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Resultaten EKE IHC 2020-2

De Wiest Bart

11 december 2020

OLV Ziekenhuis Aalst

EKE IHC 2020-2

Antistoffen:

- ✓ CD3: n= 67
- ✓ Chromogranine: n= 67
- ✓ Desmine: n= 65

Panel:

- ✓ Scoring 07/07/2020 (UZ Gent)
- ✓ Onafhankelijk, in consensus
- ✓ 3 pathologen
  - Dr. V. Schelfhout (AZ St-Maarten)
  - Dr. A. Dendooven (UZ Gent)
  - Dr. A.M. Dierick (Sciensano)

Protocol CD3

- Gebruikte clone:
  - Conc.: n=8

    - DAKO polyclonaal op Ventana stainer UV/OV (n=2)

      - 1/200 32’ ; HIER CC1 36’ UV BMK XT
      - 1/400 20’ +amp. ; HIER CC1 32’ OV BMK Ultra

    - Cell Marque MRQ-39 clone (n=2) UV BMK Ultra

      - 1/200 16’ ; HIER CC1 36’
      - 1/800 24’ +amp. ; HIER CC1 64’

Protocol CD3

- Gebruikte clone:
  - Conc. vervolg:
    - Thermo/Neomarkers SP7 clone (n=2) UV BMK Ultra
      - Zelfde protocol : 1/100 52’ +amp. bij RT ; HIER CC1 64’
    - Leica LN10 clone (n=2)
      - BondIII : 1/200 15’ RT ; HIER pH9 20’
      - Dako Omnis: 1/200 20’ 32° C +linker ; HIER pH6 30’
  - RTU ½ verdund:
    - Dako poly op Ventana GX stainer: AS 28’ ; HIER CC1 30’

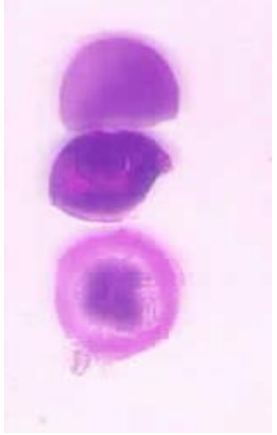
Protocol CD3

- Gebruikte clone:
  - RTU: n= 58
    - DAKO polyclonaal: n=25
      - Omnis:
        - 10’ - 20’ - 30’ RT/32° C +/- linker <> datasheet 20’
        - HIER 30’ → 1/3 pH9 <> Datasheet HIER pH6 30’
      - Autostainer: vrij gestandaardiseerd
        - 35’ RT ; HIER pH9 10’ (1/7)
        - 20’ RT ; HIER pH9 10’
        - Datasheet: HIER pH9
      - Ventana Ultra:
        - 32’ RT ; HIER CC1 36’ iView
        - 40’ bij 36° C ; HIER CC1 60’ UV

Protocol CD3

- Gebruikte clone:
  - RTU:
    - LEICA: n=2
      - LN10 clone : beiden op Bond stainer
      - Incubatietijd: 15’
      - HIER pH9 : 20’
    - VENTANA: n=30
      - 2GV6 clone
      - Incubatietijd: 12’ - 32’ RT / 36-42° C ; +/- amp.
        <> datasheet 16’ – 20’
        <> 50% gebruikt datasheet
      - HIER CC1 16’ - 72’
        <> datasheet 40’ OV of mild CC1 UV, ongeacht toesteltype
        <> slechts 1/3 gebruikt UV datasheet
        <> 0/4 OV gebruikers gebruikt datasheet

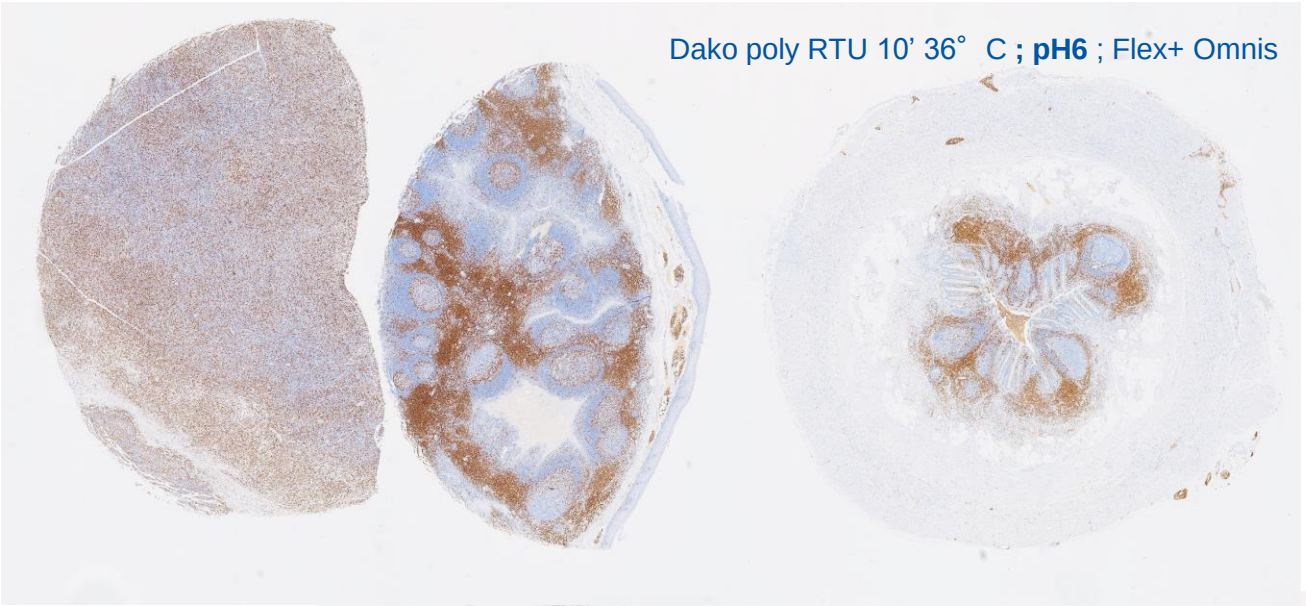
Antistof CD3



- Scoringscriteria: overwegend membranaire aankleuring
  - Appendix:
    - Matig tot sterke aankleuring van de T-cellen (intra-) epitheliaal (sterke expressor)
  - Tonsil:
    - Matig tot sterke aankleuring in kiemcentra én intrafolliculaire T-zones.
  - T-cel lymfoom:
    - Meerderheid van neoplastische cellen minstens zwak tot matig positief (controle analytische sensitiviteit)
  - Alle weefsels :
    - geen aankleuring in andere cellen, vnl. B-cellen in tonsil moeten negatief zijn (controle specificiteit)

Antistof CD3: overzicht

Optimaal



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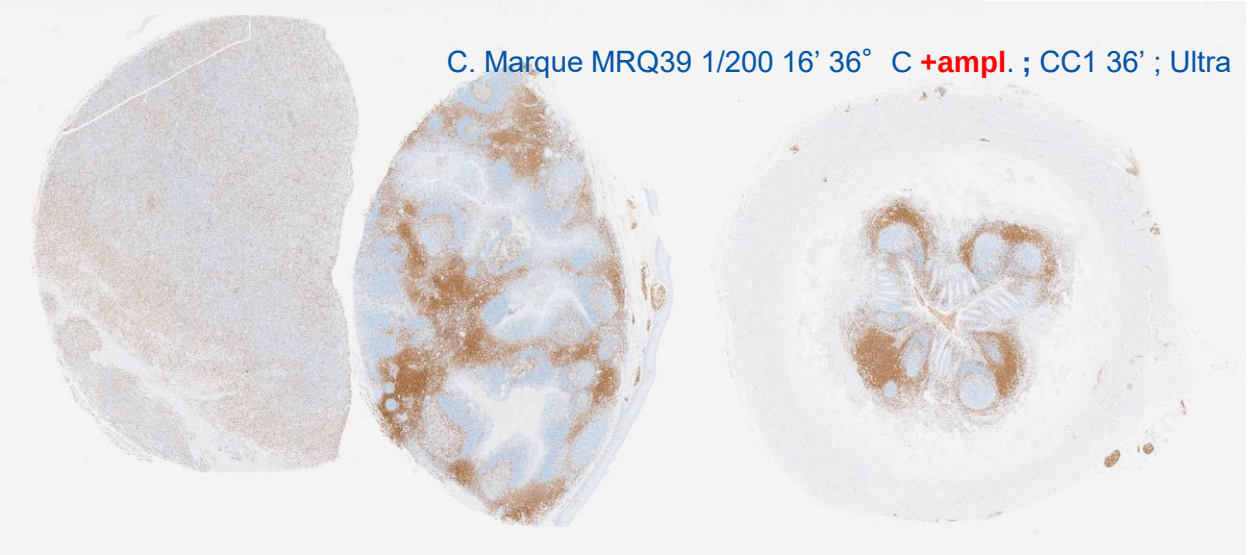
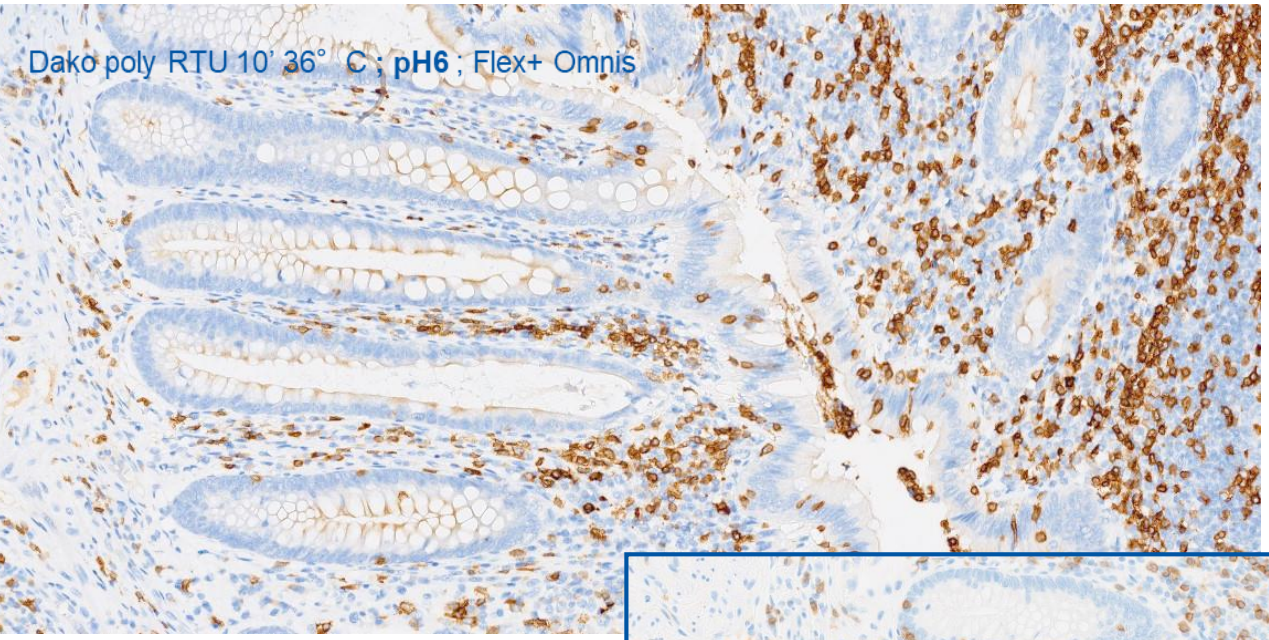


Antistof CD3: appendix

Optimaal



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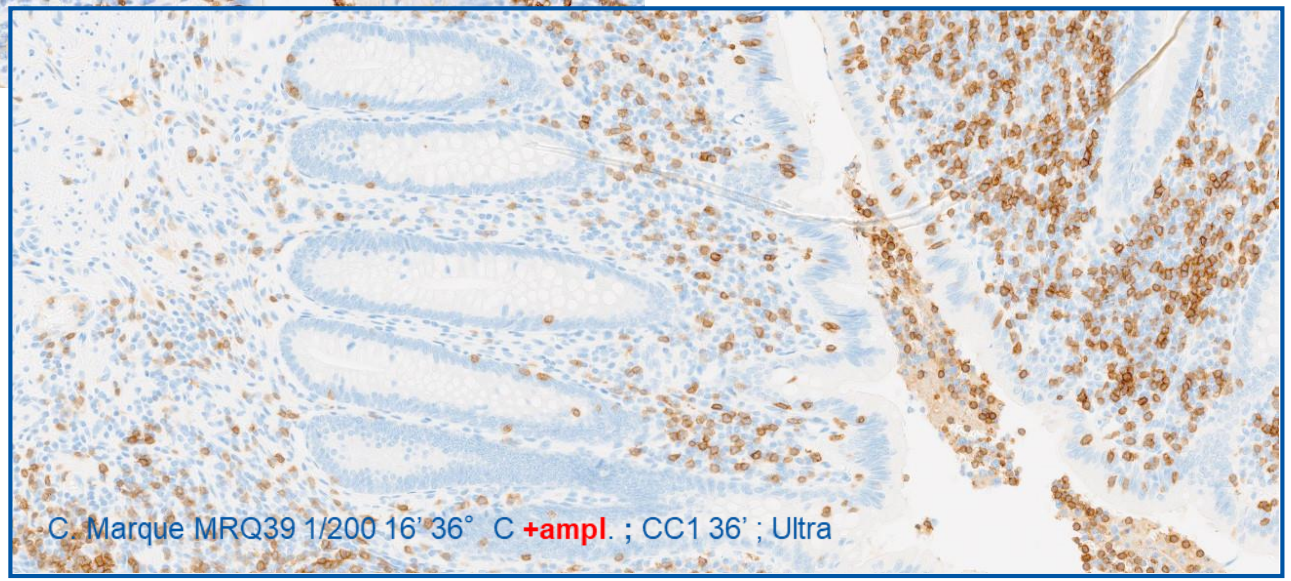
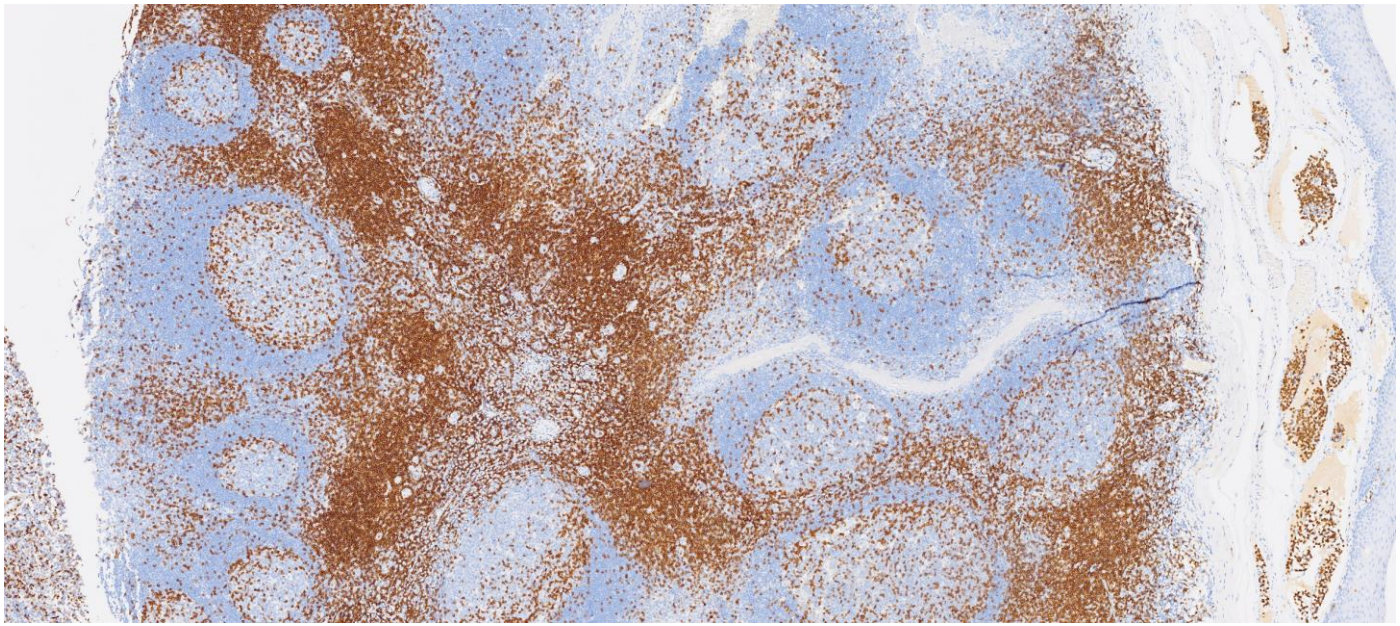


Antistof CD3: tonsil



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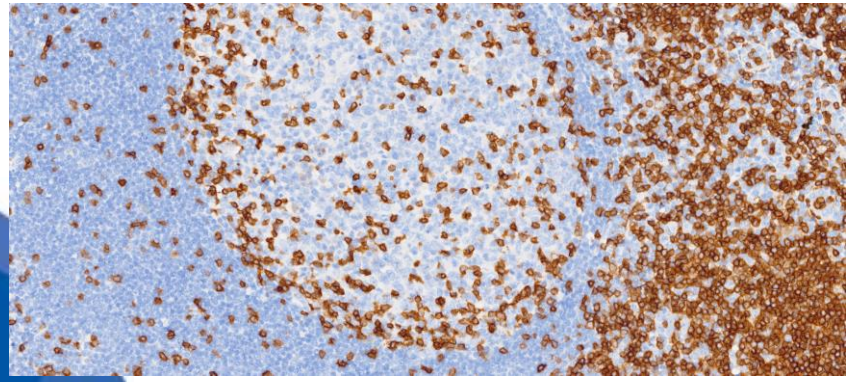
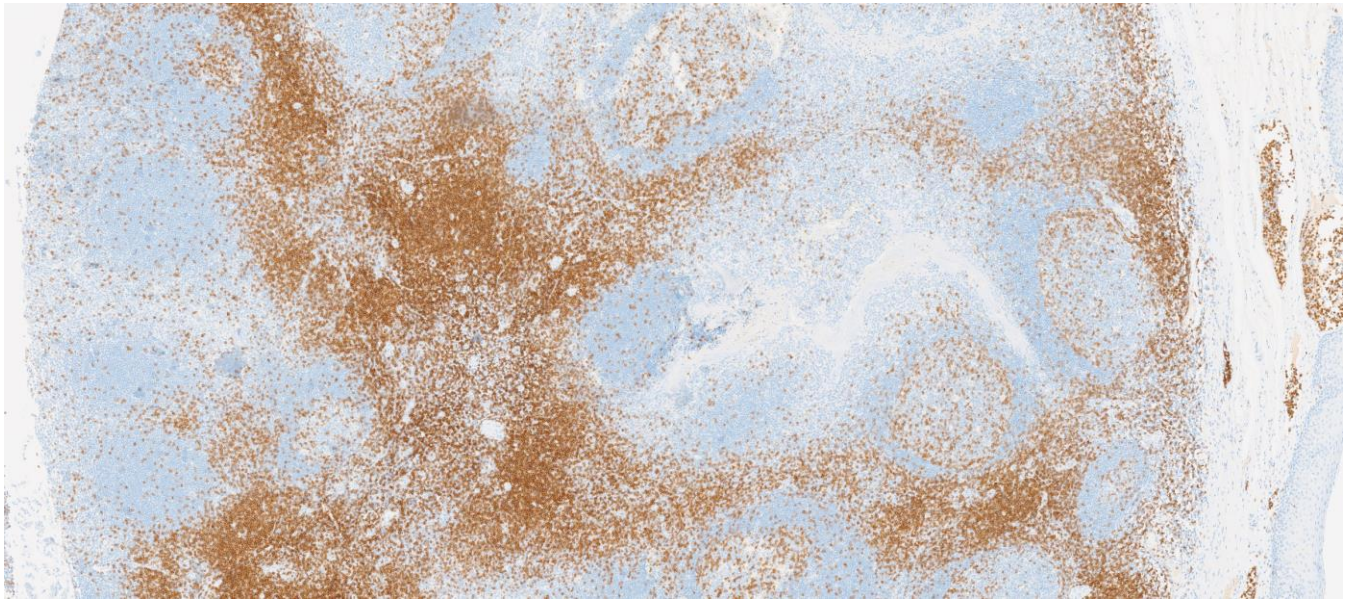


Antistof CD3: tonsil



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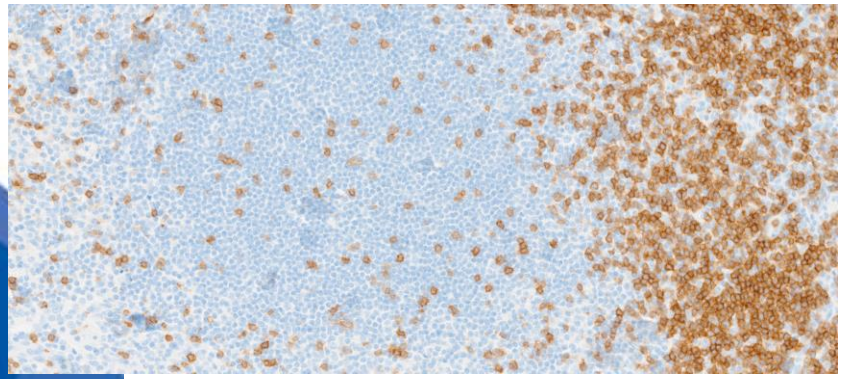
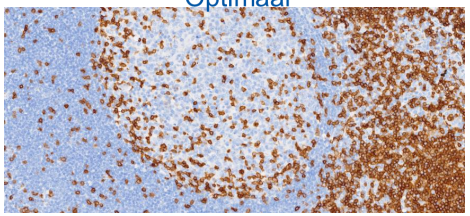
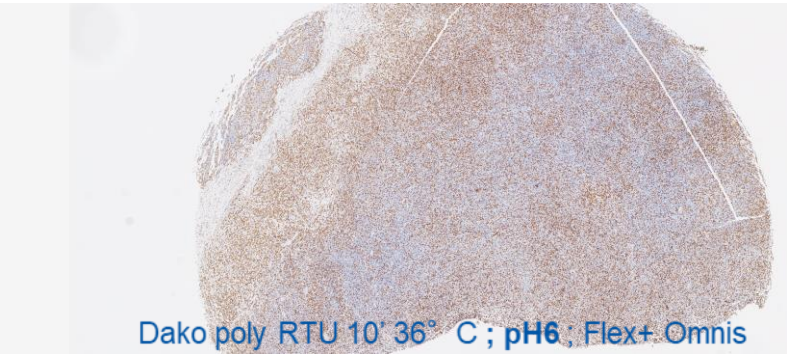


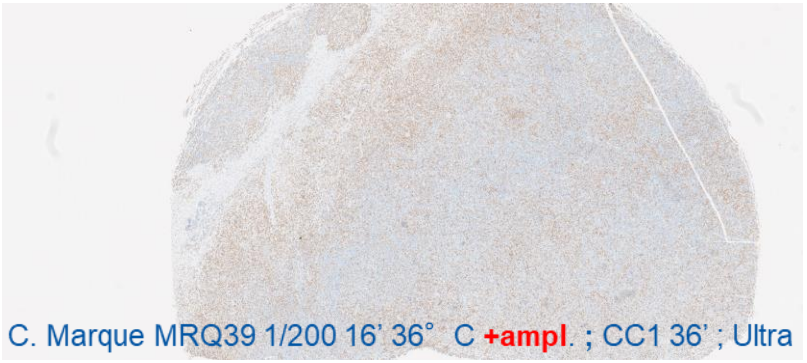
Optimaal



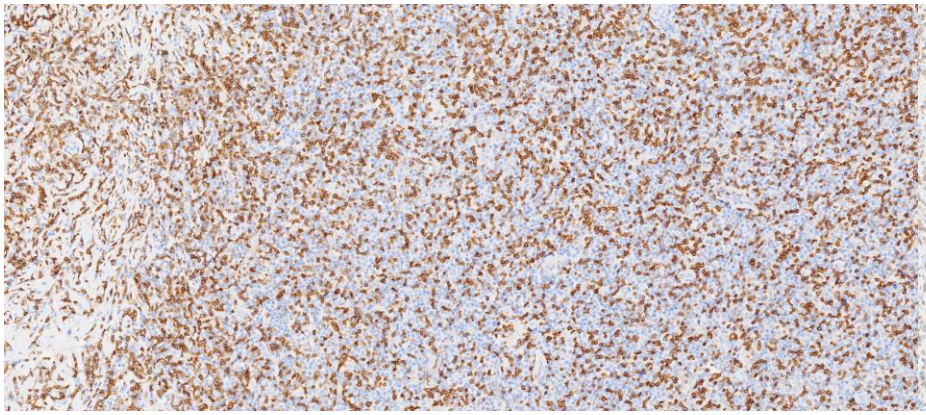
Antistof CD3: T-cel lymfoom



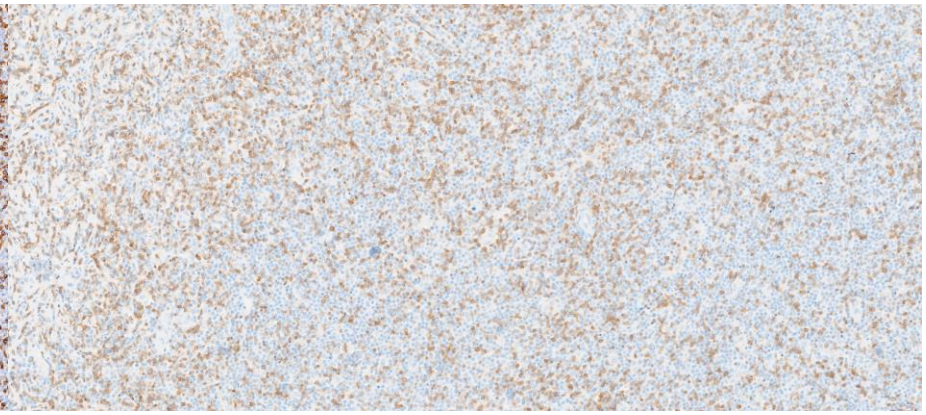
Dako poly RTU 10' 36° C ; **pH6** ; Flex+ Omnis



C. Marque MRQ39 1/200 16' 36° C **+ampl**. ; CC1 36' ; Ultra



Optimaal



Goed

Resultaatbespreking CD3

- Algemeen
  - n=67
    - Suff. 100 %

		EKE 2020-2		OLV 2015-1		NQC 2013	
	n		*suff*	*%*	*suff*	*%*	*suff*
optimaal	**53**	**79%**	***100%***	***91%***	***92%***	***66%***	***92%***
goed	**14**	**21%**		***9%***		***26%***	
Borderline	0	-		-		*8%*	
Onvoldoende	0	-		-		-	

Resultaatbespreking CD3

- Opgesplitst per clone producent
  - RTU: 84% optimaal ; suff. 100 %

n=54	*Dako* poly RTU*	*% suff*	*Ventana 2GV6 RTU*	*% suff*	*Leica LN10 RTU*	*% suff*
	n=25		n=30		n=2	
optimaal	**19**	***76%*** **	**27**	***90%***	**2**	**-**
goed	**6**	***24%***	**3**	***10%***	**0**	**-**
borderline	0	-	0	-	0	-
onvoldoende	0	-	0	-	0	-
Totaal suff	***100%***		***100%***		-	
NQC 2013	*Autostainer 97%*		*97%*		*100%*	

* RTU ½ verdund: 'goed (achtergrond)'

** Autostainer: 100% suff. , 72% optimaal / Omnis 100% suff. , 88% optimaal

Resultaatbespreking CD3

- Opgesplitst per clone producent
  - Concentrated: 50% optimaal ; suff. 100 %

n=8	*Dako* poly	*% suff*	*Leica LN10*	*% suff.*	*Thermo/ Neomarkers SP7*	*% suff*	*Cell Marque MRQ-39*	*% suff.*
	n=2		n=2		n=2		n=2	
optimaal	**2**	**-**	**1**	**-**	***1***	**-**	***0***	**-**
goed	**0**	**-**	**1**	**-**	***1***	**-**	***2***	**-**
borderline	0	-	0	-	*0*	-	*0*	-
onvoldoende	0	-	0	-	0		0	-
Totaal suff.	***100%***		***100%***		***100%***		***100%***	
NQC 20163	*93%*		*83%*		*85%*		*-*	

Resultaatbespreking CD3

Besluit:

- Suff. 100% (~NQC2013: 92%)
- Specificiteit en sensitiviteit OK
  - Geen vals positieven (B-cellen negatief)
  - Alle deelnemers kunnen T-cellymfoom voldoende aankleuren
- Resultaat 'goed':
  - Downgrade o.w.v. te zwakke aankleuring in T-cel lymfoom
  - O.w.v. te zwakke tegenkleuring die interpretatie bemoeilijkt (Ventana)
  - RTU ½ verdund: goed maar achtergrond
- Geen verschil tussen aanbevolen protocol en LDT
- Geen verschillen tussen producenten, format: robuuste test
- HIER:
  - pH type en tijd (zeer kort tot lang): geen invloed op resultaat
  - 1 deelnemer (manuele techniek): combinatie HIER + enzym: optimaal resultaat
  - Langere pretreat: geen intensere kleuring
- Amplifier: niet noodzakelijk voor optimaal resultaat
  - Dako Omnis: 17% linker: 1 deelnemer te sterke aankleuring → downgrade
  - Ventana: 23% amplifier

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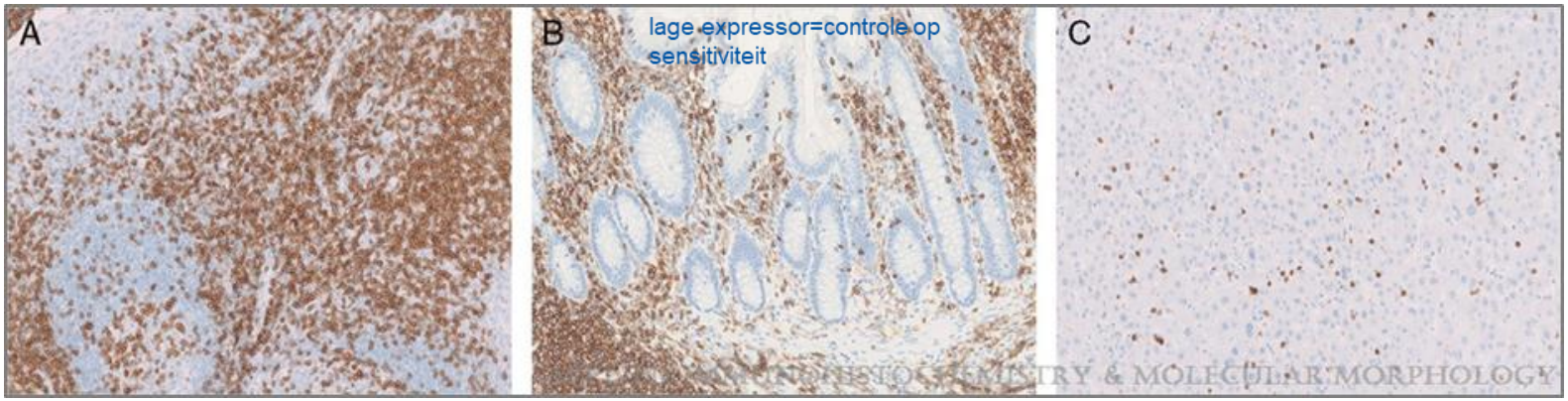
Resultaatbespreking CD3

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Torlakovic, Emina E.; Nielsen, Søren; Francis, Glenn; Garratt, John; Gilks, Blake; Goldsmith, Jeffrey D.; Hornick, Jason L.; Hyjek, Elizabeth; Ibrahim, Merdol; Miller, Keith; Petcu, Eugen; Swanson, Paul E.; Zhou, Xiaoge; Taylor, Clive R.; Vyberg, Mogens
Applied Immunohistochemistry & Molecular Morphology. 23(1):1-18, January 2015.
doi: 10.1097/PAI.0000000000000163



Hoge expressor=controle op keuze juiste AS

FIGURE 13. **CD3 iCAPC**.

A, Tonsil: virtually all T cells, both in the interfollicular T zones and scattered within the germinal centers must show a moderate to strong, distinct predominantly membranous staining reaction.

B, Appendix: dispersed intraepithelial T cells must show an at least weak to moderate, distinct predominantly membranous staining reaction (LLOD).

C, Liver: dispersed T cells must show an at least weak to moderate, distinct predominantly membranous staining reaction (LLOD). iCAPC indicates immunohistochemistry critical assay performance controls; LLOD, low limit of detection.

lage expressor=controle op sensitiviteit

45% gebruikt controle

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Protocol Chromogranine

n= 67

Conc.: n=23

- LK2H10 n=18
- LK2H10+PHE5 n=1
- DAK-A3 n=3
- Polyclonaal n=1

RTU: n=44

- LK2H10 Ventana n=39
- LK2H10 Neomarkers n=1
- 5H7 Novocastra n=2
- Polyclonaal n=2

Protocol Chromogranine

- Conc. AS: clone LK2H10
  - Thermo/Neomarkers
    - Dako Autostainer: 1/800 - 1/4000 10-20’ RT
    - Dako Omnis: 1/500 en 1/1000 20’ bij 32° C

Spreiding verdunningsfactor

  - Cell Marque
    - Dako Autostainer: 1/300 20’ RT ; HIER pH9
    - Dako Omnis:
      - 1/500 20’ bij 32° C ; HIER pH9
      - 1/1000 20’ bij 32° C ; HIER pH6

Protocol Chromogranine

- Conc. AS: clone LK2H10 vervolg
  - Dako: 1/6000 20’ bij 32° C ; HIER pH9 30’ Dako Omnis
  - Hybritech: 1/5000 52’ bij 36° C ; HIER CC1 36’ BMK Ultra UV
  - Invitrogen: 1/1000 20’ bij 32° C ; HIER pH9 30’ Dako Omnis
- Conc. AS: clone LK2H10+PHE5
  - 1/800 40’ bij 36° C +amp ; HIER CC1 24’ BMK Ultra OV
- Conc. AS: Dako polyclonaal
  - 1/1500 12’ bij 36° C +amp ; HIER CC1 16’ BMK Ultra OV
- Conc. AS: Dako DAK-A3
  - 1/50 12’ -32’ bij 36° C +amp ; HIER CC1 8’ - 30’ BMK Ultra UV
  - 1/100 30’ RT; HIER pH9 20’ Dako kit manueel

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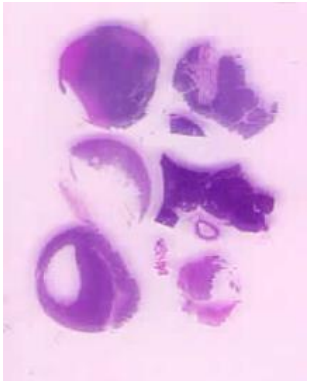
Protocol Chromogranine

- RTU: clone LK2H10
  - Neomarkers
    - 20’ bij 32° C ; HIER pH6 30’ Dako Omnis
  - Ventana
    - AS 16’ - 40’ bij RT of 36° C of 36° C +/- amp
    - HIER: geen - CC1 8’ - 72’
    - Datasheet (update 2017):
      - UV AS12’ met CC1 mild
      - OV AS4’ met CC1 24’
  - Ventana op Dako Omnis
    - AS 20’ bij RT of 32° C +/- linker
    - HIER: pH6 of pH9

Protocol Chromogranine

- RTU:
  - Leica 5H7
    - Bond III : AS 15’ RT ; HIER pH6
    - Bond Max: AS 15’ RT ; HIER pH9
  - Dako polyclonaal
    - AS 25’ RT ; HIER pH6 Dako Omnis
  - Neomarkers polyclonaal
    - AS 20’ RT ; HIER pH9 Dako Autostainer

Antistof Chromogranine



- Scoringscriteria: cytoplasmatische aankleuring
  - Ureter
    - Geen aankleuring (controle specificiteit: klierbuizen negatief)
  - Pancreas
    - Sterke aankleuring eilandjes Langerhans (hoge expressor)
    - Geen aankleuring in exocriene cellen
  - Colon normaal + Ca
    - Minstens matige tot sterke aankleuring van neuro endocriene cellen in de mucosa (controle sensitiviteit)
    - Neoplastische cellen negatief (controle analytische specificiteit)
  - Blaas kleincellig Ca (lage expressor: controle analytische sensitiviteit)
    - Minstens zwak tot matige aankleuring meerderheid neoplastische cellen
  - Schildklier medullair Ca
    - Minstens matige tot sterke aankleuring van bijna alle neoplastische cellen
  - Dundarm carcinoïd
    - Minstens matige/sterke aankleuring van bijna alle neoplastische cellen

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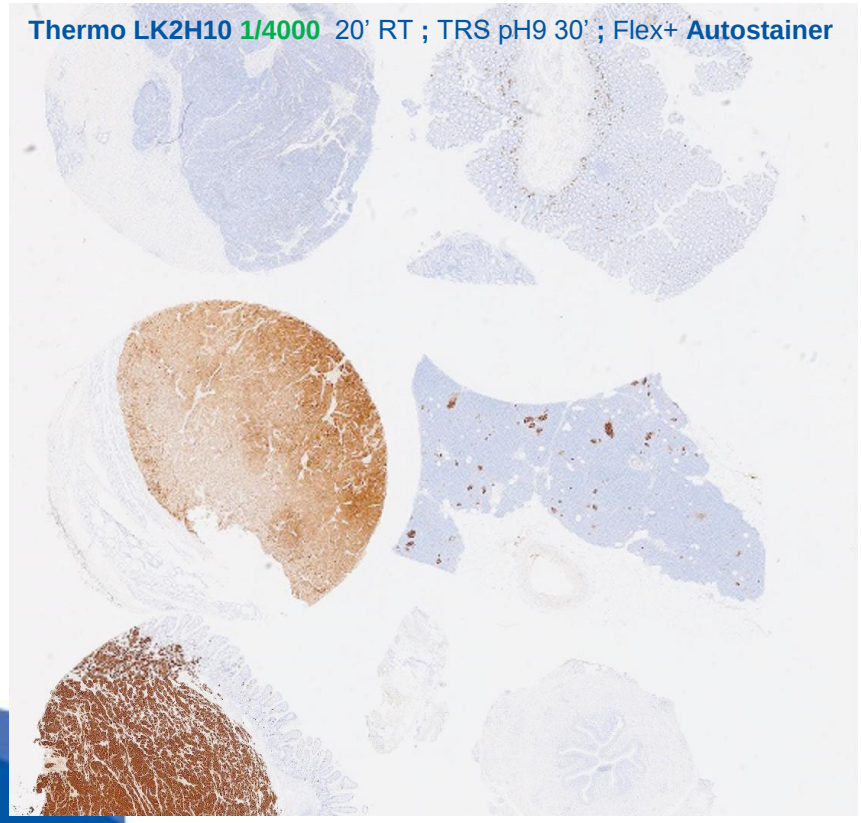
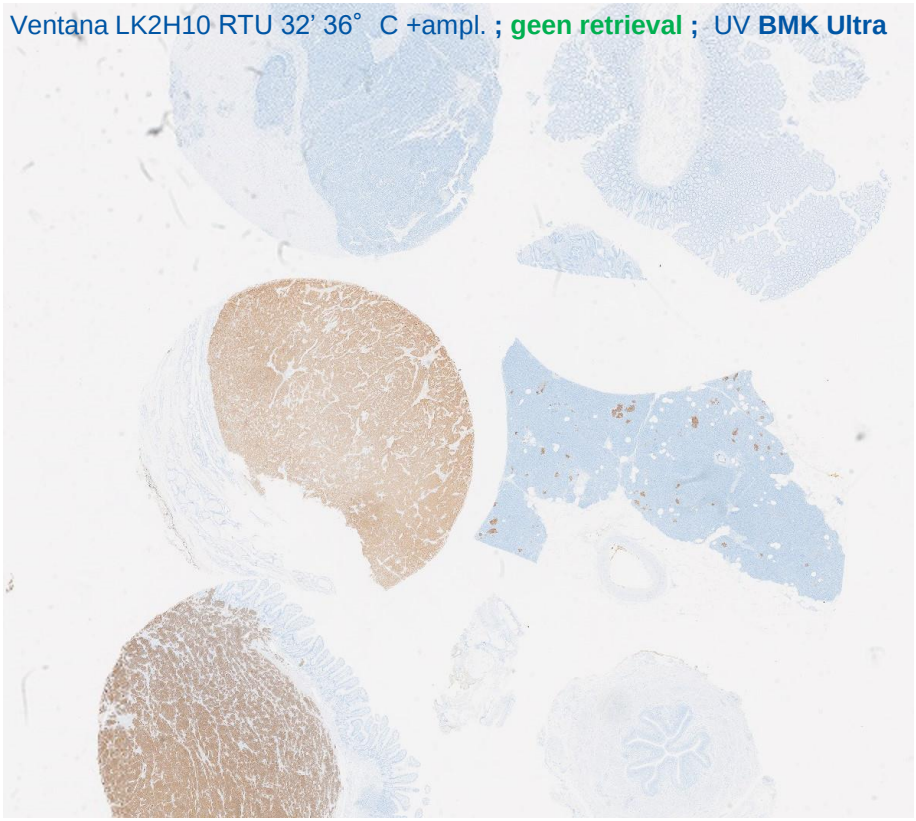
Antistof Chromogranine: overzicht

Optimaal

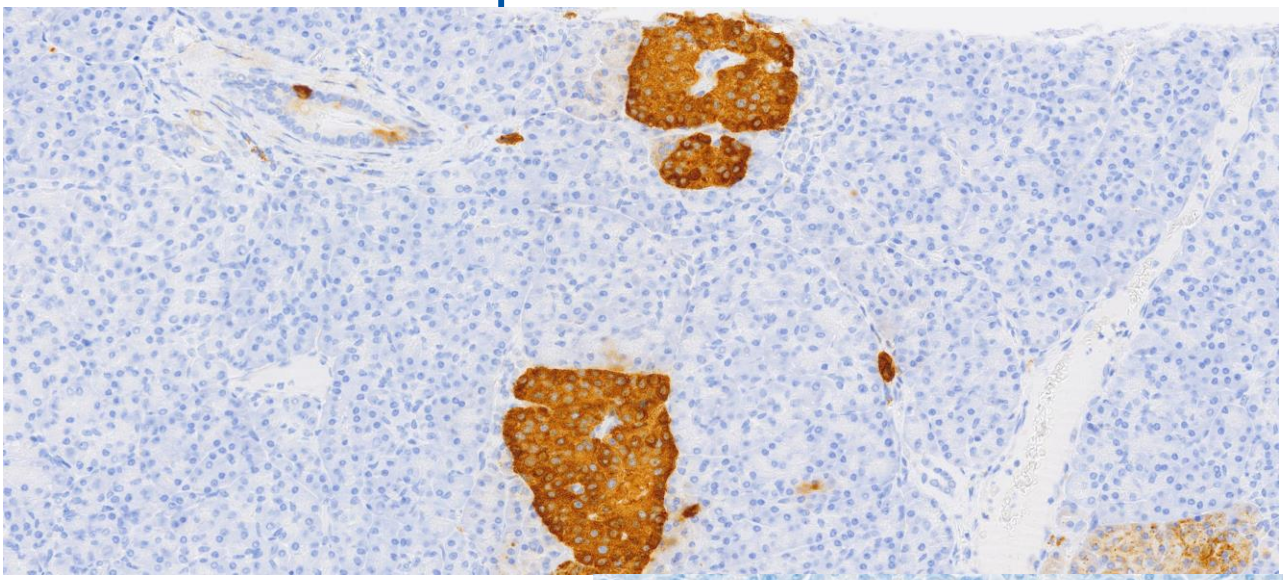


Onvoldoende



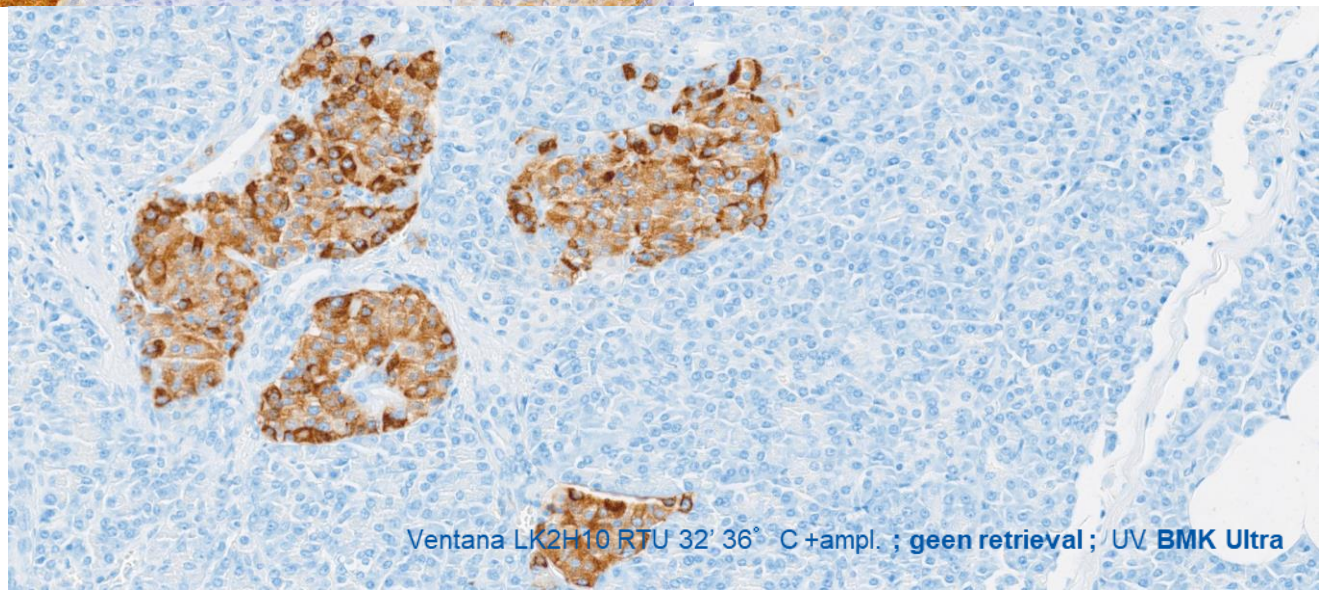
Antistof Chromogranine: pancreas

Optimaal



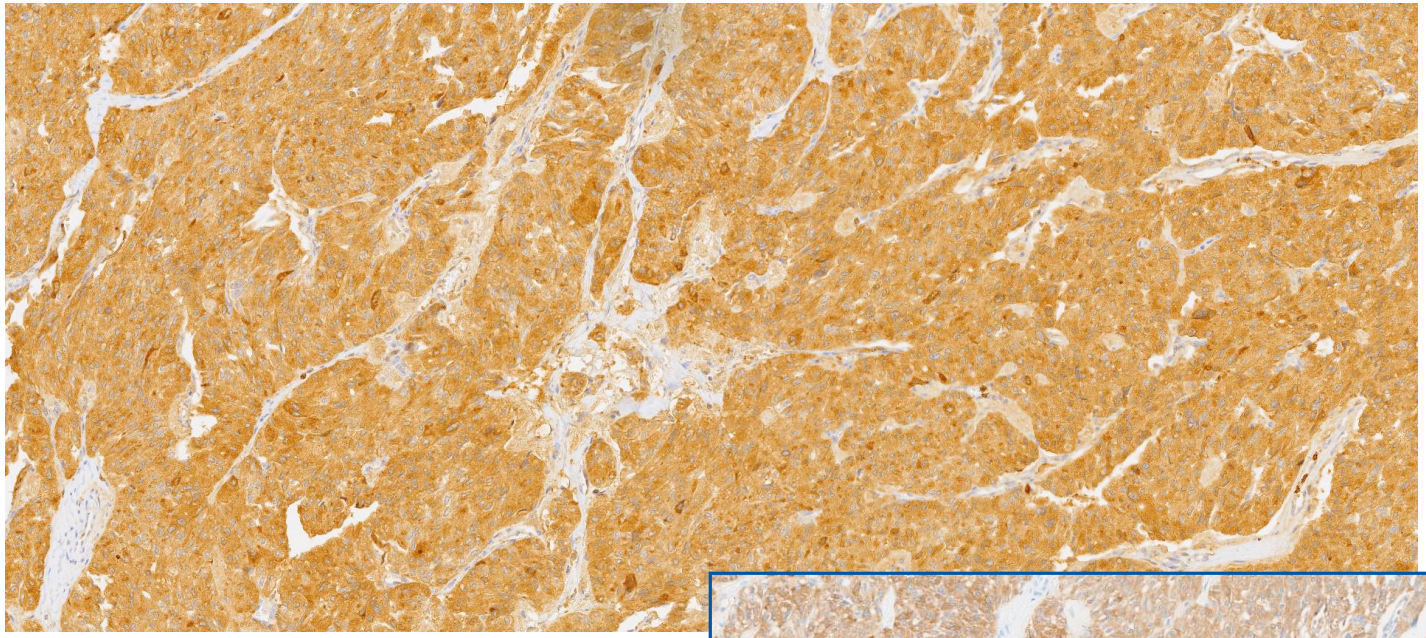
Sterke expressor

Optimaal



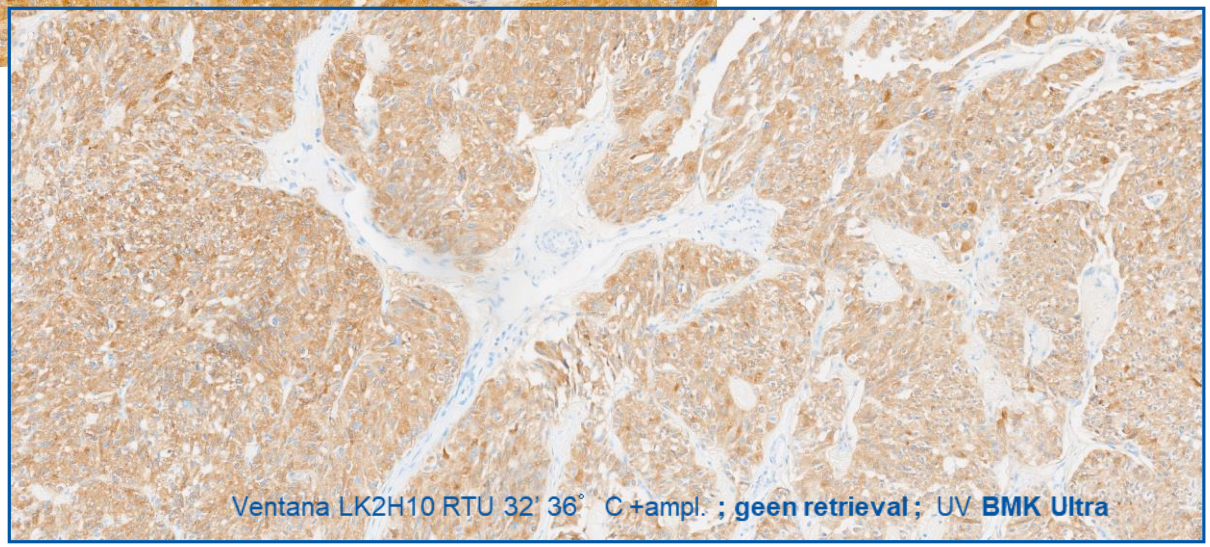
Antistof Chromogranine: medullair Ca

Optimaal ?



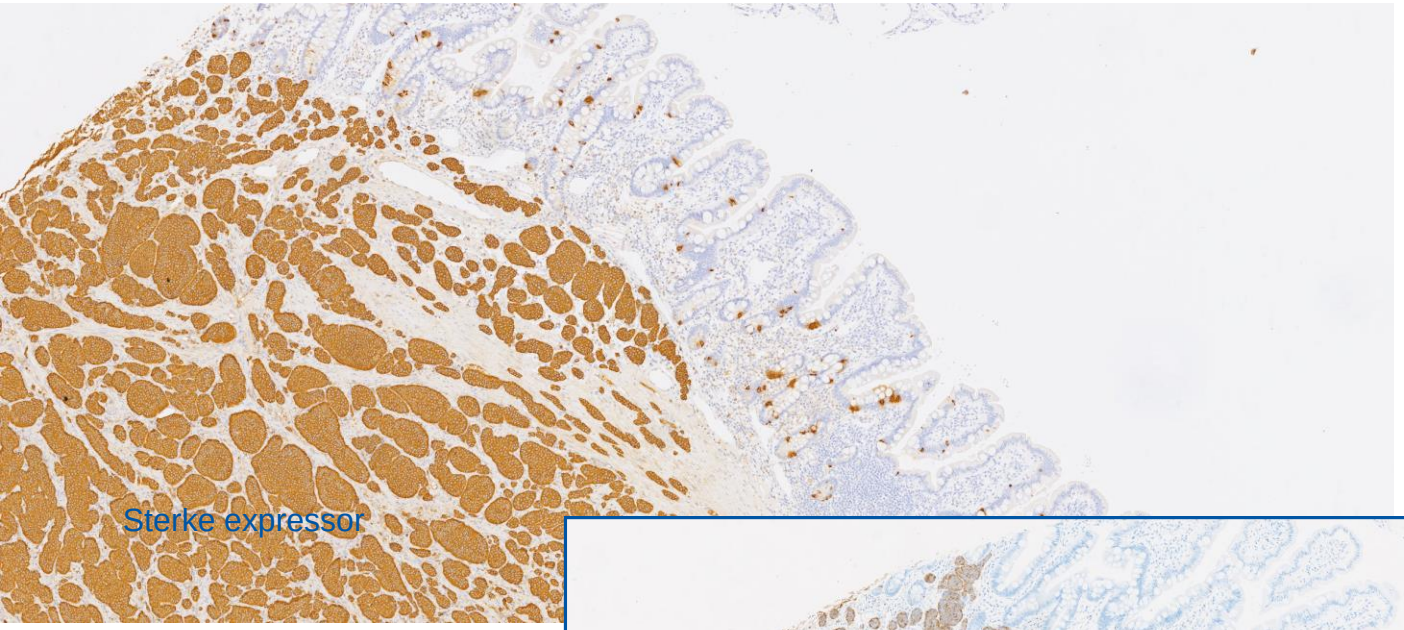
Sterke expressor

Optimaal/Onvoldoende ?

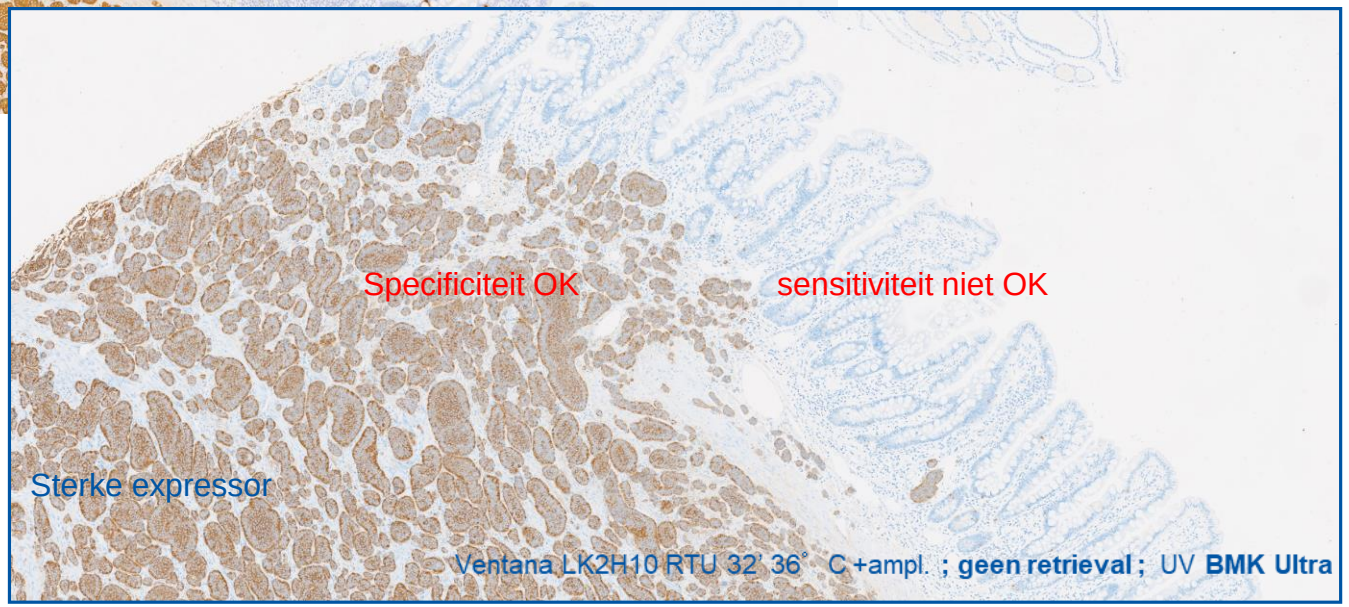


Antistof Chromogranine: NET

Optimaal

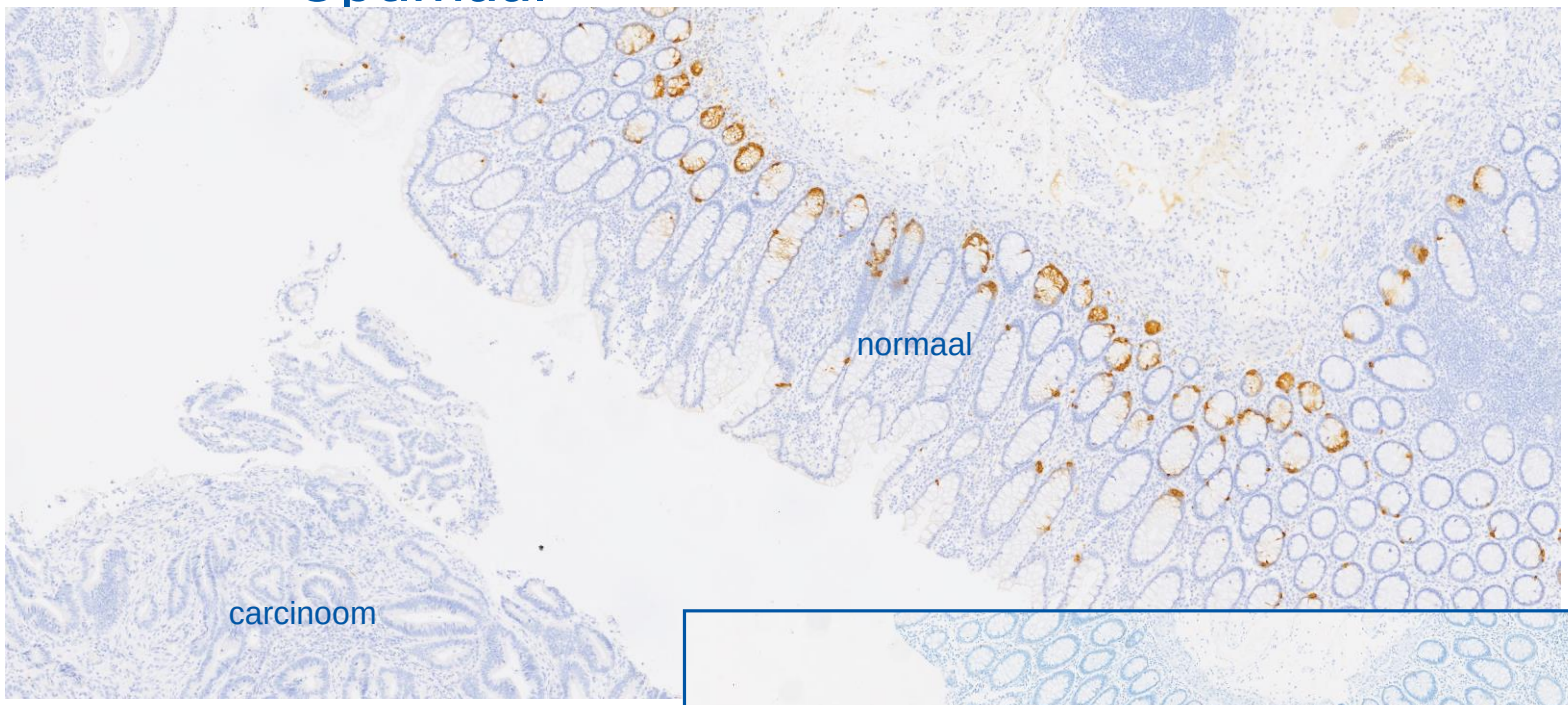


Onvoldoende



Antistof Chromogranine: colon

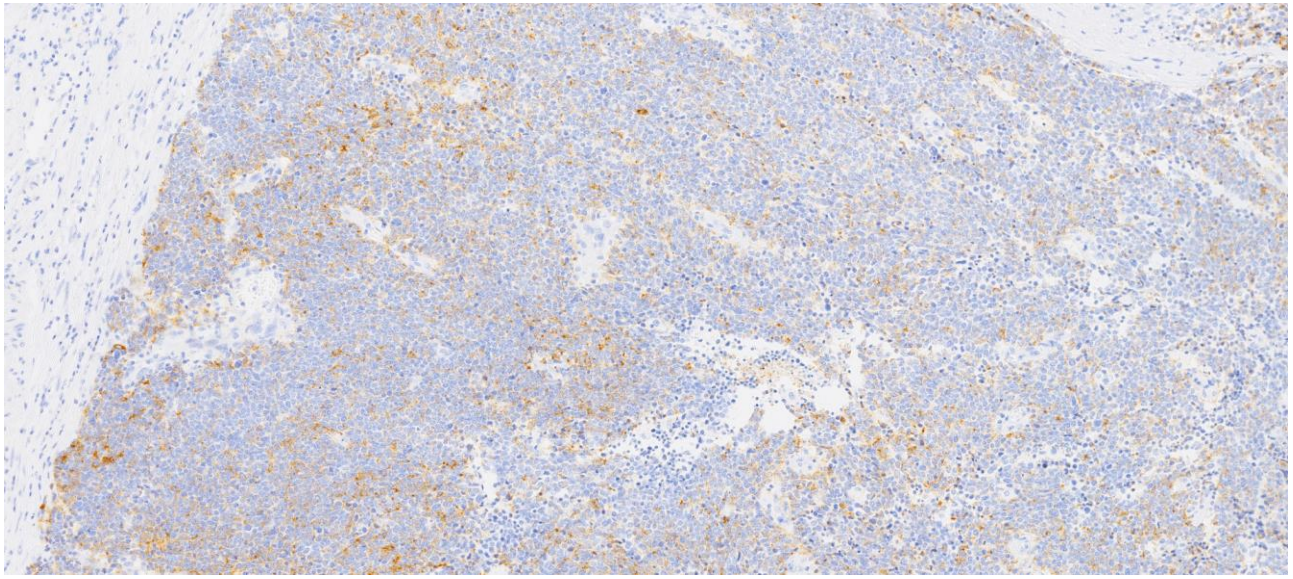
Optimaal



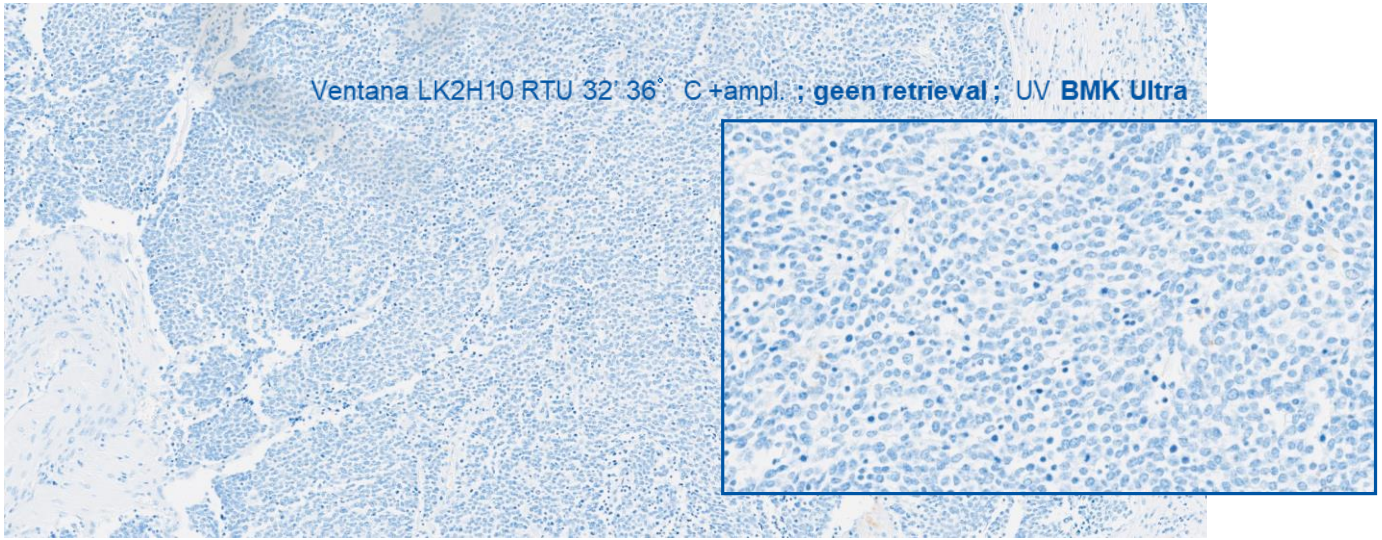
Onvoldoende

Antistof Chromogranine: blaas Ca

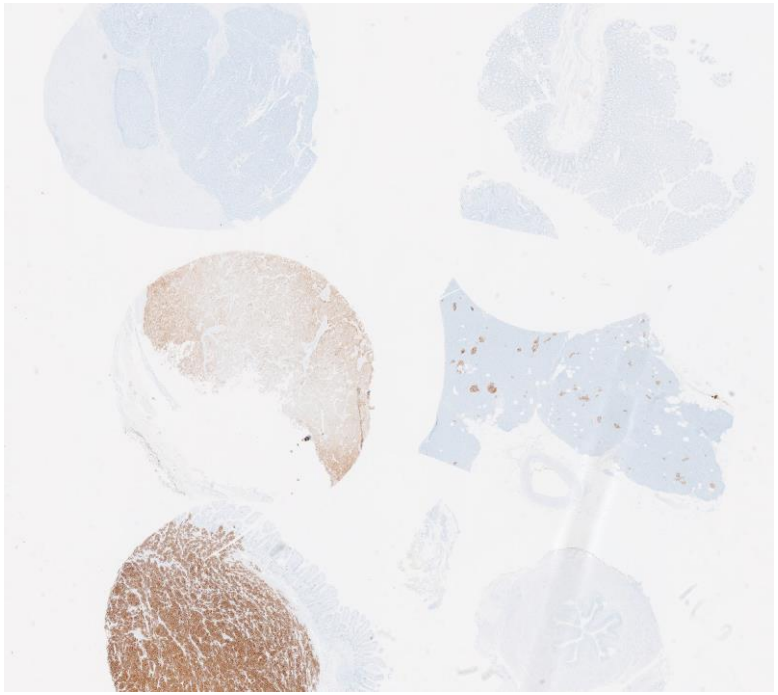
Optimaal



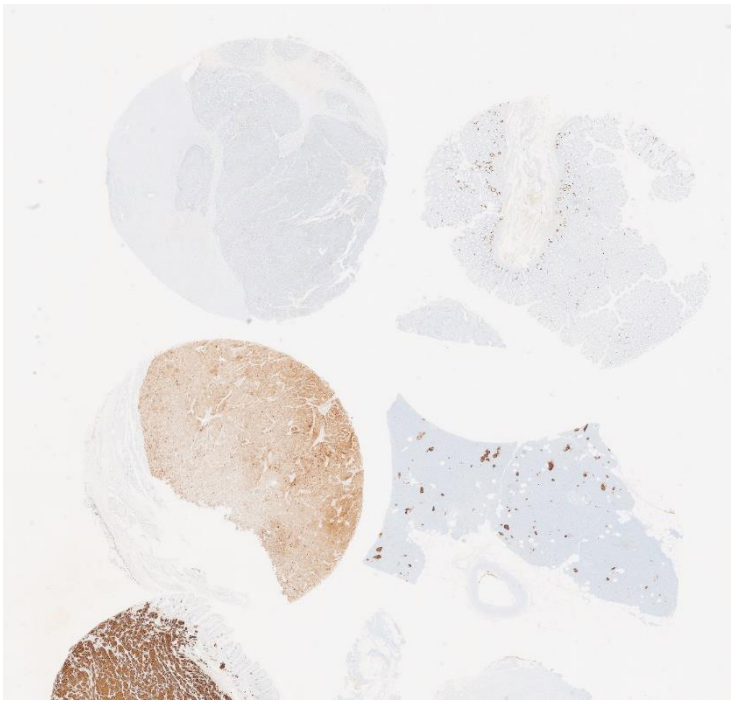
Onvoldoende



Antistof Chromogranine

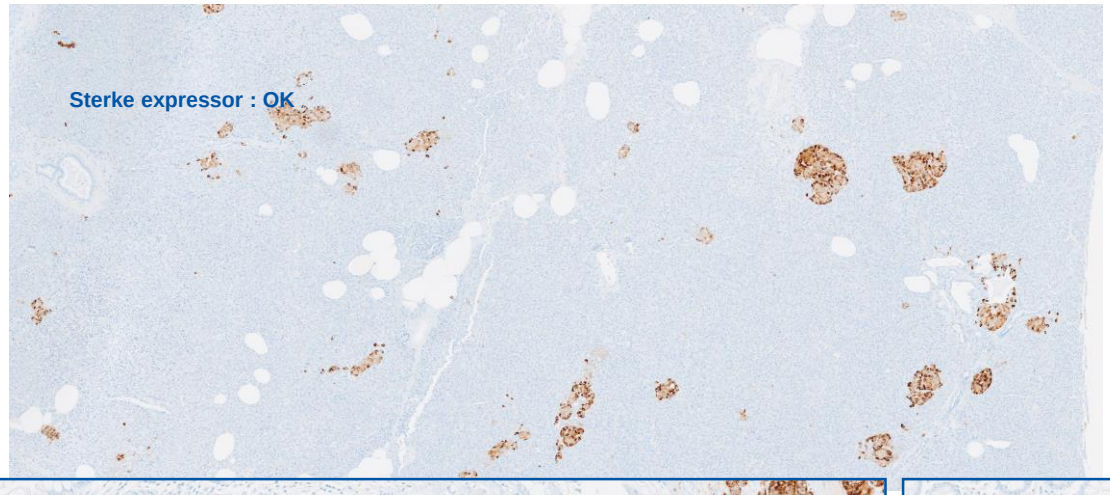


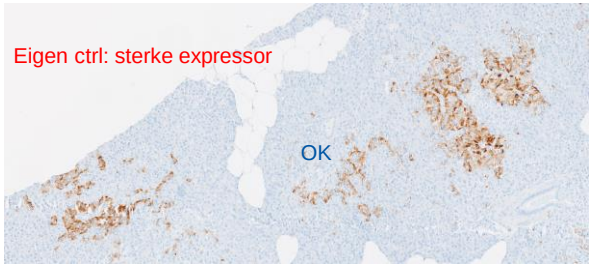
DAK-A3 1/50 12' 36° C **; HIER** CC1 8' **;** BMK Ultra ultraView

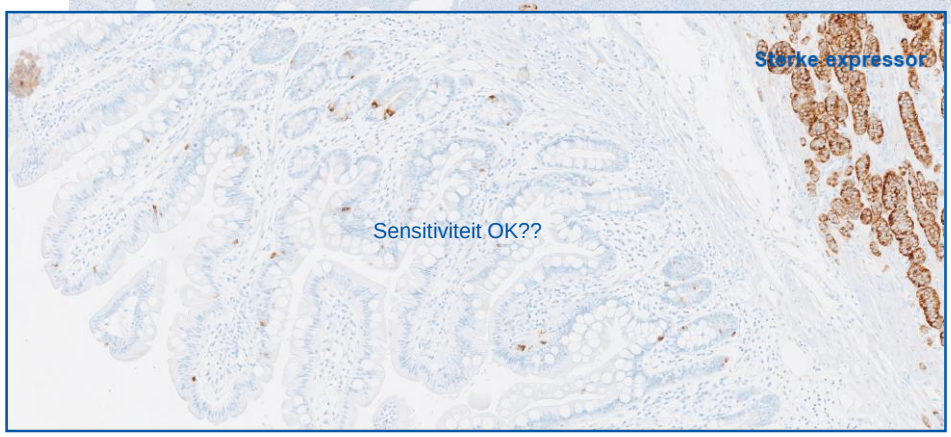


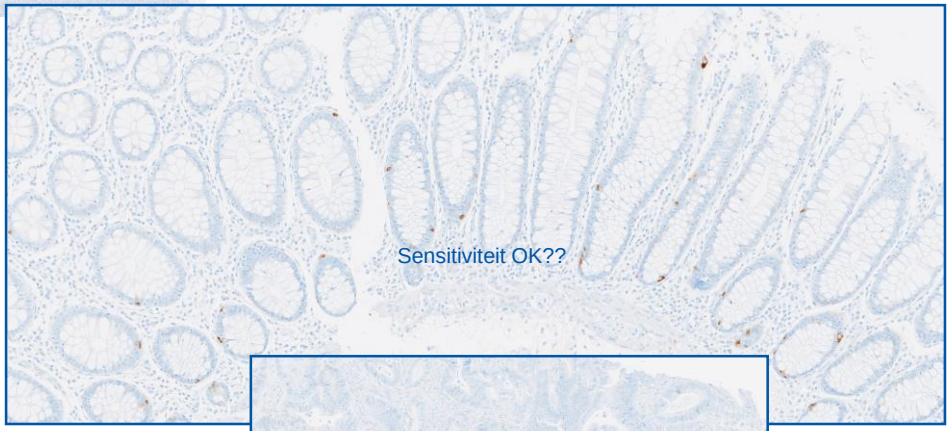
DAK-A3 1/50 32' 36° C **; HIER *CC1 30'* ;** BMK Ultra ultraView

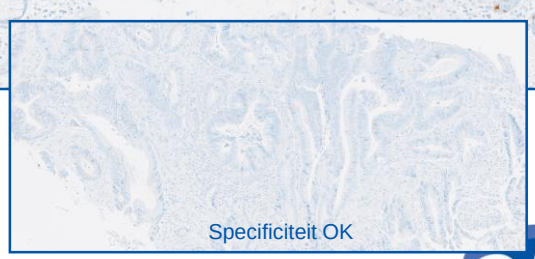
Antistof Chromogranine





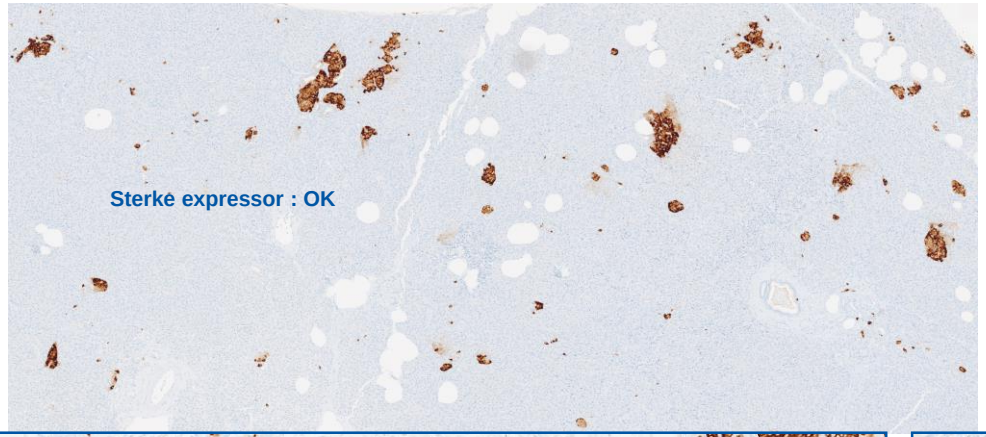


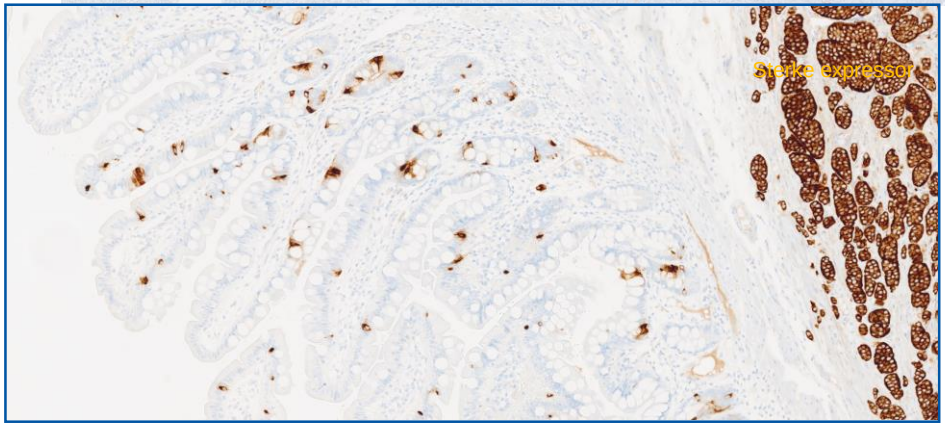


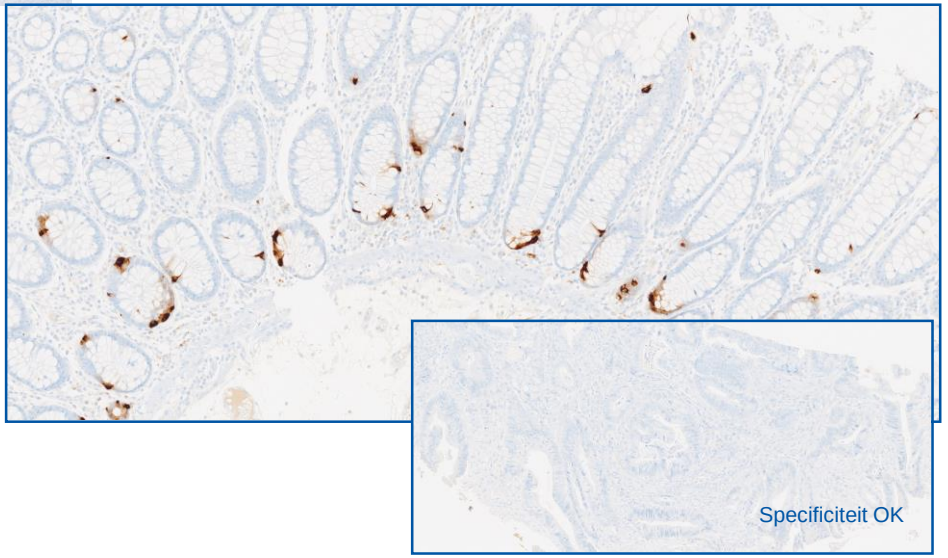


DAK-A3 1/50 12’ 36˚ C **; HIER** CC1 8’ **;** BMK Ultra ultraView

Antistof Chromogranine

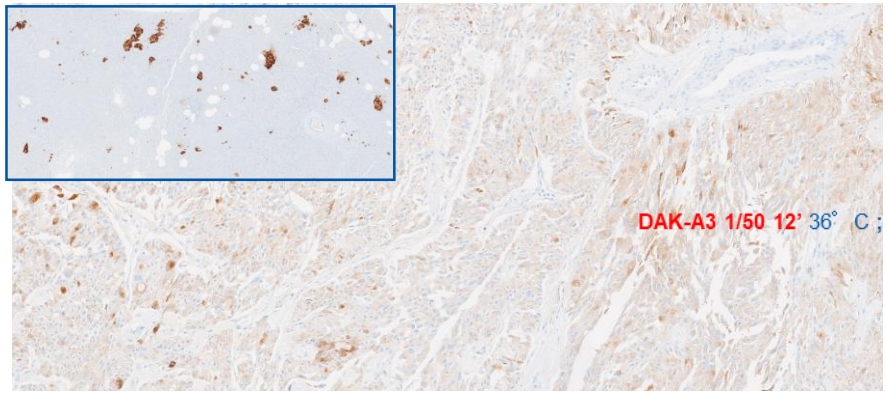




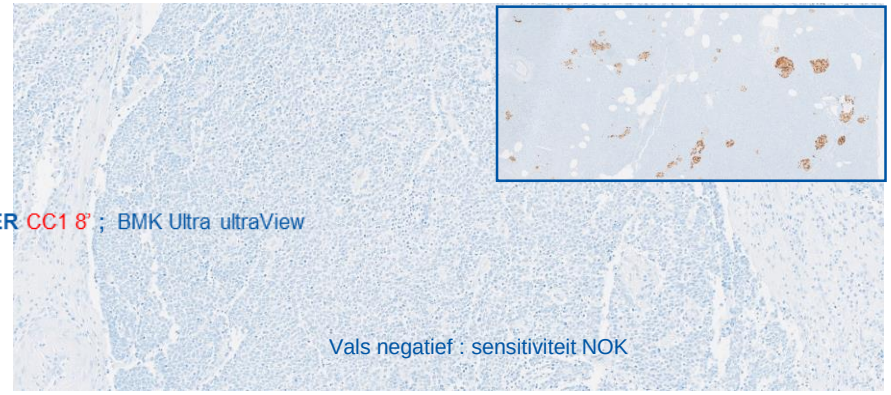


DAK-A3 1/50 32' 36° C **; HIER *CC1 30'* ;** BMK Ultra ultraView

Antistof Chromogranine

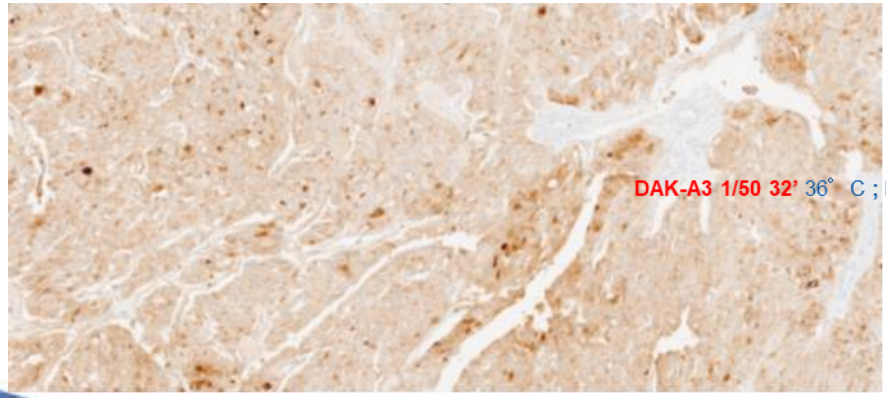


DAK-A3 1/50 12' 36° C ; HIER CC1 8' ; BMK Ultra ultraView



Vals negatief : sensitiviteit NOK

Sterke expressor



DAK-A3 1/50 32' 36° C ; HIER *CC1 30'* ; BMK Ultra ultraView

Zwak positief: sensitiviteit OK

Besluit: geen sterke expressor gebruiken voor sensitiviteit

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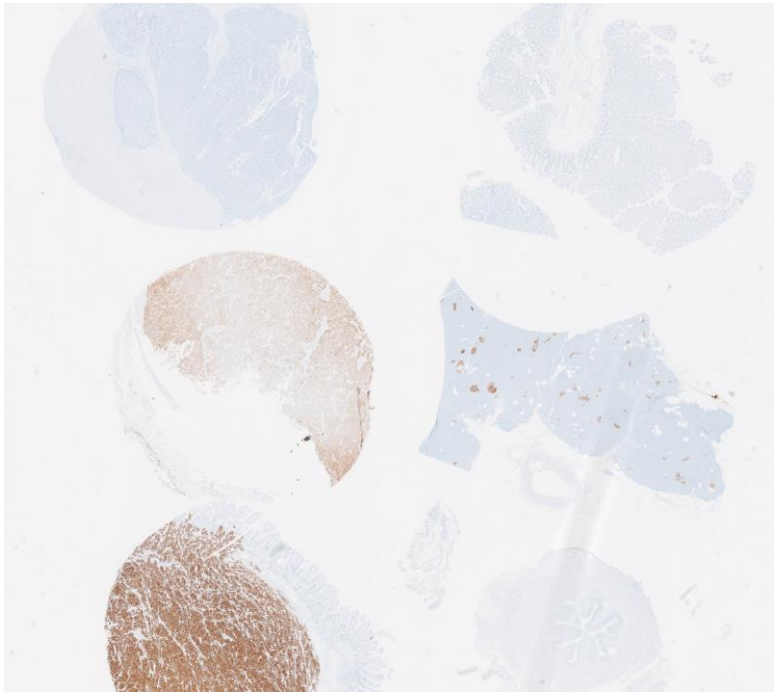
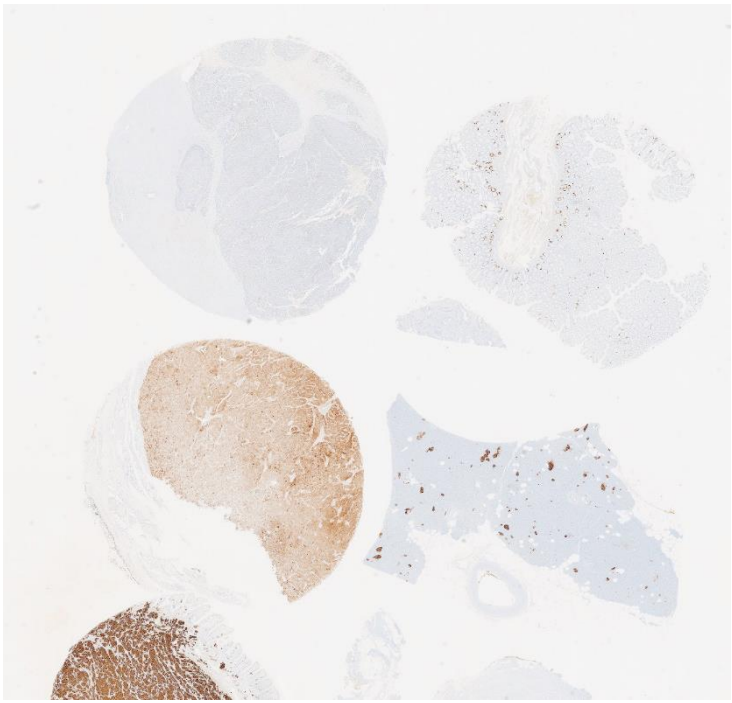
Antistof Chromogranine

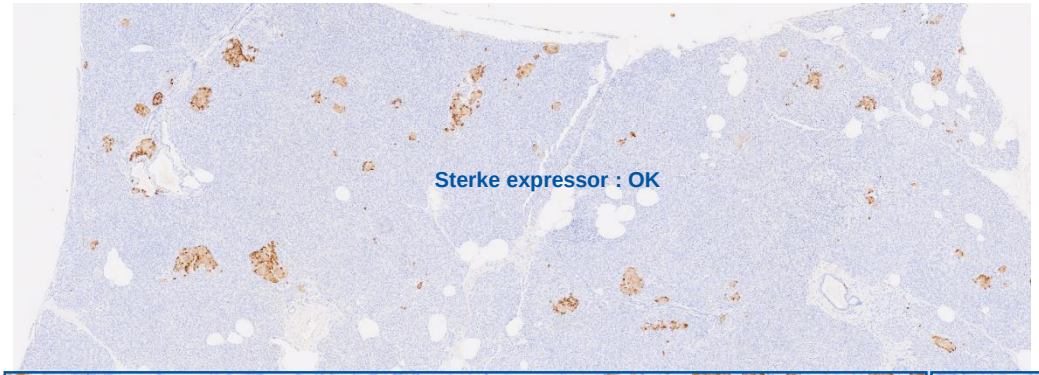


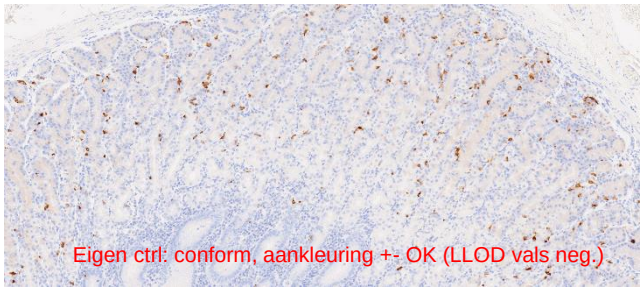
Leica 5H7 RTU 15’ RT **;** HIER **pH6** 20’ **;** **Leica** Bond III

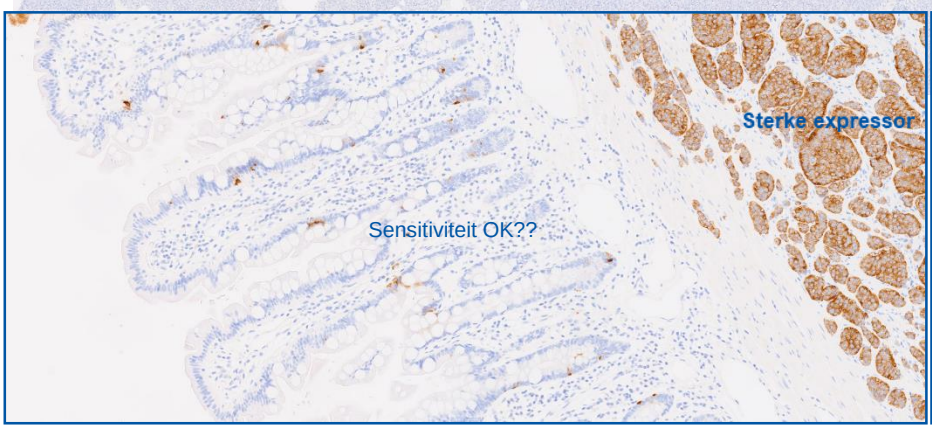


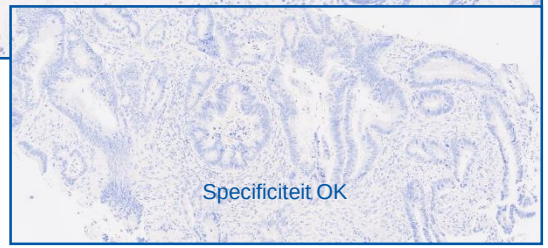
Leica 5H7 RTU 15’ RT **;** HIER **pH9** 10’ **;** **Leica** Bond Max

Antistof Chromogranine



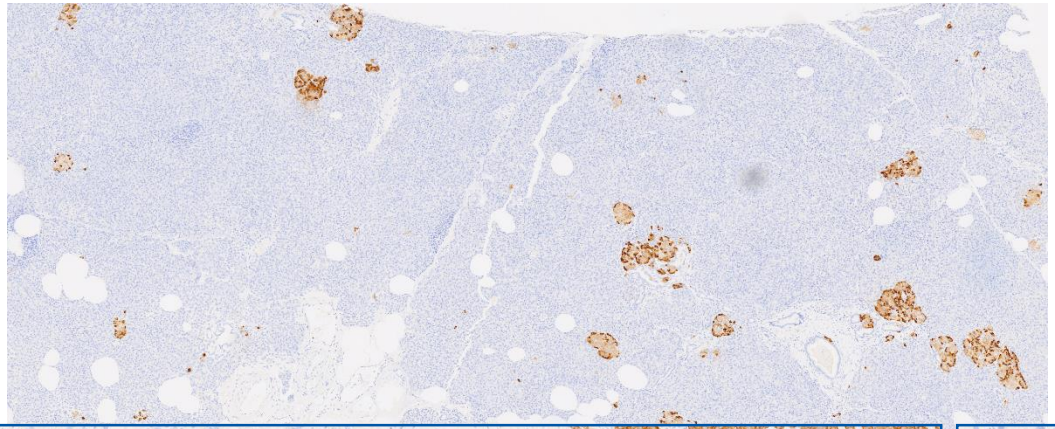


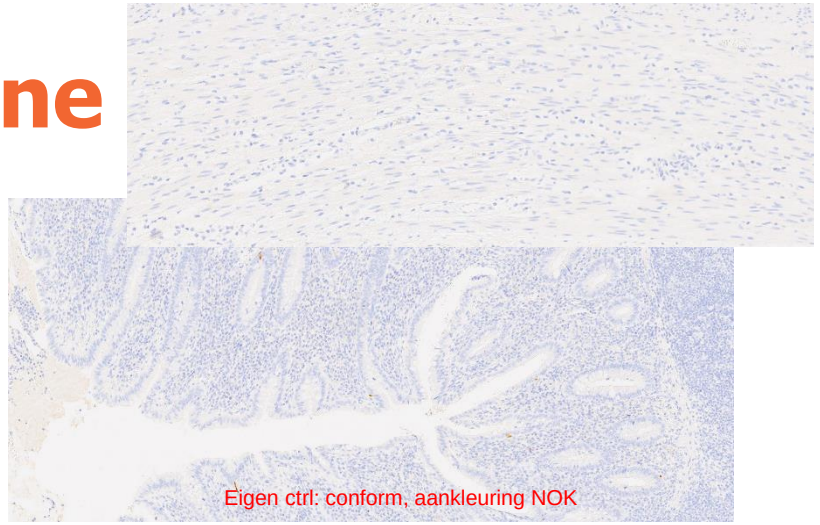


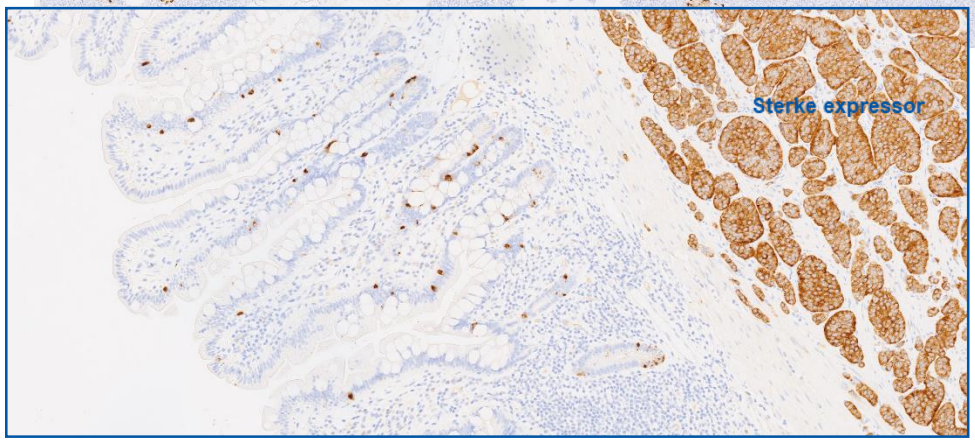


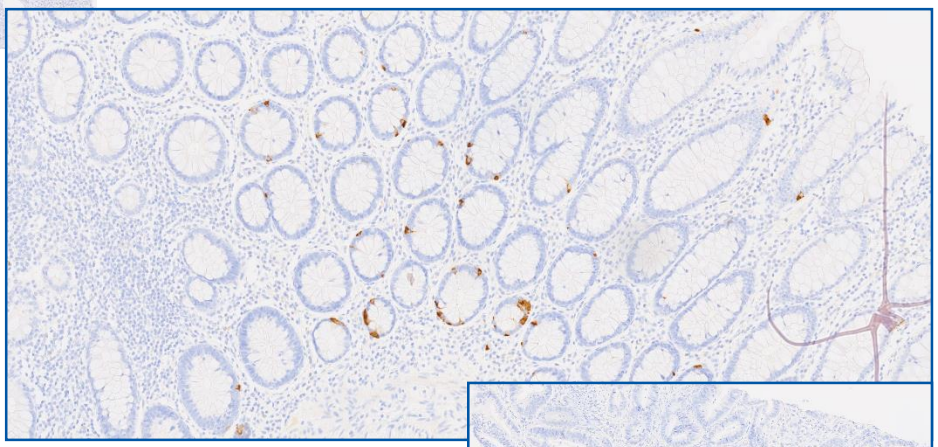
Leica 5H7 RTU 15’ RT **; HIER pH6** 20’ **;** Leica Bond III

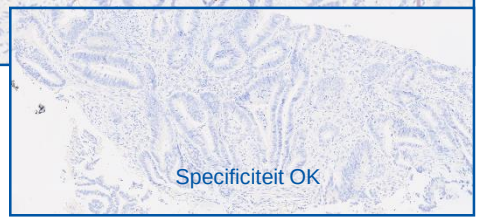
Antistof Chromogranine







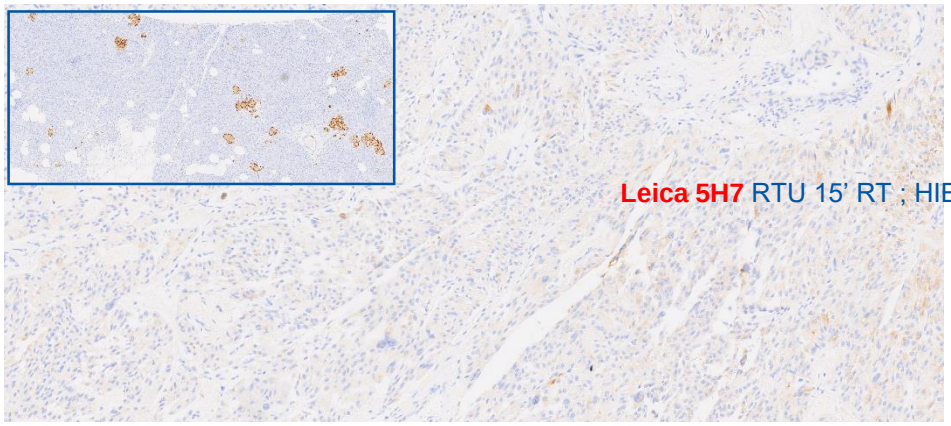




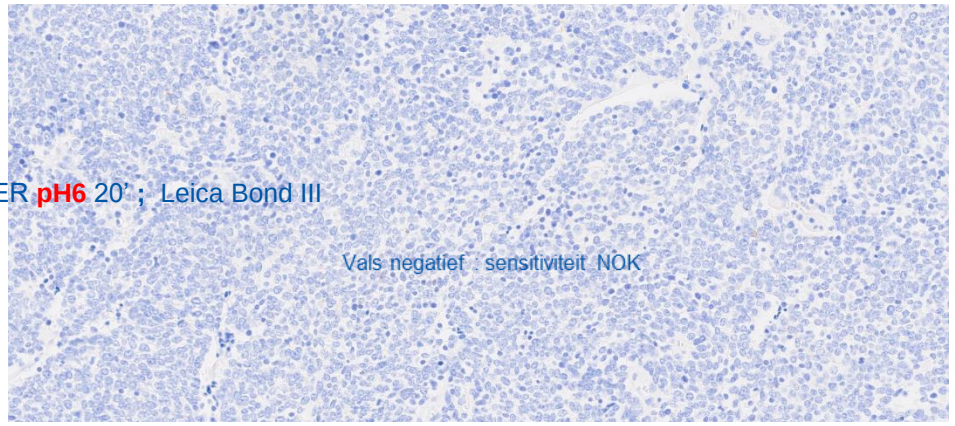
Sensitiviteit beter

Leica 5H7 RTU 15’ RT **; HIER pH9** 10’ **;** Leica Bond Max

Antistof Chromogranine

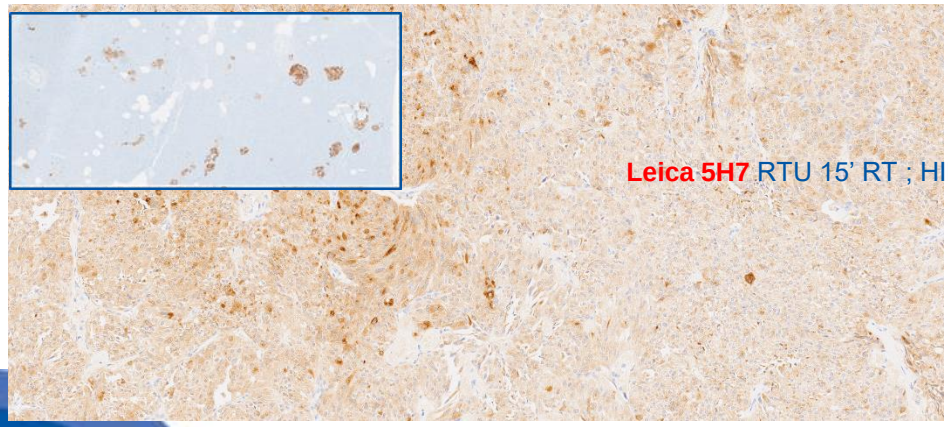


Leica 5H7 RTU 15' RT ; HIER pH6 20' ; Leica Bond III

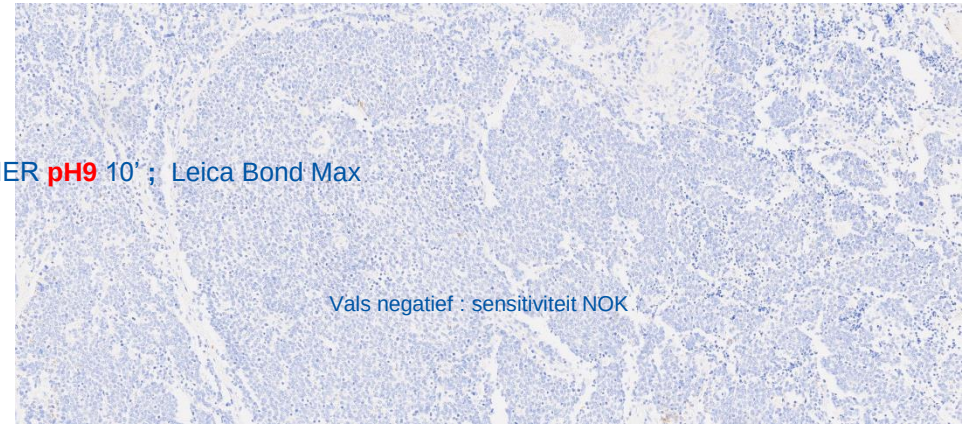


Vals negatief : sensitiviteit NOK

Sterke expressor



Leica 5H7 RTU 15' RT ; HIER pH9 10' ; Leica Bond Max



Vals negatief : sensitiviteit NOK

Besluit:
- pH selectie is kritisch, maar ook…
- Minder performante clone (sensitiviteit). NordiQC 4/6 scoort onvoldoende

'In this assessment the mAb clone 5H7 (Leica/Novocastra) showed an inferior performance both as Conc and RTU format, as 90% (9 of 10) of protocols based on this clone gave insufficient results characterized by a too weak or false negative staining reaction.'
Bron NordiQC Run 53

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Resultaatbespreking Chromogranine

- Algemeen
  - n=67
    - Suff. 79 %

		EKE 2020-2		NQC 2018		NQC 2016	
	n		*Suff.*	*%*	*Suff.*	*%*	*Suff.*
optimaal	**43**	**64%**	***79%***	***51%***	***76%***	***27%***	***67%***
goed	**10**	**15%**		***25%***		***40%***	
Borderline	8	**12%**		*14%*		*16%*	
Onvoldoende	6	**9%**		*10%*		*17%*	

Resultaatbespreking Chromogranine

- Opgesplitst per clone producent
  - RTU: 64% optimaal ; suff. 70 %

n=44	*Neomarkers LK2H10 RTU*	*% suff*	*Ventana LK2H10 RTU*	*% suff*	*Leica / Novocastra 5H7 RTU*	*% suff*
	n=1		n=39		n=2	
optimaal	**0**	**-**	**28**	***72%***	**0**	**-**
goed	**1**	**-**	**2**	***5%***	**0**	**-**
borderline	0	-	4	*10%*	1	1/2
onvoldoende	0	**-**	5	***13%***	1	1/2
Totaal suff	***1/1***		***77%***		***0/2***	
NQC 2018	*Geen data*		*87% (n=112)*		*1/6 suff. 4 onvoldoende*	
NQC 2016	*Geen data*		*80% (n=69)*		*0/6 suff. 4 onvoldoende*	

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Resultaatbespreking Chromogranine

- Opgesplitst per clone producent
  - Concentrated: 65% optimaal ; suff. 91 %

n=23	*Thermo LK2H10* conc.	*% suff*	*C. Marque H10 LK2H10 conc.*	*% suff*	*Sanbio/BioSB LK2H10 conc.*	*% suff*	*Hybritech LK2H10 conc.*	*% suff*
	n=7		n=3		n=4		n=2	
optimaal	*4*	***57%***	***2***	-	***3***	***75%***	***2***	***-***
goed	*3*	***43%***	***0***	-	***1***	***25%***	***0***	***-***
borderline	*0*	***0%***	*1*	-	*0*	***0%***	*0*	***-***
onvoldoende	*0*	***0%***	*0*	-	*0*	*0%*	*0*	-
Totaal suff.	***100%***		***2/3***		***4/4***		***2/2***	
	Resultaat clone LK2H10 : NordiQC 2018: 91% suff. (n=71) ; NordiQC 2016: 93% suff. (n=59)							

Andere clones:

- Invitrogen clone LK2H10: 1/1 goed
- Dako clone DAK-A3 en Dako polyclonaal: beiden 1/1 optimaal
- Thermo/Neomarkers clone LK2H10+PHE5: 1/1 optimaal

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Resultaatbespreking Chromogranine

Besluit:

- 79% geslaagd, 64% scoort optimaal ~ NQC2016 en 2018 ~ OLV rondzending 2012
- 9% deelnemers scoort onvoldoende (≥2 afwijkingen: colon en blaasCa)

- Sterke expressor (pancreas en carcinoïd/schildklierCa):
  - 9% te zwakke aankleuring normaal colon
  - 3% te zwakke aankleuring in sterk expresserend schildklierCa

- Zwak expresserend blaasCa:
  - 9% vals negatief resultaat
  - 20% zeer zwakke aankleuring ; 12% zwakke aankleuring maar klinisch geslaagd

- AS selectie: zelfde clone RTU <> conc. AS
  - Dako gebruikers: geen goede Dako AS (NordiQC: DAK-A3 13% geslaagd), geen alternatief

- Impact AS / format selectie:
  - RTU 73% geslaagd <> conc. meer geslaagd (91%).
  - Oorzaak: Conc. AS = HIER <> RTU 16% geen HIER

Resultaatbespreking Chromogranine

Besluit:

- Impact HIER : Ventana clone LK2H10
  - Geen HIER (18%): altijd borderline/onvoldoende ! Onafhankelijk van lotnr.
  - Korte HIER (8'): 2/5 niet geslaagd
  - UV: 2 labo's gebruiken UV datasheet: geslaagd (langere AS geeft wel betere resultaten)
  - OV: geen enkel lab HIER datasheet (opm. geen HIER = ook borderline!)

Tabel 1. Aanbevolen kleuringsprotocollen voor Anti-Chromogranin A (LK2H10)

2008-11-19

Type procedure	Platform / methode	
	ES of NexES	BenchMark of BenchMark XT
Deparaffinering	Off line	Geselecteerd
Celconditionering (antigeendemaskering)	Niet nodig	Niet nodig
Enzym (protease)	Niet nodig	Niet nodig
Antibody (primair)	16 minuten aanbevolen	16 minuten aanbevolen

Tabel 1. Aanbevolen kleuringsprotocol voor anti-Chromogranin A (LK2H10)-antilichaam met *ultra*View Universal IHC DAB detection kit.

Soort procedure	Methode		
	GX	XT	ULTRA
Deparaffinering	Geselecteerd		
Celconditionering (antigeendemaskering)	CC1, mild	CC1, mild	CC1, 36 minuten, 95 °C
Antilichaam (primair)	12 minuten, 37 °C	12 minuten, 37 °C	12 minuten, 36 °C

Tabel 2. Aanbevolen kleuringsprotocol voor anti-Chromogranin A (LK2H10)-antilichaam met OptiView DAB IHC-detectiekit.

Soort procedure	Methode		
	GX	XT	ULTRA
Deparaffinering	Geselecteerd		
Celconditionering (antigeendemaskering)	CC1 24 minuten	CC1 24 minuten	CC1, 24 minuten, 100 °C
Antilichaam (primair)	4 minuten, 37 °C	4 minuten, 37 °C	4 minuten, 36 °C

2017-11-01

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Resultaatbespreking Chromogranine

Besluit:

- Impact HIER pH keuze:
  - RTU: lage pH = 2/4 niet geslaagd
  - Conc. AS: lage pH = ½ niet geslaagd ; eerder zwakke aankleuring blaasCa
- Vergelijking resultaat conc. AS LK2H10 per stainer
  - Autostainer: 100% optimaal <> NordiQC 89%
  - Omnis:
    - pH9: 55% optimaal (9/10 geslaagd) <> NordiQC 77% (pH keuze, challenging blaasCa, HTX)
    - pH6: 0/1 optimaal (niet geslaagd) = idem NordiQC
  - BMK Ultra/XT: 83% optimaal <> NordiQC 79%
  - Leica: 0/1 optimaal (wel geslaagd), achtergrond <> NordiQC 83%

Table 3. **Proportion of optimal results for CGA for the most commonly used antibody concentrate on the four main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark 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	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **LK2H10**	16/18** (89%)	0/4	10/13 (77%)	0/1	19/24 (79%)	0/1	5/6 (83%)	1/6
mAb clones **LK2H10+PHE5**	0/1	-	2/3	-	7/9 (78%)	-	1/3	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.

Bron: NordiQC rapport Run 53, 2018

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Resultaatbespreking Chromogranine

Besluit:

46% gebruikt controle

- Aanbevolen controle
  - Appendix: ganglioncellen en axonen minstens zwak positief (=LLOD) ; neuro-endocriene cellen sterk positief. Enterocyten en gladde spier negatief.
  - Lever: geen aankleuring in hepatocyten en galwegen. Verspreide aankleuring axonen mogelijk.

In this context it must be stressed that pancreas cannot be used as positive tissue control even though recommended by some vendors. Endocrine cells in the pancreatic islets have a high level of CGA expression, which cannot reliable be used as control of sufficient sensitivity of the protocol. The low-level and limited expression of CGA in many neuroendocrine tumours and carcinomas can consequently lead to a false negative staining result in these tumours despite positive staining reaction in pancreas.

- iCAPC: critical assay performance controls (bron NordiQC)

A, Pancreas: the majority of the endocrine cells of the islets of Langerhans must show a moderate to strong cytoplasmic staining reaction.

B, Appendix: the majority of ganglion cells and axons of Auerbach's plexus must show a weak to moderate staining reaction (LLOD). The neuroendocrine cells must show a strong cytoplasmic staining reaction.

C, Liver: no staining reaction in hepatocytes and epithelial cells of bile ducts must be seen. Scattered axons can be seen.

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

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Protocol Desmine

n= 65

Conc.: n=10

- Leica DE-R-11 n=1
- Neomarkers D33 n=1
- Dako D33 n=8

RTU: n=55

- Dako D33 n=24
- VWR D33 n=1
- Ventana DE-R-11 n=28
- Leica DE-R-11 n=2

Protocol Desmine

- Conc. AS:
  - Leica DE-R-11
    - Omnis: 1/200 20’ 32° C +linker ; pH9 30’
  - Dako D33
    - Ultra: 1/50 - 1/200 bij 36-42° C +/- amp. ; CC1 30’ – 64’
    - Omnis: 1/100 20’ bij 32° C + linker 10’ ; HIER pH9 30’
    - Bond: 1/50 15’RT ; pH9 10’
  - Thermo/Neomarkers D33
    - Omnis: 1/100 28’ bij 36° C ; CC1 36’ ; UV

Spreiding protocolsettings

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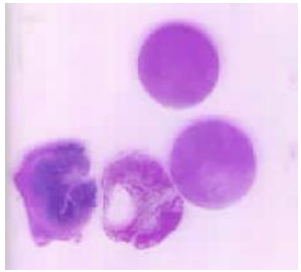
Protocol Desmine

- RTU:
  - Dako D33 clone
    - Autostainer
      - AS 15’ – 30’ RT ; geen linker
      - Enkel HIER pH9
    - Omnis
      - AS 15’ – 30’ RT / 32° C/ 36° C ; +/- linker
      - Enkel HIER pH9
    - Ventana stainers
      - AS 28’ – 30’ RT of 36° C +/- amp
  - Ventana DE-R-11 clone
    - AS 16’ – 32’ RT of 36° C +/- amp
    - HIER CC1 8’ – 72’
    - Enzymatisch: Protease 1: 4’ - 8’ RT/36° C
    - Gecombineerd: P1 of P3 + CC1 36’

Spreiding protocolsettings

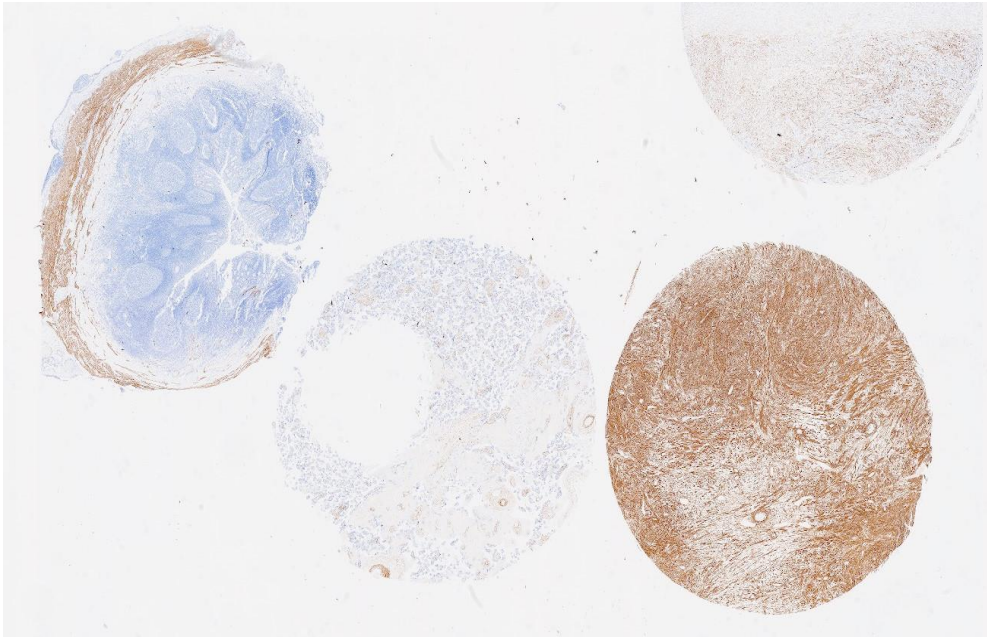
Antistof Desmine



- Scoringscriteria: cytoplasmatische aankleuring
  - Appendix
    - epitheliale cellen negatief (controle specificiteit)
    - gladde spier matig/sterk positief in muscularis propia en lamina muscularis mucosae (controle sensitiviteit - hoge expressor)
    - Myofibroblasten rond de epitheliale cellen minstens zwak positief
  - Placenta
    - cytotrofoblastische en syncytiotrofoblastische cellen negatief (controle specificiteit)
  - Maag leiomyoom
    - matig /sterke aankleuring van bijna alle neoplastische cellen (controle analytische sensitiviteit)
  - Testis leiomyosarcoom
    - matig tot sterke aankleuring van bijna alle neoplastische cellen
  - Alle weefsels:
    - gladde spiercellen in bloedvaten zwak tot matig positief

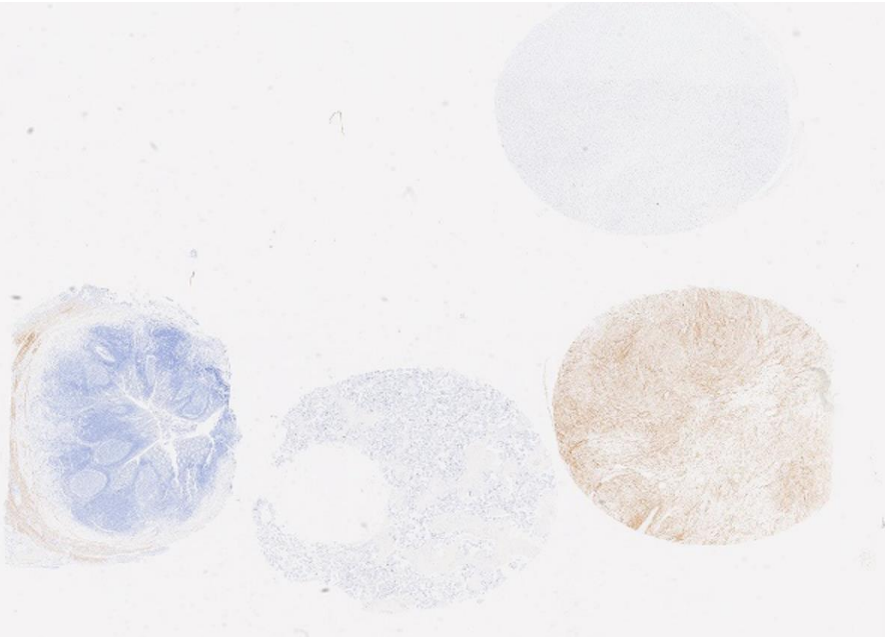
Antistof Desmine: overzicht

Optimaal



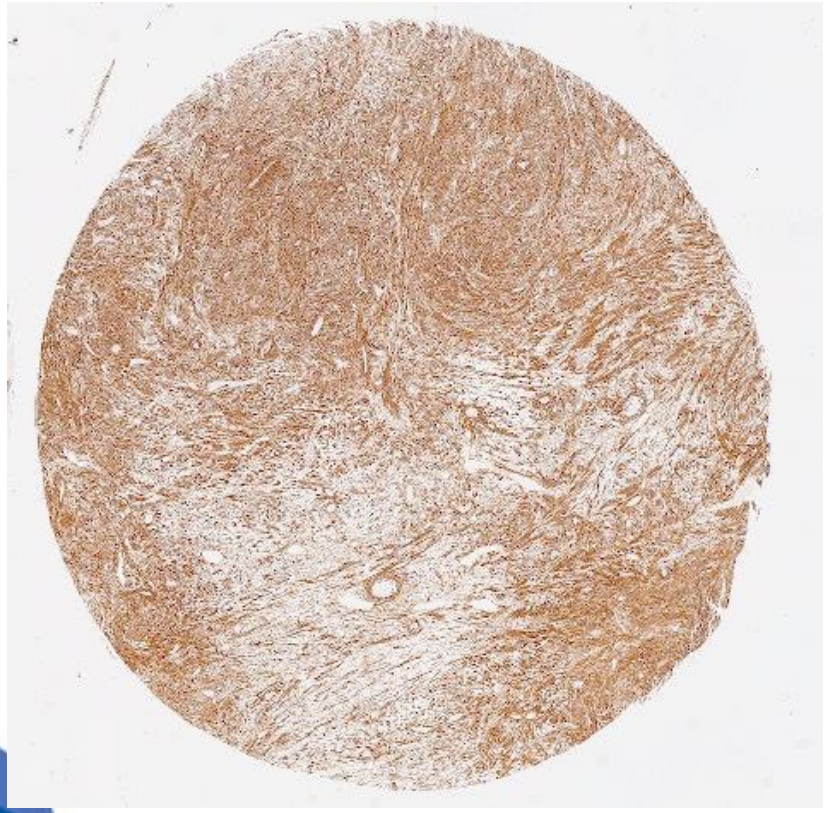
Leica DE-R-11 1/200 20’ 32˚ C **+linker ;** TRS pH9 30’ **; Dako Omnis**

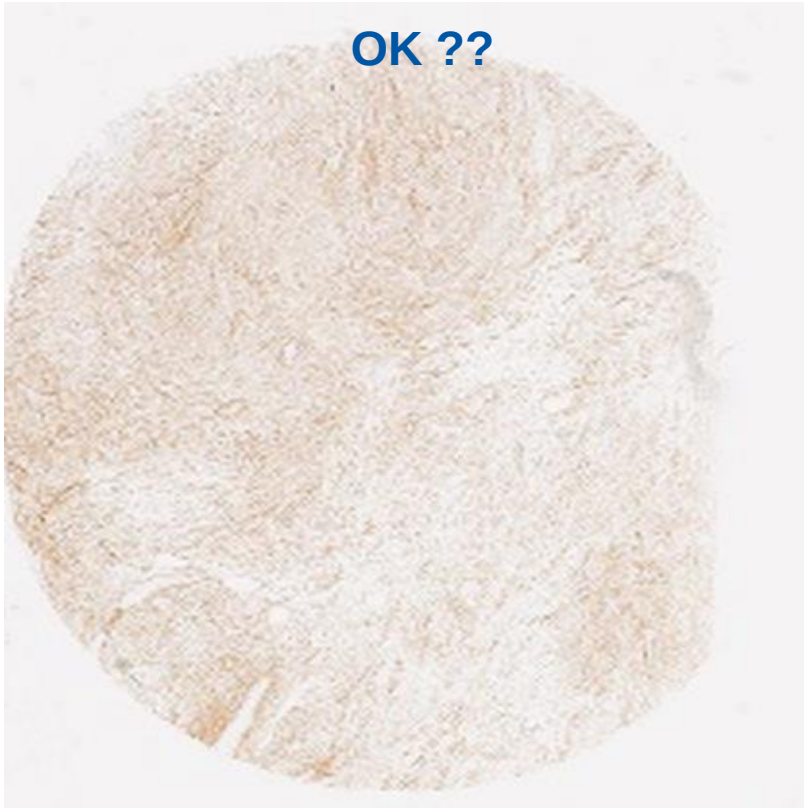
Onvoldoende



Dako D33 1/100 20’ 32˚ C **;** TRS pH9 30’ **;** Flex+ **Dako Omnis**

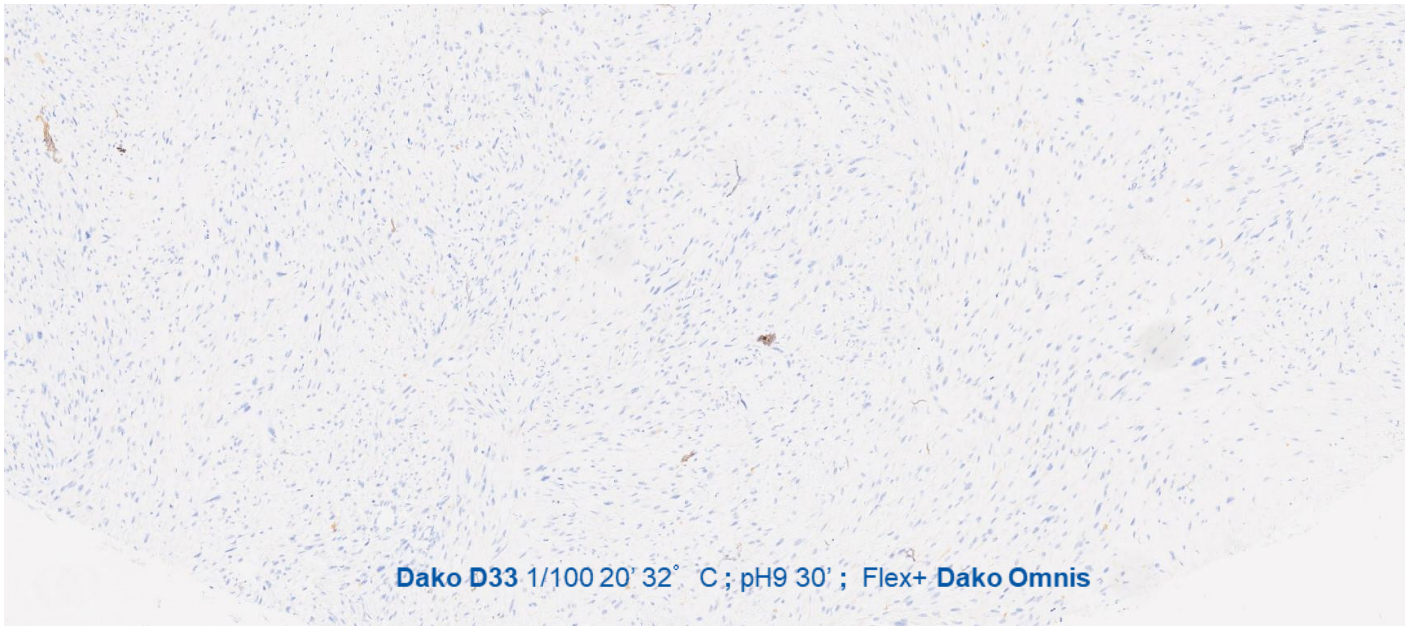
Antistof Desmine: leiomyoom



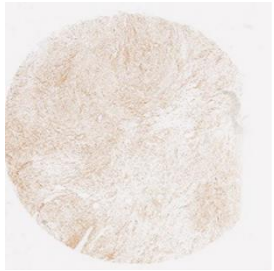


Sterke expressor

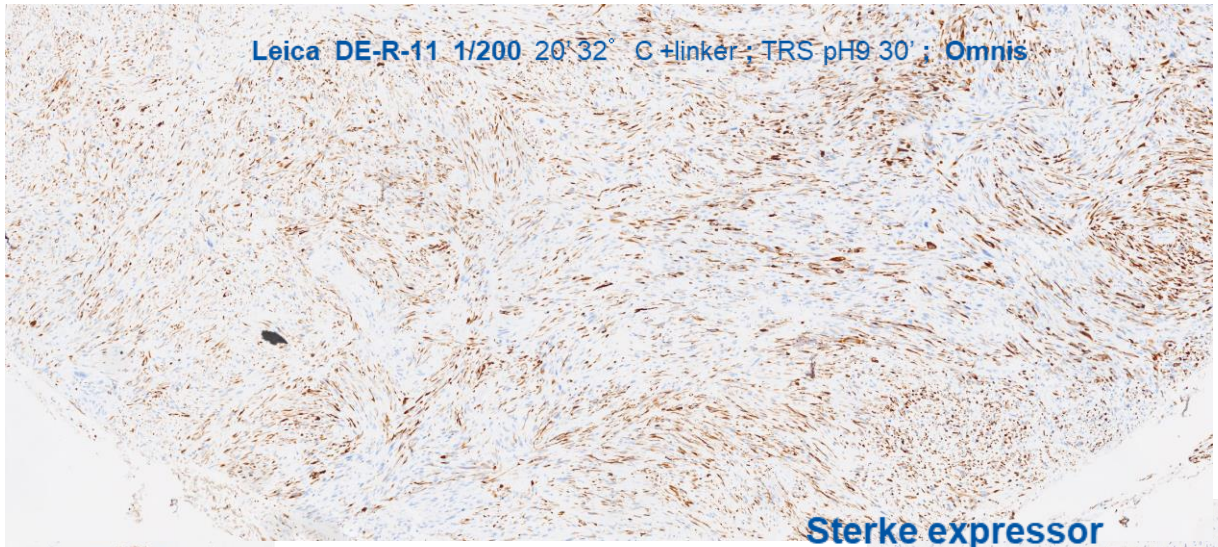
Antistof Desmine: leiomyosarcoom



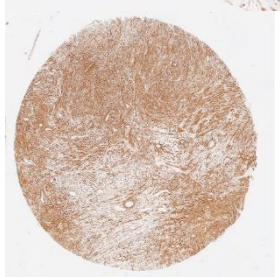
OK ??

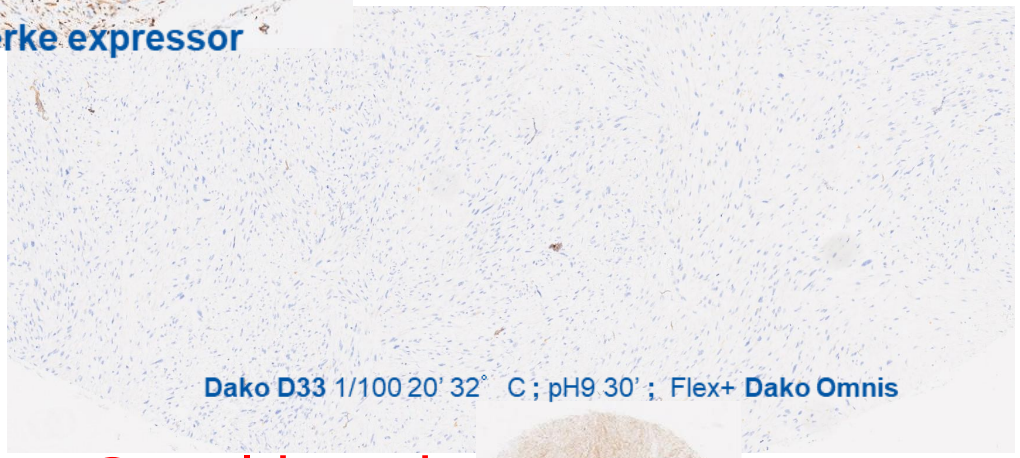


Antistof Desmine: leiomyosarcoom

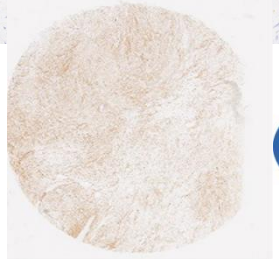


Sterke expressor



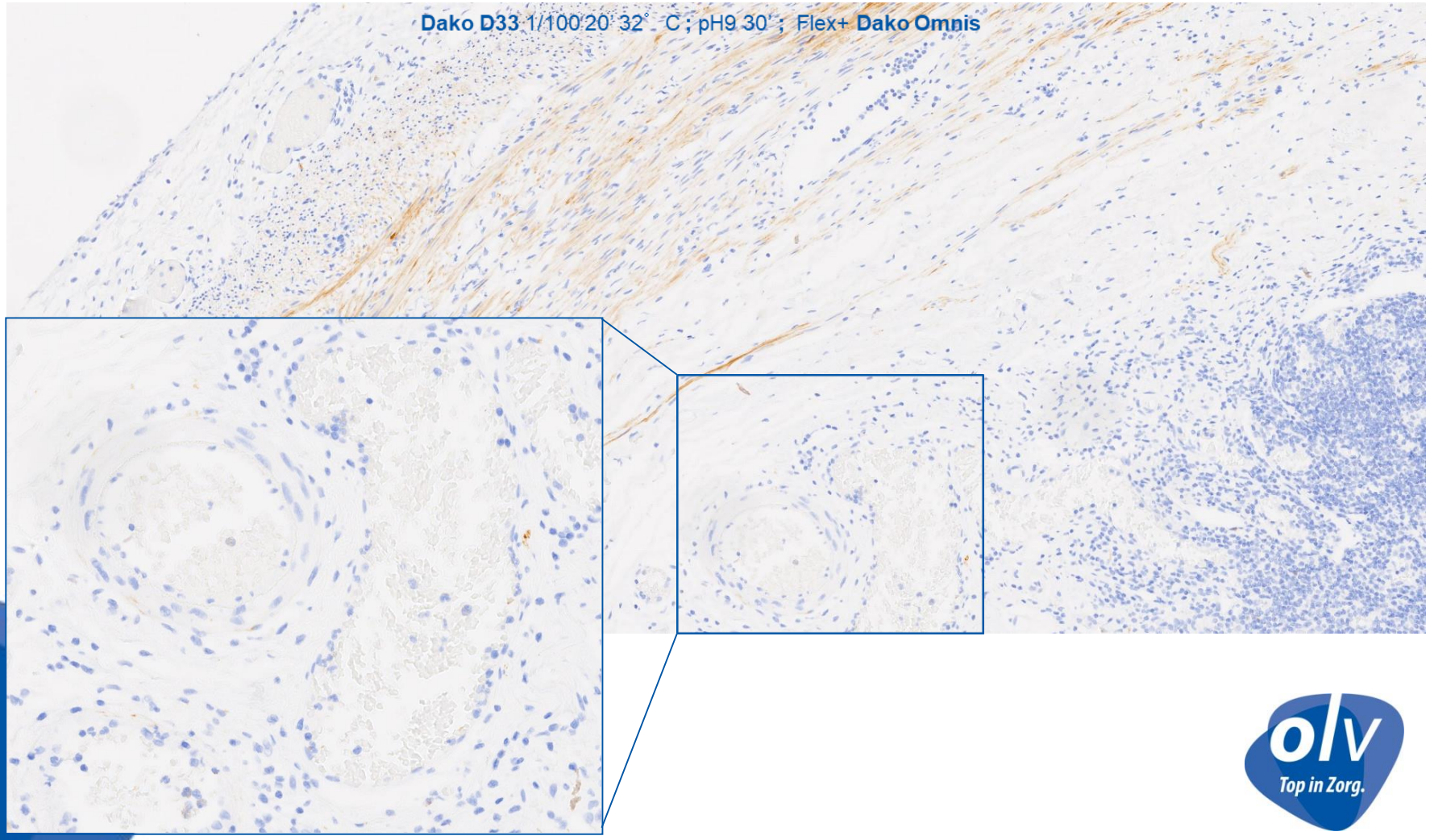


Onvoldoende



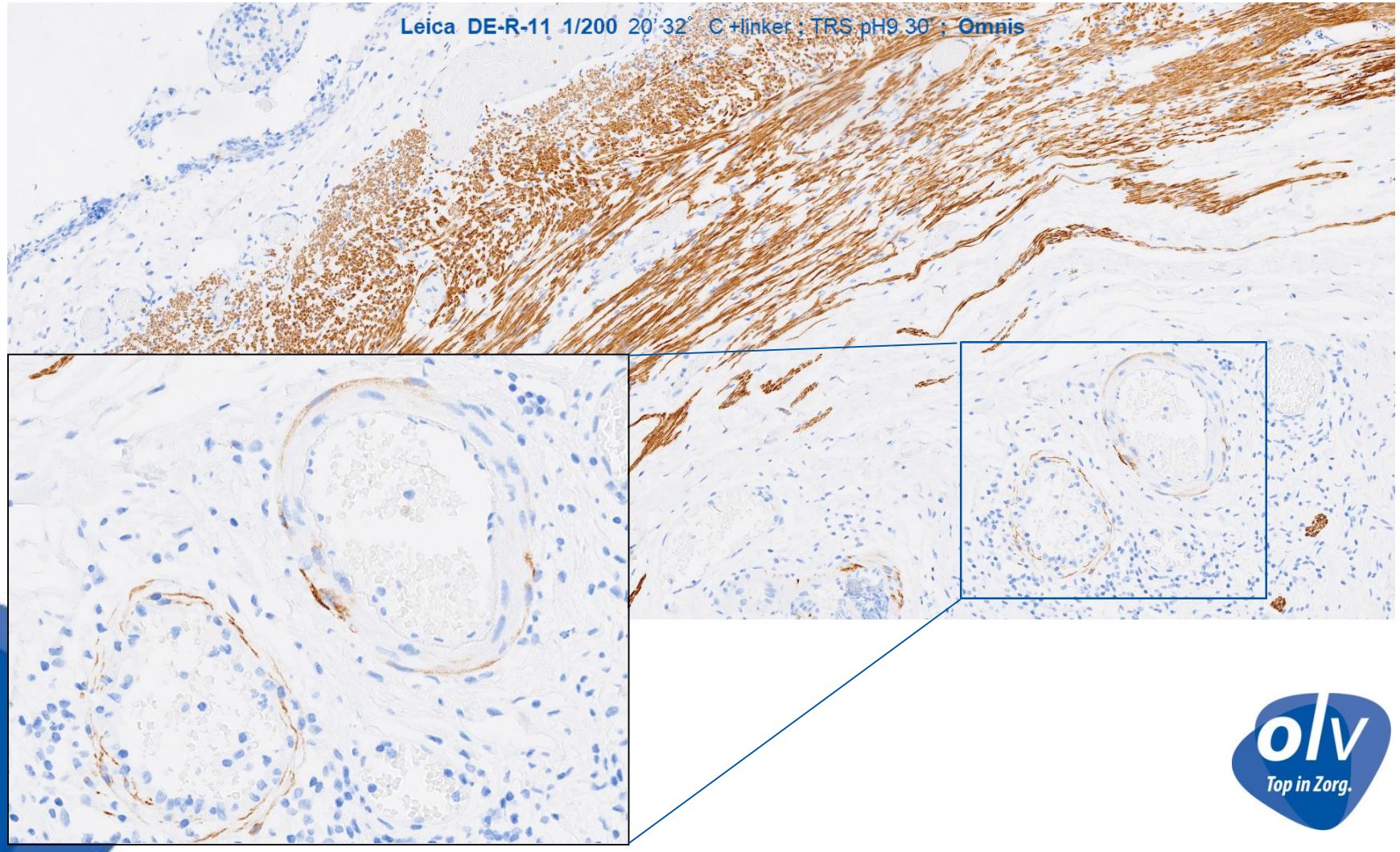
Antistof Desmine: appendix

Onvoldoende



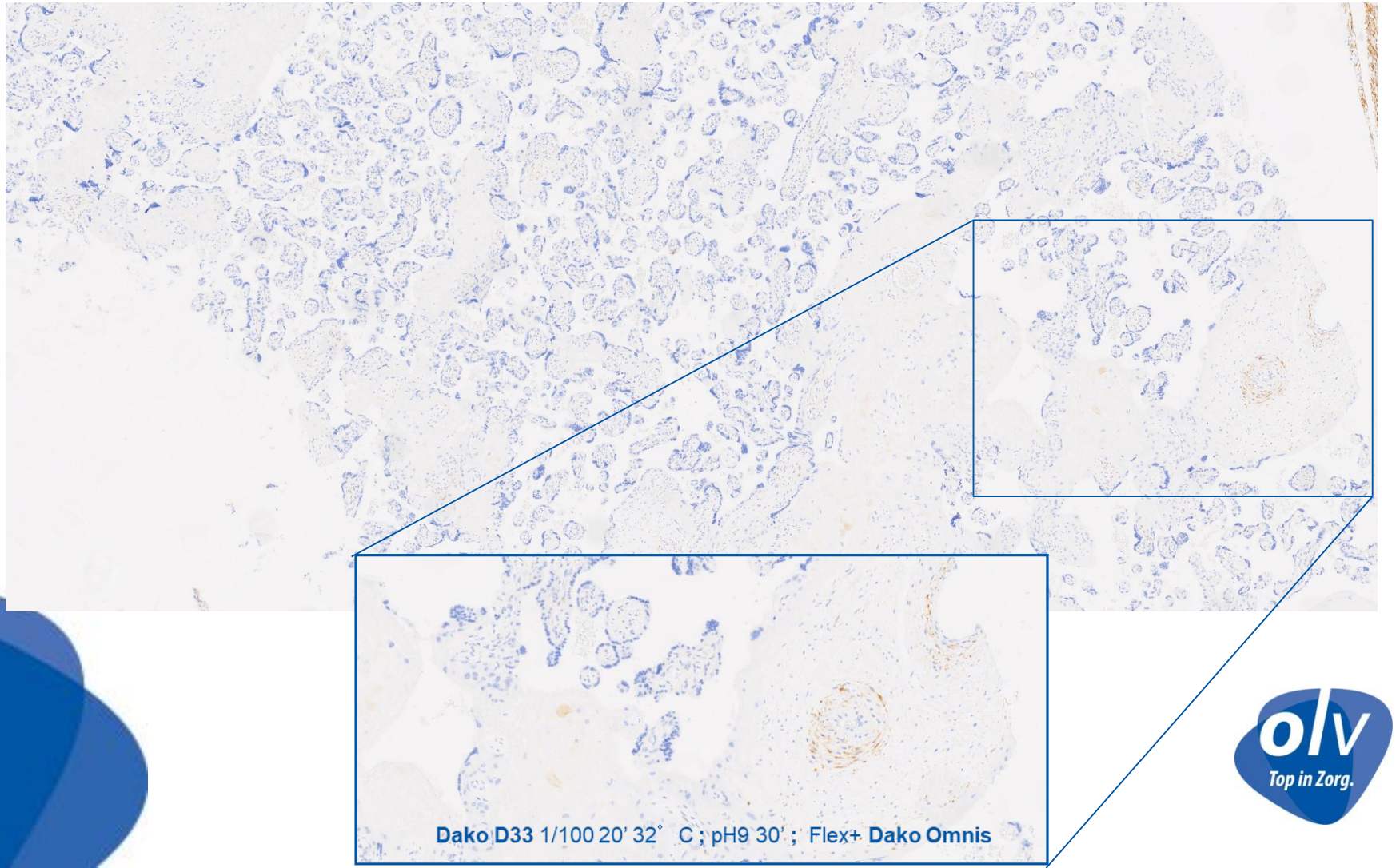
Antistof Desmine: appendix

Optimaal



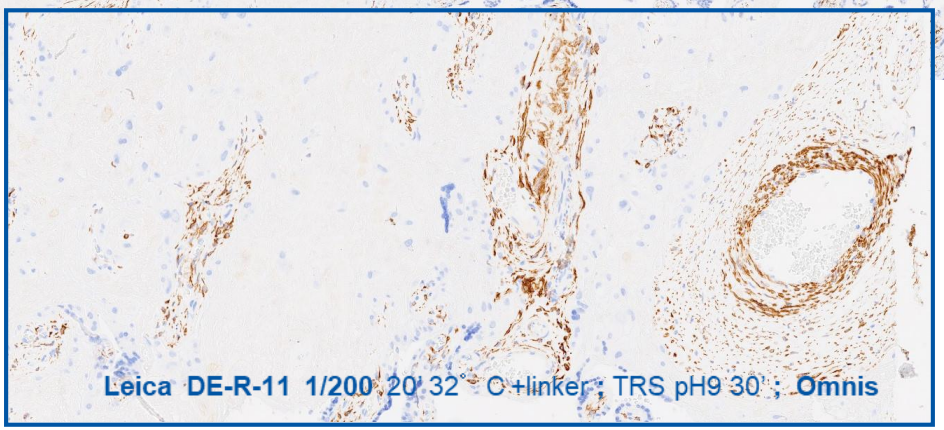
Antistof Desmine: placenta

Onvoldoende



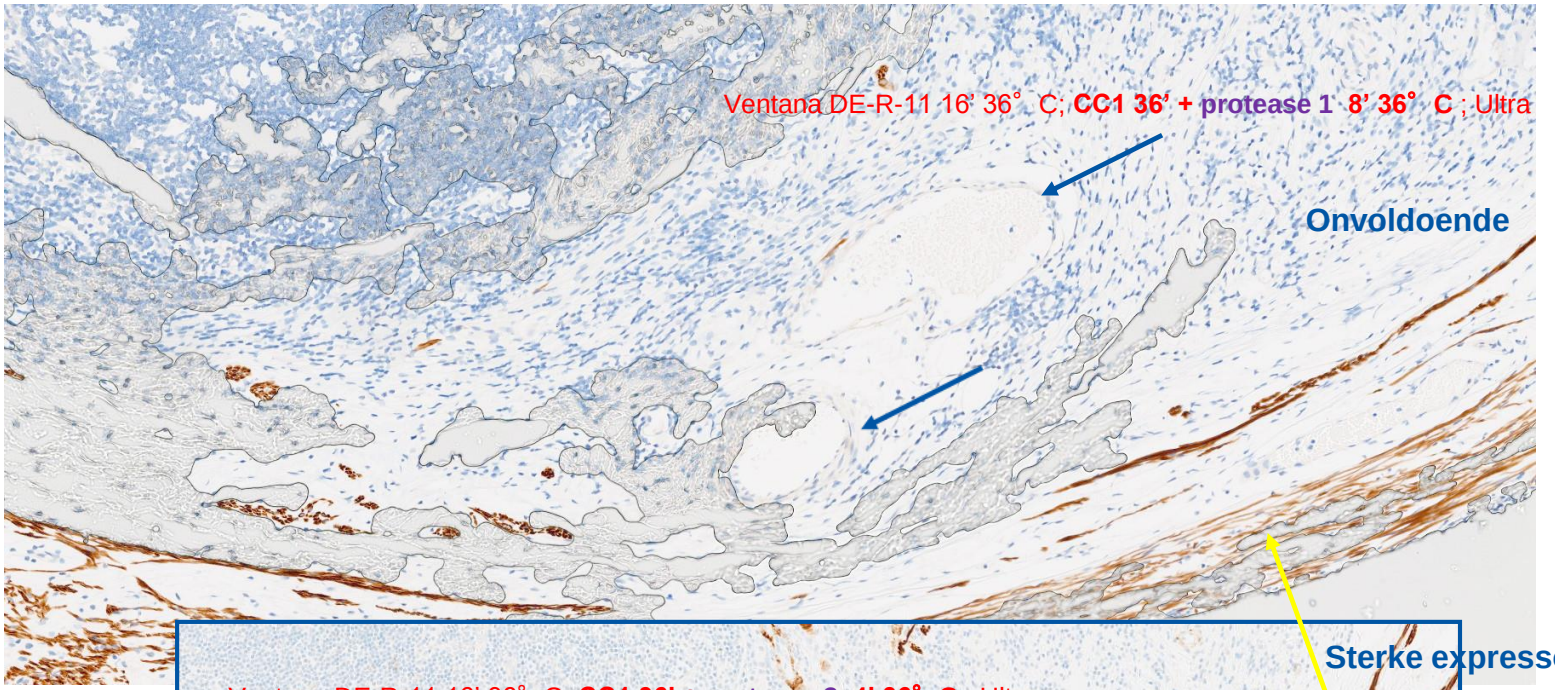
Antistof Desmine: placenta

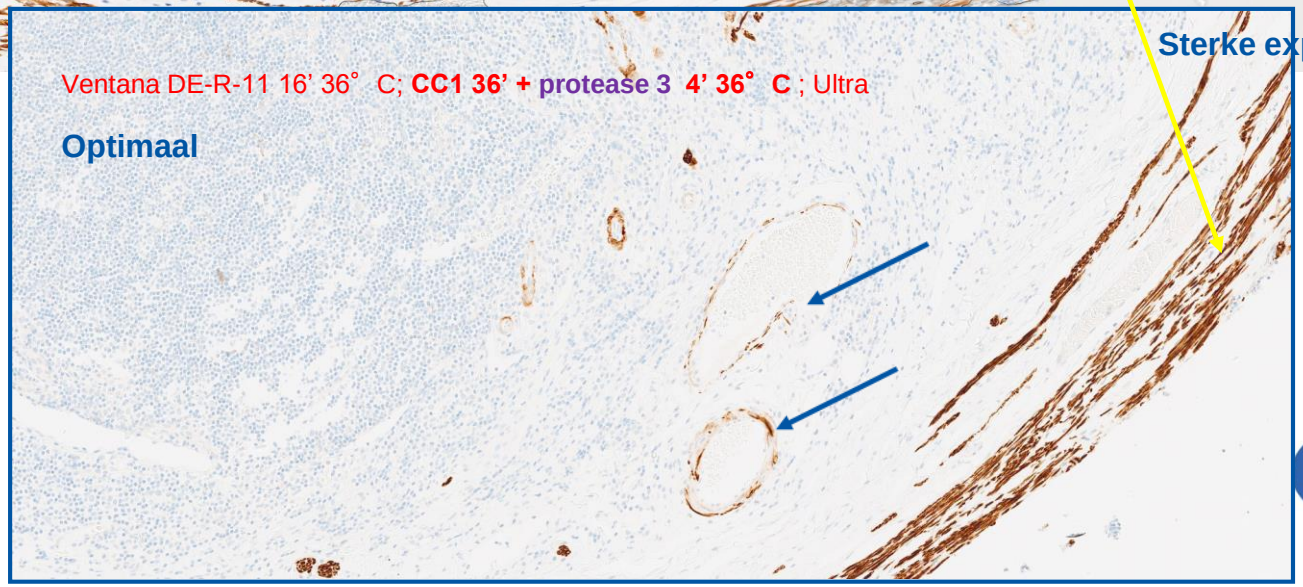
Optimaal



Antistof Desmine

Impact voorbehandeling





Antistof Desmine

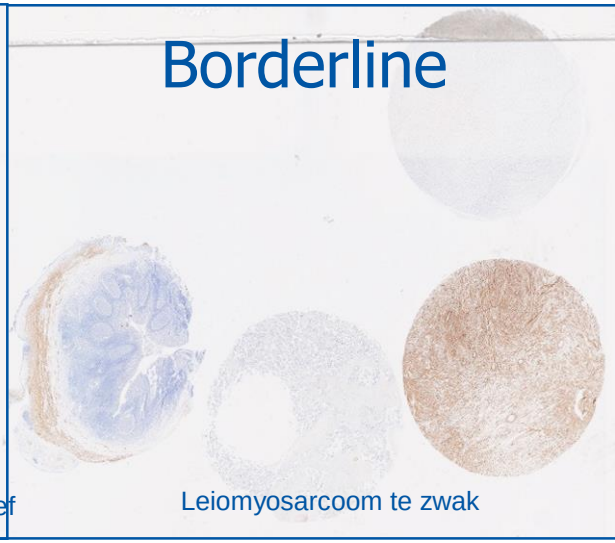
Goed

Achtergrond, tegenkleuring?

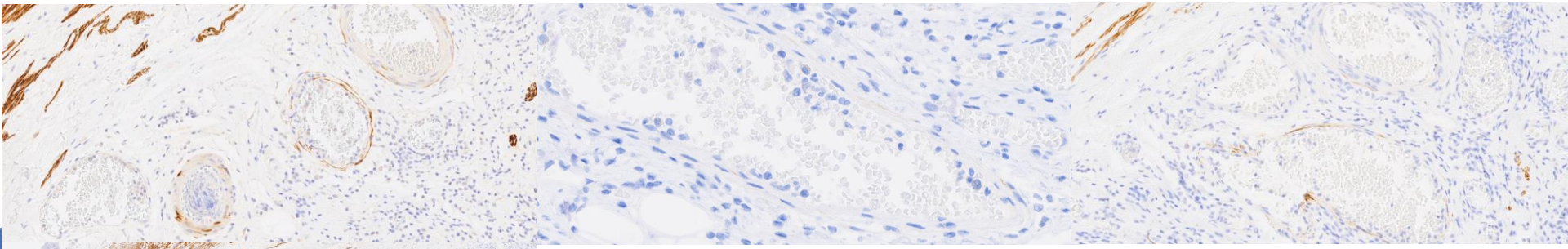
Onvoldoende

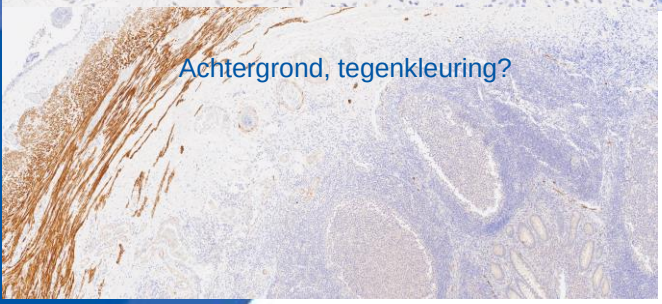
Leiosarcoom, placenta en appendix te zwak/vals negatief

Borderline



Leiomyosarcoom te zwak





Achtergrond, tegenkleuring?

Zelfde protocol: Dako D33 RTU 20' 32° C ; TRS pH9 30' ; Omnis

23% geslaagd ; geen optimaal AS ??

Resultaatbespreking Desmine

- Algemeen
  - n=65
    - Suff. 75 %

		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%*	

Resultaatbespreking Desmine

- Opgesplitst per clone producent
  - RTU: 58% optimaal ; suff. 75 %

n=55	*Dako* D33 RTU	*% suff*	*Ventana* DE-R-11 *RTU*	*% suff*	Leica DE-R-11 RTU	% suff.
	n=24		n=28		n=2	
optimaal	**8**	***33%***	**23**	***82%***	**1**	**-**
goed	**5**	***21%***	**4**	***14%***	**0**	**-**
borderline	5	***21%***	1	*4%*	0	-
onvoldoende	6	***25%***	0	***0%***	1	-
Totaal suff	***54%***		***96%***		**1/2**	
NQC 2016	*93%*		*97%*		*1/1*	

Resultaatbespreking Desmine

- Opgesplitst per clone producent
  - Concentrated: 56% optimaal ; suff. 79 %

n=10	*Dako/Thermo* D33 conc.	*% suff*	*Leica DE-R-11 conc.*	*% suff*
	n=9		n=1	
optimaal	5	***56%***	**1**	**-**
goed	2	***23%***	**0**	**-**
borderline	0	**0%**	0	**-**
onvoldoende	1	***11%***	0	-
Totaal suff.	***79%***		***1/1***	
NQC 2016	*78% (n=100)*		*94% (n=16)*	

Resultaatbespreking Desmine

Besluit:

- 75% geslaagd, 58% optimaal, merkelijk lager dan NQC
- Meeste labs kunnen sterke expressoren aankleuren
- Op alle platformen optimaal mogelijk
- Met alle clones optimaal mogelijk
- RTU scoort beter dan Conc. formats, maar…
- Onvoldoendes bij RTU format
- Resultaten RTU en conc. afhankelijk van gebruikte clone

- DE-R-11 RTU:
  - Leica: optimaal mogelijk mits voldoende HIER én AS
  - Ventana:
    - zowel HIER/enzym/combinatie
    - robuuste clone: weinig problemen ondanks spreiding protocolsettings (HIER)

- DE-R-11 conc. (n=1): op Omnis optimaal (hoge verdunning maar linker)

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Resultaatbespreking Desmine

Besluit:

- D33 conc. :
  - optimaal mogelijk mits voldoende sensitief protocol
  - Gemiddelde verdunning: 1/100
  - 4/10 gebruiken linker/amplifier

- D33 RTU:
  - Op Ventana allen optimaal ondanks spreiding protocolsettings
  - Dako Autostainer
    - Vrij gestandaardiseerd protocol (AS tijd)
    - 5/7 geslaagd
    - Gelijke settings geven wisselend resultaat (onvoldoende -> optimaal)

  - Dako Omnis
    - Spreiding protocols (AS inc. RT of ° C ; HIER gestandaardiseerd)
    - Slechts 23% geslaagd (maar achtergrond, door linker?) ; geen optimaal
    - 46% zwakke/vals negatieve appendix-placenta-leiomyosarcoom
    - Linker geen oplossing van het probleem: blijft onvoldoende
    - Oorzaak : niet eenduidig vast te stellen, over meerdere loten, AS specifiek voor Omnis??

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Resultaatbespreking Desmine

Besluit:

- Effect demaskering
  - Geen low pH
  - Enzymatisch (Enkel bij RTU gebruikers)
    - 1/3 DE-R-11 gebruikers → allen geslaagd, 50% optimaal
  - Combinatie: 8% gebruikers → P3= allen optimaal, P1 = borderline (te zwak)

- Effect detectiekit:
  - Ventana:
    - iView optimaal (n=1)
    - geen verschillen UV vs OV
    - oorzaak afwijking: suboptimale settings (verdunning..)
  - Dako:
    - Envision Flex allen niet geslaagd (Omnis)
    - Envision Flex+ : 40% geslaagd
  - Leica: te weinig data

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Resultaatbespreking Desmine

Besluit:

- Controle
  - 46% gebruikt controle
  - Aanbevolen controle (bron NordiQC)

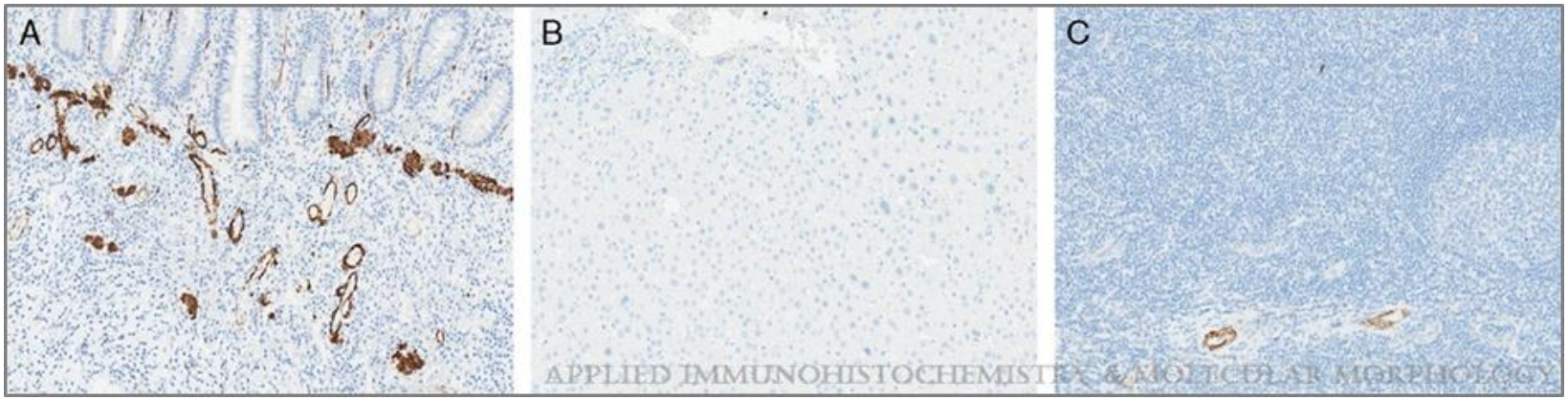
As concluded in the previous assessments for DES it is difficult to identify a robust and easy interpretable critical stain quality indicator for DES. Normal muscle cells in the muscle layers in the appendix can not be recommended, as these cells were demonstrated in virtually all protocols and have a very high expression of DES and can not be used to identify a protocol with a too low sensitivity.

The best choice seems to be the smooth muscle cells in the vessels in the appendix, which should show an as strong as possible staining reaction without any reaction in cells not expected to be demonstrated as e.g. the appendiceal epithelial cells. The staining pattern in these smooth muscle cells will typically be seen as a patchy intracytoplasmic staining reaction and not a consistent homogenous staining reaction as seen for alpha smooth muscle cell actin in these cells.

Resultaatbespreking Desmine

Besluit:

- Controle
  - iCAPC: critical assay performance controls (bron NordiQC)



Hoge expressor=controle op keuze juiste AS

lage expressor=controle op sensitiviteit

geen expressie=controle op specificiteit

A, Appendix: all smooth muscle cells in muscularis mucosae and muscularis propria of the appendix must show a moderate to strong, distinct cytoplasmic staining reaction. Most smooth muscle cells of vessels must show a weak to moderate cytoplasmic staining reaction (LLOD).

B, Liver: no staining reaction in hepatocytes must be seen. Dispersed smooth muscle cells in vessels can show a weak to moderate cytoplasmic staining reaction.

C, Tonsil: no staining reaction in epithelial cells and lymphocytes must be seen. Dispersed smooth muscle cells in vessels may show a weak to moderate cytoplasmic staining reaction. iCAPC indicates immunohistochemistry critical assay performance controls; LLOD, low limit of detection.

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

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Resultaatbespreking besluit

sufficient	2020	NQC	
CD3	100 %	92 %	
Chromo	79%	76%	
Desmin	75 %	87%	

EKE planning

Externe kwaliteitsevaluatie	EKE 2021-1	EKE 2021-2	EKE 2021-3	EKE 2022-1	EKE 2022-2	EKE 2022-3
	ER / Her2 / PR reassess 2020-3	**CD117 S100 E-Cadh.**	**PR / Her2 / ER reassess 2021-1**	**ER / Her2 / PR reassess 2021-3**	**To be defined**	**PR / Her2 / ER reassess 2022-1**
Rondzending coupes door Sciensano	15-19/02/2021	3-7/05/2020	13-17/09/2020			
Beoordeling panel	03/2021	6/2021	10/2021			
Doorsturen individueel resultaat	05/2021	10/2021	11/2021			
Resultaatbespreking / symposium	24/05/2021	nvt	?			
Doorsturen globaal rapport	06/2021	11/2021	12/2021			

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