

# Case Report: High-risk human papillomavirus (HRHPV) genotypes in clinical laboratory practice

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## Introduction

Persistent infection by high-risk human papillomavirus (HRHPV) contributes to 99% of cervical cancer cases. Accurate HRHPV genotyping helps distinguish persistent infection from transient HPV infection, which can be cleared by the immune system, therefore identifies the patient who bears true risk of developing cervical cancer. The Centers for Disease Control and Prevention (CDC) also requires HPV genotyping to assess efficacy of the HPV vaccine covering HRHPV 16/18, which represented 70% of cervical cancer cases in the U.S. Current FDA approved tests ignore one third of patients with cervical cancer caused by other HRHPV types. As a reference lab, we provided HRHPV genotyping to 3532 reflex or dual cases (with previous questionable pap or HPV test) in conjunction with pap cytology. The geographic sample collection area covers CA, CO, NY, NJ, KS, MO, and OH.

## Methods

Multiplex PCR approach by amplifying E6, E7 or L1 regions for each of 13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. specific size bands were detected by capillary gel electrophoresis.

HPV High Risk Primer Sets for multiplex:

Primer Set I: (HPV 16/45/59), 16 - 223 bp, 45 - 187 bp, 59 - 290 bp;

Primer Set II: (HPV 18/31/51), 18 - 267 bp, 31 - 288 bp, 51 - 171 bp;

Primer Set III: (HPV 52/56/35/33), 52 - 288 bp, 56 - 253 bp, 35 - 214 bp, 33 - 203 bp;

Primer Set IV: (HPV 58/68/39), 58 - 312 bp, 68 - 275 bp, 39 - 239 bp, IC beta actin - 133 bp.

The type specific amplification has been confirmed by DNA sequencing.

## Conclusions

1. Single type 16 or 18 exist in 20% of all cases; co-infection in 17%.
2. In CIN1 and above pap, 16, 18 exist 19% single, 27% multiple.
3. Multiple types account for 27% of all positive cases.
4. HPV vaccine may not protect majority of young women.

By distinguishing the persistent from transient HRHPV infection through accurate genotyping, we can identify women who present with a true high risk for cervical cancer as persistently infected with any of the 13 specific HRHPV types at the same cost as less detailed tests available today. Properly identifying these patients for referral to colposcopy will lead to more accurate and appropriate use of treatments which can reduce overall medical cost and patient anxiety.

# of 16 and/or 18 in positive specimens	
Neg pap	single 41
	Multiple 20
ASCUS	Single 51
	Multiple 53
CIN1/LSIL	single 5
	multiple 22
CIN2/3/HSIL,CIS,AGC	single 12
	multiple 2
Total type 16, 18	single 109
	multiple 94

## Results

CIS, HSIL, CIN2/3, ASC-H HPV types			
Case	Age	CIN2/3	Type
1	32	AGC, HSIL	16
2	25	2/3, HSIL, CIS	16
3	22	HSIL, ASC-H	16
4	26	HSIL, 2/3, CIS	16
5	26	HSIL	16
6	26	HSIL, 2/3, CIS	16
7	56	AGC	16
8	46	HSIL, 2/3, CIS	52
9	36	HSIL, 2/3, CIS	18
10	19	HSIL, ASC-H	16, 59
11	28	HSIL, 2/3, CIS	16
12	56	HSIL	35
13	23	HSIL, 2/3, CIS	16
14	70	HSIL, 2/3, CIS	35
15	32	HSIL, 2/3, CIS	16
16	28	HSIL	33, 35
17	20	HSIL	16, 39, 52
18	19	HSIL, 2/3, CIS	16

### Summary of HRHPV Positives vs Cytology

# of cases	Pap	Single HPV	Multiple HPV
2854	Negative	211	52
581	ASCUS	128	61
71	CIN1, LSIL	42	29
18	CIN2/3, HS	15	3
8	ACS-H	6	2
3532		402	147

### HPV types in ASC-H cases

Age	27	47	35	52	52
HPV type	51	31	51	16	16, 68

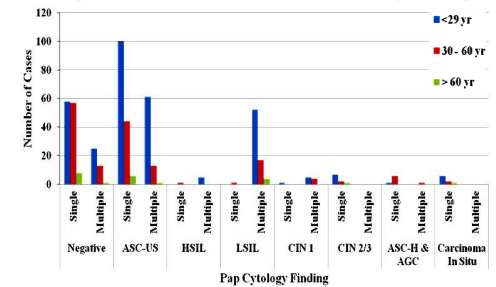
### HPV genotyping summary in patients

	2010	2009	2008	2007	2006	Total
ACS-US	80	79	88	139	195	581
ACS-US (pap+)	26	35	33	41	54	189
Total genotyping HPV+	612	475	367	1240	838	3532
HPV+	100	113	78	139	119	549

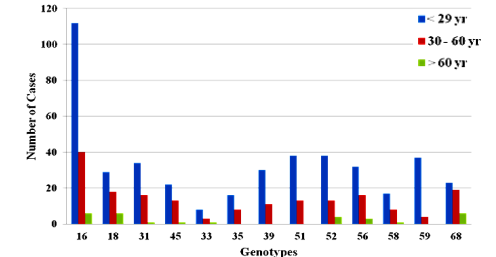
### Specificity Summary

CIN1 Above HPV Positive	89	Cancer Cases	10	HSIL+AGC Cases	8
CIN1 Above HPV Positive	89	Cancer and HPV Positive	10	HSIL HPV Positive	8

### Pap vs. HRHPV Infection in Different Age Groups



### Frequency of 13 types of HRHPV in Age Groups



Total 549 HPV positives, 402 are single HPV type, 147 are multiple infection. 16 and 18 were found in 203 specimens, (37%) including 109 single (20%), and 94 mixed with other HRHPV types (17%).

### Specific PCR Bands in HR HPV Genotyping

