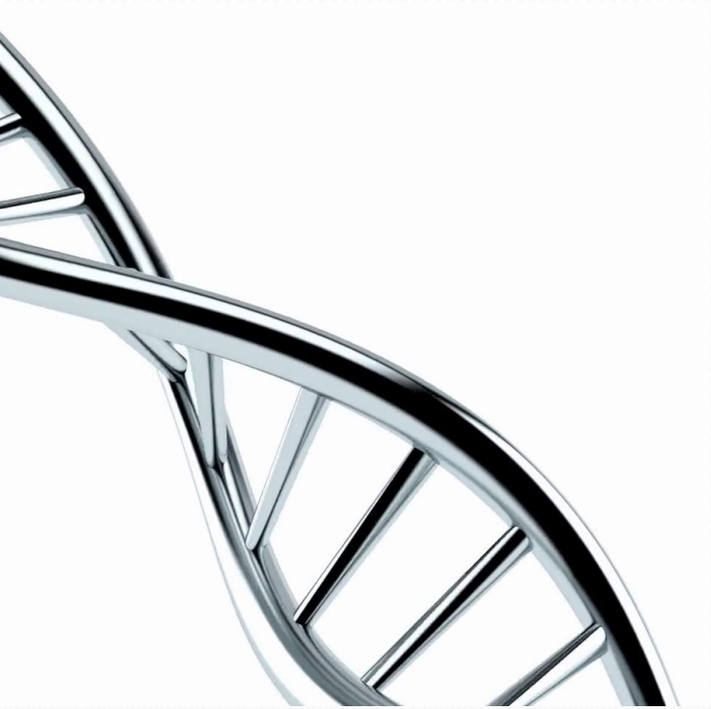
What are the clinical risks of the 12 Other (non16/18) HPV types?



Nicolas Zacchi

Snr Medical Laboratory Scientist
Pathlab Bay of Plenty
August 2022

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INDEX

- ✓ **Brief info on HPV genome and genotypes**
- ✓ **Genotypes prevalence and clinical relevance**
- ✓ **National Screening Programme**
- ✓ **HPV genotyping testing**
- ✓ **Pathlab experience with extended genotyping**
- ✓ **HPV Vaccination**
- ✓ **Final discussion**

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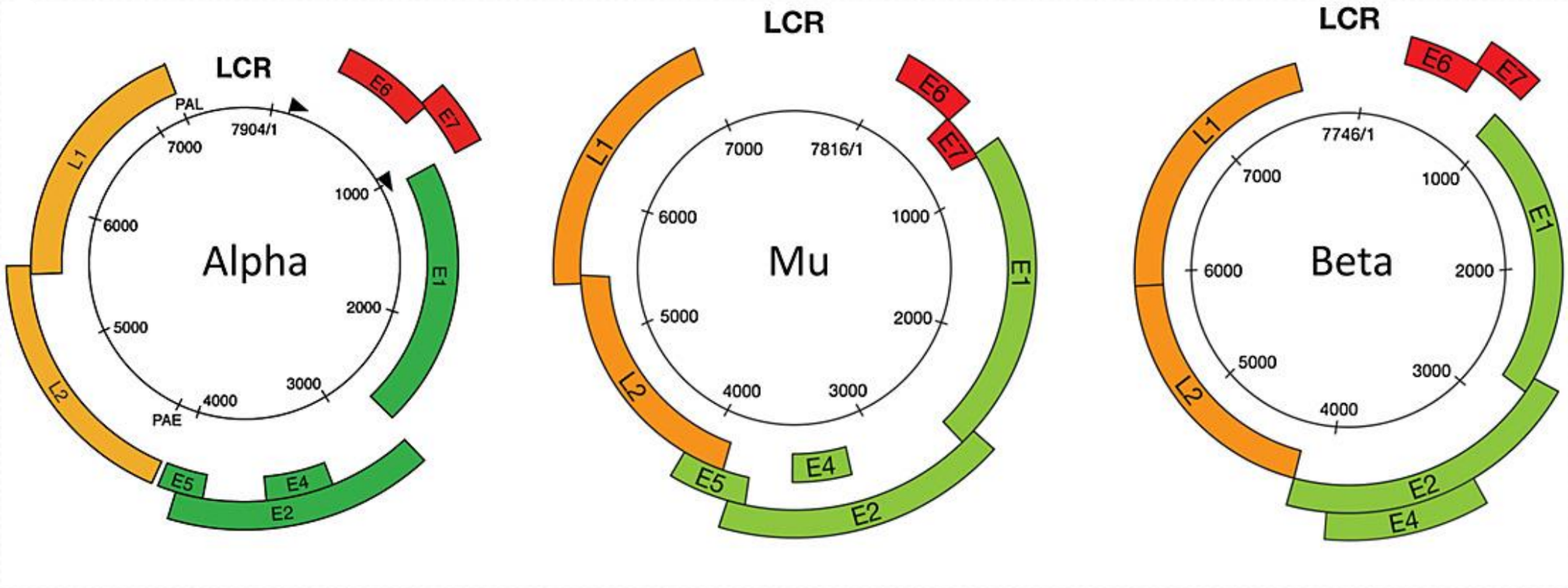
HPV Classification

Non-enveloped viruses with ds-DNA circular genomes (7–8 kbp)

Classified into 5 genera: Alpha, Beta, Gamma, Mu and Nu



HPVs cause wide range lesions, from benign to invasive tumours

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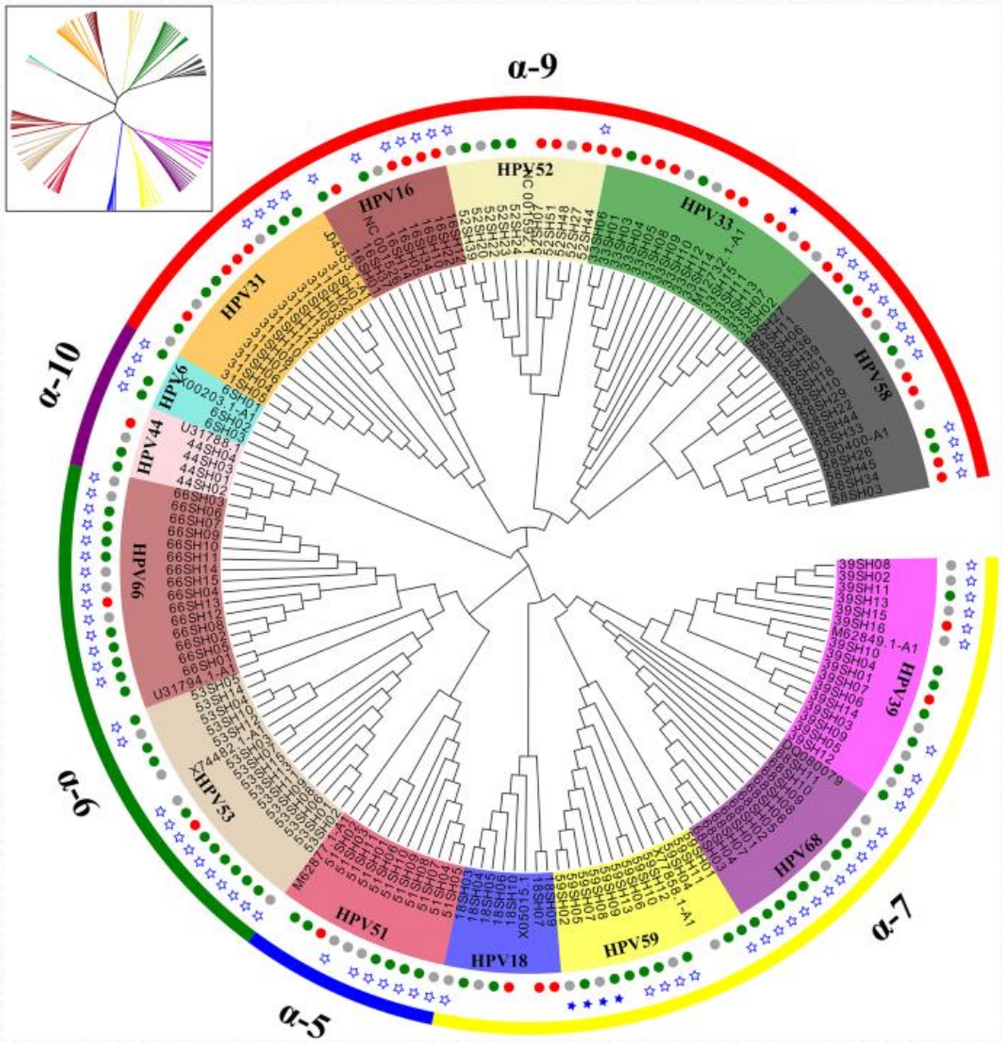
HPV Classification

All HPV that are cervical carcinogens are part of the Alpha genus

Alpha-HPV infects cutaneous and mucosal epithelia



Zhao et al, https://doi.org/10.1186/s13027-019-0250-9

HPV-mediated Oncogenesis

Persistent infection, with expression of onco-proteins E6 and E7

αHPV	HR-HPV
α-9	16, 31, 33, 35, 52, 58
α-7	18, 39, 45, 59, 68
α-5	51
α-6	56, 66

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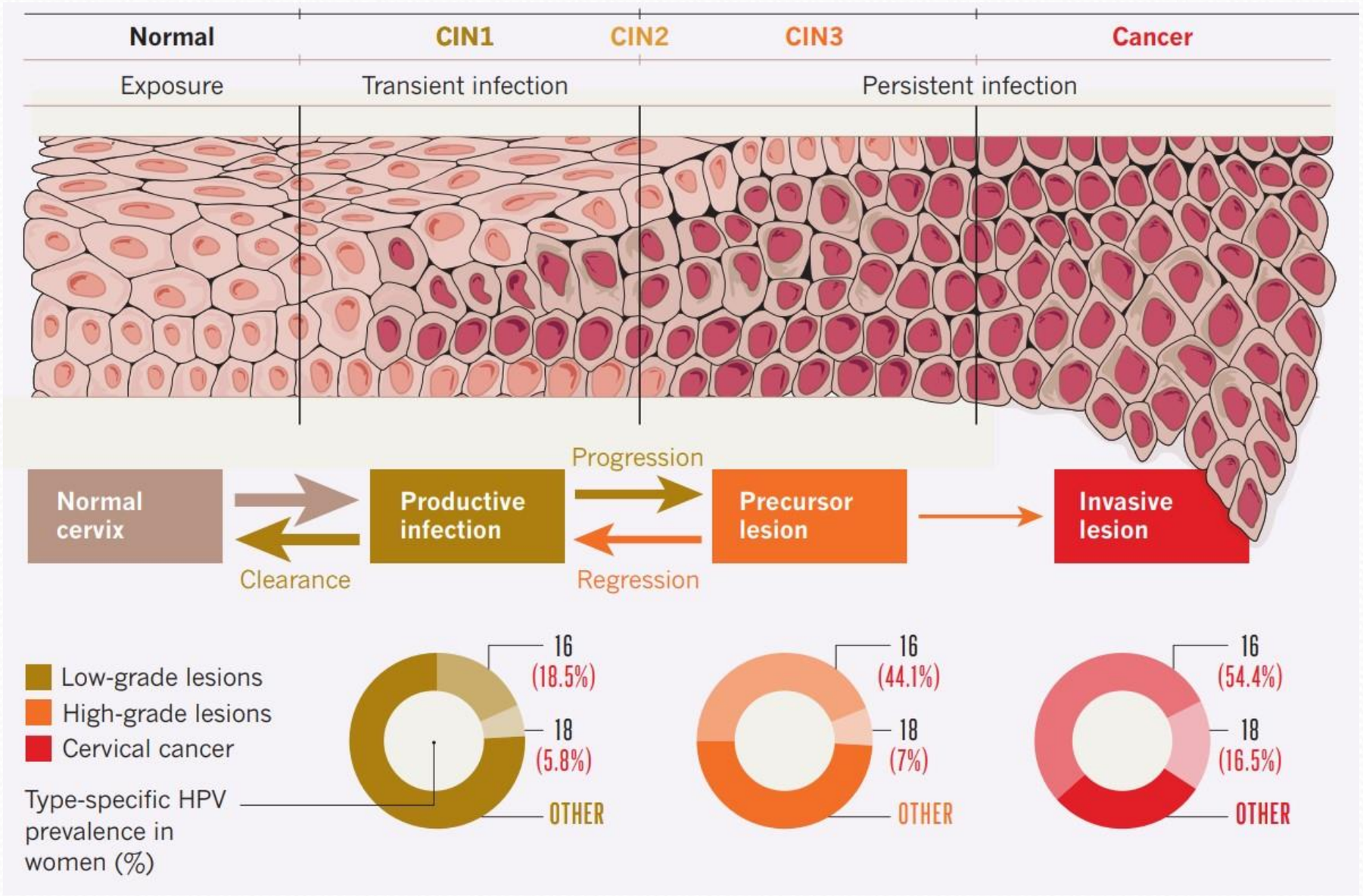
HPV Pathogenesis



Integration of HPV into the host genome results in an un-controlled oncogene expression[1]

HPV oncogenes can activate the cellular methylation machinery

The prevalence of integrated forms varies with the infecting HPV genotype

1. Woodman et al, https://doi.org/10.1038/nrc2050
2. Crow, J. HPV: The global burden. Nature 488, S2–S3 (2012). https://doi.org/10.1038/488S2a

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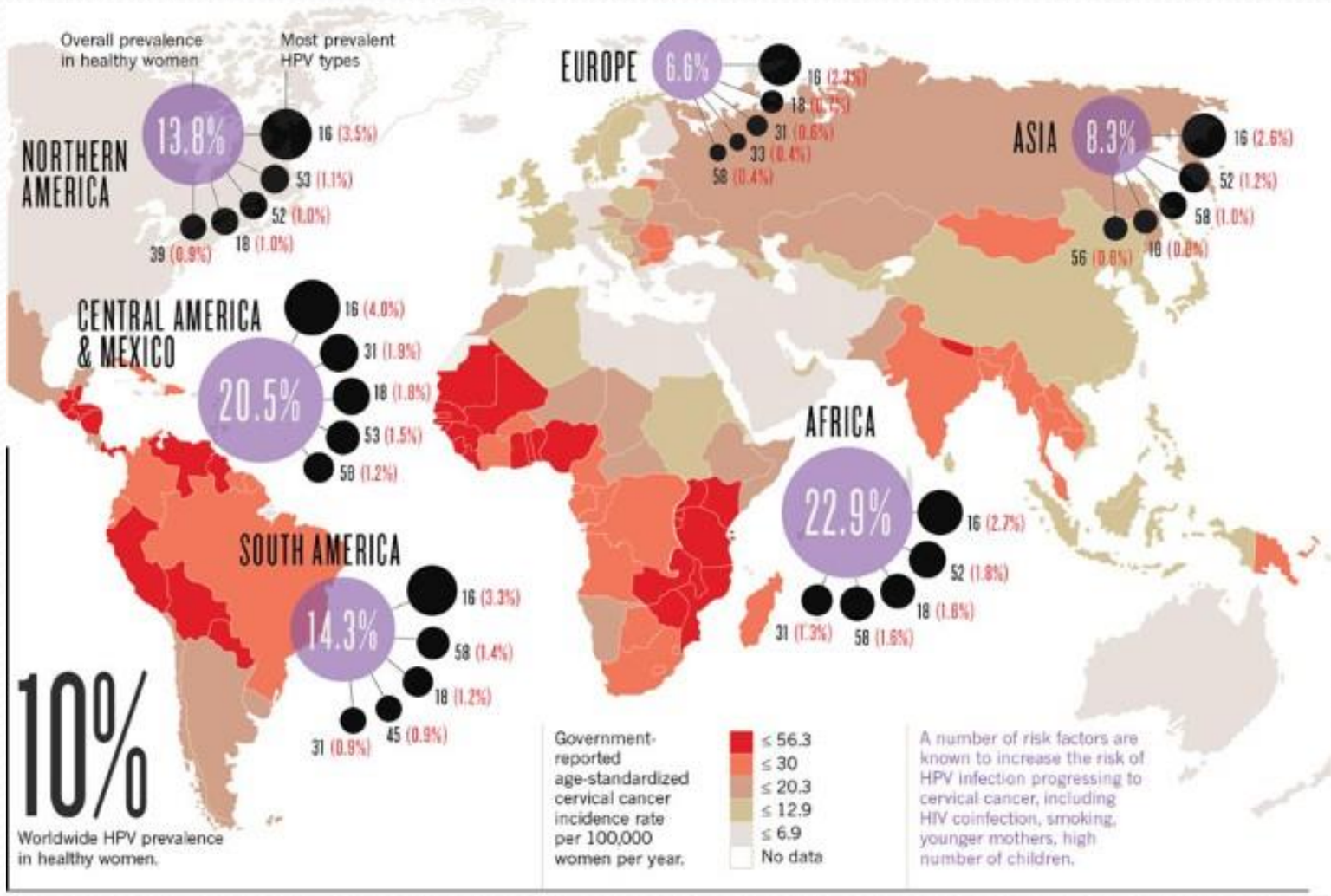
HPV Genotypes & Prevalence

- Persistent HPV infection, especially high-risk strains like HPV 16/18, can lead to cervical cancer.
- The prevalence of HPV genotypes varies by geographical regions
  - HPV 31/33 more common in Europe,[1] compared to HPV 52/58 in Asia.[1]
- Risk association of HPV genotype to CIN2+ can differ by geographical location
  - HPV 31 had a higher relative risk than HPV 33/58 in Hong Kong,[3] vice versa for US.[4]
  - HPV 56/59/66 and HPV 51 much lower relative risk than pooled HPV genotyping in Hong Kong,[3] compared to US.[4]



1. Crow et al, Nature volume 488, pagesS2–S3 (2012).
2. Wong OGW, et al. *J Clin Microbiol*. 2019;57(12):e00997-19. Published 2019 Nov 22.
3. Wright TC Jr, et al. *Am J Clin Pathol*. 2014;142(1):43-50.
4. Bonde JH, et al. J Low Genit Tract Dis. 2020 Jan;24(1):1-13.

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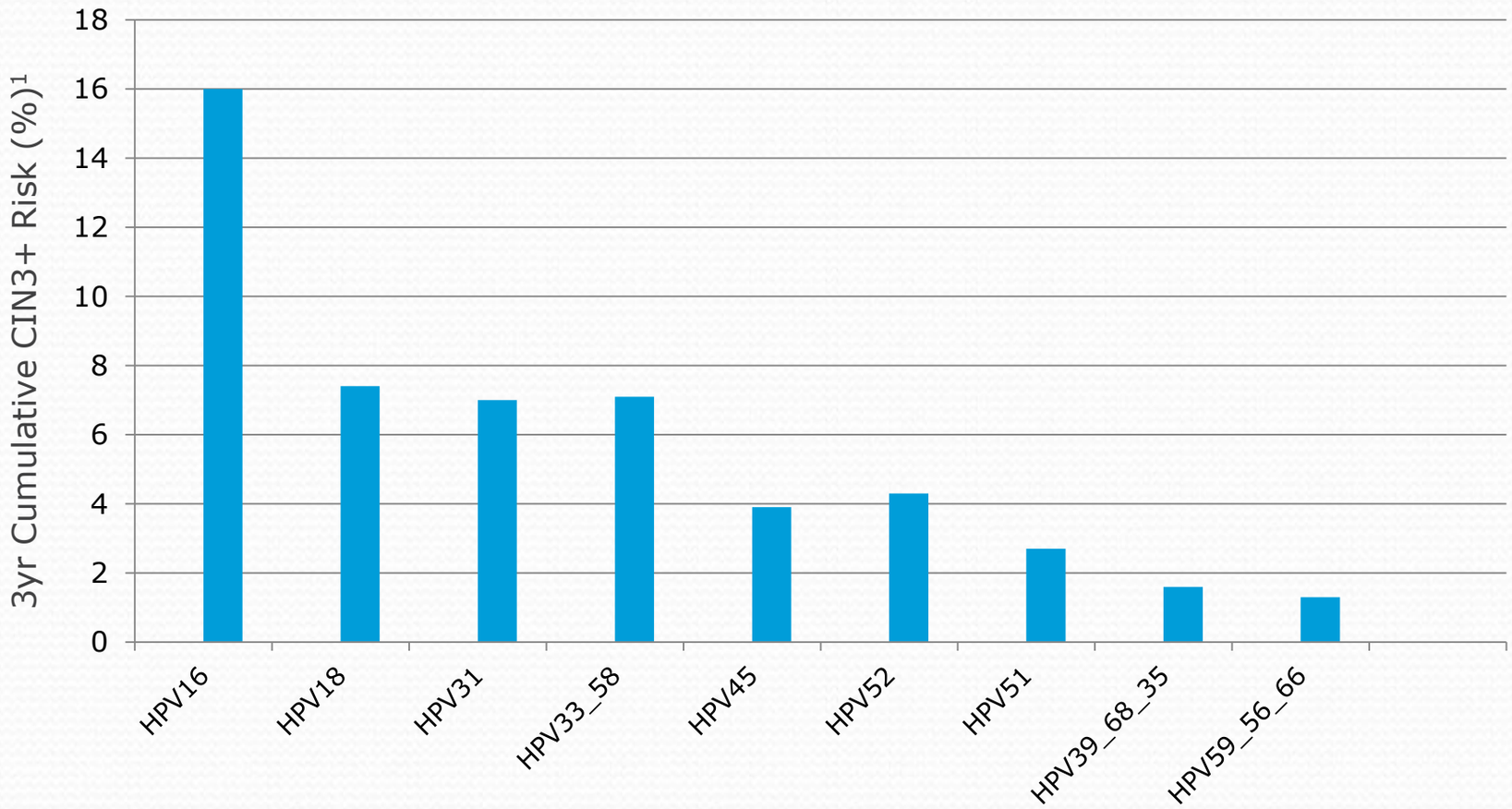
HPV Genotypes & Prevalence

Among these 14 high risk types, the risk of developing cancer is not equally distributed



It's important to identify which genotypes are most indicative of high risk pre-cancerous lesions

40% of all HPV+ ASC-US cases fall into the lower risk category

Reference: 1. Schiffman et al. Gynecologic Oncology 138 (2015) 573-78

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HPV Genotypes & Prevalence

Cancer Epidemiol Biomarkers Prev. 2019 November ; 28(11): 1816–1824.
doi:10.1158/1055-9965.EPI-19-0239.

Role of HPV Genotype, Multiple Infections, and Viral Load on the Risk of High-Grade Cervical Neoplasia

Rachael Adcock[1], Jack Cuzick[1], William C. Hunt[2], Ruth M. McDonald[2], Cosette M. Wheeler[2], New Mexico HPV Pap Registry Steering Committee

[1]Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, United Kingdom.

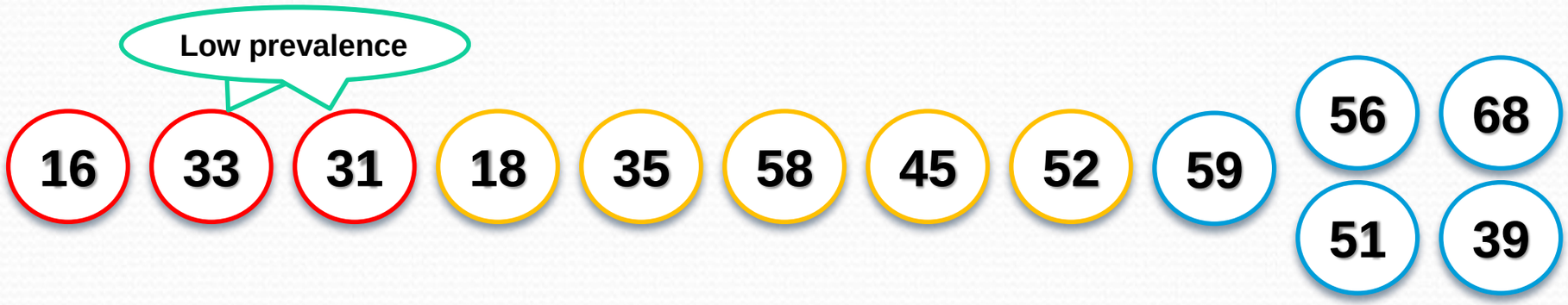
[2]Center for HPV Prevention, University of New Mexico Health Sciences Center, Albuquerque, New Mexico.

47,120 women undergoing cervical screening in New Mexico, were tested for 13 high-risk HPV genotypes. PPV for CIN2+ and CIN3+ over 3 years of follow-up were estimated for each HPV genotype and viral load.

multiple HPV infections added little to risk prediction

PPV for CIN2/3+ is more important than prevalence for clinical risk assessment

Hierarchy of HPV genotypes based on PPV for CIN3+:



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HPV Genotypes & Prevalence

Prevalence of HPV 16, 18, 31, 33 and positive predictive value (PPV) or relative risk (RR, where indicated) for CIN2+ and CIN3+ in different studies.

Study [ref]	HPV type	Population prevalence % (95% CI)	CIN2+		CIN3+	
			PPV (95% CI)	rank	PPV (95% CI)	rank
ATHENA (N=40 901) [[3], (Table 3)]	16	2.1 (1.9, 2.2)	19.5 (16.8, 22.3)	1	14.7(12.2, 17.3)	1
	18	.82 (.73, .91)	8.4 (5.64, 11.9)	4	6.9 (4.4, 10.2)	4
	31	1.0 (.92, 1.1)	15.2 (11.9, 19.0)	2	8.0 (5.5, 11.0)	2
	33 4	.28 (.23, .33)	9.7 (4.96, 16.8)	3	7.1 (3.1, 13.5)	3
NMHPVPR (N=47 617, 3y FU) [[13], Table 1; [14], Table 3]]	16	3.5 (3.3, 3.6)	10.9 (6.1, 12.7)	1.5	8.0 (6.5, 9.6)	1
	18	1.2 (1.1, 1.3)	5.7 (3.4, 7.9)	4	2.9 (1.5, 4.3)	4
	31	1.8 (1.7, 1.9)	7.3 (4.7, 9.9)	3	3.1 (1.9, 4.4)	3
	33 4	.50 (.43, .57)	10.9 (5.8, 16.0)	1.5	5.2 (1.8, 8.6)	2
FUTURE I (N=1694, 3y FU) [[15], Table 1]	16	13.9 (12.4, 15.5)	9.2 (6.0, 13.2)	2	1.7 (0.0, 3.8)	3
	18	5.8 (4.8, 6.9)	4.4 (1.4, 10.0)	4	0 (-)	–
	31	8.0 (6.8, 9.3)	8.9 (5.0, 14.5)	3	3.2 (0.0, 8.8)	2
	33 4	2.9 (2.2, 3.7)	14.0 (6.26, 25.8)	1	4.1 (0.0, 10.5)	1
KPNC (3y FU) (N=18 810, HPV+, cyto neg, >30y) [[6], Tables 2 and 3)]	16	14.7 (14.2, 15.2)	16.7 (15.5, 17.9)	1	10.6 (.89, 11.4)	1
	18	6.3 (6.0, 6.7)	9.4 (8.3, 10.7)	4	5.9 (5.2, 6.7)	2
	31	10.1 (9.7, 10.5)	10.2 (9.3, 11.3)	3	4.5 (4.1, 5.0)	4
	33 6	2.2 (2.0, 2.4)	8.9 (7.1, 11.0)	6	5.9 (4.8, 7.2)	3
			HPV 52-2nd; HPV35-5th			
Predictors 2 (referral) N=1067 [[4], Table 3 and new data[a]]	16	30.2 (27.4, 33.0)	57.8 (52.2, 63.2)	2	42.3 (37.0, 47.8)	1
	18	5.4 (4.1, 7.0)	29.3 (18.1, 42.8)	4	15.2 (6.34, 28.9)	6
	31	7.6 (6.1, 9.4)	39.5 (28.8, 51.0)	3	22.2 (13.7, 32.8)	3
	33 2	7.7 (6.2, 9.4)	59.8 (48.3, 70.4)	1	31.0 (20.5, 43.1)	2
					HPV35-4th; HPV52-5th	
New Mexico [[16], Table 2 and new data for CIS only[a]] (RR) N=5020	16	7.4 (6.6, 8.3)			5.8 (5.2, 6.3)	1
	18	2.3 (1.8, 2.8)			1.8 (1.5, 2.2)	4
	31	2.9 (2.4, 3.5)			2.7 (2.4, 3.1)	3
	33 4	.92 (.65, 1.3)			3.4 (3.0, 3.8)	2
Sweden 14y risk (N=11 685) [[17], Tables 1, 2 and 4]	16	2.4 (2.18, 2.74)	42.8 (36.4, 49.8)	2	34.5 (28.4, 41.5)	1
	18	.62 (.49, .79)	39.4 (28.4, 52.8)	4	29.7 (19.6, 43.4)	3
	31	1.0 (.84, 1.22)	41.9 (31.1, 54.6)	3	28.4 (18.2, 42.7)	4
	33 4	.38 (.27, .51)	54.2 (37.6, 72.5)	1	34.1 (19.2, 55.6)	2
Controls in Vaccine trials (15–26y) (N=17 590) [[18], Tables 1 and 3]	16	8.8 (8.4, 9.3)	26.3 (24.1, 28.6)	1	15.5 (13.7, 17.4)	1
	18	3.6 (3.4, 3.9)	12.5 (10.0, 15.3)	4	5.6 (4.0, 7.7)	4
	31	4.4 (4.1, 4.8)	18.3 (15.7, 21.2)	3	8.6 (6.7, 10.8)	3
	33 4	2.0 (1.8, 2.2)	23.5 (19.2, 28.2)	2	13.4 (10.1, 17.4)	2
POBASCAM Baseline- (N=44 102) [19, Tables 1 and 2]	16	1.6 (1.5, 1.8)	20.8 (17.9, 24.0)	1	17.1 (14.4, 20.0)	1
	18	.42 (.37, .49)	7.4 (4.1, 12.3)	3	5.3 (2.6, 9.6)	3
	31	.64 (.57, .72)	7.1 (4.4, 10.8)	4	5.0 (2.8, 8.2)	4
	33 4	.27 (.22, .32)	14.4 (8.6, 22.1)	2	12.7 (7.3, 20.1)	2
Denmark [[20], Tables 1 and 2] (N=40 382)	16	5.4 (5.1, 5.9)	15.7 (14.1, 17.3)	1	13.2 (11.8, 14.7)	1
	18	2.4 (2.2, 2.5)	10.2 (8.4, 12.3)	3	8.1 (6.5, 10.0)	3
	31	3.8 (3.6, 4.0)	9.3 (7.9, 10.9)	4	6.3 (5.1, 7.6)	4
	33 4	1.7 (1.6, 1.9)	13.2 (10.8, 16.0)	2	9.0 (7.0, 11.3)	2

Cuzick and Wheeler; Papillomavirus Research Vol2 (2016) 112-115

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HPV Genotypes & Clinical Relevance

Other factors should also be considered: methylation, biomarkers (p16) and viral load

	PPV (%) by viral load			
	CIN3+			
HPV type	Low	Intermediate	High	*P* value for trend[a]
Any hrHPV[b]	0.98	1.90	4.66	5.3E-105
16	2.11	3.58	10.56	9.2E-56
33	1.00	6.20	3.93	0.01
31	1.75	4.32	1.77	0.05
18	1.25	0.95	2.52	0.01
35	0.85	1.97	2.50	0.01
58	0.22	0.29	2.20	1.5E-05
45	1.25	1.23	1.07	0.71
52	0.26	0.84	2.55	1.4E-09
59	0.42	0.65	1.51	8.7E-04
51	1.11	0.78	0.55	0.11
39	0.15	0.56	0.83	4.1E-03
56	—	—	0.45	
68	—	—	0.27	

	PPV (%) by viral load			
	CIN2+			
HPV type	Low	Intermediate	High	*P* value for trend[a]
Any hrHPV[b]	2.38	4.46	9.28	5.3E-169
16	3.90	6.61	17.62	1.2E-72
33	4.43	9.41	10.29	1.1E-03
31	4.08	8.93	10.76	1.2E-12
35	3.62	6.03	10.10	1.2E-05
18	2.07	2.83	7.70	2.5E-09
58	1.18	2.22	6.20	4.0E-09
51	2.31	4.40	4.50	1.2E-04
45	1.32	2.35	3.38	0.01
39	1.99	2.78	2.11	0.63
52	0.40	1.16	4.57	6.4E-15
59	0.93	1.09	2.80	9.4E-05
56	0.17	2.45	1.32	0.01
68	1.02	0	0.82	0.74

High viral loads for HPV 18, 35, 52 and 58 carried more CIN3 risk than low viral loads for HPV 16, 31 and 33

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HPV Genotypes & Prevalence

Research article | Open Access | Open Peer Review

Type-specific oncogenic human papillomavirus infection in high grade cervical disease in New Zealand

Leonardo M Simonella ✉, Hazel Lewis, Megan Smith, Harold Neal, Collette Bromhead and Karen Canfell

BMC Infectious Diseases 2013 **13**:114
https://doi.org/10.1186/1471-2334-13-114
Received: 10 September 2012 | **Accepted:** 20 February 2013 | **Published:** 3 March 2013

594 women (20-69 years) with ASC-H / HLIS were tested for 37 HPV types (Linear Array). Genotype specific prevalence was estimated.

Hierarchy of HPV genotypes by prevalence in CIN2

16 31 33 52 58 18 39 35 51 56 68 45 59

Hierarchy of HPV genotypes by prevalence in CIN3

16 52 18 31 51 33 58 45 39 59 56 68 35

Reference:

- > 12%
- 12% - 5%
- < 5%

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HPV Genotypes & Prevalence

TO SUMMARIZE:

- ✓ **Persistent hrHPV (14 genotypes) are linked to high-grade lesions and cervical cancer.**
- ✓ **Prevalence of hrHPV genotypes varies between regions.**
- ✓ **All studies agree: HPV16 has the highest risk for HSIL and cancer.**
- ✓ **Although some differences between regions, HPV33 and HPV31 show higher risk than HPV18 and HPV45 for low and high grade lesions**
- ✓ **New Zealand has a high prevalence of HPV52 and HPV58.**
- ✓ **Other factors also to be considered, like methylation; viral load and tumour biomarkers (p16)**
- ✓ **Knowing the PPV and/or prevalence of the HPV genotypes in your region can improve the clinical management of the patient.**

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NCSP

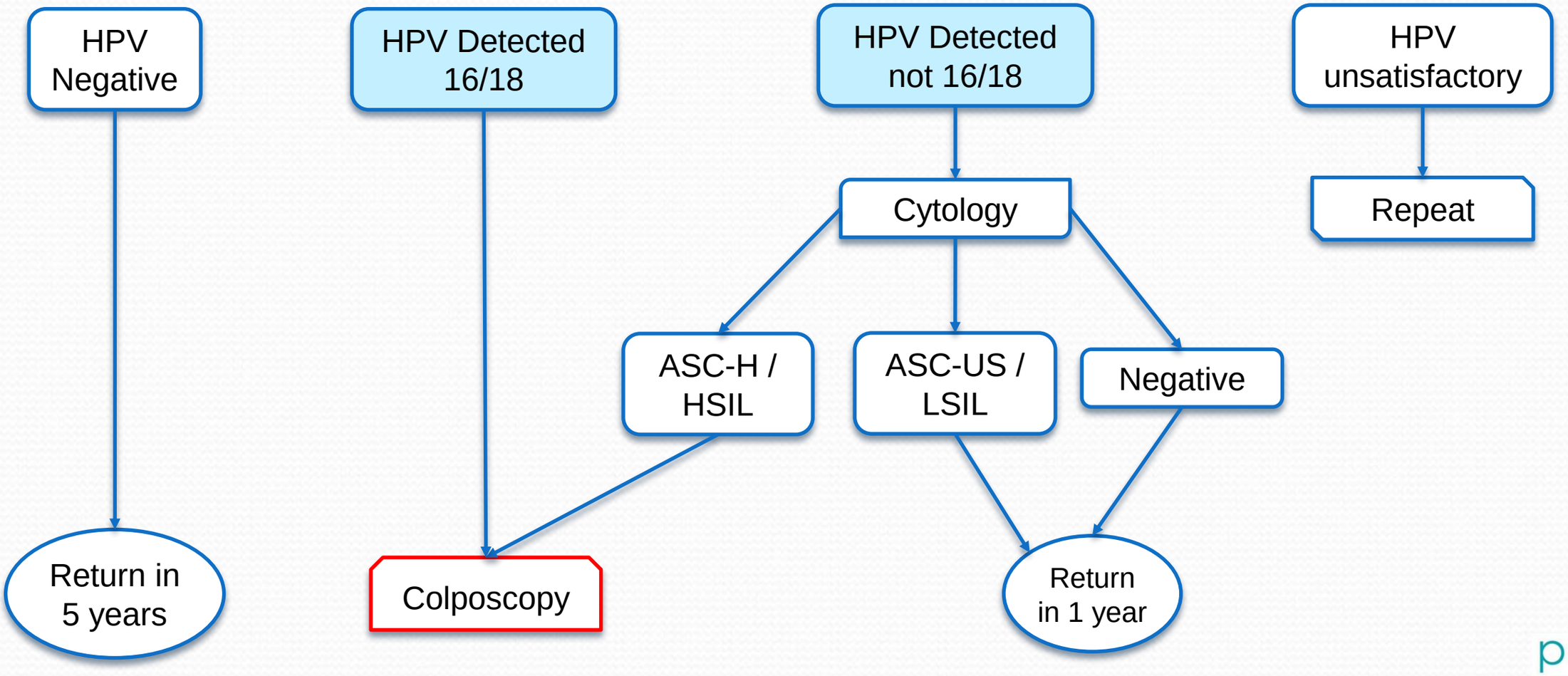
Current hrHPV testing:

- >30 years old; ASC-US/LSIL and no abnormal report within 5 years; HSIL >3years ago (re-entering screening).

If POS refer to colposcopy; no extended genotype.

If NEG repeat in 1 year.

HPV test Primary Screening



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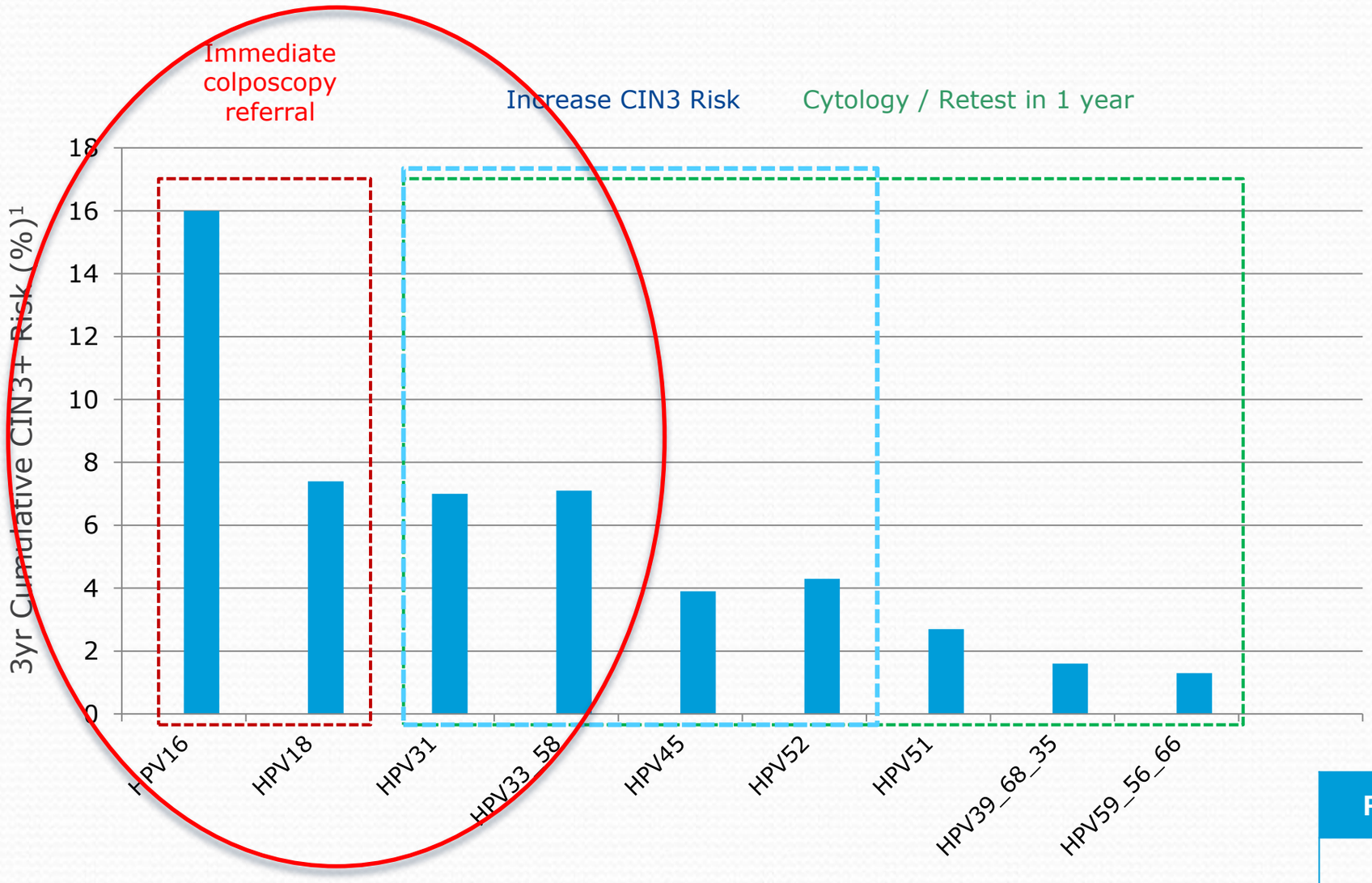
HPV Genotypes & Prevalence

Among these 14 high risk types, the risk of developing cancer is not equally distributed



ASC-US linked to HPV16, HPV18, HPV31, or HPV33/58 warrants immediate colposcopy.

Optimal management of women with HPV52 or HPV45 is uncertain.

Stratification of genotype-specific risk could be an affective approach

Risk Tier	Type of risk
16	Very high risk
18, 31, 33	High risk
45, 52, 58	Medium risk
35, 39, 51, 56, 59, 66, 68	Low risk

Reference: 1. Schiffman et al. Gynecologic Oncology 138 (2015) 573-78

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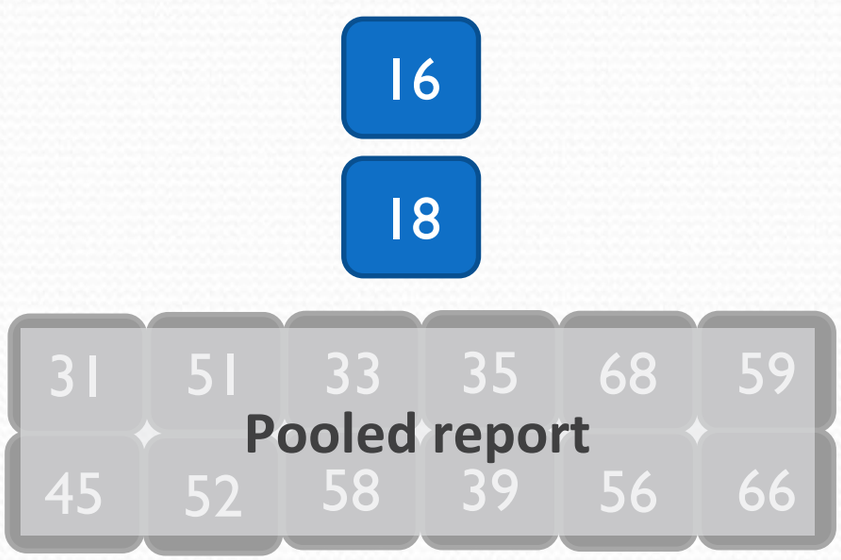
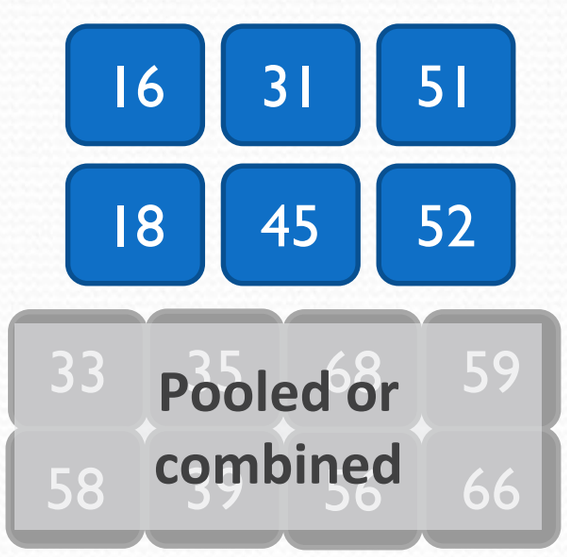
HPV Genotypes Detection

- Several HPV tests have been approved by the FDA to detect 14 HPV genotypes that have oncogenic potential.
- The assays can be broadly classified as partial, extended or full genotyping depending on the identification of individual HPV genotypes.



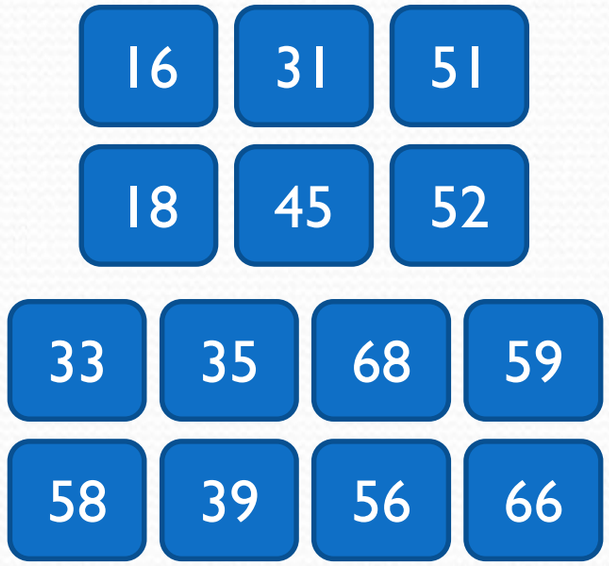
Partial

Roche Cobas 4800 or 6800
GX HPV (16 & 18/45)



Extended

BD Onclarity
Abbott Alinity m



Full

HC2 Qiagen
Seegene Anyplex II

Bonde JH, et al. J Low Genit Tract Dis. 2020 Jan;24(1):1-13

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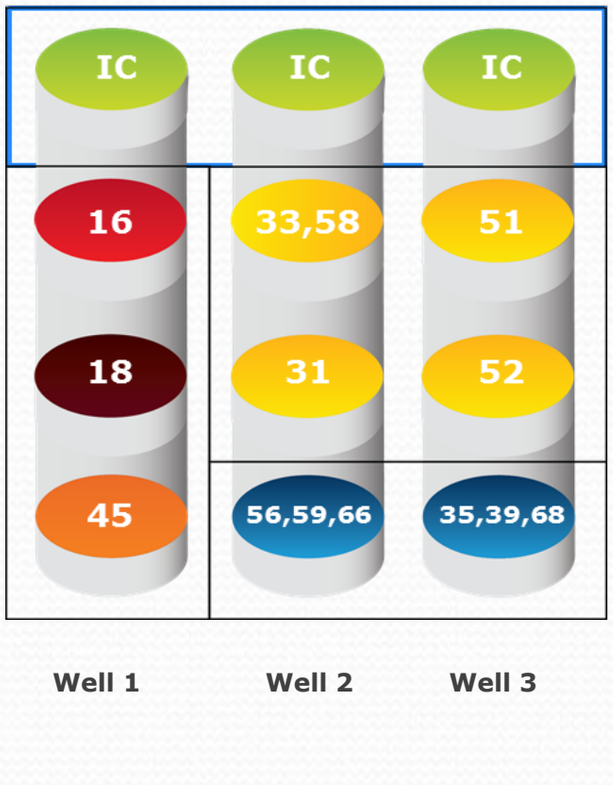
BD Onclarity

BD Onclarity assay Real-tIme PCR (on VIPER LT or BD-COR)



- **BD COR™ PX : Pre-Analytical Module**
- **BD COR™ GX: HPV Module**

Medium to high throughput

Assay Results*	GX
8 hrs	120
16 hrs	330
24 hrs	540

** Throughput may vary based on assay and specimen type*

Full automation with **integrated sample pre-analytic**

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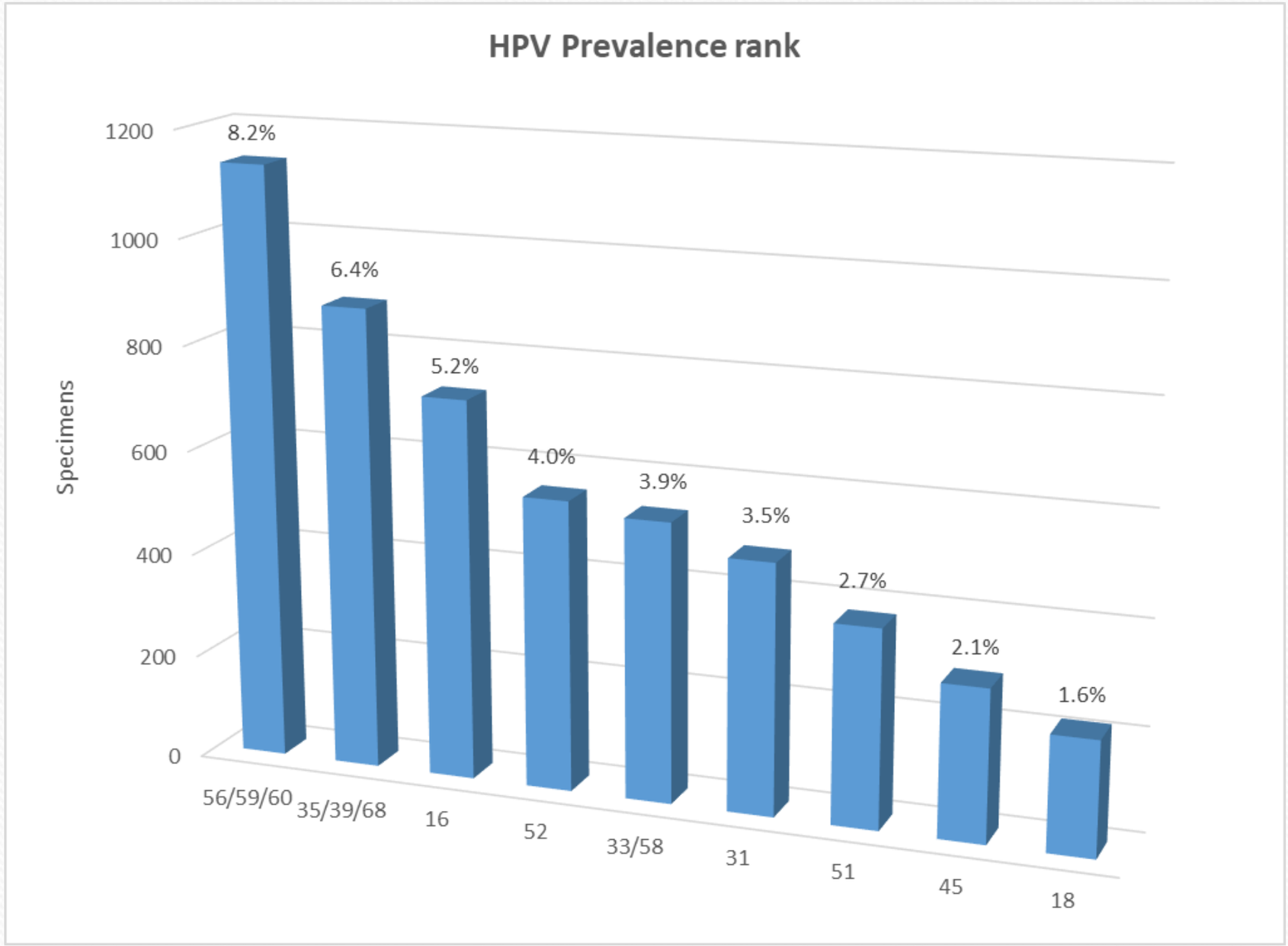
Pathlab HPV Data (2019-2022)



Data from 2019 to 2022 (June)
Total specimens = 13830

	Samples	Rate
HrHPV +ve	4014	29.0%
HrHPV -ve	9795	70.8%
HrHPV inv	21	0.2%

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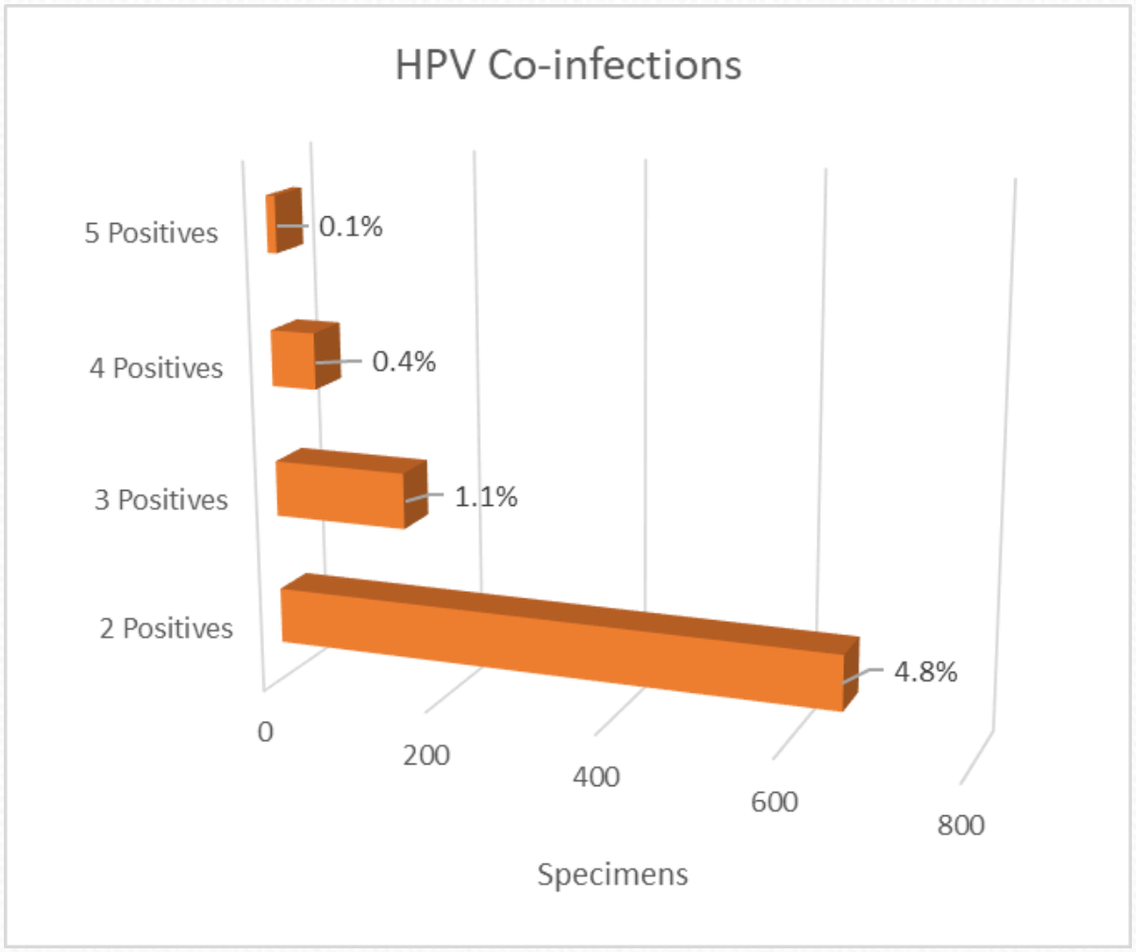
Pathlab HPV Data (2019-2022)



Co-infections with two HPV genotypes:

	16	18	45	33/58	31	56/59/60	51	52	35/39/68	TOTAL
16		3	10	19	24	31	14	20	28	149
18	3		6	4	6	10	9	8	12	58
45	10	6		7	8	26	6	9	16	88
33/58	19	4	7		16	34	13	18	35	146
31	24	6	8	16		17	8	19	24	122
56/59/60	31	10	26	34	17		33	43	65	259
51	14	9	6	13	8	33		9	19	111
52	20	8	9	18	19	43	9		32	158
35/39/68	28	12	16	35	24	65	19	32		231

- HPV 16 / 18 co-infections are rare.
- HPV 35/39/68; 56/59/60 and 33/58 are present in most co-infections.

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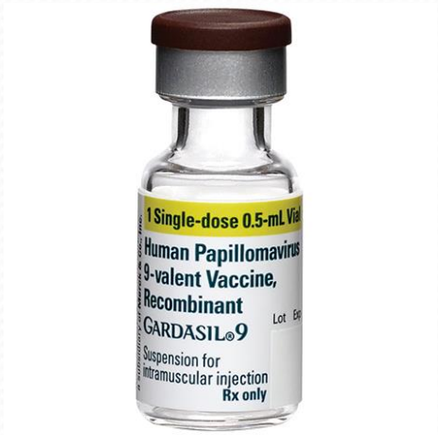
HPV Vaccines



Gardasil-9 covers hrHPV: 16, 18, 31, 33, 45, 52, 58
Previous Gardasil covered only 16, 18

Not covered: **51**, 35, 56, 59, 66, 39, 68

- Gardasil-4 introduced in NZ in 2008 for young women born in 1990
- Gardasil-9 introduced in 2017 for all aged 9-26
- NZ Vaccination coverage approx. 70%
- Vaccinated women are now starting to be screened.
- Effect of Gardasil-9 might start to be seen from around 2030 (aged 12 onwards)

Genotype distribution of HPV is essential for estimating the future impact of Gardasil-9 on cervical cancer.

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Final Discussion

- ✓ **High-grade lesion and Cervical Cancer is caused by hrHPV persistent infection.**
- ✓ **HPV16 is the genotype with highest risk for CIN2/3+ and cervical cancer.**
- ✓ **HPV33 and HPV31 show higher risk than HPV18 and HPV45 for low and high grade lesions**
- ✓ **New Zealand has a high prevalence of HPV52 and HPV58 in high-grade lesions. (α-9 group is 16, 31, 33, 35, 52, 58).**
- ✓ **HPV extended genotyping can improve patient management by reducing colposcopy referral or cytology reflex testing.**
- ✓ **By identifying the non16/18 genotypes we can estimate the impact of the vaccines in our population.**
- ✓ **More data is needed and therefore a wider use of extended genotyping is recommended.**

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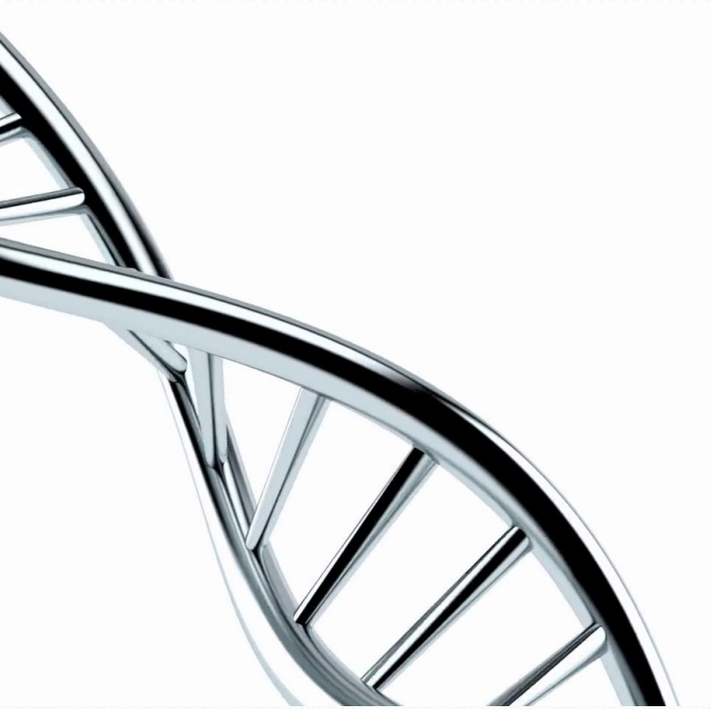
THANKS



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