Beyond Primary HPV Screening

L. C. Thompson

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UCSOM

- No COI
- AI was not used in the development of this presentation

Learning Objectives

- Implement Primary HPV screening
- Triage positive HPV screen per cytology
- Use Dual Stain to triage HPV positive screening
- Follow Extended Geno-typing for risk stratification

Updated 2025:
Includes self collection q 3 yrs

Draft 2024:
Includes self collection 30-65

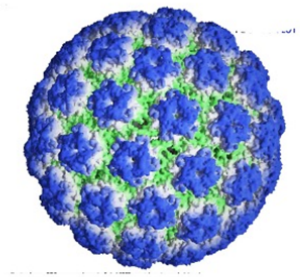
Age Range	2020 ACS	2012 ACS	2018 USPSTF
Age 21 – 24	No screening	Pap test every 3 years	Pap test every 3 years
Age 25 – 29	HPV test every 5 years (preferred) HPV/Pap co-test every 5 years (acceptable) Pap test every 3 years (acceptable)	Pap test every 3 years	Pap test every 3 years
Age 30 – 65	HPV test every 5 years (preferred) HPV/Pap co-test every 5 years (acceptable) Pap test every 3 years (acceptable)	HPV/Pap co-test every 3 years (preferred) Pap test every 3 years (acceptable)	HPV test every 5 years (Preferred) HPV/Pap co-test every 5 years or Pap test every 3 years
Age 65 +	No screening if a series of prior tests were normal	No screening if a series of prior tests were normal	No screening if a series of prior tests were normal and not at high risk for cervical cancer

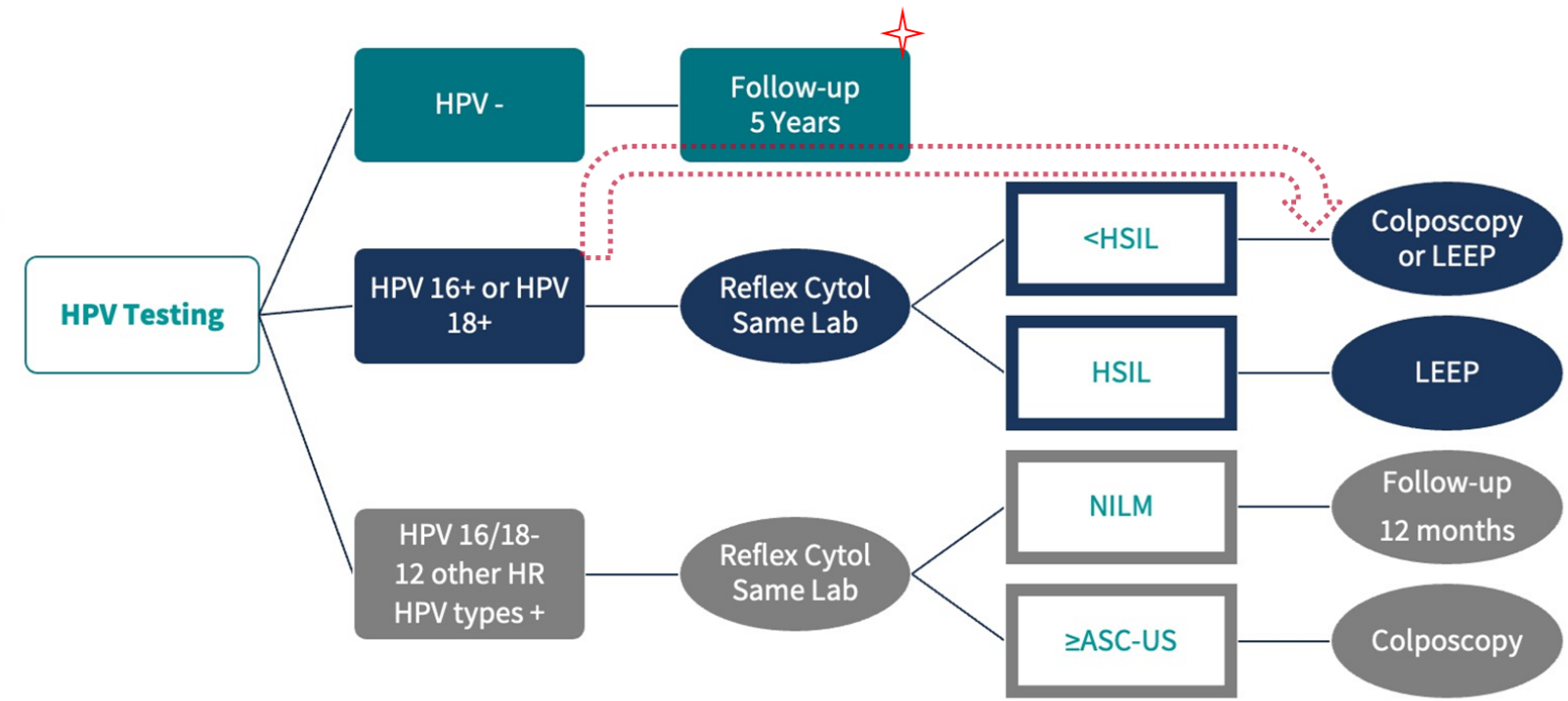
ASCCP “supports”

No longer supported, -Primary HPV

ASCCP “endorses”

Algorithm for Primary HPV Screening

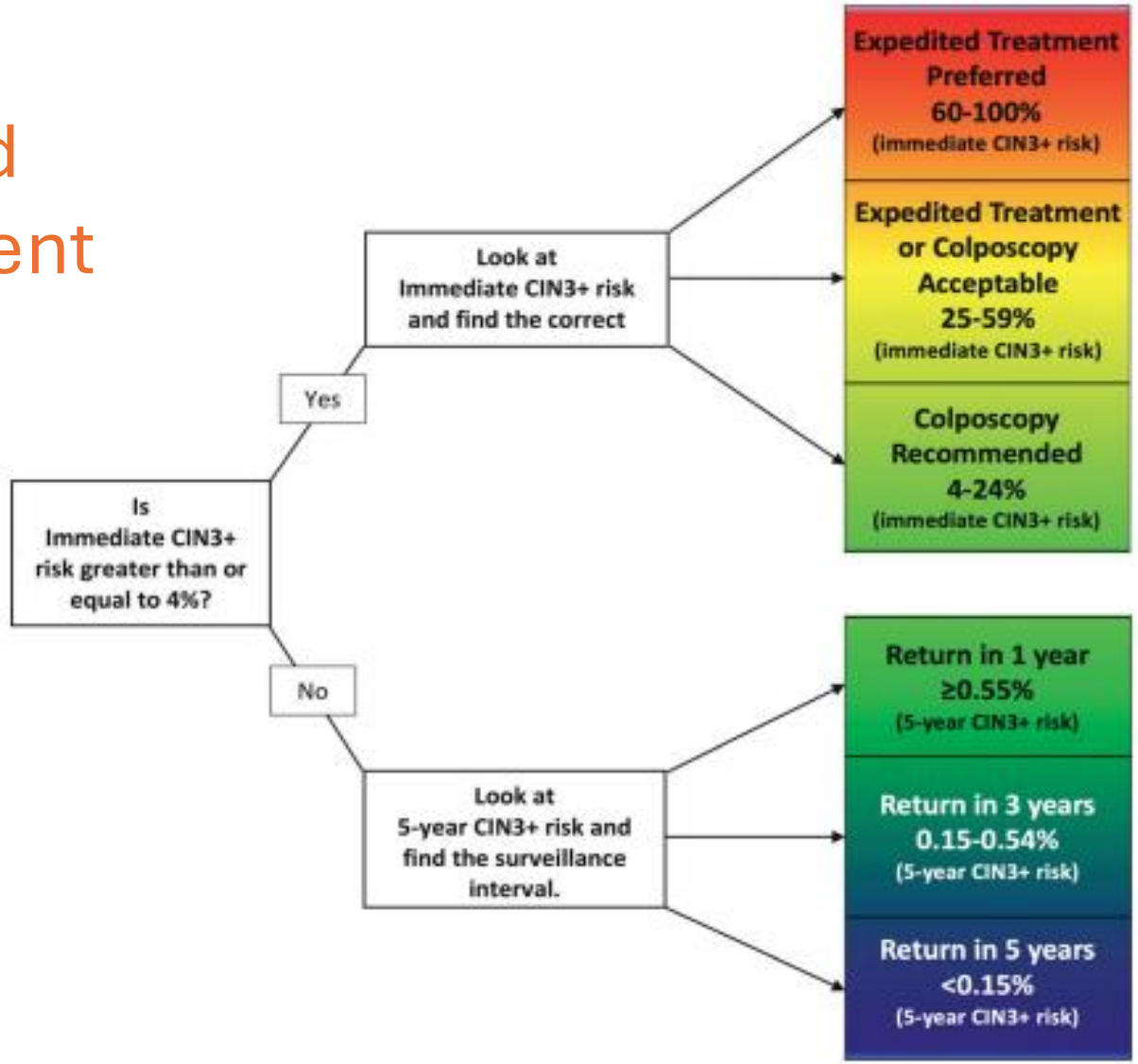




Huh WK, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance. *Obstet Gynecol*. 2015;125(2):330-337.

Perkins RB, et al. 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors. *J Low Genit Tract Dis*. 2020;24(2):102-131.

Risk Based Management



Regulatory approval of new testing

- P16/Ki67 Dual Stain
  FDA approved 2020 and included in 2024 ASCCP Risk Management Guidelines

- Extended genotyping
  FDA approved 2020 and included in 2025 ASCCP Risk Management Guidelines

- Alternative to cytology triage for HPV positive screening

P16/Ki67 Dual Stain

- FDA approval 2020 for single commercial platform
- Only for positive HPV results from Primary HPV or CoTesting screening
- Very high NPV
- P16 bio-marker for tumor suppression- Brown cytoplasm.
- Ki67 bio-marker for cell proliferation- Red nucleus.
- Together signal cell dysregulation indicates cell transformation
- Not **stand alone** test, nor **triage for cytology,** nor for **HPV negative**.
- Asymptomatic screening, **Not self collection, Clinician obtained**.
- Manual screening, labor intensive
- KPNC and STRIDES data

KPNC and STRIDES data

- KPNC (IRIS) dataset
  - >70,000 Northern California pts. 110,000 specimens
  - 25-65, mostly employer insured
  - 44% White, 20% Hispanic, 20% Asian, 10% Black

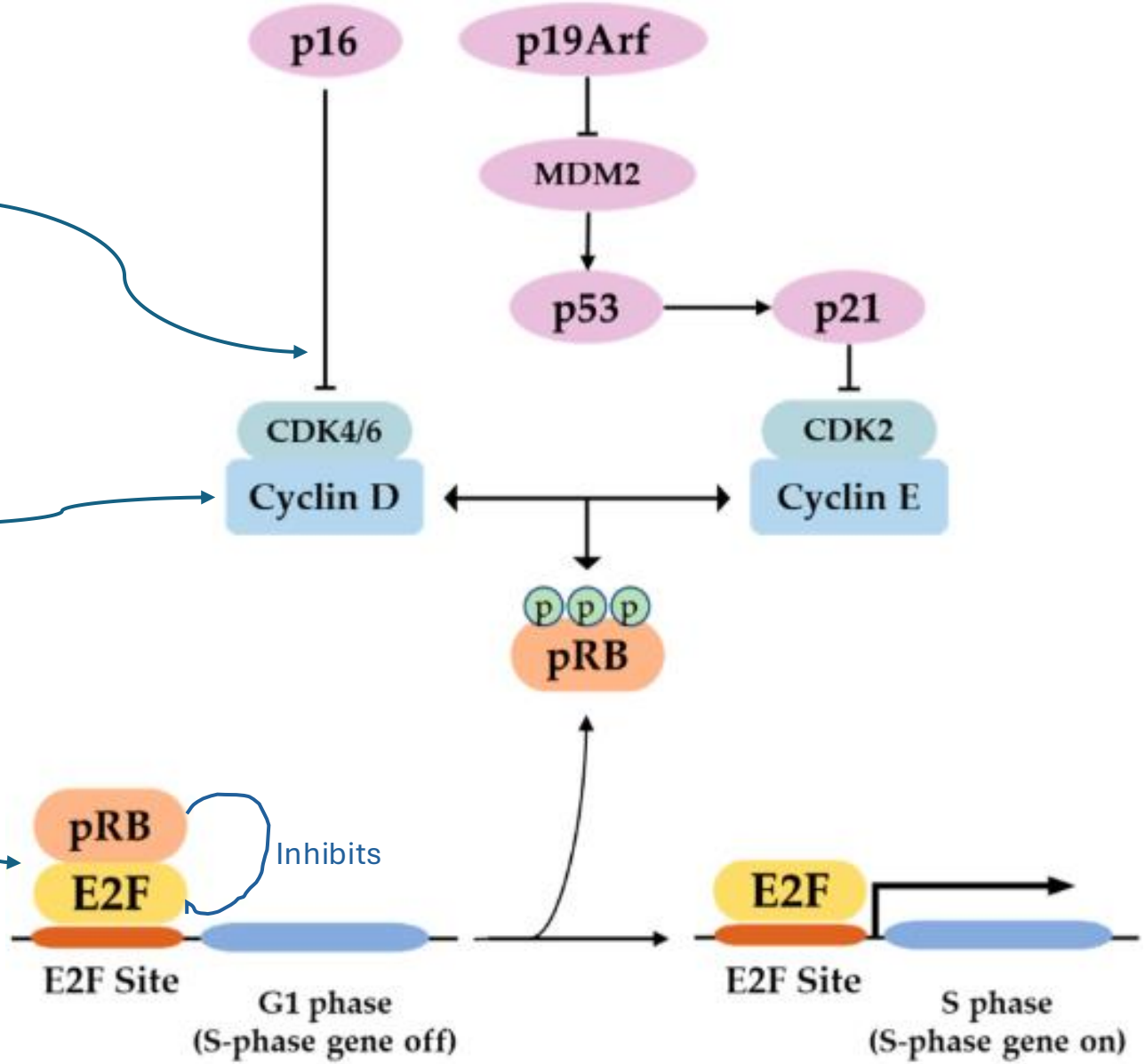
- STRIDES
  - 25,000
  - ≥21, health disparate population
  - 60% Black, 26% White, 7.5% other, 6% missing

“Step on the Breaks”

P16 inhibits CDK
With unchecked proliferation cell overexpresses p16.

CDK also inhibits pRb

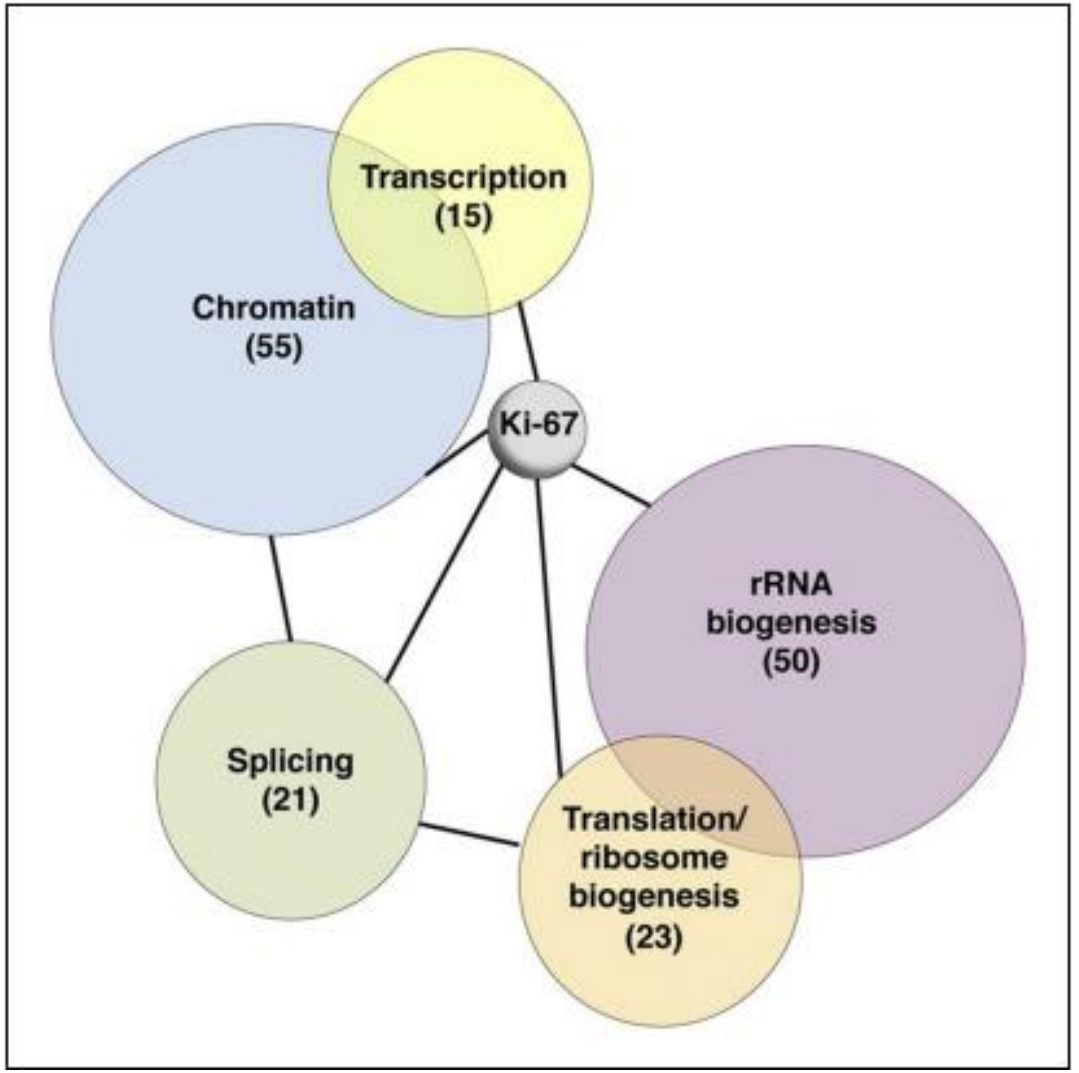
pRb inhibits cell progress
E7 (HPV) inactivates pRb
Leading to cell division

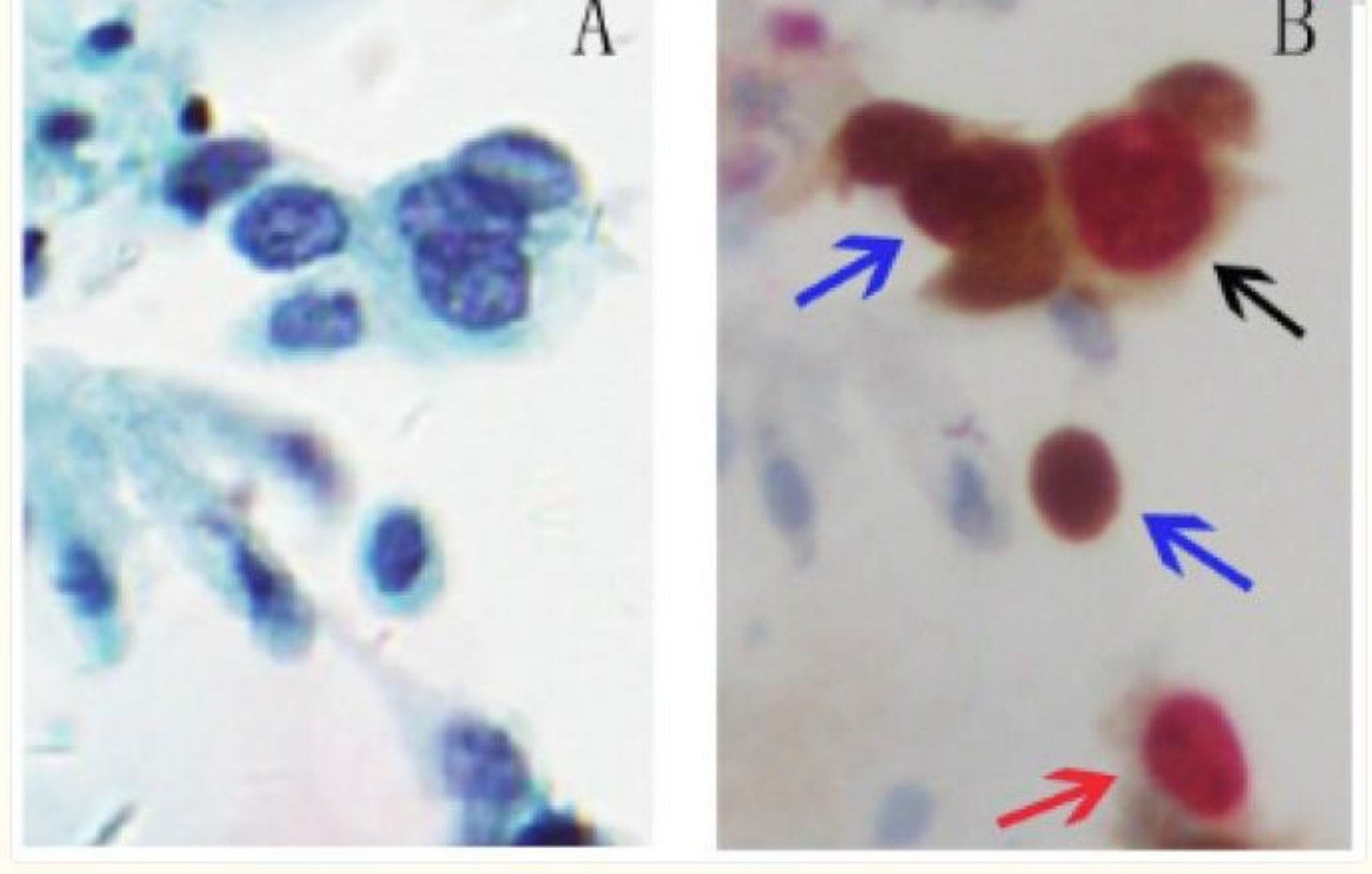


Safwan-Zaiter et al. P16INK4A-More Than a Senescence Marker. Life (Basel). 2022 Aug 28;12(9)

“Step on the Gas”

- Ki-67 Nuclear protein expressed in active phase of cell cycle, marker of active cell cycle
- Maintain chromatin organization and segregation of chromosome in mitosis
- Promotes ribosomal biosynthesis for protein production for cell growth
- Interacts with other cellular proteins that regulate cell cycle





Doesn't make sense to step on breaks and gas pedal at same time
“Like coming to a stoplight with both Green and Red light on”

Yu et al. J Cancer. 2019 Jun 2;10 (12)

P16/Ki67 Dual Stain for HPV+ w/o Genotyping

- **HPV + and DS +** **Colposcopy**
  - Immediate CIN3+ risk 9.5-11.5%
- **HPV+ and DS -** **1 year follow-up HPV based testing**.
  - Immediate CIN3+ 0.75-0.7%, (3 yr 1.5% KPNC)
- Prior HPV negative status improved the risk but did not change the threshold

Clark et al. J Low Gen Tract Dis 2024;28

P16/Ki67 Dual Stain for HPV+ w/ Limited GT

- **HPV 16 or 18** **Colposcopy**
  - DS+ HPV 16 23-24% HPV 18 11-5.6% (immediate risk)
  - DS - HPV 16 2.6-1.8% HPV 18 1.1-0% (N84) **recommend colposcopy*
- **Intermediate HPV + and DS +** **Colposcopy**
  - Immediate CIN3+ risk 5.6-7.9%
- **Intermediate HPV+ and DS -** **1 year follow-up HPV based testing.**
  - Immediate CIN3+ 0.53-.5% (3 Yr 1.1% KPNC)
- Prior HPV negative status improved the risk but did not change the threshold

Clark et al. J Low Gen Tract Dis 2024;28

P16/Ki67 Dual Stain for HPV/Cytology in Co-Testing

- **HPV+ (s GT)**
  - WNL, ASC-US, LSIL and +DS **Colposcopy** 4.1-8% (CIN 3 immediate risk)
  - WNL, ASC-US, LSIL and –DS **1 yr follow-up** 0-1.1% (0.92-1.6% 3 yr)
- **HPV+ (Limited GT)**
  - + Intermediate HPV: WNL, ASC-US, LSIL and +DS **Colposcopy** 5.6-7.9%
    WNL, ASC-US, LSIL and –DS **1 yr follow-up** 0.5% (1.1)%
  - If HPV 16/18 **Colposcopy**
- **ASC-H, AGC and HSIL** Recommend colposcopy (DS not recommended)

Clark et al. J Low Gen Tract Dis 2024;28

Dual Stain post Abnl Screen, Colpo or Tx (KPNC)

• Post Abnormal screen, Colposcopy or Tx		
• HPV 16 or 18, AGC, ASC-H or HSIL	**Colpo or Expedited Tx**	
• Post Colposcopy		
• Intermediate HPV+ DS +	**Colpo**	7.9 % Immediate
• Intermediate HPV + DS –	**1 year follow-up HPV**	.39%
• Post Tx		
• Intermediate HPV + DS+	**Colpo**	18%
• Intermediate HPV + DS-	**1 yr follow-up**	0% (N 32)
• Post screen with HPV + DS-		
• HPV + DS+	**Colposcopy**	
• HPV + DS-	**1 yr follow-up**	1.1%
• >2 yearly results HPV+ DS –	**consider colposcopy**	

Clark et al. J Low Gen Tract Dis 2024;28

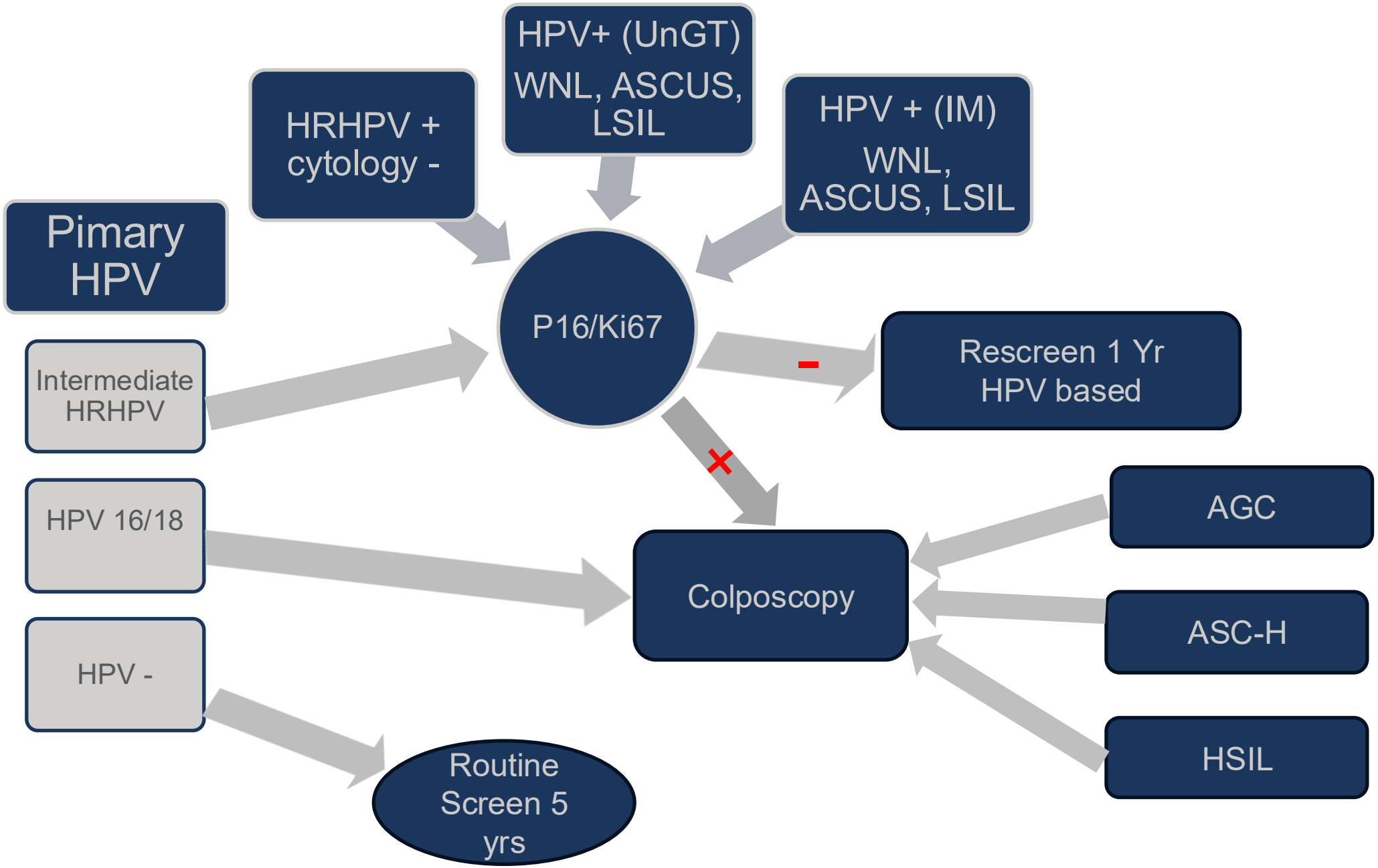
P16/Ki67 Dual Stain: Unsatisfactory

- If unsatisfactory due to sampling repeat at pt convenience and within 4 mos.
- If other actionable results (ie. HPV 16 or 18, or higher-grade abnormal cytology) manage accordingly.

Clark et al. J Low Gen Tract Dis 2024;28

P16/Ki67 Dual Stain:

- DS is an option for risk stratification for individuals with + HPV results.
- Cost-effectiveness has not been evaluated
- Requires optimal cytology preparation, May be limited with debris or atrophic smears.
- ? false positives, Only one cell as threshold, ? detect LSIL
- Dual Stain is included in ASCCP management guidelines and algorithm
- Potential for deep learning algorithm to automate dual stain.


Pimary HPV
HRHPV + cytology -
HPV+ (UnGT) WNL, ASCUS, LSIL
HPV + (IM) WNL, ASCUS, LSIL
Intermediate HRHPV
HPV 16/18
HPV -
P16/Ki67
-
Rescreen 1 Yr HPV based
+
Colposcopy
AGC
ASC-H
HSIL
Routine Screen 5 yrs

Extended Genotyping

- HPV testing **w/o Genotype**: 14 strains; **Limited Genotype**: separate 16 and 18 individually and group remaining "intermediate HRHPV" aka HPV12
- **Extended Genotyping**: Further stratify HPV strains:16, 31, 18, 33/58, 52, 45, 51, 35/39/68, 59/56/66 and 16, 18, 45, 31/33/52/58, 35/39/51/56/59/66/68
- Predicts HSIL lesions with good sensitivity and specificity
- Persistent and multiple HPV infection increases risk for dysplasia and progression
- Allows more strategic follow-up with increased fidelity of risk stratification

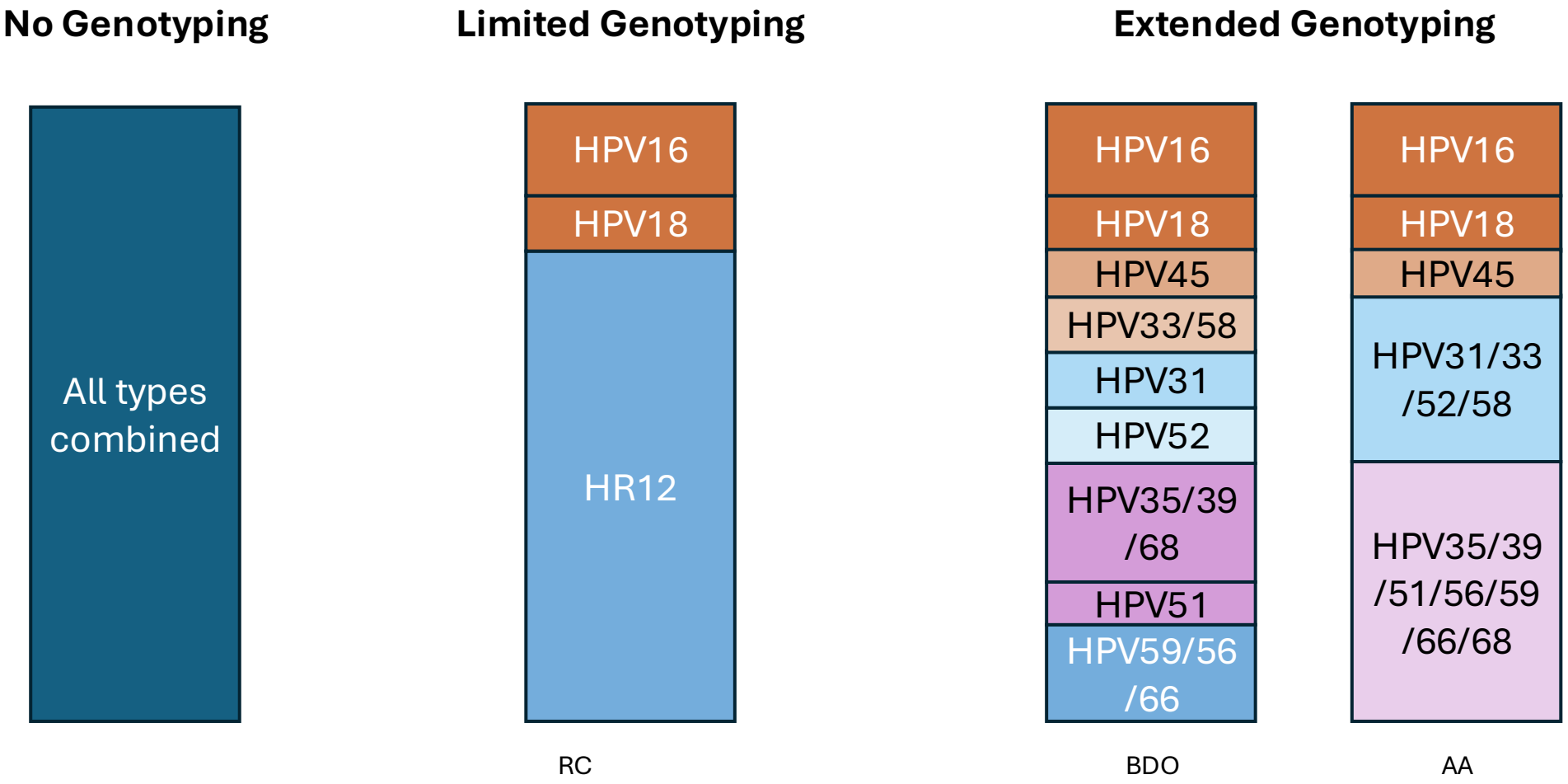
Massad et al. J Low Gen Tract Dis. 2025;29(2)

Carcinogenic HPV type	% of Cervical Cancers	9-year risk of progression to CIN3+ of incident HPV infection	Risk Group
16	60.3	6.3	16
18	10.5	3.0	18/45
45	6.1	2.2	18/45
33	3.7	4.5	16-related
31	3.6	2.2	16-related
52	2.7	2.2	16-related
58	2.2	1.9	16-related
35	2.0	2.8	16-related
39	1.6	1.1	Other
51	1.2	1.1	Other
59	1.1	0.9	Other
56	0.9	0.8	Other
68	0.6	1.0	Other

Extended Genotyping

- 2 FDA approved assays: Approved 2020 and 2023
- General Principles apply to both
  - Extended Genotyping acceptable to guide clinical management
  - For asymptomatic individuals undergoing screening or surveillance
  - Unclear performance following Hysterectomy for High grade dysplasia or cancer
  - Applicable to following strategies: Primary HPV, CoTesting and ASC-US triage
  - When multiple types reported use highest risk category for management

Massad et al. J Low Gen Tract Dis. 2025;29(2)



Journal of Lower Genital Tract Disease. 2025. *Guidelines for Extended HPV Genotyping*.

E.G. w Cotesting or Cytology Triage of Primary HPV

	Current HPV	Current cytology	Past results	Management
HPV 16/18	16	HSIL[1]	N/A[2]	Treatment preferred; colposcopy acceptable
	16	ASC-H[3]	N/A	Treatment or colposcopy
	16	NILM,[4] ASC-US,[5] LSIL,[6] AGC[7], or no cytology	N/A	Colposcopy[8] *with collection of cytology if not already done*
	18	HSIL	N/A	Treatment or colposcopy
	18	NILM, ASCUS, LSIL, ASC-H, AGC, or no cytology	N/A	Colposcopy[8] *with collection of cytology if not already done*
HPV 45,33/58, 31, 52/35/39/68, 51 **Untyped or "other" types when 16 and 18 are not present**	45,33/58, 31, 52/35/39/68, 51 or untyped/other	HSIL, ASC-H, AGC	N/A	Colposcopy[8,9]
	45,33/58, 31, 52/35/39/68, 51	ASC-US or LSIL	N/A	Colposcopy
	Untyped/other	ASC-US or LSIL	Documented HPV negative screen in past 5 years or colposcopy <CIN2[10] in past year	Repeat HPV test in 1 year
	Untyped/other	ASC-US or LSIL	Any history other than above	Colposcopy
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	NILM	Normal[11] or colposcopy <CIN2 within past year	Repeat HPV test in 1 year
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy
HPV 59/56/66	59/56/66	ASC-H, AGC, or HSIL[12]	N/A	Colposcopy[8]
	59/56/66	NILM, ASC-US, LSIL or no cytology[12]	Normal or colposcopy <CIN2 within past 1 year	Repeat HPV test in 1 year
	59/56/66	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy

Massad et al. J Low Gen Tract Dis. 2025;29(2)

E.G w Dual Stain

	Current HPV	Current DS result	Past history	Management
HPV 16/18	16 and/or 18	N/A[1]	N/A	Colposcopy *with collection of cytology if available*
HPV 45,33/58, 31, 52/35/39/68, 51 **Untyped or "other" types when 16 and 18 are not present**	45,33/58, 31, 52/35/39/68, 51 or untyped/other	DS Positive[2]	N/A	Colposcopy
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	DS Negative[3]	Normal[4] or colposcopy <CIN2[5] within past 1 year	Repeat HPV test in 1 year
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy
HPV 59/56/66	59/56/66	N/A	Normal or colposcopy <CIN2 within past 1 year	Repeat HPV test in 1 year[2]
	59/56/66	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy

Massad et al. J Low Gen Tract Dis. 2025;29(2)

Extended Genotyping

- **Specific Recommendations for 2020 approved platform**
  - HPV 16 or 18 require at least colposcopy (Additional reflex test preferred, See & Treat)
  - Triage testing for HPV type (31, 33/58, 35/39/68, 45, 51, 52) w/ cytology or Dual Stain
    - If DS negative or Cytology NILM Repeat HPV testing 12 mos
    - If DS + or Cytology ≥ASC-US refer to Colposcopy
    - For pts with negative DS or NL cytology F/U at 12 mos w any + HPV or cytology ≥ASC-H refer to Colposcopy
    - For pts with negative DS or NL cytology F/U at 12 mos w – HPV or cytology ≤LSIL recommend HPV based testing 12 mos
  - 56/59/66 only repeat HPV typing in one yr. Even if ASC-US or LSIL CoTest
    - Colposcopy if ASC-H, AGC or HSIL
    - Any + HPV at 12 mos or cytology ≥ASC-H refer to colposcopy

Massad et al. J Low Gen Tract Dis. 2025;29(2)

Challenges for Genotyping: Immediate CIN 3 risk for specific types varies from study and population

- 35/39/68 1.7% immediate risk IRIS vs. 5.9% STRIDES
  - Increased CA attribution for 35 in African ancestry- reflected in STRIDES cohort
  - 35 is “HPV 16” related, 39/68 “other grouping”
  - Triage recommended “Current standard for HPV12”

- HPV 51 1.5% IRIS vs. 6.3% STRIDES
  - Limited data set with few HPV 51 infections
  - Further triage “Current Standard for HPV12”

Massad et al. J Low Gen Tract Dis. 2025;29(2)

Challenges for Genotyping: Immediate CIN 3 risk for specific types varies from study and population

- HPV 31 2019 data: NILM and +31= 7.5% Immediate risk CIN 3
  - Unconfirmed other studies
  - ? Colposcopy?
  - Manage as Non-16/18 with triage

Massad et al. J Low Gen Tract Dis. 2025;29(2)

Self-Collection

- Multiple effectiveness studies and patient acceptability studies have shown that self-collection is effective, is cost-effective, and is acceptable to women, especially among under-screened populations
  - Sensitivity comparable to clinician-obtained samples with PCR-based HPV tests
  - A positive test requires a physician collected specimen for triage
    - At least DS and cytology
  - 4 FDA approved devices for self collection of vaginal HPV
    - 3 approved for “clinical setting”
    - 1 approved for home use
  - Requires Rx

Arbyn M, et al. Detecting cervical precancer and reaching underscreened women by using HPV testing on self-samples: Updated meta-analyses. *BMJ*. 2018; 363:k:4823.

Bottom Line

- Primary HPV screening works comparably to Co-Testing
- Less complicated
- Potentially fewer exams and tests
- Potential to increase access and improve patient participation- Self collection
- Can be cost efficient
- Need transition and preparation before widely available
- Any screen is better than no screen