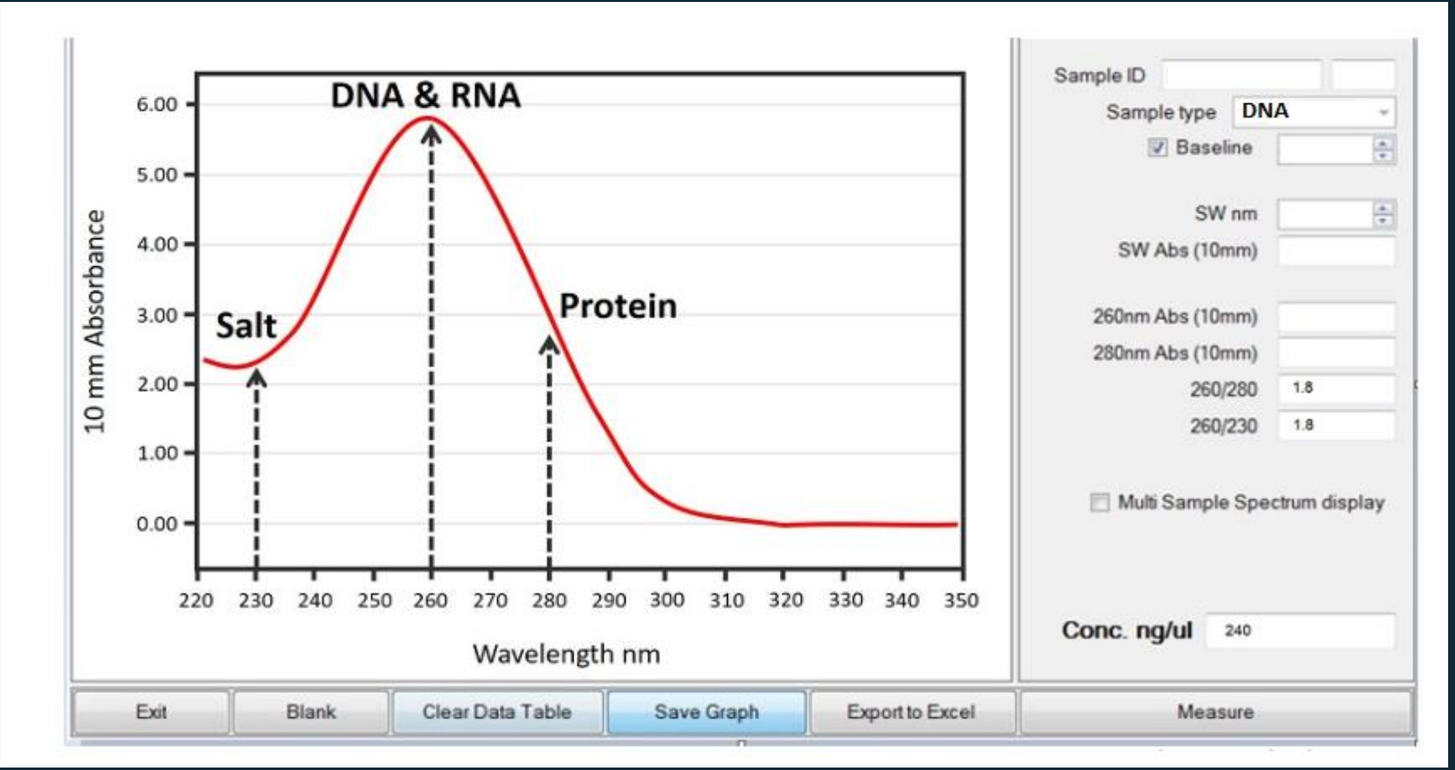
Storage and Transport

Samples are stored in a refrigerator at 2-8 °C for up to 24 hours or frozen at -20/80 °C for long-term storage. Transport regulations must be followed.

DNA extraction and quality control

Gel electrophoresis

Nano Drop



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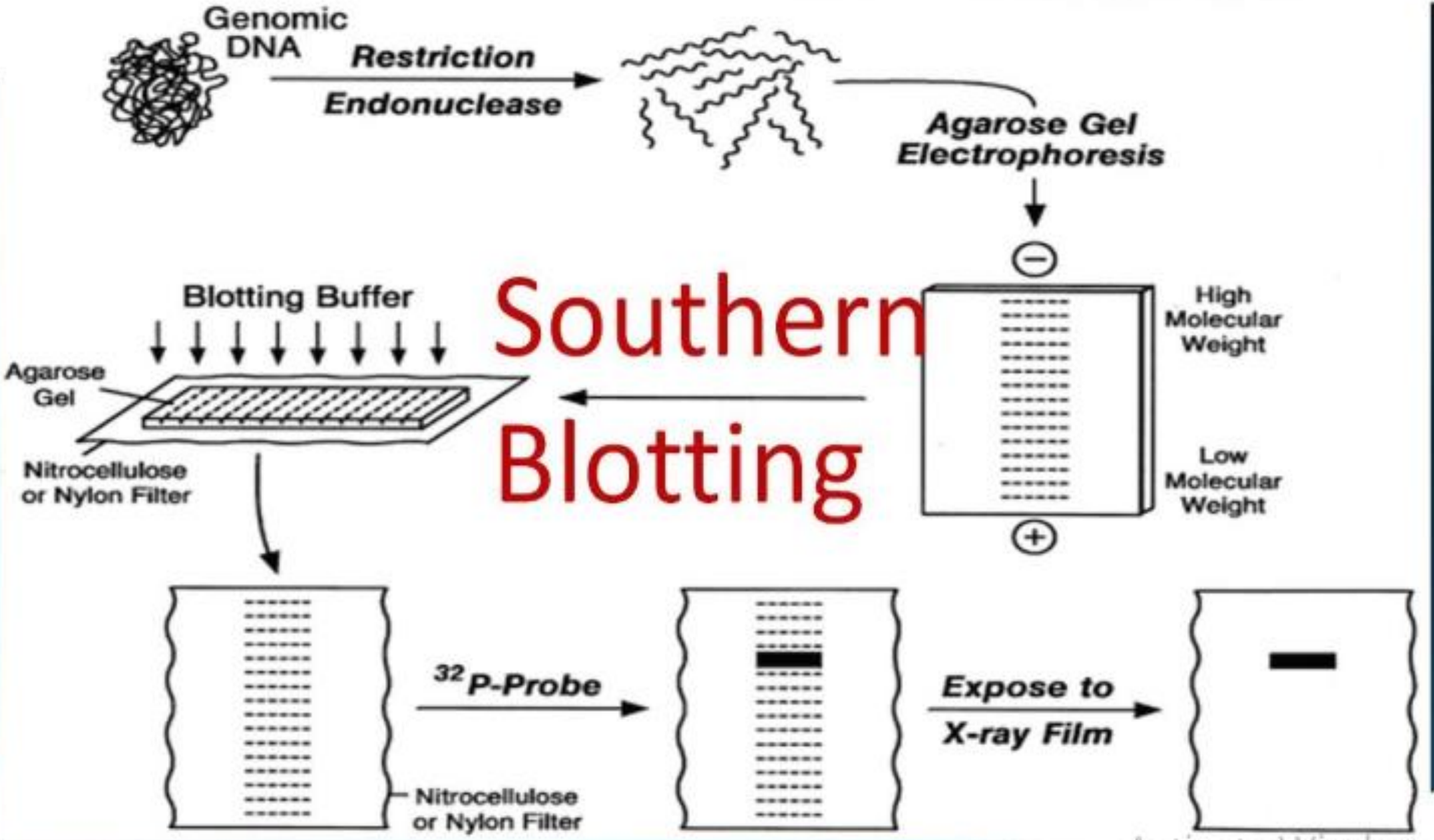
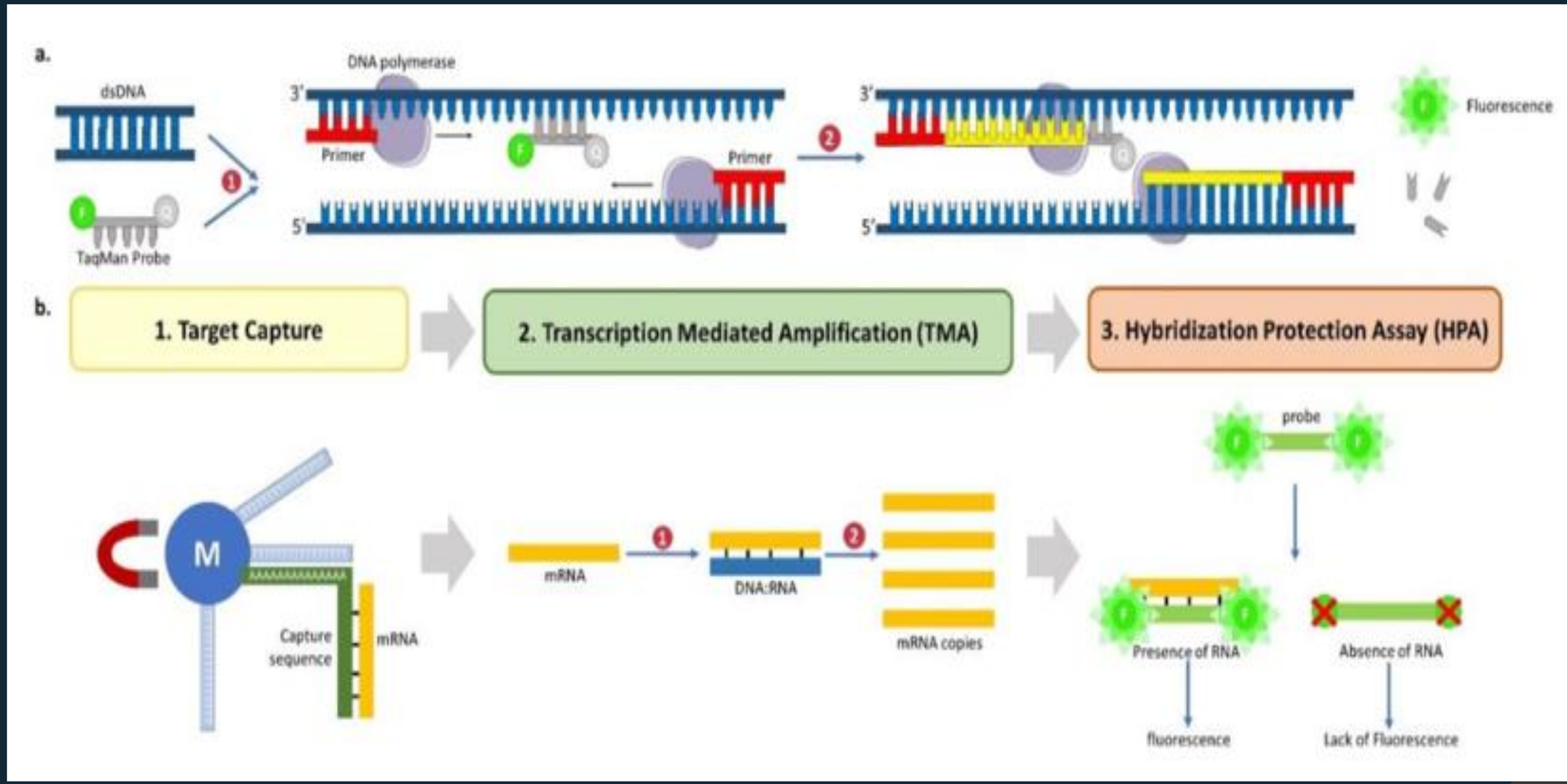
Classification of HPV tests

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 blotting, • in situ hybridization • dot-blot hybridization		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
• PCR • PCR-RFLP • Real Time PCR	Very high sensitivity	Contamination with previously amplified material can lead to false positives
	Multiplex analysis	



SIGNAL AMPLIFICATION ASSAY



HPV Genotyping with Real-TimePCR

1

Sample Collection & Preparation

Samples are collected and prepared for DNA extraction.

2

Real-Time PCR Setup

Real-time PCR (qPCR) is performed using primers and probes specific to HPV DNA.

3

Data Analysis & Interpretation

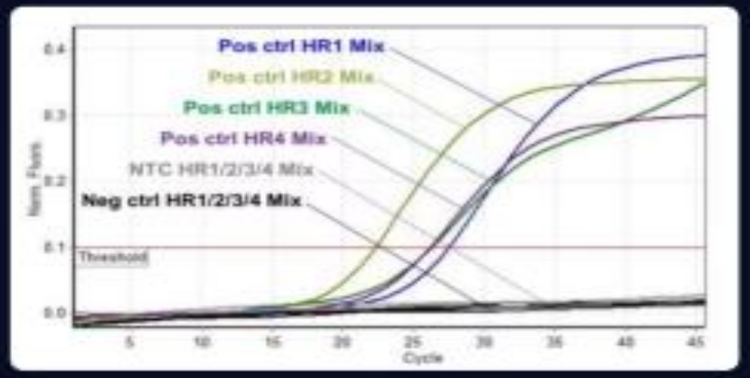
qPCR results are analyzed to detect and quantify specific HPV genotypes.

4

Clinical Application & Public Health

Results inform clinical care and support large-scale studies and screening programs.

HPV Real-Time PCR Result Analysis: Interpreting Data and Informing Clinical Decisions

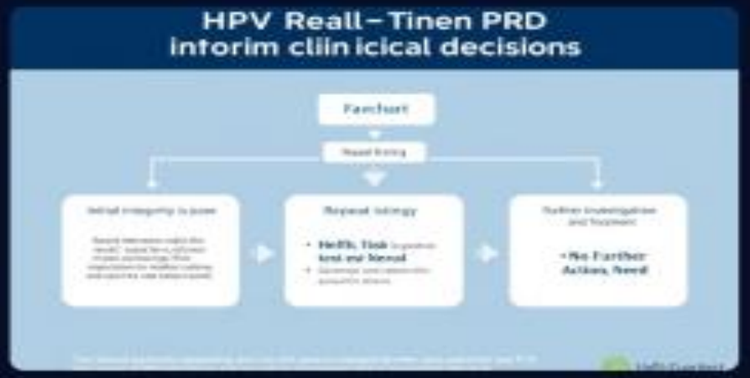


Interpreting Ct Values

Lower Ct values indicate higher viral load, suggesting a more advanced infection. However, accurate interpretation requires considering sample quality. Recall that inadequate DNA extraction, insufficient yield, or degradation can lead to false negatives. Therefore, samples should be handled according to the storage and transport guidelines outlined previously.

Addressing Ambiguities

Ambiguous results, such as Ct values near the threshold, require careful review of the entire process. Assess sample quality—was the DNA extraction sufficient? Was there any evidence of degradation? Re-testing with a new sample, prepared rigorously according to the previously described methods, may be necessary to ensure reliable results.



Clinical Decision-Making

Positive results, particularly those with low Ct values, may prompt further investigations such as colposcopy or other diagnostic procedures. Negative results, accompanied by confirmation of high sample quality, guide clinical management toward a less aggressive approach. However, always remember that sample integrity, as emphasized earlier, is crucial for the reliability of the results and subsequent clinical decisions.

Southern blotting troubleshooting

Faint Bands

*Insufficient DNA transfer to the membrane can lead to faint bands or no visible marker. This can be caused by issues with the transfer buffer, transfer time, or the gel itself.
*Check the transfer buffer and ensure it's fresh and properly prepared.
*Extend the transfer time to allow for complete transfer.
*Examine the gel after transfer to confirm no DNA remains.

Spotty Membrane

*Uneven distribution of solutions, such as blocking or hybridization reagents, during the process can result in a spotty appearance on the membrane.
*Ensure sufficient solution volume to fully cover the membrane.
*Use rocking or shaking to ensure even distribution.

Prevent membrane layers from overlapping.

Spots in DNA Lanes

*Bubbles trapped between the membrane and gel during transfer can cause spots within the DNA lanes.
*Carefully roll the membrane and Whatman paper to eliminate any air bubbles.
*Ensure a tight seal between the membrane and gel.

Real Time PCR troubleshooting for HPV genotyping

Sample Issues

Inadequate DNA extraction, insufficient yield, or degradation can lead to false negatives. Improper storage or transport can compromise sample integrity.

qPCR Setup

Troubleshooting includes checking for non-specific amplification, primer-dimer formation, inconsistent baseline fluorescence, and inaccurate quantification.

Verification

Verify sample quality and DNA integrity after extraction. Ensure reagent quality, including primer and probe concentrations. Perform instrument calibration and maintenance.

Data Analysis

Carefully perform data analysis, correctly setting the baseline and threshold. Use validated software and analysis parameters. Use positive and negative controls to validate the process.

با تشکر از توجه شما