



Prevalence of human papillomavirus and *Chlamydia trachomatis* infection in paired urine and cervical smear samples of Palestinian young women.

Walid Salim Basha, PhD

Faculty of Human Medicine

An-Najah National University



Chlamydia trachomatis

A nonmotile, gram-negative obligate intracellular bacterial pathogen, classified serologically into 15 serovars.

Pathogenesis



- | | |
|------------------------------------|----------------------------|
| 1- Trachoma | serovars A-C |
| 2- Genital infections | serovars D-K |
| 3- Lymphogranuloma venereum | serovars L1, L2, L3 |



Diagnosis

- Fluorescent antibody assay
- Frei test (delayed hypersensitivity) for LGV
- Growth in tissue culture
- DNA probe test
- PCR



C. trachomatis new detection method

PCR-SB Cryptic plasmid –PCR hybridization assay

DNA purification

Cervical and **Urine** Samples



PCR

(DNA amplification)



Hybridization

(Oligo-probes)



PCR

(DNA amplification)

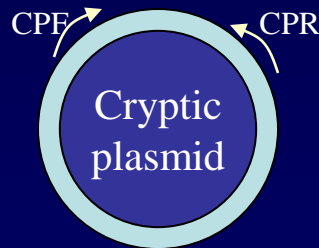
Two primers named

CT-CPF: 5'-TGATTGTACAAGGGATCCGTAAGT-3' (start at nt. 7089) and

CT-CPR: 5'-TCGATGAAAGACAGGAAATACG-3' (end at nt. 7465)

(X07547, GenBank)

Amplify 376 bps



homology to some other genes of
Human, Drosophila melanogaster and *S.cerevisiae*.



Hybridization

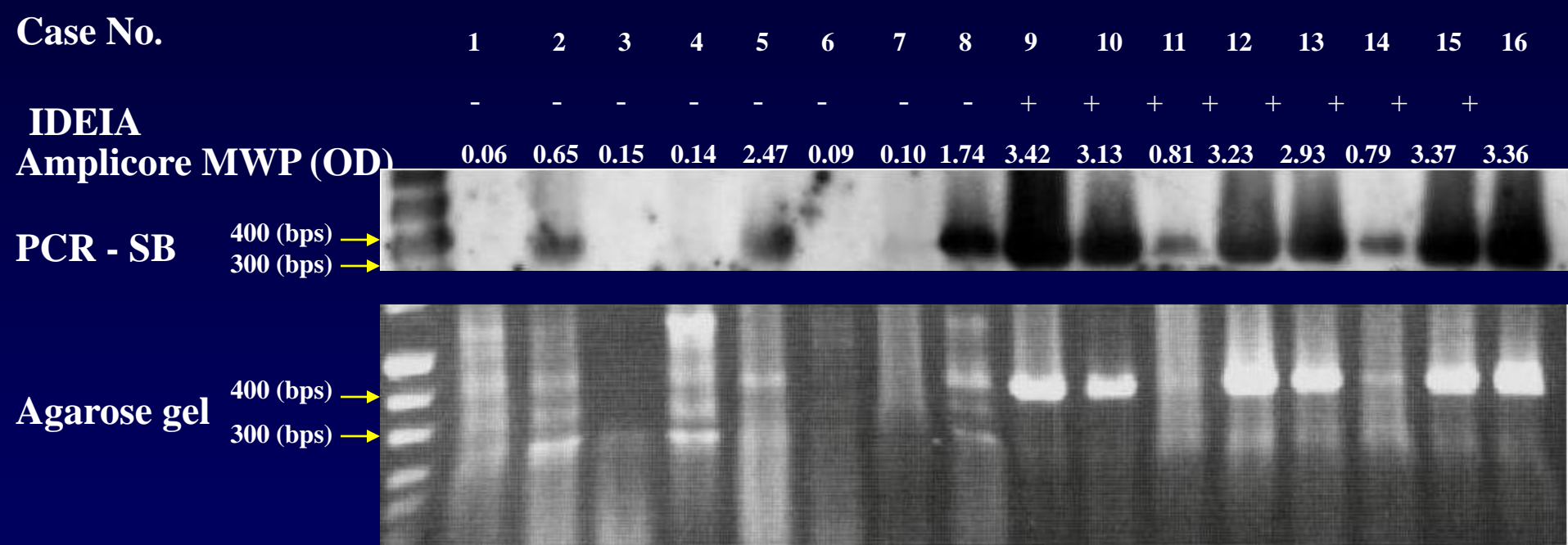
(Oligo-probes)

C. trachomatis-specific anti-sense probe named CP35
(nt.7335- nt. 7360)

The oligoprobe, CP-35 has also sequence homology in many sequences of human.



Comparison of the results with IDEIA, PCR-MWP and PCR-SB assays in 16 samples from urethritis patients





Human papillomavirus

Non-enveloped small double-stranded DNA viruses, classified into more than 100 genotypes.

Pathogenesis

1- Warts



HPV 1, 2, 3 & 7

2- Condyloma acuminata



HPV 6 & 11

3- Intraepithelial neoplasia

HPV 11, 16, 31 & 52

4- Cervical cancer

HPV 16 & 18





HPV TYPES

Low-risk HPV types

- Develop genital wart
- Common,
- Causes no symptoms
- HPV-6 or HPV-11

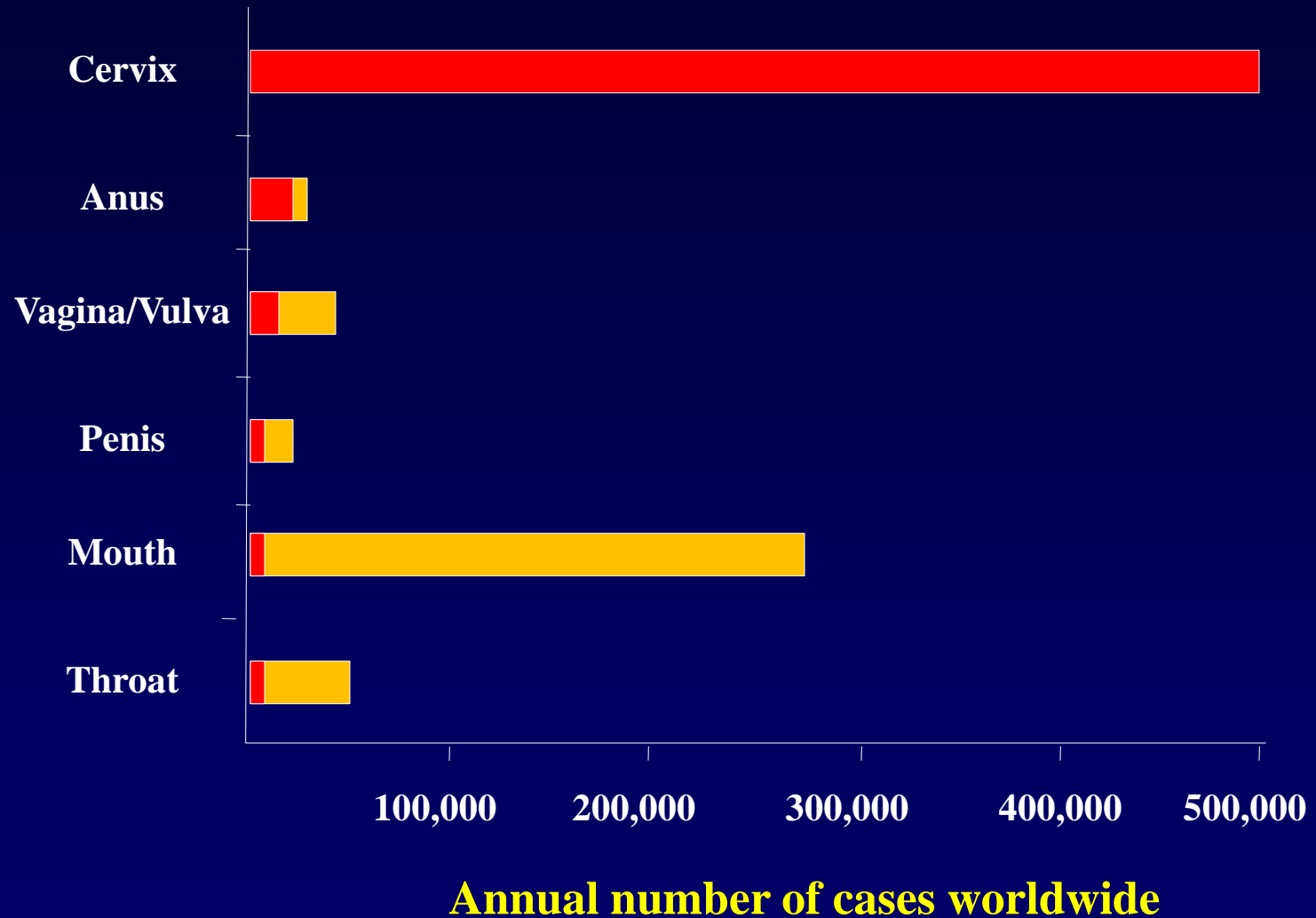
High –risk HPV types

- Develop cervical intraepithelial neoplasia (CIN)
- Common regress spontaneously

- Minority of lesions progress to high-grade dysplasias
- Then to carcinomas in situ before becoming invasive cancers.
- HPV-16 or HPV-18

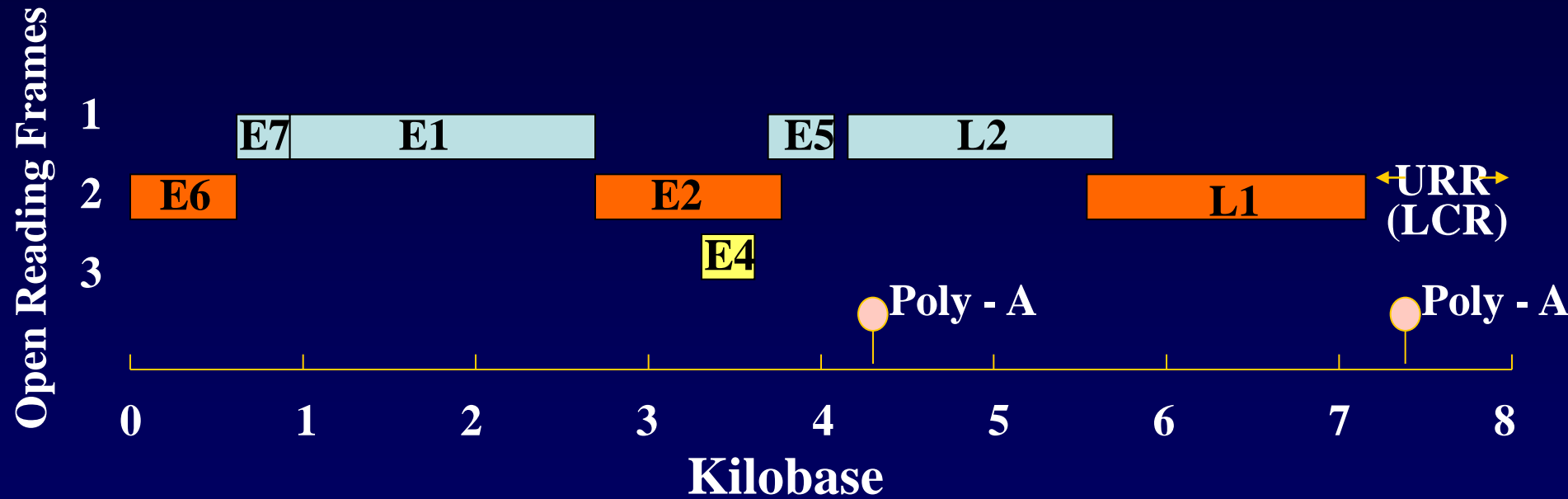


HPV-induced cancers





HPV genome - schematic representation





Noncoding

Upstream Regulatory Region

Contributes to the control of DNA replication and transcription of ORFs

Early region 6

E6 protein of oncogenic HPVs binds to the p53 tumor suppressor gene product and abrogates its activity by accelerating its degradation

Early region 7

E7 protein of oncogenic HPVs binds to the tumor suppressor gene product Retinoblastoma protein (RB) and related proteins, thus inhibiting their function

Late region 1

L1 protein is the major capsid protein

Late region 2

L2 protein is the minor capsid protein

Early region 5

E5 protein is located in the cellular membrane, prevents acidification of endosomes, and can stimulate the transforming activity of epidermal growth factor receptor and contribute to oncogenicity

Early region 4

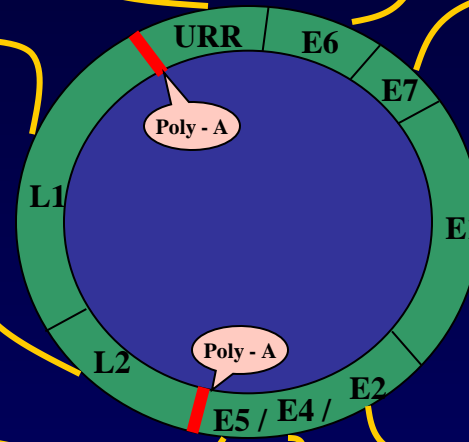
E4 proteins form filamentous cytoplasmic networks and share the same cellular distribution. They appear to play a role in viral replication

Early region 1

E1 protein involved in viral plasmid replication

Early region 2

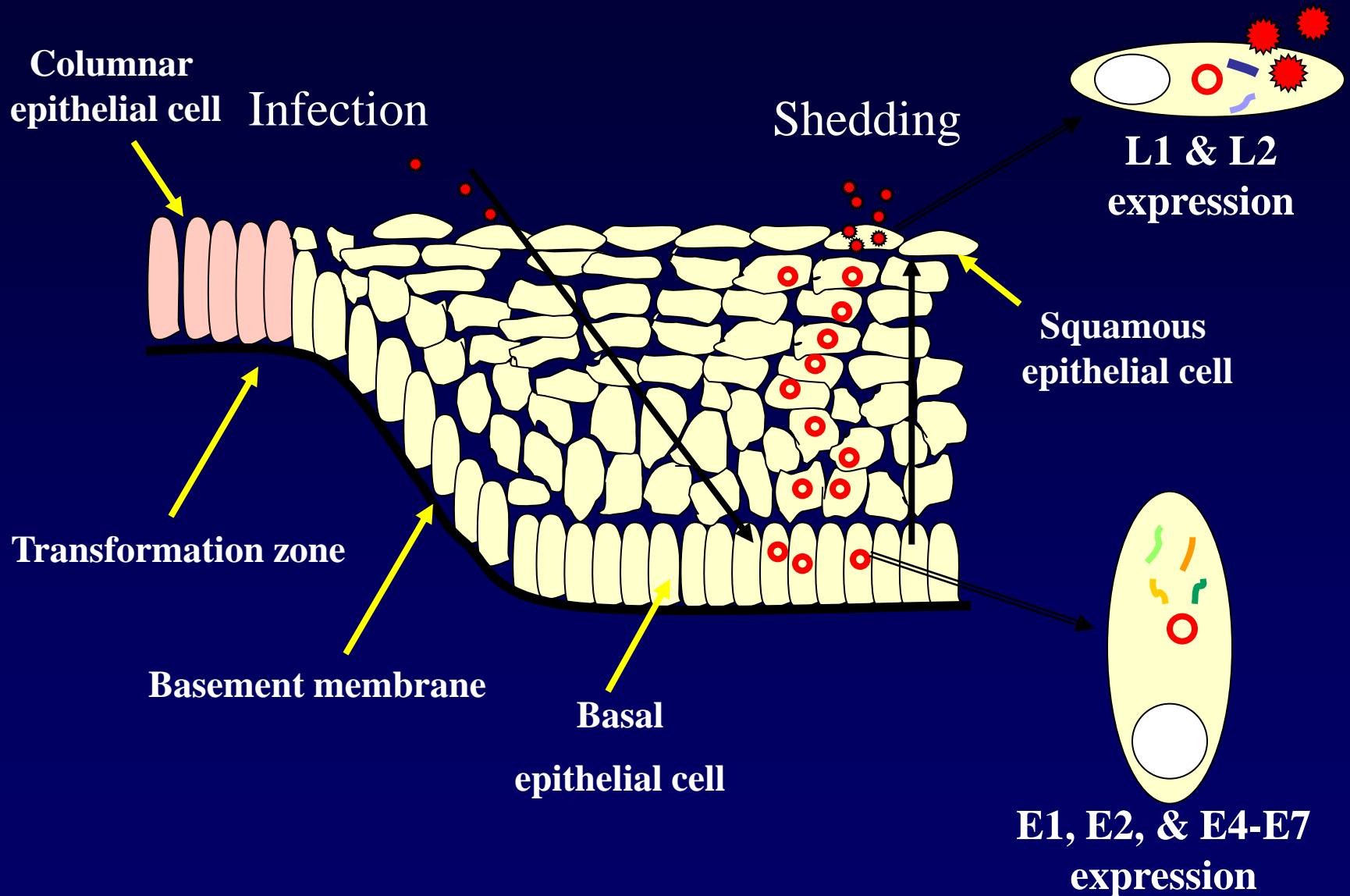
E2 protein is an important modulator of viral transcription and plays a role in viral replication

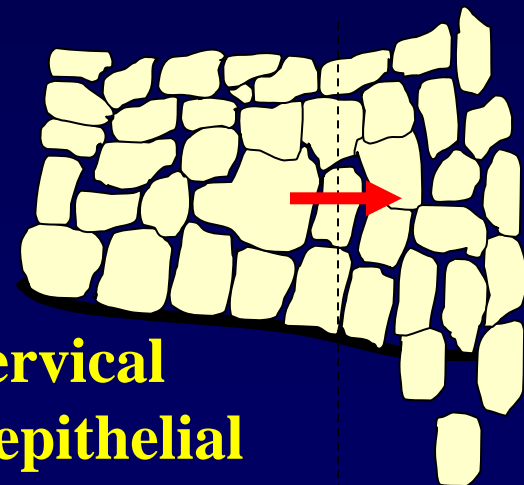


HPV Genome Structure



HPV infection and replication in cervical epithelial cells





Cervical intraepithelial neoplasia (CIN)

Invasive cervical cancer



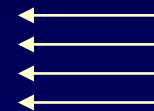
PCR

(DNA amplification)

LCR - E7 PCR

LCR-1,2,3,4

E7R-1,2,3,4



LCR

E6

E7

The primers and their related HPV sequences in LCR E7-PCR^a

HPV				HPV			
No.	Type	Group	5'-position	LCRF-primers	No.	Type	Group
				LCRF-1			
1	6b	A10	25	W W A R G G W G T R A C C G A A A A C G G -3'			
2	11	A10	25	A A A G G A G G A G G A C C G A A A A A C G G G			
3	13	A10	26	A A A G G A G G T G G A C C G A A A A A C G G G			
4	16	A9	25	T A A G G G G G T A A C C G A A A A A C G G G			
5	18	A7	32	A A A G G G G A G T A A C C G A A A A A C G G G			
6	26	A5	20	A A A G G G G T G T A A C C G A A A A A C G G G			
7	34	A11	22	A T A G G G G T G T A A C C G A A A A A C G G G			
8	35	A9	14	A G A G G G A G T A A C C G A A A A A C G G G			
9	39	A7	33	A A A G G G G T A A C C G A A A A A C G G G			
10	40	A8	24	A A A G G G G T A A C C G A A A A A C G G G			
11	42	A1	34	T A A G G G A G T A A C C G A A A A A C G G G			
12	45	A7	29	A A A G G G T G T A A C C G A A A A A C G G G			
13	51	A5	36	A A G G G T A T G A C C G A A A A A C G G G			
14	52	A9	28	A T A G G G T G T A A C C G A A A A A C G G G			
15	53	A6	42	A T A G G G T A G G A C C G A A A A A C G G G			
16	57	A4	32	A T A G G G G G T A A C C G A A A A A C G G G			
17	58	A9	30	A A G G G T G T A A C C G A A A A A C G G G			
18	59	A7	-13	A A A G G G T G T A A C C G A A A A A C G G G			
19	72	A3	28	A T A A G A C A A G A C C G A A A A A C G G G			
20	73	A11	22	A A A G G G T G T A A C C G A A A A A C G G G			
				LCRF-2			
6	A10		25	W W W G G G T C S A A C C G A A A A A C G G -3'			
11	A10		25	A A A C G G G T T C A A C C G A A A A A C G G G			
13	A10		26	A A A C G G G T T T A A C C G A A A A A C G G G			
31	A9		42	A A A G G G T G A A C C G A A A A A C G G G			
32	A1		29	T A T C G G T T T A A C C G A A A A A C G G G			
42	A1		49	A T T C G G G T T T A A C C G A A A A A C G G G			
44	A10		43	T T C G G T C C A A C C G A A A A A C G G G			
54	A7		25	T A A G G G A G G G A C C G A A A A A C G G G			
55	A10		40	T T C G G G T C C A A C C G A A A A A C G G G			
70	A7		31	A A A G G G A G G G A C C G A A A A A C G G G			
73	A11		22	A A A G G G T G T A A C C G A A A A A C G G G			
				LCRF-3			
27	30	A6	42	T A G G T T T T A G G A C C G A A A A A C G G -3'			
51	A5		36	A A A G G G T T A T G A C C G A A A A A C G G G			
53	A6		42	A A A G G G T T A A G G A C C G A A A A A C G G G			
56	A6		43	A G G G T T T A A G G A C C G A A A A A C G G G			
66	A6		43	T G G G T T T A A G G A C C G A A A A A C G G G			
68(ME188)	A7		3889	A A C G G T C A T G A C C G A A A A A C G G G			
				LCRF-4			
31	33	A9	44	G T A R G G G Y R A G A C C G A A A A R C G G -3'			
61	A3		22	G T A G G T T G G A C C G A A A A A C G G G			
72	A3		28	G T A A G A C A G A C C G A A A A A C G G G			
				E7R-primers			
				E7R-1			
1	6b	A10	5'	T C M K C C T C T T K C Y T T C T G A G G Y T G T -3'			
2	11	A10	2	T C C A C C C T C T T C T C T T C T G A G C T G T 642			
3	13	A10	3	T C C A C C C T C T T C T C T T C T G A G C T G T 642			
4	34	A11	4	T C A T C C C T C T T C C T C T G A G C T G T 638			
5	40	A8	5	T C A T C C T T C T T G A G C T G T 660			
6	44	A10	6	T C C A C C C T C T T C T C T T C T G A G C T G T 633			
7	55	A10	7	T C C A C C C T C T T C T C T T C T G A G C T G T 642			
8	57	A4	8	T A A G T A T C T C T C T T C T G A G C T G T 639			
9	61	A3	9	T C C A C C C T C T T G A C T C T G A G C T G T 639			
10	72	A3	10	T C C A C C C T C T T G A C T C T G A G C T G T 627			
11	73	A11	11	T C A T C C T C A C T C T G A G C T G T 633			
				E7R-2			
12	16	A9	5'	T C M T C M T C H T C R T C T G A G C T G T -3'			
13	26	A5	12	T C A T C C C T C T C T C A T C T G A G C T G T 671			
14	30	A6	13	T C A T C C C T C T C T C A T C T G A G C T G T 680			
15	31	A9	14	A C A T C C C T C T C T C A T C T G A G C T G T 674			
16	32	A1	15	T C A T C C A T C T C T C A T C T G A G C T G T 669			
17	33	A9	16	G C T T C A T C C T C T C A T C T G A G C T G T 682			
18	34	A11	17	T C A T C C C T C A T C C A T C T G A G C T G T 682			
19	35	A9	18	T C T C C T C T C T C A T C T G A G C T G T 660			
20	42	A1	19	T G G T C C A T C T C A T C T G A G C T G T 671			
21	51	A5	20	T C A T C C C T C T C A T C T G A G C T G T 657			
22	52	A9	21	G T A T C C C T C T C A T C T G A G C T G T 669			
23	53	A6	22	T C A T C C C T C A T C C C T C T G A G C T G T 662			
24	56	A6	23	T C A T C C C T C A T C C C T C T G A G C T G T 678			
25	58	A9	24	A T T T C A T C C T C G T C T G A G C T G T 681			
26	66	A6	25	T C A T C C C T C A T C C C T C T G A G C T G T 683			
27	73	A11	26	T C A T C C C T C A T C C C T C T G A G C T G T 681			
				E7R-3			
26	18	A7	5'	C A C W A N A T T K T G T G A C G C T G T G -3'			
27	59	A7	26	C A C A A C A T T T G T G T G A C G C T G T G 777			
28	45	A7	27	C A C A A A A T T T G T G T G A C G C T G T G 788			
29	59	A7	28	C A C A A A A T T T G T G T G A C G C T G T G 777			
30	68(ME188)	A7	29	C A C A C C A A T T G T G T G A C G C T G T G 741			
31	70	A7	30	C A C T G G A A T T G T G T G A C G C T G T G 4629			
				E7R-4			
32	54	A7	5'	C A T C A G A G T C T T C T A A T T G C T C -3'			
68(ME188)	A7		32	C G T C T G A A T C T C T A A T T G C T C 632			
70	A7		31	T G T C T G A A T C T C T A A T T G C T C 4538			
42	A1		42	C A T C T G A A C T G T C C A A T T G C T C 706			

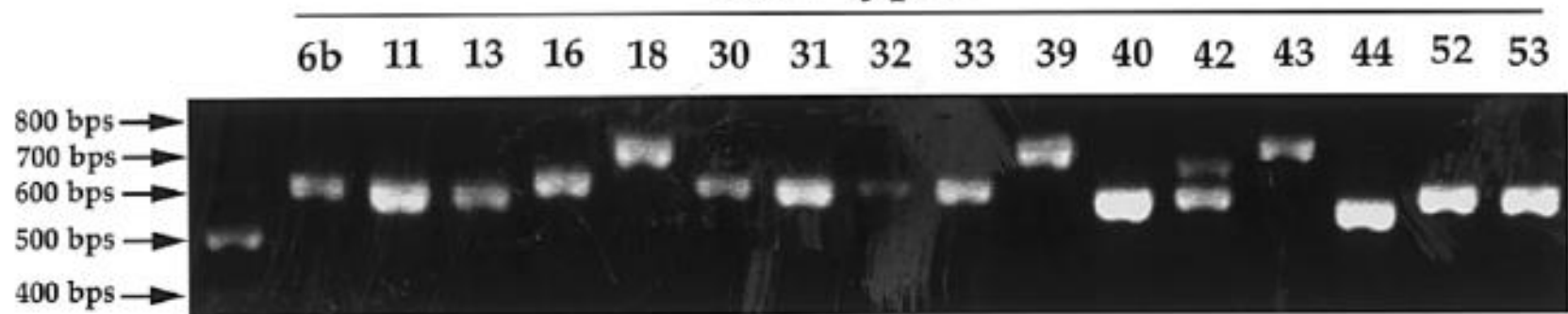
: mismatch sequence

^a R, A/G; W, A/T; Y, C/T; K, G/T; S, G/C; V, G/A/C; H, A/T/C; B, G/T/C; N, A/G/C/T.

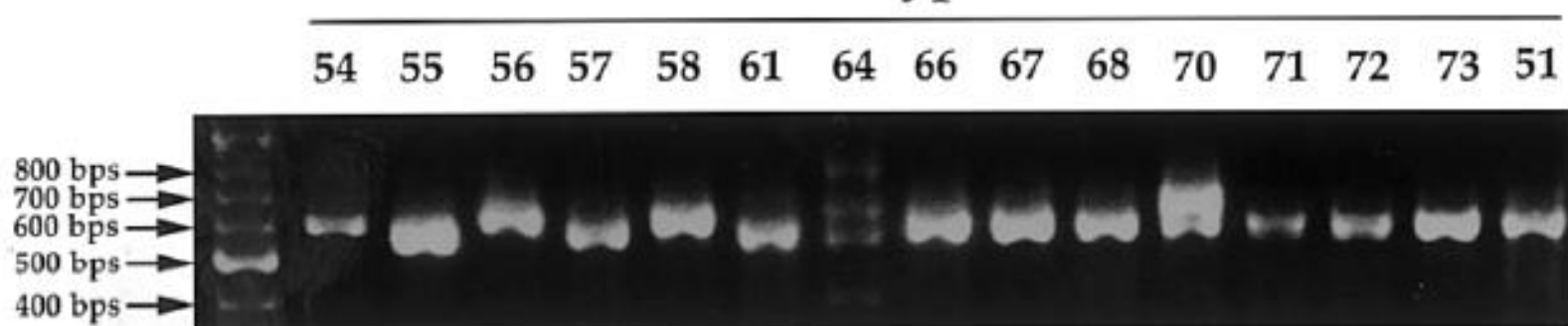


Detectable HPV types with LCR-E7 PCR test

HPV types



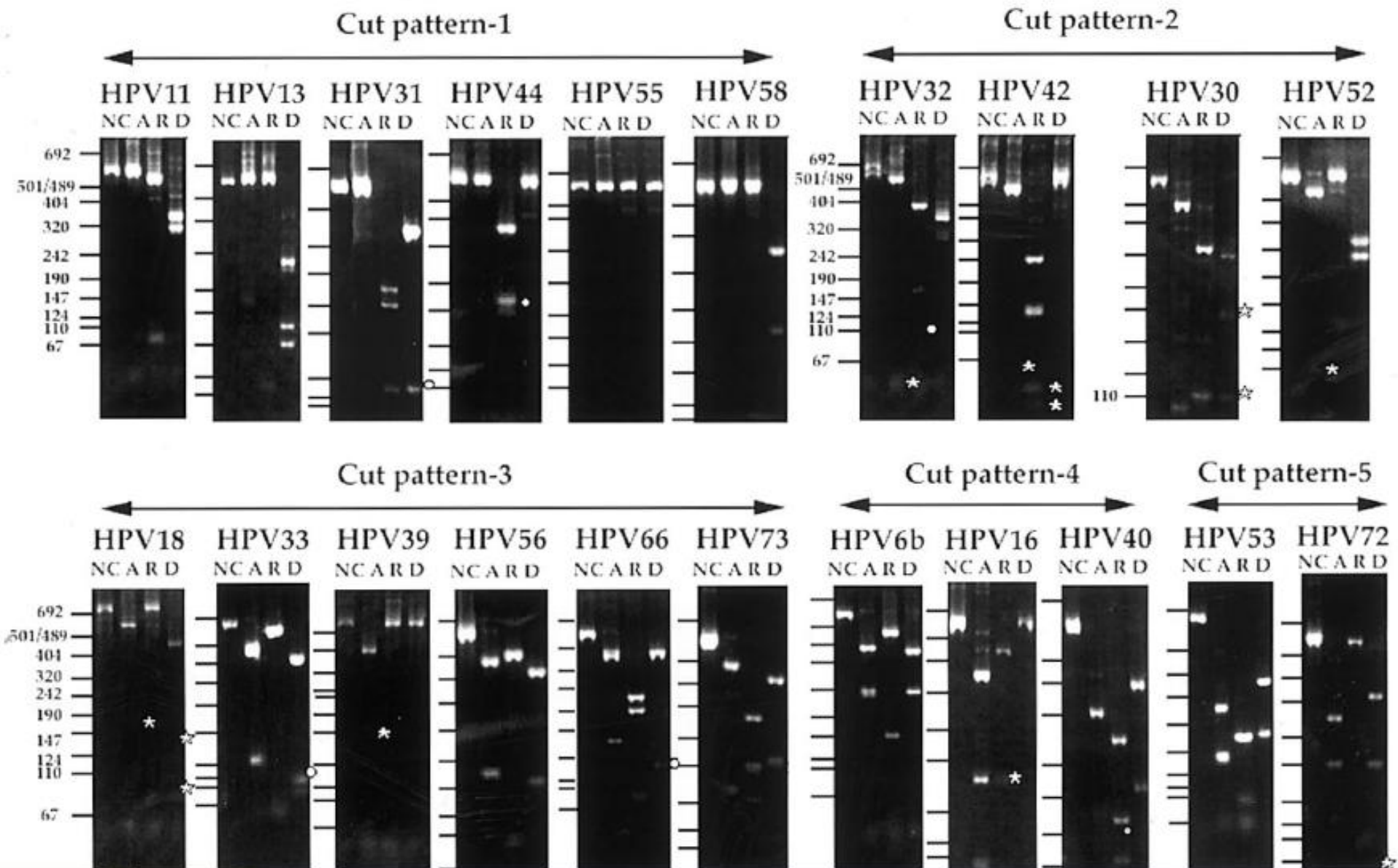
HPV types



HW typing by restriction-fragment-length polymorphism (RFLP)

Type	Size	<i>Ava</i> II	<i>Rsa</i> I	<i>Dde</i> I	<i>AC</i> CI	<i>Bam</i> HI
<i>Cut pattern-1^a</i>						
HPV26	661 ^b	661	325/176/122/38	419//225/17	661	575/86
HPV58	654	654	654	367/199/71/17	512/142	654
HPV51	650	650	332/1171/96/51	633/17	650	650
HPV51	628	628	255/243/130	426/109/43/33/17	470/1158	628
HPV44	600	600	375/179/146	583/17	600	600
HPV11	618	609/19	540/178	323/278/17	618	618
HPV55	600	600	600	583/17	600	535/65
HPV13	613	604/9	613	279/169/148/17	569/44	613
<i>Cut pattern-2</i>						
HPV32	655	598/57	378/170/83/124	344/275/36	655	655
HPV42	624	56/163	233/133/127/52/42/37	607/17	339/285	624
HPV35	658	553/105	633/25	641/17	470/188	658
HPV52	635	532/103	635	332/286/17	436/199	552/83
HPV30	633	463/99/71	337/112/63/43/23/22/17	320/185/111/17	633	633
<i>Cut pattern-3</i>						
HPV45	748	564/184	472/276	607/141	426/322	748
HPV18	746	540/181/25	746	425/144/89/88	496/250	658/88
HPV39	756	474/190/51/25/16	756	756	488/268	756
HPV70	670	501/102/51/16	670	758	529/103/38	670
HPV59	755	499/187/69	504/251	516/239	755	666/89
HPV68	650	482/102/50/16	414/236	241/223/109/77	612/38	650
HPV73	638	478/160	296/195/147	419/202/17	638	638
HPV56	639	463/176	521/96/22	435/144/43/17	639	639
HPV34	639	478/161	296/148/137/58	525/97/17	639	639
HPV33	639	487/152	585/54	438/105/79/17	639	639
HPV66	639	454/176/9	278/243/96/22	478/111/33/17	340/160/139	639
HPV57	608	465/143	547/61	287/231/73/17	608	608
<i>Cut pattern-4</i>						
HPV54	608	381/126/92/15	381/84/76/37/30	608	491/117	608
HPV16	647	386/173/88	474/173	630/17	647	647
HPV6b	618	385/224/9	470/148	368/233/17	594/24	618
HPV40	610	331/96/87/76/20	278/1661/29/37	396/197/11/6	486/124	610
<i>Cut pattern-5</i>						
HPV61	606	282/224/60/24/16	390/179/37	327/219/43/17	404/202	606
HPV72	606	282/190/114/20	569/37	339/190/60/17	606	606
HPV53	637	278/179/170/10	220/219/96/60/23/19	363/220/37/17	637	637

RFLP analysis for HPV typing on LCR-E7 PCR product





A. Hybridization result for HPV screening



B. Hybridization result for HPV cutting pattern

Sample No.	B			H			I			
HPV type	(HPV16)			(Unknown)			(HPV16 - 33)			
Cutting enzymes	M	AvaII	RsaI	DdeI	AvaII	RsaI	DdeI	AvaII	RsaI	DdeI





Aim of work

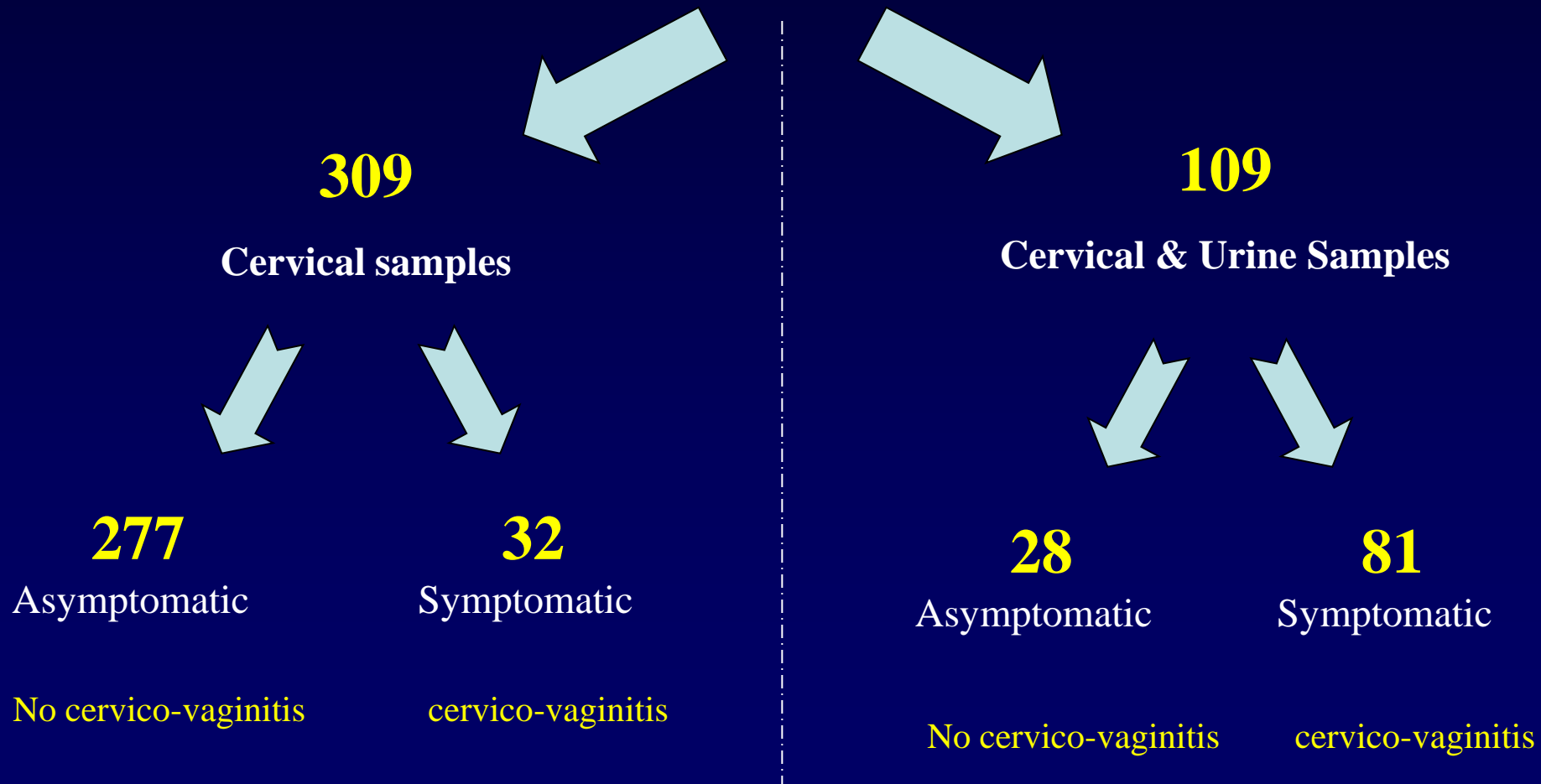
- To determine the prevalence of HPV infection in Palestine
- To determine the age group considered as a risk factor
- Is there a correlation between *C. trachomatis* and HPV infection
- Is there a correlation between HPV infection and cervical abnormalities in West Bank
- Genotyping for detected HPV in West Bank
- Recommendations in order to improve women's health care services (quality of care).



Subjects

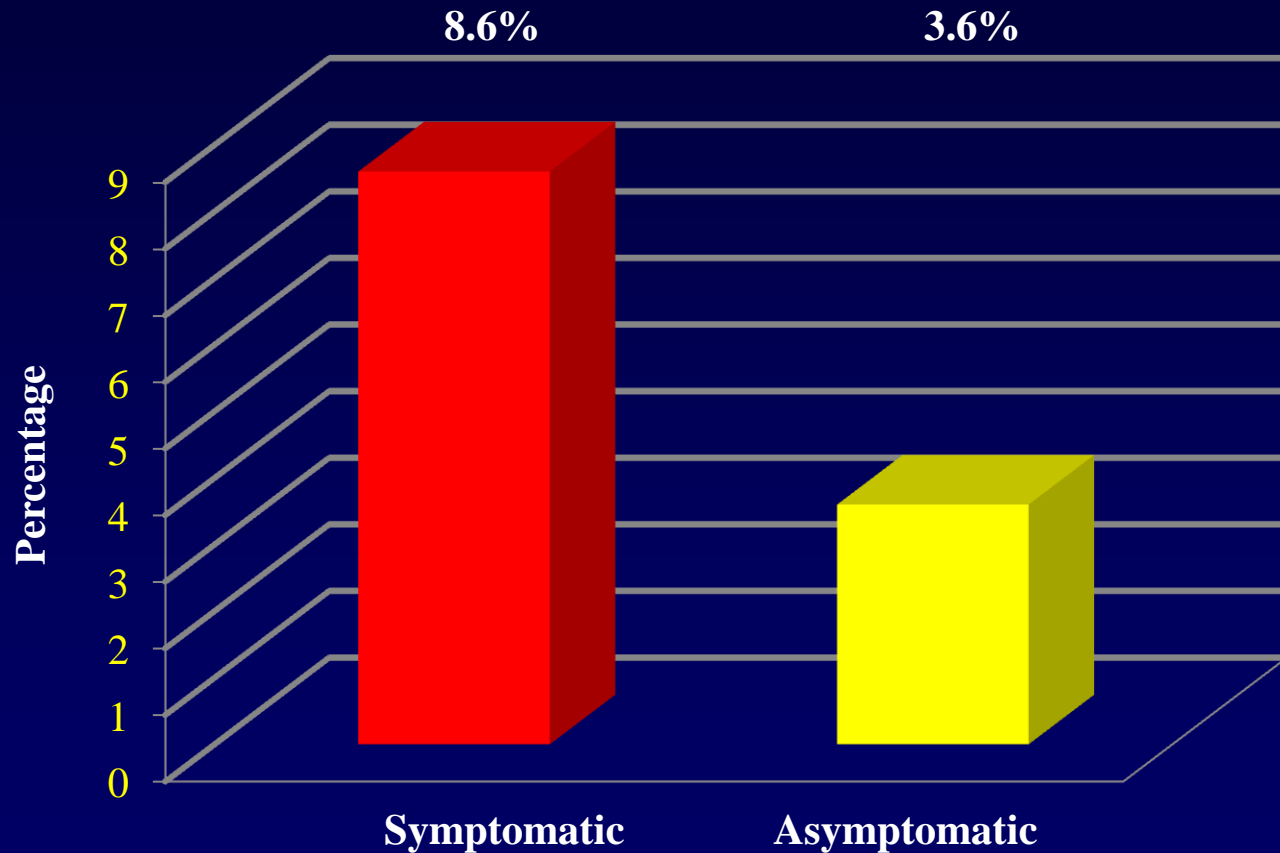
418 women

visited governmental or private obstetrics and gynecology clinics for various reasons in the West Bank



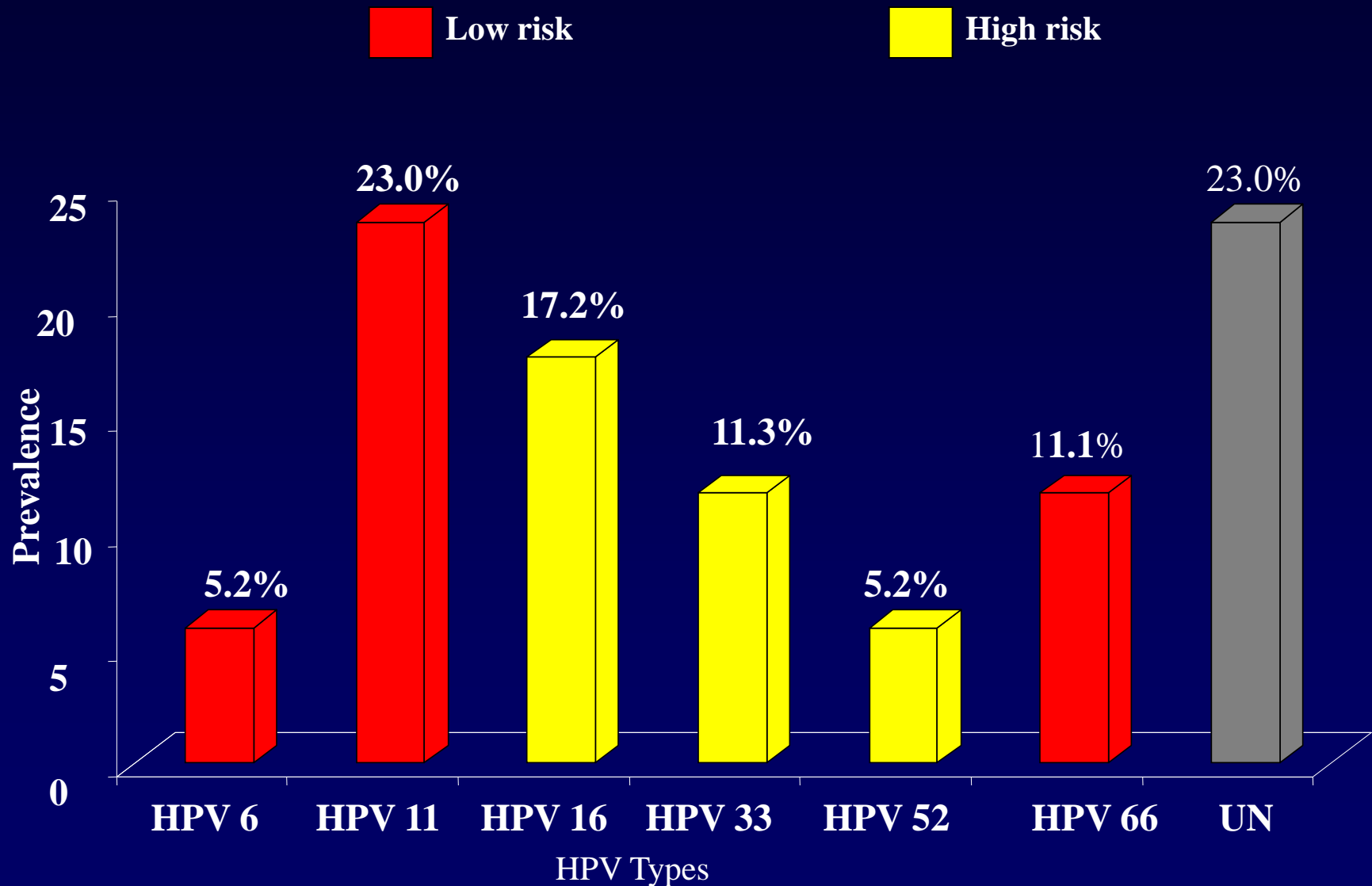


Prevalence of of HPV infection among symptomatic and asymptomatic women



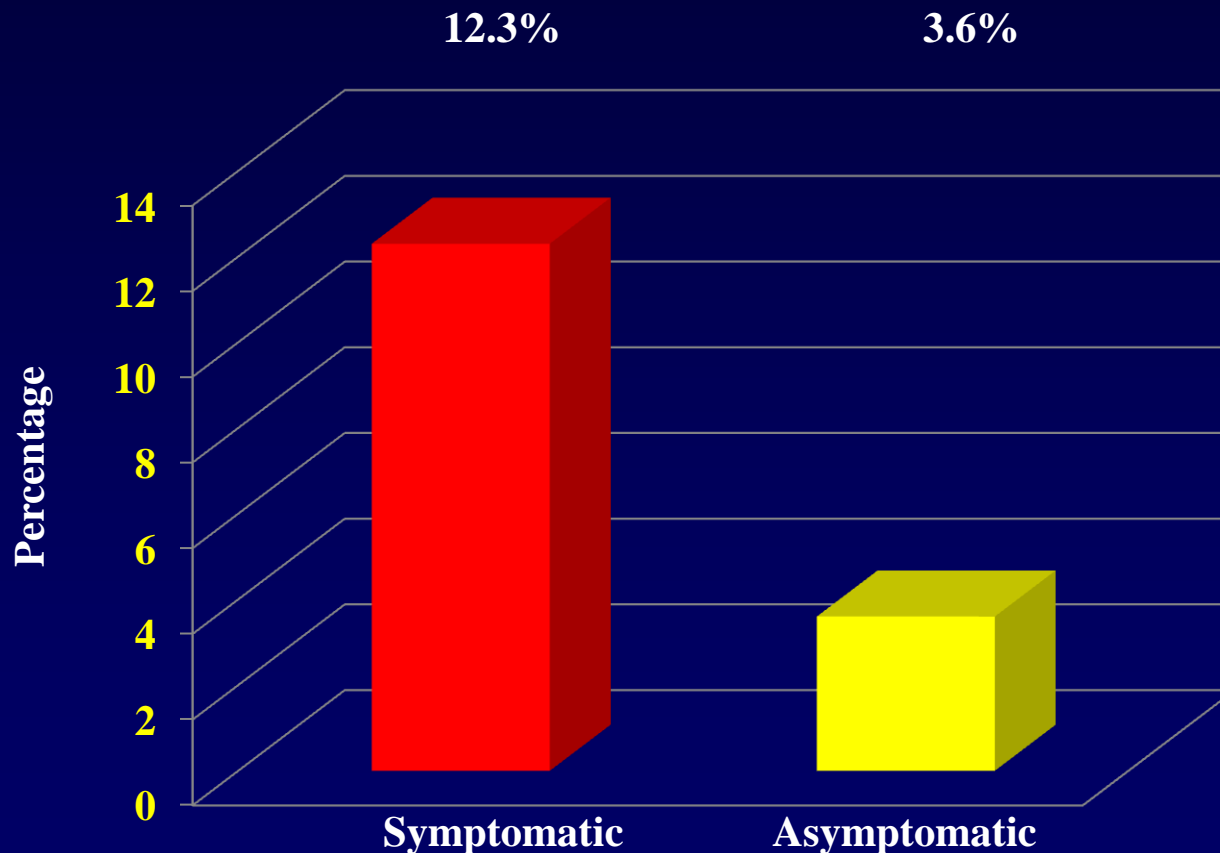


Prevalence of HPV Types



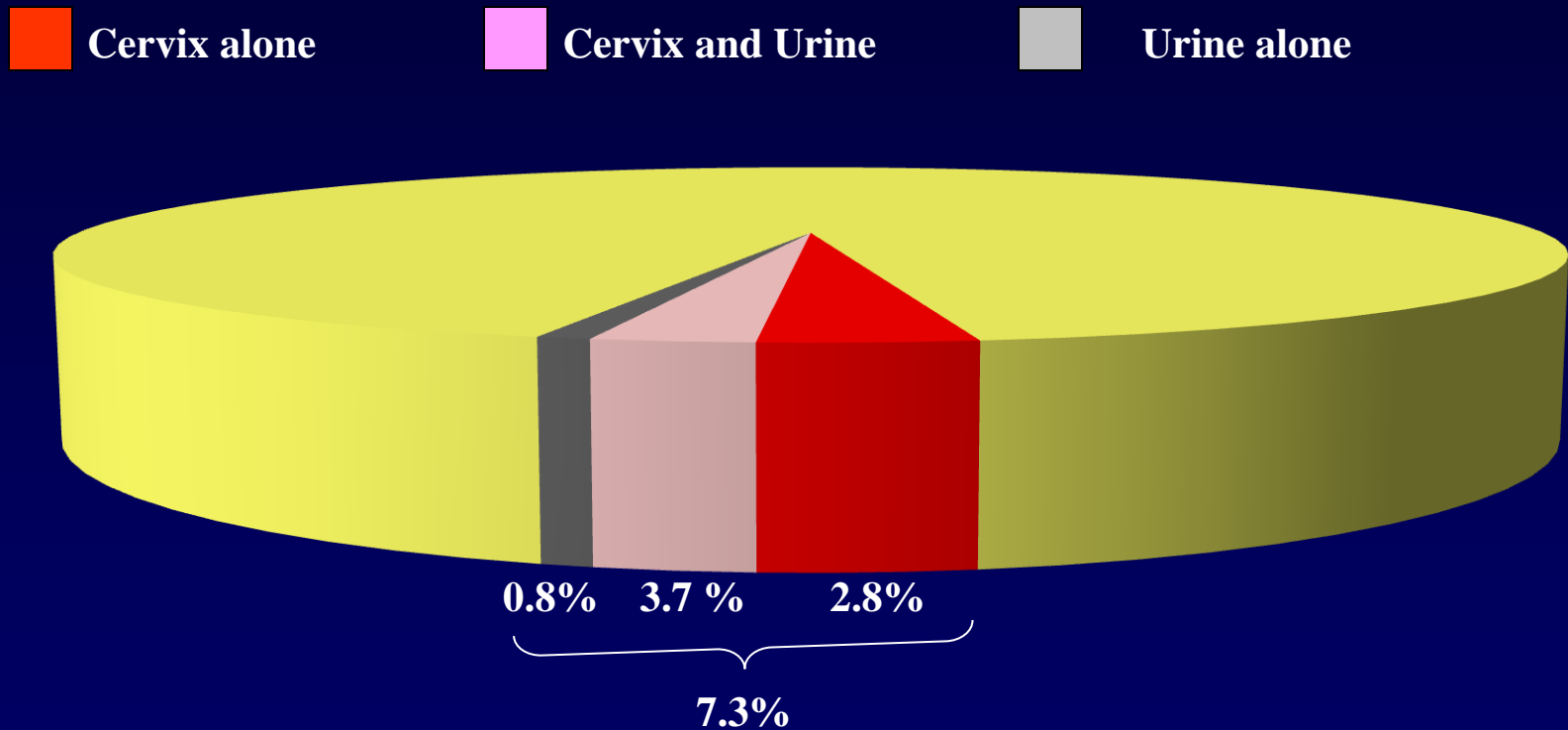


Prevalence of *C. trachomatis* among symptomatic and asymptomatic women



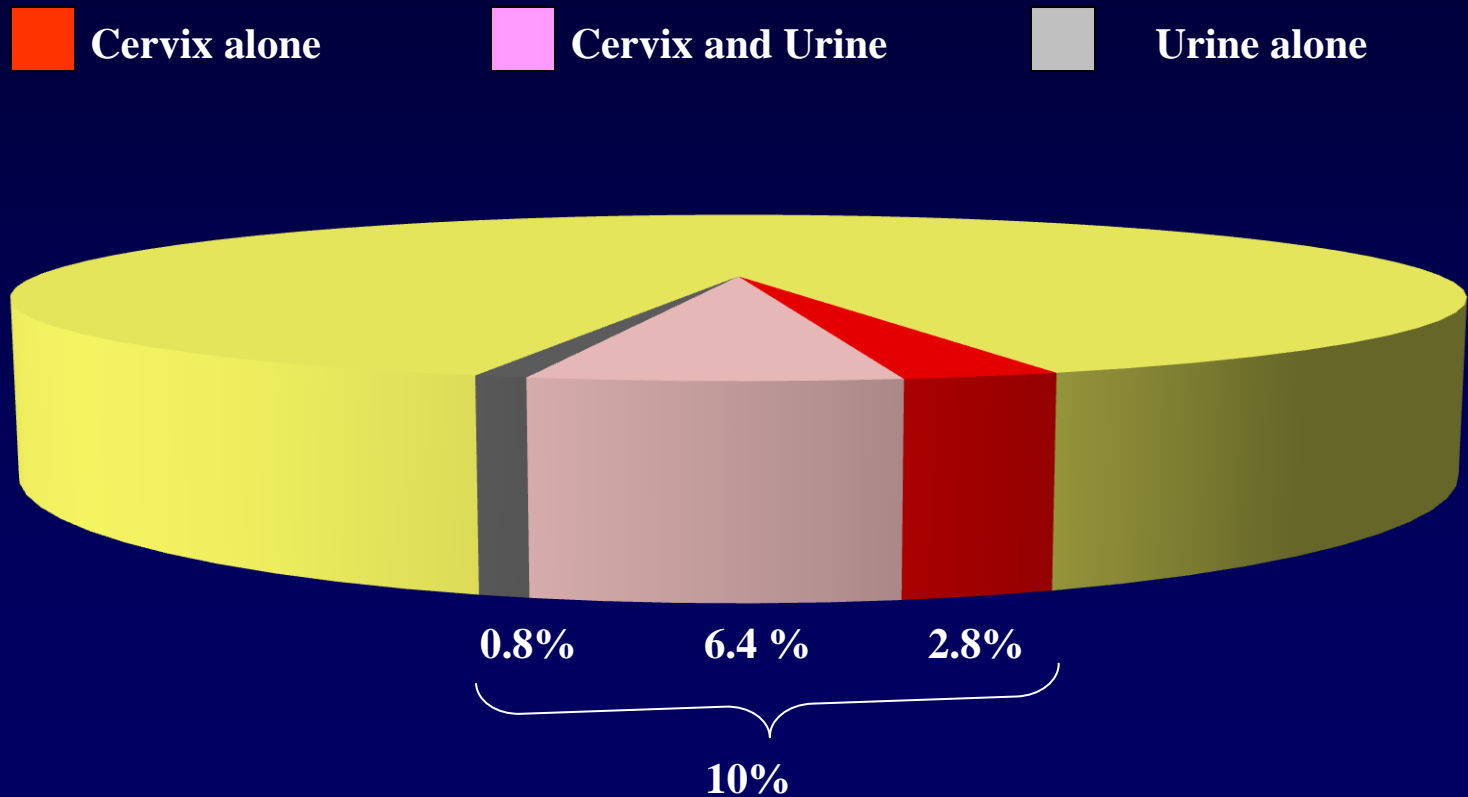


Comparison between cervical and urine samples for HPV detection





Comparison between cervical and urine samples for *Chlamydia trachomatis* detection





Recommendations

- STDs are a public health problem in Palestine
- Dealing with STDs should receive more attention by MOH and other health providers.
- Health education programs should be developed, carried out, and implemented by national health education committee.
- Medical and community awareness should be increased by all means.



Recommendations

- Introduction of HPV/DNA testing greatly facilitate the identification of women at risk for cervical cancer. This strategy minimizes unnecessary follow-up visits and invasive procedures without compromising the detection of disease.
- The urine samples can't be an alternative test for cervical samples for HPV detection but could be for CT screening

