p53 and p16 interpretation and quality control

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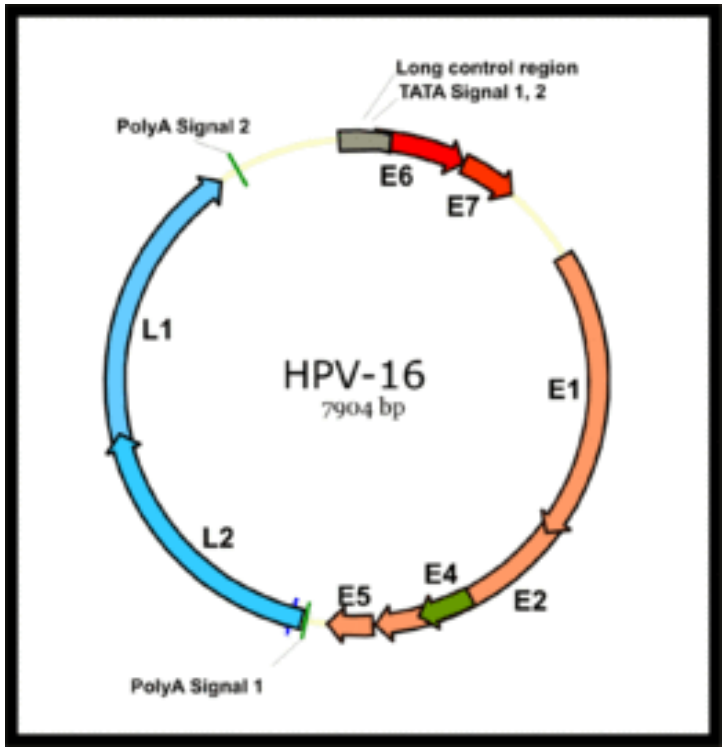
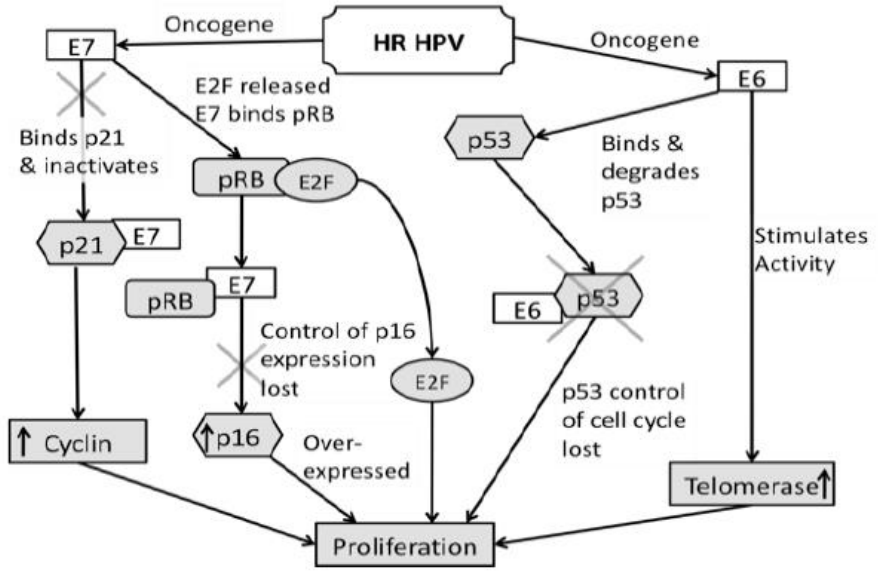
BELGIAN WORKING GROUP FOR GYNAECOLOGICAL PATHOLOGY

Persistent HR-HPV infection→increased E6 and E7



- HPV oncogenes E6 and E7 disrupts cell cycle



- IHC of expression patterns of p53, pRB, $p16^{INK}$, $p21^{WAF1}$, cyclin D1 and Ki67

courtesy Annika Antonsson

p16

- Major function in the normal cell is to inhibit CDK4 and CDK6, required to phosphorylate the retinoblastoma protein, pRb

→**CELL CYCLE BLOCKADE**: blocks inappropriate cell division

→Marker of **AGING**/cell **SENESCENCE**

- Role of p16 in cancer is COMPLEX:

• Classical role is to maintain state of cell cycle arrest: TUMOUR SUPPRESSOR

• Other roles: APOPTOSIS / INVASION/ANGIOGENESIS

• **INACTIVATED** in about 50% of all human cancers (variety of mechanisms)

• **OVEREXPRESSED** in some tumours (mechanism best understood in HPV)

Can be understood as attempt by the cell to inhibit uncontrolled proliferation.

Naveena Singh, BAGP 2018

Interpretation of p16 Immunohistochemistry In Lower Anogenital Tract Neoplasia

BRITISH ASSOCIATION OF GYNAECOLOGICAL PATHOLOGISTS

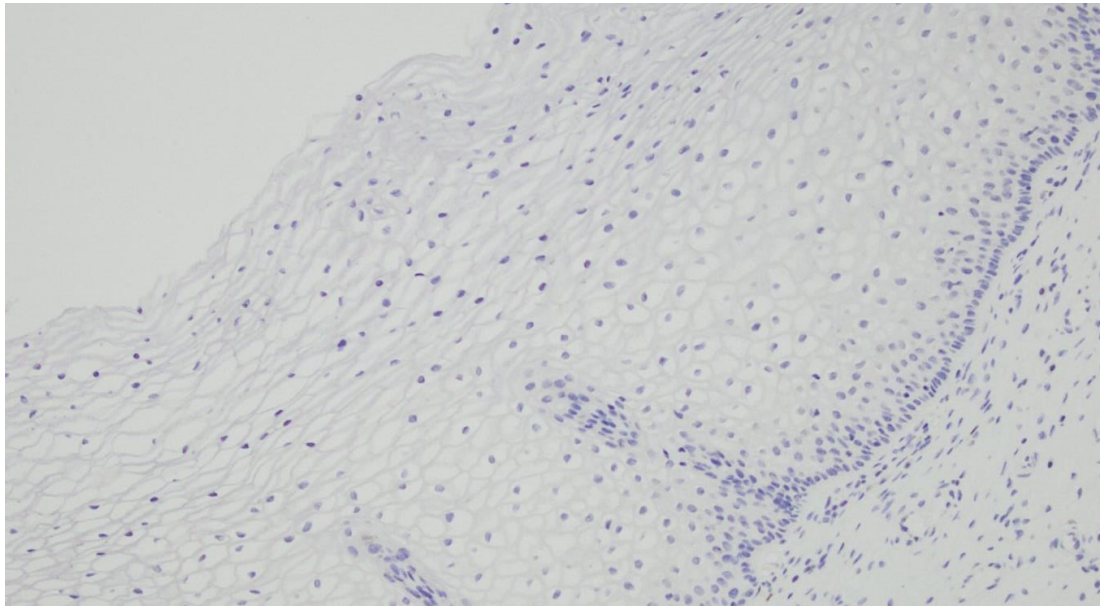
HOW TO INTERPRET P16 IMMUNOREACTIVITY SO THAT IT BECOMES A SURROGATE FOR HR-HPV INFECTION WITH THE POTENTIAL OF NEOPLASTIC TRANSFORMATION?

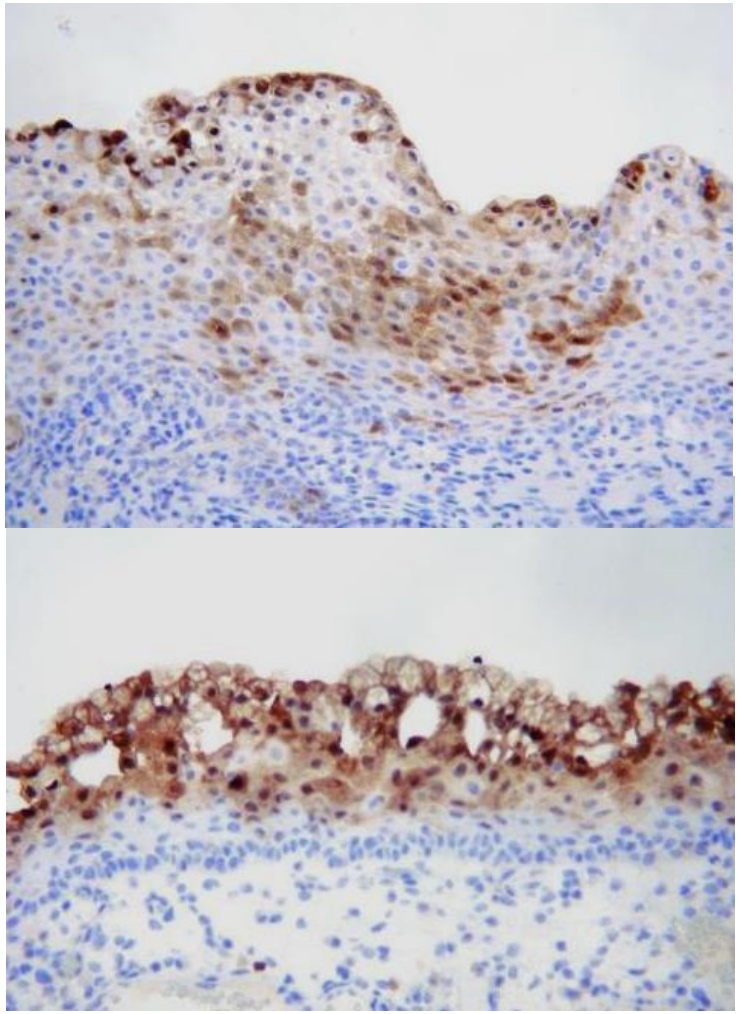
Lower Anogenital Squamous Terminology (LAST) consensus group put forward guidance for the use of p16. Darragh TM et al. Int J Gynecol Pathol 2013 Jan;32(1):76-115.

Naveena Singh, C Blake Gilks, Richard Wing-Cheuk Wong, W Glenn McCluggage, C Simon Herrington

BAGP Guidance document: p16 IHC reporting in anogenital neoplasia version 1.0, dated August 2018

p16: normal/reactive expression pattern in squamous epithelium

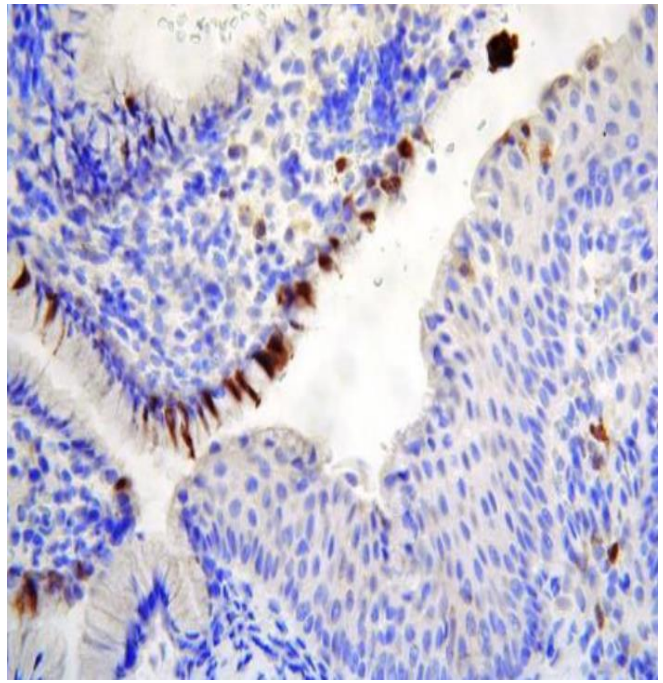


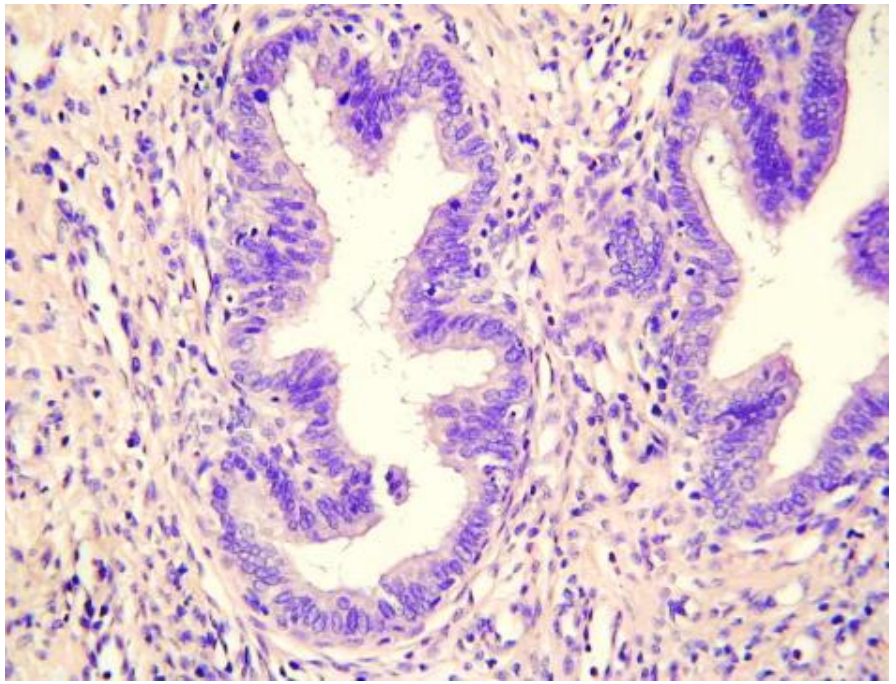


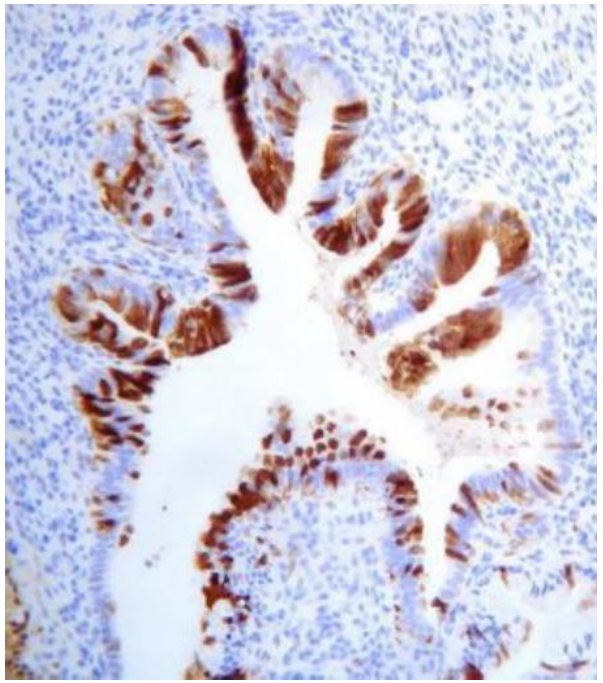
- Normal squamous epithelium generally shows completely absent expression.
- In immature metaplastic epithelium, occasional scattered, weakly staining cells may be seen. The p16 staining in the immature metaplastic squamous epithelium is typically patchy with sparing of the basal layer.

P16: normal/reactive expression pattern in glandular epithelium

- Normal endocervical epithelium usually shows completely negative or absent staining.
- In reactive epithelium occasional scattered positive cells may be observed.
- Tubo-endometrial metaplasia and lower uterine segment endometrial epithelium generally show patchy staining.



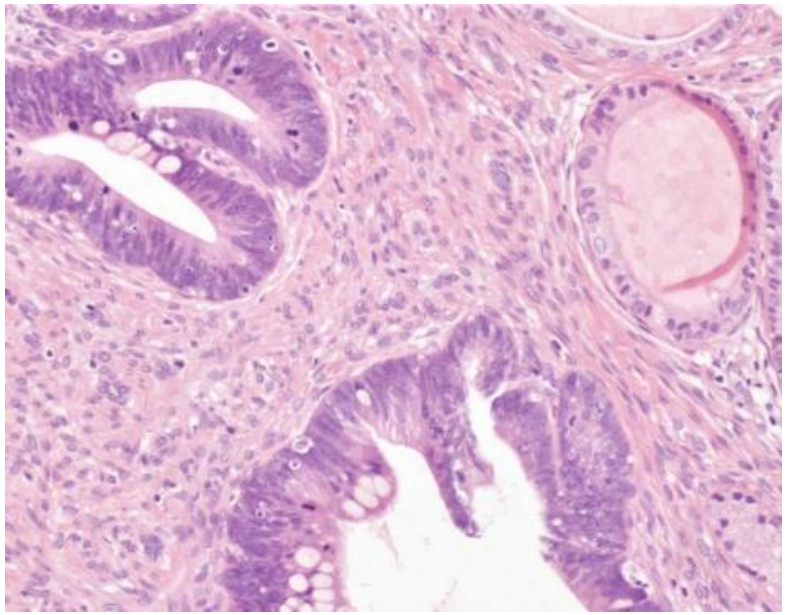


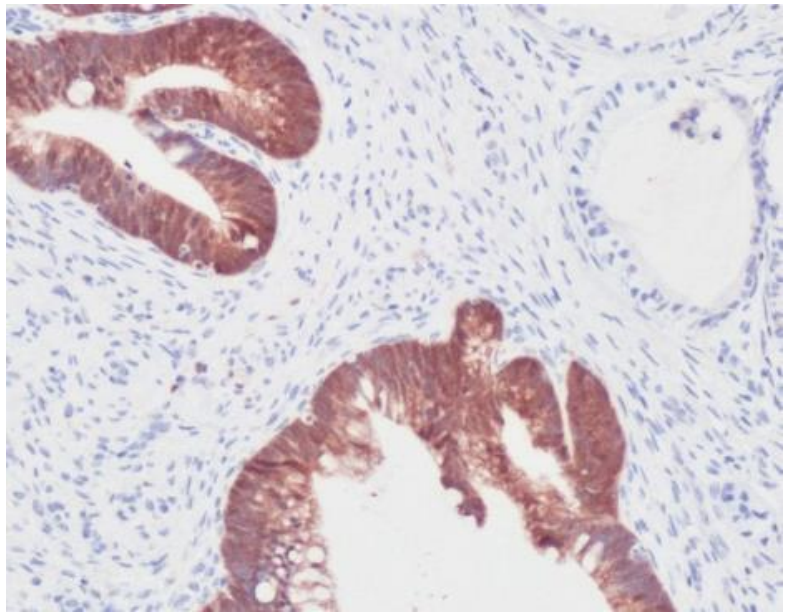


p16 abnormal expression pattern in glandular epithelium

- = strong and continuous DIFFUSE positive staining in glandular epithelial cells.
- Staining may be nuclear or more commonly nuclear and cytoplasmic.
- Do not use the term ‘block-type’ for glandular lesions as this term relates specifically to squamous lesions.
- Report as presence versus absence of abnormal diffuse positivity.

Endocervical adeno-carcinoma in situ AIS



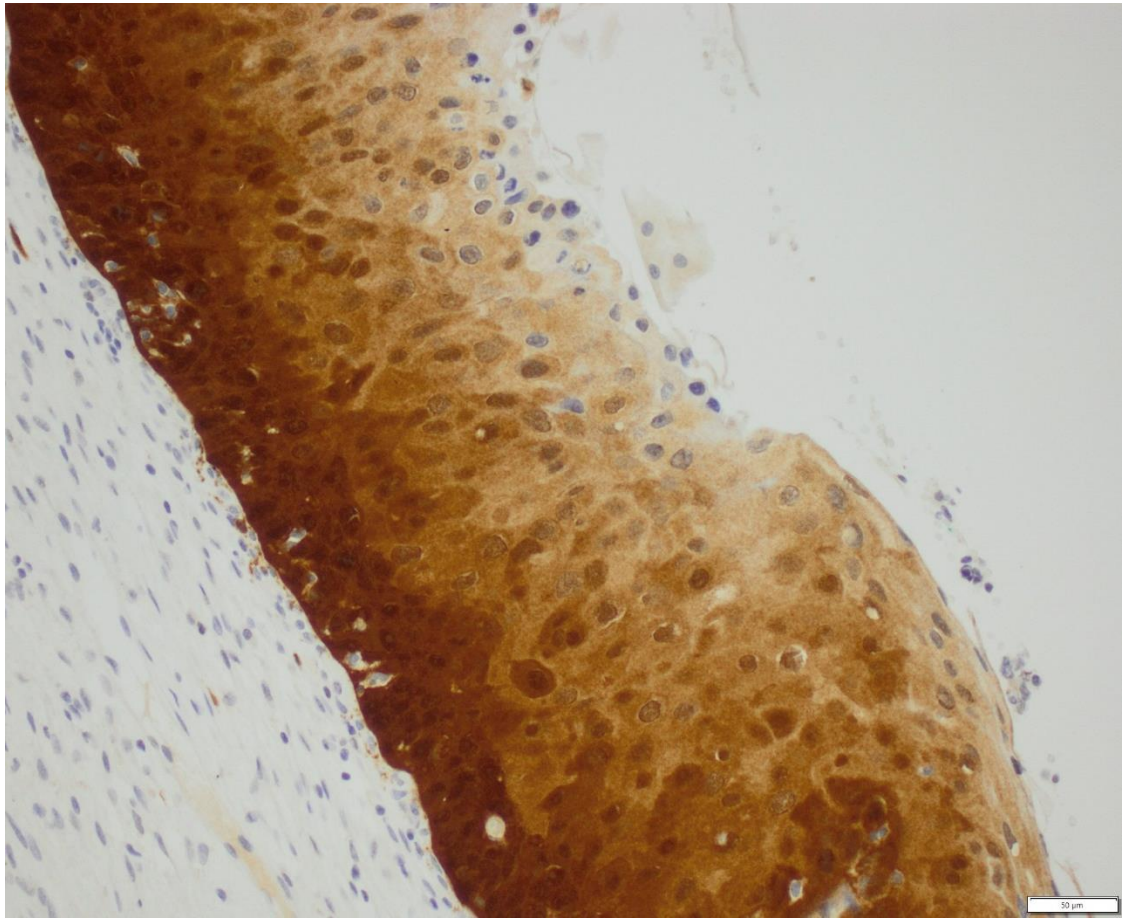


Always put immunostaining in the correct context and correlate with morphology.

Abnormal expression in squamous epithelium: BLOCK POSITIVE →correlates with HR-HPV infection with the potential of neoplastic transformation

- = Strong and continuous nuclear OR more typically nuclear and cytoplasmic expression in all epithelial cells of basal and parabasal layers with upward extension. Cytoplasmic staining only = normal.
- Upward extension must involve at least the lower one-third of the epithelial thickness.
- Abnormal expression must extend for at least 6 cells across.
- the criteria defining the horizontal and upward extent are arbitrary but serve to improve specificity

Abnormal expression in squamous epithelium: DIFFUSE BLOCK POSITIVE



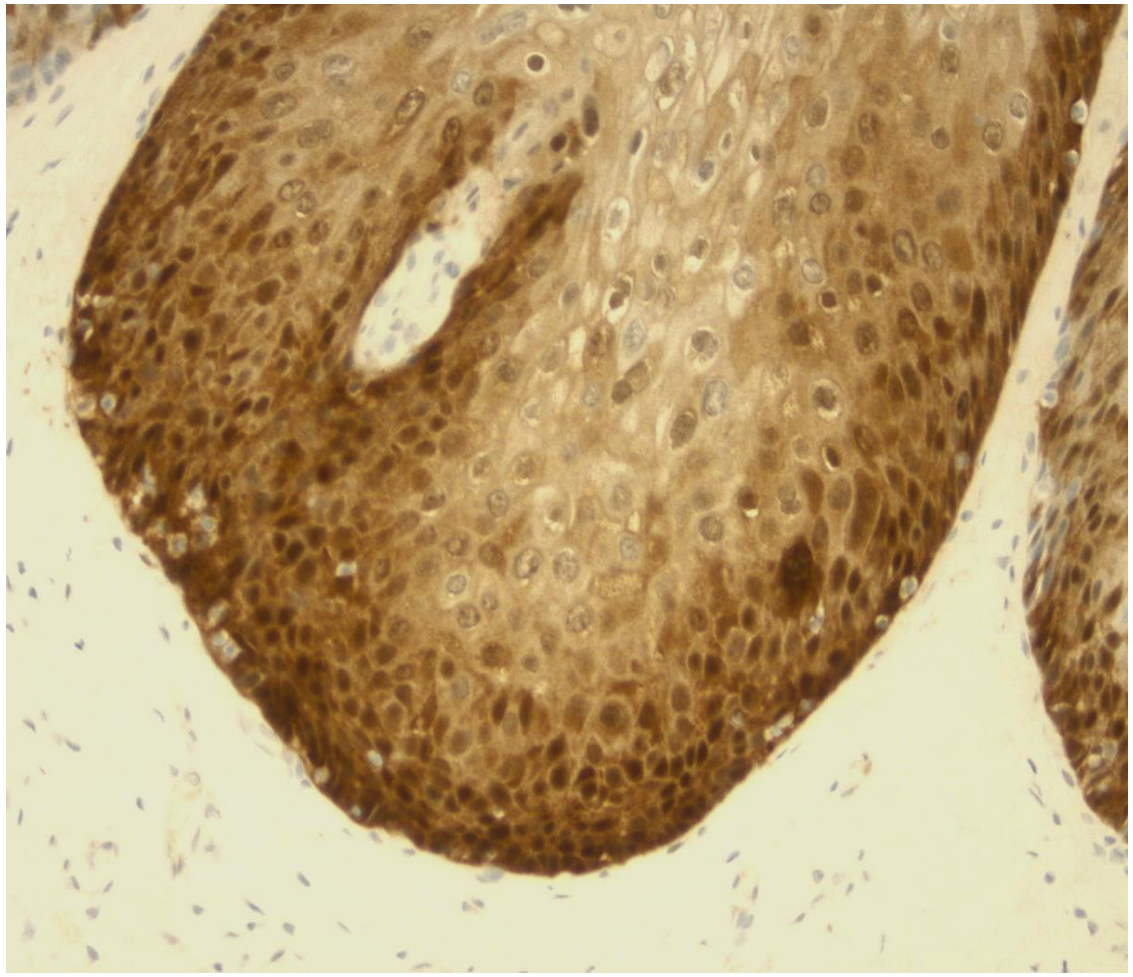
Use of the word ‘positive’ is not recommended in pathology reports.

ABNORMAL DIFFUSE BLOCK POSITIVE EXPRESSION

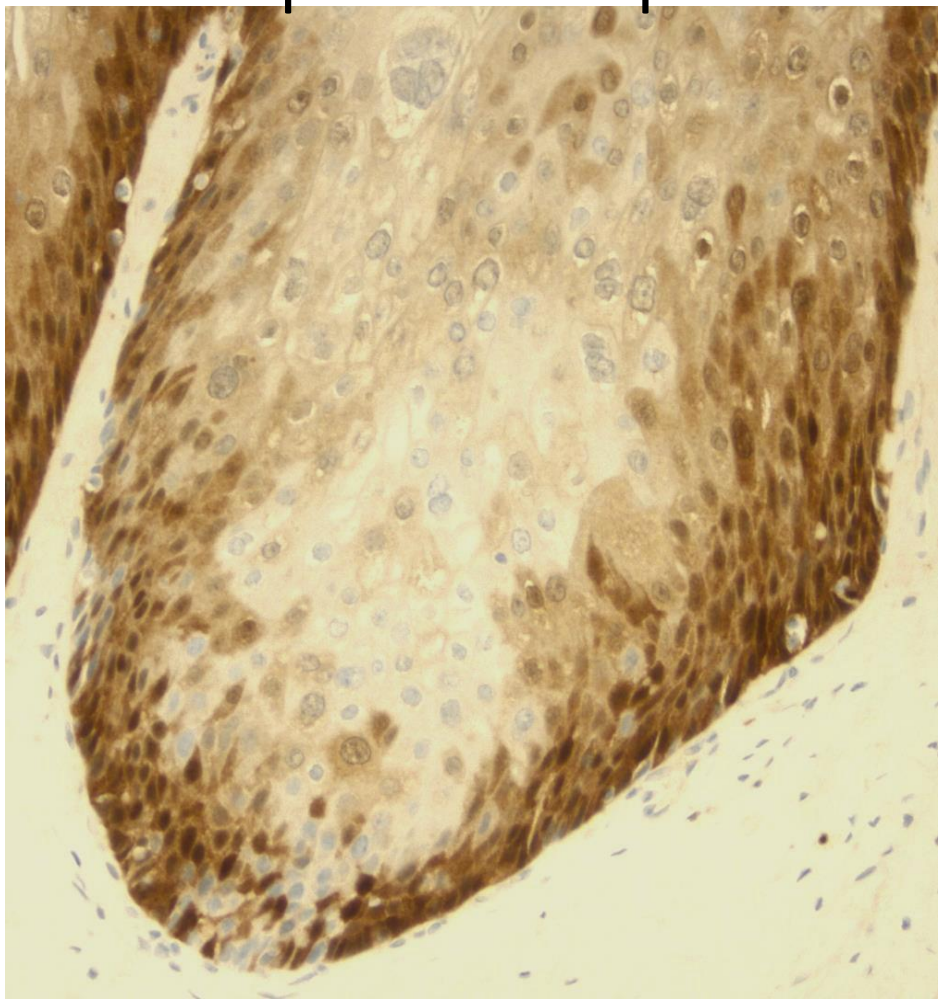
Versus

NEGATIVE/NORMAL/REACTIVE expression = ABSENCE of DIFFUSE BLOCK POSITIVITY

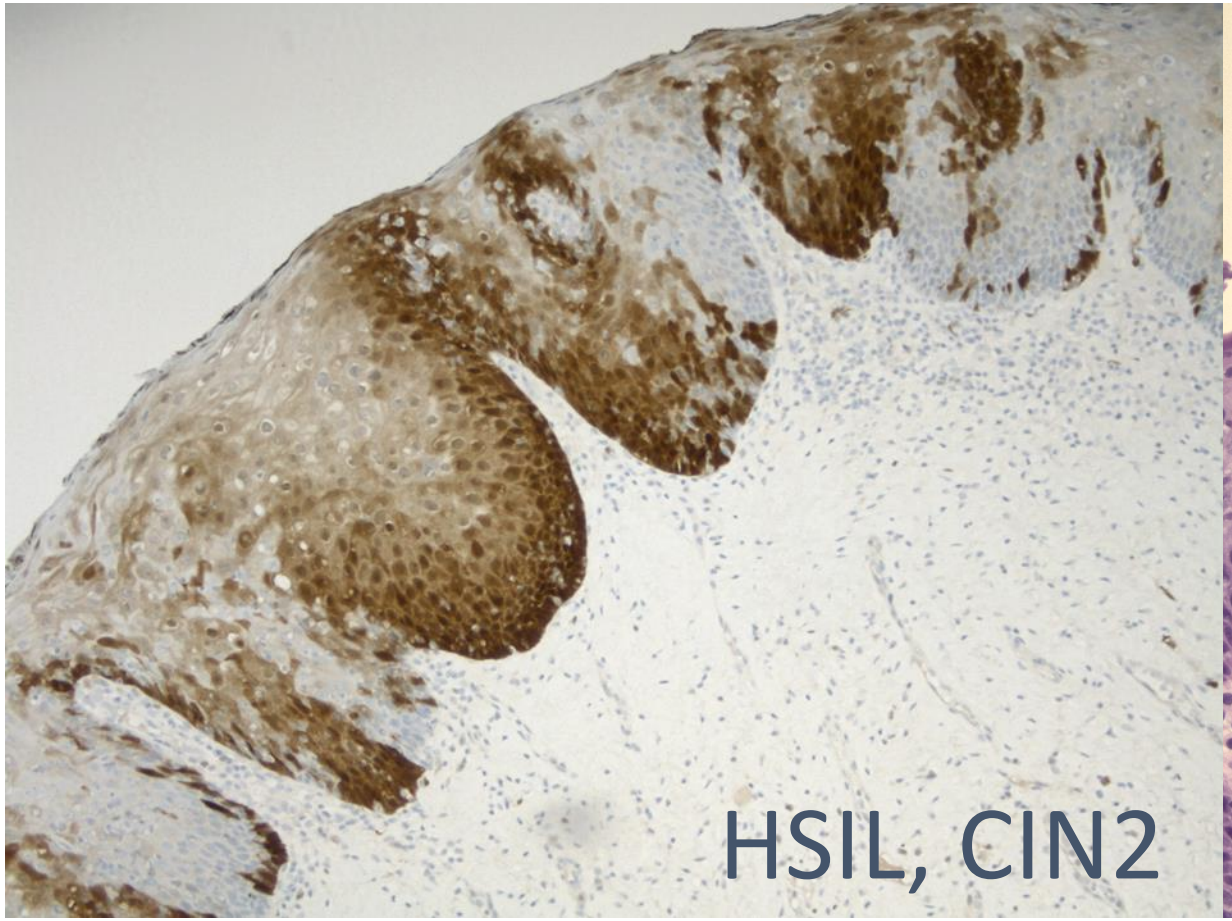
diffuse block positive p16

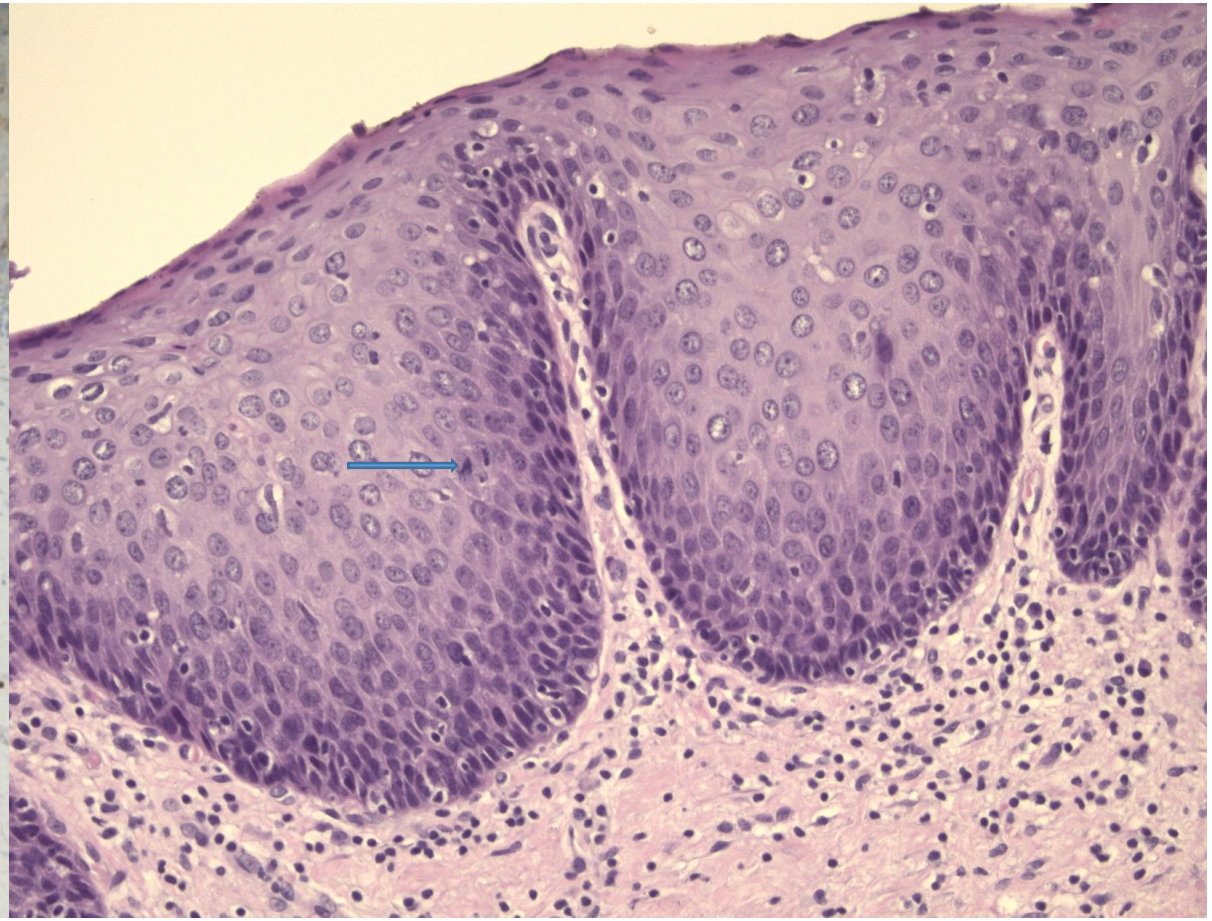


absence of diffuse block positive p16

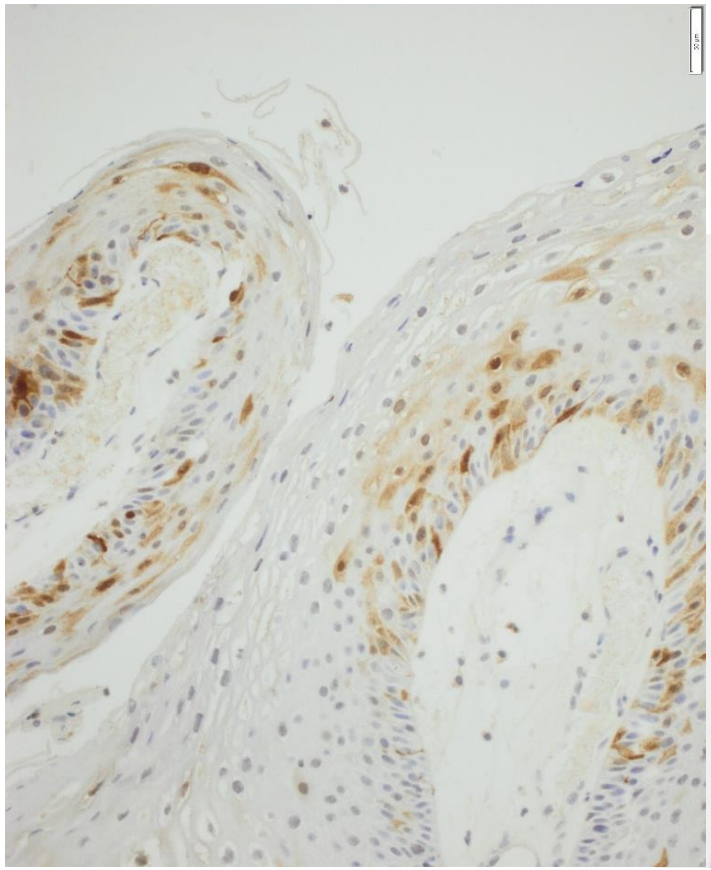


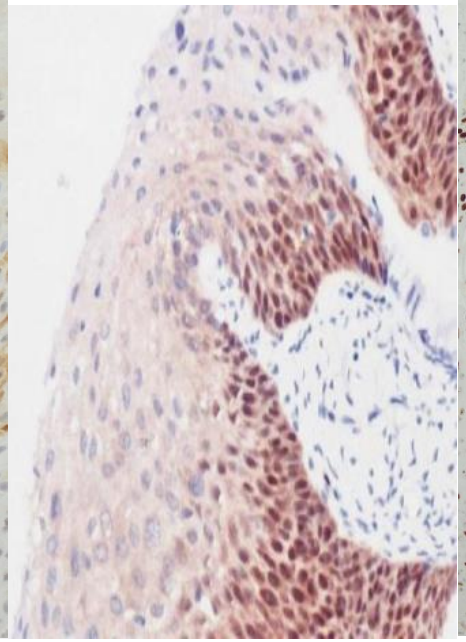
Abnormal expression in squamous epithelium:
BLOCK POSITIVE: correlate with H&E morphology.
Up to 50% of LSIL/CIN1 are p16 diffuse block positive!

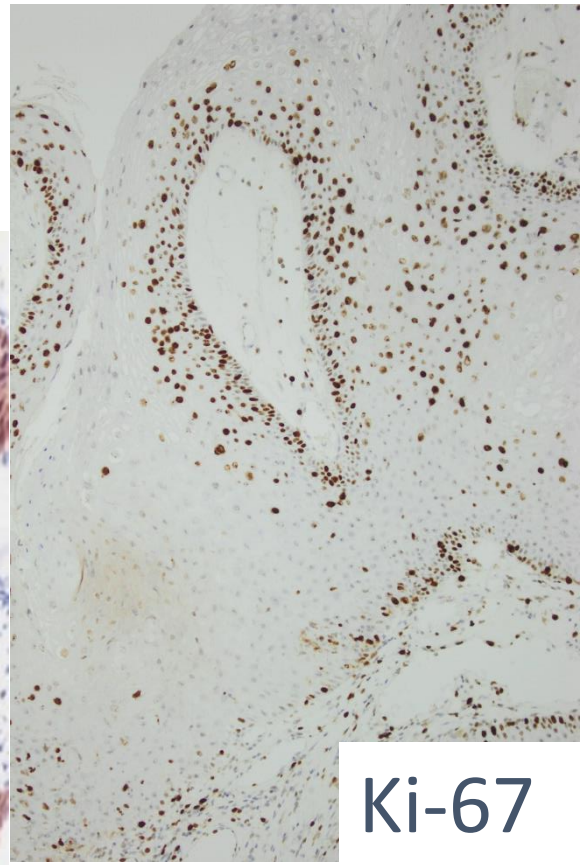


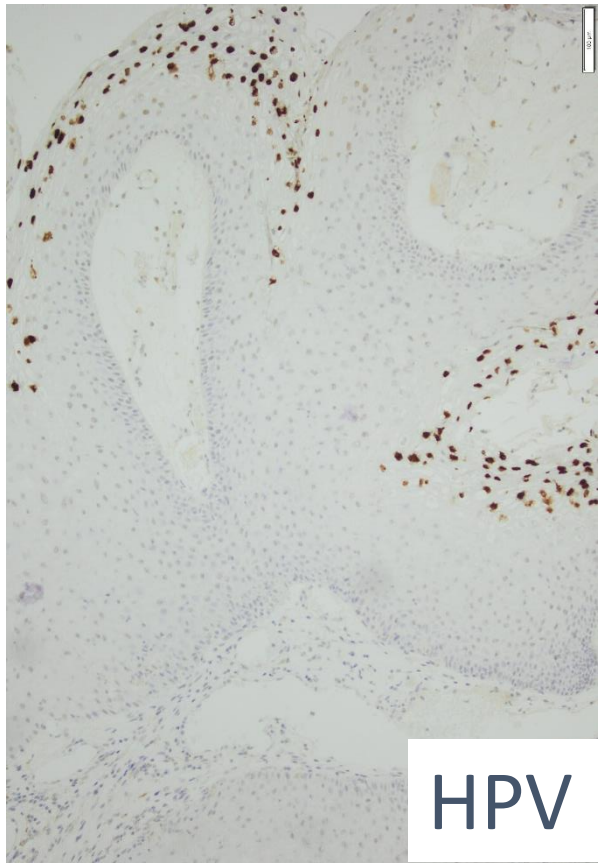


Up to 50% of LSIL/CIN1 are p16 diffuse block positive!







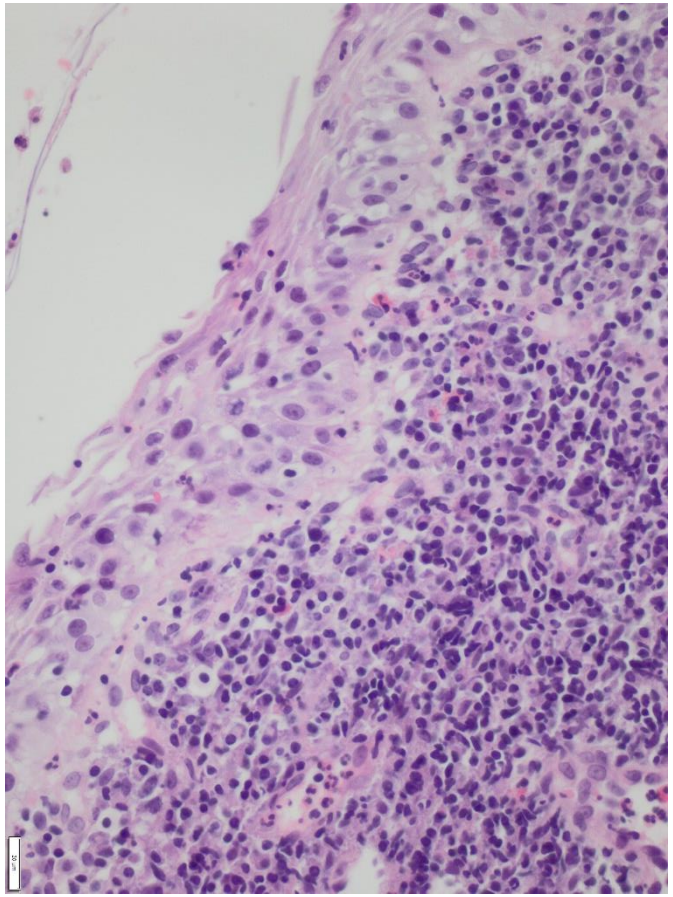


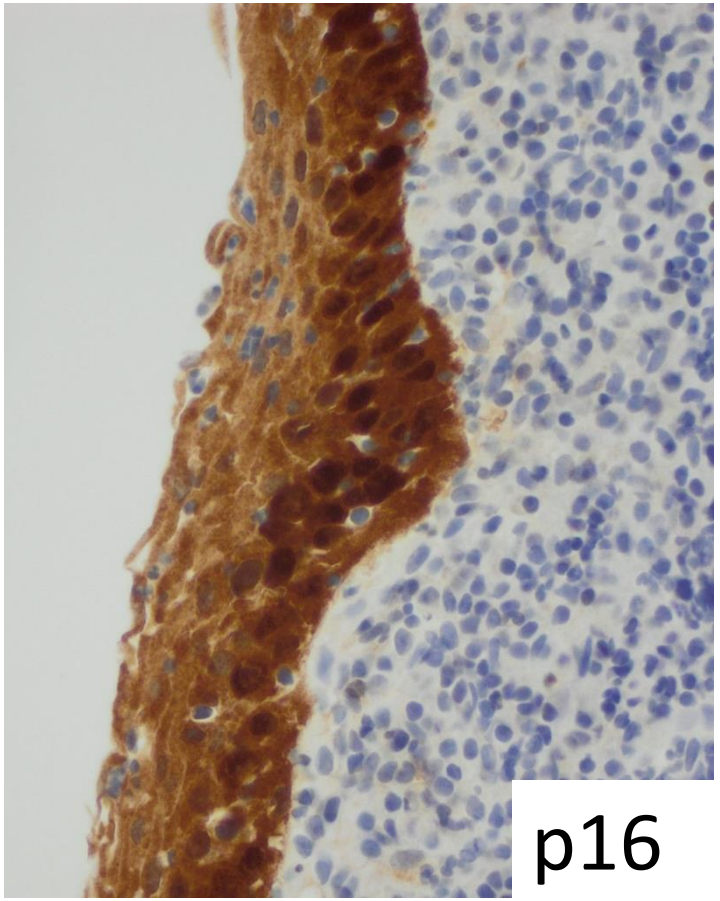
- p16 diffuse block positivity in LSIL/CIN1 does not predict progression to HSIL.
- Use of other biomarkers than p16 is not recommended/helpful.
- Grading should be based on morphological criteria.

LAST recommendations for use of p16 IHC

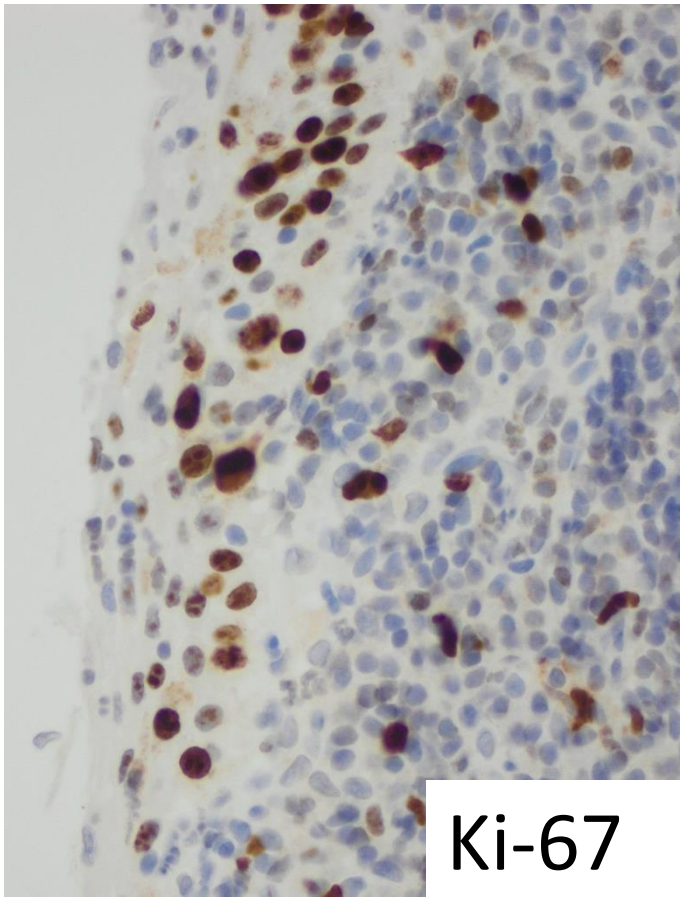
Int J Gynecol Pathol 2013 Jan;32(1):76-115.

1. when H&E morphological differential diagnosis is between precancer (HSIL; –IN 2 or –IN 3) and a mimic of precancer (e.g. immature squamous metaplasia, atrophy, reparative epithelial changes, …).





p16



Ki-67

LAST recommendations for use of p16 IHC

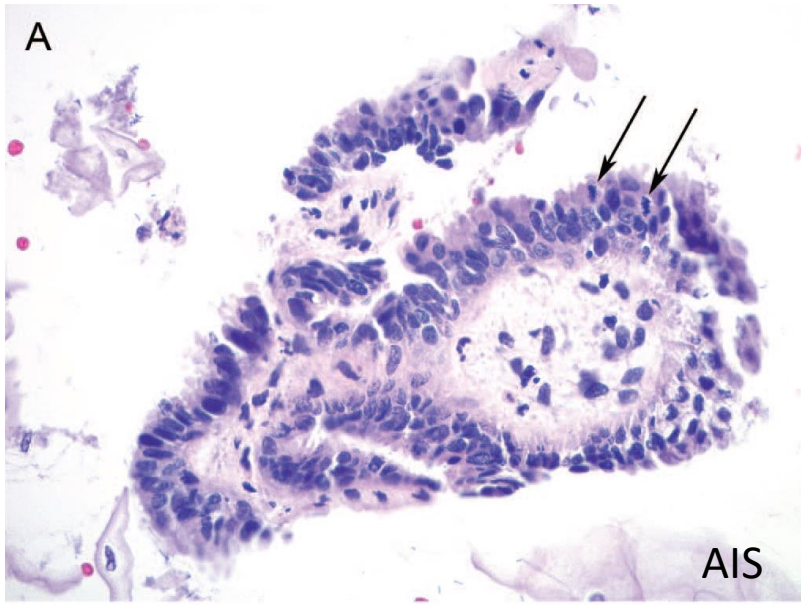
2. If the pathologist is entertaining an H&E morphological interpretation of HSIL/–IN 2, but cannot rule out LSIL. Grading should be based on morphological features; the value of p16 is in exclusion of HSIL in the absence of a diffuse block positive stain.

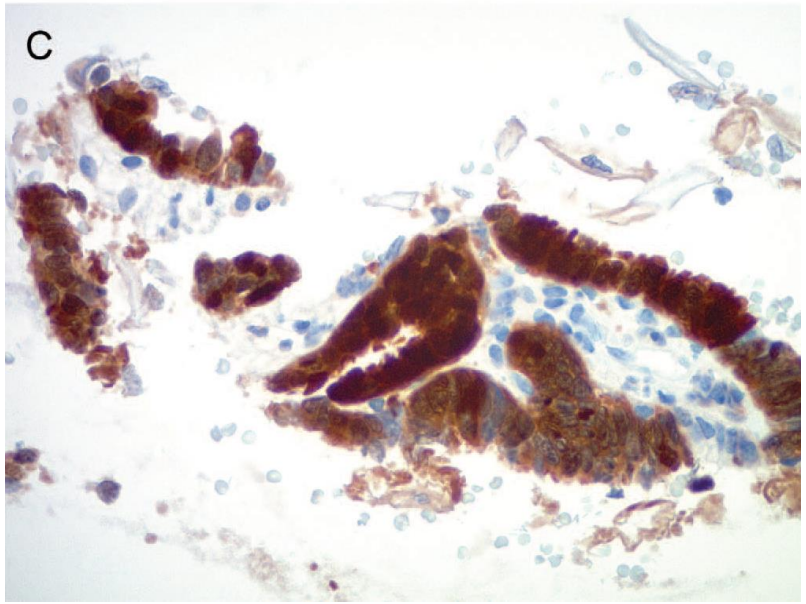
3. p16 is recommended for use as an adjudication tool for cases in which there is a professional disagreement in histological specimen interpretation, in which the differential diagnosis includes a precancerous lesion (HSIL/–IN 2 or –IN 3).

4. The group recommends against the use of p16 IHC as a routine adjunct to histological assessment of biopsy specimens with H&E morphological interpretations of negative, LSIL–IN 1, and HSIL–IN 3 (8-28% of HSIL/CIN3 lesions are not p16 diffuse block positive!).

4a: SPECIAL CIRCUMSTANCE: p16 IHC is recommended as an adjunct to morphological assessment for biopsy specimens interpreted as LSIL/–IN 1 or less that are at high risk for missed high-grade disease, which is defined as a prior cytological interpretation HSIL, ASC-H, ASC-US/HPV-16, or AGC (NOS).

→*following these recommendations: p16 IHC in 20% of cervical biopsies*

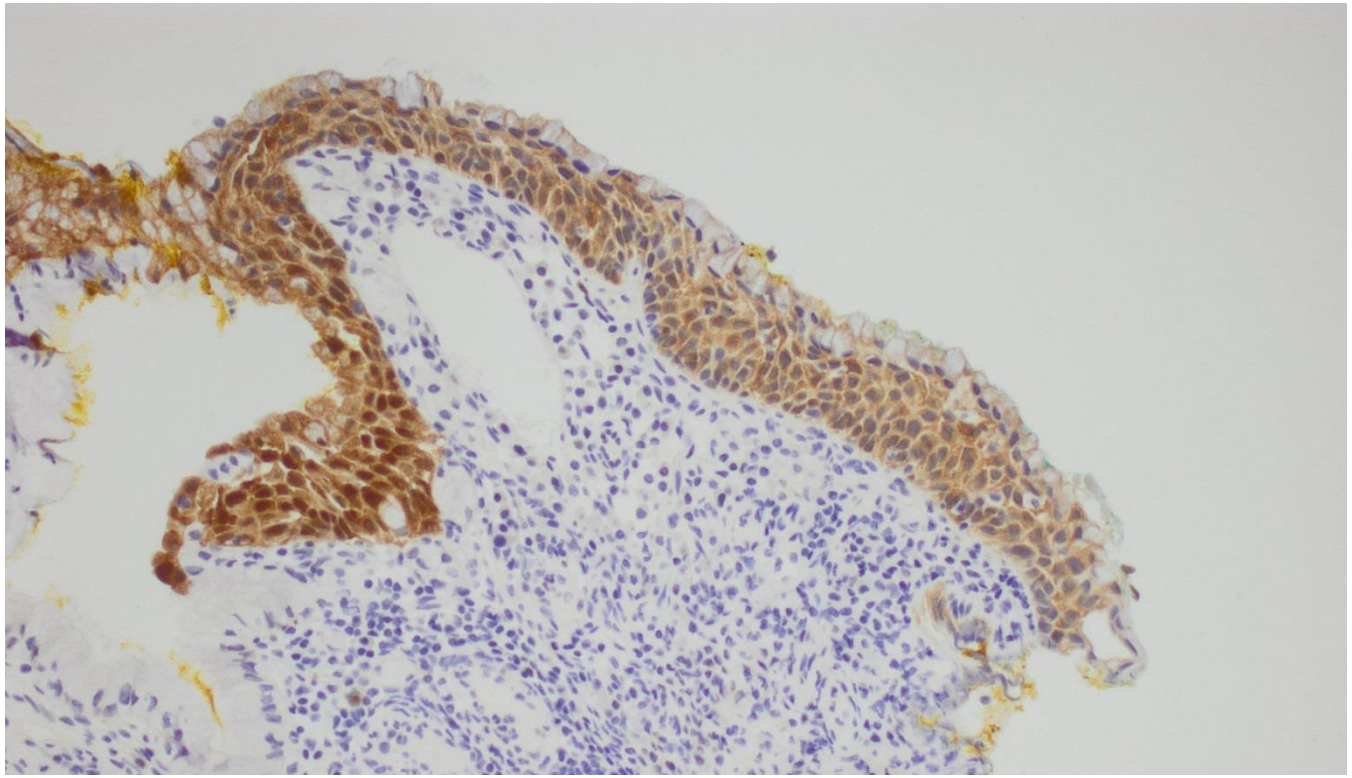




Use of p16: evaluation of

small tissue fragments in curettings

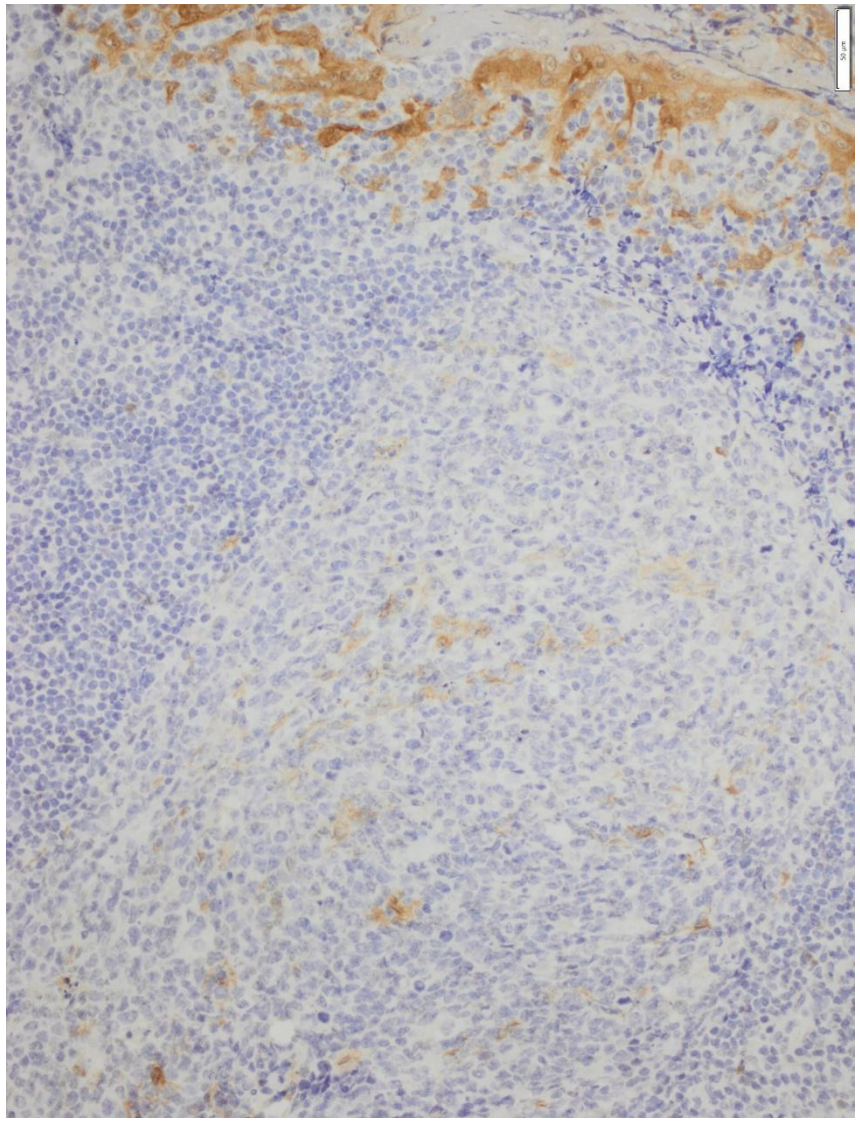
cauterized cone biopsy margins

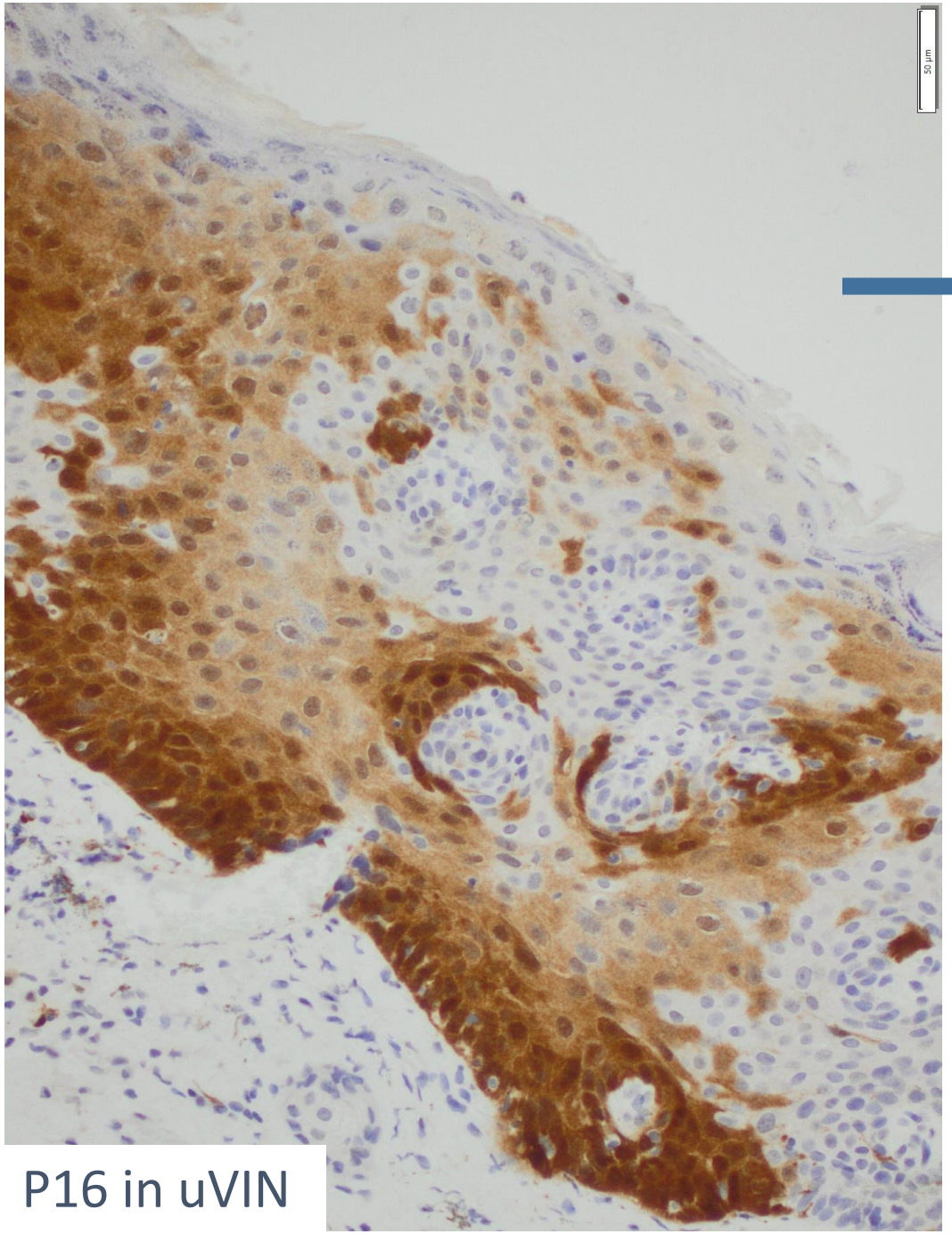


Buza N, Arch Pathol Lab Med. 2017

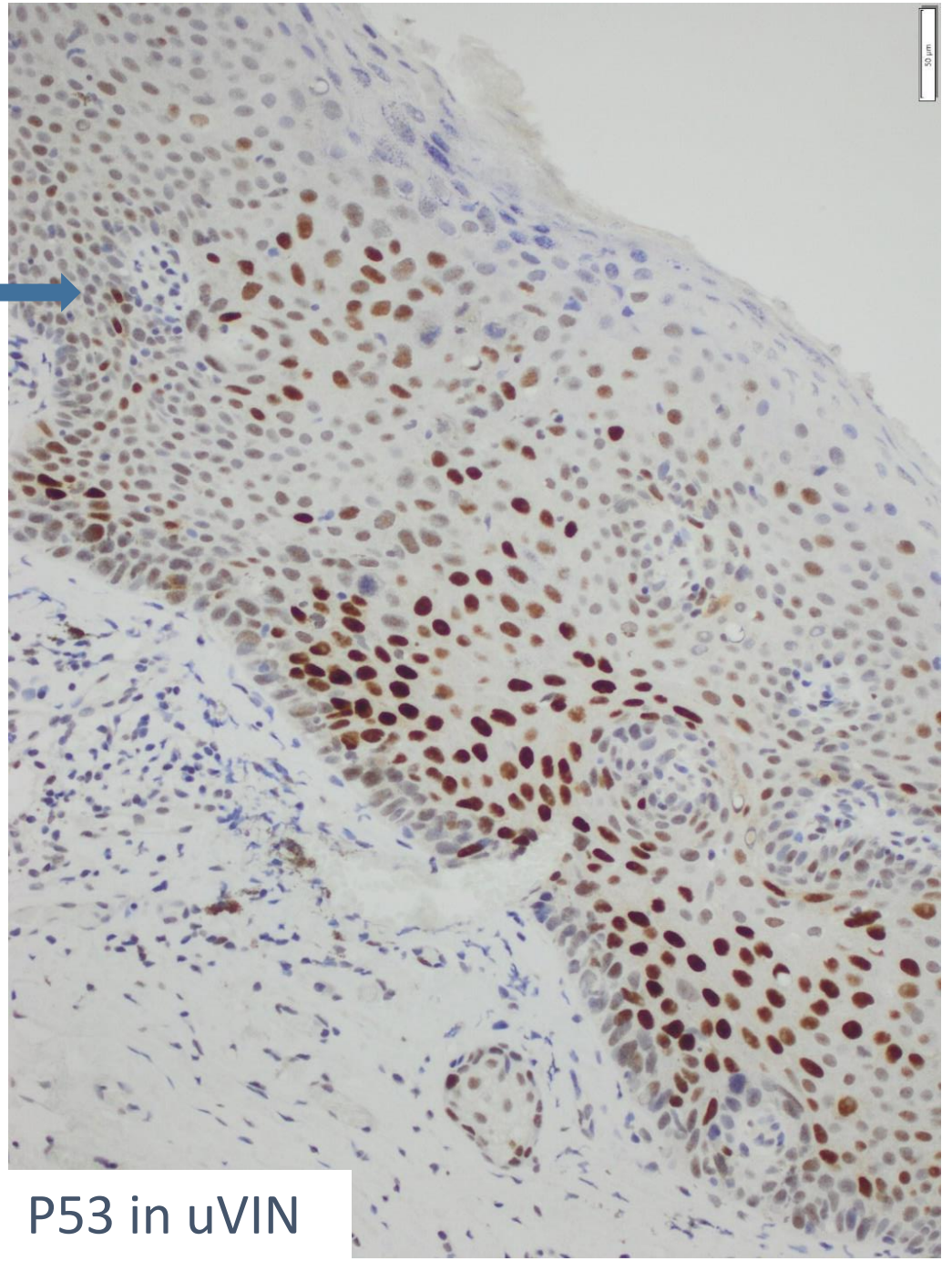
P16 immunohistochemical quality control

- https://www.nordiqc.org/
- Last assessment of p16 in 2009
- 96 laboratories participated in this assessment: 90 % sufficient result.
- Tonsil appears to be a recommendable control: critical quality indicators for p16 staining are:
  - the follicular dendritic cells must show an at least moderate nuclear and cytoplasmic staining
  - germinal centre B-cells should be negative
  - patchy staining of the squamous epithelium



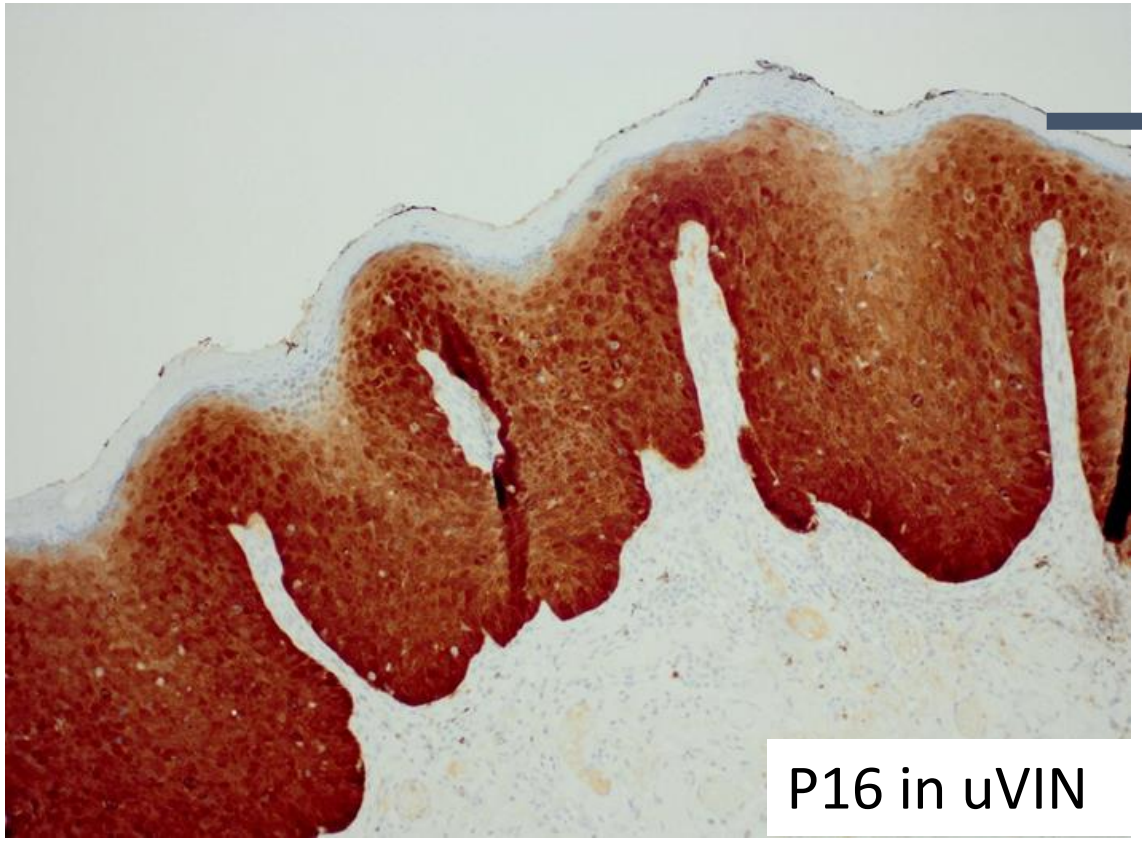


P16 in uVIN

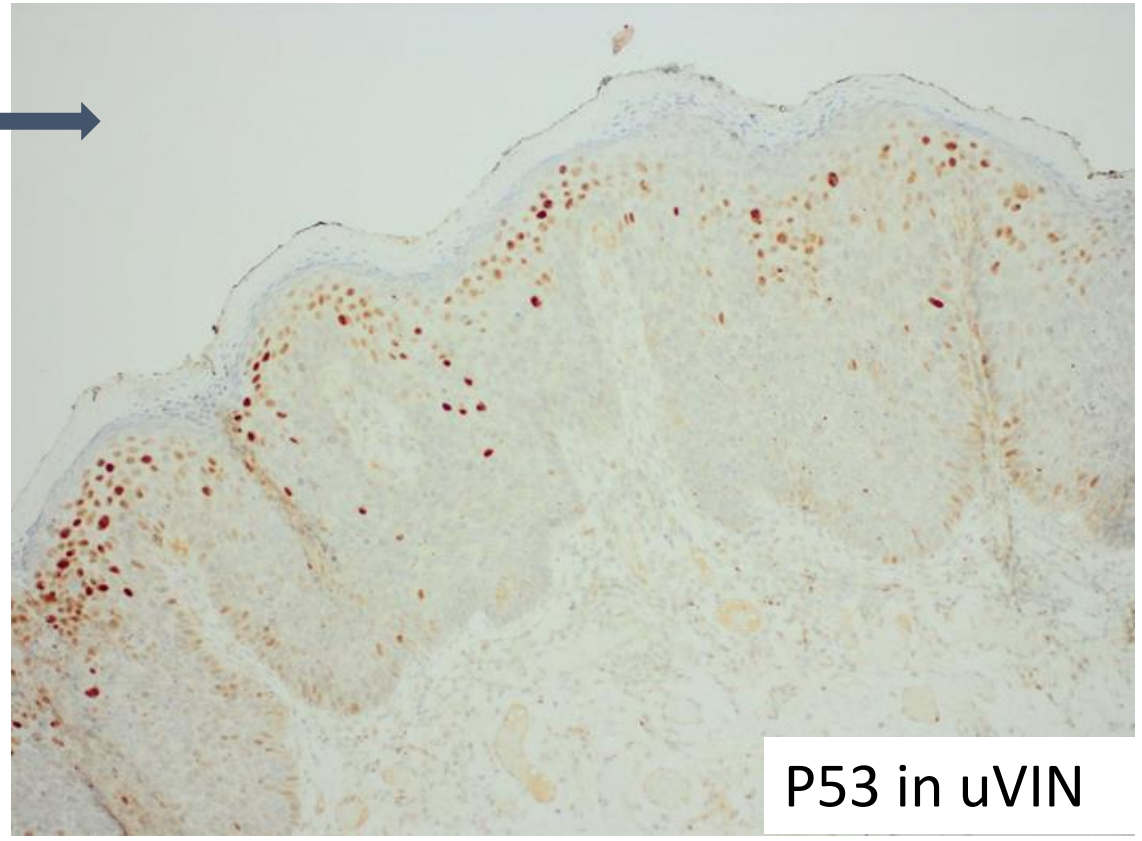


P53 in uVIN

P53 interpretation in non-HPV pre(cancers)



P16 in uVIN



P53 in uVIN

Non-mutational upregulation of p53 may be seen in HPV associated (pre)cancer and reactive conditions <-> p53 expression associated with *TP53* mutations in non-HPV pre(cancers).

Courtesy Koen Van de Vijver

THE BRITISH ASSOCIATION OF GYNAECOLOGICAL PATHOLOGISTS

Interpretation of p53 Immunohistochemistry In Tubo-Ovarian Carcinoma: Guidelines for Reporting
Author: Martin Köbel. Co-authors: W Glenn McCluggage, C Blake Gilks, Naveena Singh.

p53 immunohistochemistry pattern and interpretation

Pattern	p53 IHC Interpretation	*TP53* mutation type	% in HGSC
		***TP53* MUTATION ABSENT**	
Wild type	Normal	No mutation	0
		***TP53* MUTATION PRESENT**	
Overexpression	Abnormal	Non-synonymous (missense); also in-frame deletion, splicing	66%
Complete absence/ null	Abnormal	Indels, stopgains, splicing mutations	25%
Cytoplasmic	Abnormal	Indels and stopgains with disruption of the nuclear localization domain	4%
Wild type	Normal*	Truncating mutation	5%

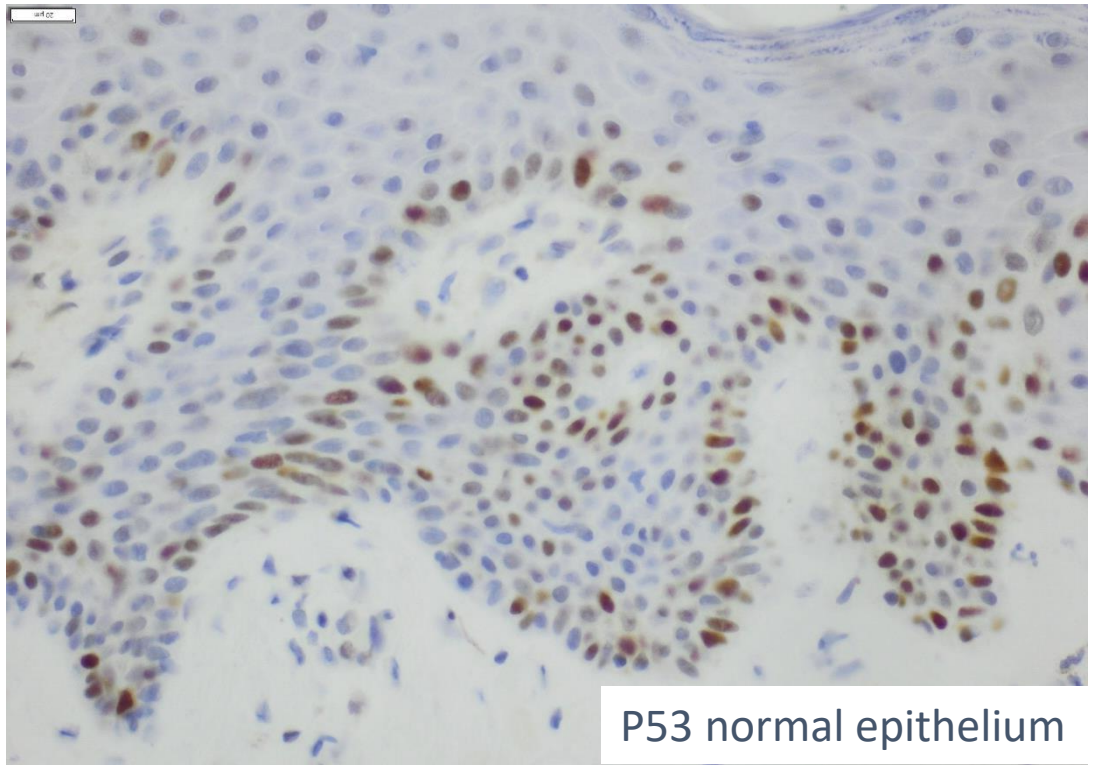
HGSC- high-grade serous carcinoma

Guidance document: p53 IHC reporting in tubo-ovarian carcinoma version 1.0, dated October 2016

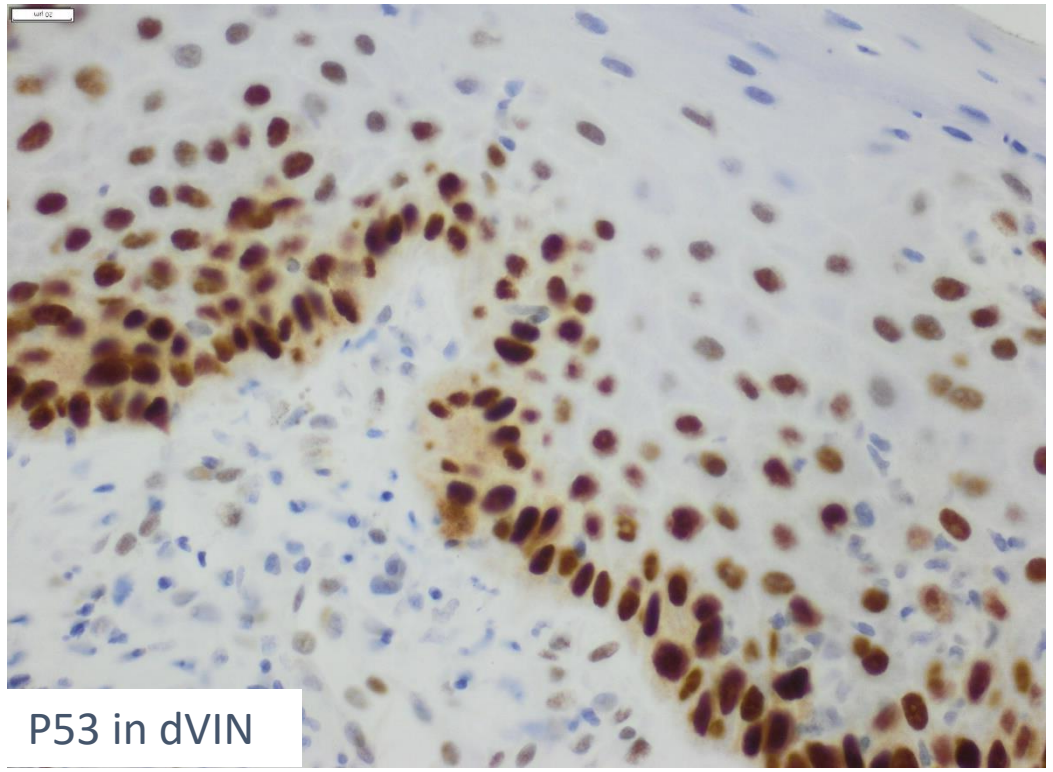
p53 interpretation in non-HPV (pre)cancers?

- Surrogate for *TP53* mutation:
  - intense nuclear p53 positivity
  - or less commonly, complete absence of p53 staining (‘‘p53 null’’ phenotype)

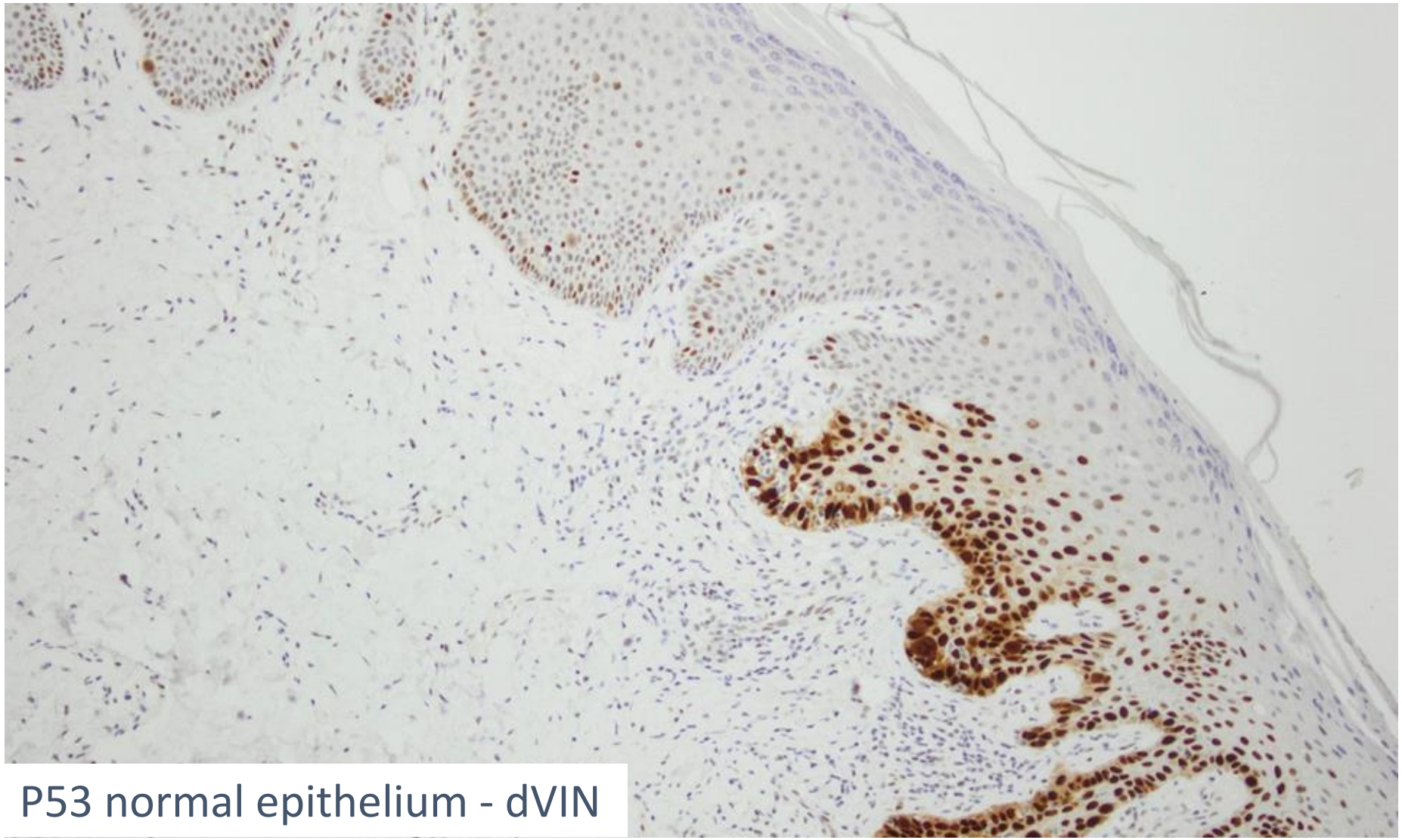
  extending from the basal cell layer to the suprabasal cells, involving one-third to full thickness of the epidermis. *Buza, Hui, Arch Pathol Lab Med. 2017;141:1052*



P53 normal epithelium



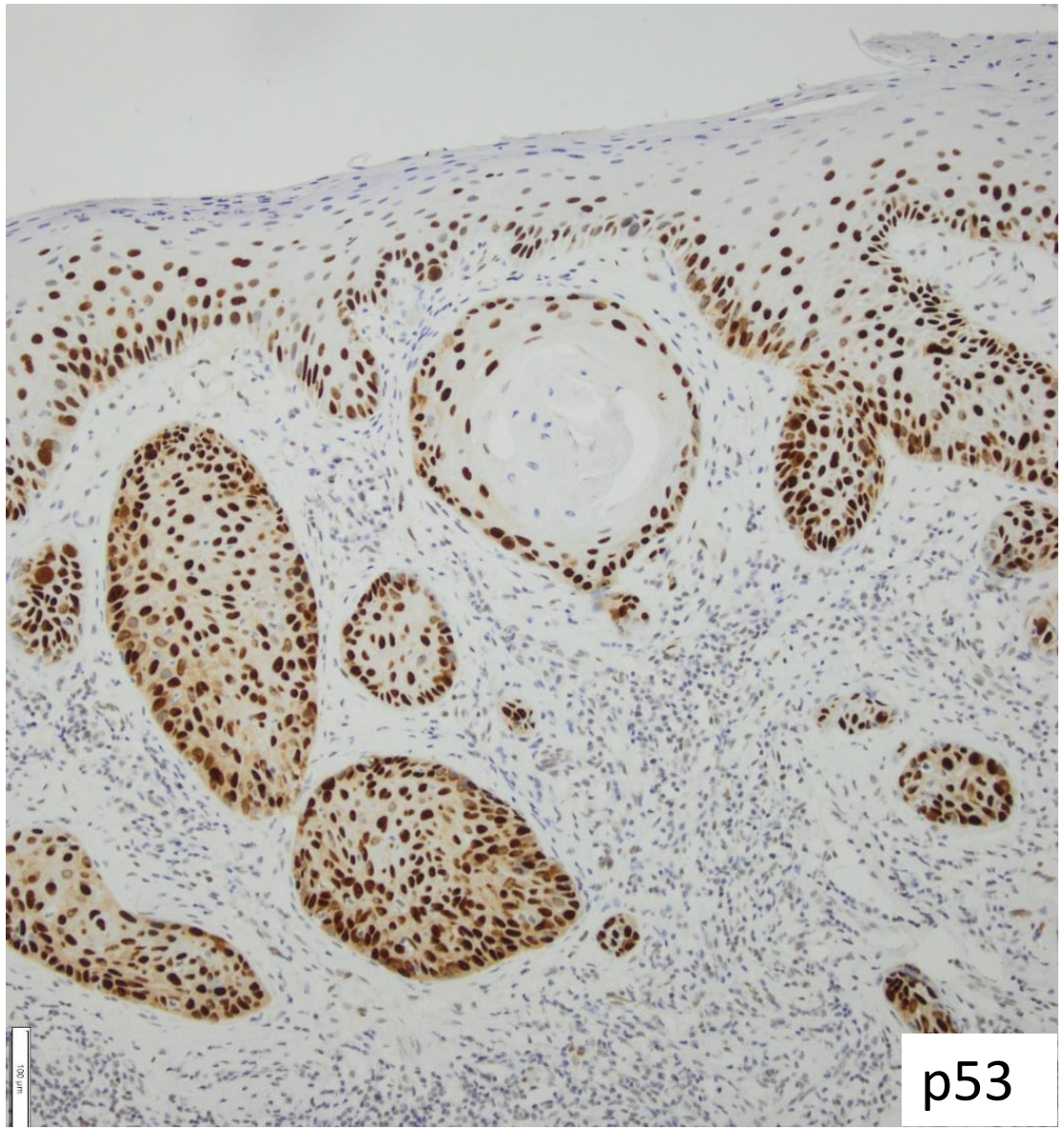
P53 in dVIN

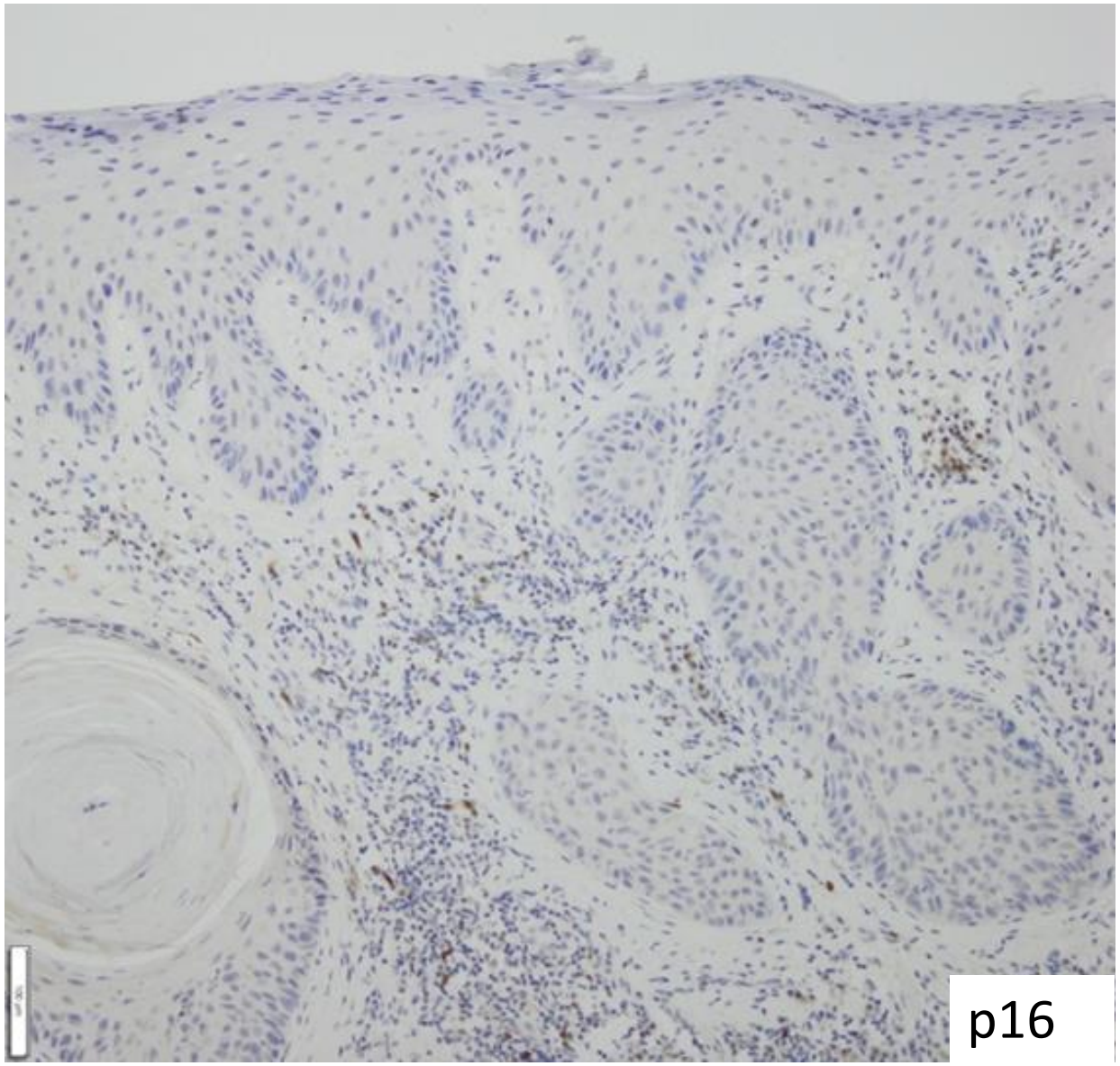


P53 normal epithelium - dVIN

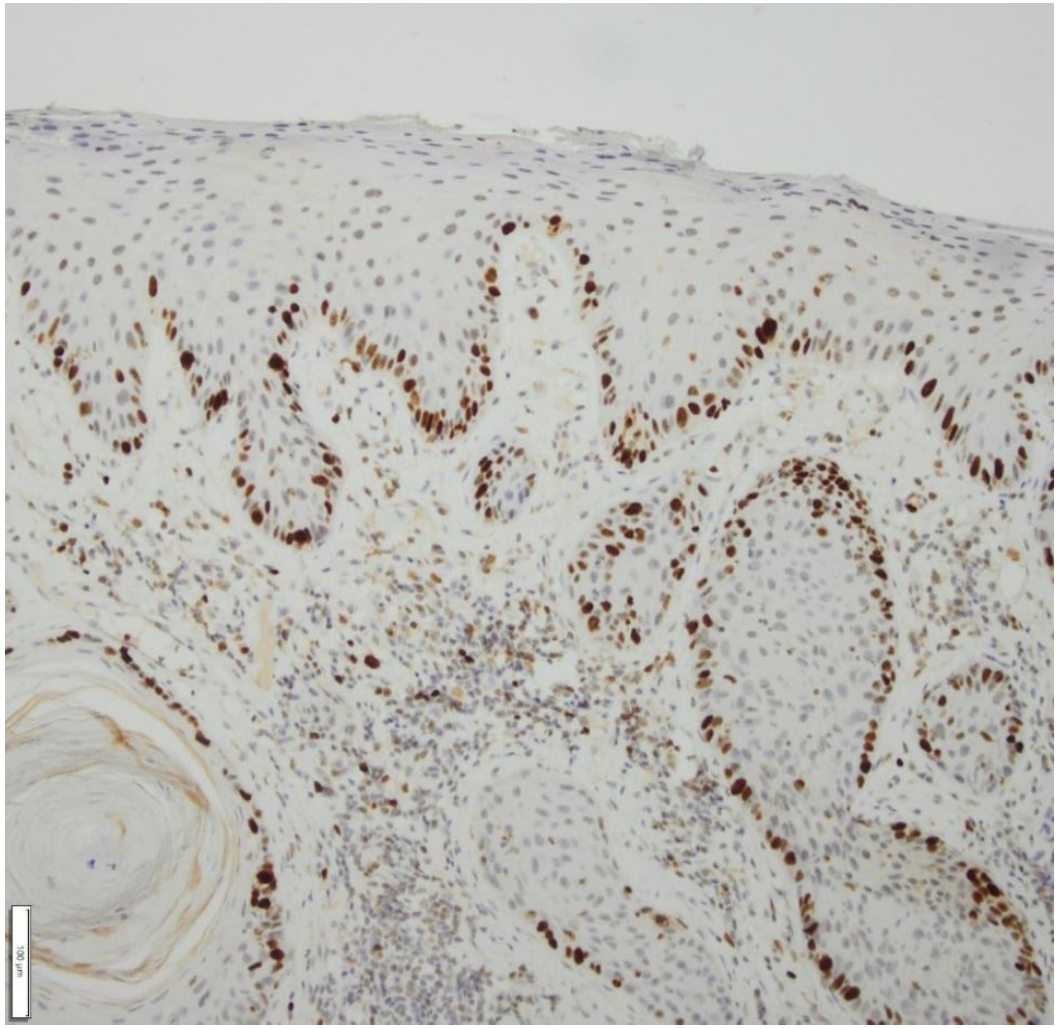
In normal epithelium, intensity and extent of p53 nuclear staining is associated with the proliferation index, e.g., basal keratinocytes of normal skin show variable p53 staining while the mitotically inactive superficial keratinocytes are negative. Stromal fibroblasts and intratumoral lymphocytes also show wild type pattern and are used as intrinsic control.

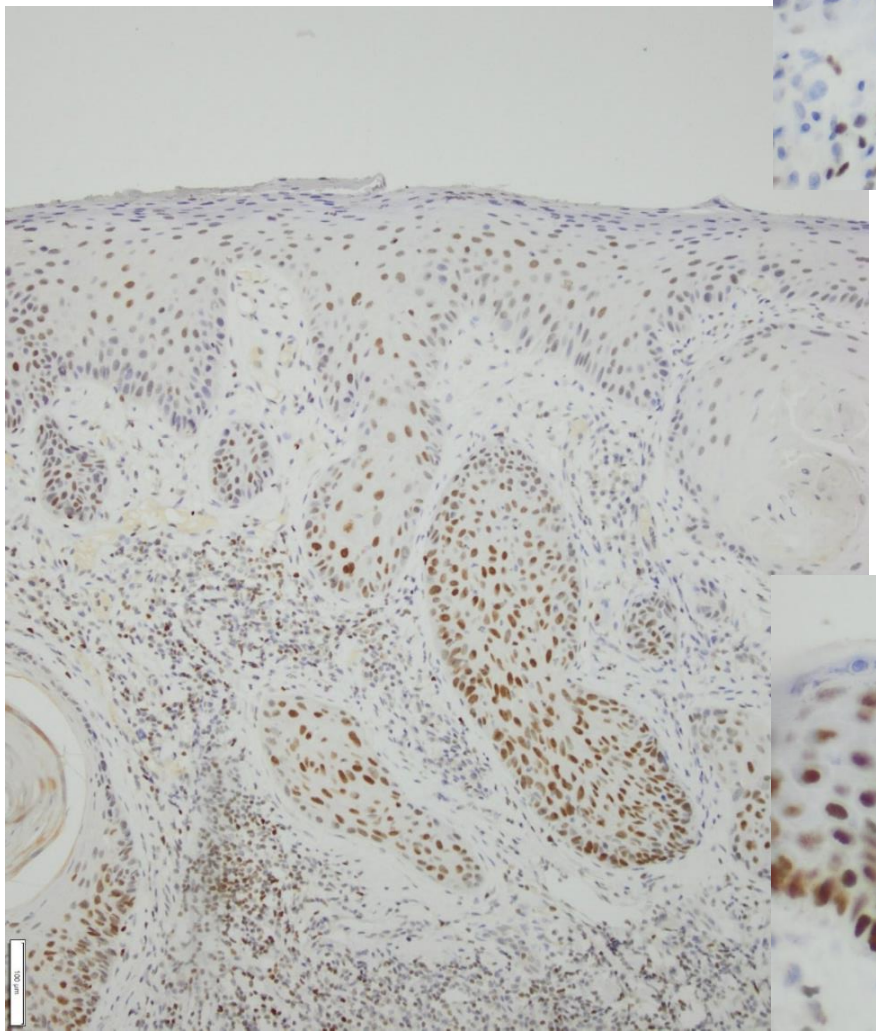
dVIN with invasive squamous cell carcinoma

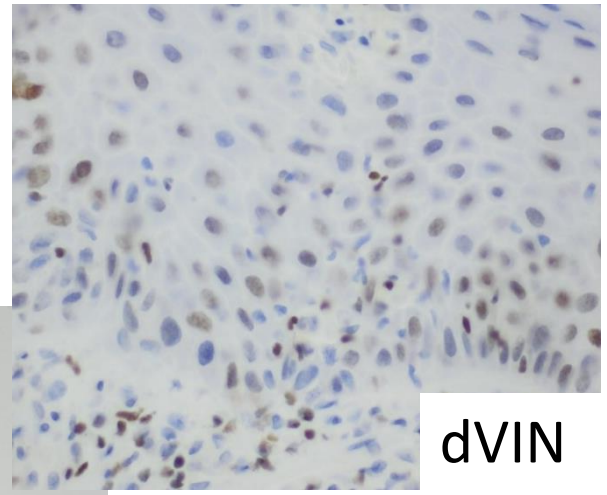




dVIN: Ki-67 basal





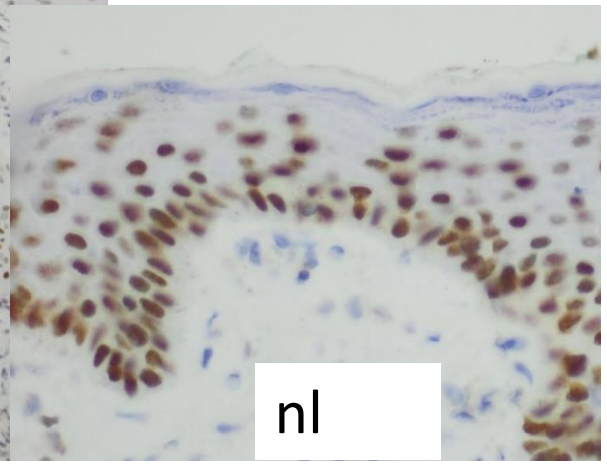


dVIN

GATA3:

lost in dVIN

present in invasive ca



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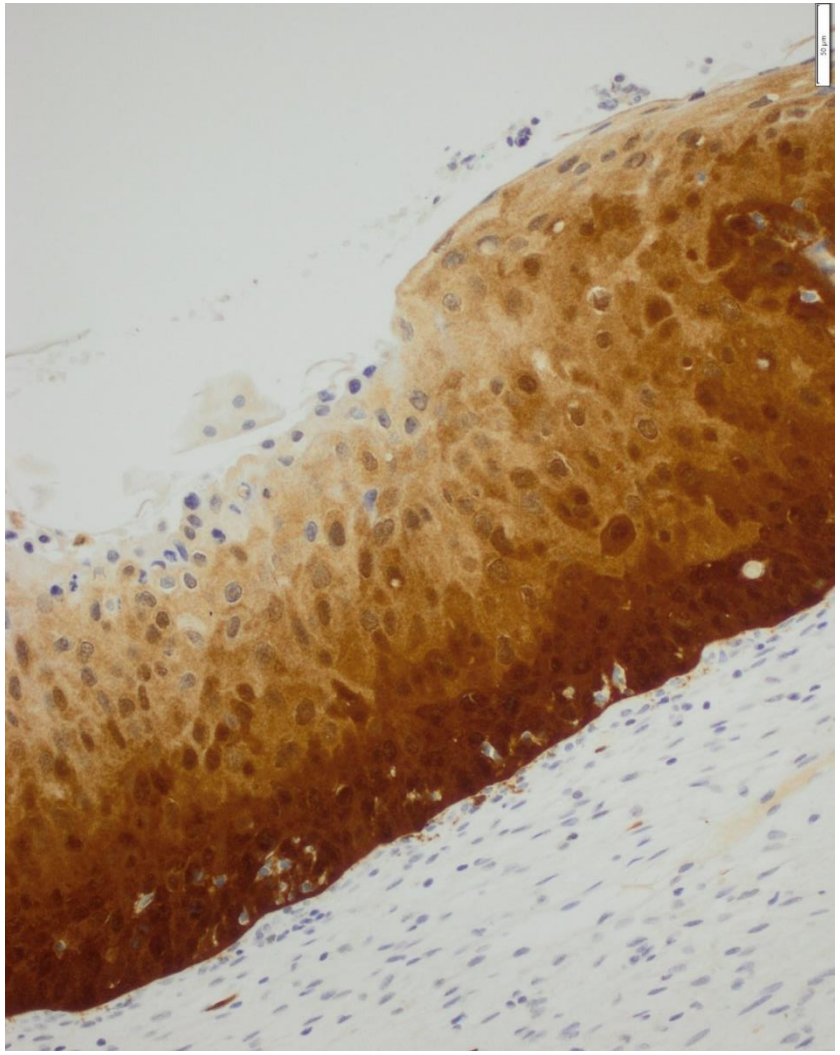
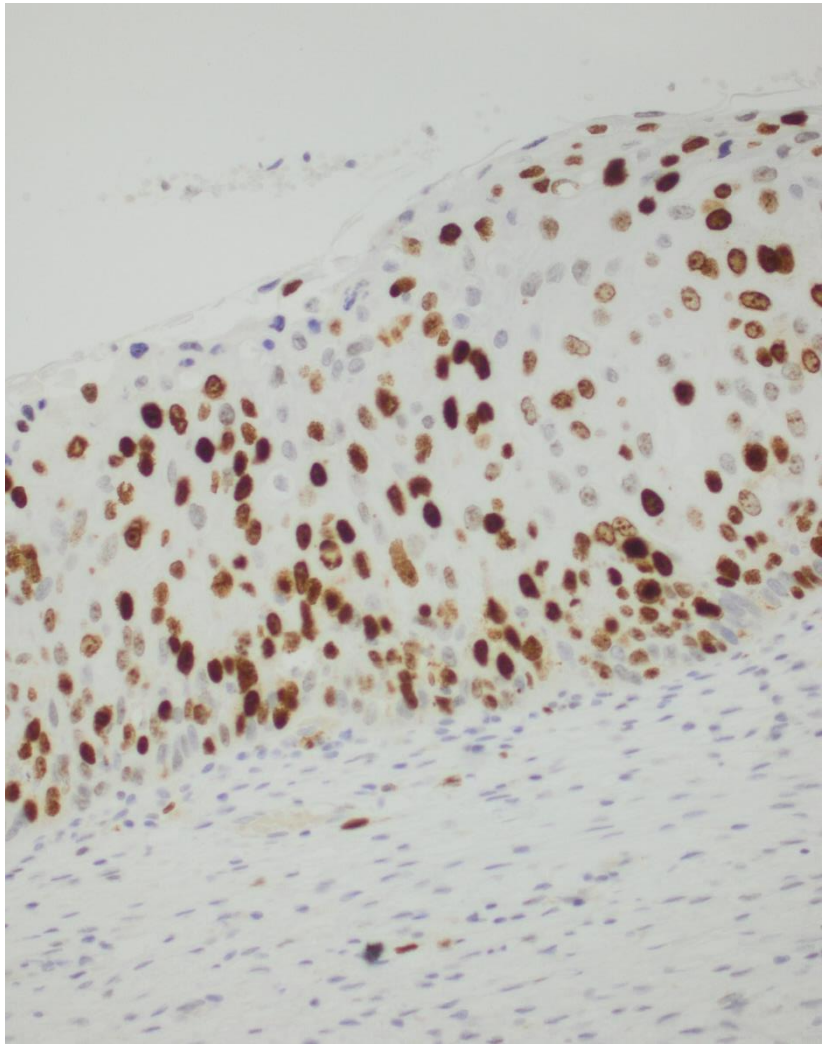
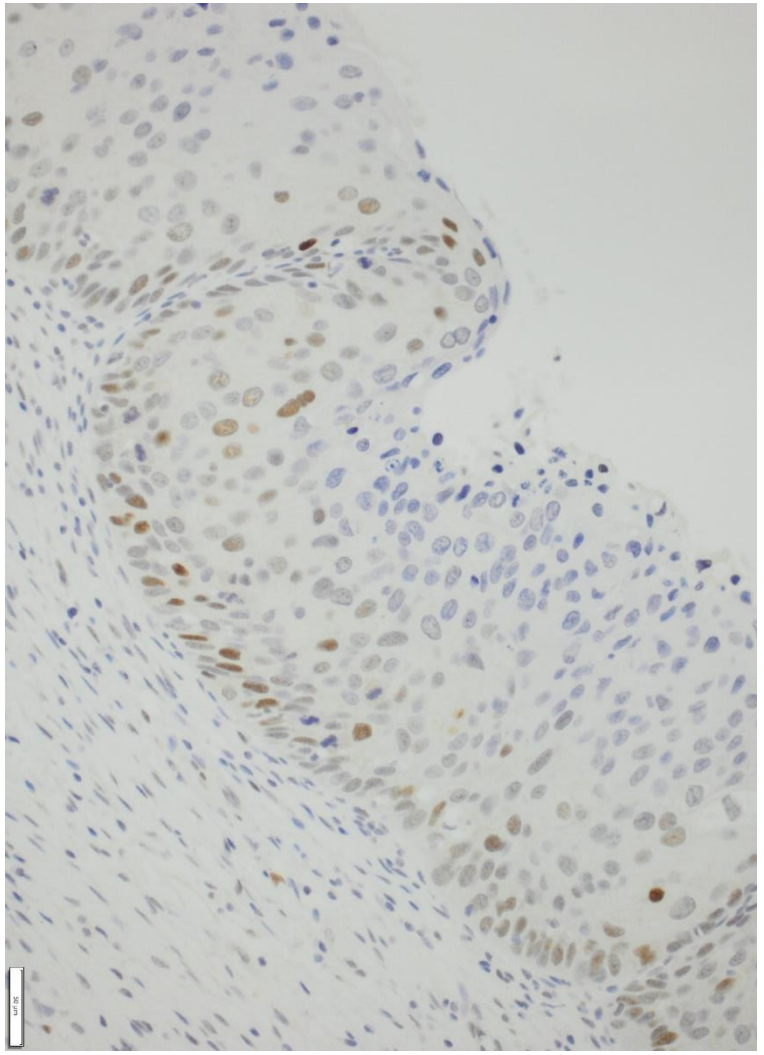
uVIN: p16 block pos., Ki-67 in upper 2/3, p53 wild type







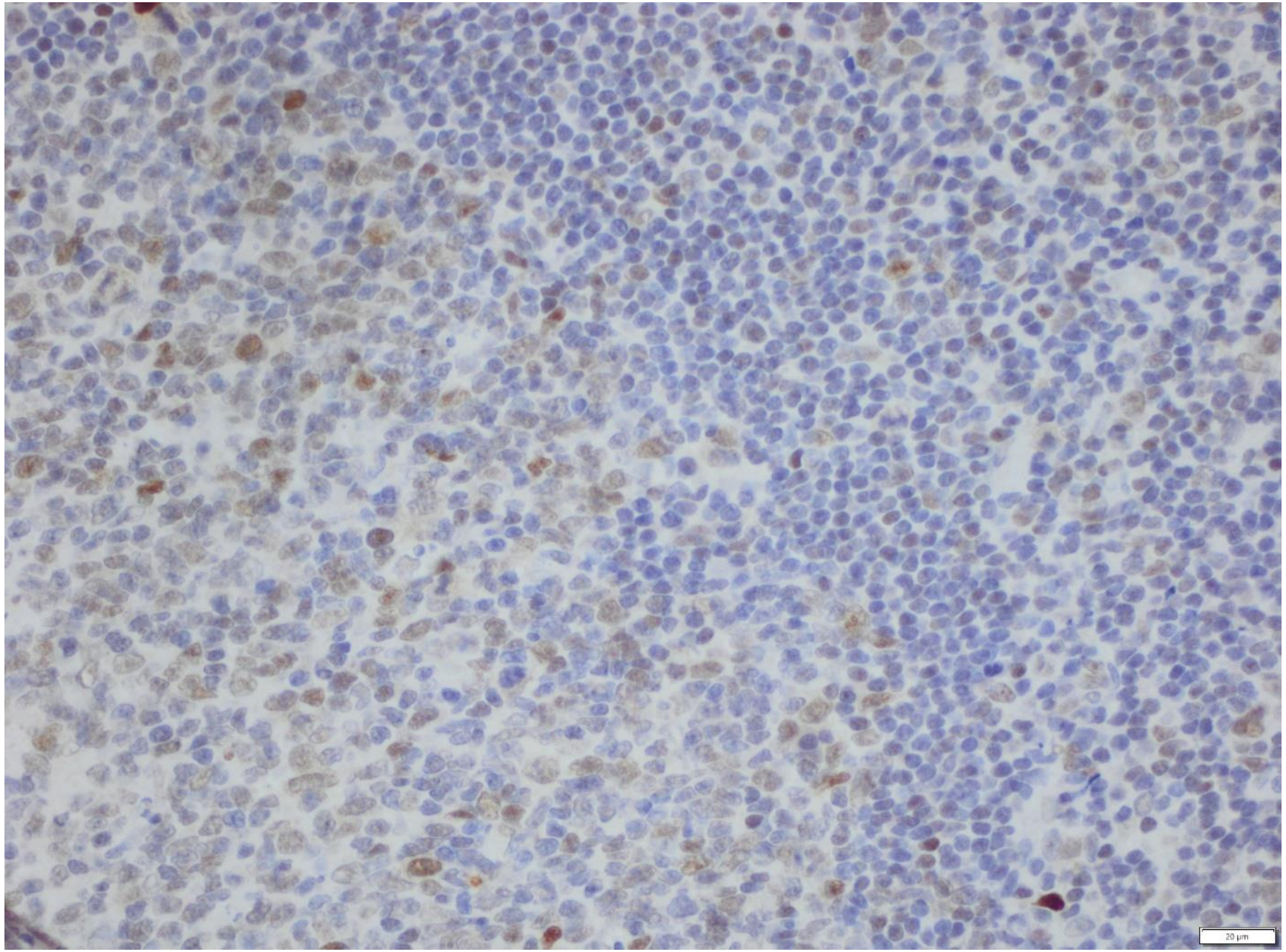
P53 immunohistochemical quality control

Last **Assessment Run 38 2013**

- 218 laboratories participated in this assessment. 79 % achieved a sufficient mark (optimal or good)
- In this assessment tonsil and colon were identified as the most recommendable positive and negative tissue controls.
- In tonsil, more than 20 % of germinal centre B-cells must show a weak to moderate nuclear staining reaction, while less than 10 % of the mantle zone B-cells should be demonstrated.
- In colon, dispersed epithelial cells in the basal parts of the crypts must show a weak to moderate nuclear staining reaction, while the luminal epithelial cells must be negative.

Optimal p53 staining of the tonsil

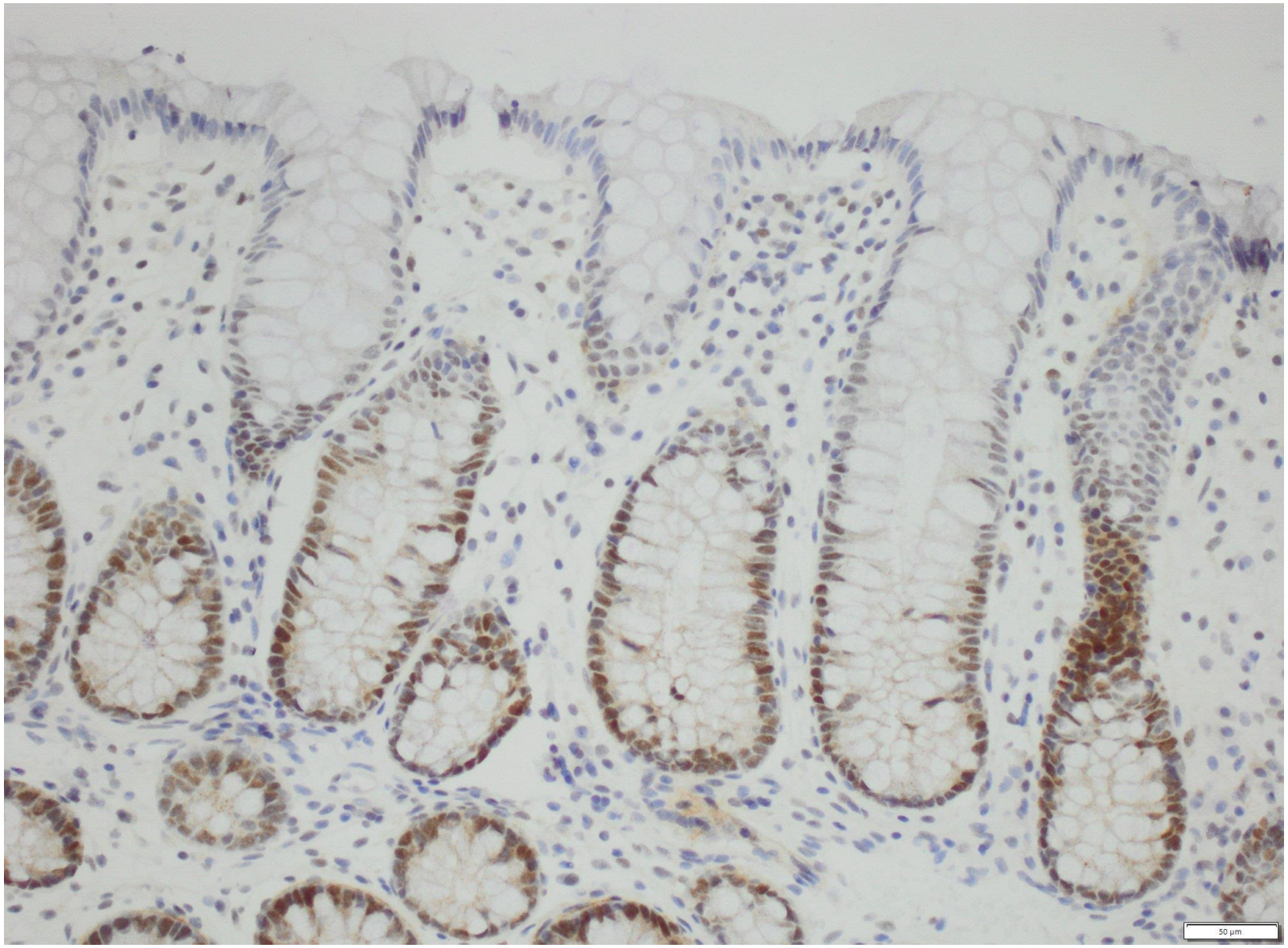
mAb clone DO-7.
More than 20 % of germinal centre B-cells must show a weak to moderate nuclear staining reaction, while less than 10 % of the mantle zone B-cells should be demonstrated.



Optimal p53 staining of colonic mucosa

mAb clone DO-7

Dispersed epithelial cells in the basal parts of the crypts must show a weak to moderate nuclear staining reaction, while the luminal epithelial cells must be negative.



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