

# The use of biomarkers in the diagnosis of preinvasive and invasive lesions of the cervix uteri

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- Cervical cancer is the fourth most common cancer in women
- An estimated 528,000 new cases in 2012
- An estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths
- Almost 87% of cervical cancer deaths occur in underserved regions of the world




4,440 new cervical cancer cases are diagnosed annually in Germany

Cervical cancer ranks as the 13th cause of female cancer in Germany.

Cervical cancer is the 3rd most common female cancer in women aged 15 to 44 years in Germany.

Reduction of incidence and mortality by 70% since introduction of screening program

- 
- Great success overall, but still problems in:
    - Correct evaluation of biopsy specimens
    - Secondary prevention

# Agenda

- Role of biomarkers in histology
- Role of biomarkers in cytology

# Histological Diagnosis - The Challenges

- **Tissue samples difficult to differentiate between normal, low grade and high grade disease:**
  - Scant sample
  - Atrophy
  - Special types of metaplasia
  - High grade mimickers
  - CIN2
- **Resulting in:**
  - High rates of discordance among pathologists
  - Low level of inter- and intra-observer agreement in diagnosing cervical lesions
- **Leading to:**
  - Diagnostic accuracy issues which have substantial impact on appropriate patient management

# Diagnosing Cervical Intraepithelial Neoplasia (CIN)

- Subjective procedure
- Histopathologic interpretation of cervical biopsies as HSIL (CIN2 or 3) often leads to excisional or ablative therapeutic intervention
- Accuracy of diagnosis is critical to distinguish between low-grade and high-grade lesions
- Germany: CIN 2 is not regarded as HSIL and therefore is not being treated

# Reader Variability

- Morphologic criteria for grading of CIN have been well defined; nevertheless often difficult to apply
- Lack of objectivity
- Various studies indicating substantial variation between readers in the interpretation of CIN
- Kappa coefficients as a measure of agreement among observers corrected by chance typically found in the 0.4 – 0.5 range

# Clinical Relevance

## ➤ Risk of overtreatment of False-positive cases

- Many low-grade lesions have the potential to spontaneously regress
- Increasing evidence for regression of a substantial proportion of CIN2 lesions as well
- Risk of negative impact of ablational therapy on reproductive outcome of women

## ➤ Lack of treatment for False-negatives

- Missed high-grade CIN cases may progress to invasive disease

# Adjunctive Methods

- To enhance the **diagnostic accuracy** of morphology based interpretation of CIN
- To increase the **diagnostic agreement** between observers

# Biomarkers

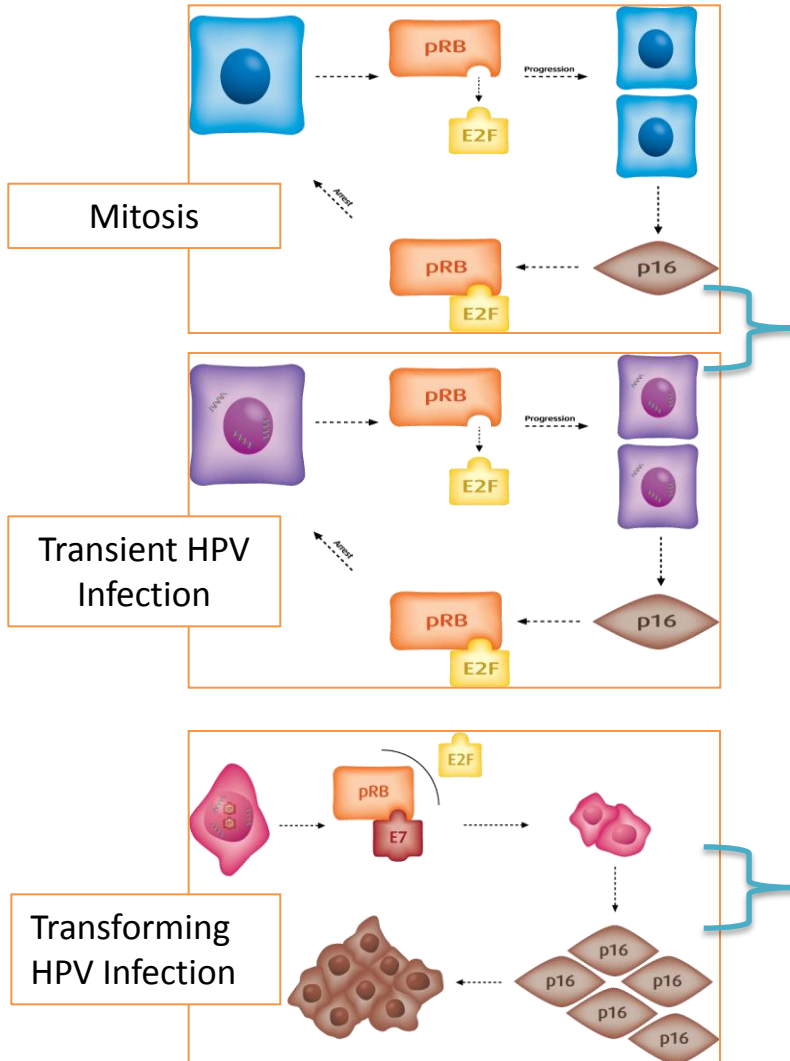
- p16INK4a
- Ki-67
- Pro-Ex-C
- HPV L1 capsid
- Stathmin-1
- Claudin-1

# The p16<sup>INK4a</sup> Biomarker

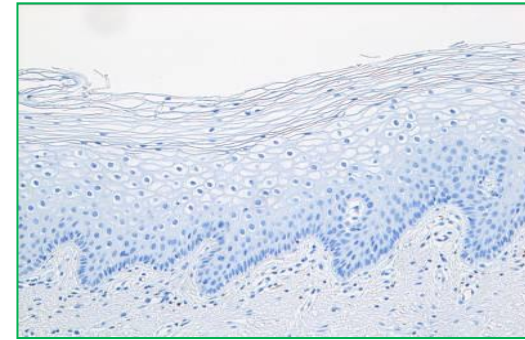
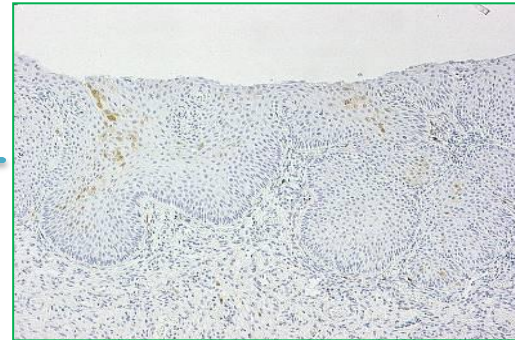
- A cellular protein involved in cell-cycle control
- Over-expression of p16<sup>INK4a</sup> can be used as a biomarker for pre-cancerous and cancerous cervical lesions
  - Surrogate for inactivation of the tumor suppressor protein pRB by high-risk HPV E7 oncoproteins
- Direct link between over-expression of p16<sup>INK4a</sup> and pathogenetic process of cervical dysplasia
- Measures the carcinogenic activity of human papillomavirus (HPV) at the cellular level
  - Patient age independent
  - HR-HPV type independent

# p16 in Histology

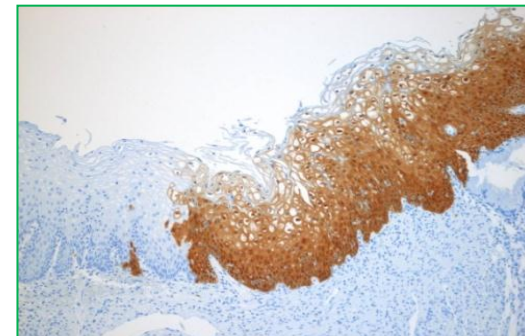
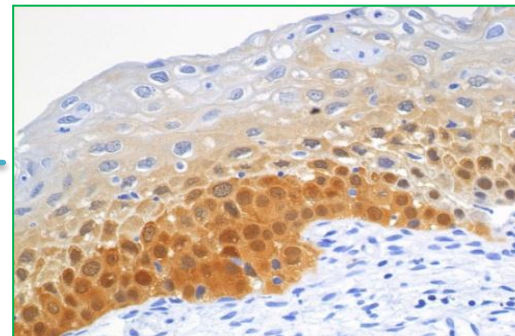
## *Staining pattern*



p16 staining in this case is expected to be **Focal** or **Negative**

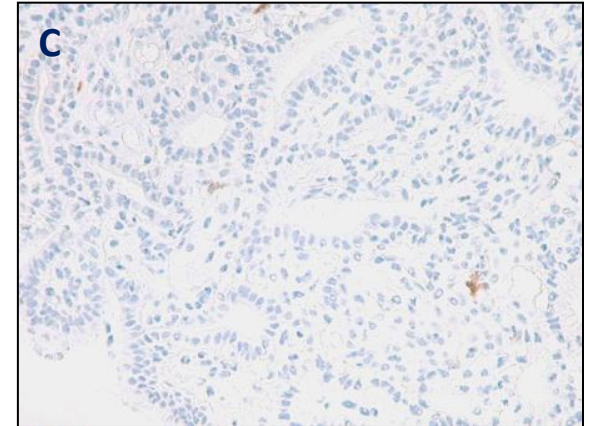
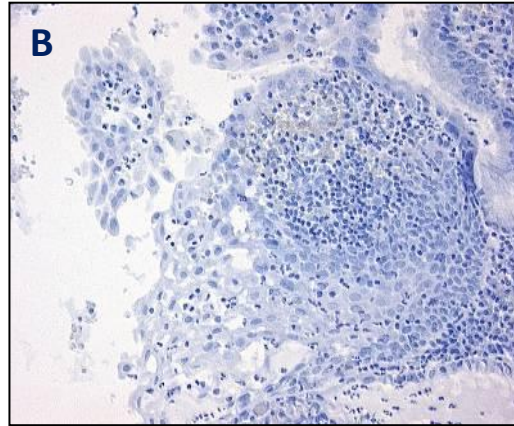
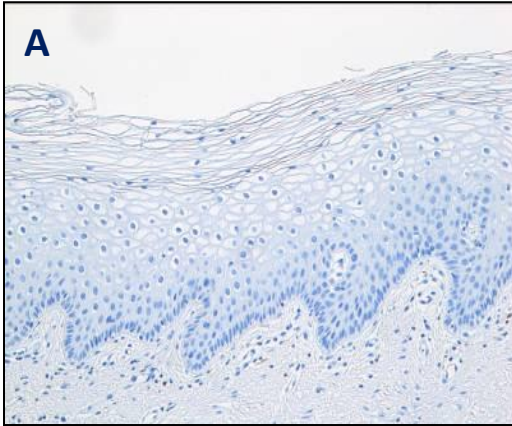


p16 staining in this case is expected to be **Diffuse** and called **Positive**



# P16 negativ

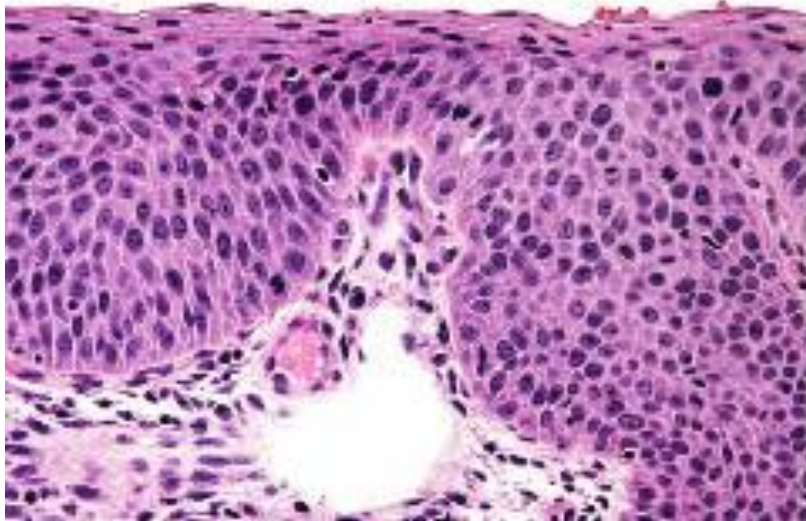
*No staining*



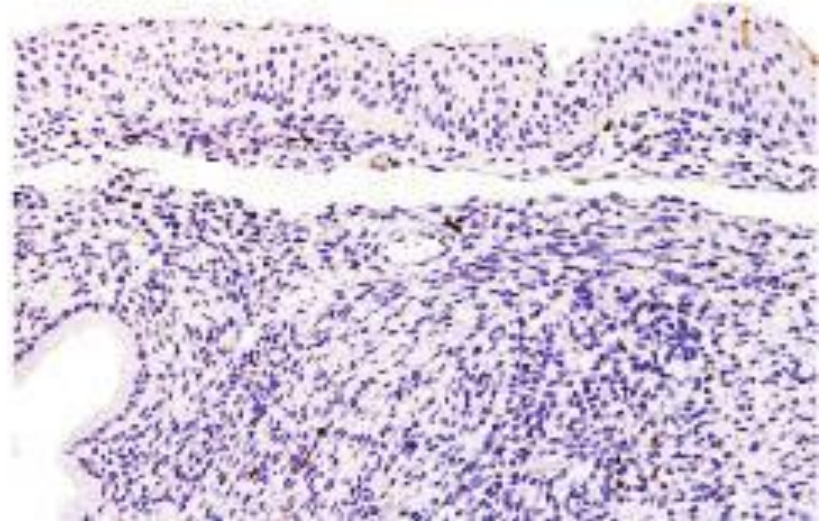
- No staining
  - The rating “**negative**” will be assigned if the p16 stained slide shows no staining reaction
    - Panels A and B represent cervical squamous epithelium tissue specimens
    - Panel C represents an endocervical tissue specimen. Glandular cells are negative for the p16 stain.

# P16 negative Atrophy

(A)

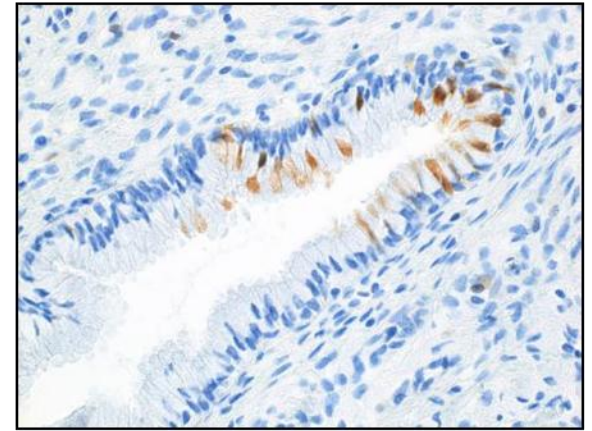
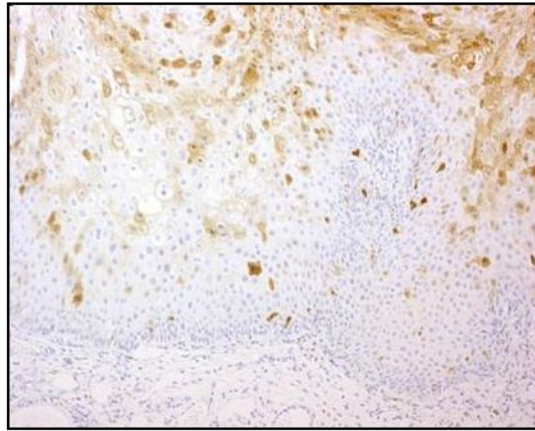
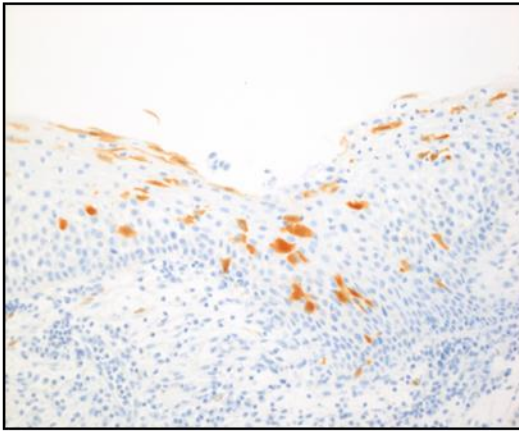


(B)

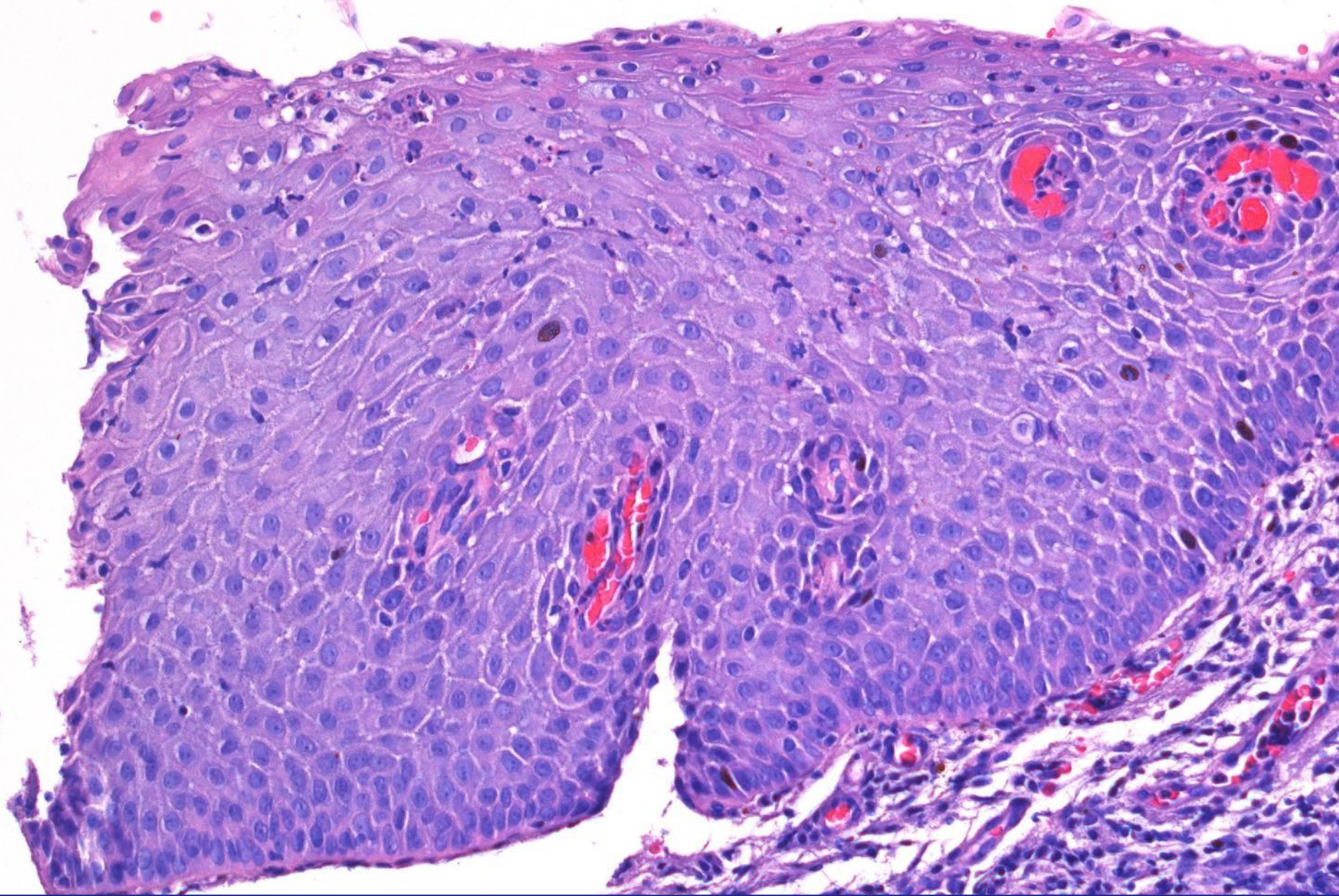


# CINtec® Histology negative

## *Focal staining pattern*

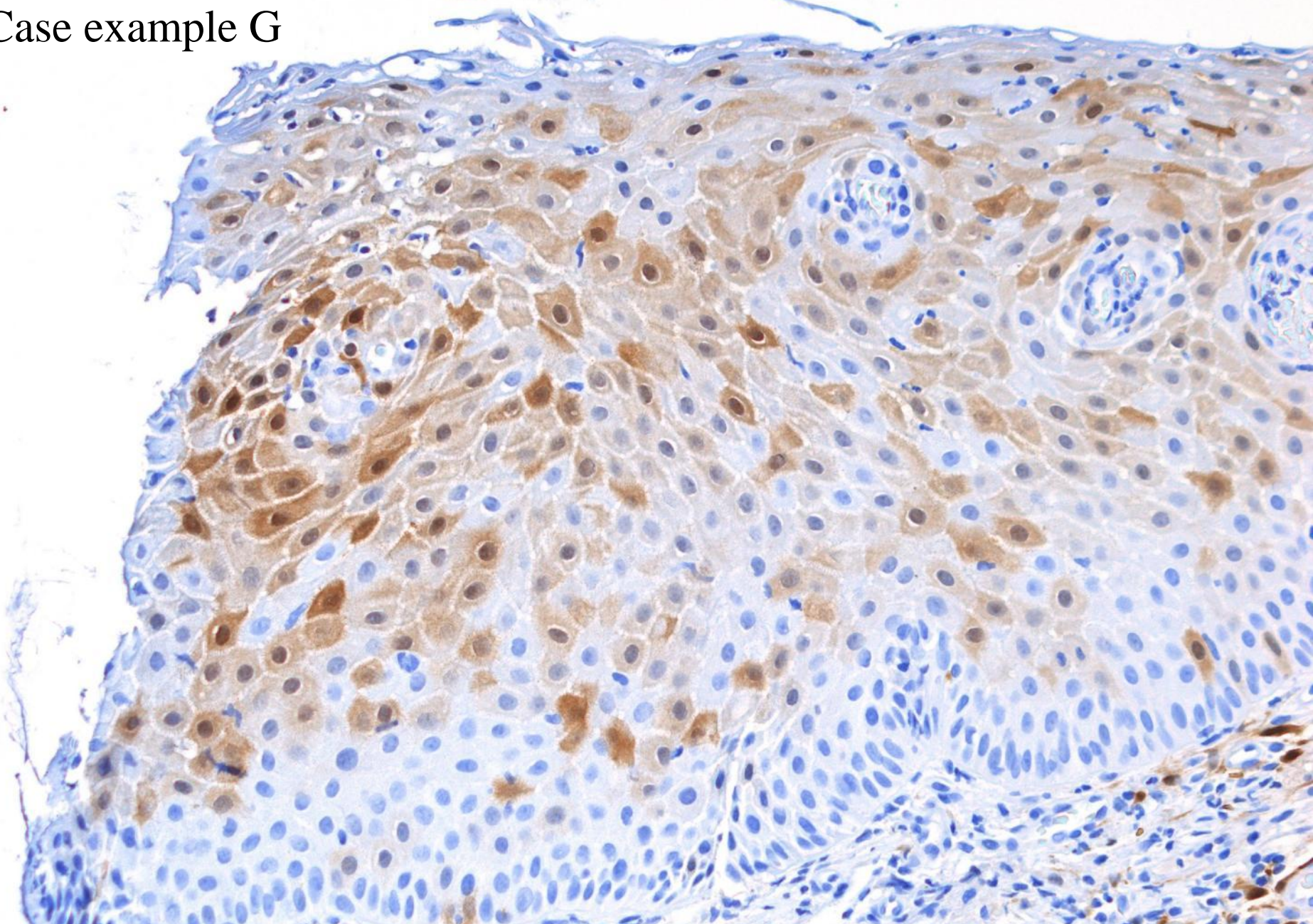


- Focal staining
  - Either a staining of isolated cells or small cell clusters; i.e. a non-continuous staining, particularly not of the basal and parabasal cells.
  - Slides that stain focal are assigned a “**negative**” rating
- Endocervical (right panel)
  - Individual cells or small groups of neighboring glandular cells may show p16 immuno-reactivity



**Squamous metaplasia; H&E stain**

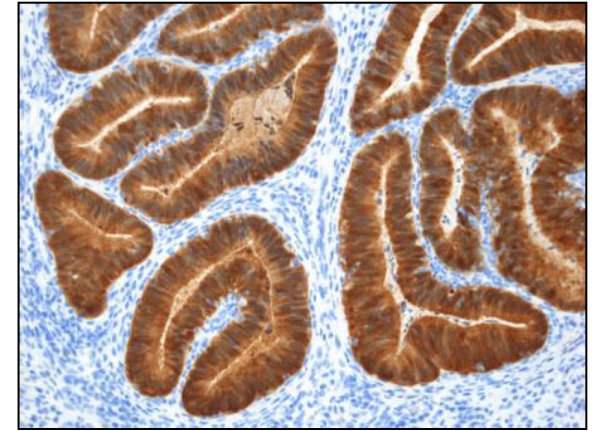
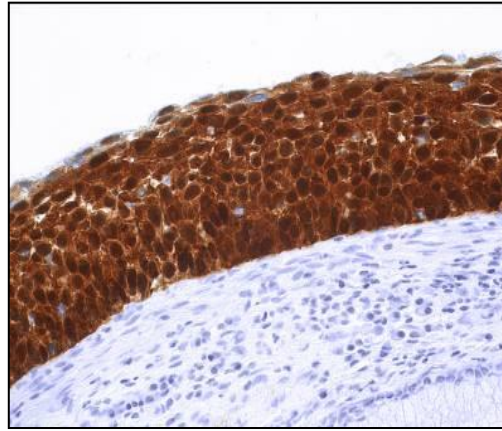
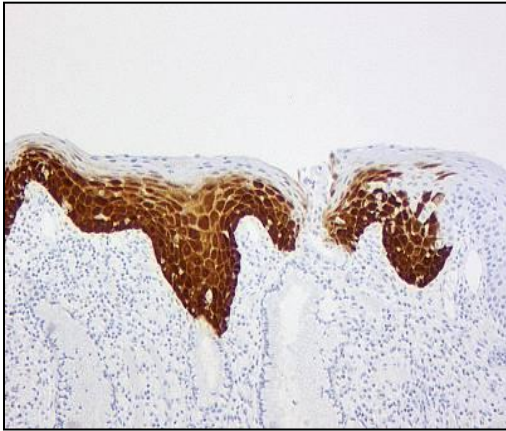
Case example G



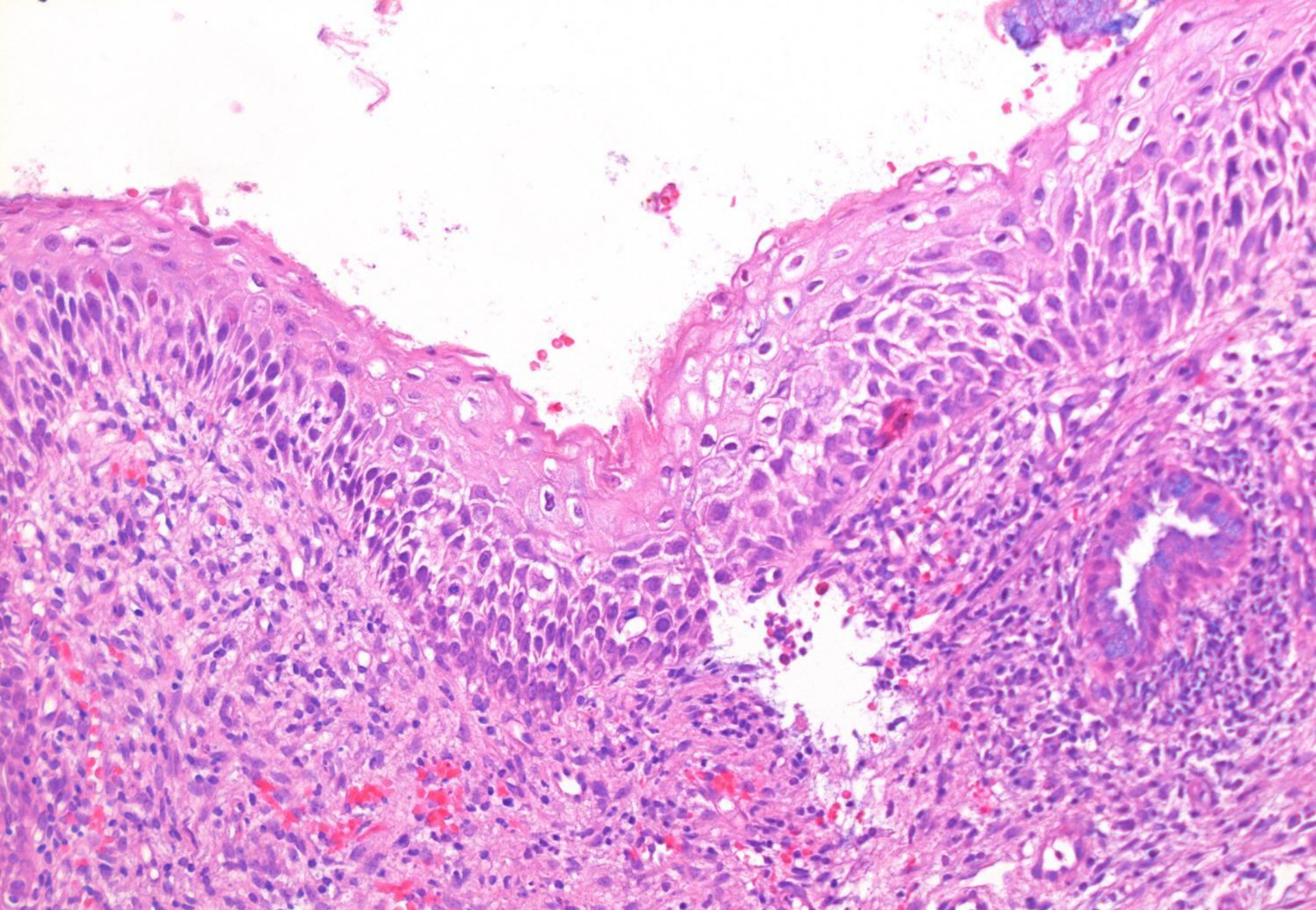
**Squamous metaplasia; Focal p16 stain**

# P16 positive

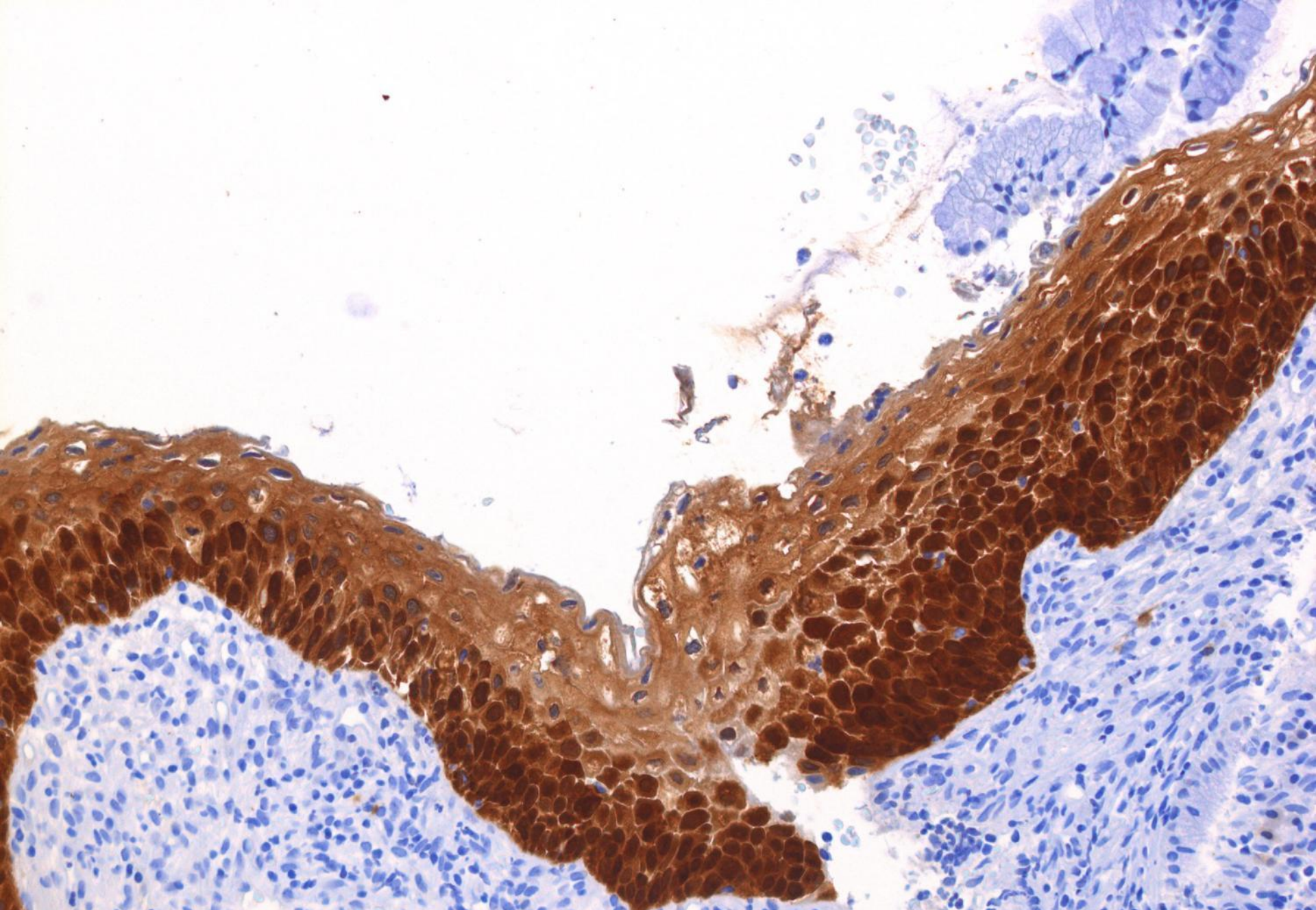
## *Diffuse staining pattern*



- Diffuse staining
  - A continuous staining of cells of the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of cells of superficial cell layers.
  - Slides that stain diffuse are assigned a “**positive**” rating
- Endocervical (right panel)
  - All cells in the gland must stain for p16



**CIN 1; H&E stain**

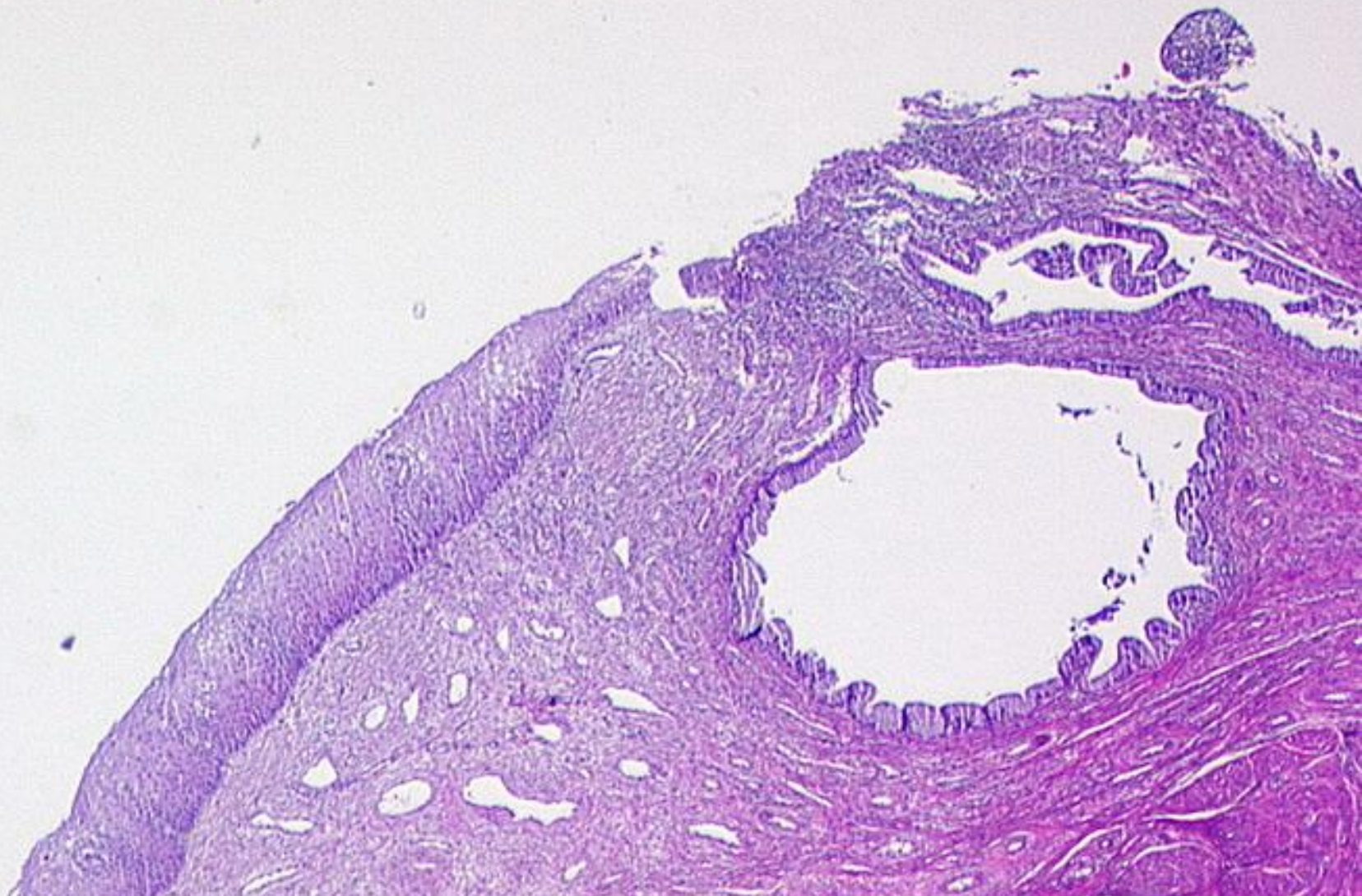


**CIN 1; Diffuse p16 stain**

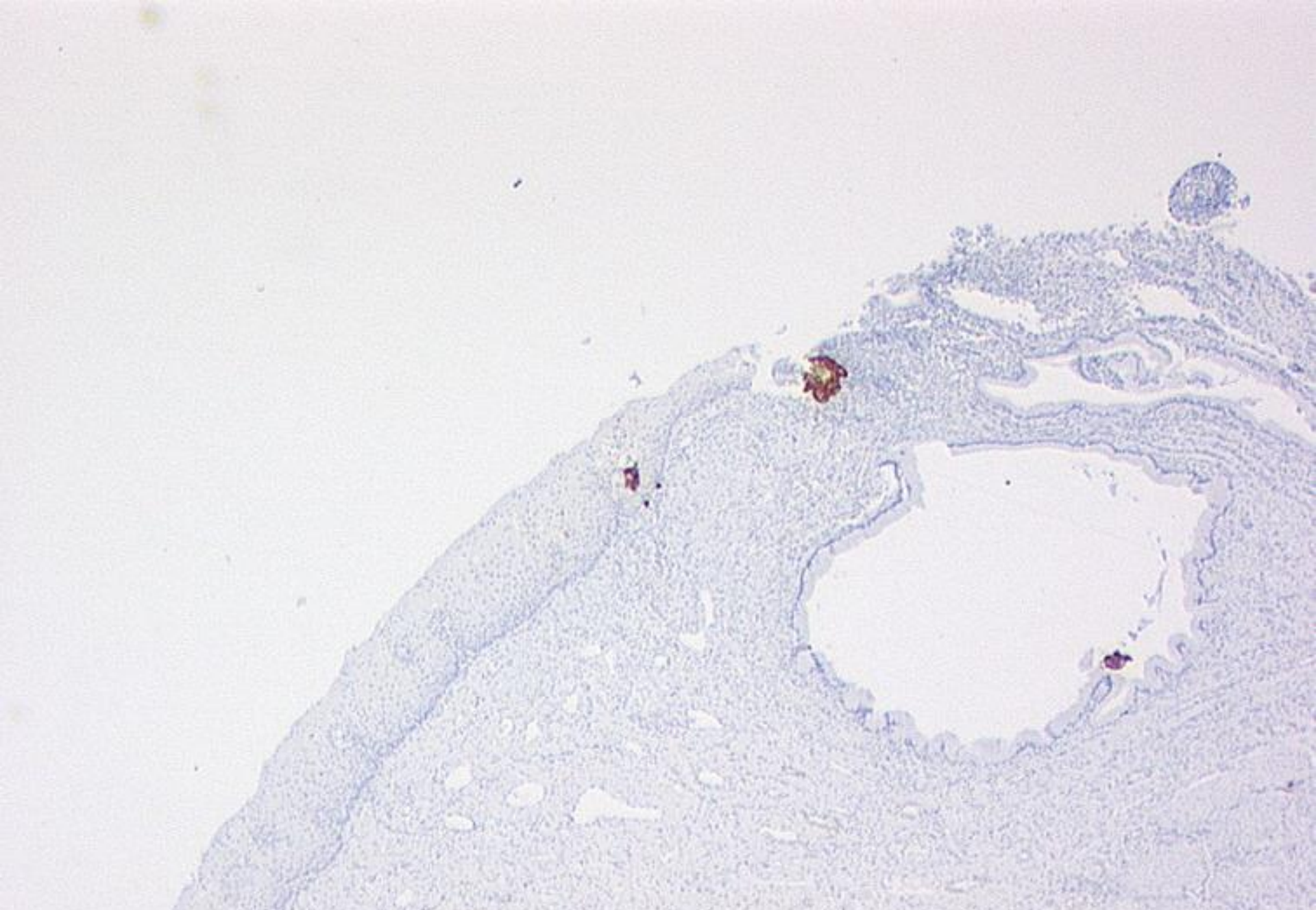
# p16 positivity

## Selected literature

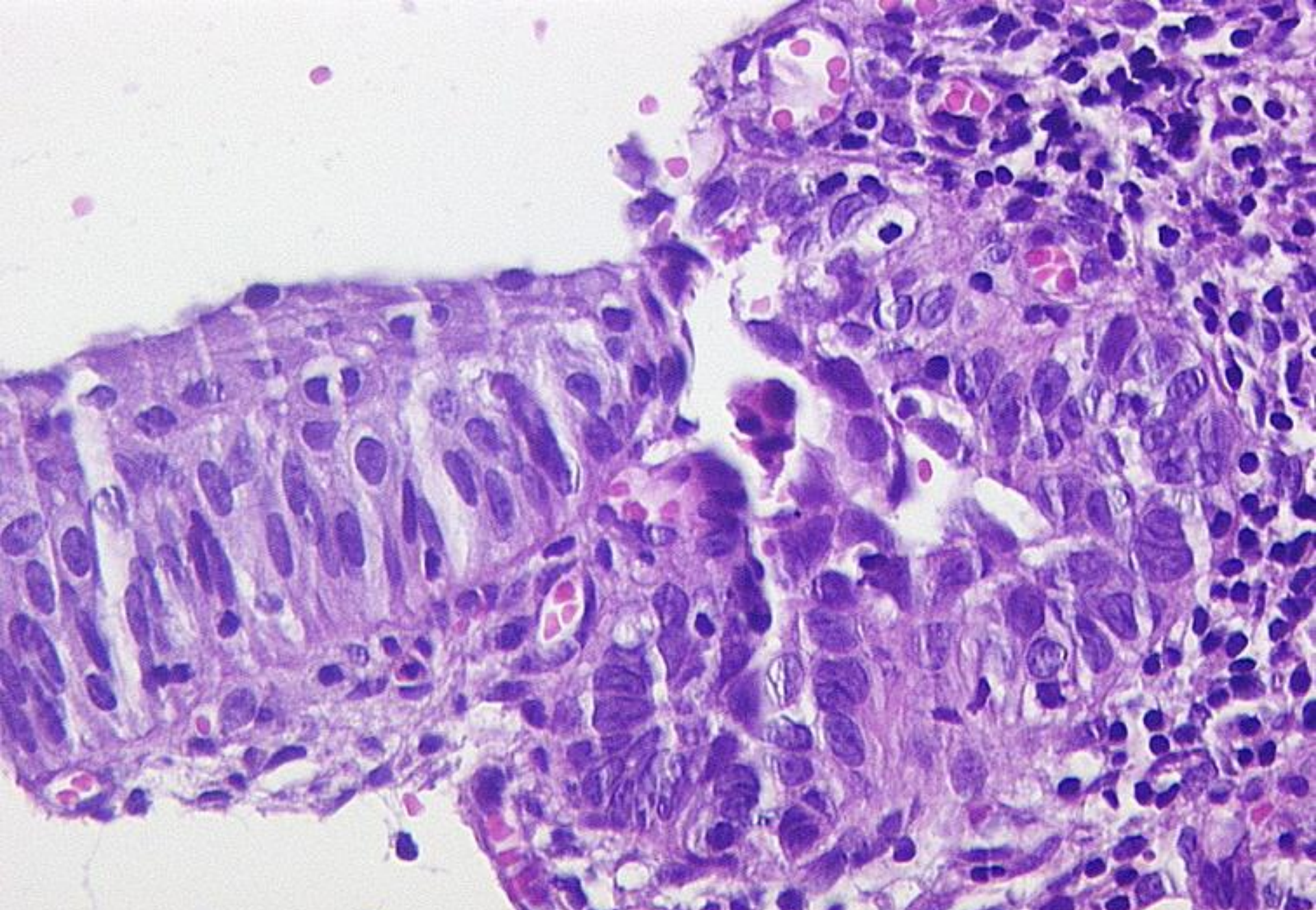
Author	CIN 1	CIN 2+
Shi et al. 2007	77%	100%
Badr et al. 2008	35%	93%
Pinto et al. 2008	ND	84%
Halloush et al. 2008	92%	100%
Conesa-Zamora et al. 2009	63%	87%
Howitt et al. 2013	71%	96%



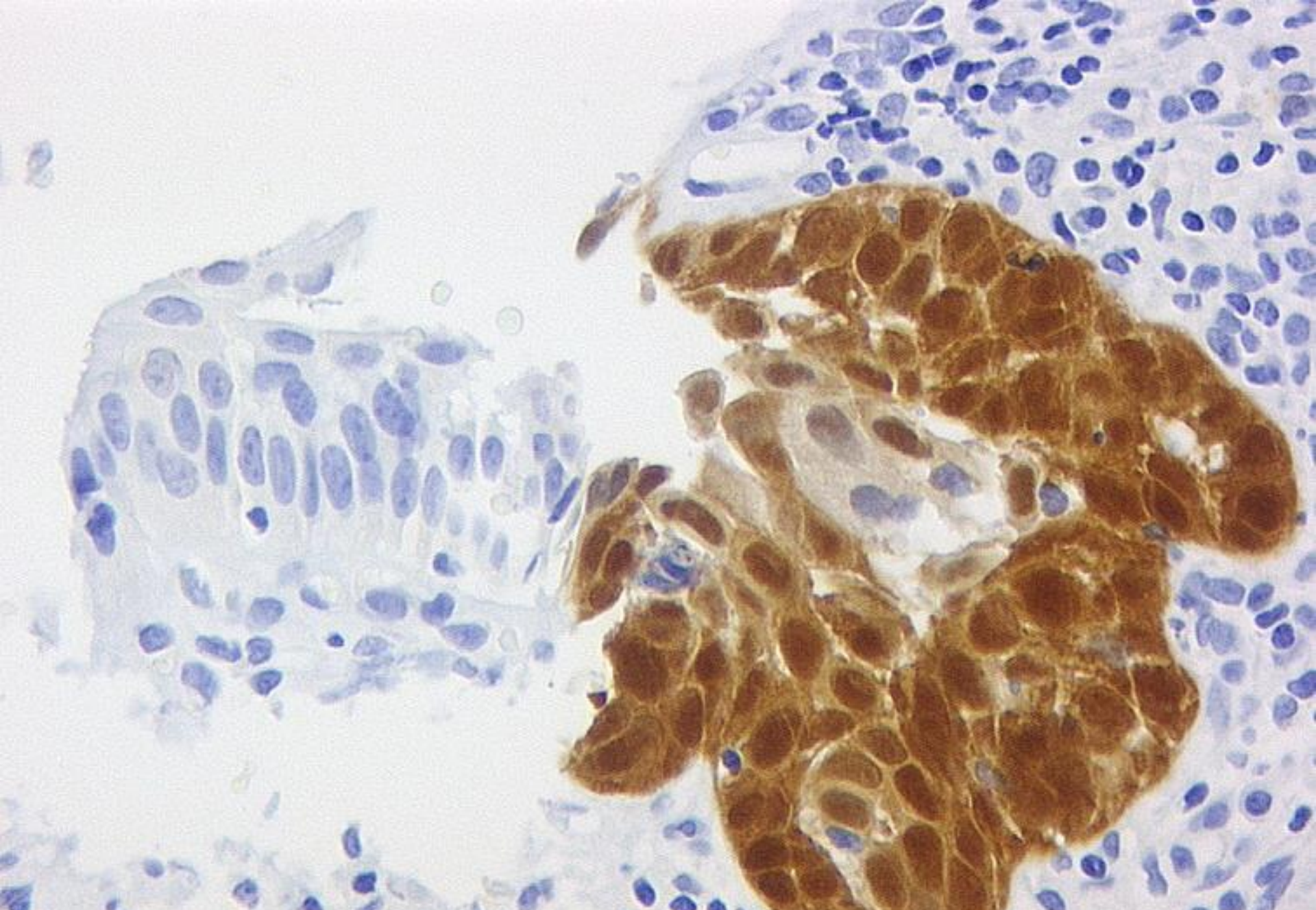
**Small CIN lesion; H&E stain**



**Small CIN lesion; Diffuse p16 stain**



**Small CIN lesion (Further magnified); H&E stain**



**Small CIN lesion; Diffuse p16 stain**

# **Conjunctive p16<sup>INK4a</sup> Testing Significantly Increases Accuracy in Diagnosing High-Grade Cervical Intraepithelial Neoplasia**

*Bergeron et al.*

*American Journal of Clinical Pathology*

*2010; 133:395-406*

# Study objectives

- Evaluate the impact of adding p16 to H&E conjunctively in diagnosing high-grade disease in a community-based setting
  - Impact on diagnostic accuracy
  - Impact on level of agreement between community-based pathologists
  - Reliability of p16-staining pattern interpretation

# Study design

- “Gold Standard” diagnoses established
  - 500 H&E cases were independently read by 3 expert gynecopathologists
    - Comparable number of punch biopsies and cone biopsies
  - Discrepant results were subjected to adjudication
  - Majority consensus diagnoses were included as final Gold Standard

# Study design

- 12 community-based pathologists collectively performed 12,000 readings on Gold Standard slides
  - All 12 independently diagnosed all 500 H&E slides
  - Washout period of at least 4 weeks
  - All 12 established diagnoses on the same set of 500 H&E slides, conjunctively with the matched p16 stained slides
  - All 12 were blinded to case numbers, original diagnoses, and Gold Standard diagnoses
  - Complete data sets for 482 of these 500 cases were available and used in the analysis

## Gain in sensitivity for CIN2+:

*Adding p16-stained slides to H&E vs. expert consensus*

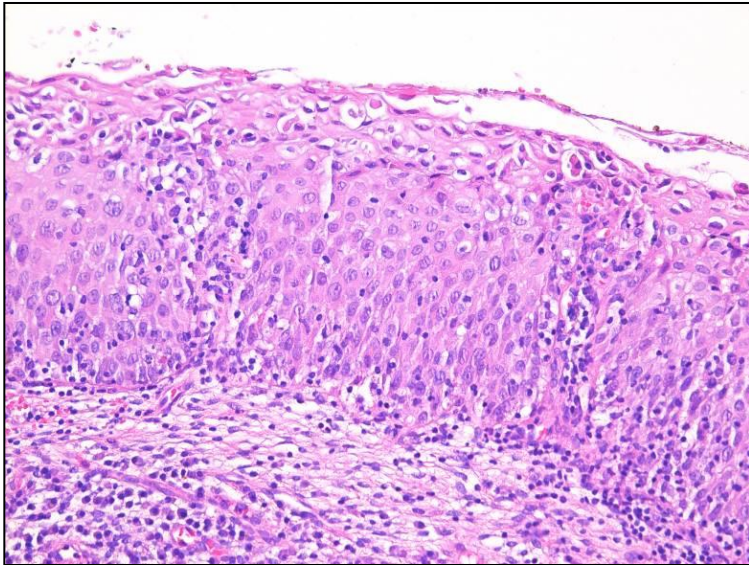
Diagnostic Category	Sensitivity HE	Sensitivity HE+p16	Improvement
CIN2+	77%	87%	13%
CIN2 Only	62%	75%	21%
CIN3 Only	86%	95%	11%

(p=0.0004)

- 30% of all cases were re-classified after reviewing the H&E plus p16 stained slides
- Reduced the rate of false-negative almost in half

# Morphology Case Example

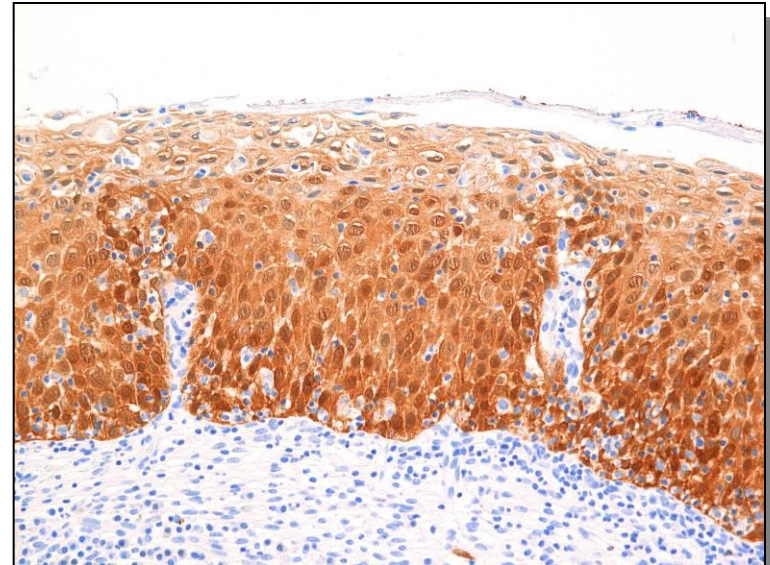
**Goldstandard: CIN2**



**Panel H&E only:**

**8  $\leq$  CIN 1**

**4 CIN 2+**

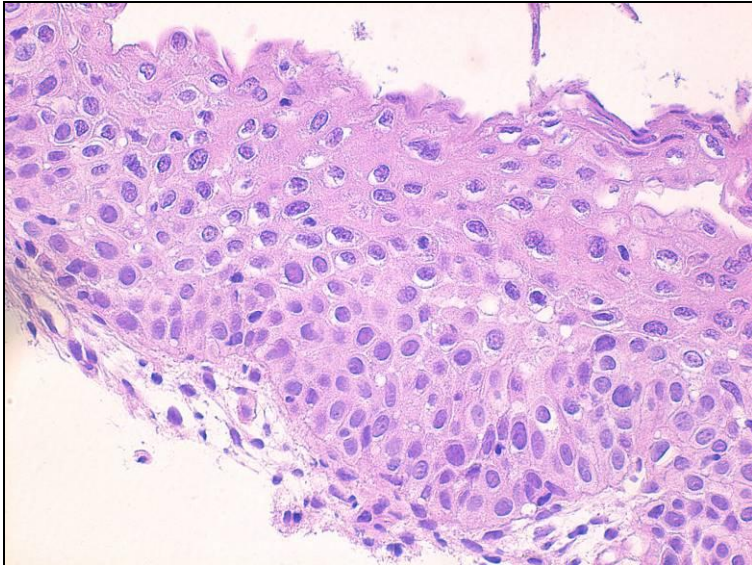


**Panel H&E+ p16:**

**12 CIN 2+**

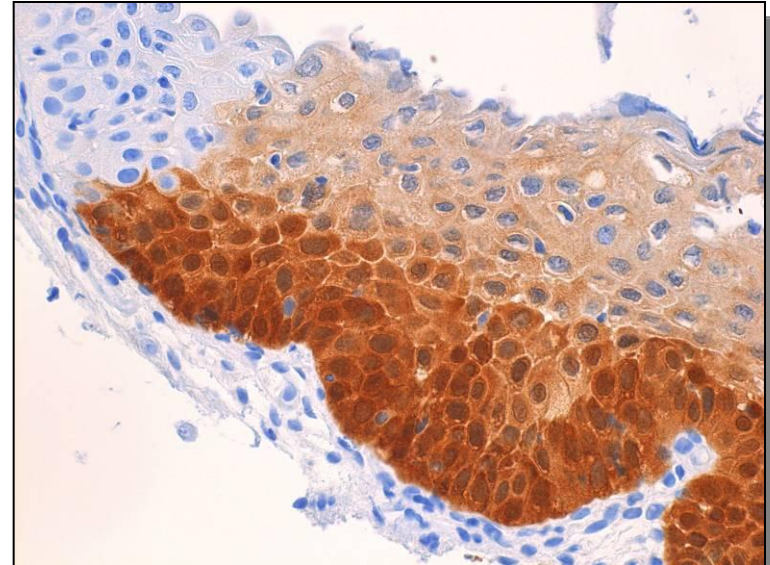
# Morphology Case Example

**Goldstandard: CIN2**



**Panel H&E only:**

**5 CIN 1  
7 CIN 2+**

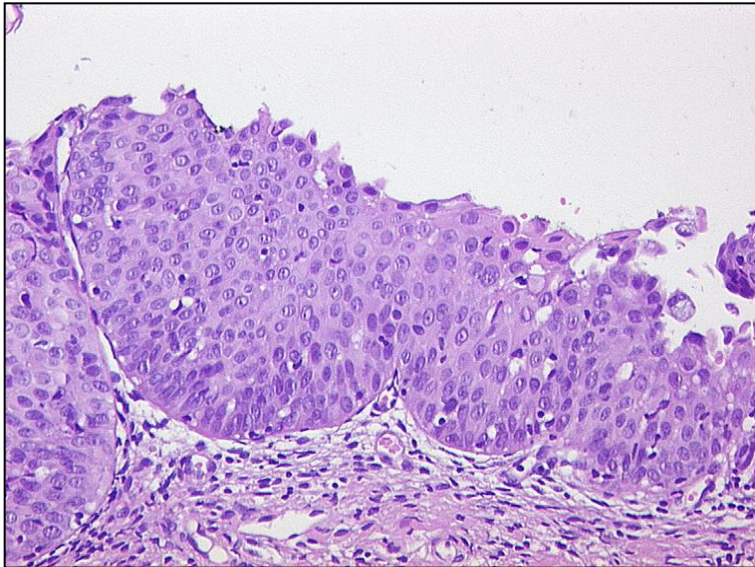


**Panel H&E+ p16:**

**12 CIN 2+**

# Morphology Case Example

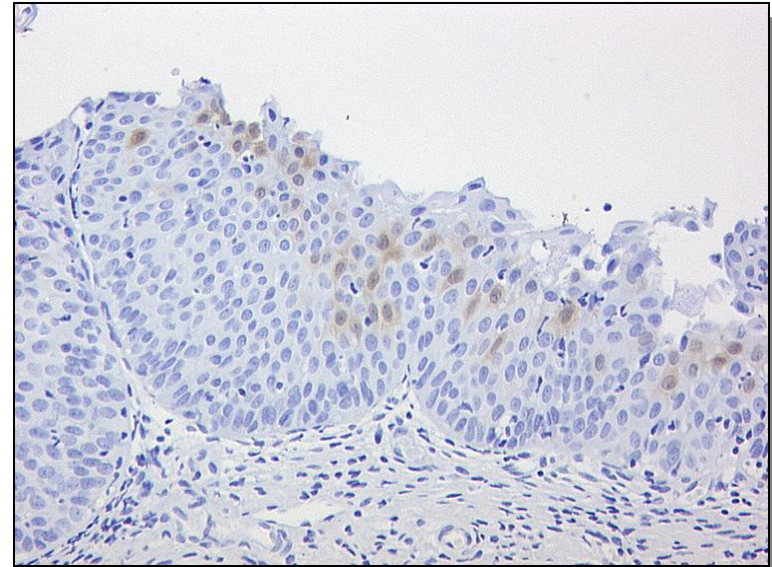
**Goldstandard: Negative for CIN**



**Panel H&E only:**

**10  $\leq$  CIN 1**

**2 CIN 2**



**Panel H&E+ p16:**

**12  $\leq$  CIN 1**

# Increased inter-observer agreement in diagnosing CIN2+

- A 30% overall increase in inter-observer agreement for diagnosing CIN2+
  - H&E only  $\kappa = 0.580$
  - H&E+p16  $\kappa = 0.756$
- Highly statistically significant ( $p < 0.0001$ )
- Same improvement seen for punch biopsies and cone specimens

# Reproducibility of p16 immuno-staining interpretation

- High level of reproducibility for rating a p16 immuno-staining pattern:
  - Mean weighted  $\kappa$  value for rating p16 pattern as diffuse, focal or negative = 0.803
  - Mean weighted  $\kappa$  value for rating p16 pattern as either positive (diffuse) or negative (focal/neg) = 0.899
  - Conclusion on reproducibility of rating:
    - Almost 100% consistent in this study

# Overall Findings

- Statistically significant increase in Diagnostic Accuracy for diagnosing CIN2+ ( $p=0.0004$ )
  - 95% Detection of CIN3
  - Overall 13% Increased Detection of CIN2+
  - Reduced the rate of false-negative results almost in half
- Statistically significant increase in Inter-Observer Agreement for diagnosing CIN2+ ( $p<0.0001$ )
- High reliability of p16 Histology stain interpretation
  - Reproducibility of p16 stain interpretation was excellent

# Conclusions

- The study has demonstrated that the conjunctive use of p16 stained cervical tissue slides:
  - Significantly increases the diagnostic accuracy for diagnosing CIN2+
  - Significantly increases the Inter-observer agreement for diagnosing CIN2+
- Agreement in rating p16 patterns is very high
- Conjunctive interpretation of p16<sup>INK4a</sup>-stained slides could significantly improve the routine interpretation of cervical histopathology

# p16 Histology data summary

## *High Sensitivity for CIN2+*

### Studies demonstrate strong data for adjunctive use of CINtec® Histology

Bergeron	<ul style="list-style-type: none"> <li>500 cervical biopsies (Total of 12,000 interpretations - 12 community-based pathologists)</li> <li>Multinational, multicenter, retrospective diagnostic study</li> <li>Number of high-grade lesions that were missed by H&amp;E alone was reduced by 45%</li> </ul>
Galgano	<ul style="list-style-type: none"> <li>1455 cervical biopsies</li> <li>US-based retrospective study</li> <li>Comparative analysis of p16 IHC alone vs other markers (Ki-67, HPV L1) vs. in combination</li> </ul>
mtm US clinical trial	<ul style="list-style-type: none"> <li>1695 cervical biopsies (Total of 62-community-based pathologists)</li> <li>US-based retrospective study</li> <li>Statistically significant improvement in CIN2+ diagnostic sensitivity without loss to specificity</li> </ul>

CINtec® Histology	Sens of H&E alone	Sens of H&E + CINtec®
Bergeron	77%	87%
Galgano	69%	87%*
mtm registrational trial	86%	91%

\*interpretation using CINtec p16 Histology staining alone

# p16<sup>INK4a</sup> as a Biomarker for CIN 2,3

## *2012 CAP / LAST guidelines*

- p16 IHC **is recommended** when the differential diagnosis is between CIN 2,3 and a "mimic" such as atrophy, immature metaplasia, reparative change, and tangential cutting
- Whenever a pathologist is considering a diagnosis of CIN2, p16 IHC **is recommended**
- p16 IHC **is recommended** whenever there is a professional disagreement as to CIN 2,3

## **p16<sup>INK4a</sup> as a Biomarker for CIN 2,3**

### *2012 CAP / LAST guidelines*

- p16 IHC is **recommended** as an adjunct to morphological assessment for biopsy specimens diagnosed as  $\leq$ CIN 1 in women with a prior cytological diagnosis of HSIL, ASC-H, AGC(NOS), or ASC-US / HPV 16 (+).

# p16 IHC: Detection of Occult CIN Lesions

*Int J Gyn Pathol 2009; 28:90-97*

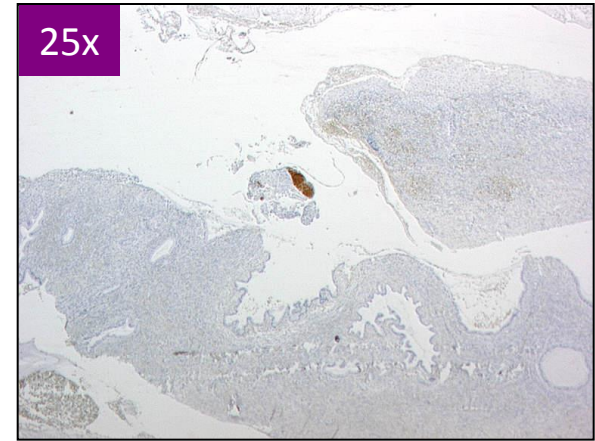
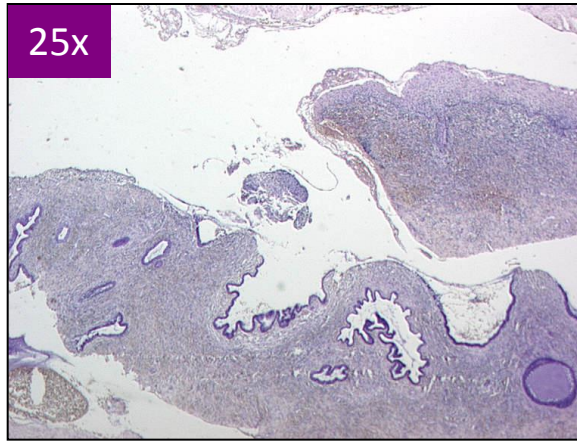
Original Article

## p16<sup>INK4a</sup> Immunostaining Identifies Occult CIN Lesions in HPV-positive Women

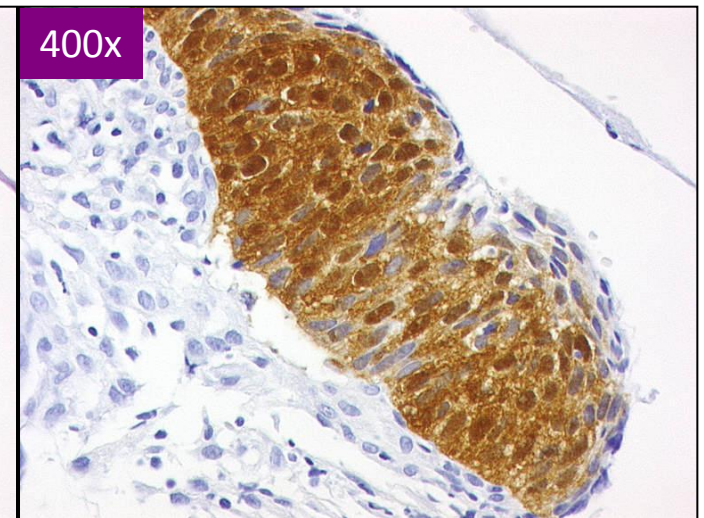
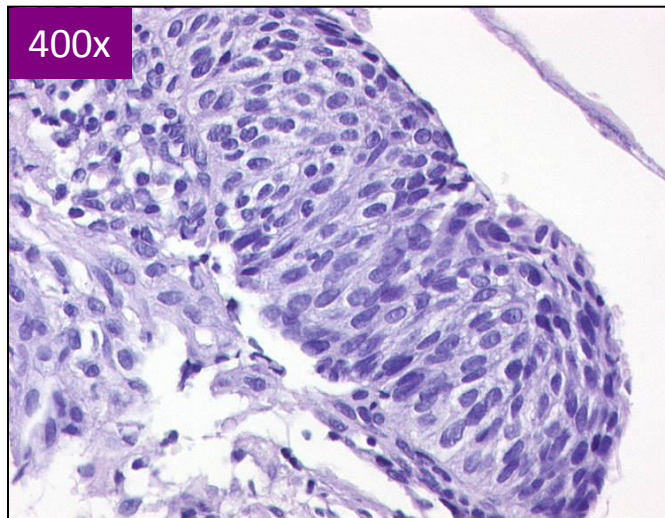
Jaume Ordi, M.D., Sònia Garcia, M.D., Marta del Pino, M.D., Stefania Landolfi, M.D.,  
Immaculada Alonso, M.D., Llorenç Quintó, M.D., and Aureli Torné, M.D.

# Increased Reproducibility in Diagnosing Cervical Lesions by p16<sup>INK4a</sup> IHC

Consensus  
diagnosis:  
no lesion



High-grade lesion  
detected by  
p16<sup>INK4a</sup> IHC



# Study Results: Final Diagnosis after Re-evaluation in the Group positive for HR-HPV, negative initial Biopsy

TABLE 3. p16<sup>INK4a</sup> immunostaining categories, final diagnosis after slide review in the group of cases for HR-HPV but with negative initial biopsy

p16 <sup>INK4a</sup>	n	Biopsy final evaluation			p
		No lesion (n=107)	CIN1 (n=13)	CIN2/3 (n=19)	
Negative	105	103 (98%)	2 (2%)	0 (0%)	<0.001
Focal	10	4 (40%)	6 (60%)	0 (0%)	<0.001
Diffuse	24	0 (0%)	5 (21%)	19 (79%)	<0.001

- If there was no diffuse p16 staining, **NPV** for having no CIN 2/3 was found to be **100%**
- If there was diffuse p16 staining, **PPV** for having a CIN 2/3 was found to be **79%**

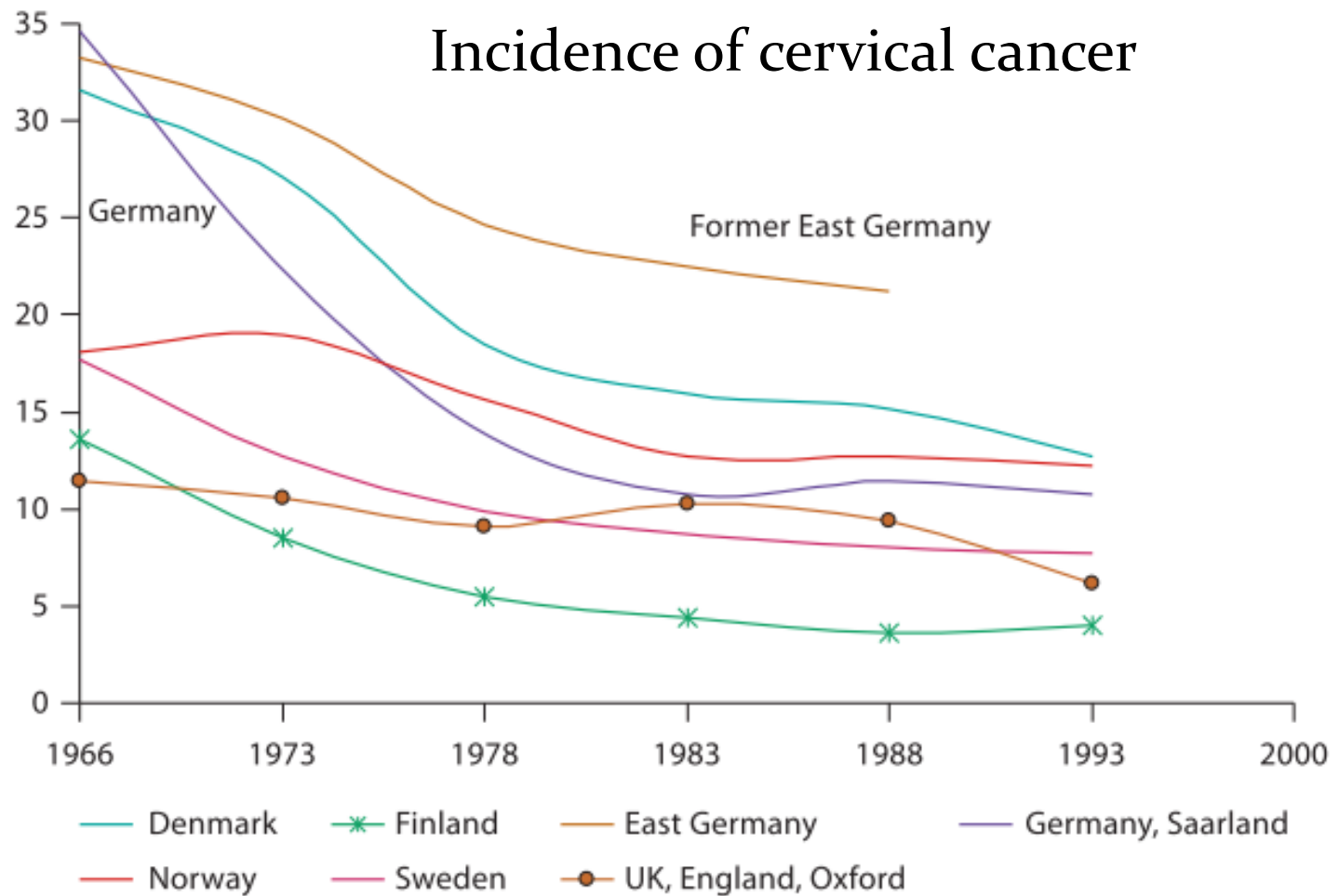
# Clinical utility of p16

## *Summary*

### ▪ Histology

- Significant increase in overall accuracy diagnosing CIN2+/CIN3+ lesions
  - Detection of occult lesions
  - Significant increase in inter-observer agreement
  - Prognostic utility in CIN1
  - Recommendation is for routine use with H&E
- 
- Bergeron et al. American Journal of Clinical Pathology, 2010
  - Galgano et al. American Journal of Surgical Pathology, 2010
  - Dijkstra et al. Journal of Clinical Pathology, 2010
  - Ordi et al. International Journal of Gynecological Pathology, 2009
  - Del Pino et al. American Journal of Obstetrics & Gynecology, 2009

## Incidence of cervical cancer



Marquardt et al., 2011

# Cervical cancer screening

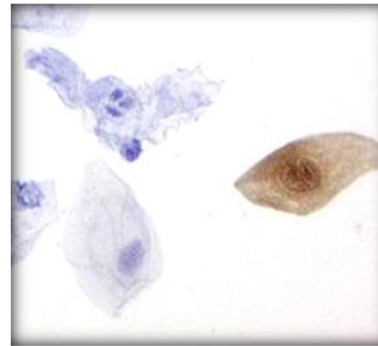
## *Opportunities to improve patient management*

Primary screening	<ul style="list-style-type: none"><li>• Cytology has low sensitivity (50-70%) and poor interobserver reproducibility</li><li>• HPV has high false positive rate with women &lt;30</li></ul>
ASC-US triage	<ul style="list-style-type: none"><li>• Current algorithms vary – repeat cytology / colposcopy / HPV</li><li>• Repeat cytology increases patient anxiety</li><li>• Colposcopy is invasive and has false positives</li></ul>
LSIL triage	<ul style="list-style-type: none"><li>• Current standard of care is colposcopy, which is invasive and expensive</li><li>• No standard triage test exists</li></ul>
Triage of HPV+	<ul style="list-style-type: none"><li>• Opportunity to decrease unnecessary colposcopies</li><li>• No standard triage test exists</li></ul>
Adjunct (Pap - / HPV +)	<ul style="list-style-type: none"><li>• Current standard of care is repeat cytology</li><li>• No standard triage test exists</li></ul>

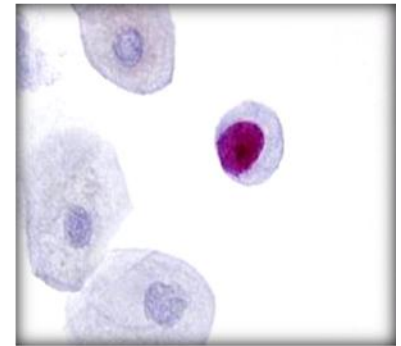
## CINtec *PLUS* Cytology

p16/Ki-67 cocktail & dual color detection packaged together

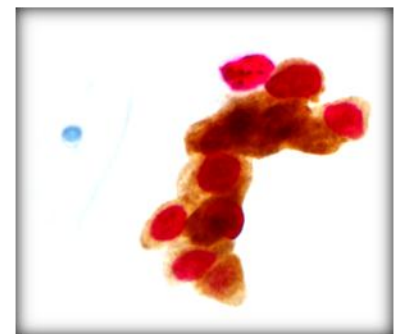
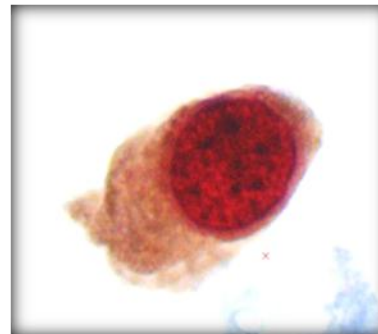
- Detection of the co-localization of p16 and Ki-67
- Indicator of presence of cell cycle deregulation, independent of morphology
- Expression of anti-proliferative p16 protein and proliferation marker Ki-67 should be mutually exclusive



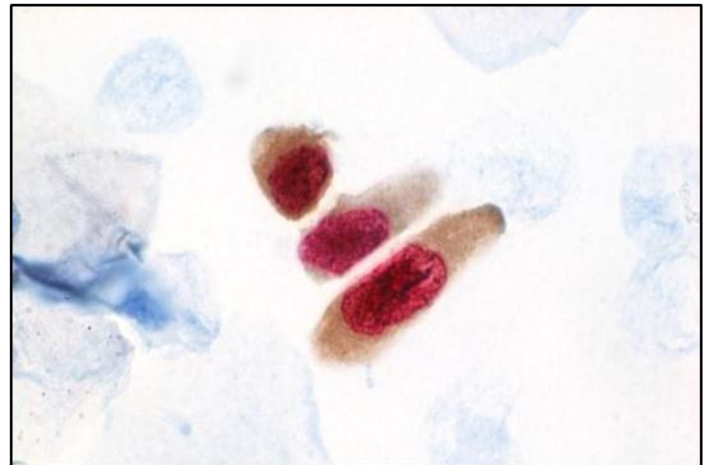
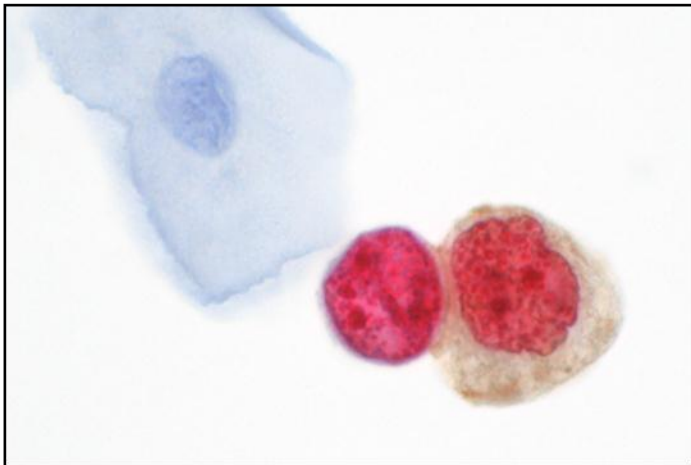
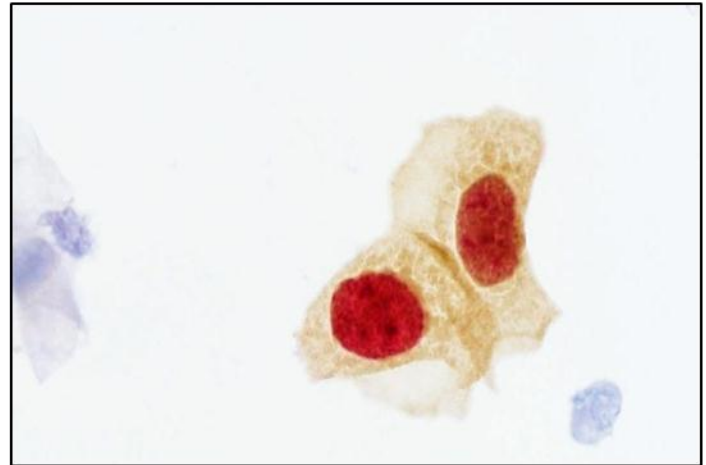
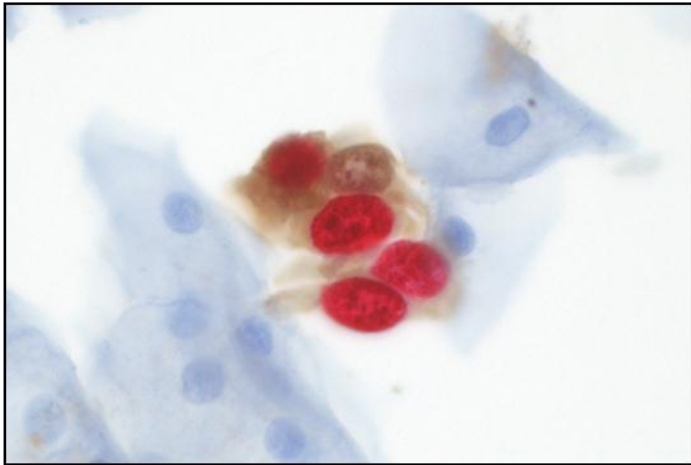
Single p16



Single Ki-67



Dual stained cells



# CINtec *PLUS* Cytology data summary

*High Sensitivity PLUS High Specificity for CIN2+*

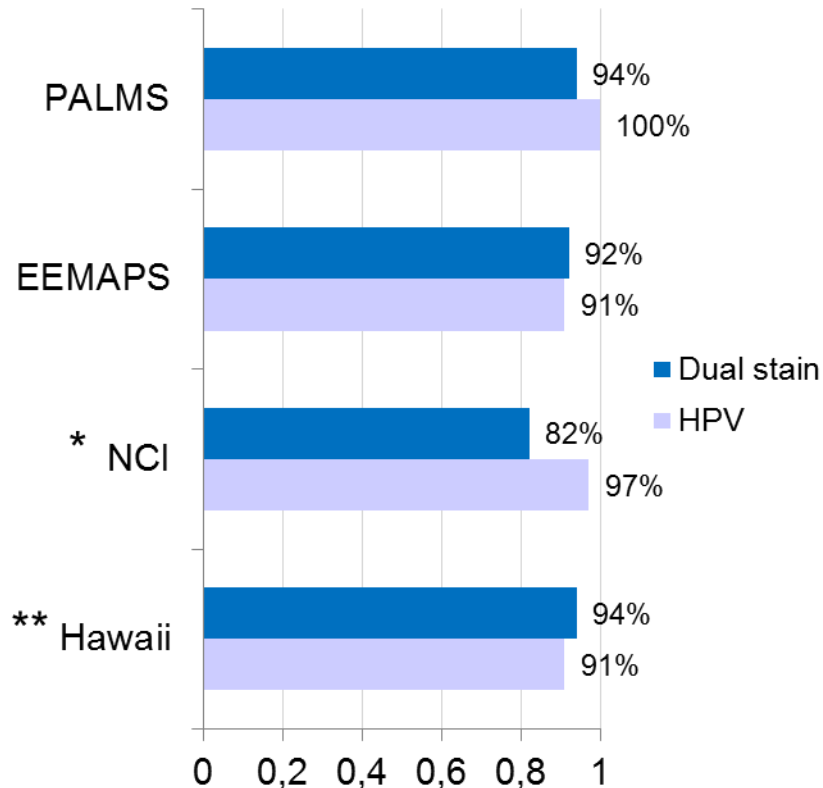
## Pan-European trials demonstrate strong clinical data

PALMS	<ul style="list-style-type: none"> <li>• Primary screening, ASC-US, LSIL Marker Study</li> <li>• 27,000 women</li> <li>• Multinational, multicenter, prospective diagnostic study</li> </ul>
EEMAPS	<ul style="list-style-type: none"> <li>• European Equivocal or Mildly Abnormal Pap Cytology Study</li> <li>• Over 200 cases of biopsy confirmed high-grade disease from a total of 777 cases of ASC-US and LSIL</li> </ul>
Wolfburg	<ul style="list-style-type: none"> <li>• The Wolfsburg Pap/HPV Co-Testing Study</li> <li>• Over 4,400 women aged 30 and older</li> </ul>

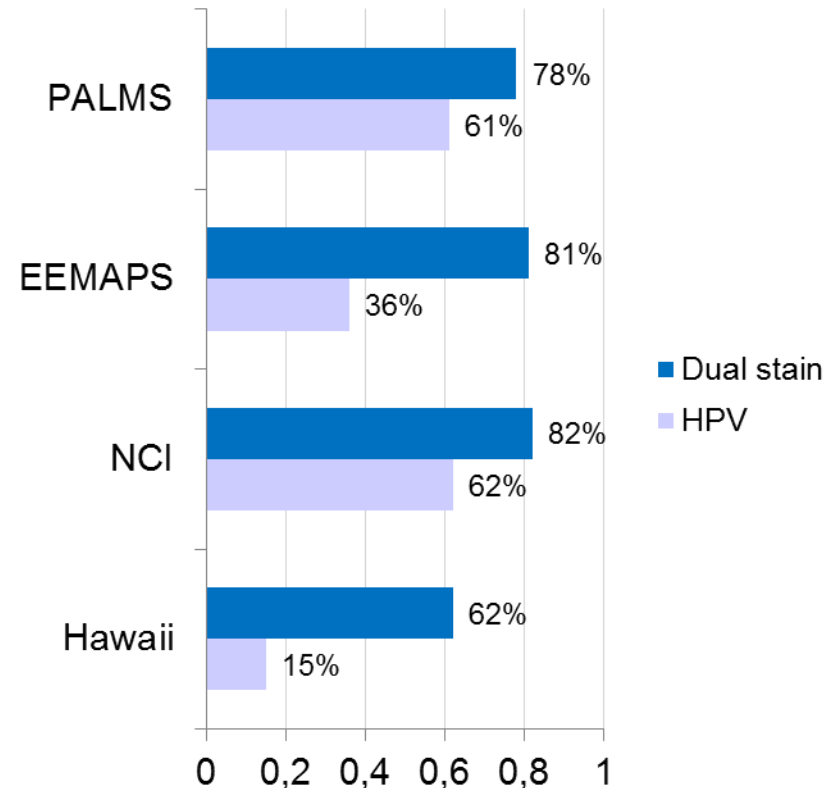
CINtec <i>PLUS</i> Cytology	Sensitivity	Specificity
Screening	90 - 93%	95 - 97.5%
LSIL triage	85 - 94%	54 - 68%
ASCUS triage	92 - 94%	78 - 81%
Pap negative / HR-HPV positive triage	92%	85%

# Summary: Clinical validation data, ASC-US triage CINtec *PLUS* Cytology vs. HPV for $\geq$ CIN2

## Sensitivity



## Specificity

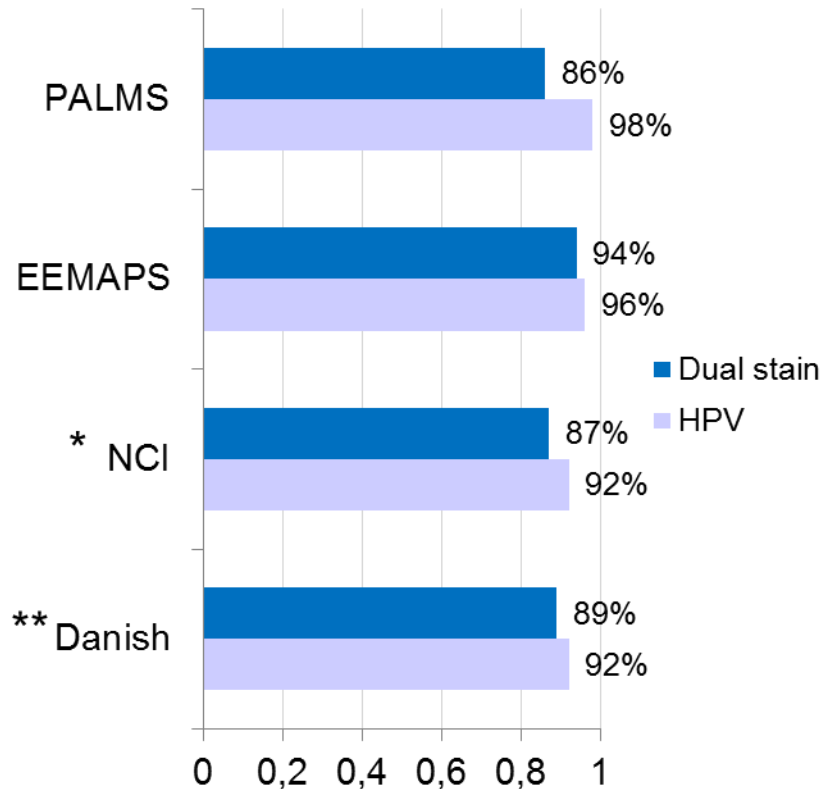


\* Triage of HPV(+) ASC-US, Wentzensen et al. Clinical Cancer Research 2012

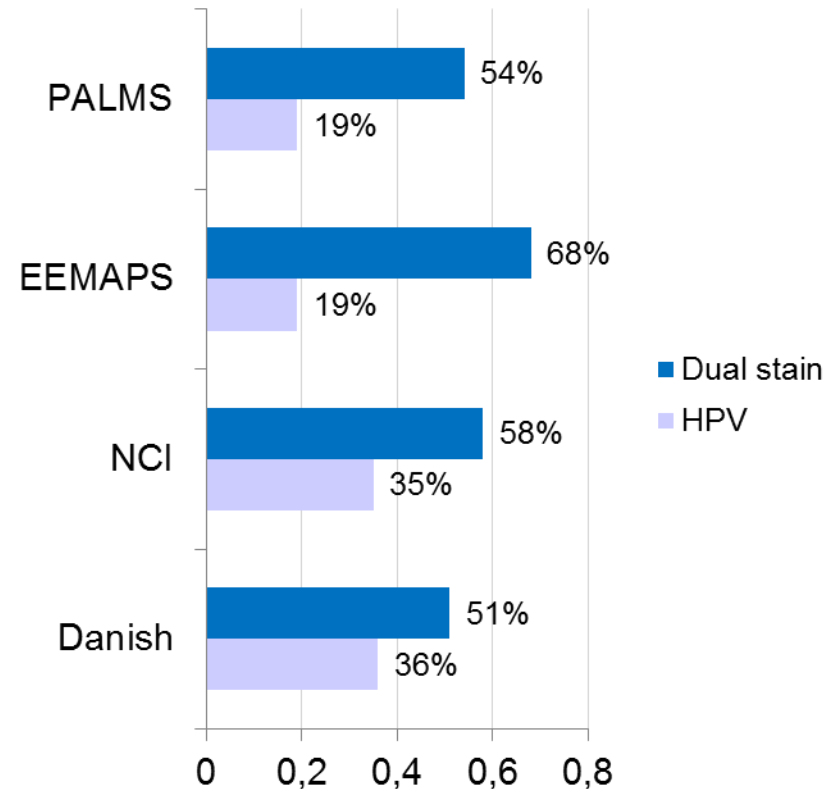
\*\*Triage of  $\geq$ ASC-US, Killen, et al; Journal of Lower Genital Tract Disease, 2013

# Summary: Clinical validation data, LSIL triage CINtec PLUS Cytology vs. HPV for $\geq$ CIN2

## Sensitivity



## Specificity

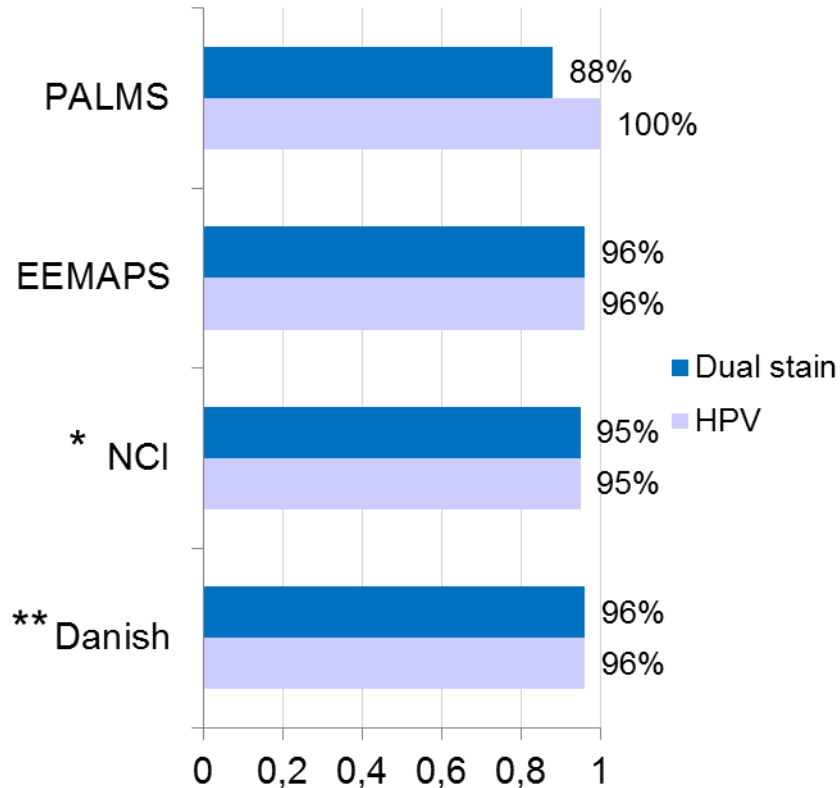


\* Wentzensen et al. Clinical Cancer Research 2012

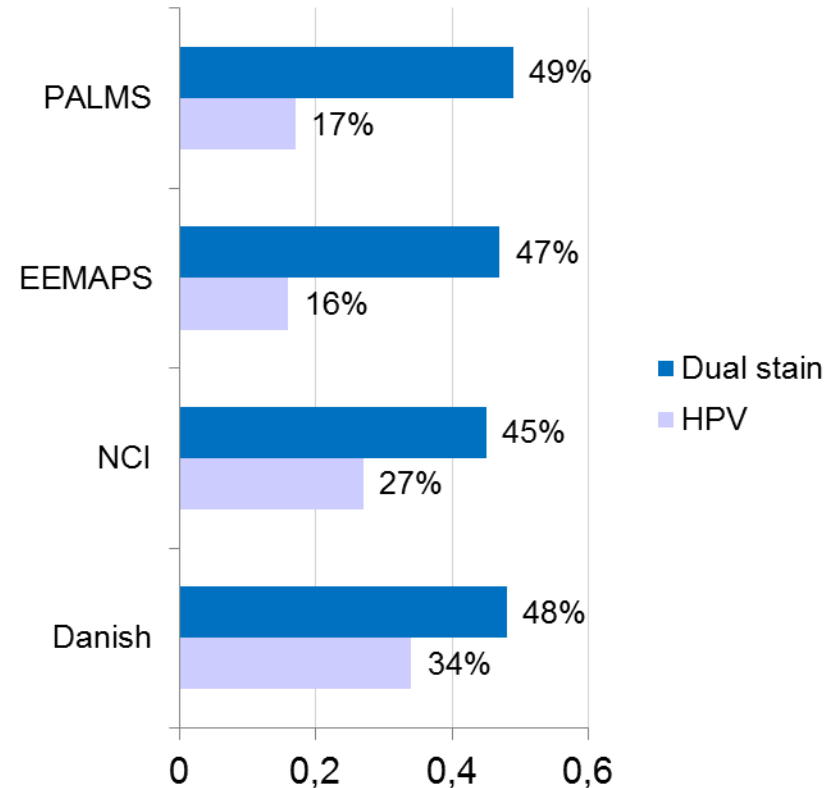
\*\* Waldstrom et al; Cancer Cytopathology 2012

# Summary: Clinical validation data, LSIL triage CINtec *PLUS* Cytology vs. HPV for $\geq$ CIN3

## Sensitivity



## Specificity



\* Wentzensen et al. Clinical Cancer Research 2012

\*\* Waldstrom et al; Cancer Cytopathology 2012

**Clinical validation data, NILM/HPV(+) triage**  
**CINtec *PLUS* Cytology performance, women**  
 **$\geq 30$**

	<b>Petry et al. 2011</b>	<b>PALMS Sub-analysis</b>
Dual stain positivity rate	108/425 (25%)	225/1,023 (22%)
Sensitivity for CIN2+	92%	82%
Specificity for CIN2+	82%	79%

RESEARCH ARTICLE

Open Access

# p16<sup>INK4a</sup>/Ki-67 dual stain cytology for cervical cancer screening in Thika district, Kenya



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**Table 2** Agreement between VIA/VILI, HPV results and p16<sup>INK4a</sup>/Ki-67 cytology. Shown are absolute numbers of positive and negative samples for the three tests

		p16 <sup>INK4a</sup> /Ki-67		VIA/VILI	
		Negative	Positive	Negative	Positive
HPV DNA	Negative	373	4	365	12
	Positive	65	35	97	3
VIA/VILI	Negative	425	37		
	Positive	13	2		

## Summary: Clinical validation data

CINtec *PLUS* Cytology in triaging ASC-US/LSIL, NILM/HPV(+) and screening

- Similar to somewhat lower sensitivity for CIN2+ as compared to HPV testing in ASC-US & LSIL triage
  - Comparable sensitivity at CIN3+ threshold
  - NCI study; all HPV16 associated HG CIN identified in colposcopy referral population
- Specificity significantly higher in triaging ASC-US and LSIL
- High sensitivity & high specificity in triaging NILM/HPV(+) cases
  - Majority of disease within the group of 20-25% of NILM/HPV(+) women that is Dual stain(+)
- Opportunity for screening, especially in younger women
  - Significant higher sensitivity than Pap, with specificity unchanged



**Thank you for your  
attention**

