

Moving
away from

Beyond Pap Morphological Triage: p16/ki67 Dual Staining

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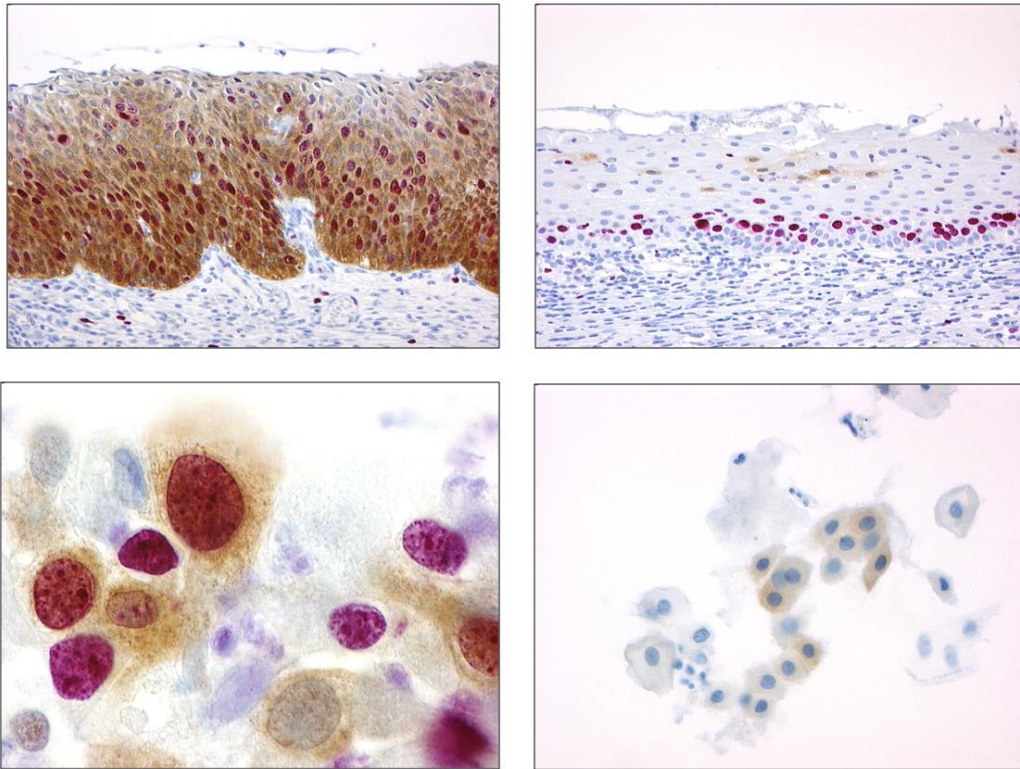


Disclosures

- I am evaluating new assays for cervical cancer screening that are in part donated or purchased at reduced cost from various companies. Otherwise, I have no conflict of interest.
- These are personal opinions and not official NCI statements



p16 and Ki-67

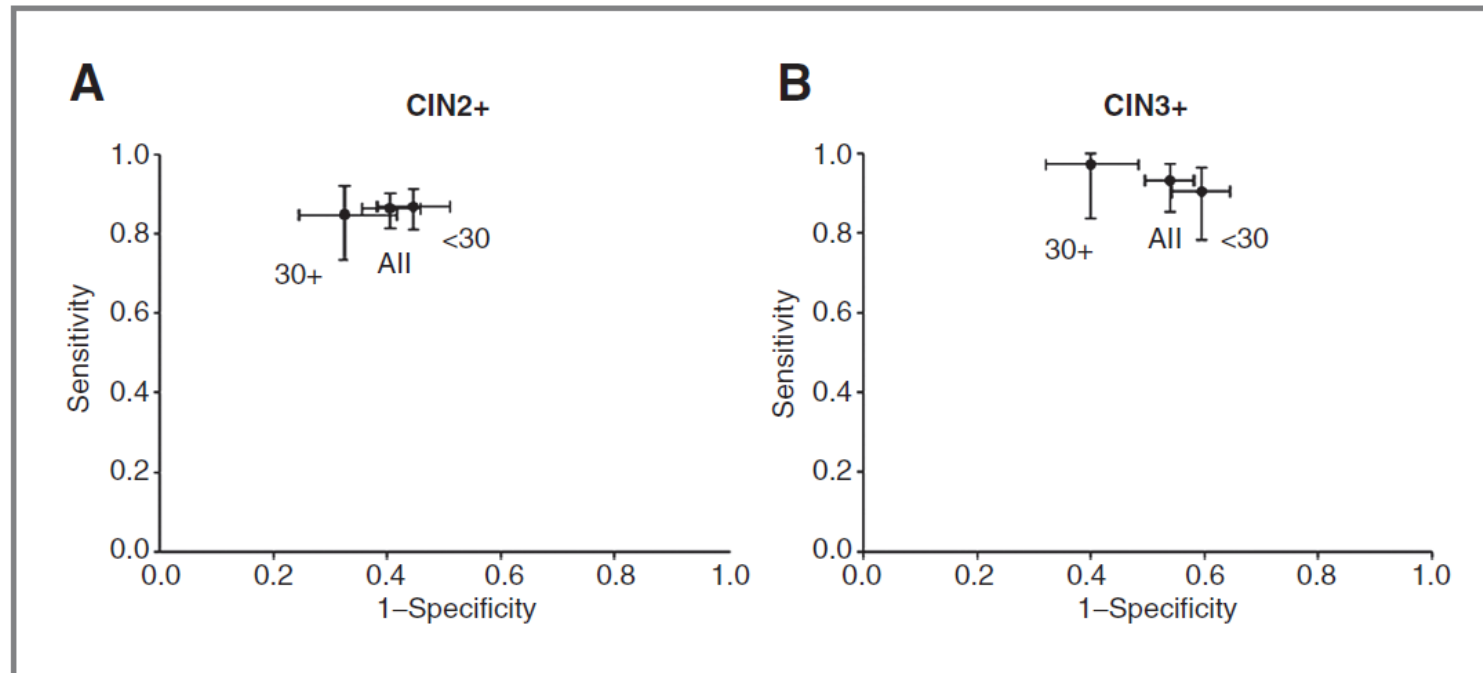


Red nuclear stain: Ki-67 / Brown cytoplasmic stain: p16

- In HPV-transformed cells, E7 oncoprotein leads to accumulation of p16
- Strong diffuse p16 staining is a marker of high grade CIN



p16/Ki-67 cytology in a colposcopy referral population

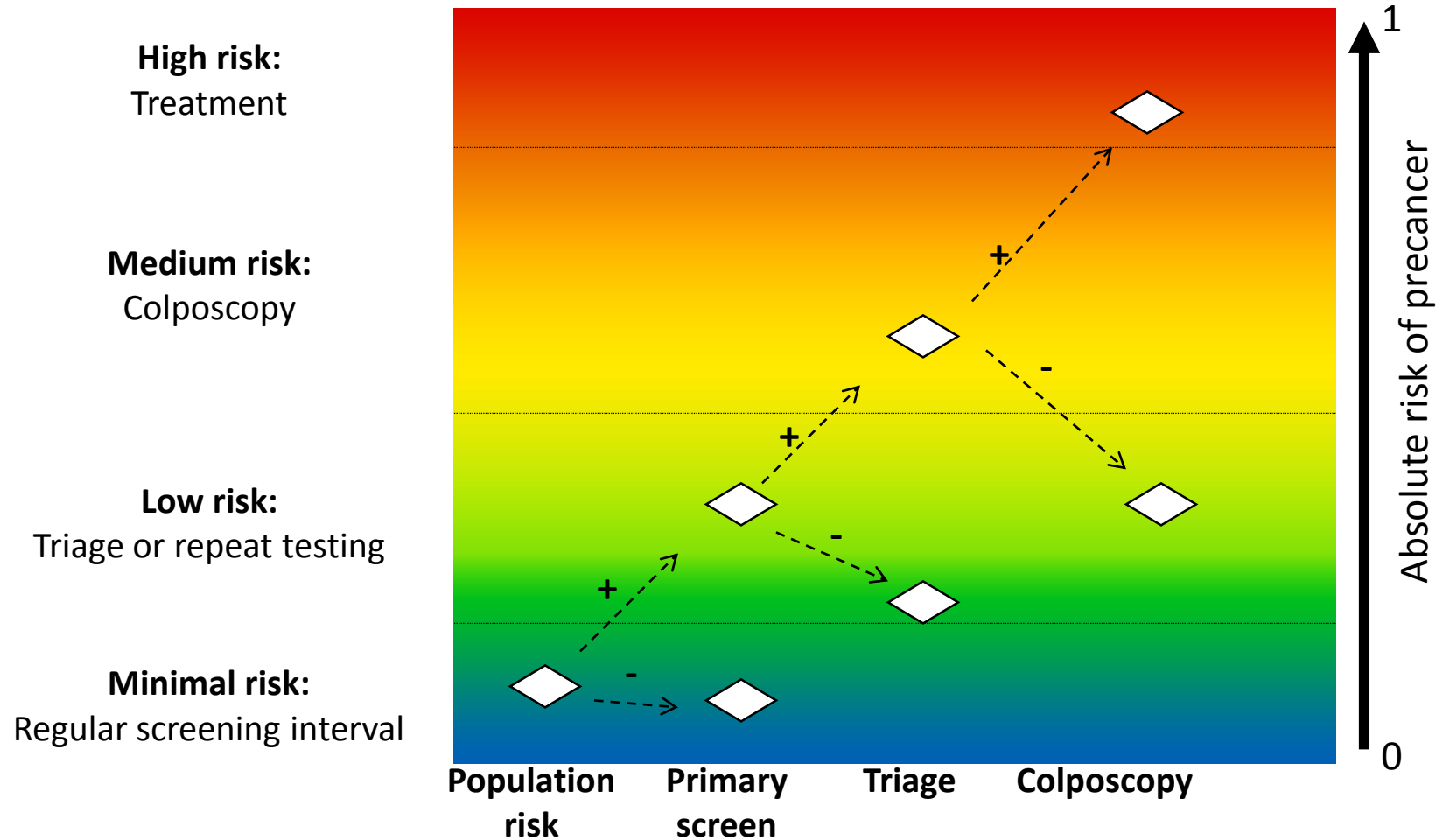


Wentzensen Clin Cancer Res 2012

- High sensitivity for CIN2+, CIN3+
- In this population, referral could be reduced by almost half

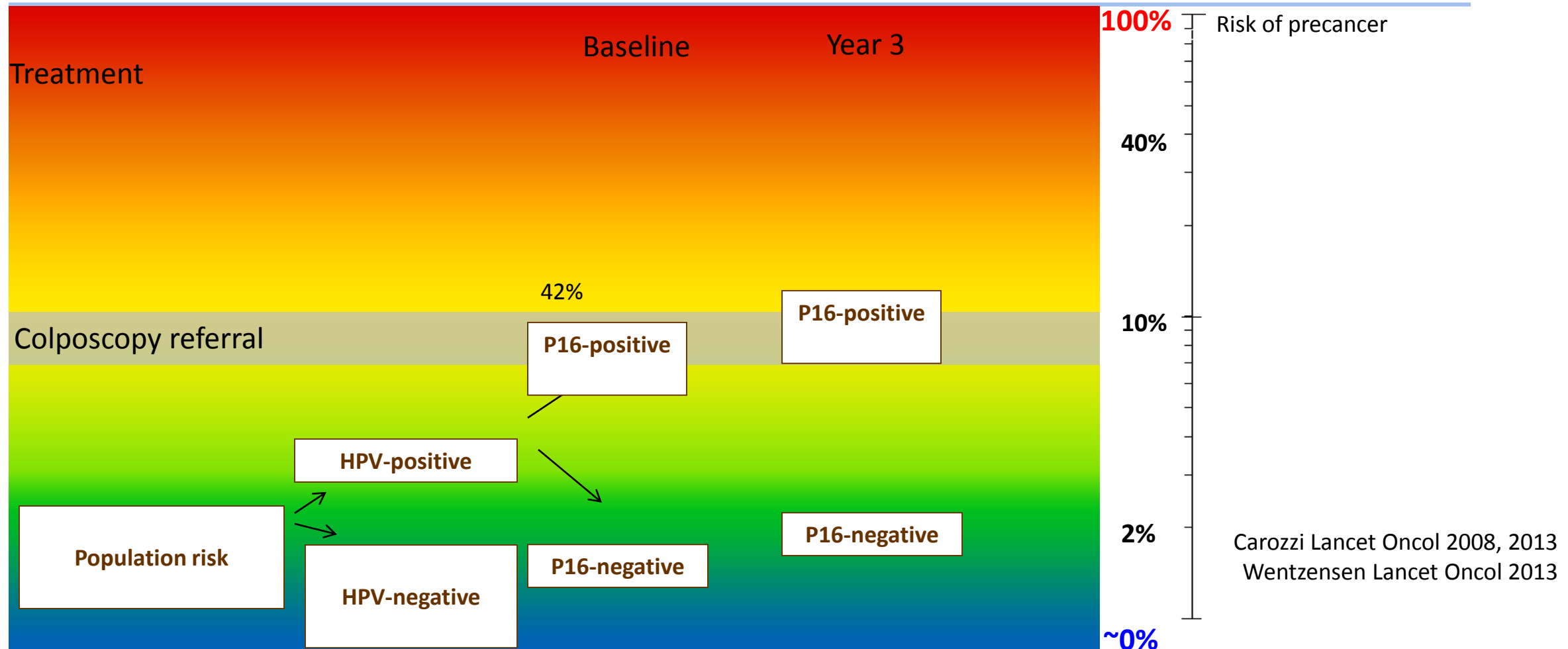


Risk-based approach to screening and management



Wentzensen JCV 2016

Evaluation of p16 triage in the Italian screening trial (NTCC)



KPNC p16/Ki-67 triage study

- Kaiser Permanente Northern California (KPNC) Regional Laboratory
- 2400 HPV-positive women (hybrid capture 2)
- p16/Ki-67 dual stain (CINtec Plus) on residual Surepath samples
- Semi-quantitative evaluation of p16/Ki-67-stained cells by cytotechnologist
- Pap cytology: Focal Point Slide Profiler followed by cytotechnologist review with knowledge of HPV status; full review of all HPV+/NILM slides



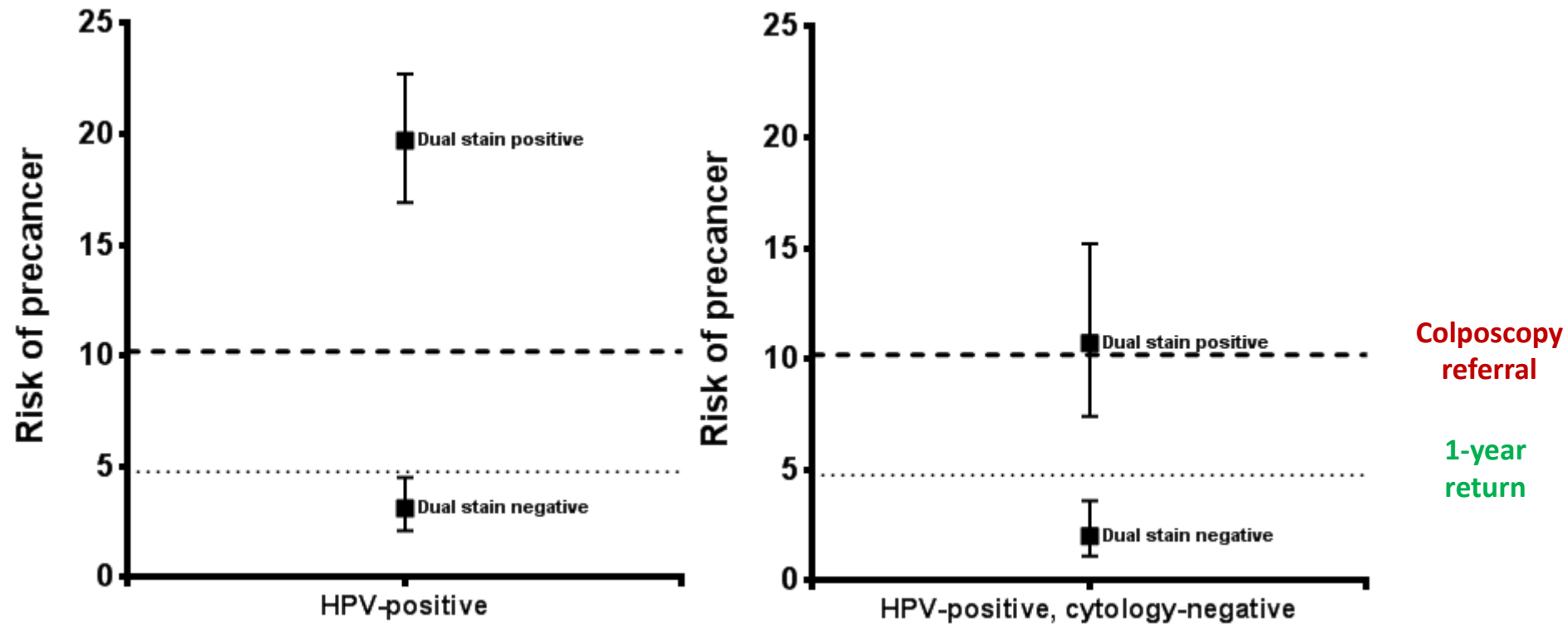
Performance of dual stain and cytology for triage of HPV-positive women

	p16/Ki-67 dual stain (95% CI)	Cytology ASCUS+ (95% CI)	P-value
Positivity	694 (45.9%)	806 (53.4%)	<0.0001
Detection of CIN2+ (n=175)			
Sensitivity	83.4% (77.1 - 88.6)	76.6% (69.6 - 82.6)	0.1
Specificity	58.9% (56.2 - 61.6)	49.6% (46.9 - 52.3)	<0.0001
PPV	21.0% (18.1 - 24.3)	16.6% (14.1 - 19.4)	<0.001
NPV	96.4% (94.9 - 97.6)	94.2% (92.2 - 95.8)	0.03
Detection of CIN3+ (n=99)			
Sensitivity	86.9% (78.6 - 92.8)	83.8% (75.1 - 90.5)	0.7
Specificity	56.9% (54.2 - 59.5)	48.7% (46.1 - 51.4)	<0.001
PPV	12.4% (10.0 - 15.1)	10.3% (8.3 - 12.6)	0.002
NPV	98.4% (97.3 - 99.1)	97.7% (96.3 - 98.7)	0.3

Wentzensen JNCI 2015

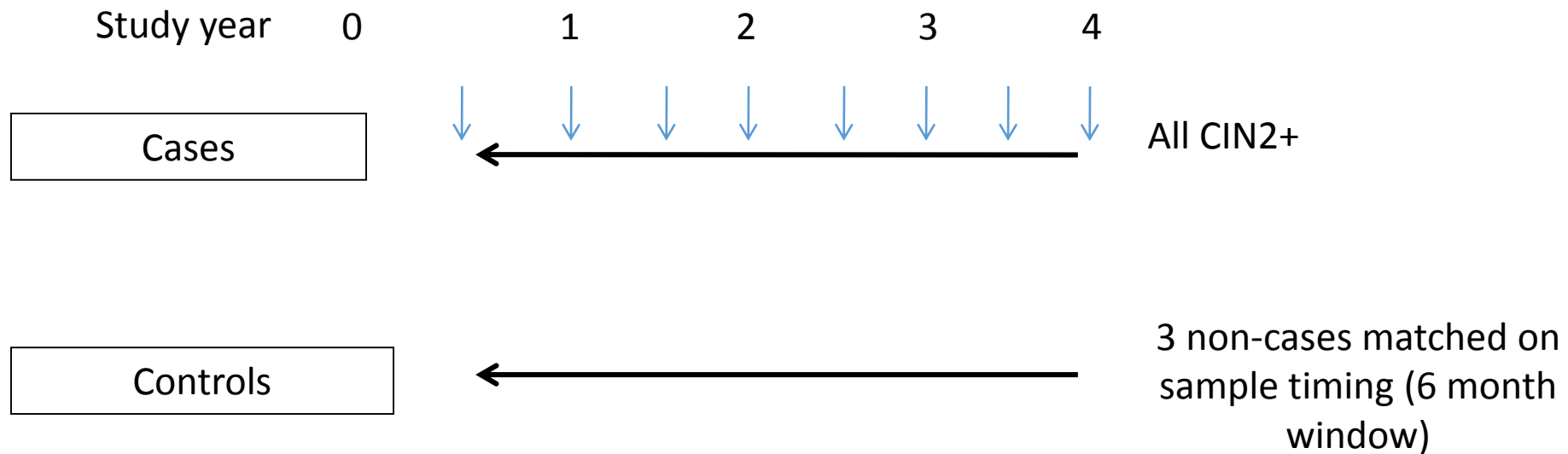


Dual stain for triage of HPV-positive women



- Risk stratification by DS suggests different clinical management: Colposcopy referral of DS-positives, extended interval (>1 year) in DS-negatives

p16/Ki-67 dual stain in vaccinated women



HPV vaccine trial (Bivalent HPV vaccine vs. Hepatitis A)

7,466 women age 18-25



p16/Ki-67 dual stain performance

	HPV arm	Control arm	p value
Positivity	284 (37.2 %)	613 (54.1 %)	0.02
CIN2+ n	19	41	0.18
Sensitivity	89.5% (65.9-97.4)	90.2% (76.5-96.3)	0.93
Specificity	67.7% (55.2-78.2)	51.1% (40.8-61.4)	0.04
PPV	20.8% (12.7-32.3)	21.0% (15.7-27.5)	0.98
cNPV	1.5% (0.4-5.7)	2.7% (1.0-6.9)	0.48

- p16/Ki-67 stained on slides from residual cells in PreservCyt
- Dual stain results weighted back to the full population



p16/Ki-67 dual stain by HPV status

	Control	HSIL	CIN2	CIN3+
HR-HPV-	526	5	19	8
%DS	4%	60%	37%	63%
HR+/HPV16-	218	26	66	54
% DS	23%	88%	70%	85%
HPV16+	31	10	36	37
% DS	52%	80%	89%	100%

High dual stain positivity in HPV16-positive controls



Reproducibility of p16/Ki-67 cytology

- 2-day training
- After completion of training and competency evaluation, 12 reviewers participated in reproducibility study (2 dropouts)
- 480 slides were distributed, each slide was reviewed 4 times, each reviewer evaluated 160 slides
- Compared to reference evaluation



Reproducibility and accuracy

Reviewer Type	Kappa
All KPNC evaluators (n=10)	0.70
Cytotechnologist evaluators (n=6)	0.73

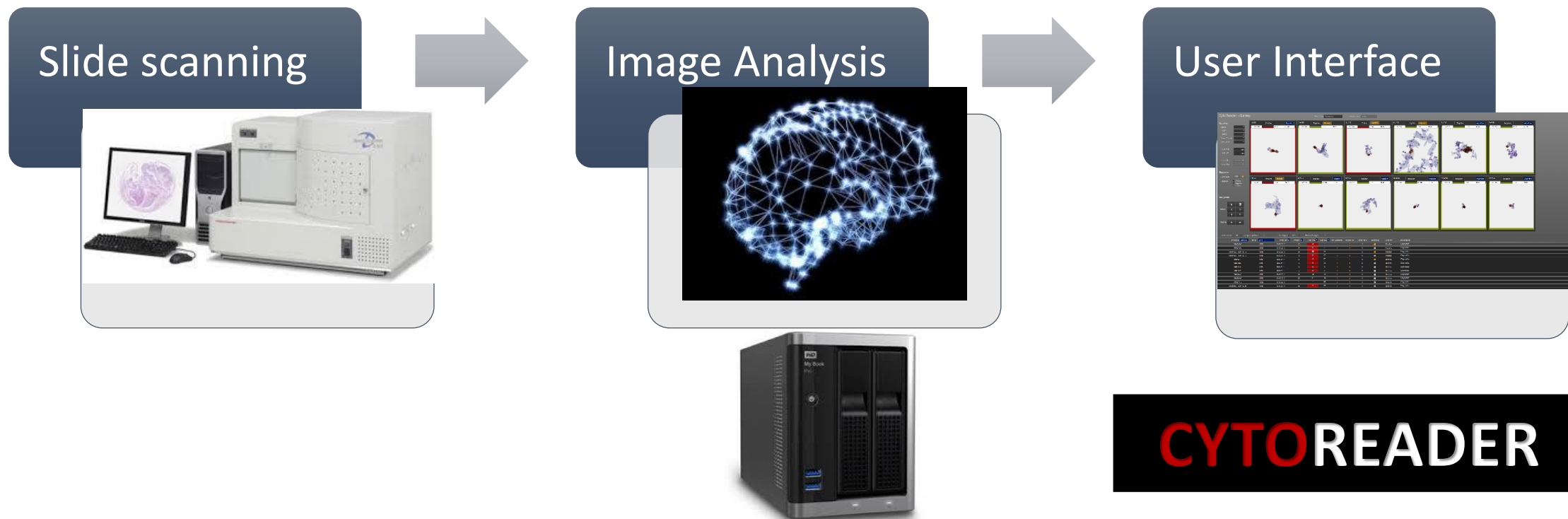
Reader	Sensitivity	Specificity
All KPNC evaluators	82.0% (73.1-88.4)	63.9% (60.0-67.5)
Reference evaluation	84.0% (63.1-94.7)	62.5% (56.6-68.0)

- 10 KPNC reviewers participated in reproducibility study
- 480 slides were distributed, each reviewer evaluated 160 slides

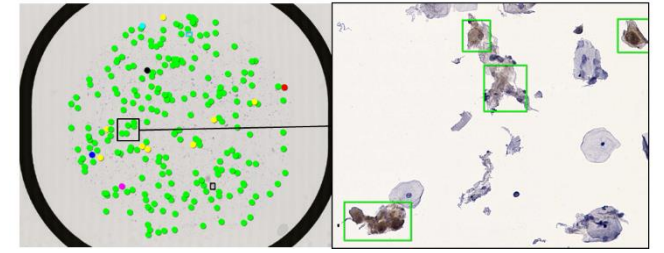
Wentzensen Cancer Cytopath 2014



Automated analysis of p16/Ki-67 dual stain



Application scenarios



- **Fully automated:** computer calls slides positive, negative, presents cells for review
- **Ranked review:** All slides are reviewed on the screen, computer ranks events for each slide
- **Exclusion review:** A subset of slides is determined negative and is not reviewed, equivocal and positive slides are reviewed on screen with ranked events



Assisted evaluation: Image gallery

CytoReader - Gallery

Select User: Diagnostics Current user: admin

Statistics

- Strong: 7
- Singles: 0
- Clusters: 0
- Smear Clusters: 0
- Low stained: 6
- Single Ki67: 1,069
- Single p16: 1,153
- Human Pos: 2
- Human Neg: 2

Diagnosis

Completed: YES ☐

Diagnosis: ☐ Undone ☐ Negative ☐ Positive

Navigation

Images:

Patients:

1 of 35 Positive Algorithm 227149 P: 77 N: 23

2 of 35 Negative Human 227146 P: 75 N: 25

3 of 35 Positive Human 227147 P: 60 N: 40

4 of 35 Negative Human 227148 P: 59 N: 41

5 of 35 Negative Algorithm 227151 P: 32 N: 68

6 of 35 Negative Algorithm 227150 P: 31 N: 68

7 of 35 Positive Human 227152 P: 29 N: 71

8 of 35 Negative Algorithm 227158 P: 18 N: 82

9 of 35 Negative Algorithm 227157 P: 15 N: 85

10 of 35 Negative Algorithm 227156 P: 10 N: 90

11 of 35 Negative Algorithm 227154 P: 2 N: 98

12 of 35 Negative Algorithm 227153 P: 2 N: 98

Total patients: 54 Completed patients: 4 Total images: 3,419 Classified images: 7

Patient ID	Show All	Batch	CIN3	EntryDate	Images	Algo Pos	Algo Neg	Hum Classified	Human Pos	Human Neg	Completed	Diagnosis	AccountName
54664500		CIN3		13.07.2016	35	4	31	4	2	2	☐	Positive	Diagnostics
54664580		CIN3		13.07.2016	5	0	5	0	0	0	☐	Negative	Diagnostics
54664920 - 2014-07-19		CIN3		13.07.2016	35	10	25	1	1	0	☐	Positive	Diagnostics
54664960 - 2014-07-19		CIN3		13.07.2016	17	0	17	0	0	0	☐	Positive	Diagnostics
54665210		CIN3		13.07.2016	42	5	37	1	1	0	☐	Undone	Diagnostics
54665460		CIN3		13.07.2016	12	0	12	0	0	0	☐	Undone	Diagnostics
54665850		CIN3		13.07.2016	5	0	5	0	0	0	☐	Undone	Diagnostics
54665920		CIN3		13.07.2016	4	2	2	0	0	0	☐	Undone	Diagnostics
54666340		CIN3		13.07.2016	52	20	32	0	0	0	☐	Undone	Diagnostics
54666460		CIN3		13.07.2016	55	17	38	0	0	0	☐	Undone	Diagnostics
54667910		CIN3		13.07.2016	71	31	40	0	0	0	☐	Undone	Diagnostics
54668480 - 2014-07-28		CIN3		13.07.2016	56	7	49	0	0	0	☐	Undone	Diagnostics

Interim results

Thinprep

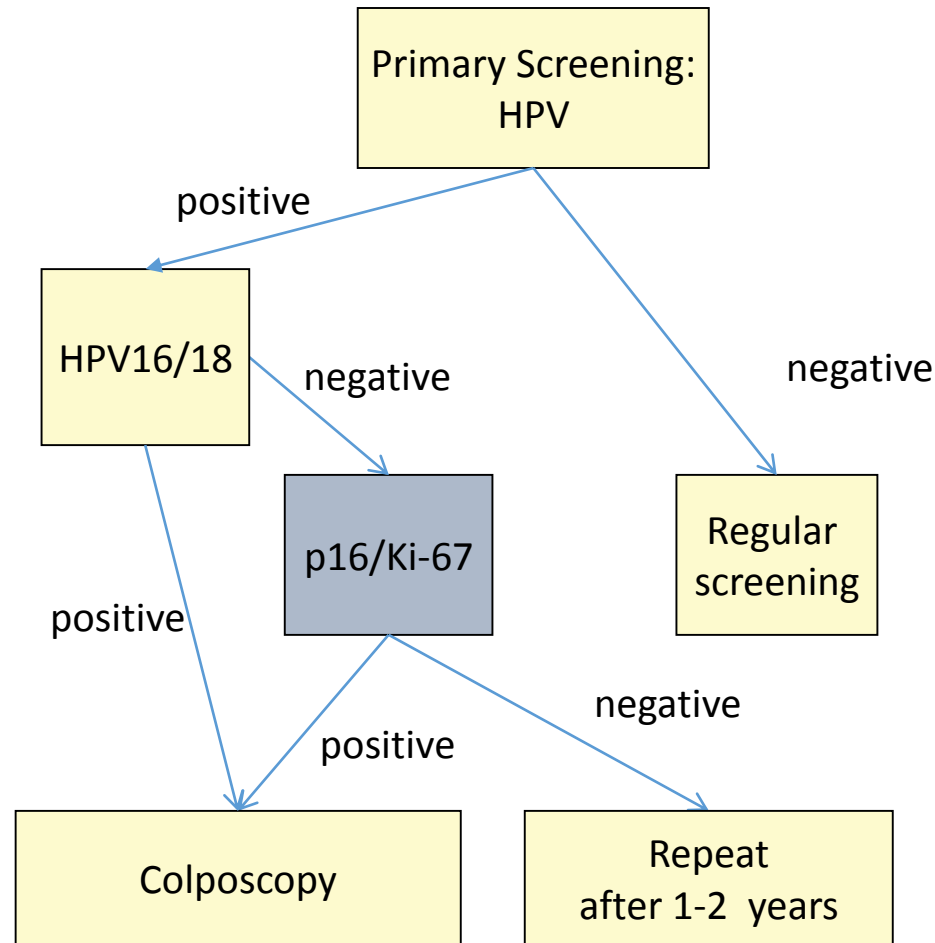
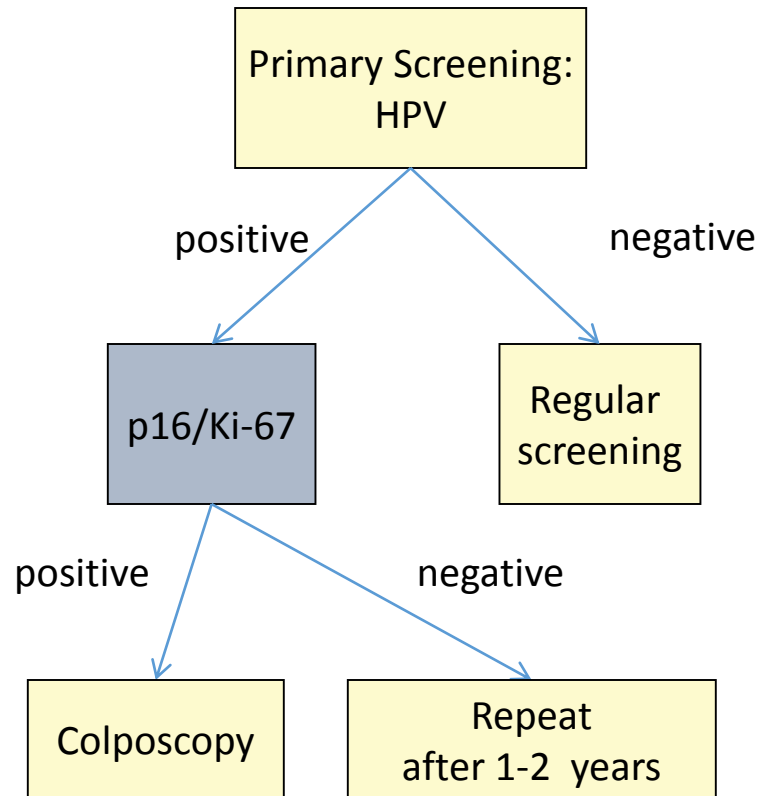
Detection of CIN2+ (n=145)	Stain Positive	Sensitivity	p value	Specificity	p value
Conventional	64.6	85.5% (78.5-90.6)	--	50.0% (42.8-57.2)	--
Automated					
1 or more DS+ cells	75.4	90.3 (84.0-94.4)	0.08	35.7% (29.1-42.9)	0.0001
2 or more DS+ cells	67.5	86.9% (80.0-91.7)	0.66	46.9% (39.8-54.2)	0.39
4 or more DS+ cells	56.7	79.3% (71.6-85.4)	0.15	60.2% (53.0-67.0)	0.01
Morphology	62.9	86.2% (79.3-91.2)	0.82	54.6% (47.3-61.7)	0.38

Surepath

Detection of CIN3+ (n=1584)	Sensitivity	Specificity
Conventional	80.2 (71.1-87.5)	59.7 (57.2-62.3)
Automated		
SP2: 1 or more DS+	80.2 (71.1-87.5)	49.3 (46.7-51.9)
SP2: 2 or more DS+	76.2 (66.7-84.1)	61.9 (59.4-64.4)
SP3: 2 or more DS+	77.2 (67.8-85)	59.4 (56.9-61.9)



How to integrate in cervical cancer screening?



**KPNC
implementation
study**

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Questions?