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Resultaten EKE IHC 2022-2

De Wiest Bart

16 december 2022

OLV Ziekenhuis Aalst

Procedure panelbeoordeling

- Literatuurstudie m.b.t. antilichaam: NordiQC website, datasheets, guidelines, …
  - Wat moet / kan aankleuren?
  - Wat mag zeker niet aankleuren?
- Vastleggen criteria aankleuring per AS
- Beoordeling
  - Consensus binnen het panel
  - Reden voor afwijkend resultaat bij borderline/onvoldoende
  - Evt. aanbevelingen protocol

  → eindbeoordeling

EKE IHC 2022-2

Antistoffen:

- ✓ CD5: n=63
- ✓ panCK: n=64
- ✓ MLH-1: n=59

Panel:

- ✓ Scoring 07/07/2022 (Sciensano)
- ✓ Onafhankelijk, in consensus
- ✓ 2 pathologen
  - Dr. V. Duwel (AZ Klina, Brasschaat)
  - Dr. M.A. V. Caillie (St Lucas, Brugge)

CD5

- 1 labo geen protocolsettings
- Gebruikte clone: 4C7
  - RTU: n=25
  - Dako 4C7 op BMK Ultra (2)
    - ultraView: HIER CC1 36’ ; AS 28’
    - optiView: HIER CC1 20’ ; 32’ RT (?)

CD5

- 1 labo geen protocolsettings
- Gebruikte clone: 4C7
  - RTU: n=25
  - Dako Omnis/Autostainer
    - Autostainer
      - AS 20’ RT ; geen linker
      - HIER pH9 10’ of 30’
    - Omnis
      - AS 10’ - 15’ - 20’ - 27’30” RT/32° C
      - 1 linker
      - HIER pH9 30’
  - Leica Bond III/Max
    - Bond Max: 15’ RT; HIER pH9 20’
    - Bond III: 15’ RT; HIER pH6 10’ of pH9 20’

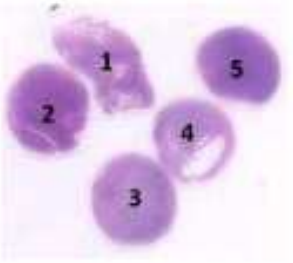
Protocol CD5

- Gebruikte clone: 4C7
  - Conc.: n=7
  - Leica BondIII: 1/50 15'RT ; pH9 20'
  - Dako: Omnis? 1/200 20' 32° C ; pH9 30'
  - BMK Ultra (5)
    - ultraView:
      - 3 AS leveranciers
      - Verdunning: 1/40 – 1/50 (meest)
      - AS 24'- 32' - 120'
      - RT, 36° C of 42° C
      - +/- ampl. 8' - 16'
      - HIER CC1 36' of 64' (meest)

Protocol CD5

- Gebruikte clone: SP19
  - RTU: n=30
  - Dako Omnis:
    - leverancier VWR
    - HIER pH9 30’ ; AS 20’ RT
  - BMK Ultra/GX
    - Producent: Thermo/Neomarkers (1) en Ventana
    - ultraView: datasheet CC1 64’ ; AS 16’
      - HIER: geen (?) ; CC1 36’ - 64’ - 72’
      - AS 16’ - 32’ +- ampl.
    - optiView : geen datasheet
      - HIER: CC1 32’ - 40’ - 64’ - 72’
      - AS 16’ - 20’

Antistof CD5



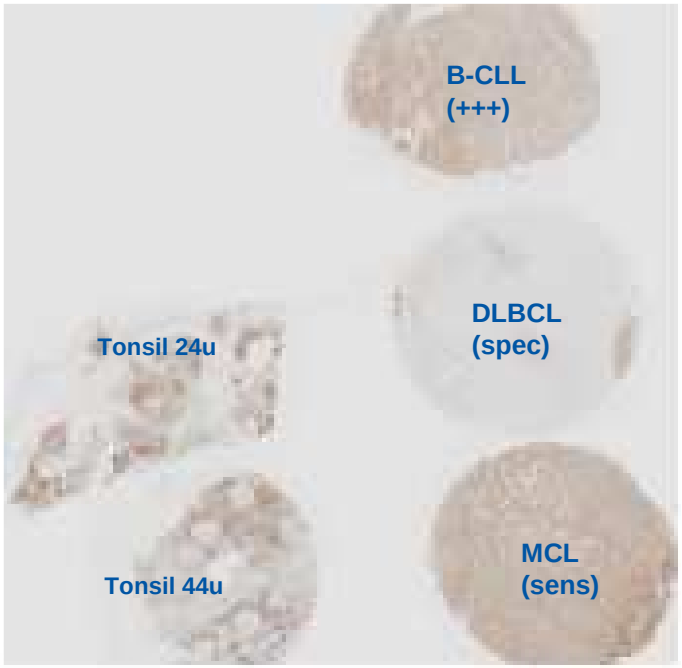
- Scoringscriteria: overwegend membranaire aankleuring
  - Tonsil § (fixatie 24u.) : controle robuustheid
    - sterke aankleuring van bijna alle T-cellen in de T zones en in de kiemcentra
    - minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones
  - Tonsil § (fixatie 44u.) : controle robuustheid
    - sterke aankleuring van bijna alle T-cellen in de T zones en in de kiemcentra
    - minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones
  - MCL :
    - minstens matige aankleuring in de neoplastische cellen (controle sensitiviteit)
    - sterke aankleuring van alle T-cellen tussen de neoplastische cellen
  - DLBCL :
    - aankleuring in de lymfocyten
    - geen aankleuring in de neoplastische cellen (controle specificiteit)
  - B-CLL :
    - sterke aankleuring in de neoplastische cellen
    - sterke aankleuring van alle T-cellen tussen de neoplastische cellen
  - Alle weefsels : geen aankleuring in de B-cellen van de kiemcentra

§ Aankleuring van plaveiselcelepitheel dat de crypten bekleedt wordt aanvaard.

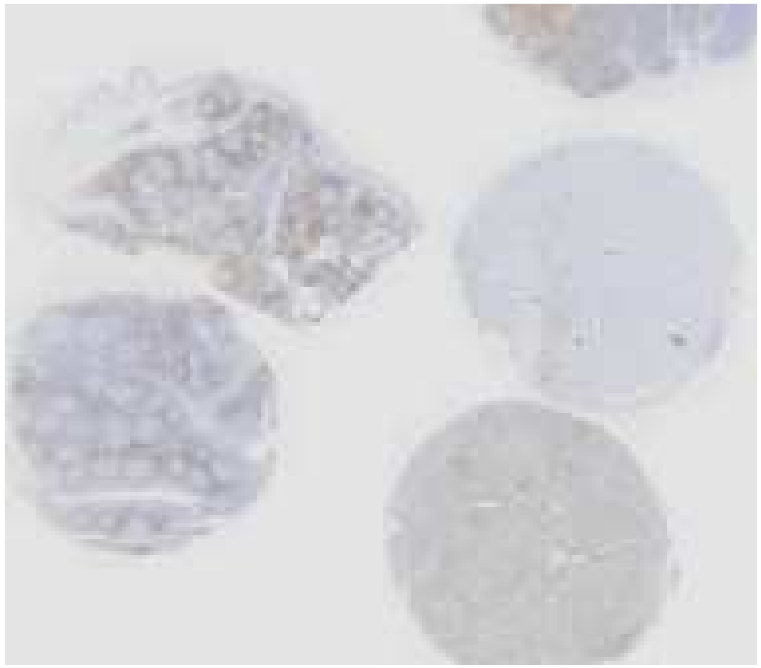
Bron: NordiQC Run 49, 2017

olv Top in Zorg.

Antistof CD5: overzicht



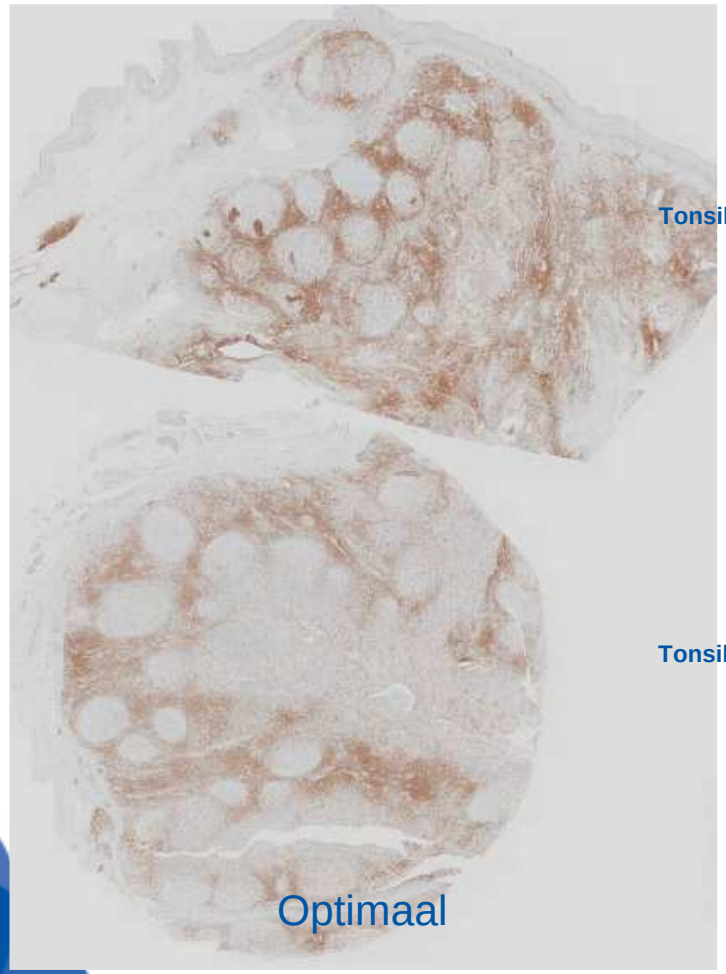
Optimaal



Borderline

Antistof CD5: tonsil

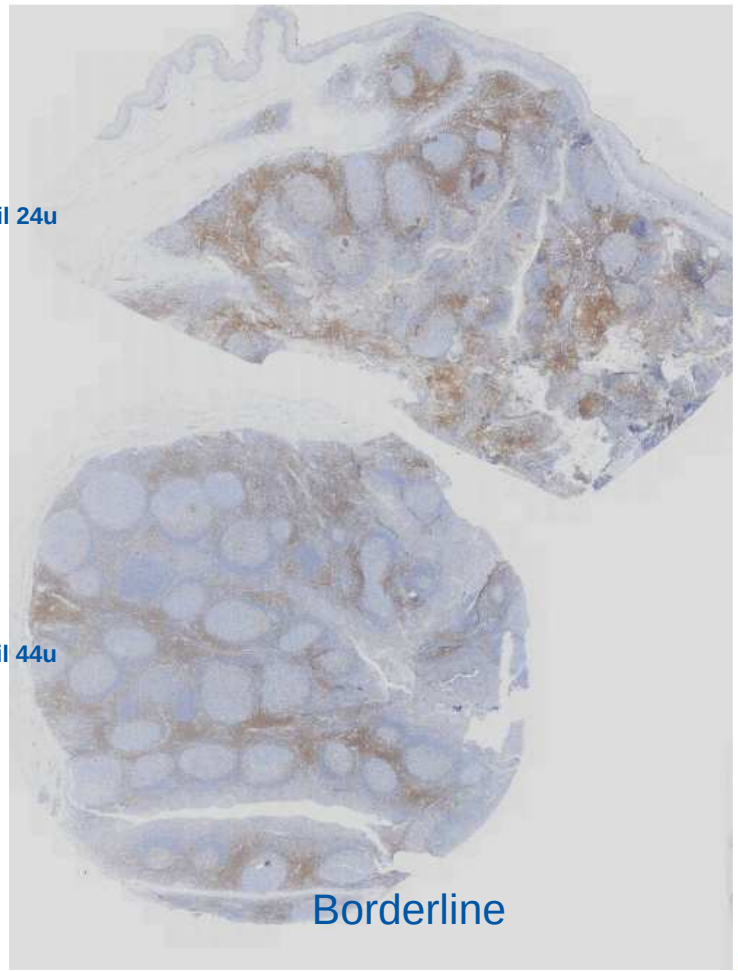
Dako 4C7 1/50 24' 36° C + amp **;** CC1 64 ; **UV ;** BMK Ultra **Dako 4C7 1/200** 20' 32° C **;** TRS pH9 30' **; Dako Flex,** toestel?



Optimaal

Tonsil 24u

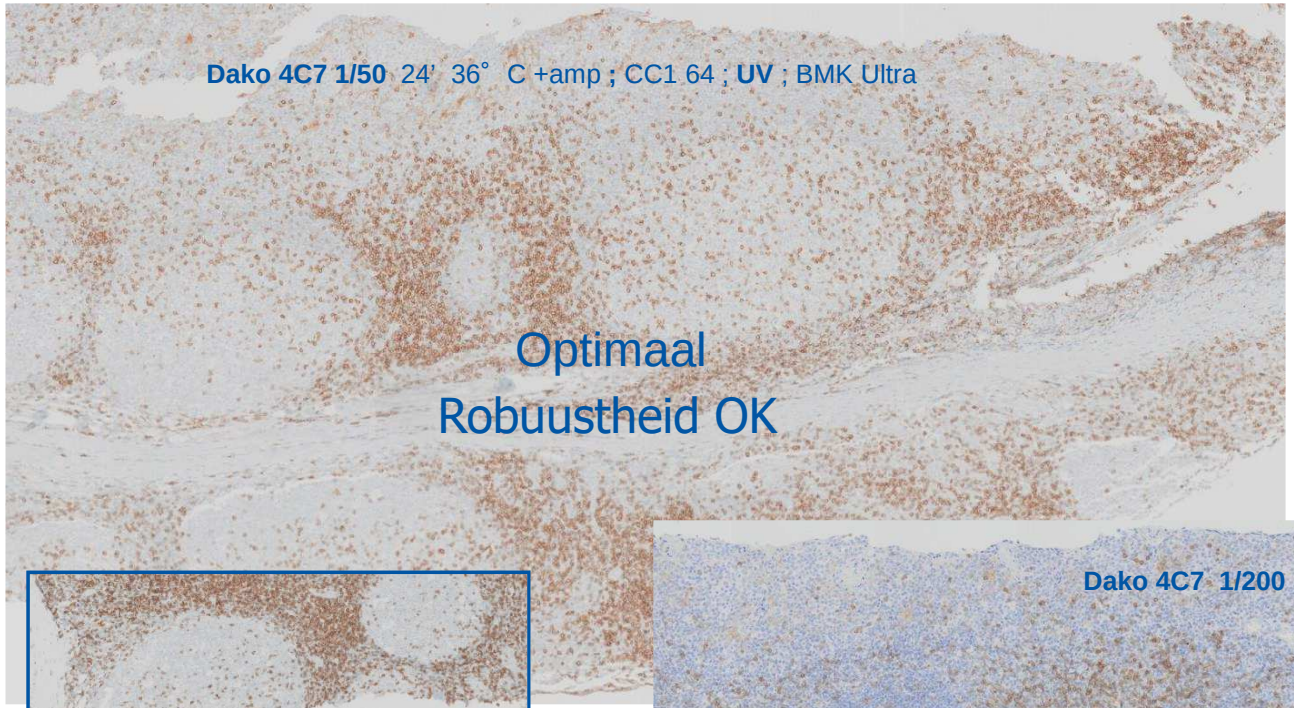
Tonsil 44u



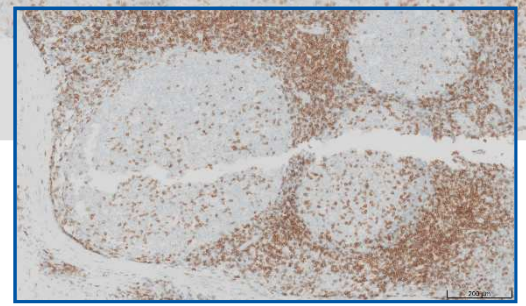
Borderline

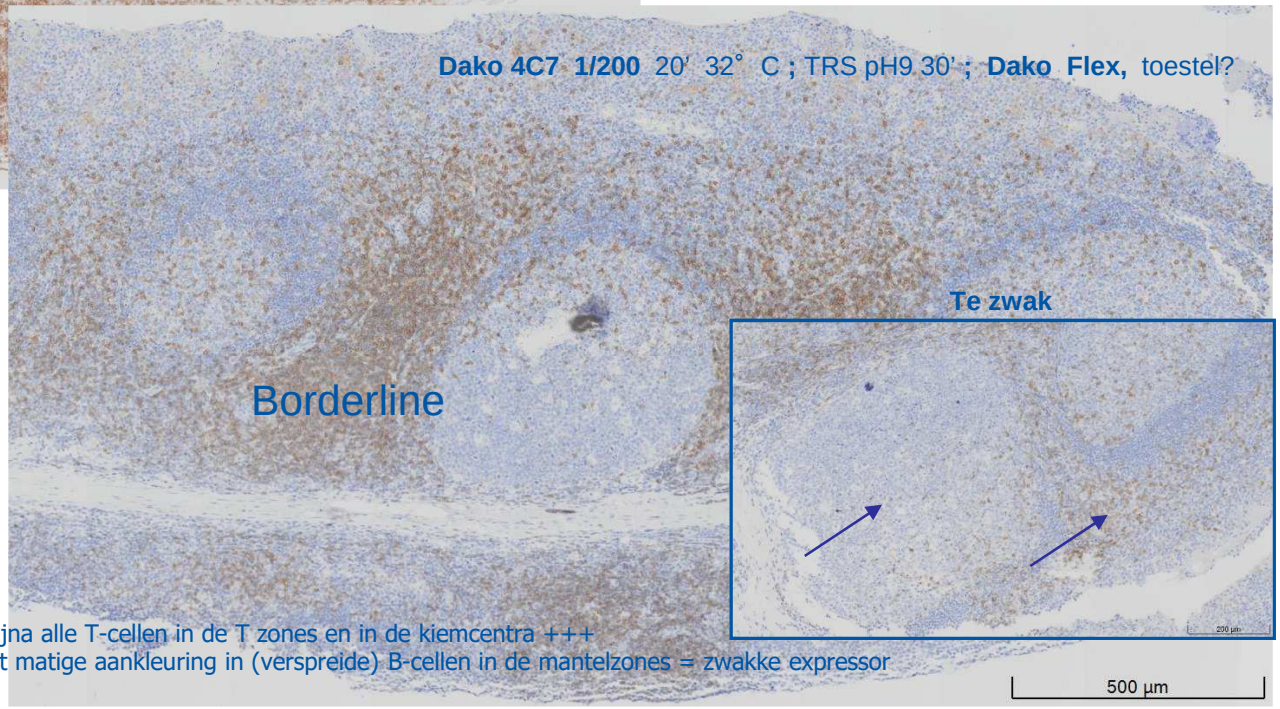
- sterke expressor: bijna alle T-cellen in de T zones en in de kiemcentra +++
- minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones = zwakke expressor

Antistof CD5: tonsil detail



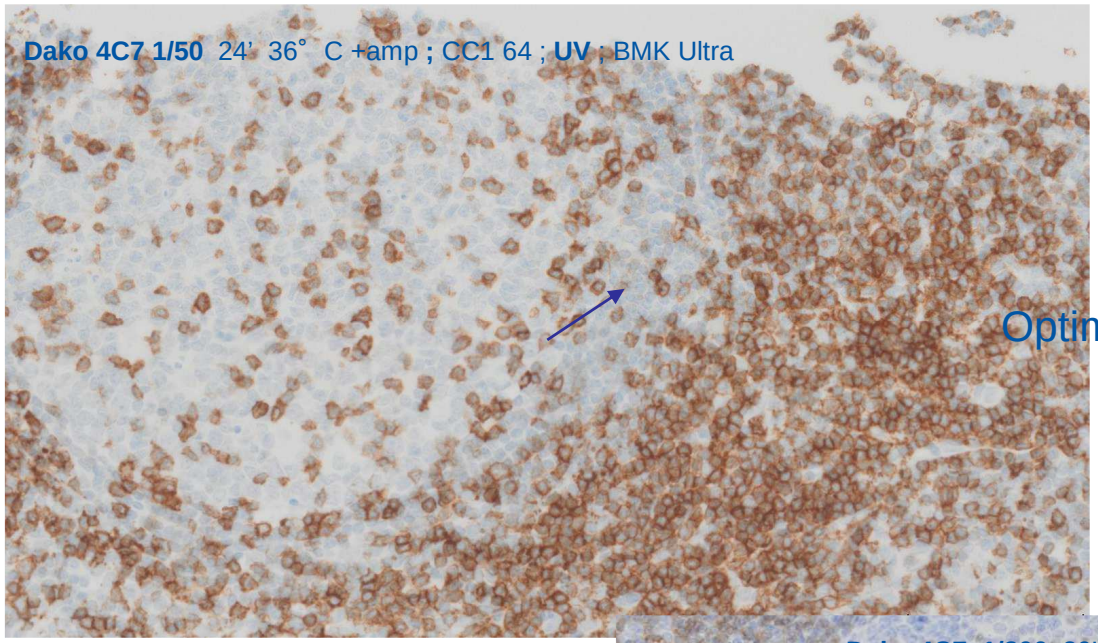
Tonsil 44u



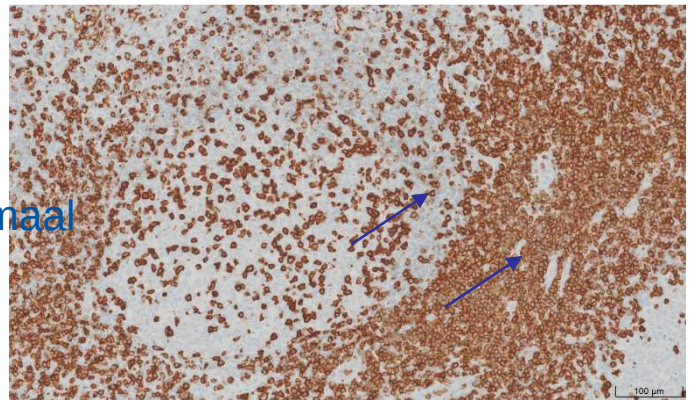


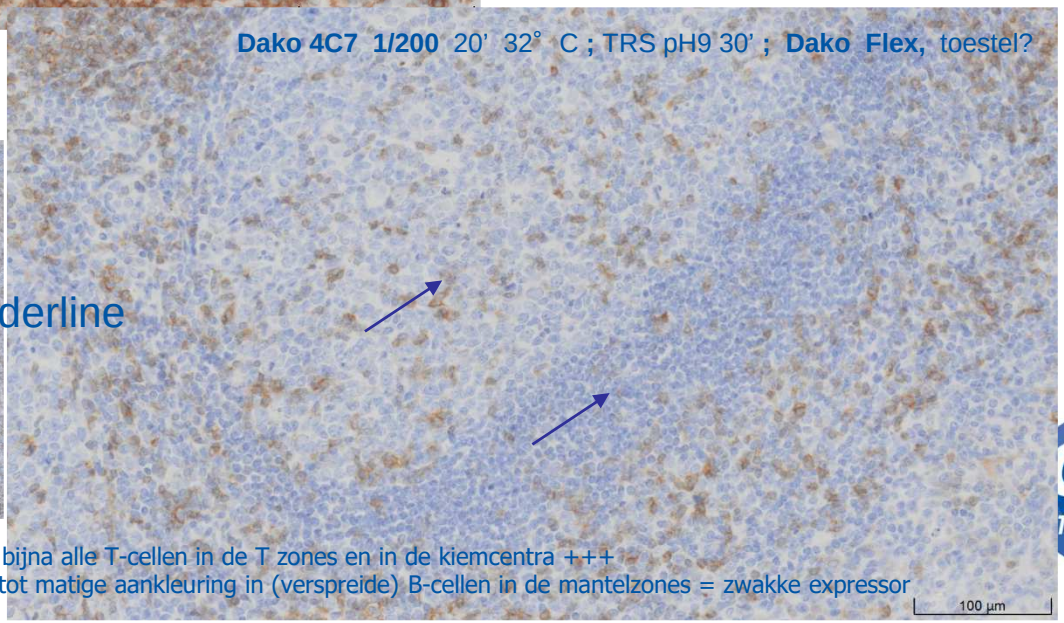
- sterke expressor: bijna alle T-cellen in de T zones en in de kiemcentra +++
- minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones = zwakke expressor

Antistof CD5: tonsil detail

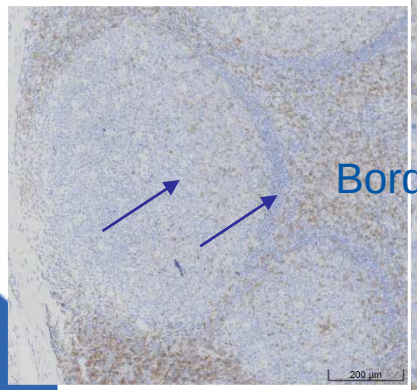


Optimaal



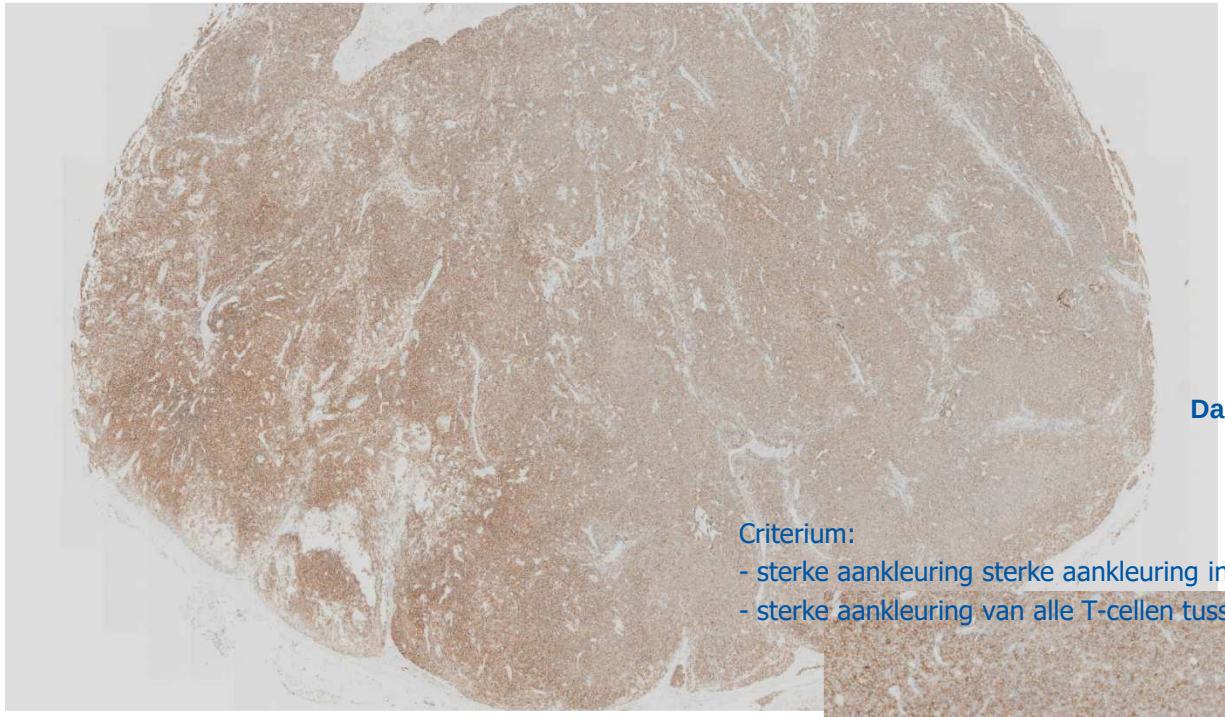


Borderline



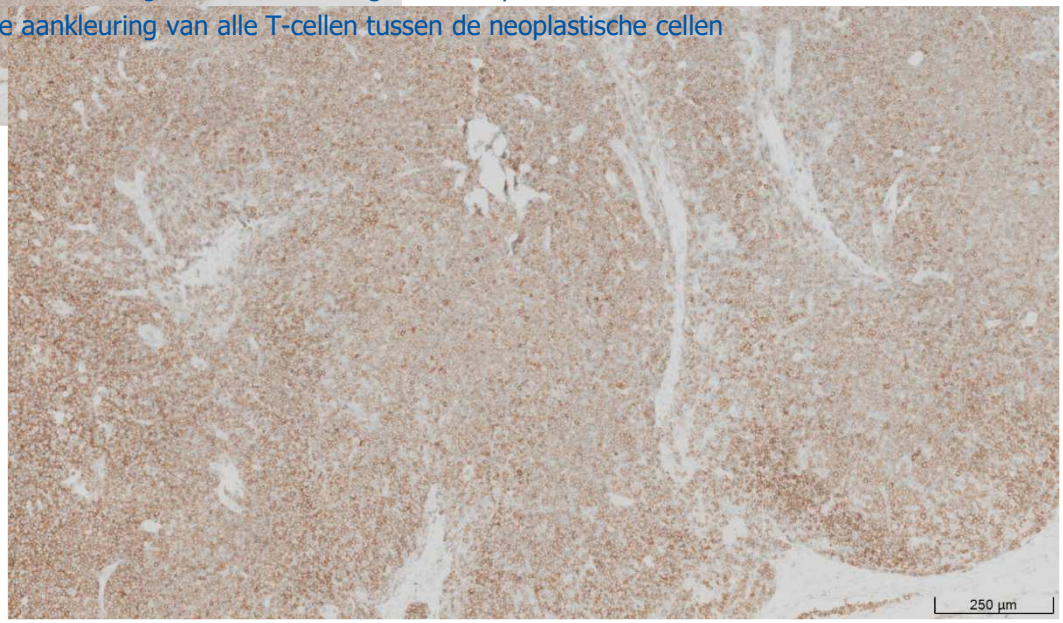
- sterke expressor: bijna alle T-cellen in de T zones en in de kiemcentra +++
- minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones = zwakke expressor

Antistof CD5: B-CLL



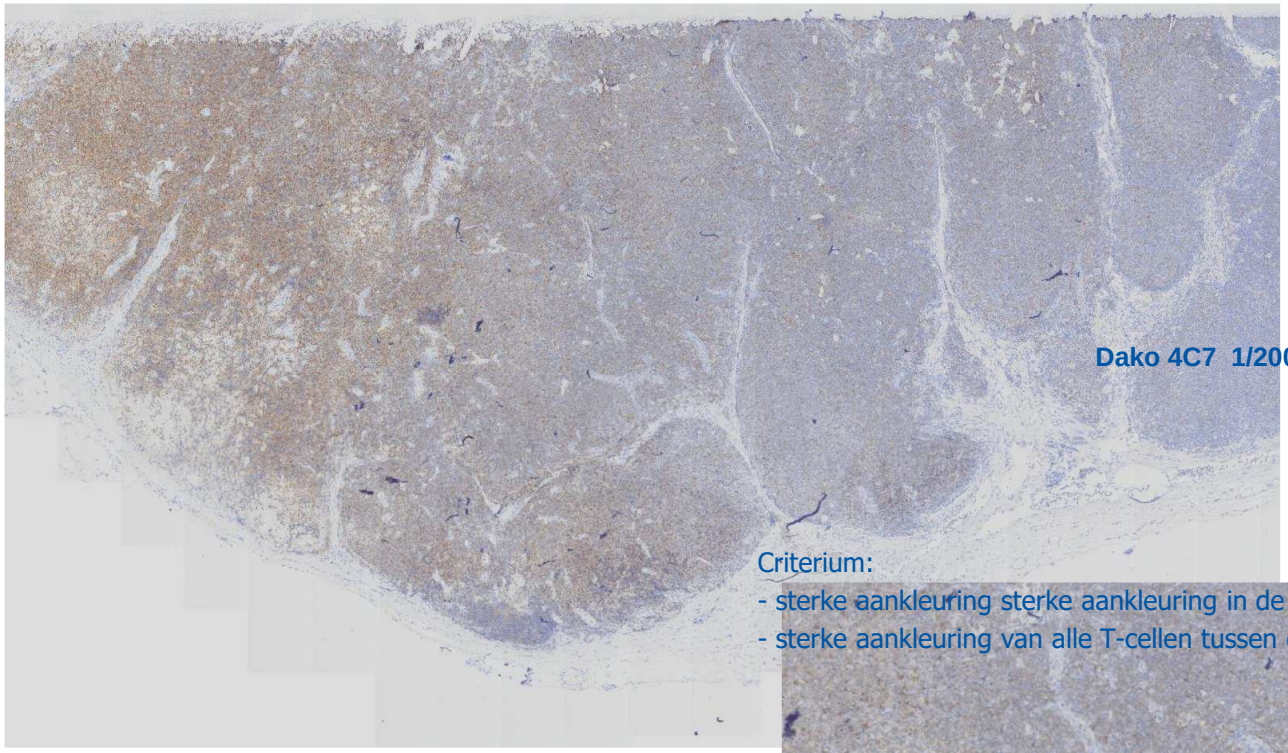
Dako 4C7 1/50 24’ 36° C +amp **;** CC1 64 ; **UV** ; BMK Ultra

Criterium:
- sterke aankleuring sterke aankleuring in de neoplastische cellen +
- sterke aankleuring van alle T-cellen tussen de neoplastische cellen



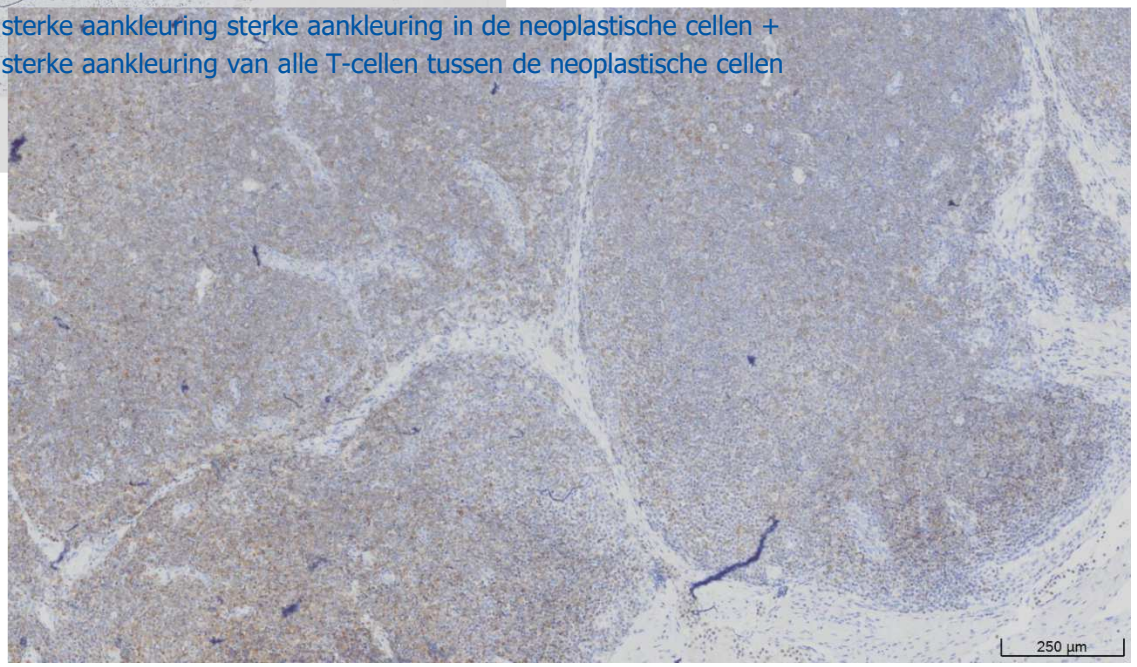
Optimaal

Antistof CD5: B-CLL



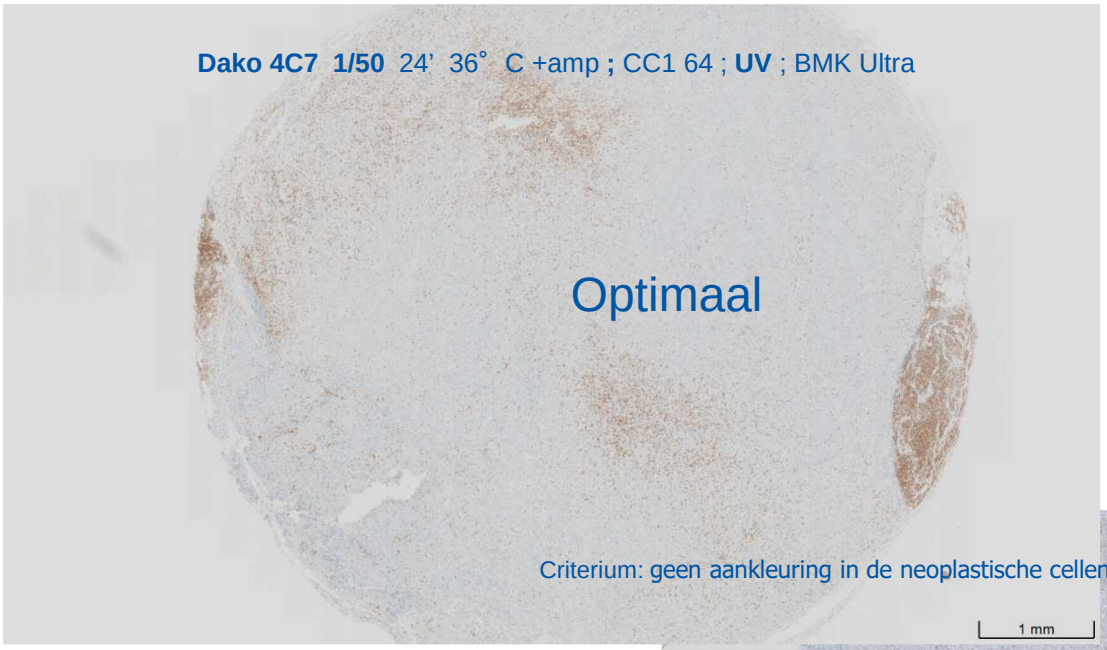
Dako 4C7 1/200 20’ 32° C ; TRS pH9 30’ ; **Dako Flex,** toestel?

Criterium:
- sterke aankleuring sterke aankleuring in de neoplastische cellen +
- sterke aankleuring van alle T-cellen tussen de neoplastische cellen

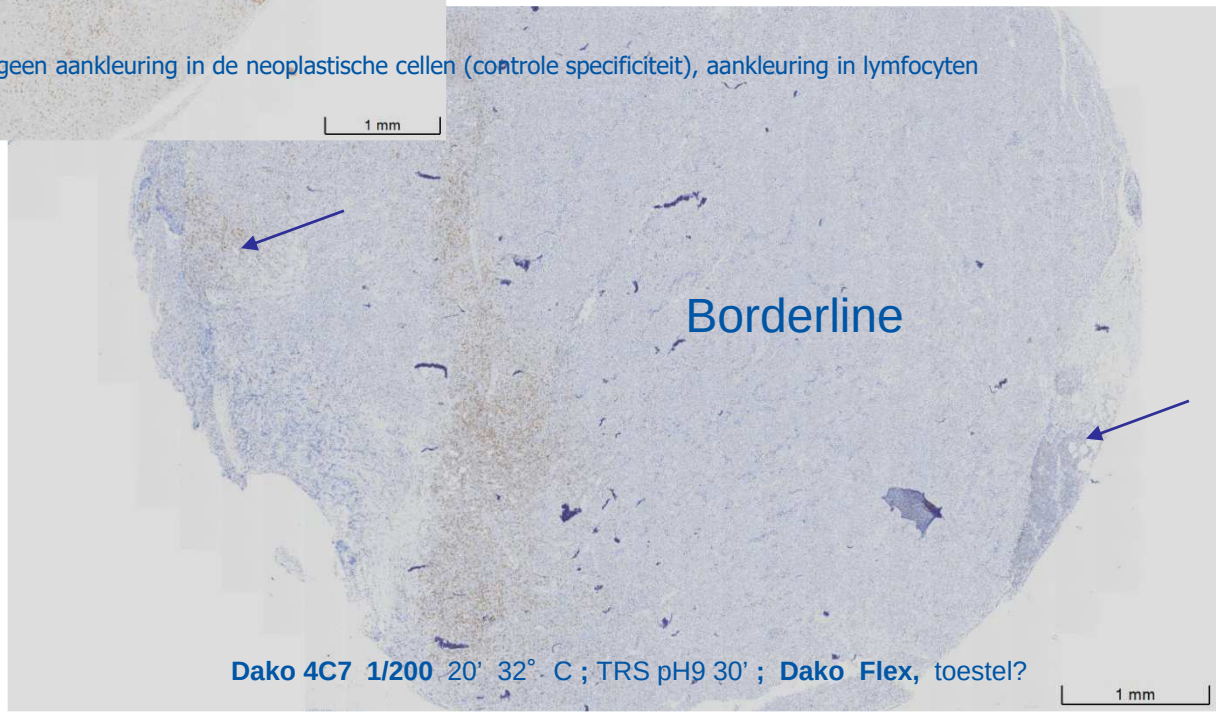


Borderline : te zwak

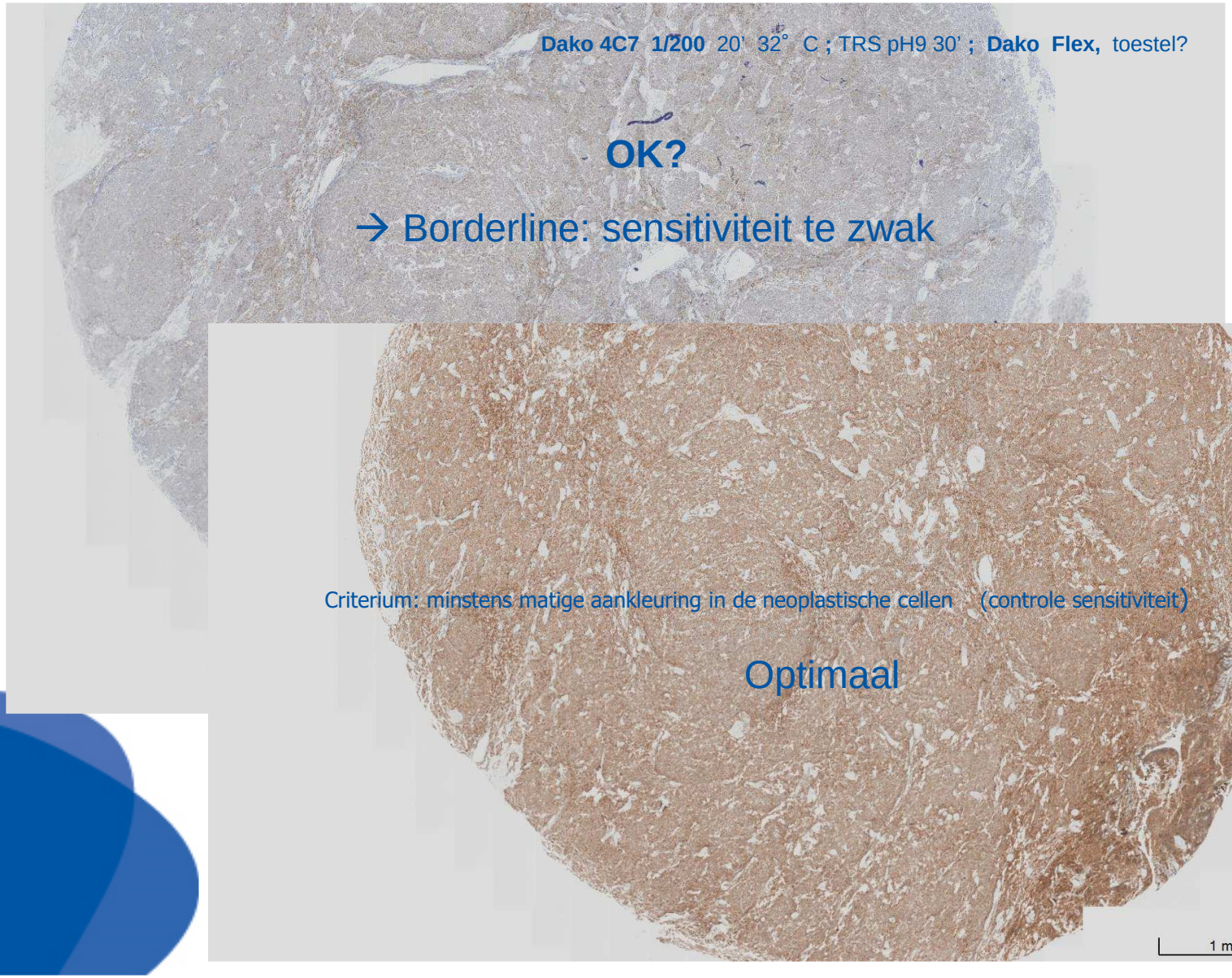
Antistof CD5: DLBCL



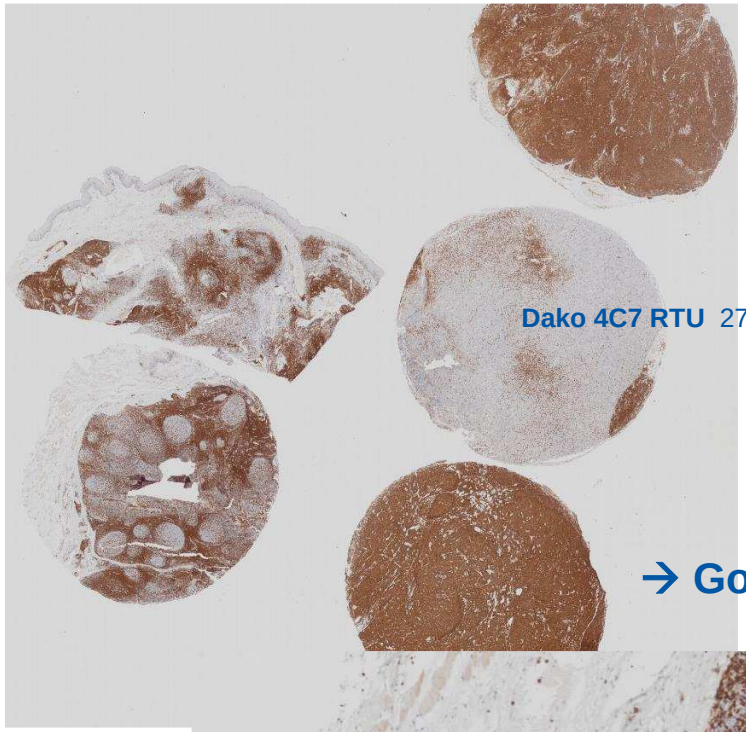
Criterium: geen aankleuring in de neoplastische cellen (controle specificiteit), aankleuring in lymfocyten

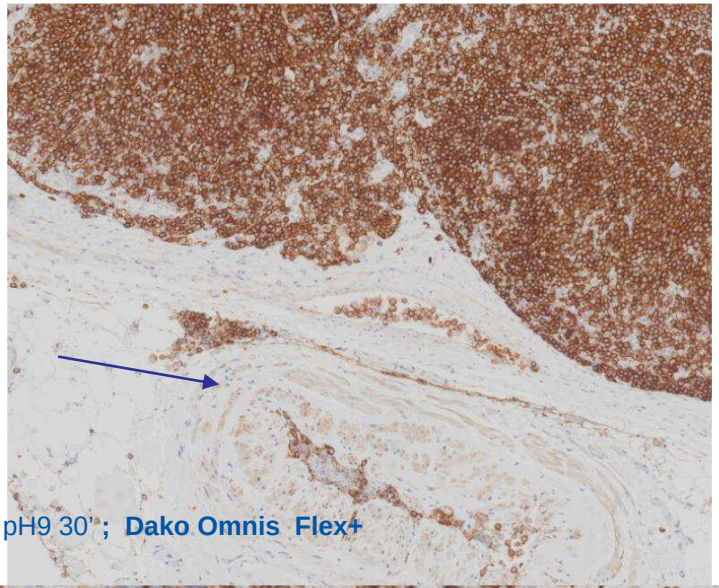


Antistof CD5: MCL



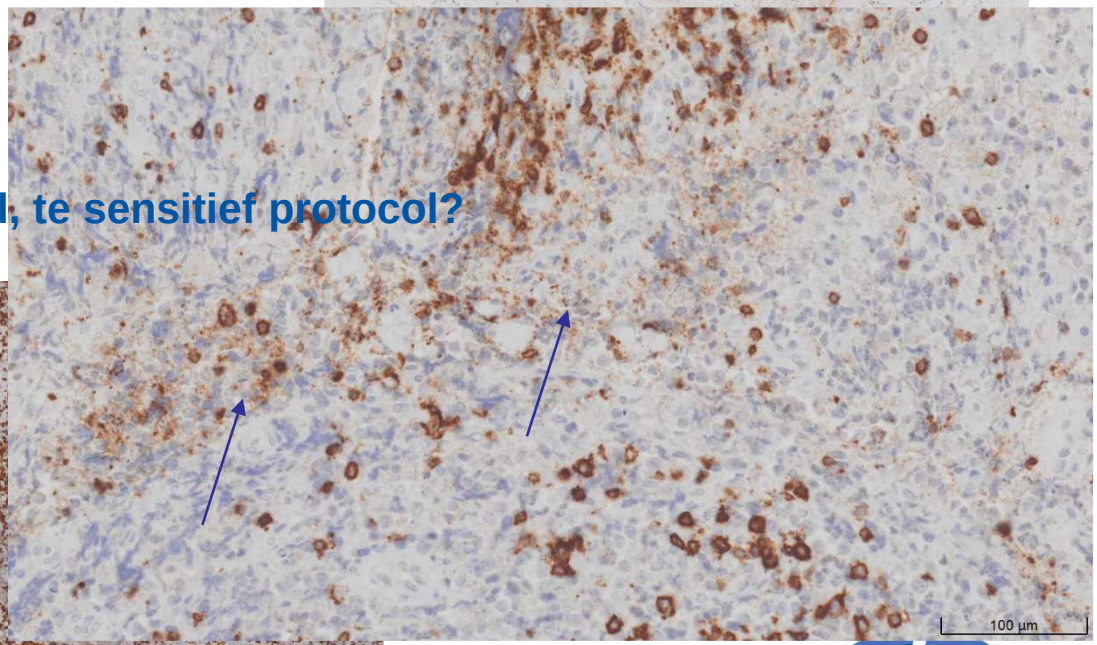
Antistof CD5

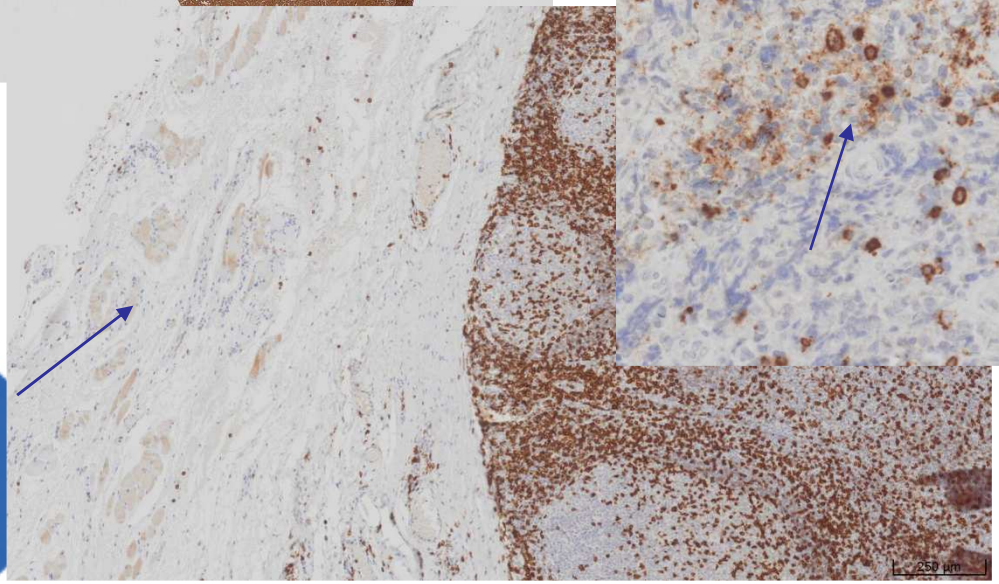




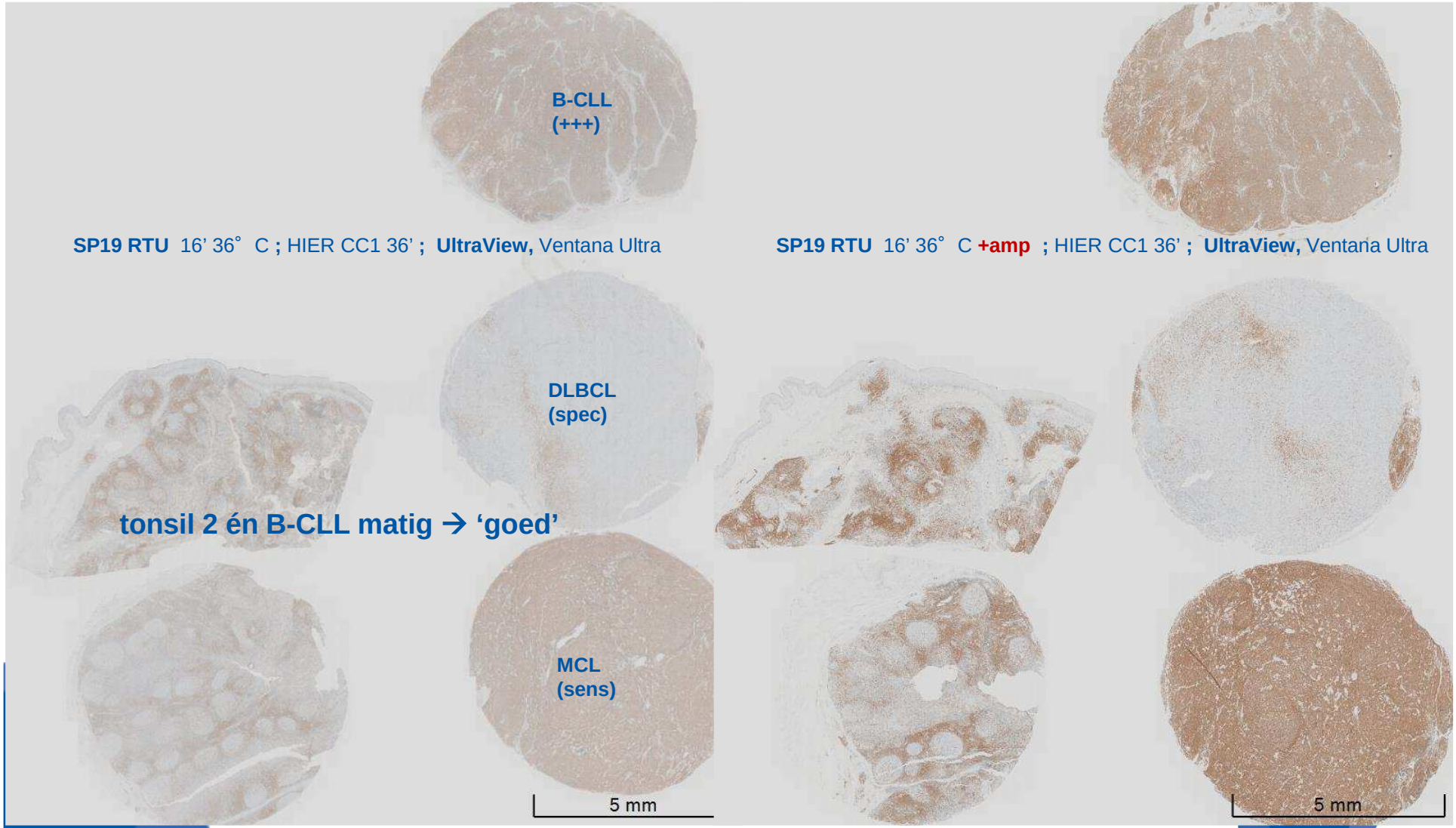
Dako 4C7 RTU 27'30" 32° C **+ linker 10' ;** TRS pH9 30' **; Dako Omnis Flex+**

→ **Goed, te sensitief protocol?**





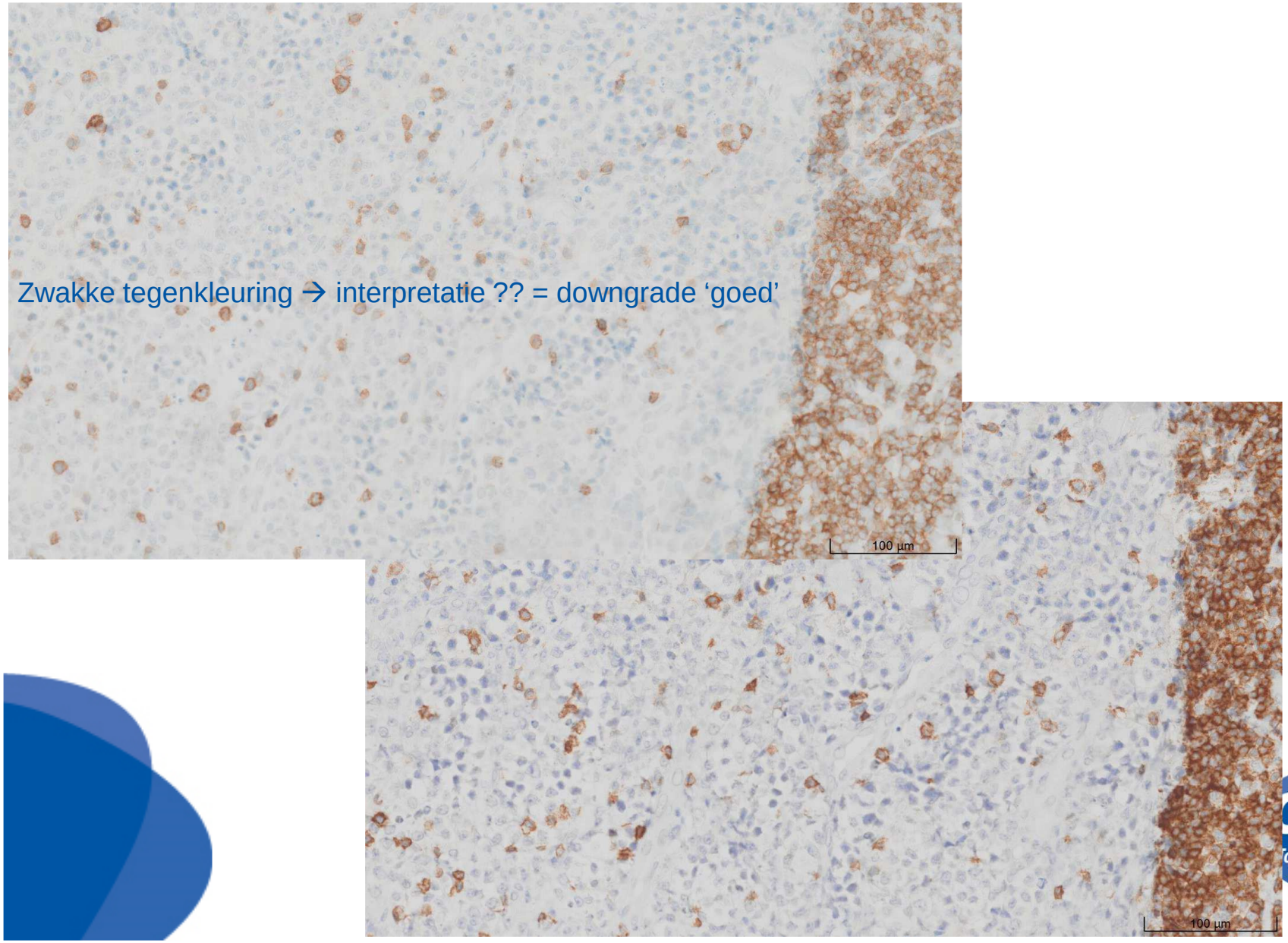
Antistof CD5



Signaalversterker = sensitiviteit OK, specificiteit blijft OK

Top in Zorg.

Resultaatbespreking CD5



Resultaatbespreking CD5

- Algemeen
  - n=63
    - Suff. 98,5 %

		EKE 2022-2		NQC 2017	
	n		*suff*	*%*	*suff*
optimaal	**44**	**70%**	***98,5%***	***68%***	***92%***
goed	**18**	**28,5%**		***24%***	
Borderline	1	1,5%		*6%*	
Onvoldoende	0	0%		*2%*	

Resultaatbespreking CD5

- Opgesplitst per clone producent
  - RTU: 70% optimaal ; suff. 100%

n=55	*Ventana SP19* **RTU**	*% suff*	*Thermo VWR SP19 RTU*	*% suff*	*Dako 4C7 RTU*	*% suff*	*Leica 4C7 RTU*	*% suff*
	n=28		n=2		n=23		n=2	
optimaal	**17**	**61%**	**1**	**-**	**20**	***87%***	***0***	**-**
goed	**11**	**39%**	**1**	**-**	**3**	***13%***	***2***	**-**
borderline	0	-	-	-	0	-	0	-
onvoldoende	0	-	-	-	0	-	0	-
Totaal suff	***100%***		***2/2***		***100%***		**2/2**	
NQC 2017	*99%*		*Geen data*		*92%*		*92%*	

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb 4C7 **IR/IS082**	94% (16/17)	71% (12/17)	95% (21/22)	68% (15/22)
Leica BOND mAb 4C7 **PA0168**	100% (3/3)	100% (3/3)	89% (8/9)	67% (6/9)
VMS Ultra/XT rmAb SP19 **790-4451**	100% (6/6)	33% (2/6)	90% (70/78)	68% (53/78)

Bron NordiQC

Resultaatbespreking CD5

- Opgesplitst per clone producent
  - Concentrated: 71% optimaal ; suff. 86%

n=7	*Dako 4C7*	*% suff*	*Biocare 4C7*	*% suff*	*Leica Novocastra 4C7*	*% suff*
	n=3		n=2		n=2	
optimaal	2	**-**	***2***	**-**	***1***	**-**
goed	0	**-**	***0***	**-**	***1***	**-**
borderline	1	-	*0*	-	*0*	-
onvoldoende	0	-	*0*	-	*0*	-
Totaal suff.	***2/3***		***2/2***		***2/2***	
NQC 2017	*89% optimaal, 93% geslaagd (n=80)*					

Concentrated antibodies	Dako/Agilent Omnis		Ventana BenchMark XT / Ultra/GX		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **4C7**	3/5 ** (60%)	-	15/26 (58%)	-	5/11 (45%)	-
rmAb clone **SP19**	2/3	-	8/11 (73%)	-	0/1	-

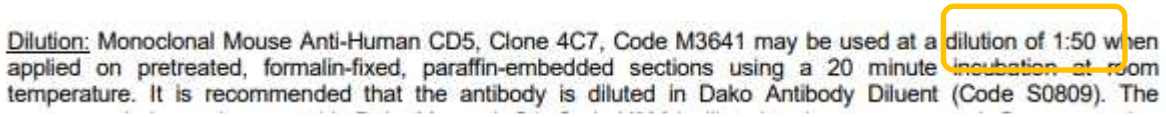
Concentrated antibodies	Dako/Agilent Autostainer Link / Classic		Thermo/LabVision Autostainer 36/48/72		Biocare Medical IntelliPATH	
	TRS pH 9.0	TRS pH 6.1	HIER buffer H pH 9.0	HIER Buffer L pH 6.0	Borg pH 9.0	Diva pH 6.2
mAb clone **4C7**	8/11 ** (73%)	-	-	-	2/2	-
rmAb clone **SP19**	0/4	-	-	-	-	-

Bron NordiQC

Resultaatbespreking CD5

Besluit:

- Suff. ~99% (NQC 2017: 92%)
- Robuustheid (obv fixatietijden): geslaagd
- Sterke expressor (T-cellen) en lage expressor (B-cel mantelzone): geen probleem maar…
- Downgrade optimaal → goed owv te zwakke aankleuring sterke expressoren (SP19) in normaal weefsel/B-CLL
- Resultaat 'borderline' (n=1): analytische sensitiviteit te laag
  → te zwakke aankleuring in alle weefsels
  - Protocol: 1/200 = bovengrens NordiQC (1/20- 1/200 afh. van totale sensitiviteit protocol)
  - Aanbevolen protocolsettings:


Dilution: Monoclonal Mouse Anti-Human CD5, Clone 4C7, Code M3641 may be used at a dilution of 1:50 when applied on pretreated, formalin-fixed, paraffin-embedded sections using a 20 minute incubation at room temperature. It is recommended that the antibody is diluted in Dako Antibody Diluent (Code S0809). The

  - Andere conc. AS: verdunning 1/40- 1/50 (op Roche/Leica stainer)

Resultaatbespreking CD5

Besluit:

- Suff. ~99% (NQC 2017: 92%)

- Result conc. (n=11%)
  - 4C7: slechts 2/7 AS gebruikt op toestel zelfde fabrikant
  - Robuuste clones: protocolspreiding geen impact op resultaat (100% geslaagd) onafh. van type stainer

- Resultaten RTU (n=89%)
  - SP19:
    - protocolspreiding geen impact op resultaat: robuuste clone, 100% geslaagd maar…
    - 8 deelnemers zwak/matige aankleuring T-cellen en B-CLL

      - Roche/Thermo AS
      - allen 2 stap (ultraView) detectie, AS 16', HIER mild/standard
      - vs 7/10 optimaal = 3 stap detectie (optiView) of 2 stap + amp.
      - 4 deelnemers te zwakke tegenkleuring (interpretatie) → downgrade 'goed'

Resultaatbespreking CD5

Besluit:

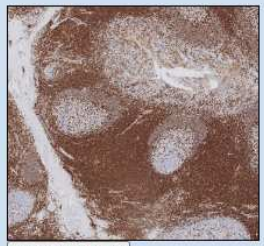
- Suff. ~99% (NQC 2017: 92%)

- Resultaten RTU (n=89%)
  - SP19:
  - Dako 4C7:
    - Op Roche stainers: downgrade owv achtergrond
    - Autostainer: allen optimaal
    - Omnis: allen geslaagd, 94% optimaal
    - ➢ vrij gestandaardiseerd muv AS tijd: geen impact
    - ➢ linker (n=1) → 'goed' owv algemene achtergrond
  - Leica 4C7:
    - Allen op Leica toestel
    - Beiden 'goed'
    - 2 Technische issues (heterogeen…), eigen controle wel OK
      → nieuwe glaasjes opvragen steeds mogelijk !

Resultaatbespreking CD5

Besluit:

- Aanbevolen controle: tonsil = pos. én neg. controle
  - ❑ 57% gebruikte controle
  - ❑ Zwak/matig expressie: B-cellen in mantelzone
  - ❑ Sterke expressor: T-cellen
  - ❑ Negatieve controle: kiemcentrum B-cellen

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Tonsil	Tonsil	Tonsil
Description	Virtually all T-cells, both in the interfollicular T-zones and within germinal centers, must show a strong and distinct predominantly membranous staining reaction.	At least dispersed B-cells in the mantle zone of the secondary follicles must display a weak to moderate and distinct membranous staining reaction. *Note in some tonsils only dispersed mantle zone B-cells are demonstrated, while in other tonsils most mantle zone B-cells are demonstrated. However, irrespectively of the proportion the B-cells must show a distinct staining reaction.*	No staining reaction should be seen in the germinal center B-cells.
Example	Click to enlarge	 Click to enlarge	Click to enlarge

NordiQC leden

Recommended controls

Search:

Epitope	Tissues	Actions
ALK (lung)	Appendix/colon, Tonsil	See controls
AMACR	Kidney, Prostate	See controls
ASMA	Appendix/colon, Liver	See controls

Bron NordiQC

panCK

n= 64

Conc.: n=9

- Leica AE1/AE3 n=1
- Dako AE1/AE3 n=7
- Sanbio (NordicBiosite?) BS5 n= 1

RTU: n=52

- Dako AE1/AE3 n=22
- Roche AE1/AE3/PCK26 n=27
- 1 onbekend

RTU en verder verdund (AE1/AE3/PCK26) n=2

Protocol panCK

- Conc. AS:
  - Sanbio clone BS5
    - Leica Bond III
    - 1/400 15’ RT
    - Voorbehandeling: pH6 20’
  - Leica AE1/AE3
    - Leica Bond III
    - 1/100 15’ RT
    - Voorbehandeling: pH6 10’
  - Dako AE1/AE3
    - Omnis:
      - 1/150 20’ 32° C ; geen linker , HIER pH9 30’, Flex
      - 1/500 20’ 32° C ; geen linker , HIER pH9 30’, Flex+
    - Ventana
      - Slechts 1 verdunning : 1/100
      - 32’ - 72’ bij 36° C
      - HIER CC1 36’ + amplifier
      - HIER CC1 64’ – 92’ zonder amplifier

Spreiding protocolsettings

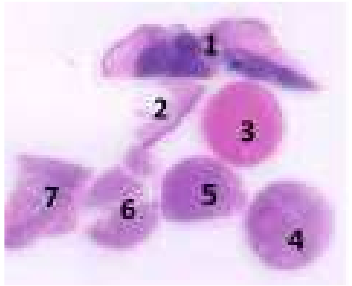
Protocol panCK

- RTU:
  - Roche AE1/AE3/PCK26: geen protocols met enkel HIER
    - UV Red
      - AS 16’ ; enzymatisch protease 1 4’
    - UV DAB enzymatisch
      - AS 20’-32’ geen amplifier ; Protease 1 8’
      - AS 8’ 36° C geen amplifier , Protease 3 4’
    - UV DAB combined enzyme/HIER
      - 10% gebruikt Protease 1, rest protease 3 ; tijd meestal 4’
      - AS 4’ – 32’ ; 20% amplifier
      - HIER 8’ – 64’
    - OV enzymatisch (1)
      - AS 4’ geen amplifier ; Protease 1 4’
    - OV combined enzyme/HIER (1)
      - AS 16’ geen amplifier ; Protease 3 4’ ; HIER CC1 32’

Protocol panCK

- RTU:
  - Dako AE1/AE3
    - Autostainer (4)
      - AS 15’ – 20’ RT ; geen linker
      - HIER pH9 10’ – 30’
    - Omnis
      - AS 10’ – 12’30” bij RT/32° C ; geen linker
      - Enkel HIER pH9 30’
    - Ventana UltraView
      - AS 32’ RT geen amplifier
      - HIER CC1 36’
      - RTU 1/2 verdund, AS 16’ , geen amplifier ; P3 4’ én CC1 36’ (n=2)
  - Leica AE1/AE3
    - AS 15’ RT geen linker ; HIER pH6 10’ ; Bond III
    - AS 20’ RT geen linker ; pH9 20’ ; Bond III/Max

Antistof panCK



- Scoringscriteria: cytoplasma/membraan aankleuring

- Tonsil :

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige aankleuring in de interstitiële reticulum cellen ('cytokeratin-positive interstitial reticulum cells' of 'CIRC') (controle sensitiviteit)
- geen aankleuring in de lymfocyten (controle specificiteit)

- Slokdarm :

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige ('patchy') aankleuring in de gladde spiercellen van de bloedvaten en van de muscularis mucosae (controle sensitiviteit)

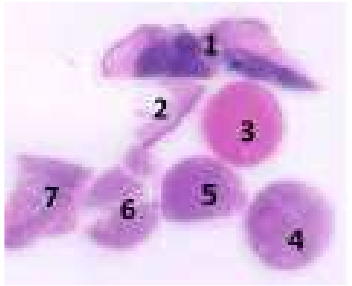
- Lever :

- sterke aankleuring in de epitheliale cellen van de galwegen
- minstens matige aankleuring in de meeste hepatocyten mét membranaire accentuatie (controle sensitiviteit)
- geen aankleuring in de stromale cellen (controle specificiteit)

Bron: NordiQC Run 59

olv Top in Zorg.

Antistof panCK



- Scoringscriteria: cytoplasma/membraan aankleuring

- Long, adenocarcinoom :

- sterke aankleuring van bijna alle neoplastische cellen

- Long, plaveiselcelcarcinoom :

- sterke aankleuring van bijna alle neoplastische cellen

- Nier, CCRCC :

- zwakke tot matige (voornamelijk membranaire) aankleuring van de meeste neoplastische cellen (controle analytische sensitiviteit)

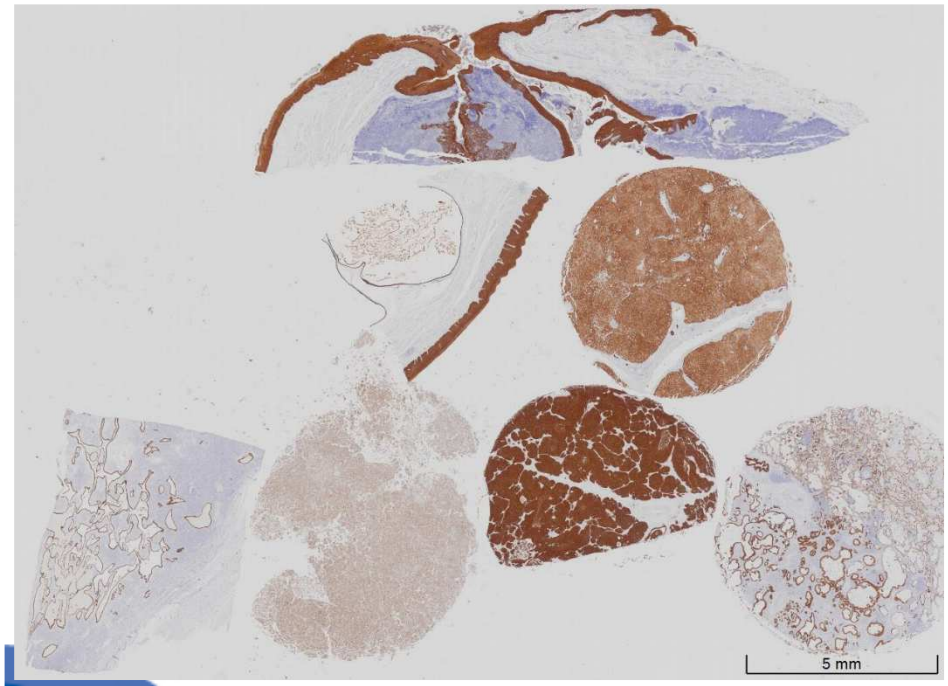
- **Testis, DLBCL :**

- geen aankleuring van de neoplastische cellen (controle analytische specificiteit)

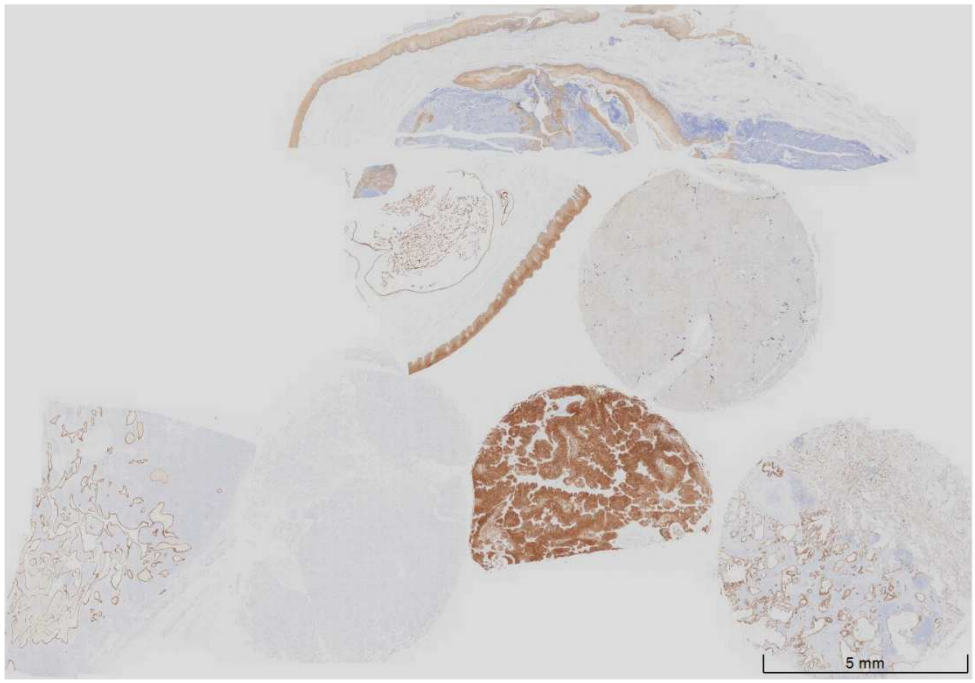
Bron: NordiQC Run 59

Antistof panCK: overzicht

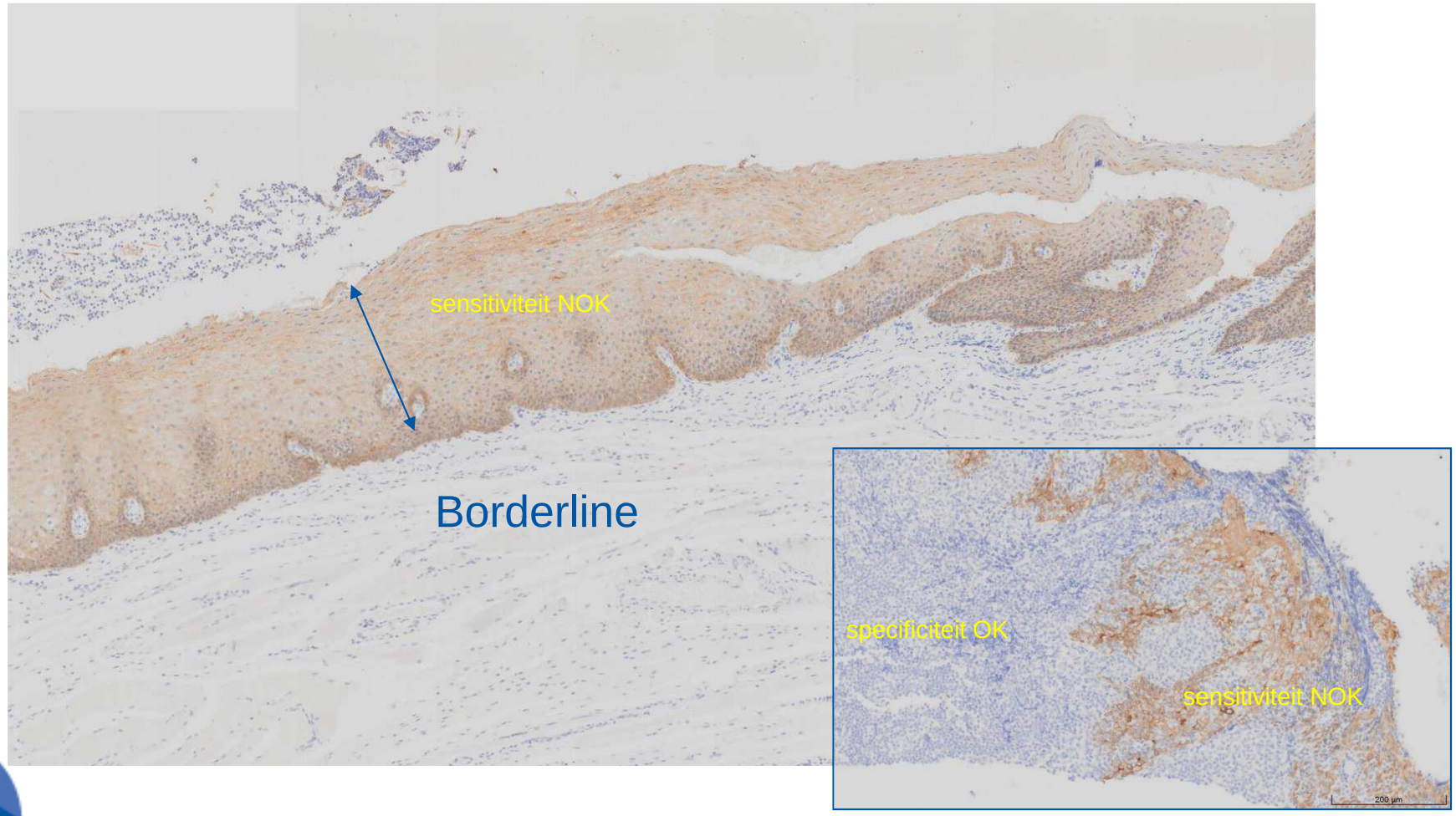
Optimaal



Onvoldoende



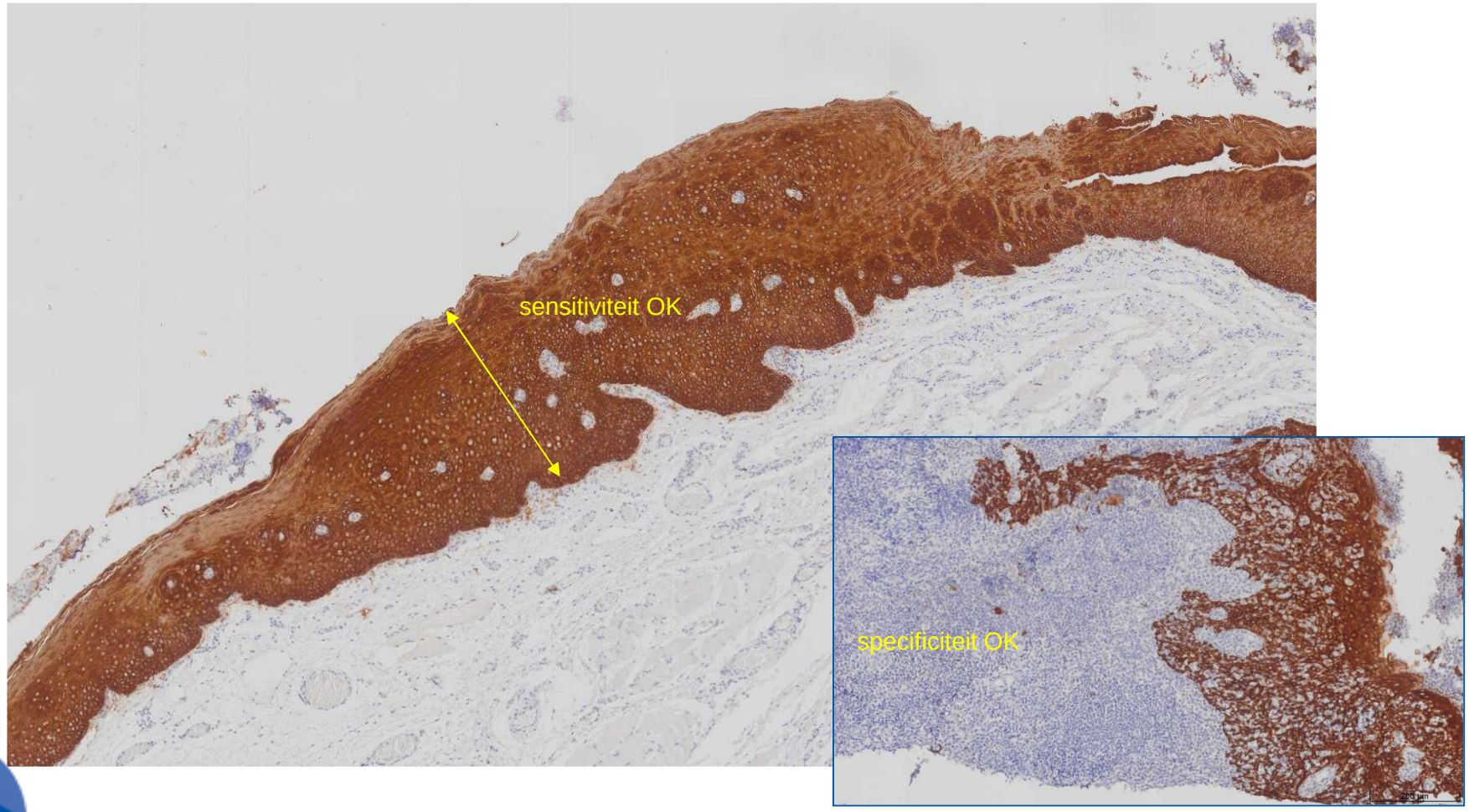
Antistof panCK: tonsil



Criterium:

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige aankleuring in de interstitiële reticulum cellen of 'CIRC') (controle sensitiviteit)
- geen aankleuring in de lymfocyten (controle specificiteit)

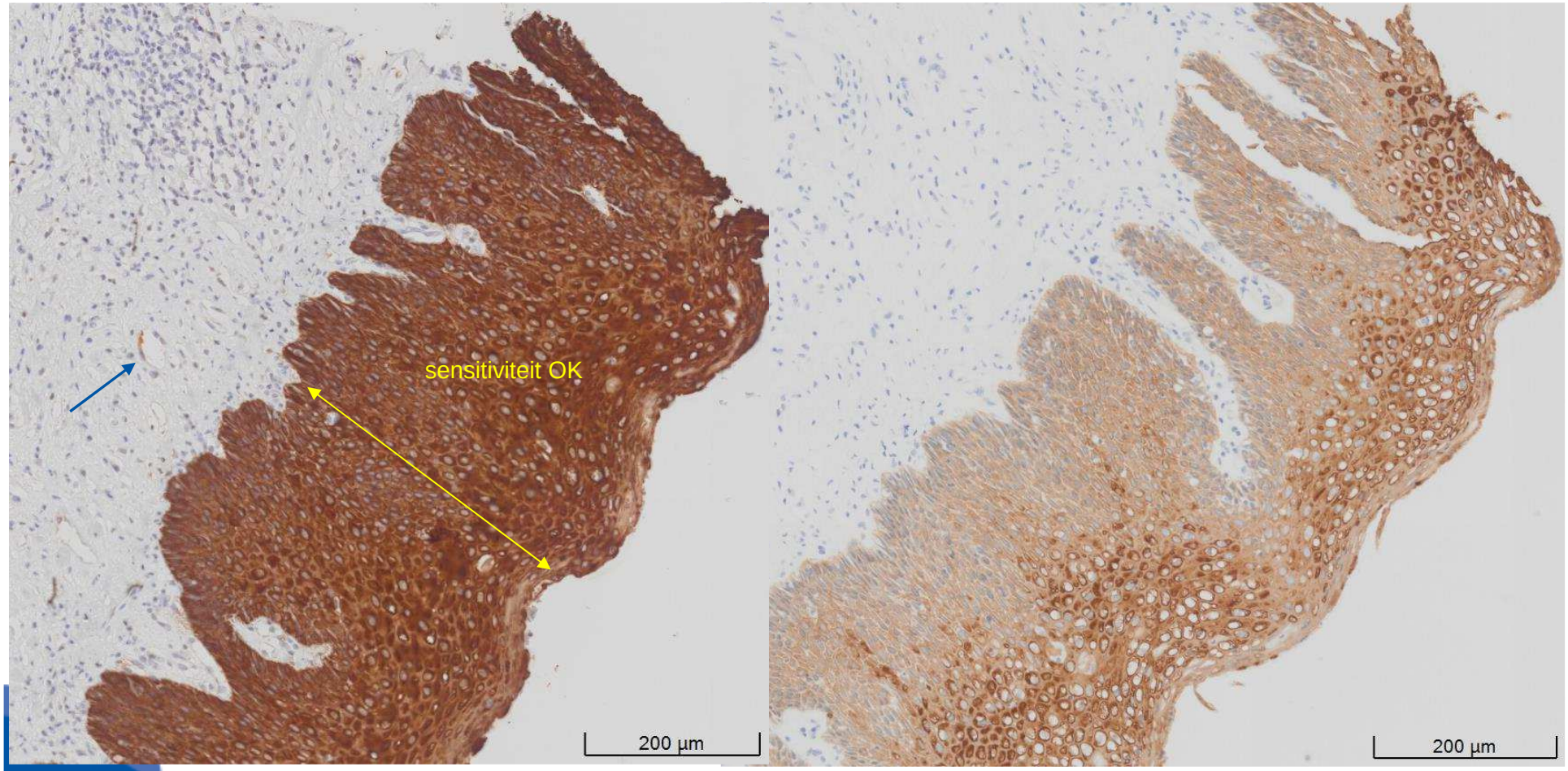
Antistof panCK: tonsil



Criterium:

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige aankleuring in de interstitiële reticulum cellen of 'CIRC') (controle sensitiviteit)
- geen aankleuring in de lymfocyten (controle specificiteit)

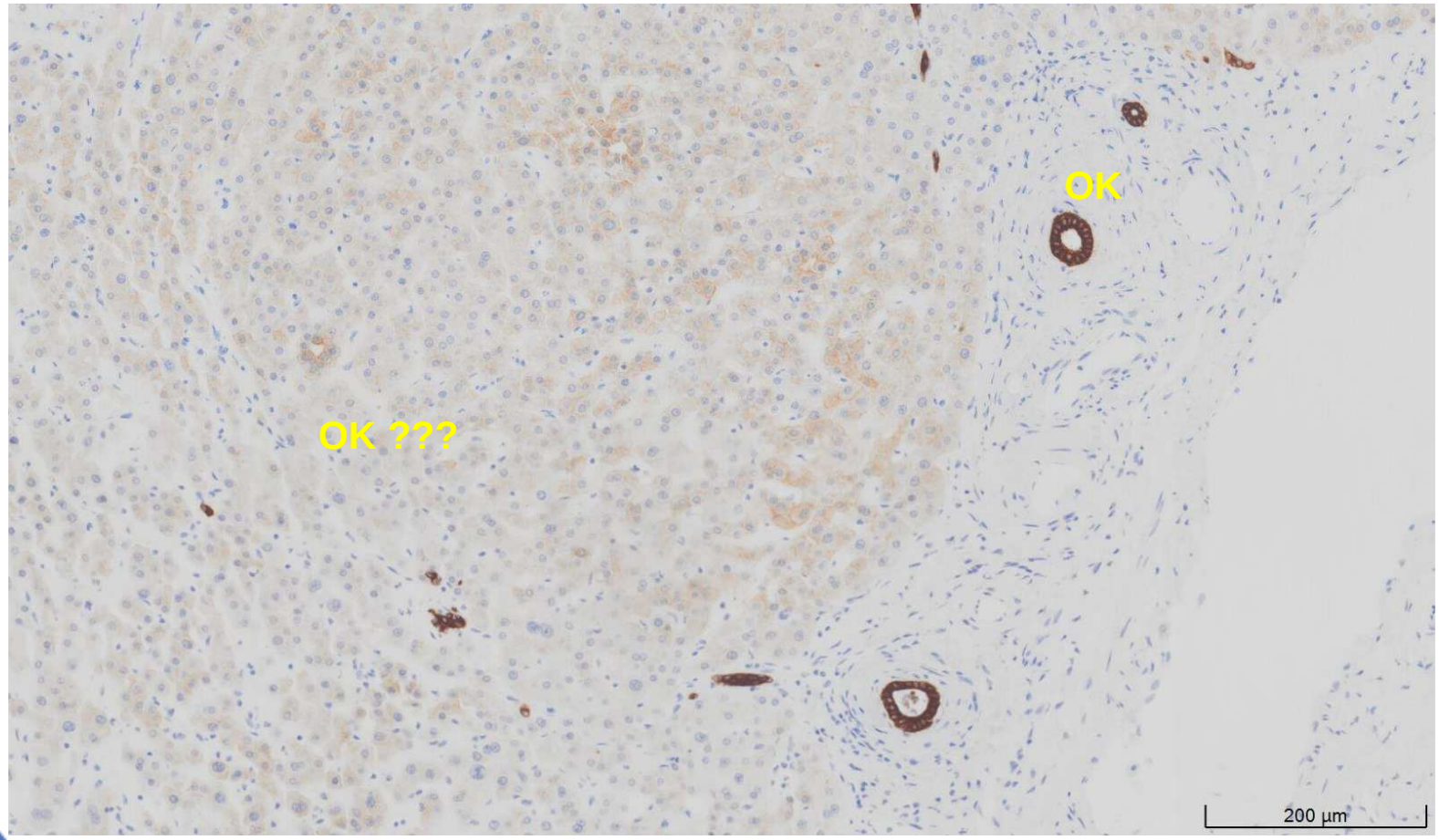
Antistof panCK: slokdarm



Criterium:

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige ('patchy') aankleuring in de gladde spiercellen van de bloedvaten en van de muscularis mucosae (controle sensitiviteit)

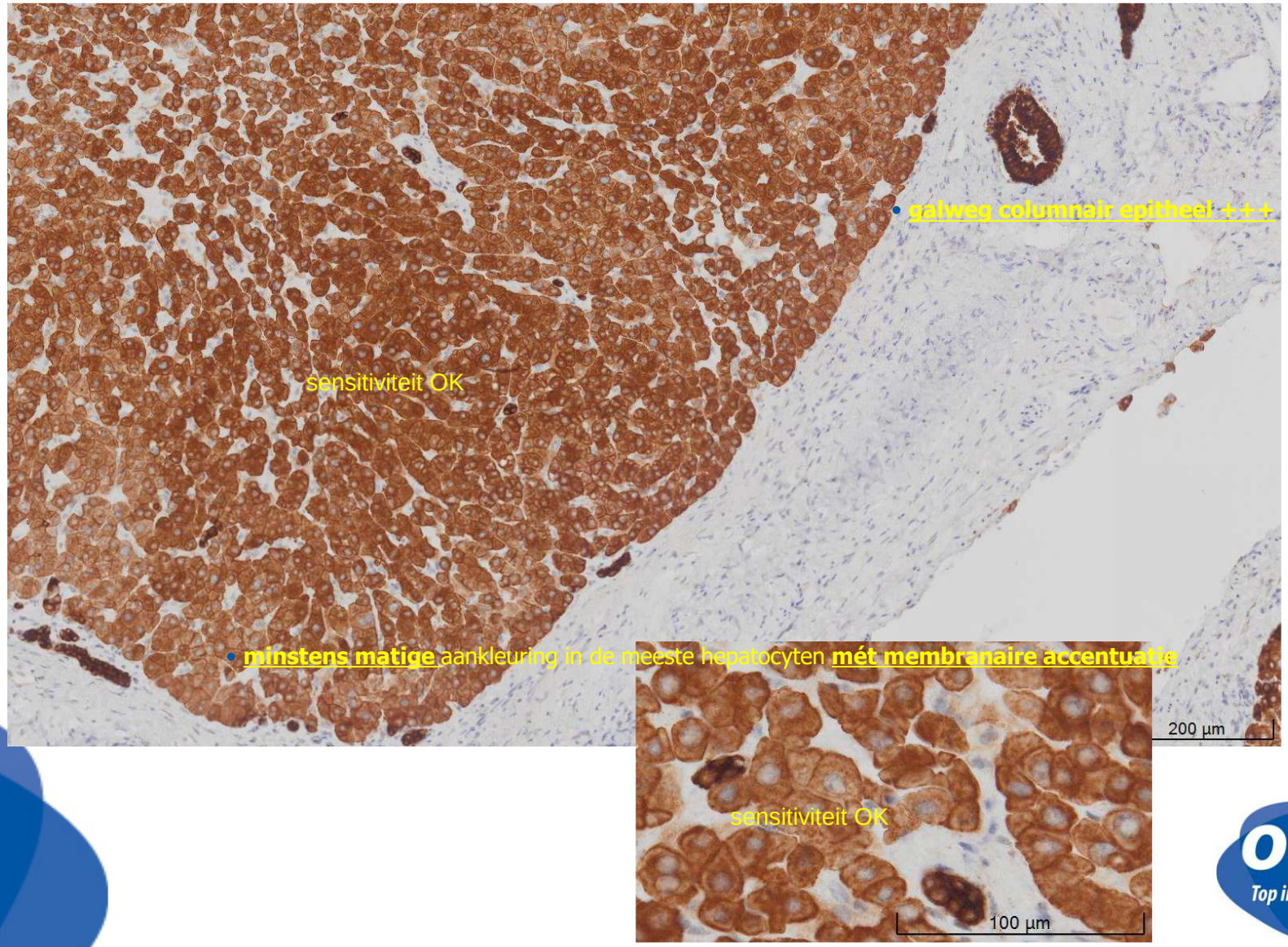
Antistof panCK: lever



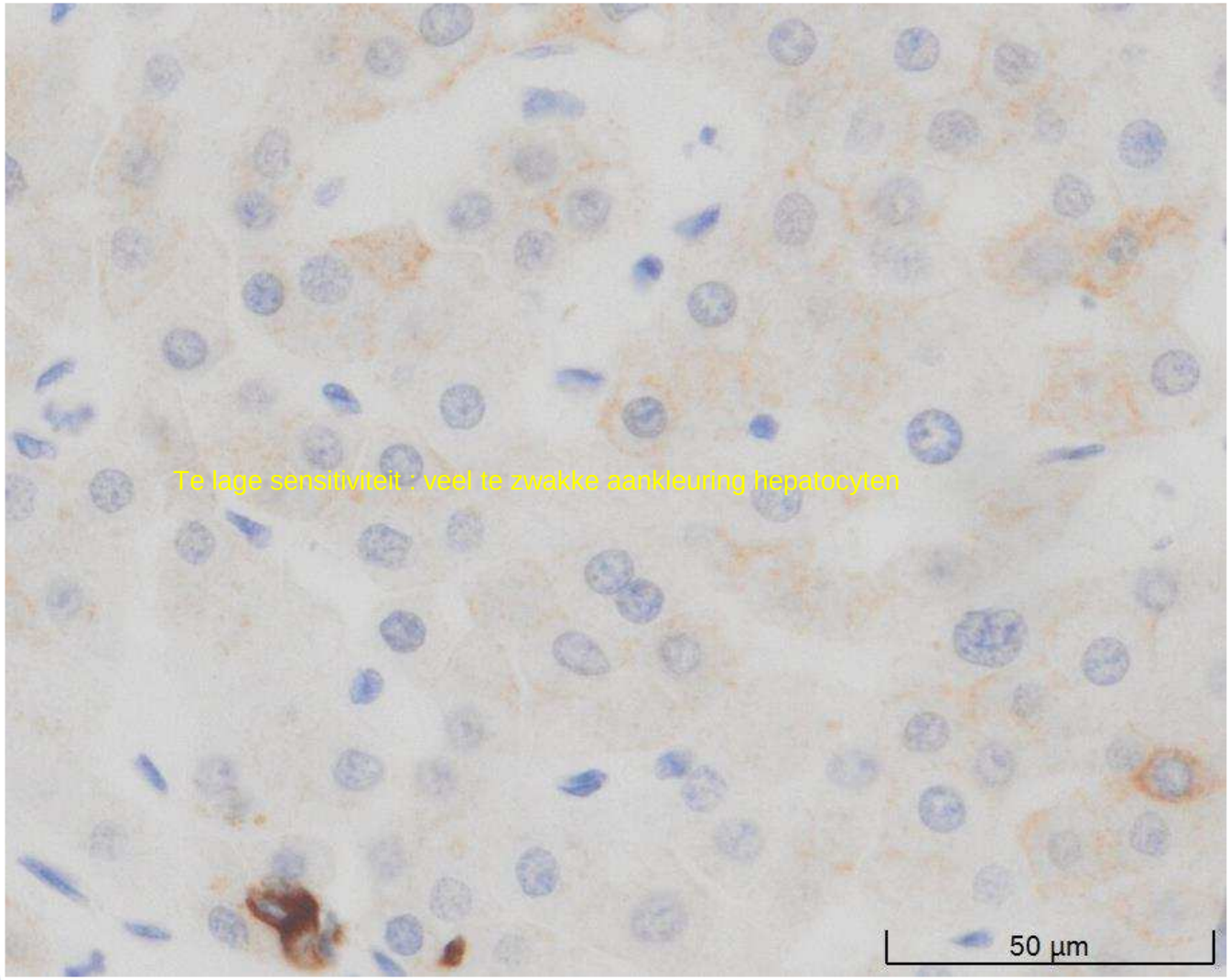
Criterium:

- sterke aankleuring in de epitheliale cellen van de galwegen
- minstens matige aankleuring in de meeste hepatocyten mét membranaire accentuatie (controle sensitiviteit)
- geen aankleuring in de stromale cellen (controle specificiteit)

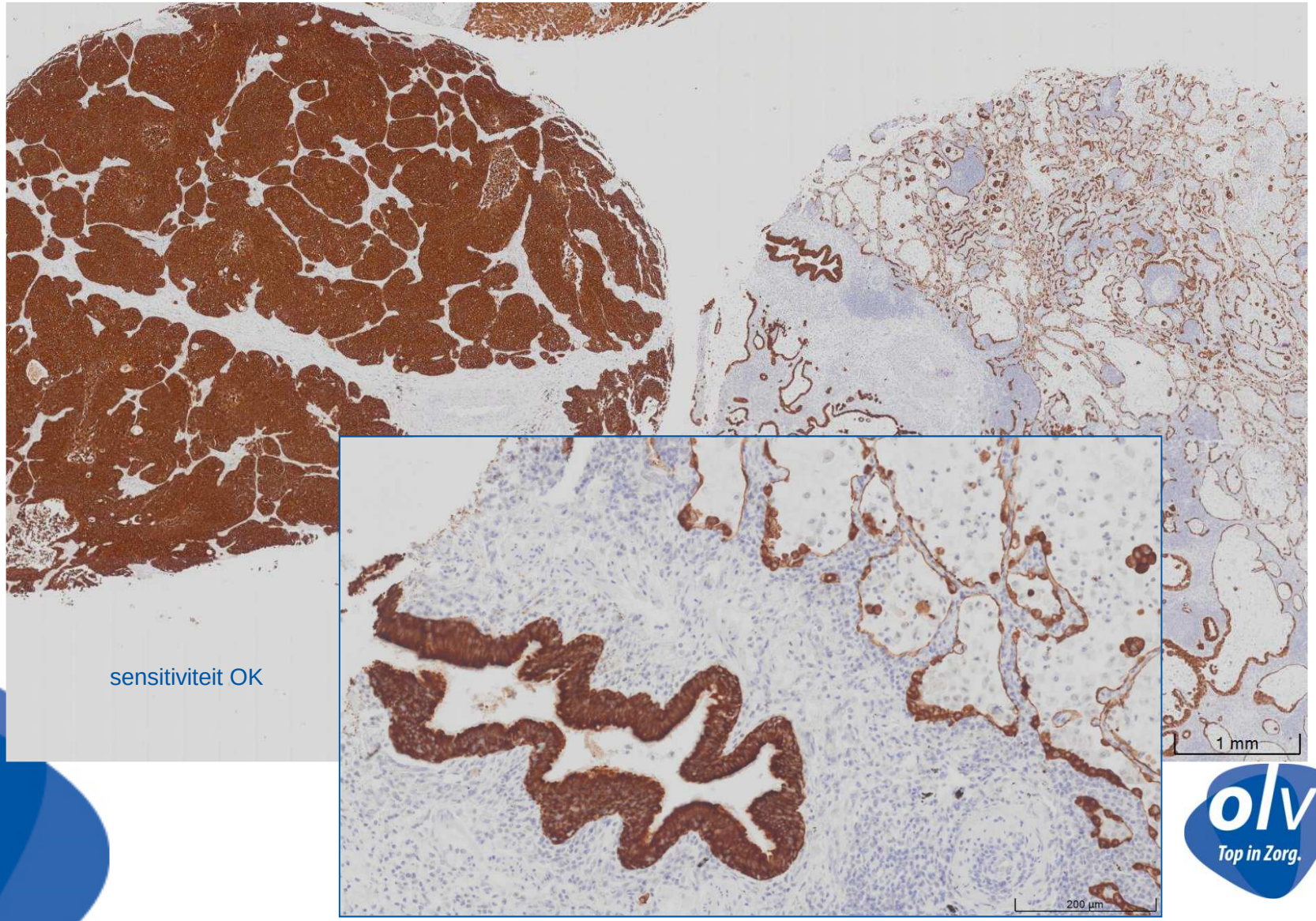
Antistof panCK: lever



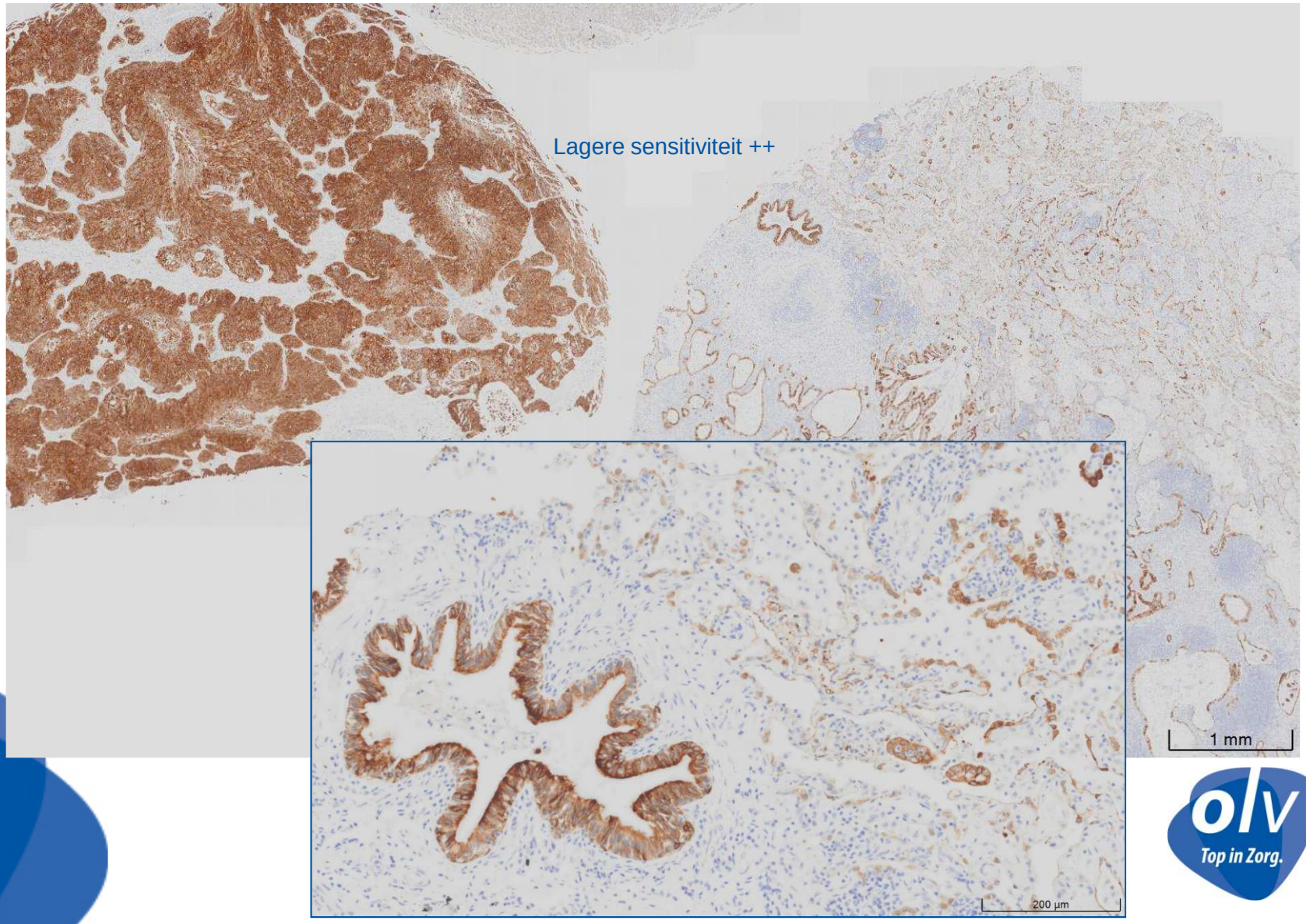
Antistof panCK: lever



Antistof panCK: long squameus Ca + AdCa

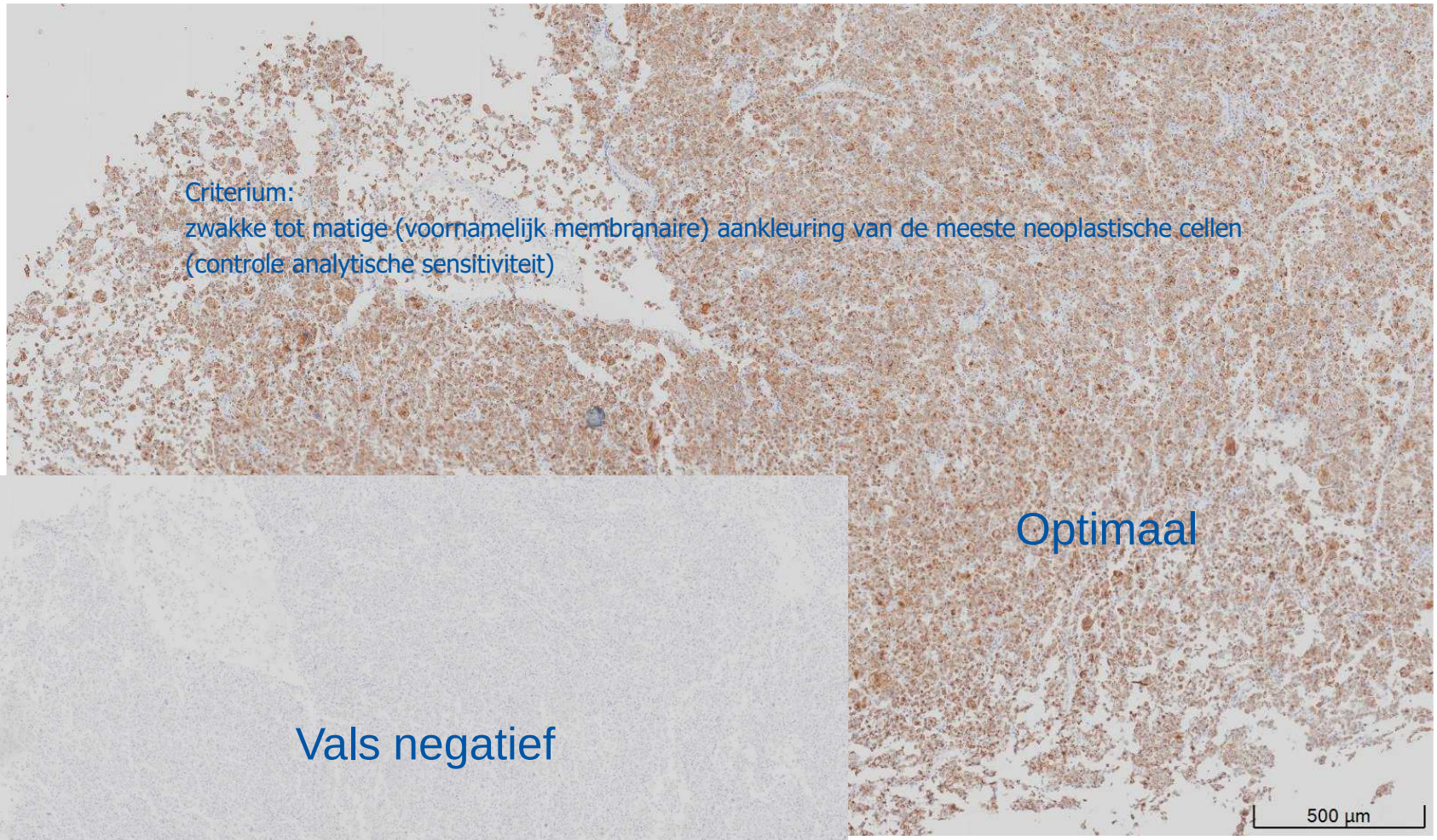


Antistof panCK: long squameus Ca + AdCa

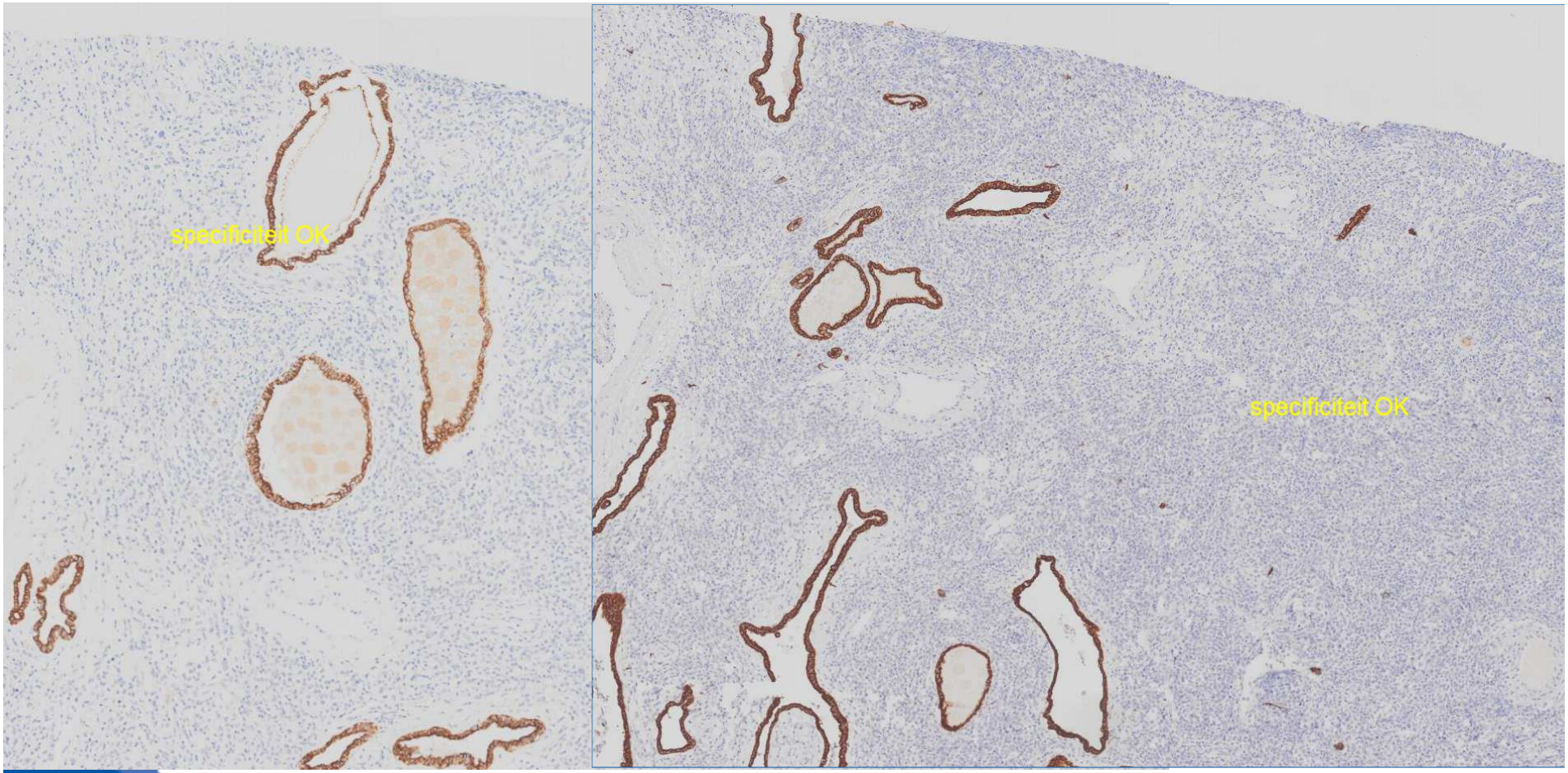


Antistof panCK: nier CRCC

Antistof panCK: nier CRCC

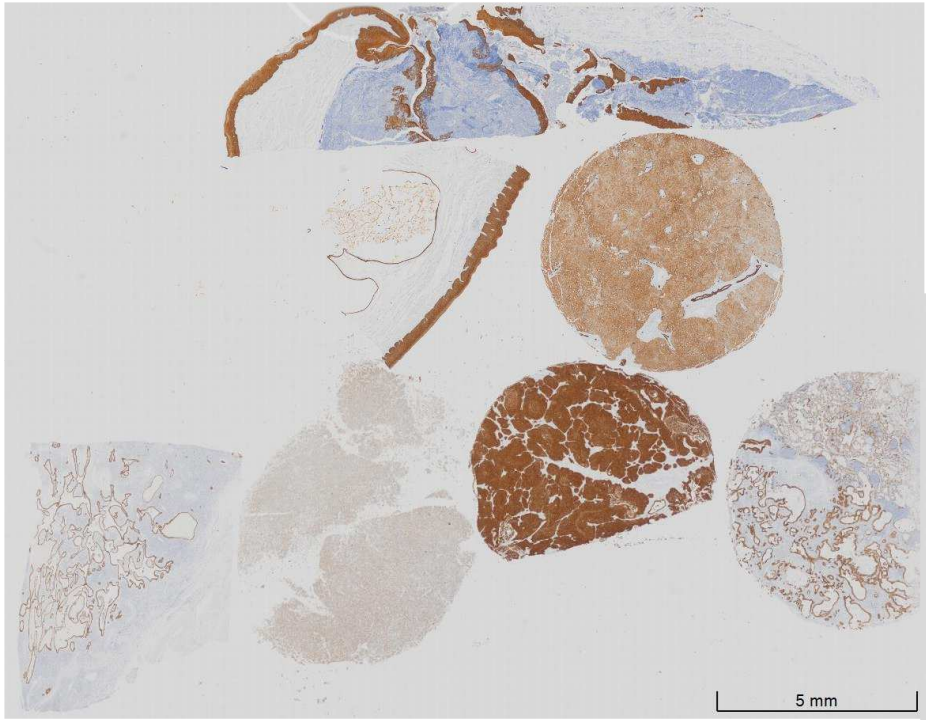


Antistof panCK: testis DLBCL

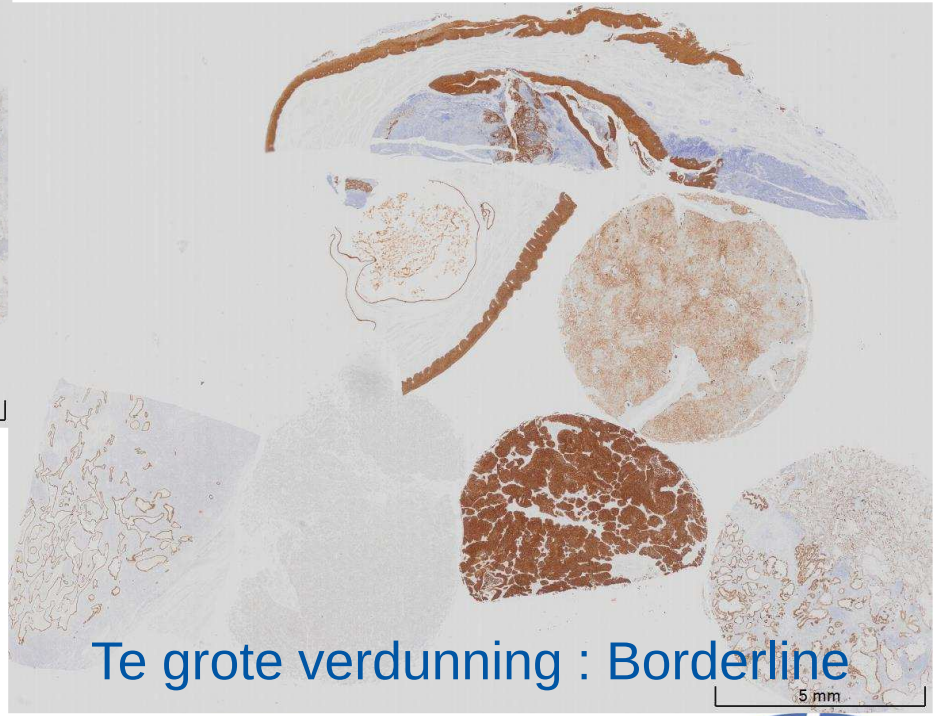


Criterium: geen aankleuring van de neoplastische cellen (controle analytische specificiteit)

Antistof panCK: verdunning



Dako AE1/AE3 1/150 20’ 32° C ; HIER pH9 ; Dako Omnis **Flex+**



Te grote verdunning : Borderline

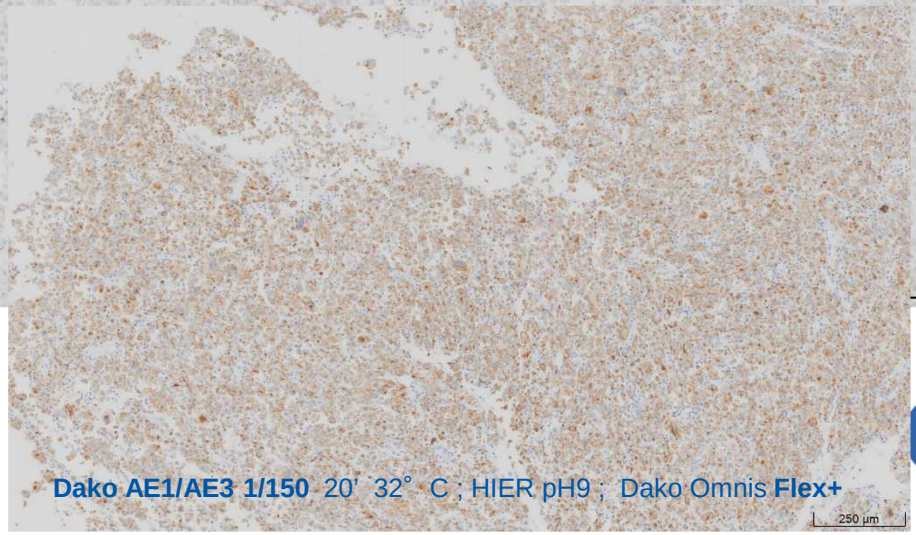
Dako AE1/AE3 1/500 20’ 32° C ; HIER pH9 ; Dako Omnis **Flex**

Antistof panCK: verdunning

Dako AE1/AE3 1/500 20’ 32° C ; HIER pH9 ; Dako Omnis **Flex**

Nier CRCC OK?

Te lage sensitiviteit: veel te zwak



Dako AE1/AE3 1/150 20’ 32° C ; HIER pH9 ; Dako Omnis **Flex+**

250 µm

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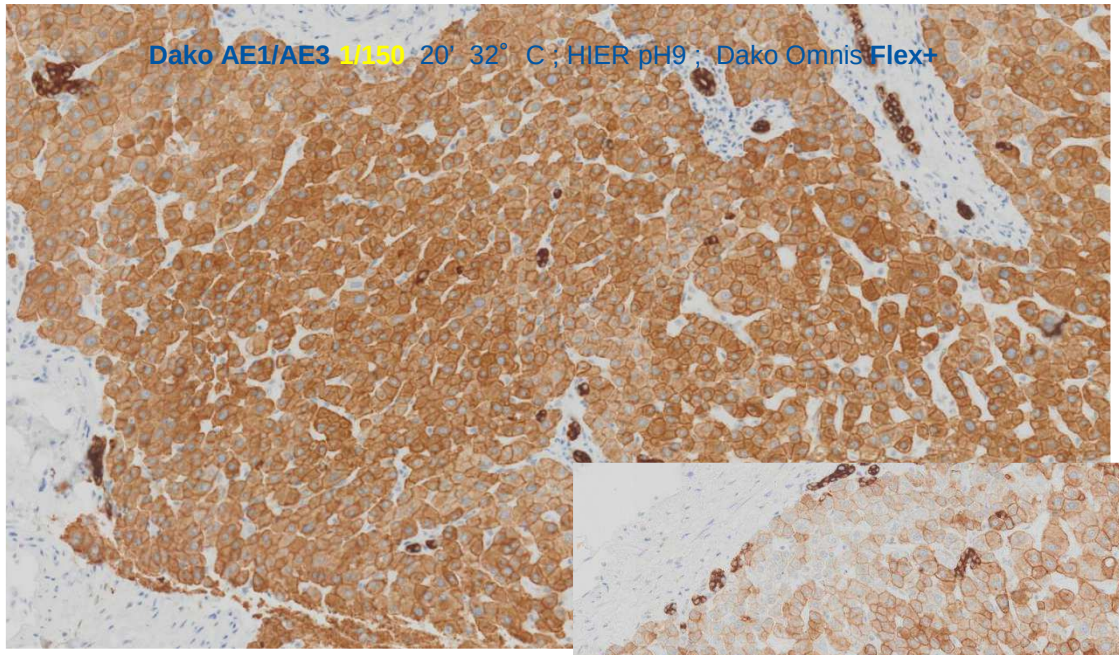
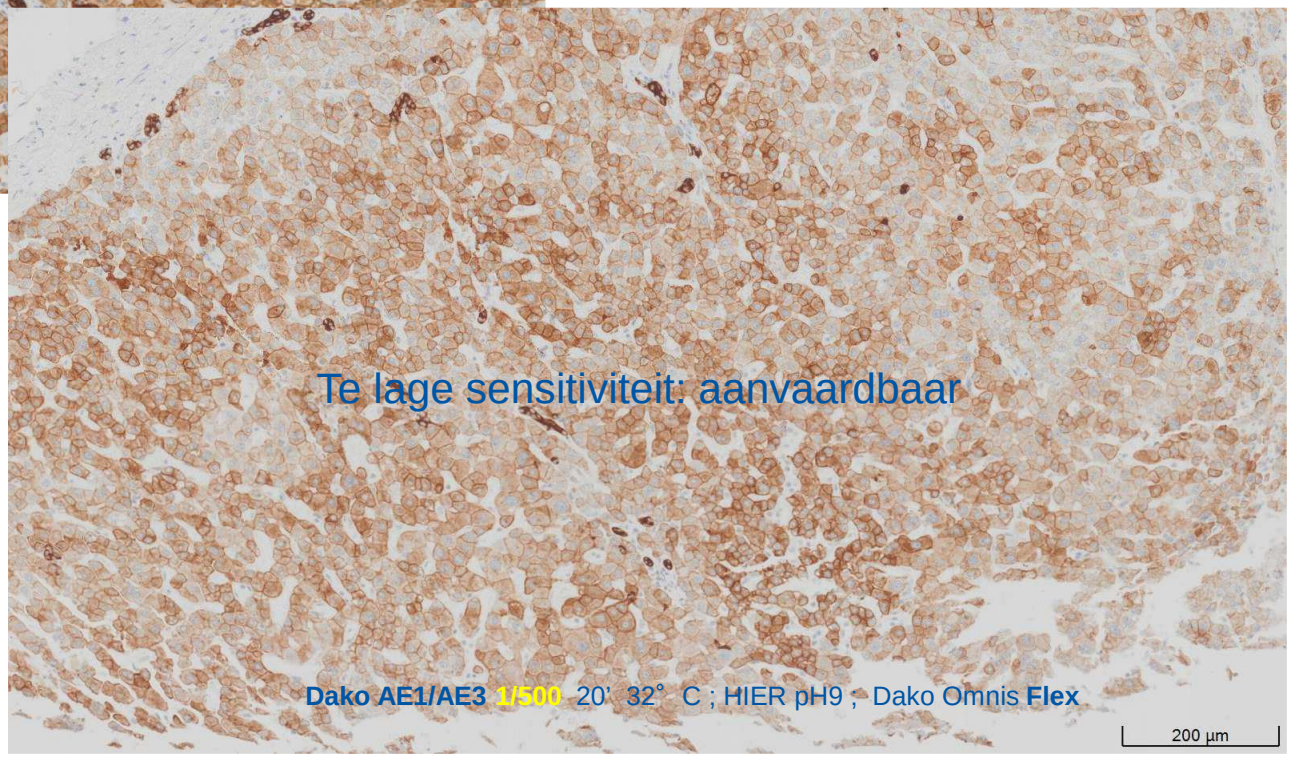
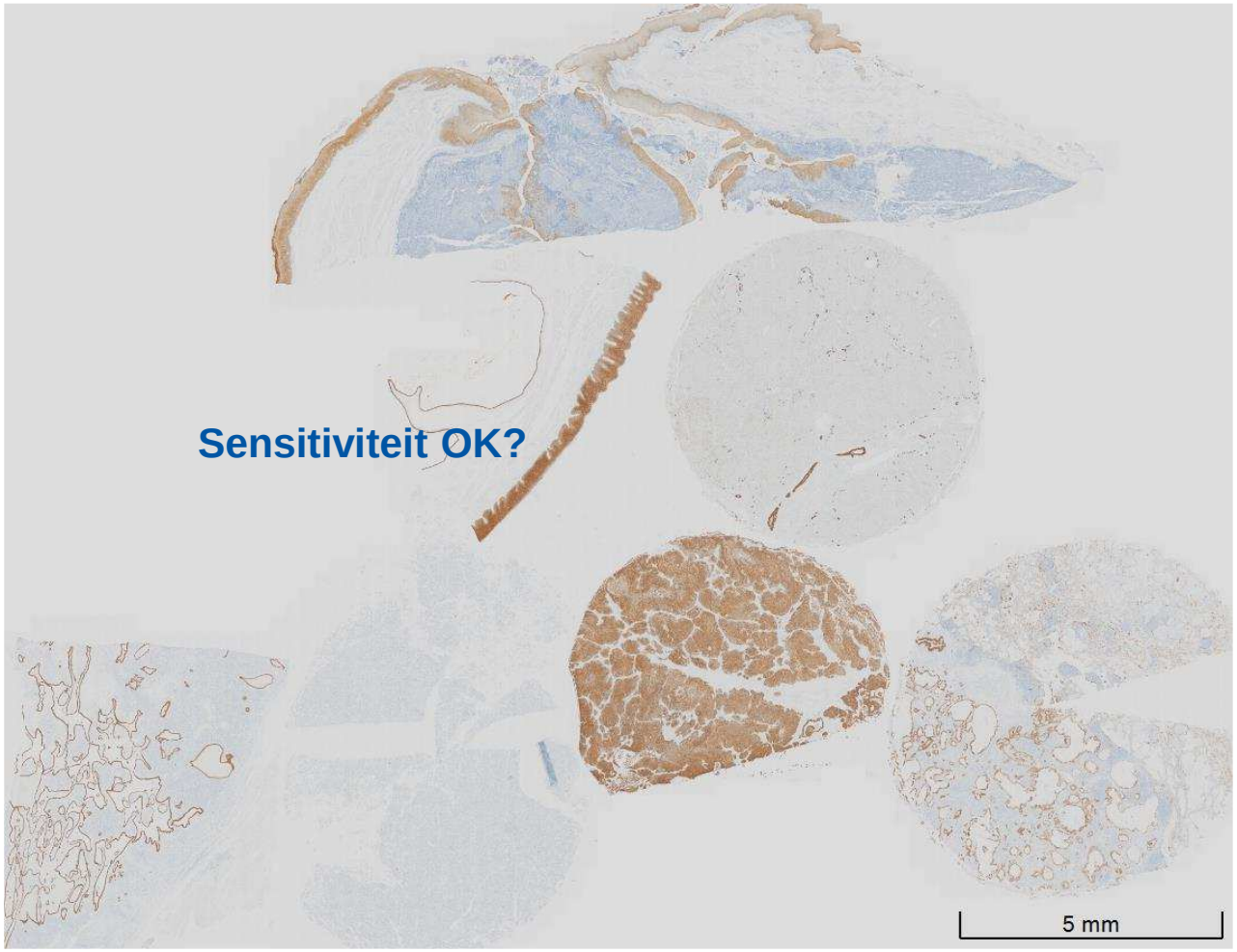
Antistof panCK: verdunning



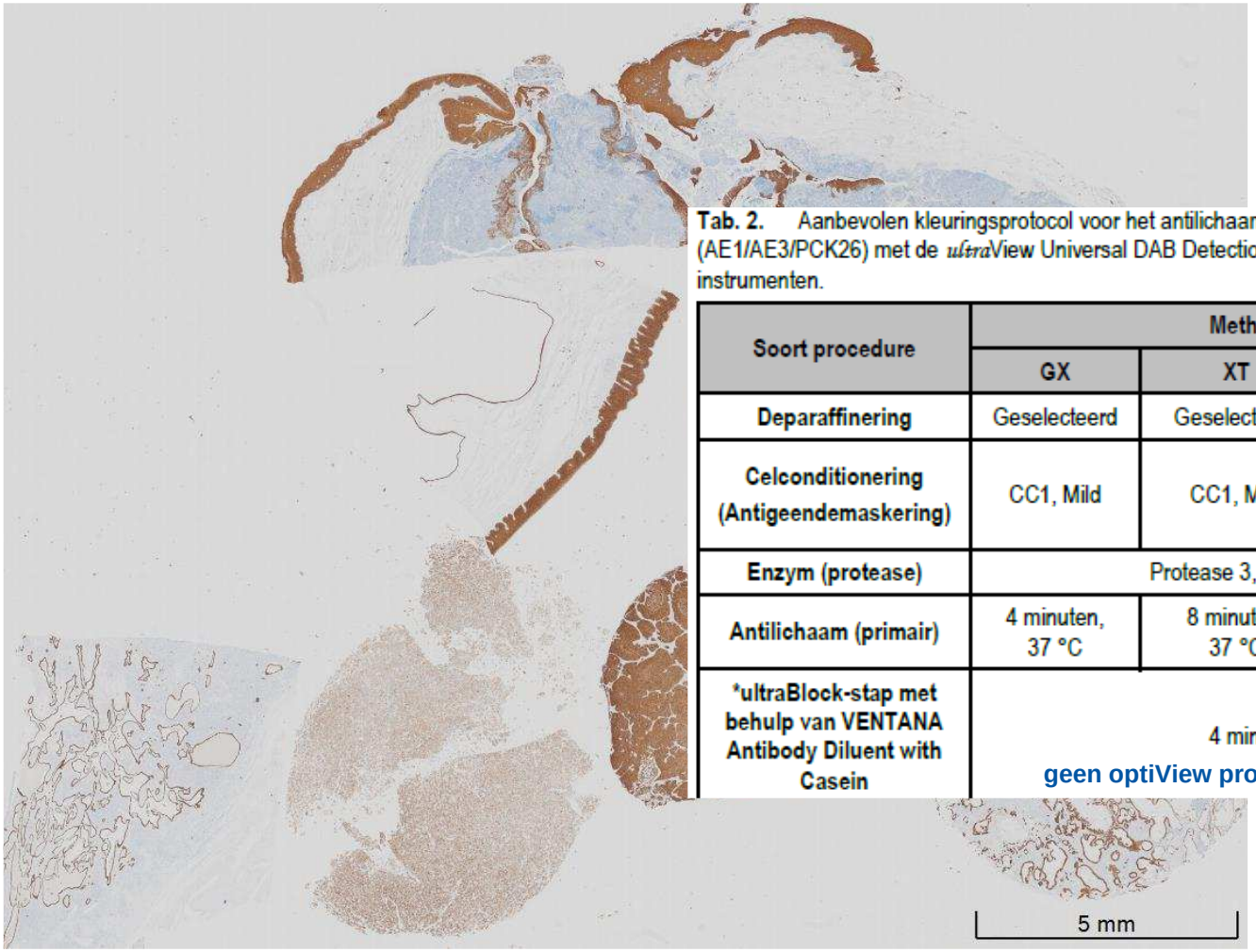


Antistof panCK: voorbehandeling



Ventana **AE1/AE3/PCK26** RTU **20'** 36˚ C ; **Protease1 8'**; **UV** Ventana Ultra

Antistof panCK: voorbehandeling

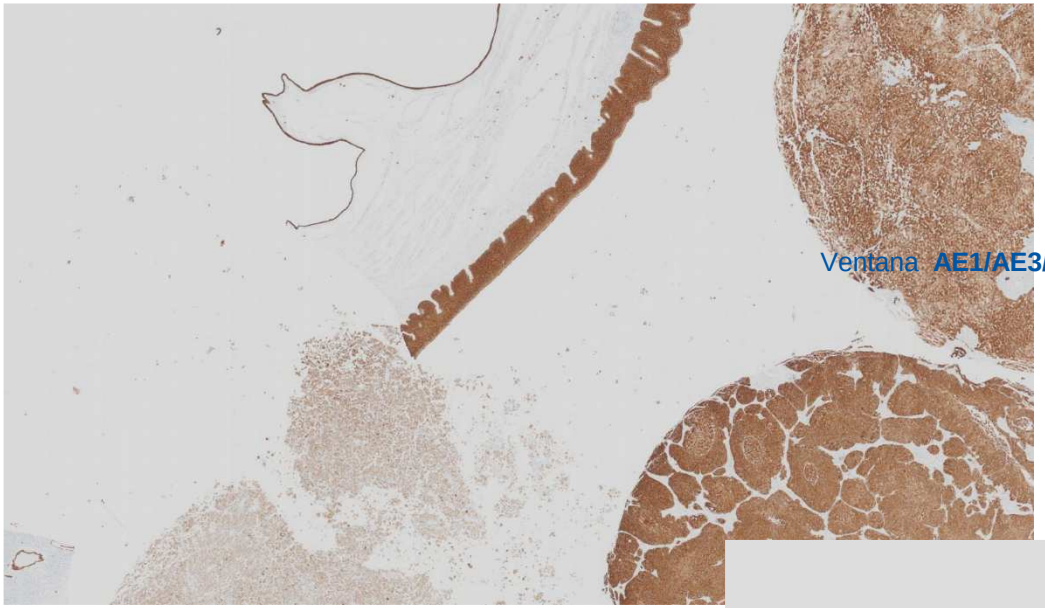


Tab. 2. Aanbevolen kleuringsprotocol voor het antilichaam Anti-Pan Keratin (AE1/AE3/PCK26) met de *ultra*View Universal DAB Detection Kit op BenchMark IHC/ISH-instrumenten.

Soort procedure	Methode		
	GX	XT	ULTRA
Deparaffinering	Geselecteerd	Geselecteerd	Geselecteerd
Celconditionering (Antigeendemaskering)	CC1, Mild	CC1, Mild	ULTRA CC1, 36 minuten, 95 °C
Enzym (protease)	Protease 3, 4 minuten		
Antilichaam (primair)	4 minuten, 37 °C	8 minuten, 37 °C	8 minuten, 36 °C
***ultraBlock-stap met behulp van VENTANA Antibody Diluent with Casein**	4 minuten **geen optiView protocol beschikbaar**		

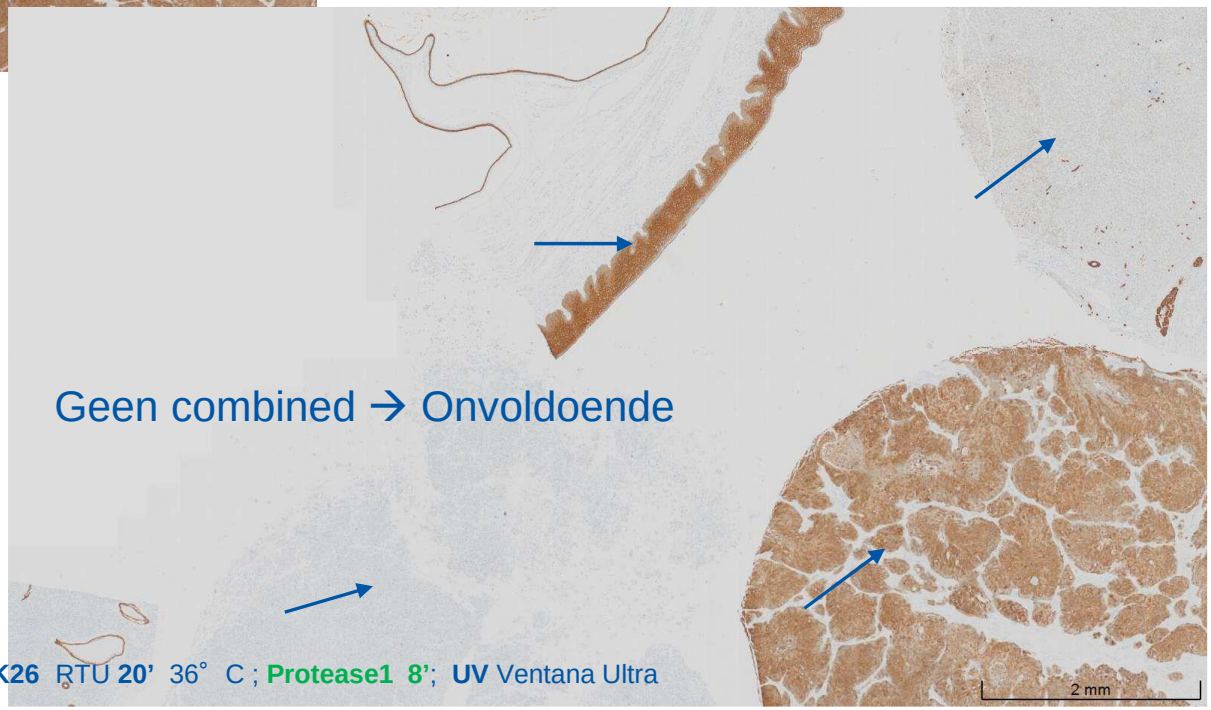
Ventana **AE1/AE3/PCK26** RTU **8'** 36° C ; **Protease3 4' + CC1 36'**; **UV** Ventana Ultra

Antistof panCK: voorbehandeling



Ventana **AE1/AE3/PCK26** RTU **8’** 36˚ C ; **Protease3 4’ + CC1 36’**; **UV** Ventana Ultra

detail

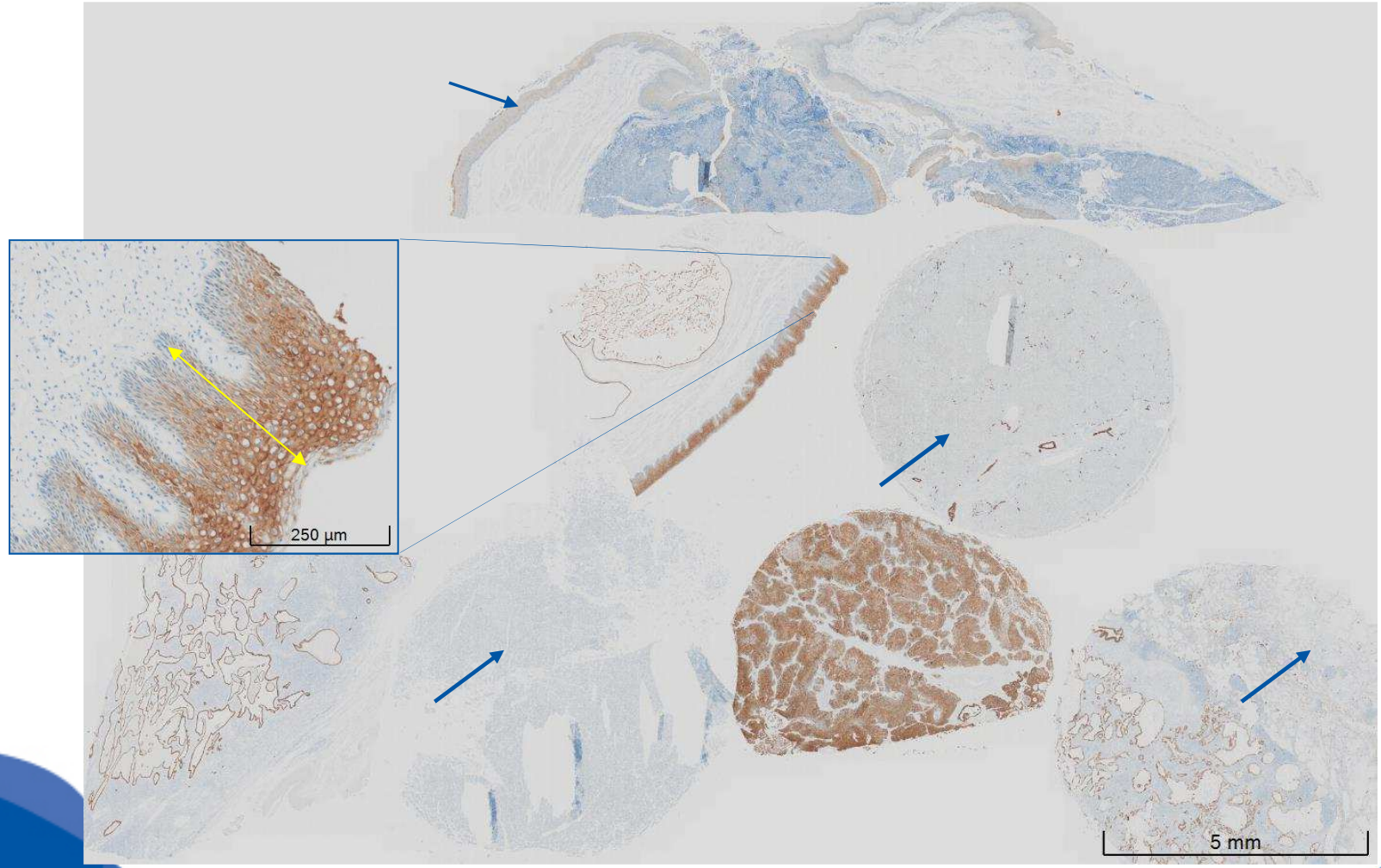


Ventana **AE1/AE3/PCK26** RTU **20’** 36˚ C ; **Protease1 8’**; **UV** Ventana Ultra

Antistof panCK: voorbehandeling

Ventana **AE1/AE3/PCK26** RTU 16’ 36° C ; **Protease3 4’**; **CC1 32’ OV ,** Ventana Ultra

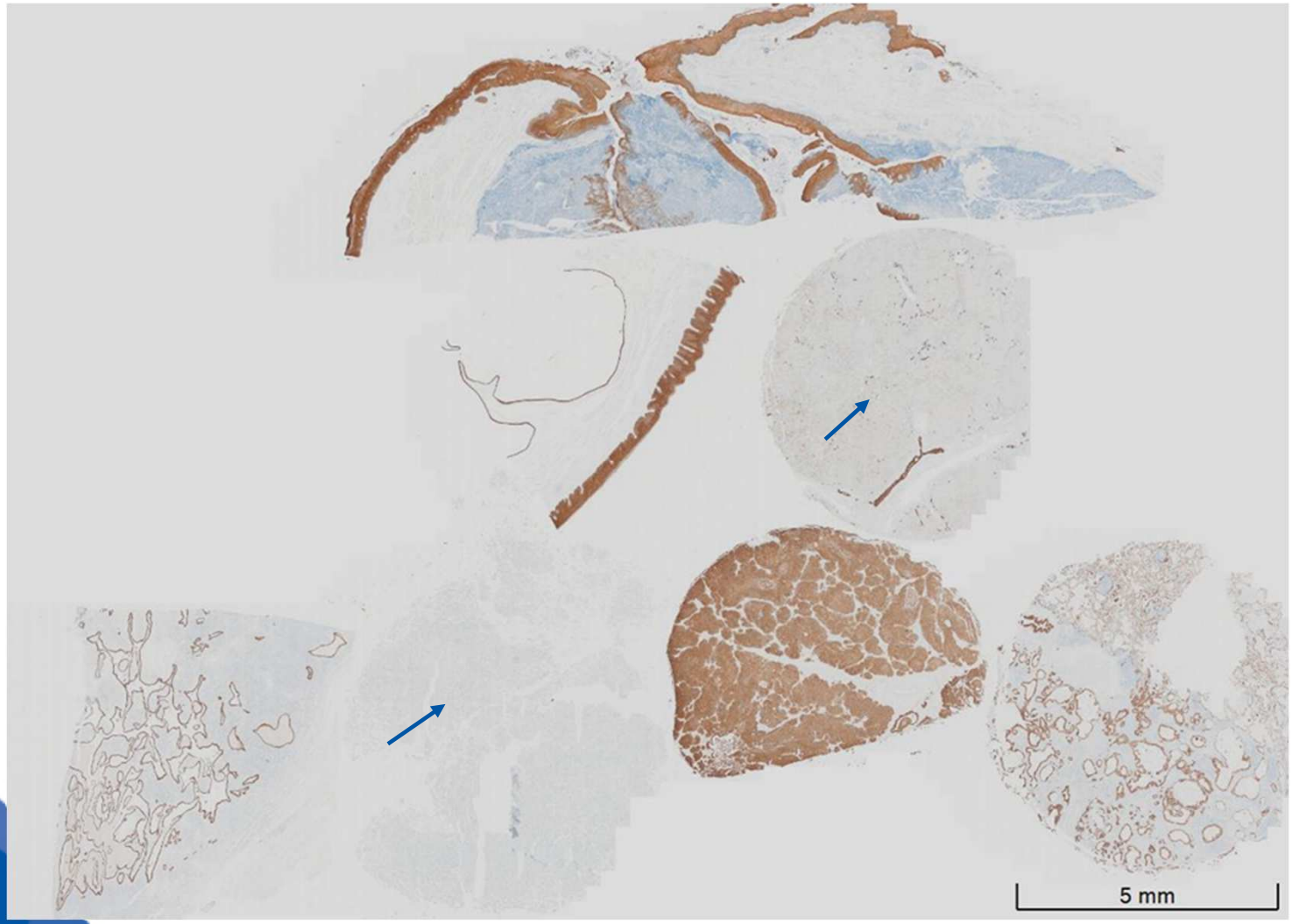
Antistof panCK: voorbehandeling



Ventana **AE1/AE3/PCK26** RTU 4’ 36˚ C ; **Protease1 4’**; **OV** Ventana Ultra

Geen combined → Onvoldoende, ook bij 3-stap detectie !

Antistof panCK: detectiesysteem



Ventana **AE1/AE3/PCK26** RTU **16'** 36˚ C **+amplifier** ; **Protease1 4'**; **CC1 8' UV** Ventana Ultra

Sensitiviteit OK ondanks amplifier en combined?

Antistof panCK: detectiesysteem

Keuze detectiesysteem niet beslissend/bepalend

Optimaal ook mogelijk met 2-stap mits voldoende sensitief (combined + AS-tijd)

Ventana **AE1/AE3/PCK26** RTU 16' 36° C ; **Protease3 4'**; **CC1 32' OV** , Ventana Ultra

Resultaatbespreking panCK

- Algemeen
  - n=64
    - Suff. 84 %

		EKE 2021-1		NQC 2020		NQC 2017	
	n		*suff*	*%*	*suff*	*%*	*suff*
optimaal	**50**	**78%**	***84%***	***52%***	***82%***	***38%***	***82%***
goed	**4**	**6%**		***23%***		***24%***	
borderline	3	**5%**		*15%*		*19%*	
onvoldoende	7	**11%**		*11%*		*19%*	

Resultaatbespreking panCK

- Opgesplitst per clone producent
  - RTU: 79 % optimaal ; suff. 83%

n=52	*Dako* AE1/AE3	*% suff.*	*Ventana* AE1/AE3 /PCK26	*% suff.*	*Leica AE1/AE3*	*% suff*
	n=22		n=27		n=2	
optimaal	21	***95,5%***	20	***74%***	0	**-**
goed	1	***4,5%***	2	***7%***	0	**-**
borderline	0	***0%***	0	***0%***	1	-
onvoldoende	0	***0%***	5	***19%***	1	**-**
Totaal suff.	***100%***		***81%***		***0/2***	
NQC 2020	*100% (LDT) 90% (datasheet) Omnis*		*70% (LDT) 76% (UV datasheet)*		*80% suff., 20% optimaal (weinig data)*	

* RTU verdund ½ (AE1/AE3/PCK26): beiden optimaal

Resultaatbespreking panCK

- Opgesplitst per clone producent
  - Concentrated: 67% optimaal ; suff. 78 %

n=9	*Dako AE1/AE3* conc.	*% suff*	*Leica AE1/AE3* conc.	*% suff*	*Sanbio BS5 conc.*	*% suff*
	n=7		*n=1*		n=1	
optimaal	6	***86%***	***0***	**-**	0	**-**
goed	0	***0%***	***0***	**-**	1	**-**
borderline	1	***14%***	***0***	**-**	0	**-**
onvoldoende	0	***0%***	*1*	-	0	-
Totaal suff.	***86%***		***0/1***		***1/1***	
NQC 2020	*74% (49% optimaal)*				*86% (57% optimaal)*	

Resultaatbespreking panCK

Besluit:

- 84% geslaagd = NQC
- 10 labo's niet geslaagd, waarvan 7 onvoldoendes.
- RTU presteert beter dan de conc. AS (14%): globaal én % optimaal
- Borderline/onvoldoende: analytische sensitiviteit te laag: in oa lever, nier CCRCC…
- Resultaten RTU
  - Dako: zeer robuuste AS, protocolspreiding geen impact op resultaat (100% geslaagd ; >95% optimaal) ; onfhankelijk van type Dako stainer en protocolsettings (enkel HIER)
  - Ventana RTU
    - Optimaal mogelijk (duidelijk lager dan bij Dako), maar zorgvuldige calibratie nodig
    - Enkel enzymatisch = veel te lage sensitiviteit → onvoldoende (1/5 geslaagd? : onjuiste settings meegedeeld??)
    - Keuze detectiesysteem is niet bepalend, wel de sensitiviteit van het protocol = ook optiView geeft onvoldoende resultaten
    - Combined pretreatment (enzym + mild HIER) is noodzakelijk voor een optimaal resultaat, niet de AS tijd
    - downgrade goed o.w.v. achtergrond/zeer zwakke tegenkleuring
  - Leica AE1/AE3:
    - Beiden niet geslaagd (onafh. van type HIER) vs NordiQC geslaagd mogelijk met pH9

Although the number of participants using the mAb clone BS5 within a LD assay was low, this primary Ab seems robust and promising, as most protocols (12 of 14) were assessed as sufficient (see Table 1). This Ab might be an alternative to the more challenging Abs (e.g. MNF116 or AE1/AE3) on the Bond (Leica) platforms where 6/7 received a sufficient mark using mAb BS5. The mAb BS5 performed optimally using a dilution in the range of 1:100-1:800 carefully calibrated according to the overall sensitivity of the detection systems applied and using HIER in an alkaline buffer (e.g. BERS2, Leica) (Figs. 7a-b).

Top in Zorg.

Resultaatbespreking panCK

Besluit:

- Resultaten conc. : slaagpercentage vergelijkbaar met RTU (robuuste Dako conc. AS)
  - Dako AS op Ventana: 1/100, settings sterk verschillend (enkel HIER) → allen optimaal
- Reden downgrade
  - Goed: matige aankleuring van BS5 op Leica stainer
  - Borderline: algemeen te zwak → te grote verdunning (Dako 1/500) ; HIER/AS tijd OK
  - Onvoldoende: verkeerde pH keuze HIER (Leica → algemeen veel te lage sensitiviteit)

NordiQC **Nordic Immunohistochemical Quality Control**
Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

Recommended protocol for CK-PAN
Obtained in run 58
20 Dec 2019

Immunostainer	
Type:	Dako Omnis
Primary antibody	
Clone:	AE1/AE3
Producer:	Dako/Agilent
Product no. / lot no.:	M3515 / 10149487
Diluent:	Renoir Red Diluent
Dilution factor:	1:100
Incubation time / temperature:	20 min. / 32°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Dako Omnis Target Retrieval Solution, High pH
Heating time at max. temp.:	30 min.
Maximum heating temp.:	97°C
Visualization system	
Producer:	Dako Omnis
Product / no:	EnVision Flex / GV800/GV823
Linker:	None
Incubation time polymer:	20 min.
Incubation temperature:	32°C
Chromogen	
Producer:	Dako Omnis
Product / no:	DAB+ Substrate Chromogen System / GV825
Incubation time / temperature:	5 min. / 32°C
Enhancement:	None

NordiQC **Nordic Immunohistochemical Quality Control**
Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

Recommended protocol for CK-PAN
Obtained in run
12 Sep 2015

Immunostainer	
Type:	Leica BOND III
Primary antibody	
Clone:	AE1/AE3
Producer:	Dako
Product no. / lot no.:	M3515 / 10079695
Diluent:	Antibody Diluent
Dilution factor:	1:75
Incubation time / temperature:	30 min. / 20°C
Epitope retrieval, HIER	
Device:	On Board / On Machin
Buffer:	Leica Bond Epitope Retrieval Solution 2
Heating time at max. temp.:	20 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	20°C
Chromogen	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time / temperature:	10 min. / 20°C
Enhancement:	None

olv Top in Zorg.

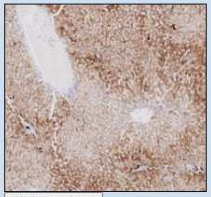
Resultaatbespreking panCK

Besluit:

- Controle
  - 61% gebruikt controle
  - Aanbevolen controle: lever en tonsil/slokdarm
    - ✓ Lever: minstens een matige (cytoplasmatische en membranaire) aankleuring aanwezig zijn in de hepatocyten; de stromale cellen in de lever mogen niet aankleuren.
    - ✓ Tonsil/slokdarm: bijna alle plaveiselcellen van het epitheel sterk positief doorheen alle cellagen + een zwakke tot matige ('patchy') aankleuring in de gladde spiercellen in de bloedvaten en in de muscularis mucosae van de slokdarm.
    - ✓ Tonsil: negatieve controle: lymfocyten mogen niet aankleuren; een zwakke tot matige aankleuring in verspreide interstitiële reticulum cellen in de tonsil ('CIRC')

131_85.pdf (nordiqc.org)

CK-PAN - Pan Cytokeratin

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Tonsil	Liver	Tonsil
Description	Virtually all squamous epithelial cells must show a moderate to strong, distinct cytoplasmic staining reaction (Demonstration of CK-HMW).	Virtually all hepatocytes must show an at least weak to moderate cytoplasmic staining reaction typically with a membrane accentuation (Demonstration of CK-LMW). *Note epithelial cells of bile ducts will show a strong cytoplasmic staining reaction and cannot be used to evaluate analytical sensitivity (Demonstration of CK-LMW).*	No staining reaction in lymphocytes. *Note interstitial/interdigitating reticulum cells may show a weak to moderate cytoplasmic staining reaction due to expression of CK 8 and 18.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

MLH1

n= 59

Conc.: n=2

- Leica ES05 mm n=1
- Dako ES05 mm n=9

RTU: n=54

- Dako ES05 mm n=26
- Leica ES05 mm n=1
- Roche M1 mm n=20
- Roche ES05 mm ??? n=1
- RTU verdund: n=2

Protocol MLH1

- Conc. AS:
  - Leica ES05
    - Leica Bond III:
      - 1/20 15’ RT; pH9 20’ BondIII
  - Dako ES05
    - Omnis:
      - 1/50 20’ bij 32° C +linker; pH9 30’; Flex+

Protocol MLH1

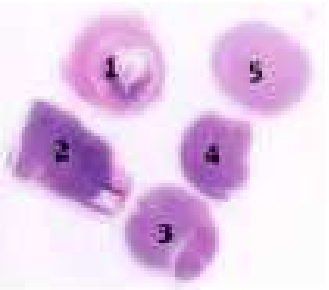
- RTU:
  - Dako ES05
    - Autostainer
      - AS 20’ – 30’ RT
      - ¾ deelnemers + linker
      - HIER pH9 30’
    - Omnis
      - AS 20’ – 25’ RT/32° C
      - 53% + linker
      - HIER pH9 30’
    - Ventana stainers
      - UV: AS 40’ 36° C +amp 16’ ; CC1 92’
      - OV: AS 12’ 36° C +amp 8’ ; HIER CC1 80’
  - Leica ES05
    - AS 15’ RT ; geen linker ; HIER pH6 20’- 40’

Protocol MLH1

- RTU:
  - Roche M1
    - OV Datasheet (rev. 2022): AS 24’ +amp 8’ ; CC1 64’
      - Slechts 3 labs volgen 100% datasheet
    - UV (4/25)
      - AS 16’ - 24’ 36° C
      - 2/4 amp.
      - HIER CC1 36’ - 64’
    - OV
      - AS 4’ - 12’ – 24’ - 32’ bij 36° C (meerderheid 4’ en 16’)
      - HIER CC1 40’ tot 92’
      - 57% amp (4’-16’)
  - Dako ES05 verdund (2)
    - AS 52’ 36° C + amp. ; HIER mild CC1; optiView

Spreiding protocolsettings

Antistof MLH1



- Scoringscriteria: kern aankleuring

- **Appendix :** zwakke tot matige aankleuring van bijna alle cellen

- Tonsil :

- matige tot sterke aankleuring van de B-cellen in de kiemcentra
- zwakke tot matige aankleuring van bijna alle B-cellen in de mantelzone

- Colon, adenocarcinoom :

- bewaarde expressie d.w.z. sterke aankleuring in de neoplastische cellen
- sterke aankleuring in de meeste andere cellen (stromale cellen, lymfocyten enz.) (controle sensitiviteit)
- sterke aankleuring in het columnair epitheel (indien aanwezig)

- Colon, adenocarcinoom :

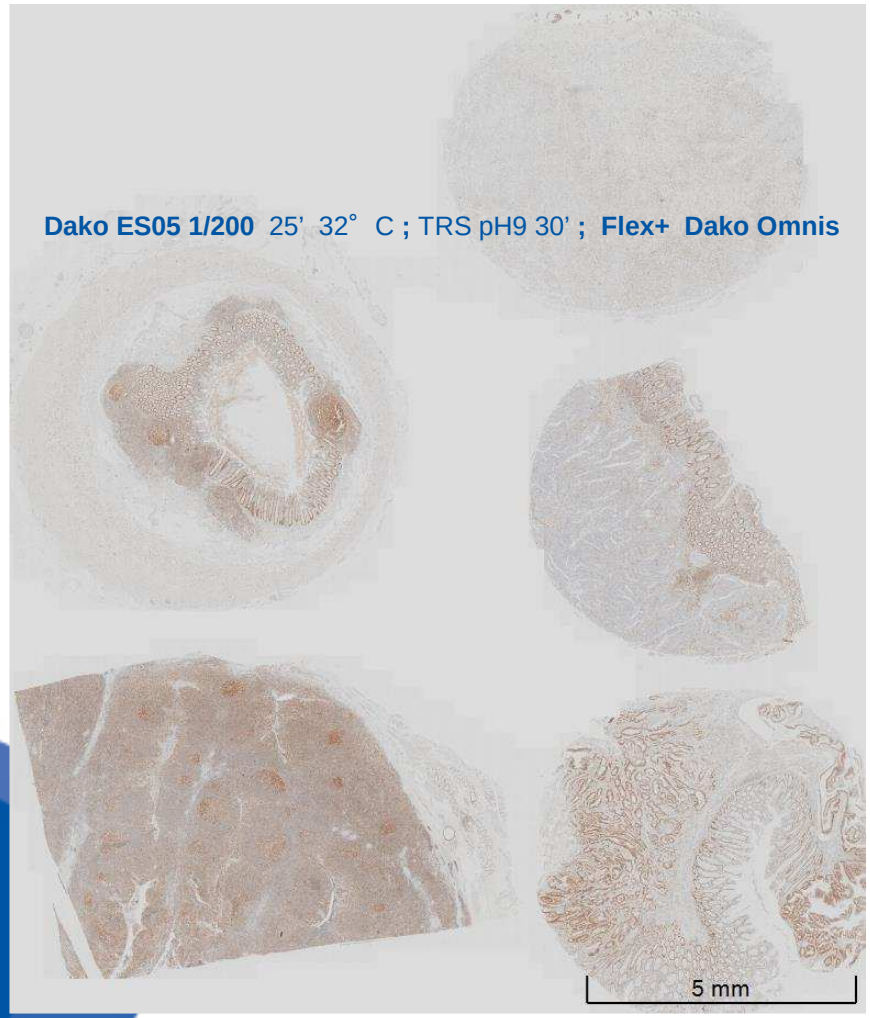
- verlies van expressie in de neoplastische cellen (controle specificiteit)
- sterke aankleuring in de meeste andere cellen (stromale cellen, lymfocyten enz.) (controle sensitiviteit)
- sterke aankleuring in het columnair epitheel (indien aanwezig)

Opmerking : zwakke cytoplasmatische aankleuring wordt aanvaard

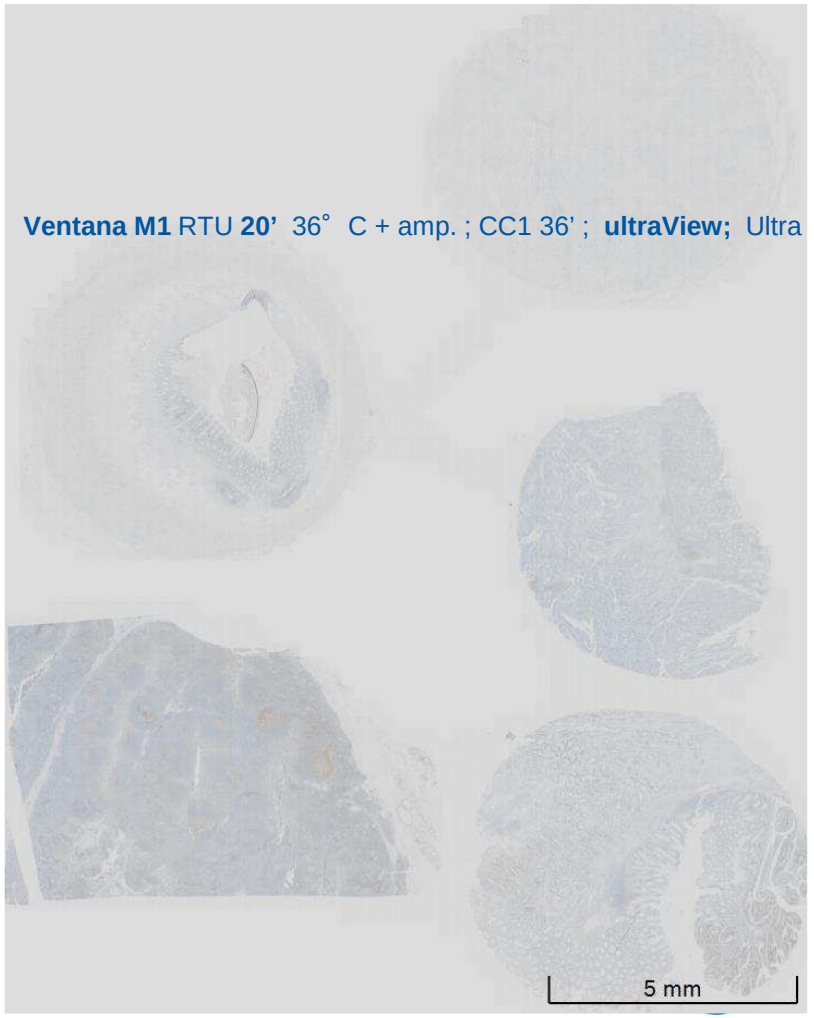
Bron: NordiQC Run 56, 2019

Antistof MLH1: overzicht

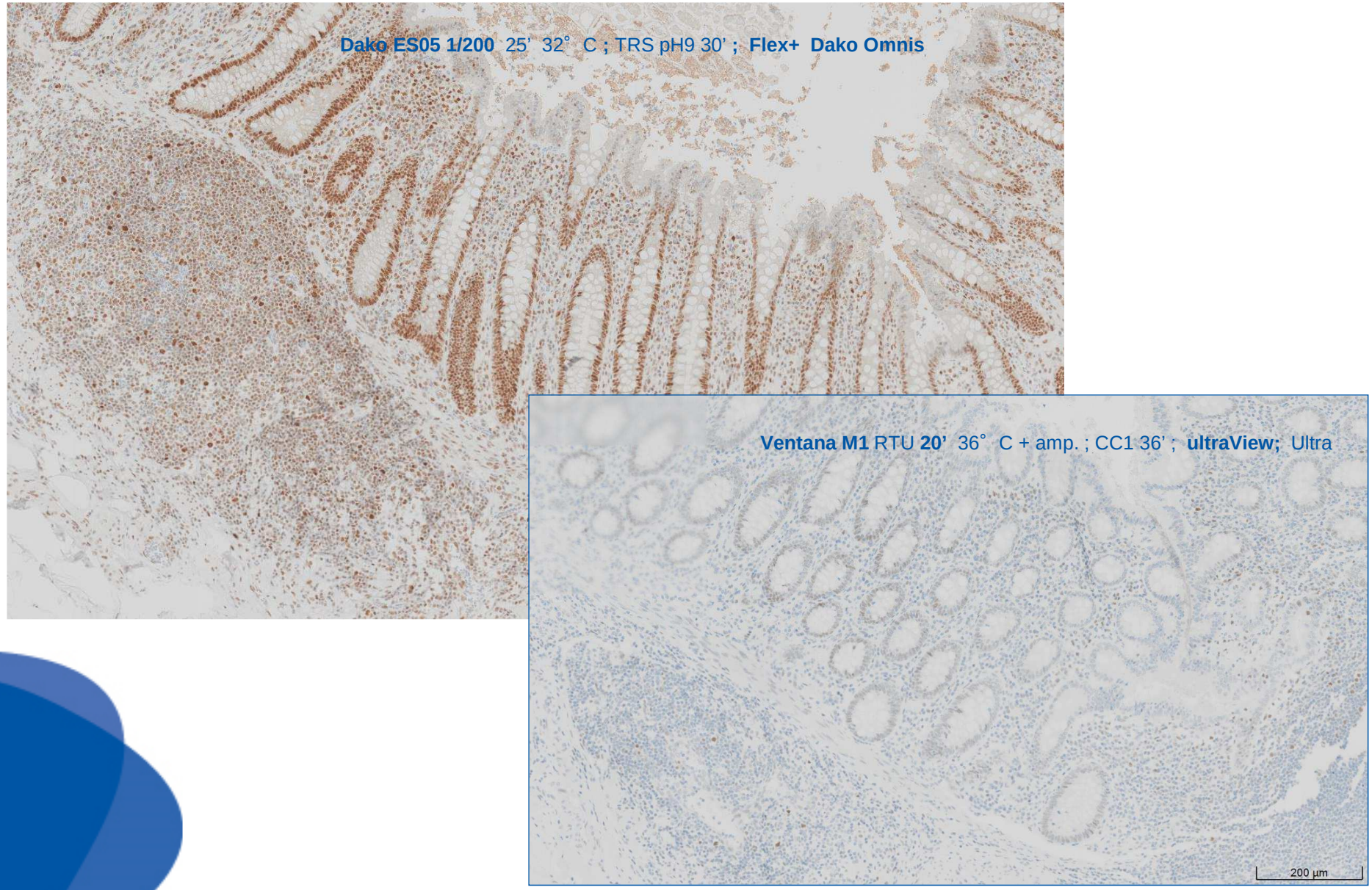
Optimaal



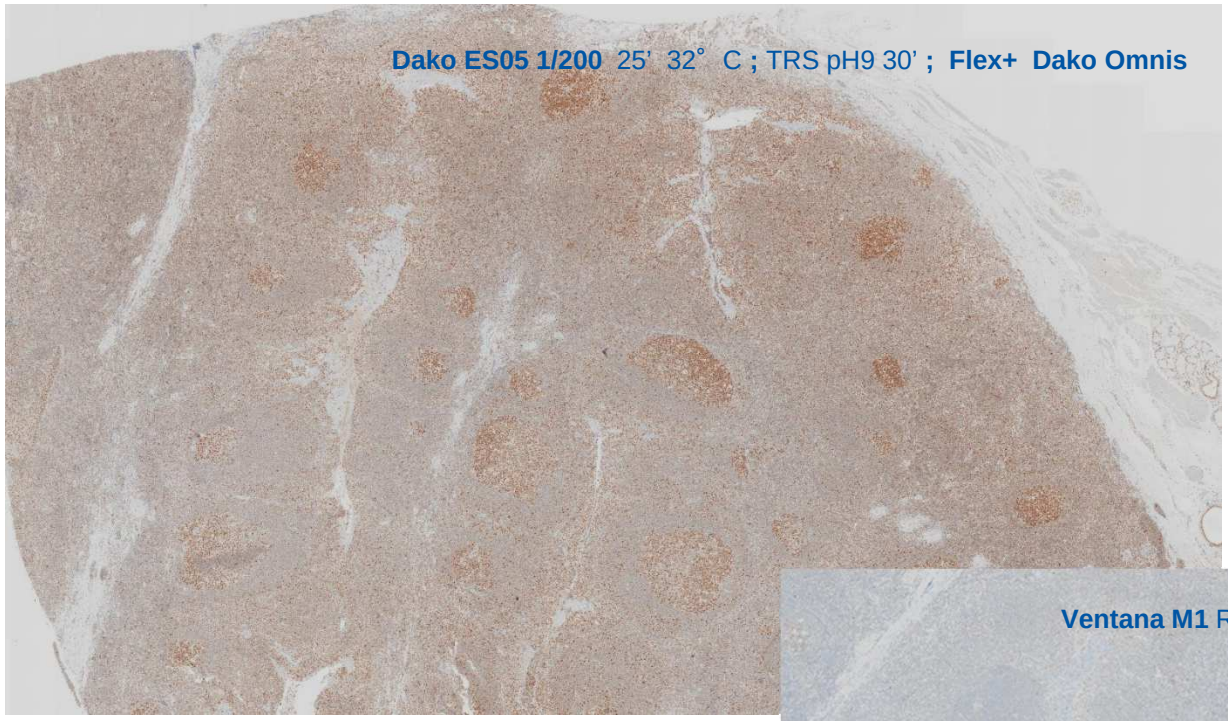
Onvoldoende

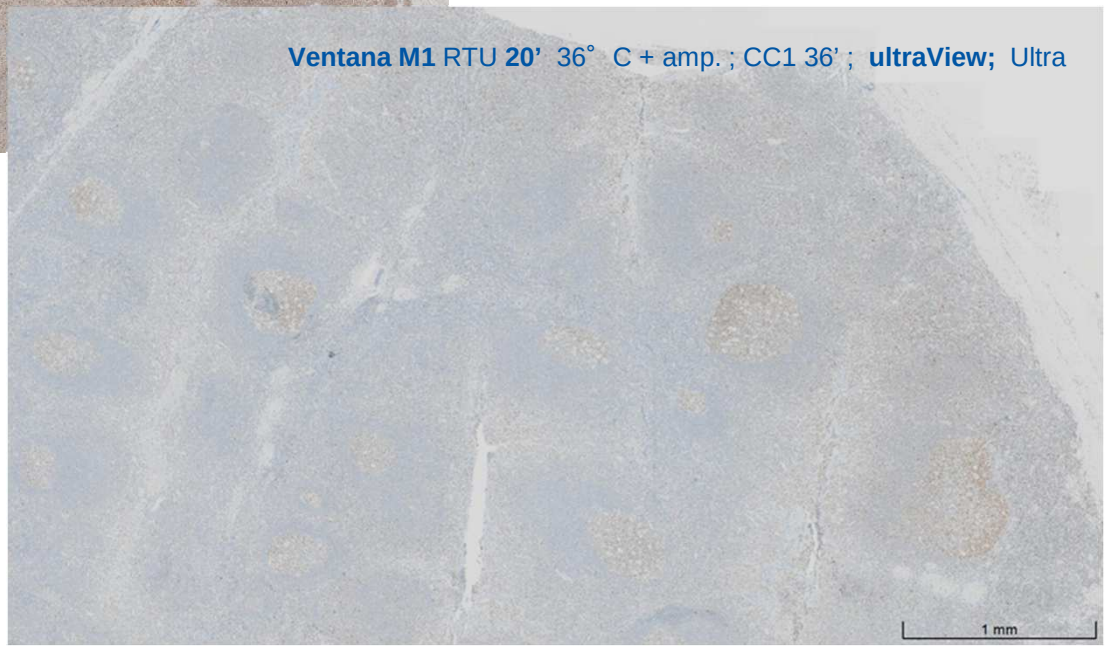


Antistof MLH1: appendix

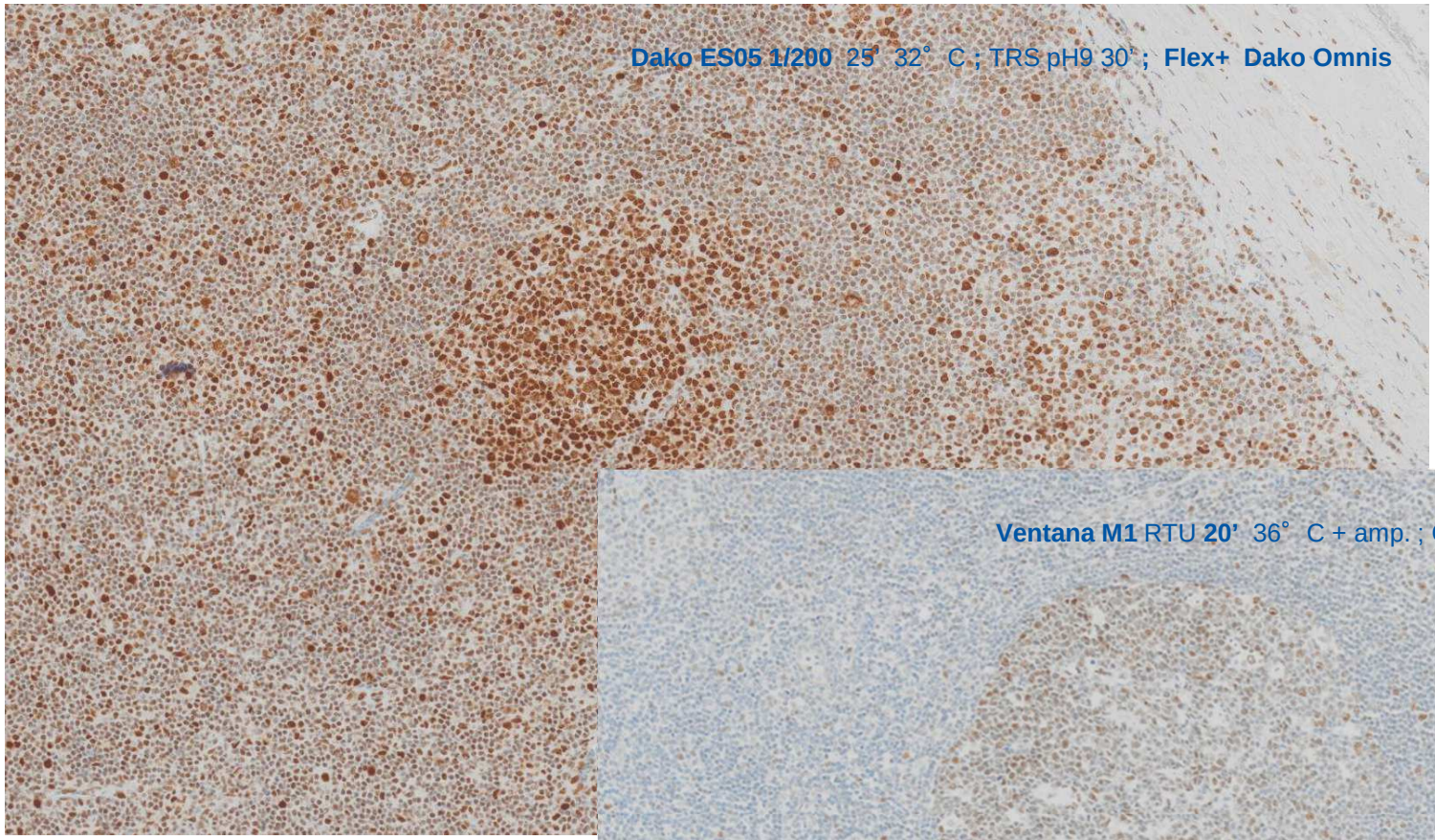


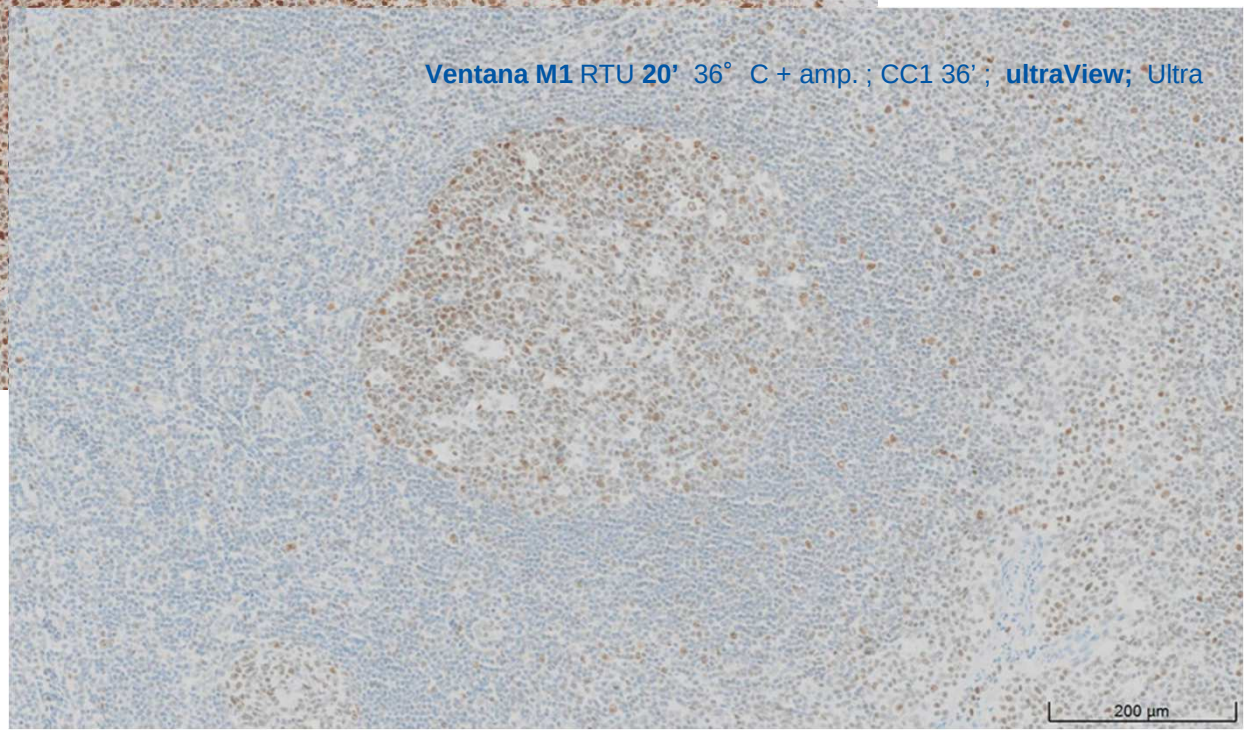
Antistof MLH1: tonsil



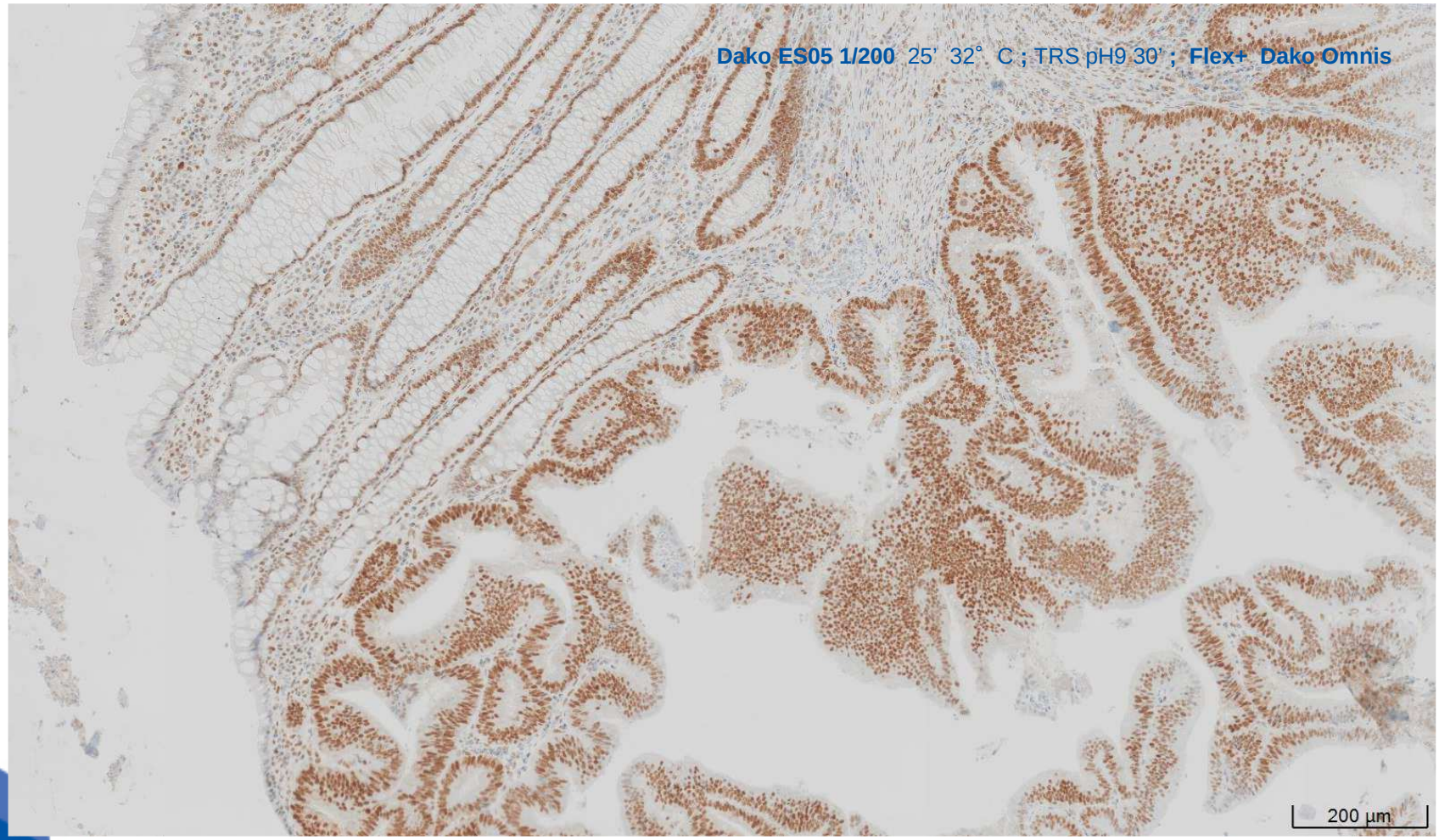


Antistof MLH1: tonsil

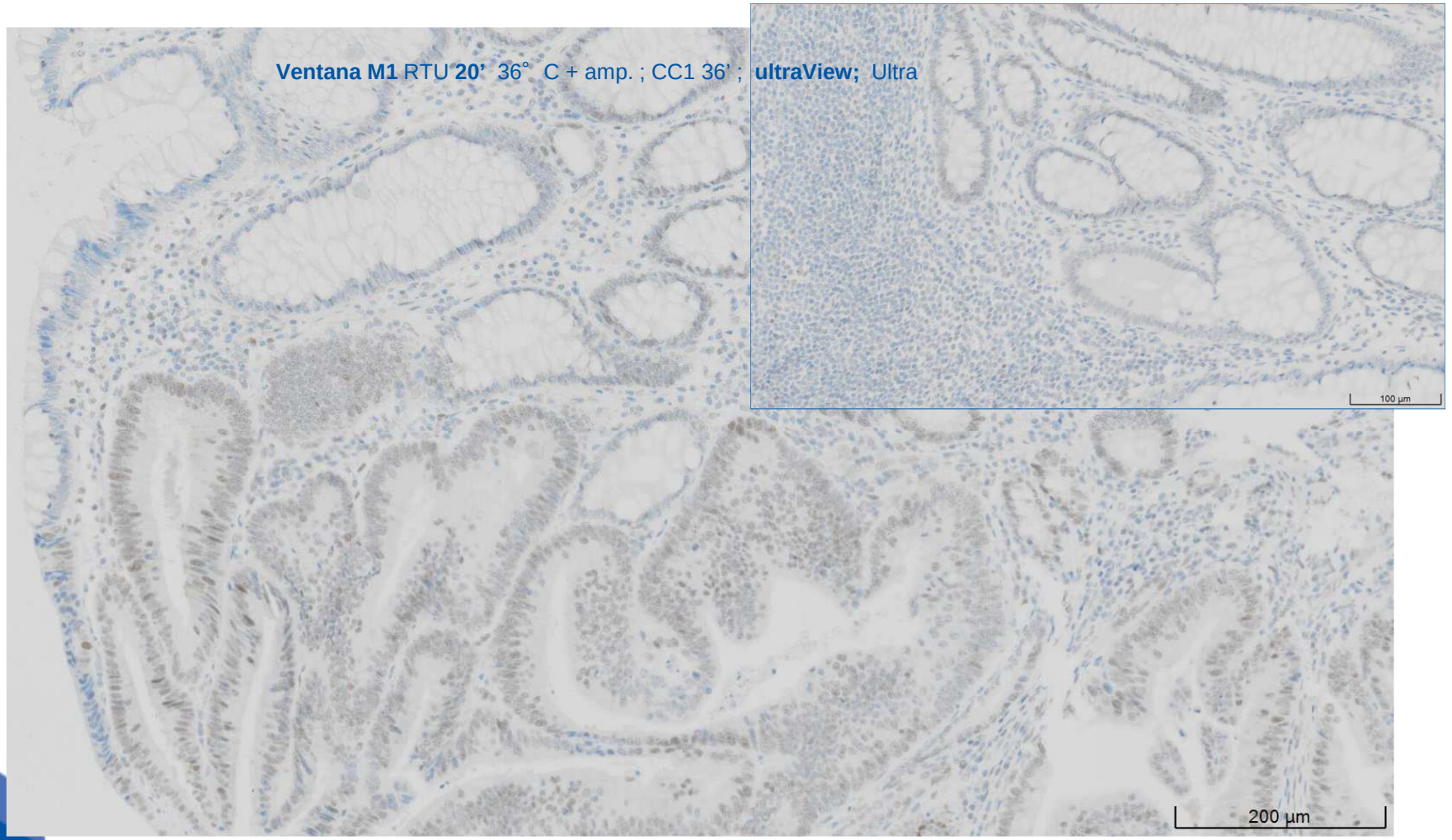




Antistof MLH1: colonCa core 3

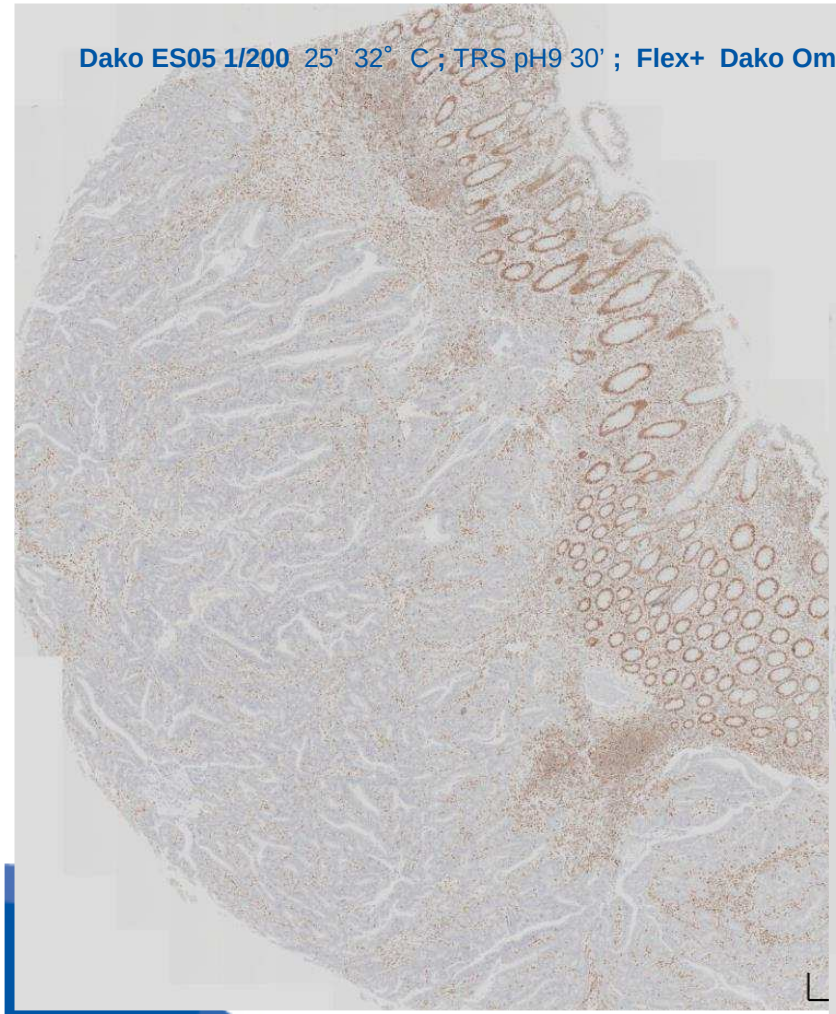


Antistof MLH1: colonCa core 3

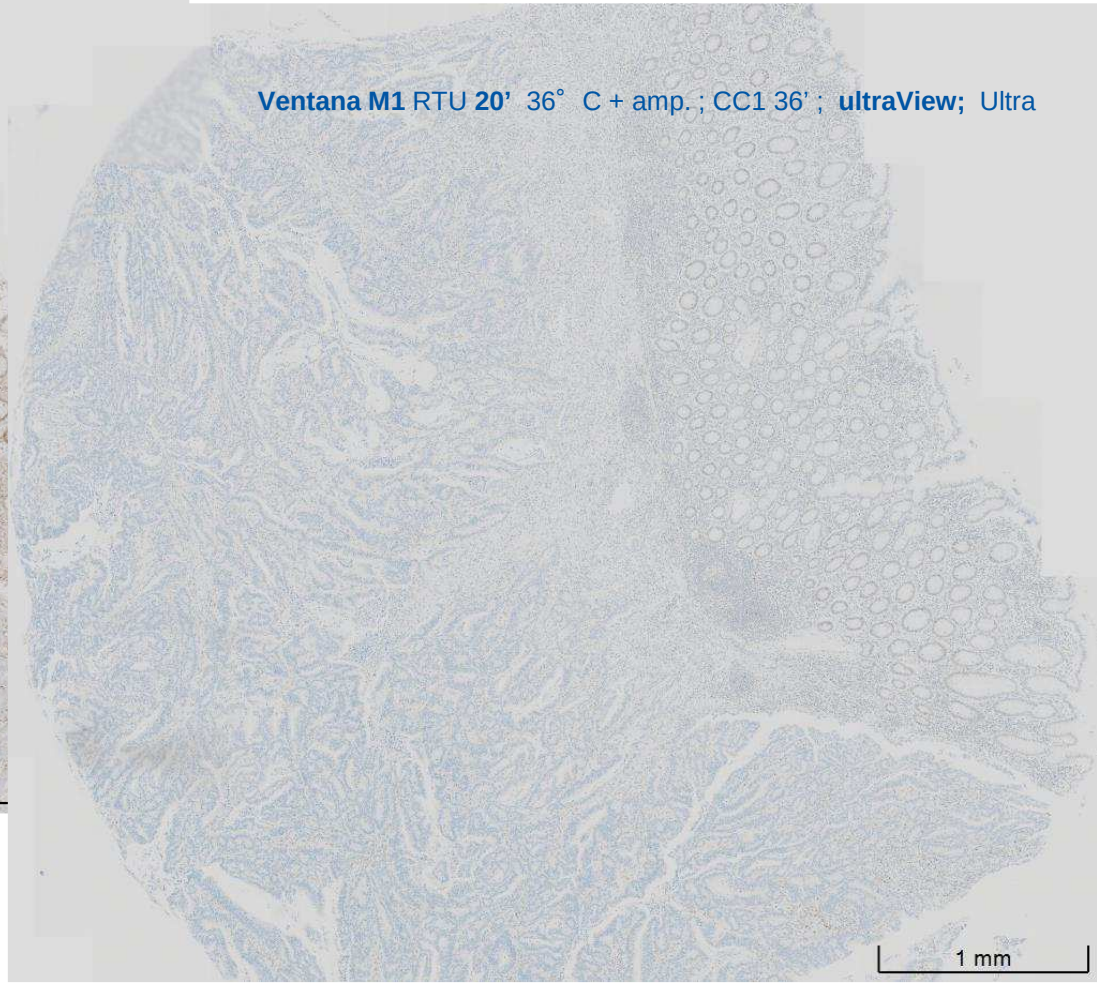


Antistof MLH1: colonCa core 4

Dako ES05 1/200 25’ 32° C ; TRS pH9 30’ ; **Flex+ Dako Omnis**

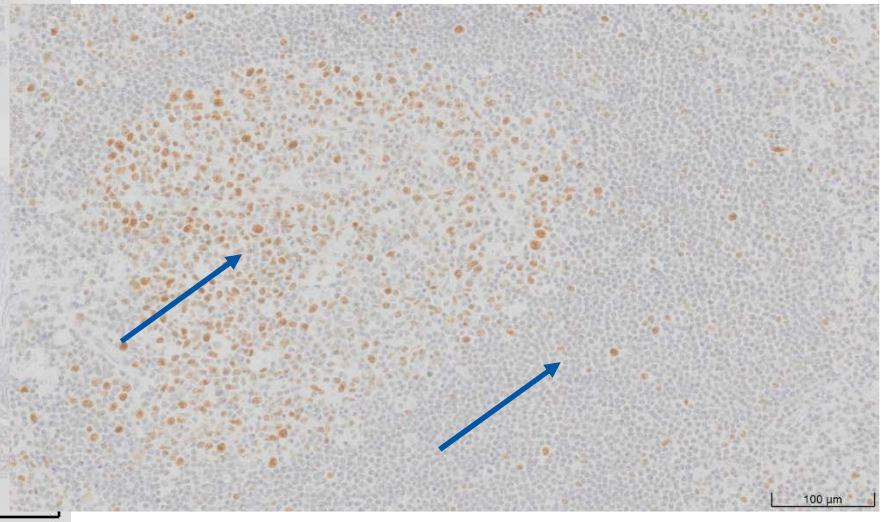


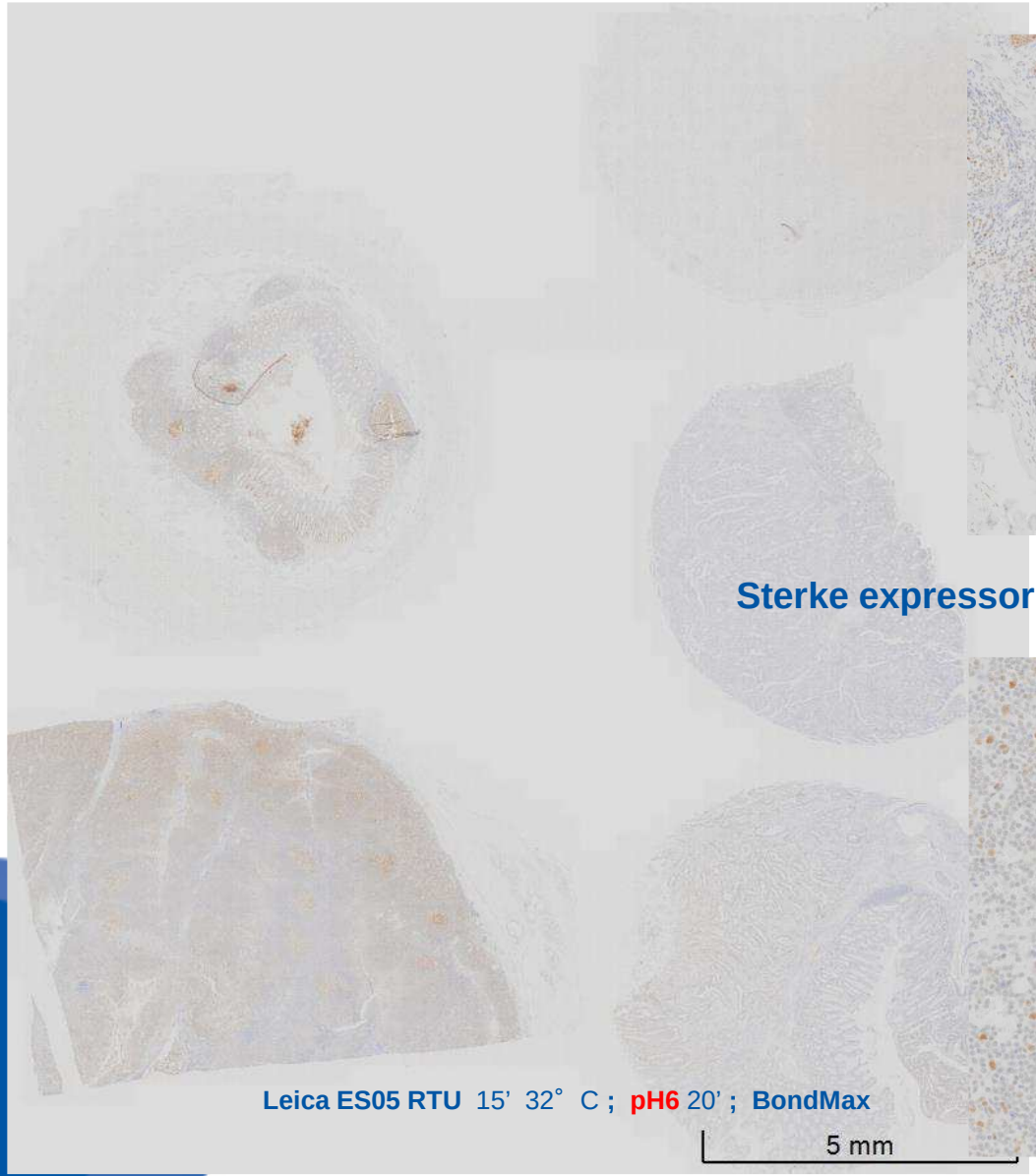
Ventana M1 RTU **20’** 36° C + amp. ; CC1 36’ ; **ultraView;** Ultra



Antistof MLH1: voorbehandeling

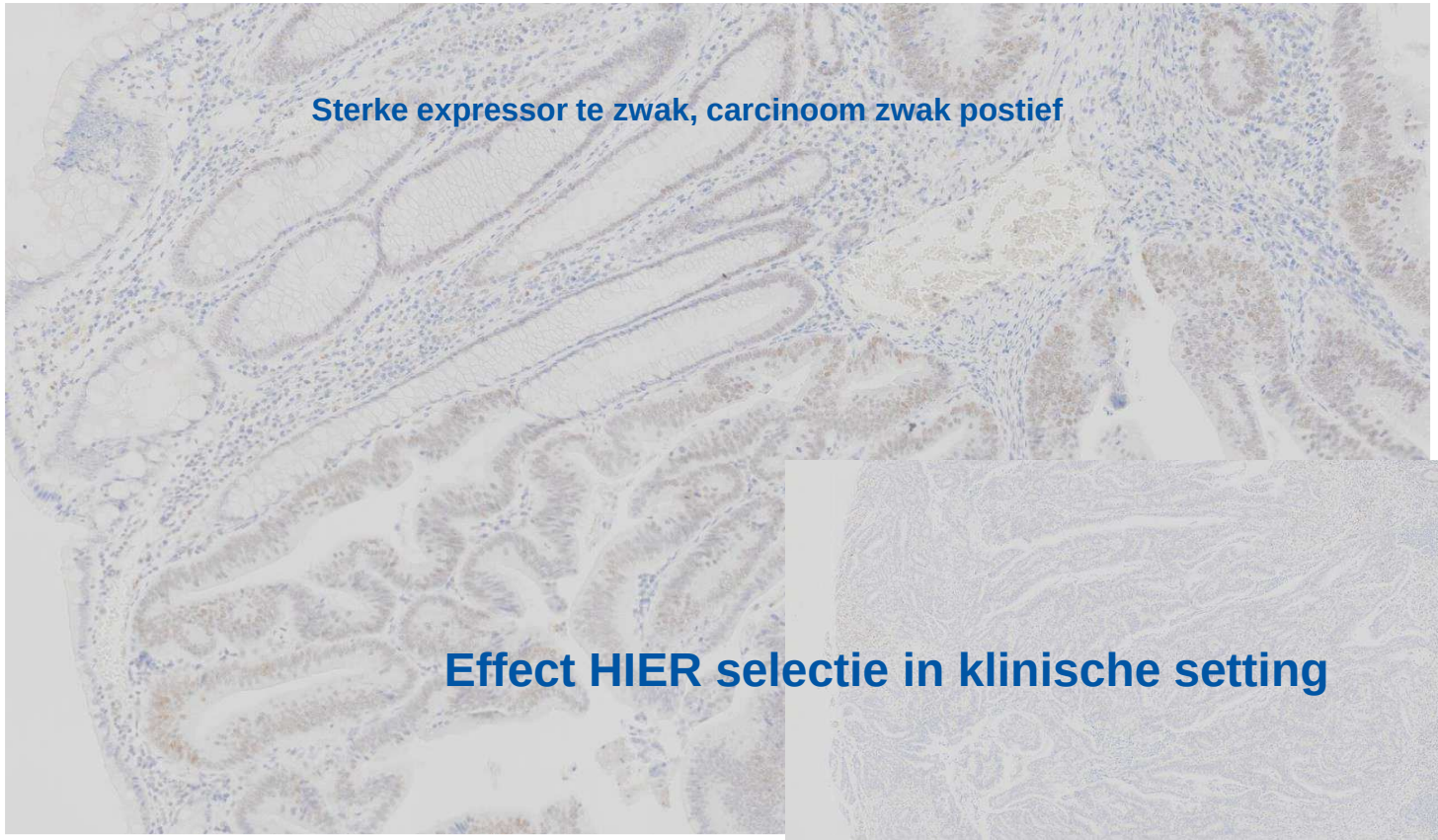
Sterke expressor te zwak, zwakke expressor bijna vals negatief





Leica ES05 RTU 15’ 32° C ; **pH6** 20’ ; **BondMax**

Antistof MLH1: voorbehandeling



Sterke expressor te zwak, carcinoom zwak postief

Effect HIER selectie in klinische setting

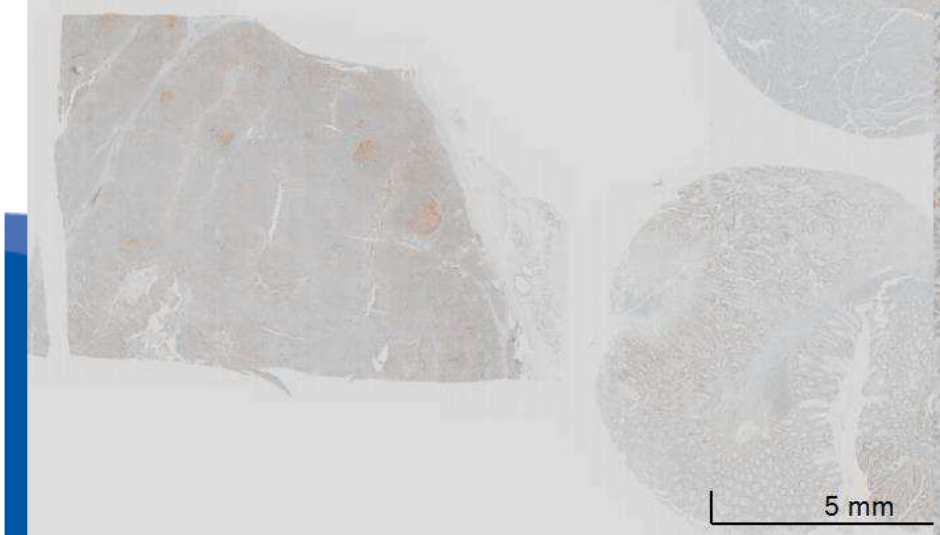
Leica ES05 RTU 15’ 32˚ C ; **pH6** 20’ ; **BondMax**

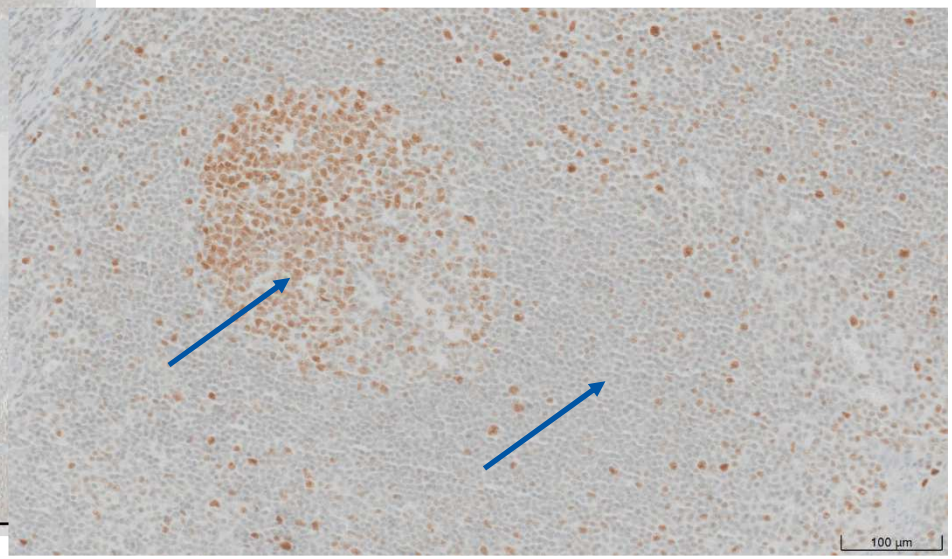
Carcinoom negatief maar interne controle vals negatief→ geen interpretatie

Antistof MLH1: detectiesysteem

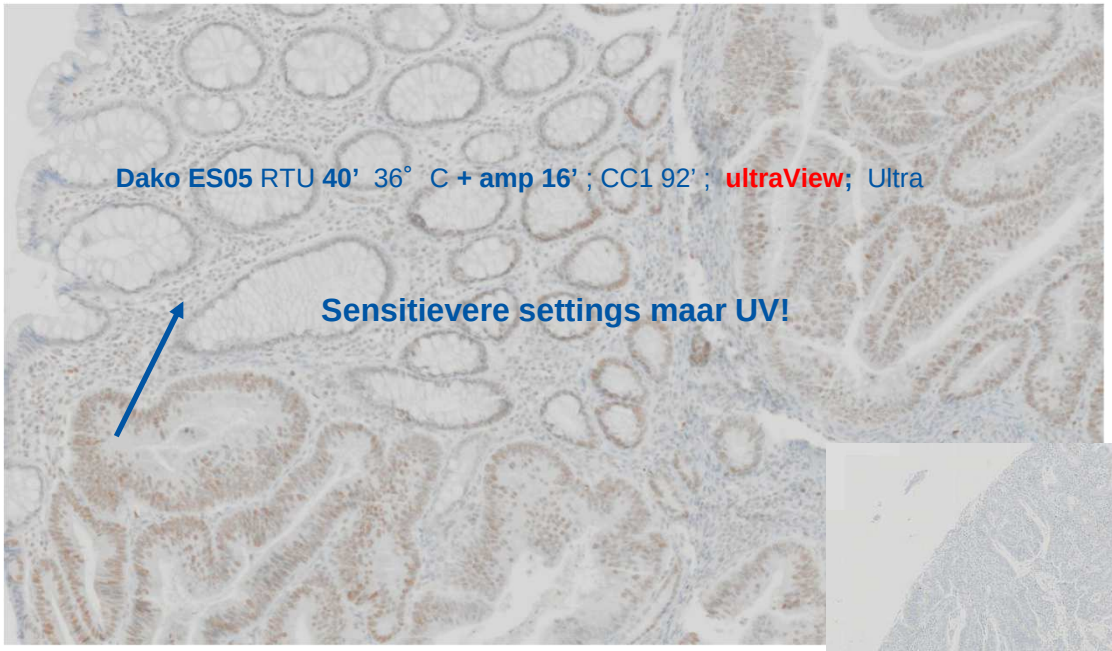
Dako ES05 RTU **40'** 36° C **+ amp 16'** ; CC1 92' ; **ultraView;** Ultra

Effect keuze detectiesysteem in weefsel gekend expressiepatroon



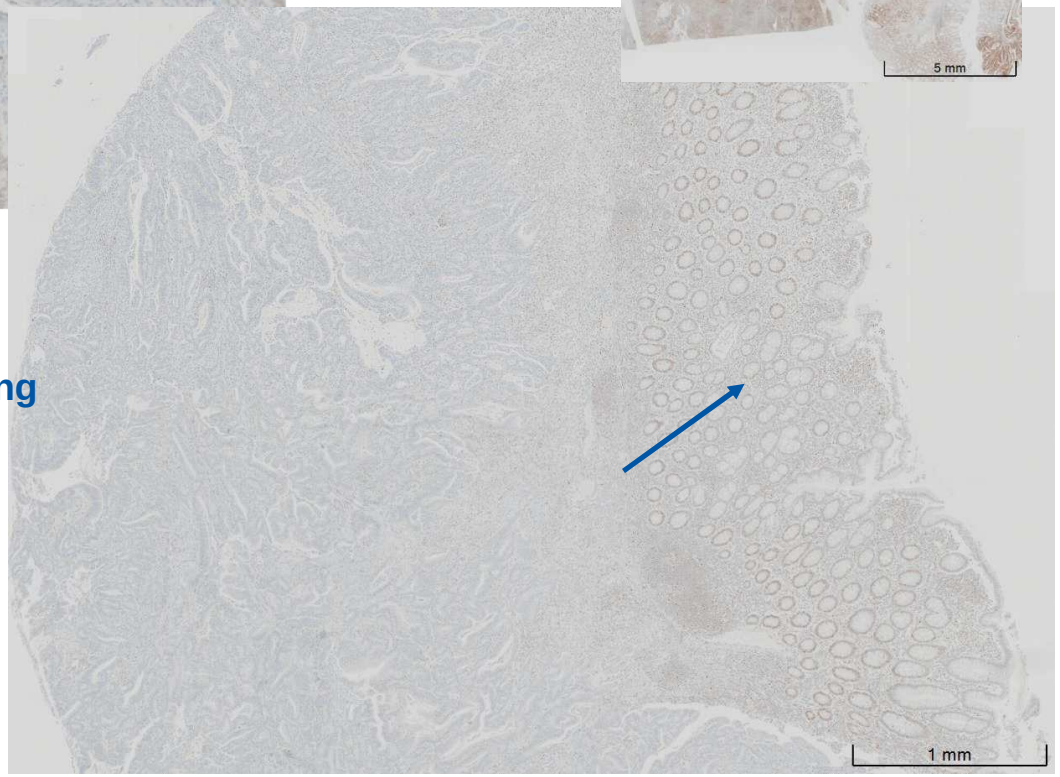


Antistof MLH1: detectiesysteem



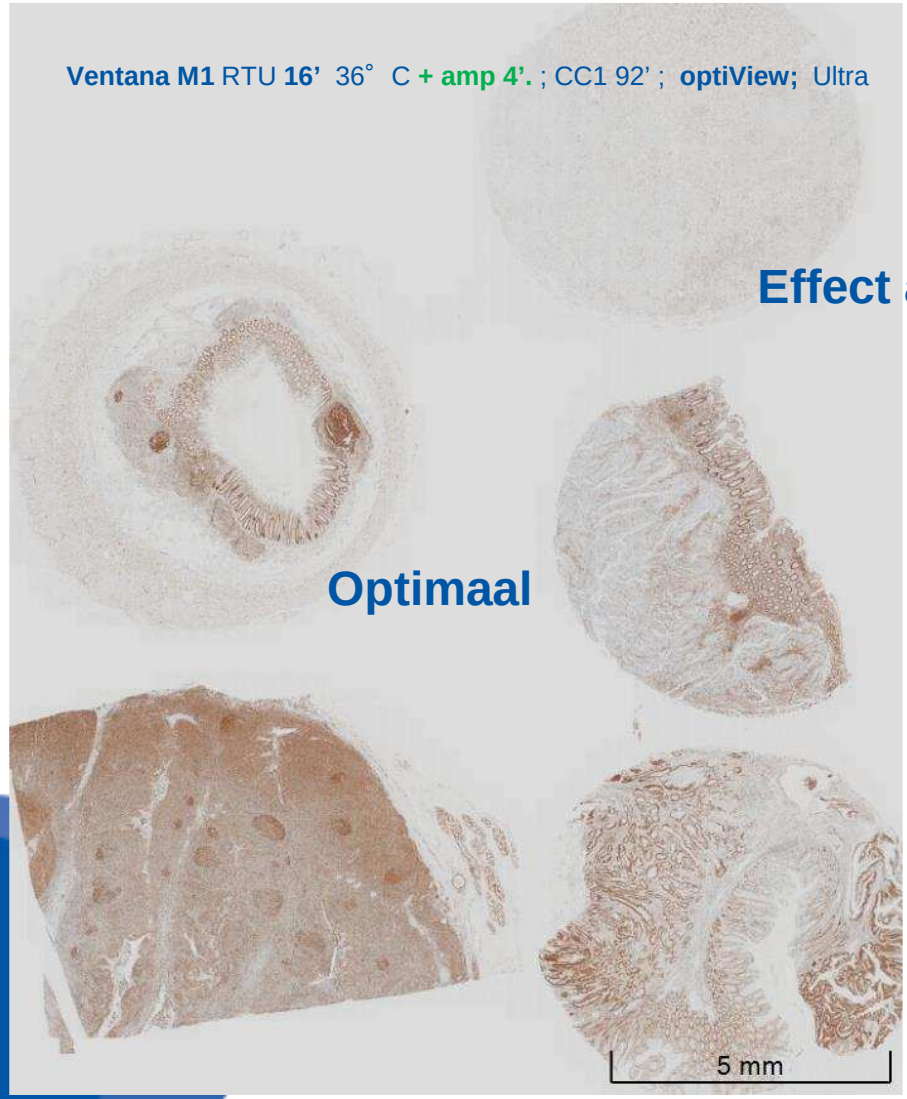
Dako ES05 RTU **12'** 36° C **+ amp 8'** ; CC1 80' ;**optiView;** Ultra

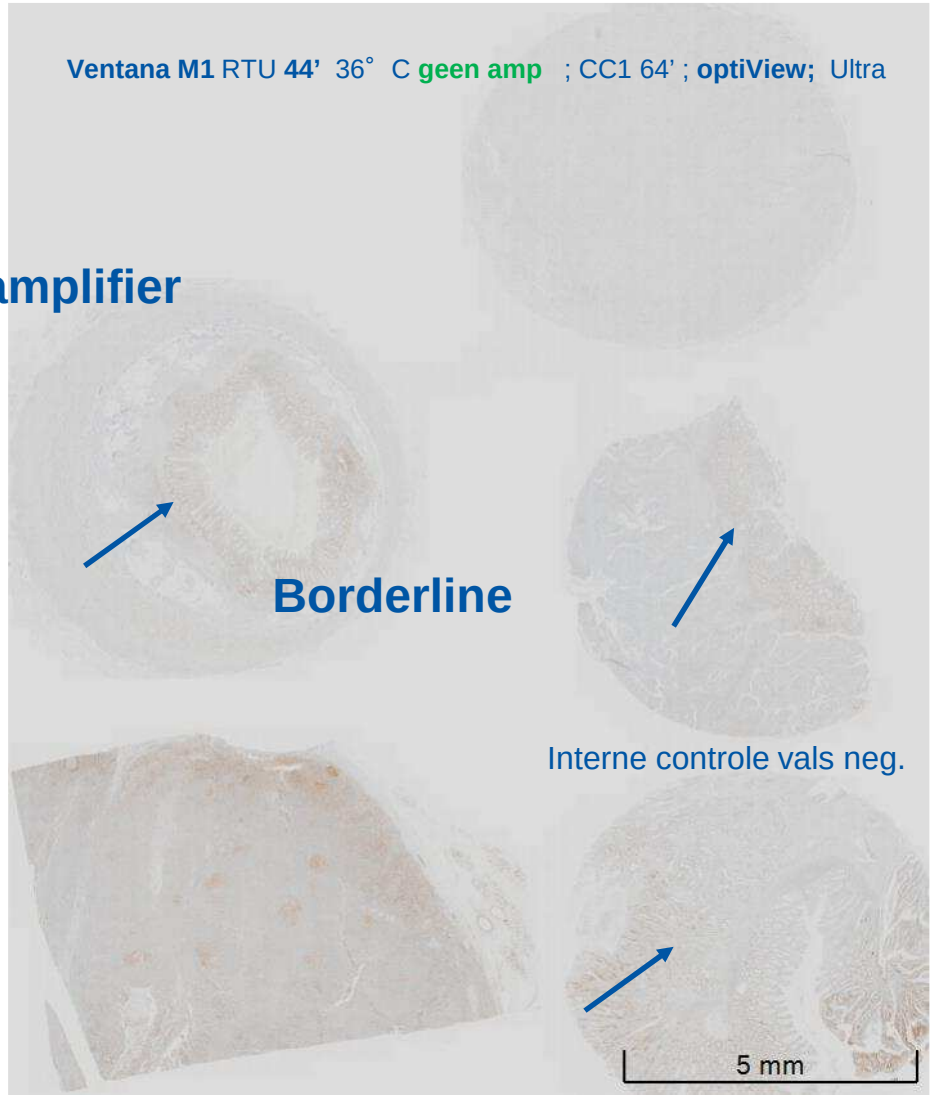
Gelijkaardig CC1 maar OV!



Effect detectiesysteem keuze in klinische setting

Antistof MLH1: signaalversterking





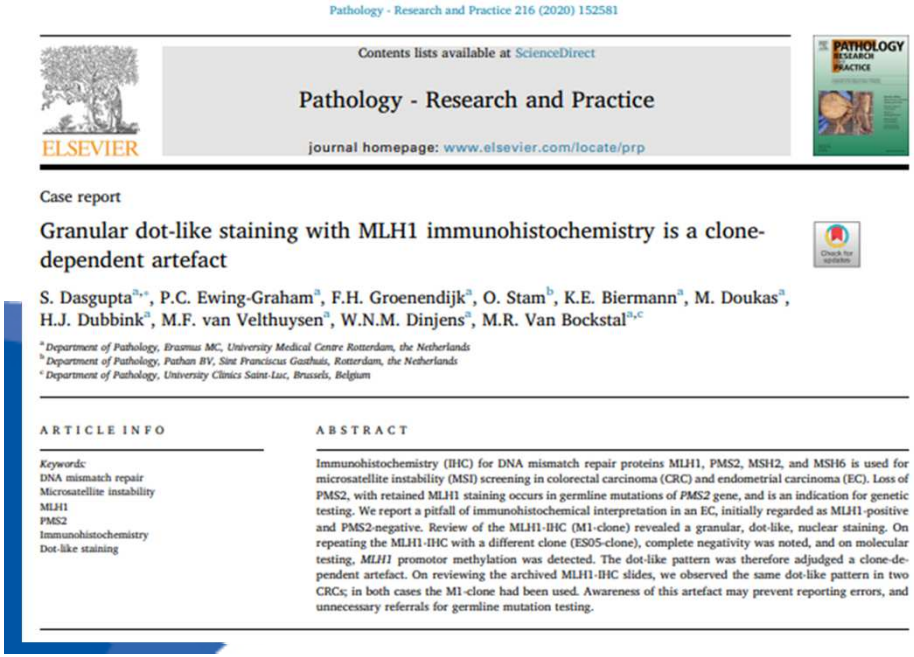
Effect amplifier

Antistof MLH1

- Vals positieve aankleuring (tov referentiecoupe) in colonCa met expressieverlies
  - 9 laboratoria, allen Ventana M1 clone
  - Enkel in core 5 (expressie verlies extern bevestigd)
  - Typisch 'granulair/dot like' waarneming naast specifieke kernaankleuring in >10% tumor

    → als een artefact gelinkt aan het gebruik van deze kloon,

    → interpretatie risico
  - Contact met Roche EMEA EQA-lab Strasbourg → nog geen verdere info



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journal homepage: www.elsevier.com/locate/prp

Case report

Granular dot-like staining with MLH1 immunohistochemistry is a clone-dependent artefact

S. Dasgupta[a,*], P.C. Ewing-Graham[a], F.H. Groenendijk[a], O. Stam[b], K.E. Biermann[a], M. Doukas[a], H.J. Dubbink[a], M.F. van Velthuysen[a], W.N.M. Dinjens[a], M.R. Van Bockstal[a,c]

[a] Department of Pathology, Erasmus MC, University Medical Centre Rotterdam, the Netherlands
[b] Department of Pathology, Pathan BV, Sint Franciscus Gasthuis, Rotterdam, the Netherlands
[c] Department of Pathology, University Clinics Saint-Luc, Brussels, Belgium

ARTICLE INFO

Keywords:
DNA mismatch repair
Microsatellite instability
MLH1
PMS2
Immunohistochemistry
Dot-like staining

ABSTRACT

Immunohistochemistry (IHC) for DNA mismatch repair proteins MLH1, PMS2, MSH2, and MSH6 is used for microsatellite instability (MSI) screening in colorectal carcinoma (CRC) and endometrial carcinoma (EC). Loss of PMS2, with retained MLH1 staining occurs in germline mutations of *PMS2* gene, and is an indication for genetic testing. We report a pitfall of immunohistochemical interpretation in an EC, initially regarded as MLH1-positive and PMS2-negative. Review of the MLH1-IHC (M1-clone) revealed a granular, dot-like, nuclear staining. On repeating the MLH1-IHC with a different clone (ES05-clone), complete negativity was noted, and on molecular testing, *MLH1* promotor methylation was detected. The dot-like pattern was therefore adjudged a clone-dependent artefact. On reviewing the archived MLH1-IHC slides, we observed the same dot-like pattern in two CRCs; in both cases the M1-clone had been used. Awareness of this artefact may prevent reporting errors, and unnecessary referrals for germline mutation testing.

'The main reason was an unexpected aberrant nuclear staining reaction of neoplastic cells in the colon adenocarcinoma tissue core no. 4, a tumour known not to express MLH1. The aberrant nuclear staining was primary seen in certain areas of the tumour. These areas generally accounted for more than 10% of the neoplastic cells. Occasionally the aberrant staining reaction was both diffuse and granular/dot-like in appearance (see Fig. 5a), whereas other cases only showed a dot-like nuclear staining reaction (see Fig. 5b). In previous NordiQC assessments aberrant nuclear staining Nordic Immunohistochemical Quality Control, MLH1 run 49 2017 Page 5 of 8 have not been observed with mAb clone M1, but the Canadian Immunohistochemistry Quality Control program (cIQc) reported in their MLH1 assessment in 2014: "Four different clones were used. Clone M1 did appear to give false positive staining of core 4, for some labs" 1 . No explanation was given for this reported false positive staining. Likewise, no explanation can be given for the aberrant nuclear staining in the neoplastic cells in colon adenocarcinoma no. 4 seen in the recent NordiQC assessment. '

Nordic Immunohistochemical Quality Control, MLH1 run 49 2017

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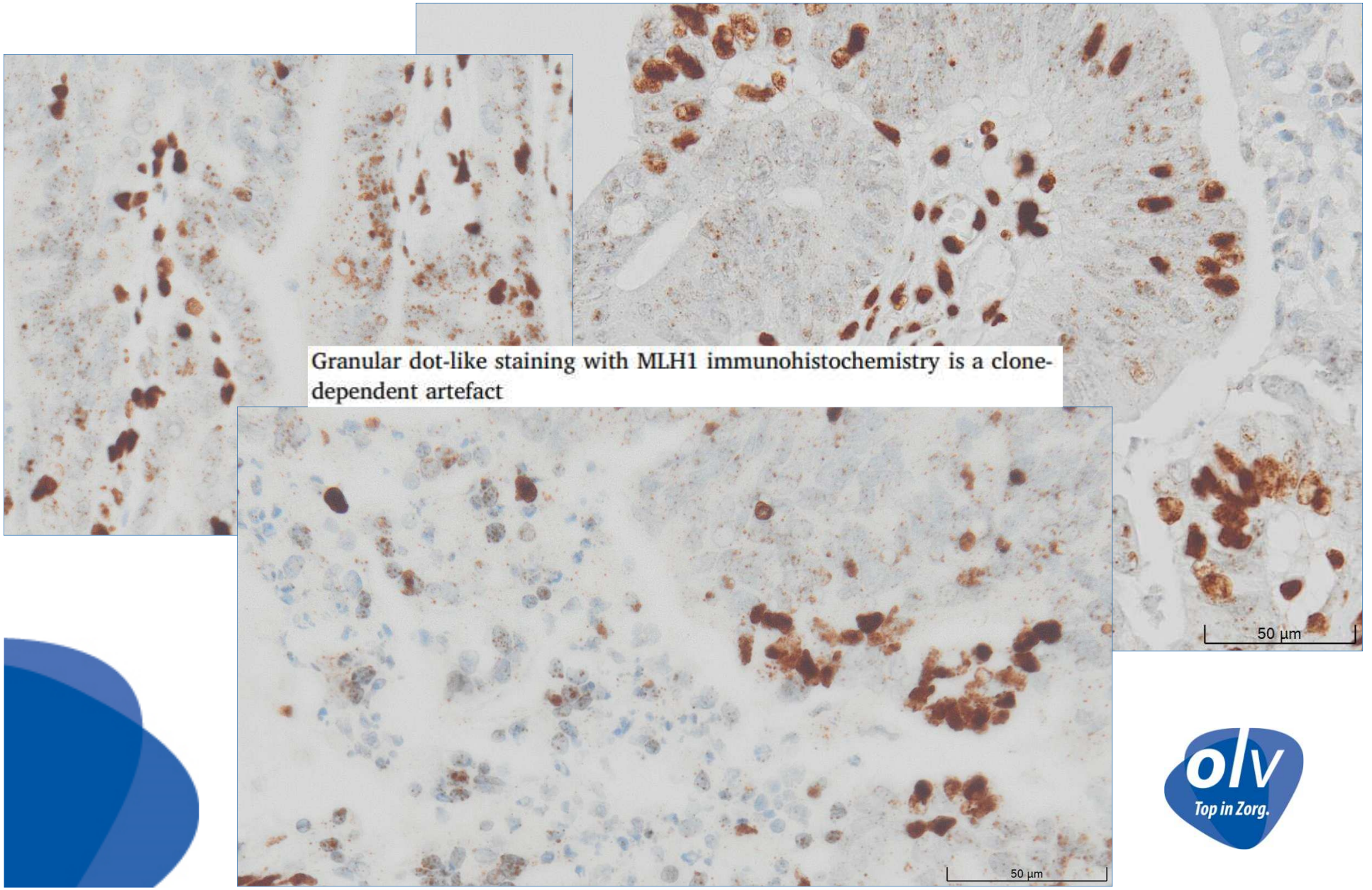
Antistof MLH1



Resultaatbespreking MLH1

- Algemeen
  - n=59
    - Suff. 46 %

		EKE 2022-2		NQC 2019		NQC 2017	
	n		*suff*	*%*	*suff*	*%*	*suff*
optimaal	**21**	**36%**	***46%***	***64%***	***90%***	***30%***	***59%***
goed	**6**	**10%**		***26%***		***29%***	
Borderline	27	**46%**		*7%*		*37%*	
Onvoldoende	5	**8%**		*3%*		*4%*	

Resultaatbespreking MLH1

- Opgesplitst per clone producent
  - RTU: 35 % optimaal ; suff. 46 %

n=54	*Dako ES05*	*% suff.*	*Ventana M1*	*% suff.*	*Leica ES05*	*% suff*		
	n=23		n=28		n=2			
optimaal	12	***52%***	7	***25%***	0	**-**		
goed	4	***17%***	2	***7%***	0	**-**		
borderline	7	***31%***	17	***61%***	1	-		
onvoldoende	0	***0%***	2	***7%***	1	**-**		
Totaal suff.	***69%***		***32%***		***0/2***			
NQC 2019	*94% (74% optimaal)*		*98% (83% optimaal)*		*Geen data*			

Resultaatbespreking MLH1

- Opgesplitst per clone producent
  - Concentrated: ½ geslaagd

n=2	*Dako ES05*	*% suff*	*Leica ES05*	*% suff*
	n=1		n=1	
optimaal	0	-	1	-
goed	0	-	0	-
borderline	1	-	0	-
onvoldoende	0	-	0	-
Totaal suff.	*0/1*		*1/1*	
NQC 2020	*89% (61% optimaal) cf onder*			

Table 3. **Proportion of optimal results for MLH1 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Dako OMNIS		Ventana BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **ES05**	4/5** (80%)	-	4/7 (57%)	-	18/28 (64%)	-	9/15 (60%)	1/2
mAb clone **G168-15**	-	-	-	-	4/11 (36%)	-	1/2	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Resultaatbespreking MLH1

Besluit:

- <50% geslaagd, beduidend lager NQC 2019 maar ~ NQC 2017
- Zowel bij RTU als conc.

- Borderline/Onvoldoende: alle platformen
  - Analytische sensitiviteit te laag : vals negatieve/ veel te zwakke aankleuring
  - Interne controle
    - Tonsil: mantelzone B-cellen (zwakke expressor) vals negatief/te zwak ; kiemcentra OK
    - Appendix: ook columnair epitheel te zwak (moet sterk pos. zijn)

  - Roche: 2-staps detectie, RTU verdund, zelfs met amplifier of combinatie

  - Leica: verkeerde selectie HIER (pH6)?

  - Dako: vrij gestandaardiseerd (+- linker) → geen duidelijke reden, zelfde settings optimaal/goed

    Opmerking NordiQC: *'These data suggest that the use of the IR079/IS079 system on the Dako Omnis platform within a laboratory developed (LD) assay, requires profound adjustment of the protocol settings and a "direct" transfer of the original Autostainer protocol cannot be applied*
    --> MLH1 Omnis released, geen data gebruik labo's (6 labs borderline)

Resultaatbespreking MLH1

Besluit:

- Dako ES05 RTU:
  - 69% geslaagd ; 52% optimaal
  - Per type stainer
    - Ventana stainer:
      sensitieve settings , slechts ½ geslaagd (optiView+amplifier)
      ultraView + amplifier = niet geslaagd
    - Dako Omnis:
      vrij gestandaardiseerd (+-linker)
      gelijke settings geven wisselend resultaat: optimaal → borderline te zwak
      1/3 niet geslaagd: oorzaak?
    - Dako Autostainer:
      ¾ geslaagd
      'eerder matige aankleuring' → 'goed'
      kortste AS tijd (20') = borderline (te zwak + heterogeen) → 25-30'=geslaagd
- Leica ES05 RTU: weinig data (2 deelnemers)
  - Geen geslaagd: te zwak tot vals negatief
  - Verkeerde pH keuze (pH6). Oorzaak?

Table 4. **Proportion of sufficient and optimal results for MLH1 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako Autostainer Classic/Link mAb ES05 **IR079/IS079**	100% 15/15	93% 14/15	90% 18/20	60% 12/20

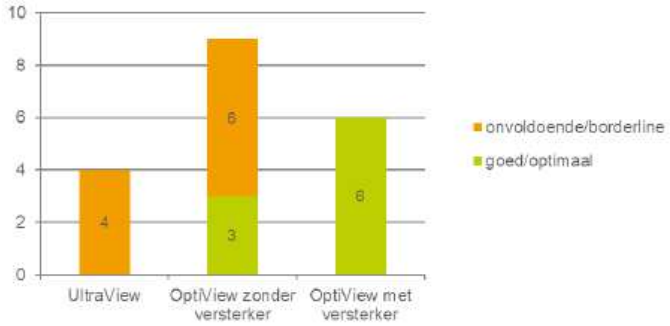
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipmen
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Resultaatbespreking MLH1

Besluit:

- Ventana M1 RTU
  - Slechts 32% geslaagd (25% optimaal)
  - 3-stap detectie met amplifier (én optimale settings) is noodzakelijk voor optimaal resultaat

    → 2-stap detectie (15%) met amplifier = niet geslaagd



  - Datasheet: slechts 3 labo's → niet geslaagd (vals positieve core 5)

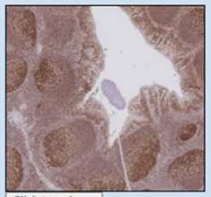
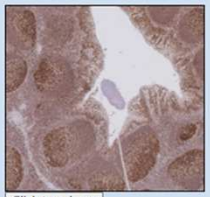
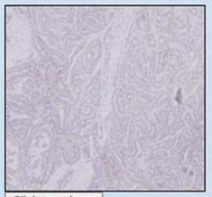
Procedure Type	Method		
	GX	XT	ULTRA
Antibody (Primary)	anti-MLH1 Mouse Mono Ab Selected Or Negative Control Selected	anti-MLH1 Mouse Mono Ab Selected Or Negative Control Selected	anti-MLH1 Mouse Mono Ab Selected Or Negative Control Selected
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes	CC1, 64 minutes	ULTRA CC1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	24 minutes, 37°C	24 minutes, 37°C	24 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

Resultaatbespreking MLH1

Besluit:

- Controle
  - 58% gebruikt controle
  - Aanbevolen controle:
    - ✓ Tonsil:
      - prolifererende B-cellen in de kiemcentra matig tot sterk positief.
      - bijna alle B-cellen in de mantelzone minstens zwak tot matig positief
    - ✓ ColonCa verlies expressie als negatieve controle
      - geen expressie in carcinoom maar…
      - duidelijke expressie in stromale cellen en lymfocyten (zo sterk mogelijk zonder achtergrond)

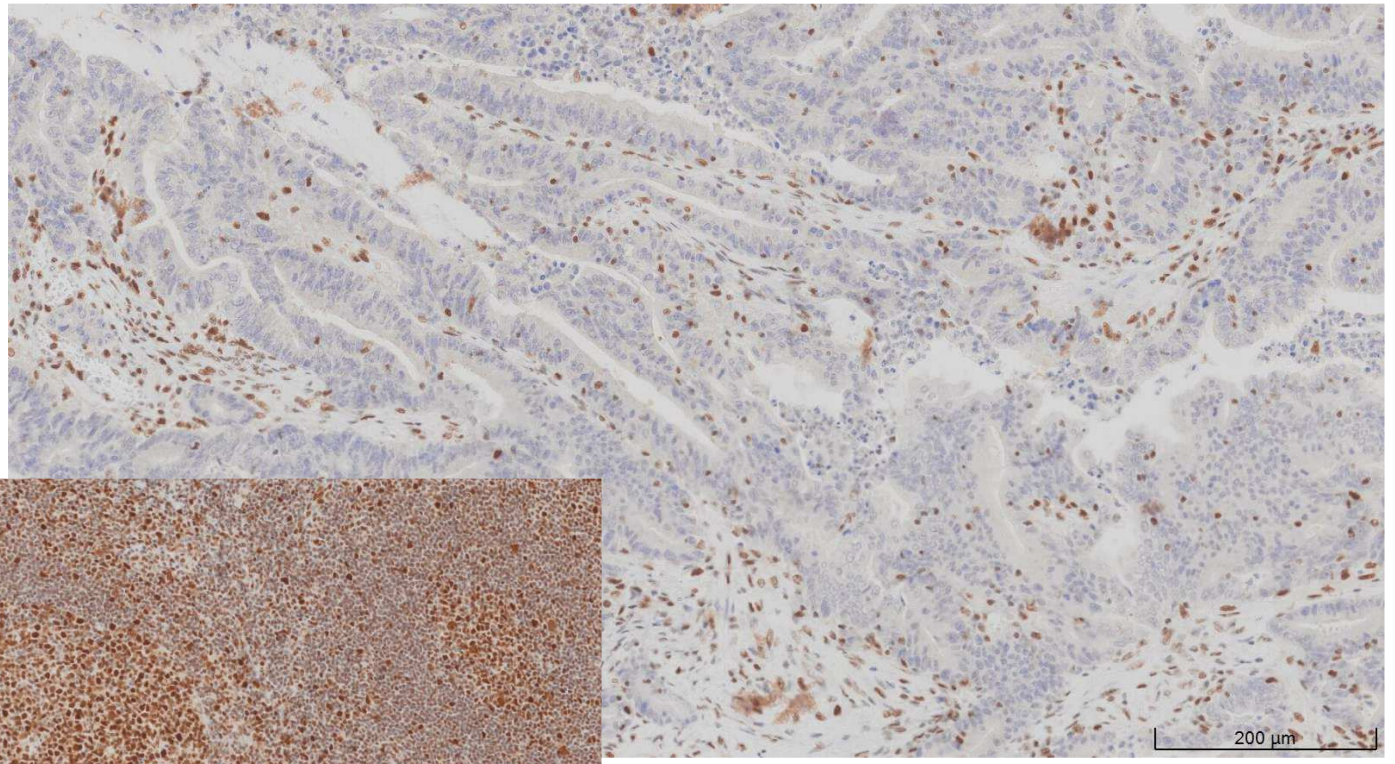
MLH1 - Mismatch repair protein MLH1

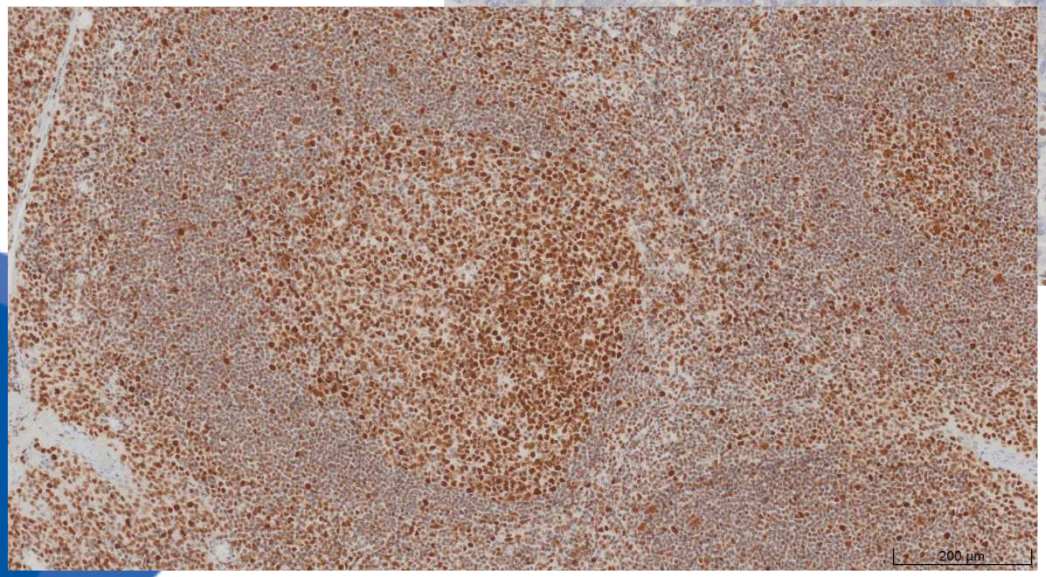
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Tonsil	Tonsil	Tumour
Description	Virtually all proliferating germinal centre B-cells must show a distinct, moderate to strong nuclear staining reaction.	Virtually all mantle zone B-cells and interfollicular lymphocytes must show an at least weak to moderate and distinct nuclear staining reaction.	Colon adenocarcinoma with validated loss of MLH1 expression is recommended as negative tissue control for MLH1. No nuclear staining reaction should be seen in the neoplastic cells. *(other solid carcinoma with confirmed and validated MLH1 loss can be used as alternative to colon adenocarcinoma)*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Resultaatbespreking MLH1

Besluit:

- Controle





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Resultaatbespreking besluit

geslaagd		2022	NQC
CD5		98%	92%
panCK		84%	82%
MLH1		46%	90%*

*2017: 59%

Reassessment MLH1

- Scoringscriteria: kern aankleuring

- **Appendix :** zwakke tot matige aankleuring van bijna alle cellen

- Tonsil :

- matige tot sterke aankleuring van de B-cellen in de kiemcentra
- zwakke tot matige aankleuring van bijna alle B-cellen in de mantelzone

- Colon, adenocarcinoom :

- bewaarde expressie d.w.z. sterke aankleuring in de neoplastische cellen
- sterke aankleuring in de meeste andere cellen (stromale cellen, lymfocyten enz.) (controle sensitiviteit)
- sterke aankleuring in het columnair epitheel (indien aanwezig)

- Colon, adenocarcinoom :

- verlies van expressie in de neoplastische cellen (controle specificiteit)
- sterke aankleuring in de meeste andere cellen (stromale cellen, lymfocyten enz.) (controle sensitiviteit)
- sterke aankleuring in het columnair epitheel (indien aanwezig)

Opmerking : zwakke cytoplasmatische aankleuring wordt aanvaard

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Reassessment MLH1

Algemeen

n=17		***reassess***	
	n	*%*	*suff*
optimaal	**10**	***59%***	***88 %***
goed	**5**	*29%*	
borderline	**2**	*12%*	
onvoldoende	**0**	*0 %*	

		EKE 2022-2		NQC 2019		NQC 2017	
	n		*suff*	*%*	*suff*	*%*	*suff*
optimaal	**21**	**36%**	***46%***	***64%***	***90%***	***30%***	***59%***
goed	**6**	**10%**		***26%***		***29%***	
Borderline	27	**46%**		*7%*		*37%*	
Onvoldoende	5	**8%**		*3%*		*4%*	

Geslaagd : 46% → 71%

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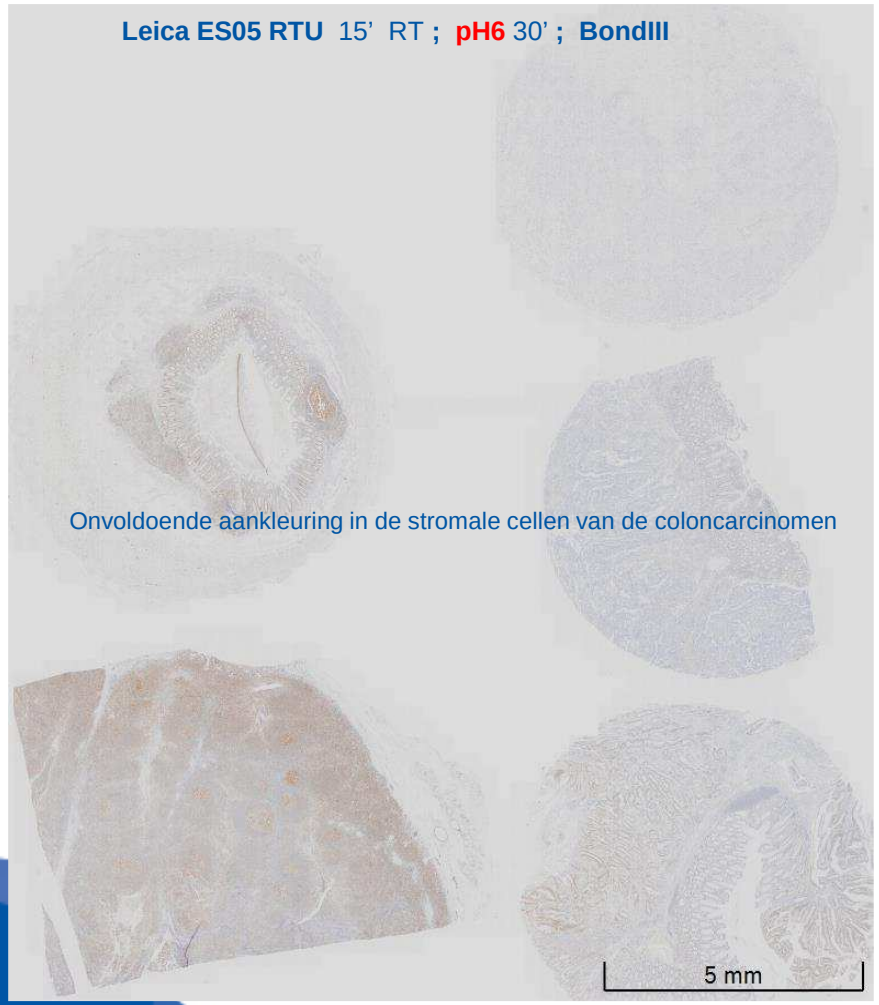
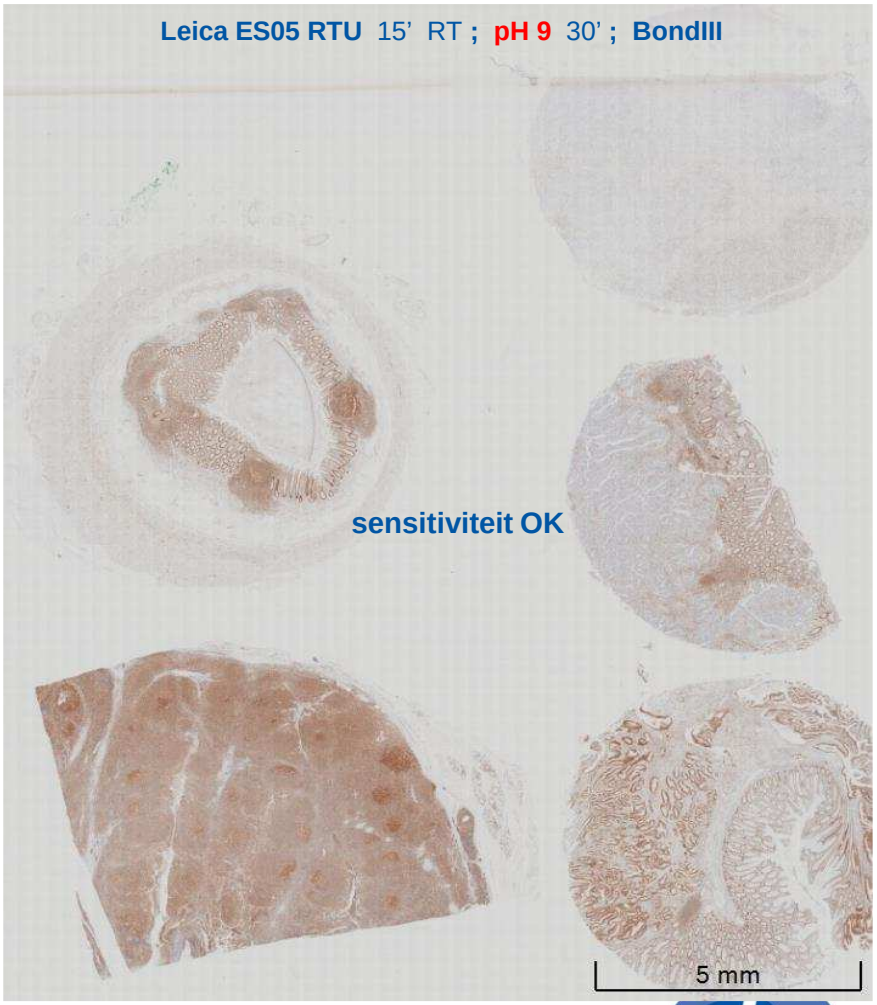
Reassessment MLH1



VOOR



NA

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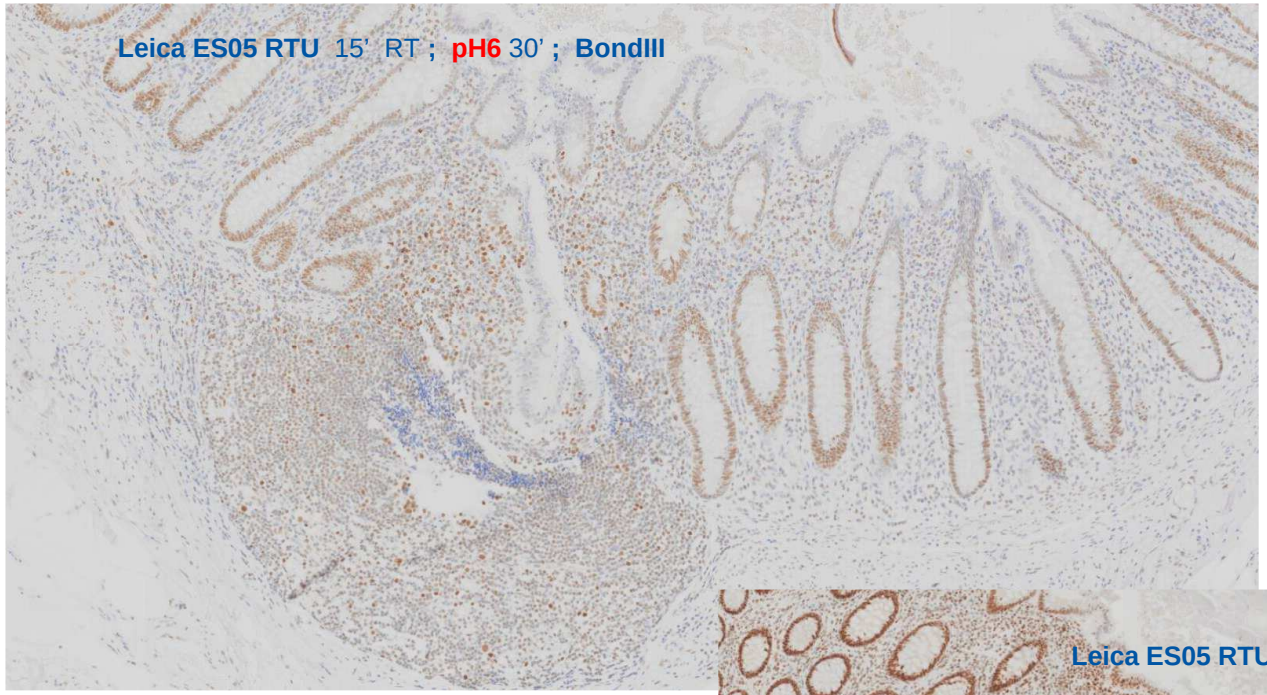
Reassessment MLH1



VOOR

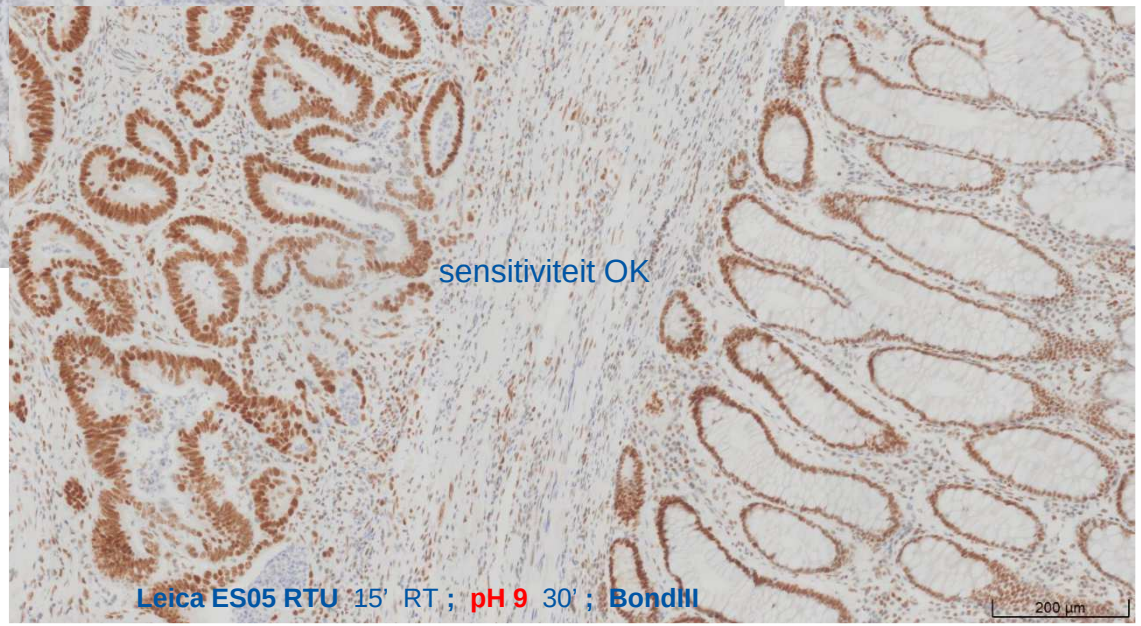
NA

sensitiviteit OK

Reassessment MLH1

Impact HIER op outcome is bepalend

NA



VOOR

Reassessment MLH1

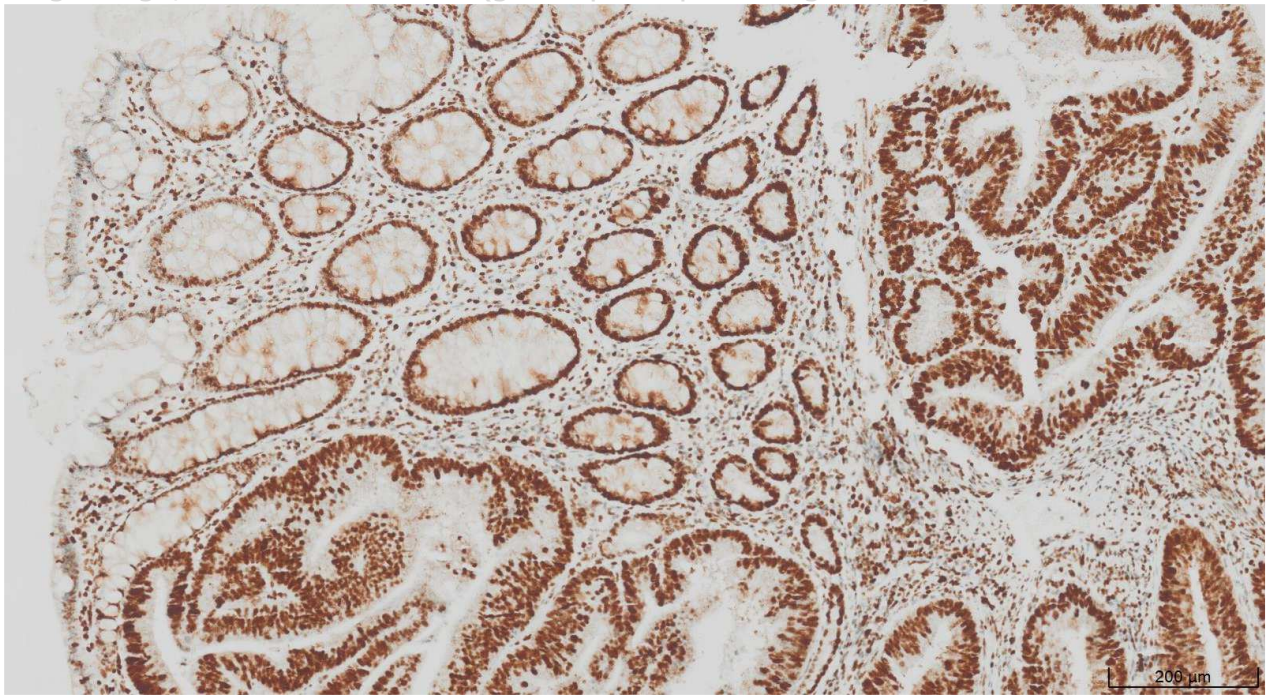
Besluit:

- 17 niet geslaagde labo's hebben een reassessment aangevraagd (niet verplichtend)
- 88% geslaagd, slechts 2 borderline (geen update protocol gekend?)
- Aktieplan:
  - 6/13 protocolwijziging: aanpassing : geen HIER → HIER en/of AS tijd (RTU)
  - 7 geen ruwe data
- Resultaten:
  - Gewijzigde protocols: 11/11 geslaagd (6 optimaal) ; maar nog steeds (beperkte) kernaankleuring in core 5 → risico op vals positieve interpretatie of te zwakke aankleuring stromale cellen (interne controle)
  - Geen data: 6/8 geslaagd (4 optimaal), maar nog steeds (beperkte) kernaankleuring in core 5 → risico op vals positieve interpretatie (in rapport)

Reassessment MLH1

Besluit:

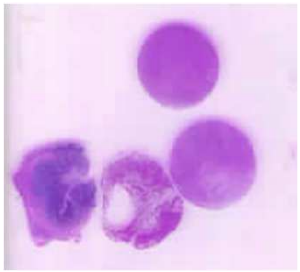
- 17 niet geslaagde labo's hebben een reassessment aangevraagd (niet verplichtend)
- 88% geslaagd, slechts 2 borderline (geen update protocol gekend?)



'a gradient of expression in the normal crypts, which fades out towards the lumen should be present'

Te sensitief protocol

Reassessment CD5



- Scoringscriteria: overwegend membranaire aankleuring
  - Tonsil § (fixatie 24u.) : controle robuustheid
    - sterke aankleuring van bijna alle T-cellen in de T zones en in de kiemcentra
    - minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones
  - Tonsil § (fixatie 44u.) : controle robuustheid
    - sterke aankleuring van bijna alle T-cellen in de T zones en in de kiemcentra
    - minstens zwakke tot matige aankleuring in (verspreide) B-cellen in de mantelzones
  - MCL :
    - minstens matige aankleuring in de neoplastische cellen (controle sensitiviteit)
    - sterke aankleuring van alle T-cellen tussen de neoplastische cellen
  - DLBCL :
    - aankleuring in de lymfocyten
    - geen aankleuring in de neoplastische cellen (controle specificiteit)
  - B-CLL :
    - sterke aankleuring in de neoplastische cellen
    - sterke aankleuring van alle T-cellen tussen de neoplastische cellen
  - Alle weefsels : geen aankleuring in de B-cellen van de kiemcentra

§ Aankleuring van plaveiselcelepitheel dat de crypten bekleedt wordt aanvaard.

Reassessment CD5

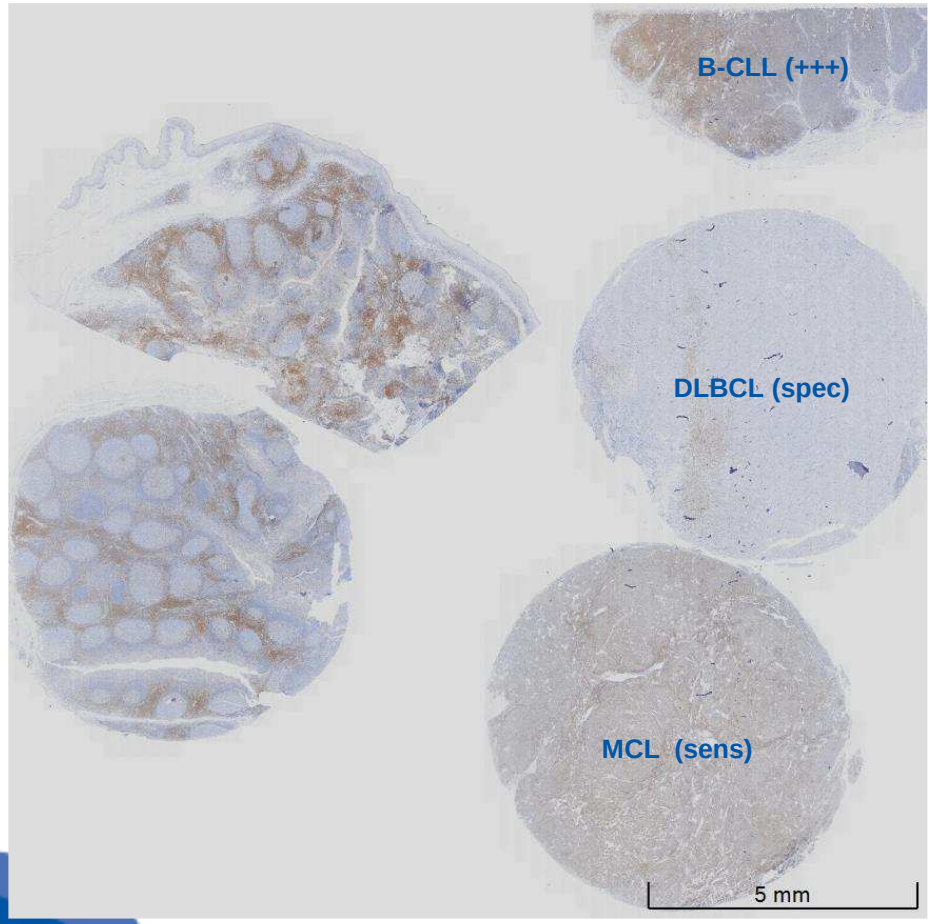
Algemeen

n=1		***reassess***	
	n	*%*	*suff*
optimaal	**0**	**-**	***1/1***
goed	**1**	*1/1*	
borderline	**0**	-	
onvoldoende	**0**	-	

		EKE 2020-2		***NQC 2016***	
	n		*suff*	*%*	*suff*
optimaal	**38**	**58%**	***75%***	***48%***	***87%***
goed	**11**	**17%**		***39%***	
Borderline	7	**11%**		*10%*	
Onvoldoende	8	**12%**		*3%*	

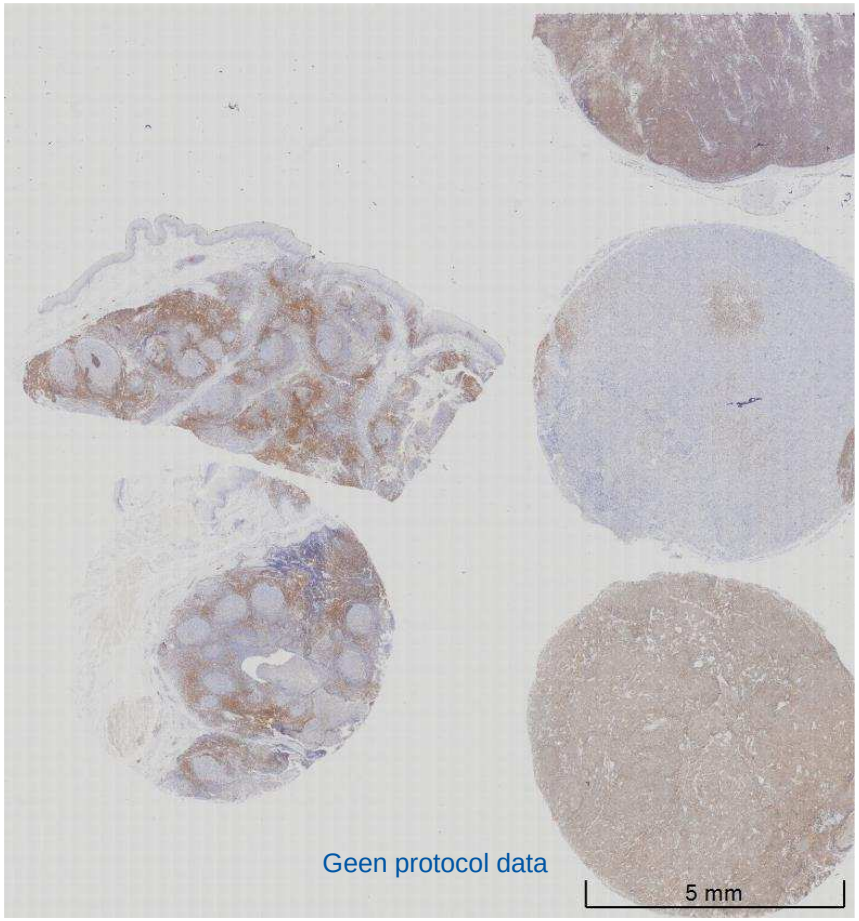
Geslaagd : 75% →78%

Reassessment CD5



Dako 4C7 1/200 20' ; pH9 30' ; Dako toestel??

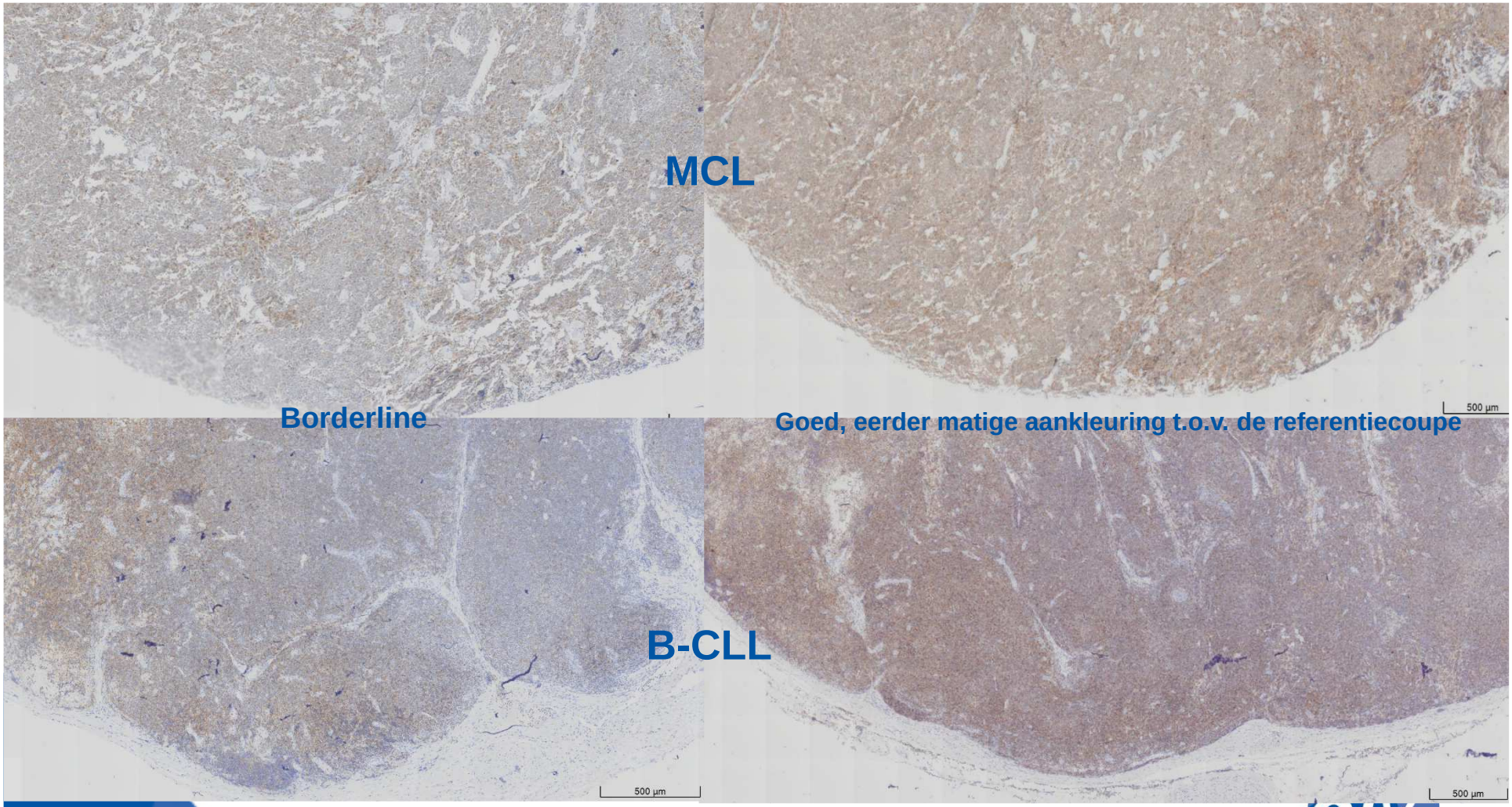
VOOR



Eerder matige aankleuring t.o.v. de referentiecoupe in alle weefsels

NA

Reassessment CD5



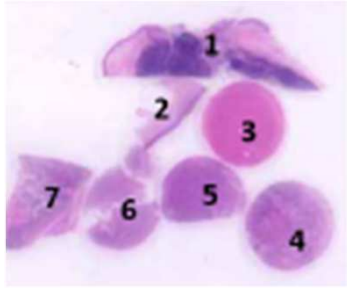
Dako 4C7 1/200 20' ; pH9 30' ; Dako toestel??

Geen protocol data

VOOR

NA

Reassessment panCK



- Scoringscriteria: cytoplasma/membraan aankleuring

- Tonsil :

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige aankleuring in de interstitiële reticulum cellen ('cytokeratin-positive interstitial reticulum cells' of 'CIRC') (controle sensitiviteit)
- geen aankleuring in de lymfocyten (controle specificiteit)

- Slokdarm :

- sterke aankleuring van het plaveiselcelepitheel doorheen alle cellagen
- zwakke tot matige ('patchy') aankleuring in de gladde spiercellen van de bloedvaten en van de muscularis mucosae (controle sensitiviteit)

- Lever :

- sterke aankleuring in de epitheliale cellen van de galwegen
- minstens matige aankleuring in de meeste hepatocyten mét membranaire accentuatie (controle sensitiviteit)
- geen aankleuring in de stromale cellen (controle specificiteit)

- Long, adenocarcinoom en plaveiselcelcarcinoom :

- sterke aankleuring van bijna alle neoplastische cellen

- Nier, CCRCC :

- zwakke tot matige (voornamelijk membranaire) aankleuring van de meeste neoplastische cellen (controle analytische sensitiviteit)

- Testis, DLBCL :

- geen aankleuring van de neoplastische cellen (controle analytische specificiteit)

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Reassessment panCK

Algemeen

n=6		***reassess***	
	n	*%*	*suff*
optimaal	**5**	***83%***	***100%***
goed	**1**	*17%*	
borderline	**0**	-	
onvoldoende	**0**	-	

		EKE 2021-1		NQC 2020		NQC 2017	
	n		suff	%	suff	%	suff
optimaal	**50**	**78%**	***84%***	***52%***		***38%***	
goed	**4**	**6%**		***23%***	***82%***	***24%***	***82%***
borderline	3	**5%**		*15%*		*19%*	
onvoldoende	7	**11%**		*11%*		*19%*	

Geslaagd : 84% →94%

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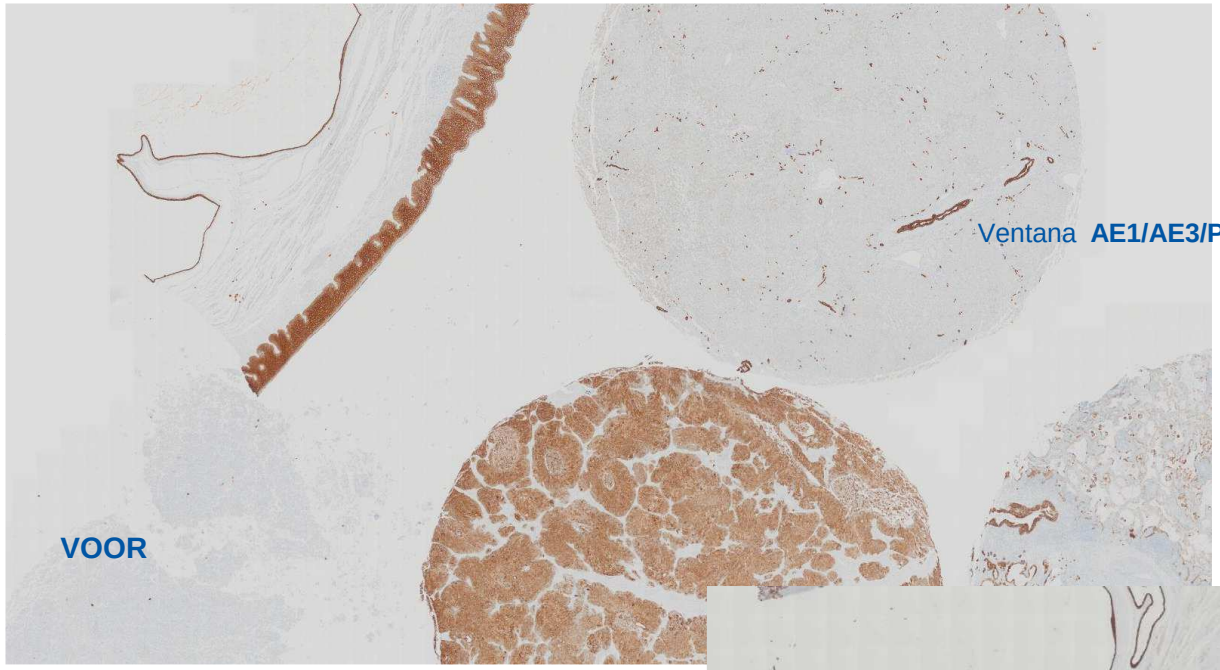
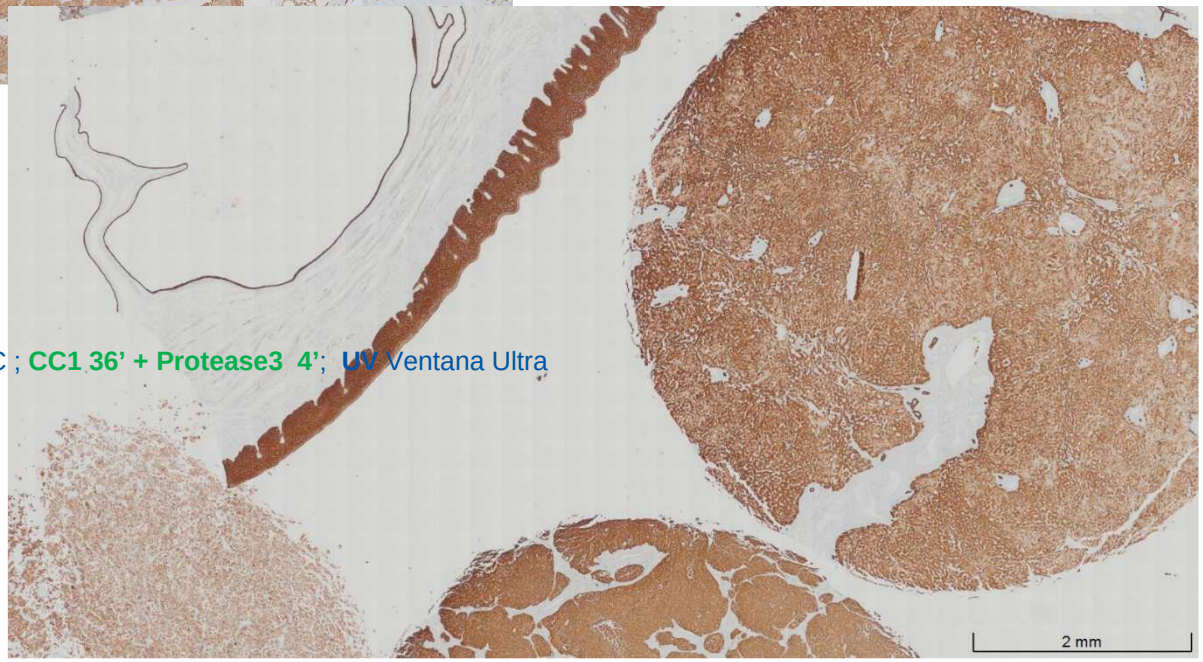
Reassessment panCK



Ventana **AE1/AE3/PCK26** RTU 32’ 36˚ C ; **Protease1 8’**; **UV** Ventana Ultra

VOOR

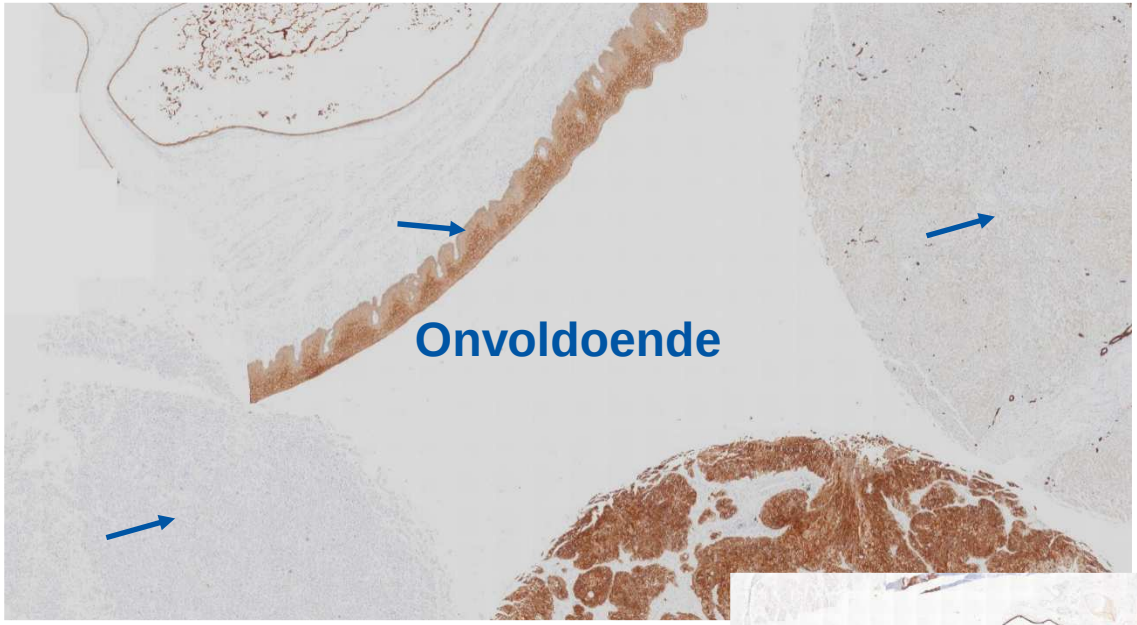


Ventana **AE1/AE3/PCK26** RTU 32’ 36˚ C ; **CC1 36’ + Protease3 4’**; **UV** Ventana Ultra

Optimaal

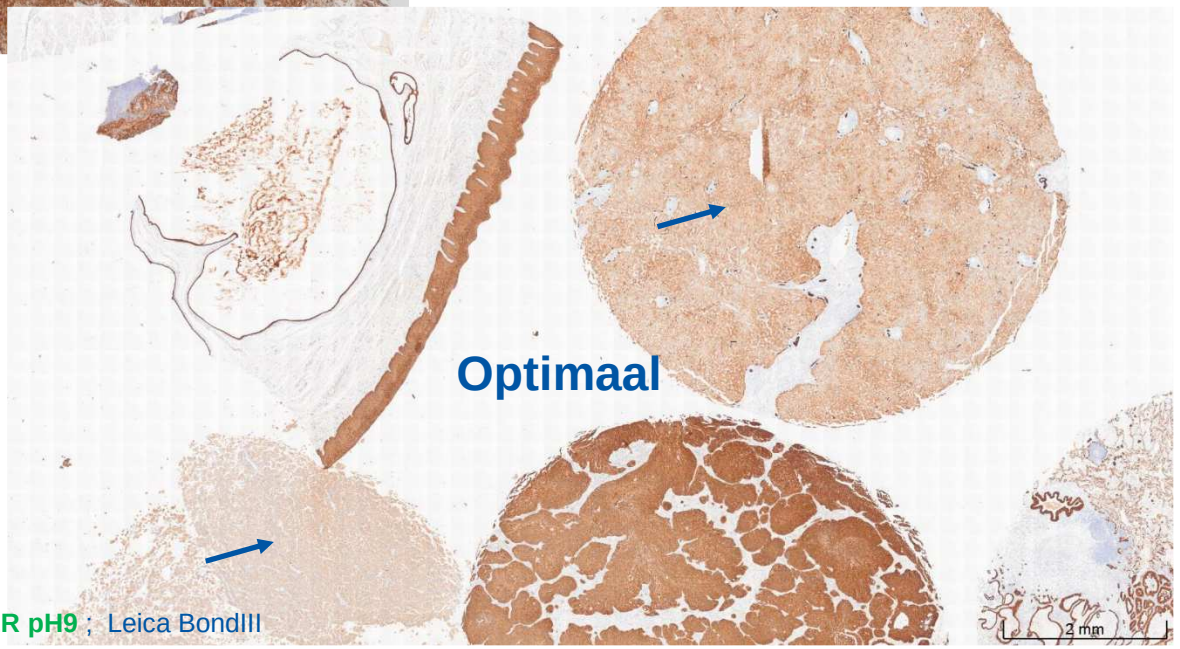
NA

Reassessment panCK



VOOR

Leica AE1/AE3 15’ RT ; **HIER pH6** ; Leica BondIII



NA

Leica AE1/AE3 15’ RT ; **HIER pH9** ; Leica BondIII

Reassessment panCK

Besluit:

- 6 niet geslaagde labo's hebben een reassessment aangevraagd (niet verplichtend)

- Aktieplan:
  - 1 lab zonder protocolwijzigingen
  - 5 labo's gekend mét wijzigingen (wijziging voorbehandeling: P1→P3/combined of type HIER pH6→pH9)
  - 1 geen ruwe data

- Resultaten:
  - 100% geslaagd, 5/6 optimaal
  - Gewijzigde protocols: allen geslaagd
  - Niet gewijzigd protocol: geslaagd
  - Geen data: onvoldoende → optimaal

EKE planning

Externe kwaliteitsevaluatie	EKE 2022-1	EKE 2022-2	EKE 2022-3	**EKE 2023-1**	**EKE 2023-2**
	ER Her2	AMACR MLH1 CD5	PR / Her2 ER reassess 2022-1	**ER Her2**	**EMA, MelanA, p63, BCL2**
Rondzending coupes	17-21/02/2020	4-8/05/2020	14-18/09/2020	2/2023	5/2023
Beoordeling panel	03/2020	6/2020	10/2020	03/2023	06/2023
Doorsturen individueel resultaat	05/2020	10/2020	11/2020		
Resultaatbespreking / symposium	?	?	?		
Doorsturen globaal rapport	?	?	?		

Symposium donderdag 25/05/2023

Symposium vrijdag 08/12/2023

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