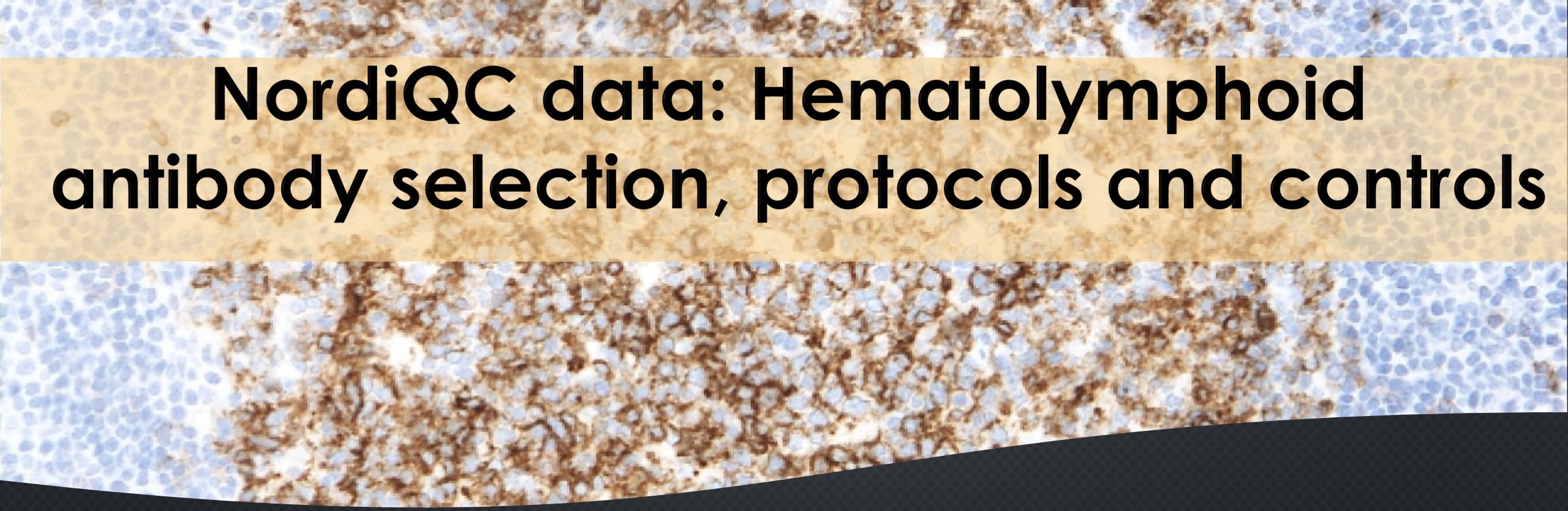
NordiQC data: Hematolymphoid antibody selection, protocols and controls



Tanya Julio

Histotechnologist

Pathology department

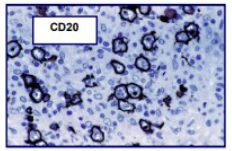
Aarhus University Hospital, DK

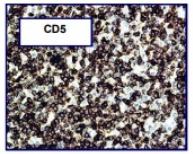
Useful antigens in haematopathology

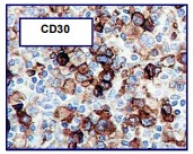
- CD45
- B-cell 'specific'
  - CD19
  - CD20
  - CD79α
  - Pax-5
  - OCT-2 / BOB1
  - Ig
- T-cell 'specific'
  - CD3
  - CD5
  - CD2
  - CD7
  - CD1a
  - CD4
  - CD8
  - PD-1/CXCL-13 (TFH)

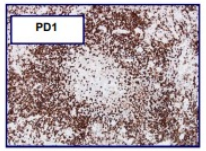
- Other
  - CD30
  - CD10
  - Bcl-2
  - Bcl-6
  - ALK
  - c-myc
  - CD21
  - CD23
  - CD15
  - TdT
  - Cyclin-D1
  - SOX-11
  - CD56
  - TIA-1, granzyme, perforin
  - PDL-1

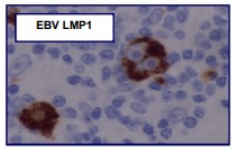
- Other
  - EBV
    - LMP1
    - EBNA2
    - (EBER)
  - CD56
  - CD57
  - EMA
  - S100
  - CD68
  - CD163
  - CD123





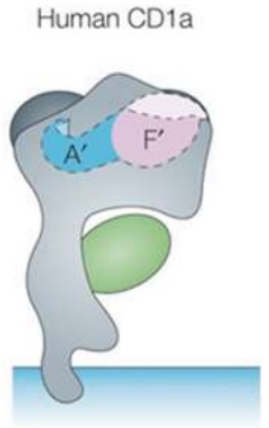




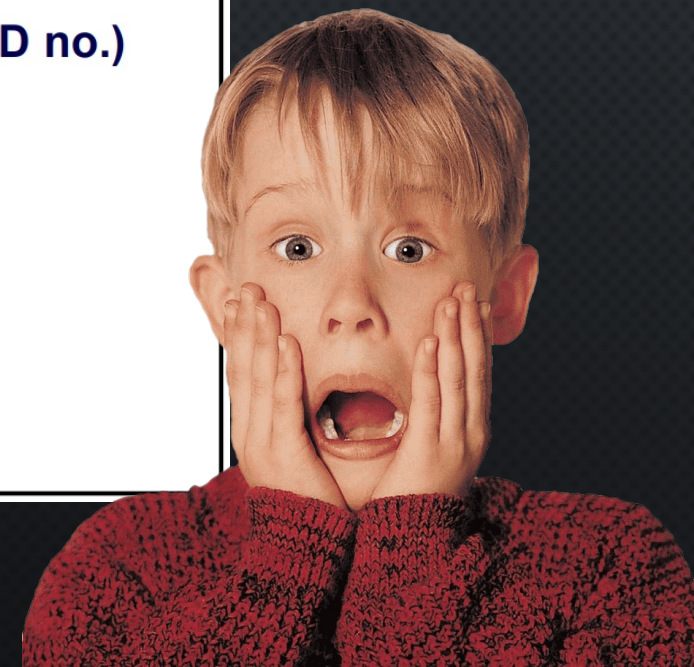


From Stephen Hamilton NordiQC workshop 2023

What are CD numbers?



- CD: ”clusters of differentiation”
- Classification system for antigens (and antibodies)
- Originally for surface antigens on leucocytes
- Now includes other cells and intracellular antigens (no CD no.)
- 10 workshops since 1982
- Currently > 350 CD antigens



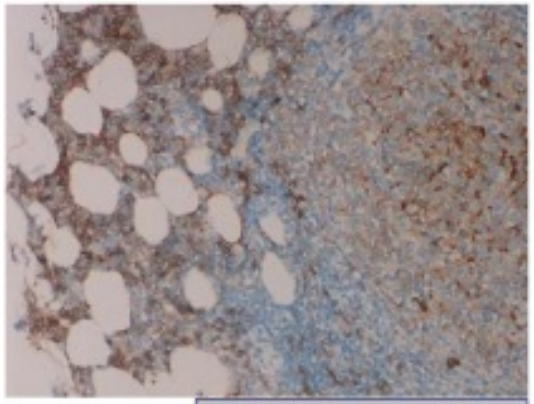
From Stephen Hamilton NordiQC workshop 2023

CD10

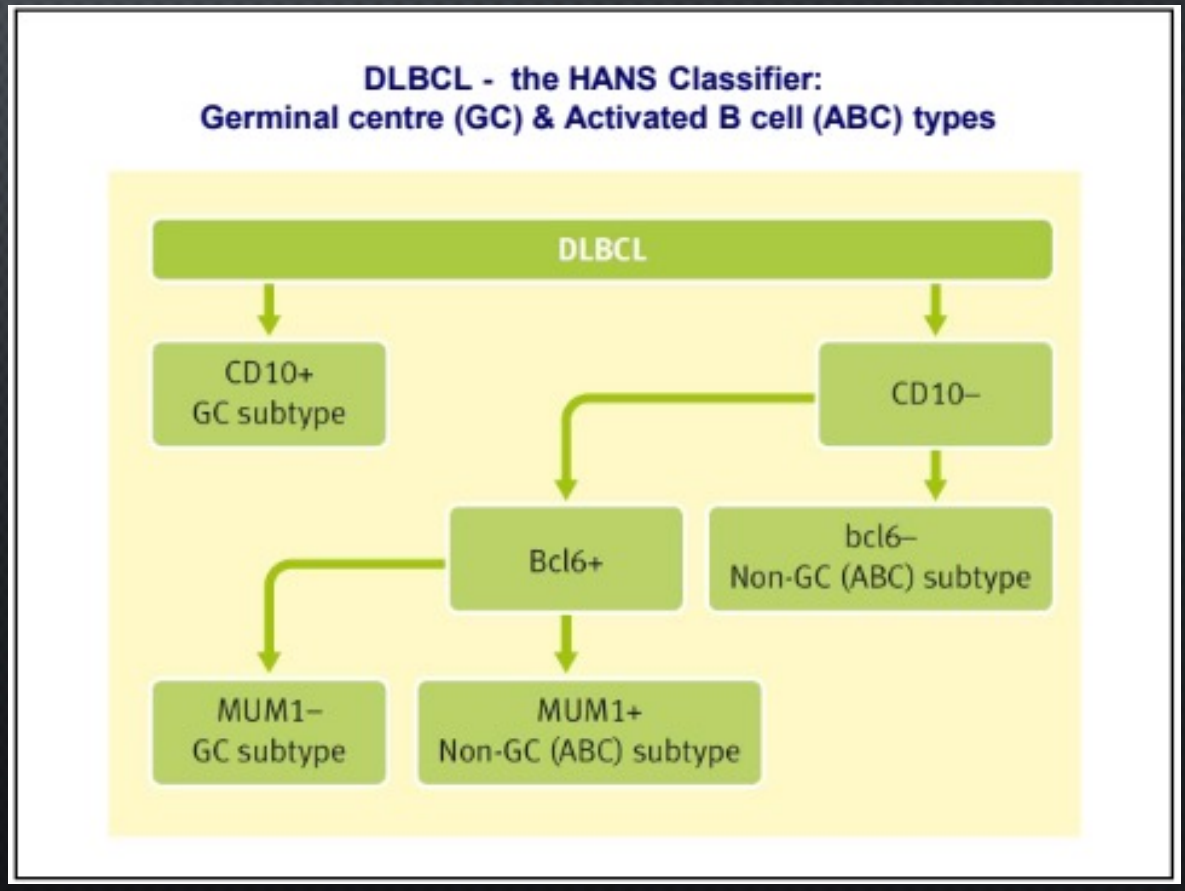
From Stephen Hamilton NordiQC workshop 2023

Secondary stain: CD10

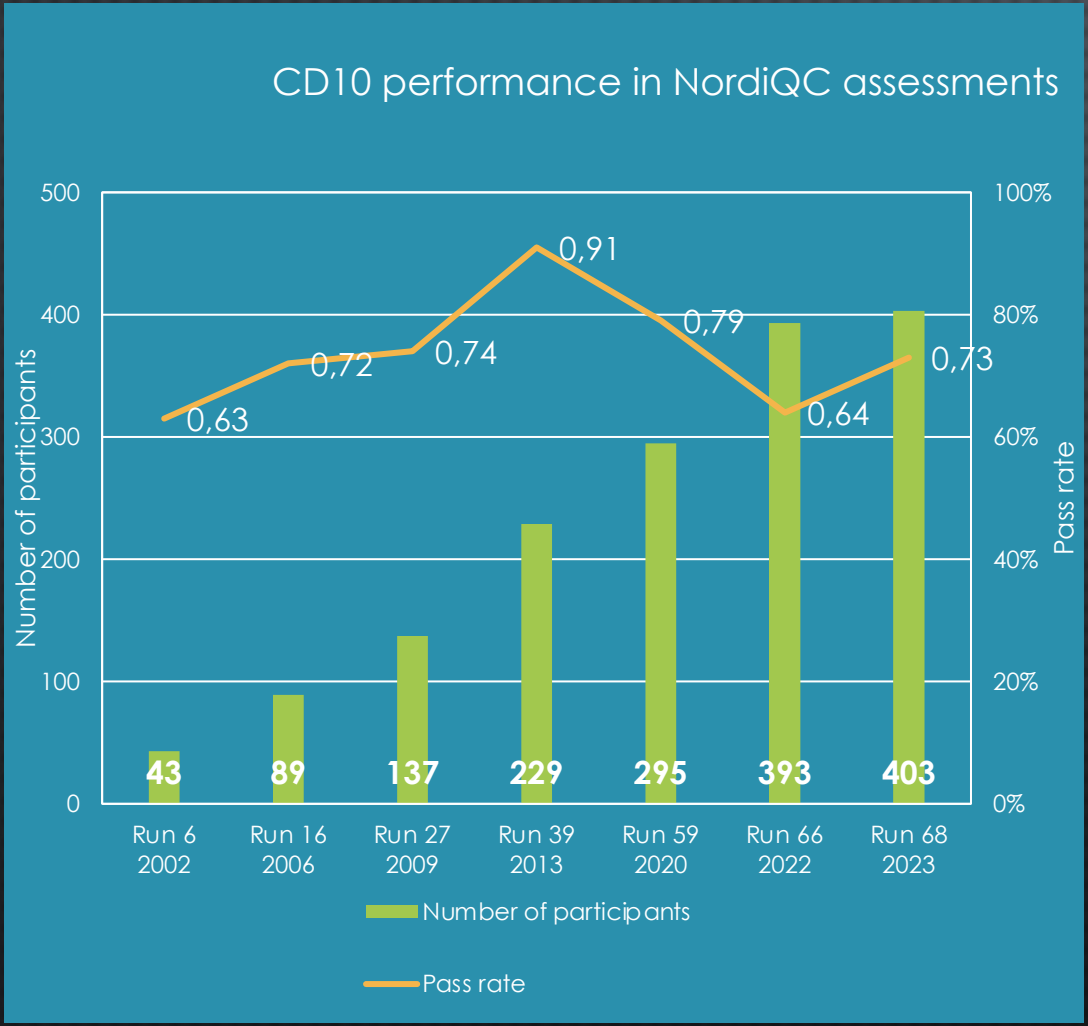
- >90% precursor B-LB (membrane & paranuclear stain)
- ca. 25% precursor T-LB
- Burkitt lymphoma
- Follicular lymphoma
  - Interfollicular CD10+ cells suggets lymphoma
- Some DLBCL
  - 'Cell of origin' algorithm in DLBCL
  - GCB *vs* ABC



- Follicular lymphoma – CD10
- Interfollicular tumour cells



CD10



Modified table 1

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **56C6**	58 7 3 2 2 1 1	Leica Biosystems Cell Marque Monosan/Sanbio Biocare Medical Thermo Scientific/Epredia Immunologic Zytomed	46	7	17	4	72%	62%
Conc total	77		46	8	18	5	70%	60%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **DAK-CD10 GA786 (VRPS)**[3]	22	Dako/Agilent	12	10	0	0	100%	55%
mAb clone **DAK-CD10 GA786 (LMPS)**[4]	33	Dako/Agilent	21	11	1	0	97%	64%
mAb clone **DAK-CD10 IR786 (VRPS)**[3]	5	Dako/Agilent	2	2	0	1	80%	40%
mAb clone **DAK-CD10 IR786 (LMPS)**[4]	15	Dako/Agilent	7	5	2	1	80%	47%
mAb clone **56C6 GA648 (VRPS)**[3]	14	Dako/Agilent	11	3	0	0	100%	79%
mAb clone **56C6 GA648 (LMPS)**[4]	18	Dako/Agilent	13	3	2	0	89%	72%
mAb clone **56C6 IR/IS648 (VRPS)**[3]	3	Dako/Agilent	0	2	1	0	-	-
mAb clone **56C6 IR/IS648 (LMPS)**[4]	13	Dako/Agilent	9	2	2	0	85%	69%
mAb clone **56C6 PA0270/0131 (VRPS)**[3]	22	Leica Biosystems	12	6	4	0	82%	55%
mAb clone **56C6 PA0270/0131 (LMPS)**[4]	27	Leica Biosystems	16	3	8	0	70%	59%
rmAb clone **SP67 790-4506 (VRPS)**[3]	18	Ventana/Roche	3	4	10	1	39%	17%
rmAb clone **SP67 790-4506 (LMPS)**[4]	115	Ventana/Roche	30	41	44	0	62%	26%
rmAb clone **QR021 8386-C010**	1	Sakura Finetek	1	0	0	0	-	-
RTU total	326		147	95	79	5	74%	45%
Total	403		193	103	97	10		
Proportion			48%	26%	24%	2%	73%	

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako Omnis mAb 56C6 **GA648**	100% (33/33)	94% (31/33)	100% (21/21)	95% (20/21)
Dako AS mAb 56C6 **IR648**	1/3	0/3	100% (13/13)	85% (11/13)
Leica Bond III/Max mAb 56C6 **RA270/0131**	100% (11/11)	91% (10/11)	90% (9/10)	70% (7/10)
ra/XT/GX 67 **06**	2/4	0/4	59% (49/83)	23% (19/83)

Table 2. Recommended staining protocol for VENTANA anti-CD10 (SP67) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 92 minutes	CC1, 92 minutes	ULTRA CC1, 92 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	32 minutes, 37°C	12 minutes, 37°C	28 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
OV AMP H2O2, OV Amplifier	8 minutes	12 minutes	8 minutes
OV AMP Multimer	8 minutes	12 minutes	8 minutes
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

Immunostainer

Type: Ventana Benchmark Ultra

Run 59 2020

Primary antibody

Clone: SP67

Producer: Ventana/Roche

Incubation time polymer: 8 min.

Incubation temperature: 36°C

"The highest proportion of sufficient results for protocols based on OptiView without amplification was achieved together with 48-64 min. in HIER and antibody incubation of 32 min., which resulted in a pass rate of **78%** (14/18 slides from 11 different laboratories), but only **14%** (3/18) were **optimal**. Both a too weak and also false positive staining was seen in the 4 insufficient results, proving again the lack of robustness of the antibody. " RUN 67

Urgent Field Safety Notice

SBN-RDS-Pathology Lab-2024-001

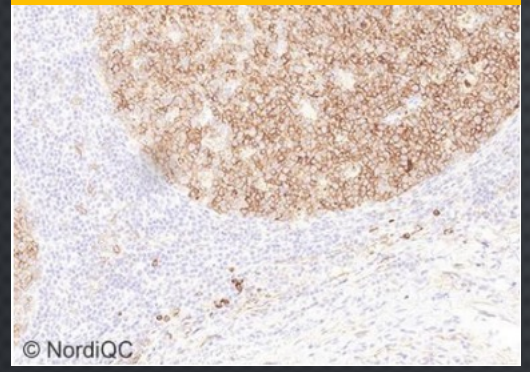
RDS / Pathology Lab
Version 2

Date: Jun-2024

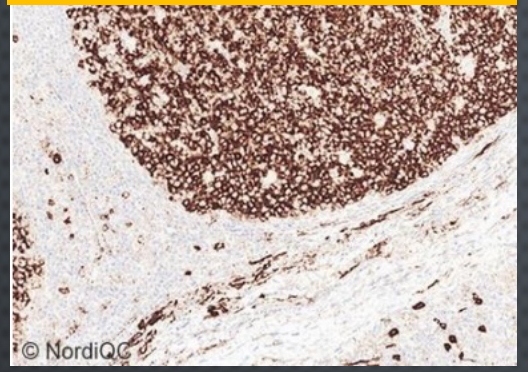
Risk of False Positive results with specific lots of VENTANA anti-CD10 (SP67) Rabbit Monoclonal Primary Antibody due to High Background

Production Identifier (Lot No./Serial No.)	J04613, J11853, J17541, J25047, J30286, K00982, K06239, K09880, K14266, K19784, K26461, and M00669

Bond, clone 56C6 RTU, VPRS



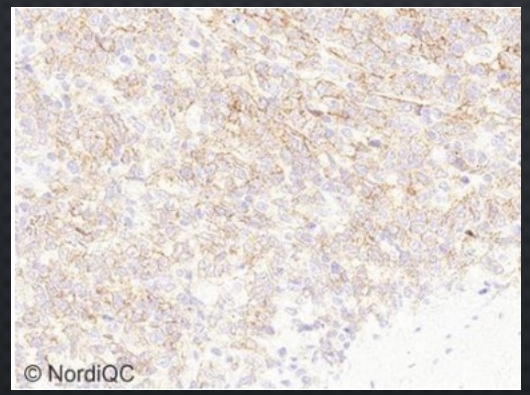
Ventana, clone SP67 RTU,VPRS (8+8)

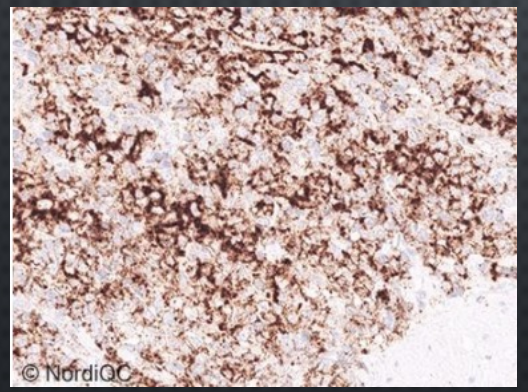


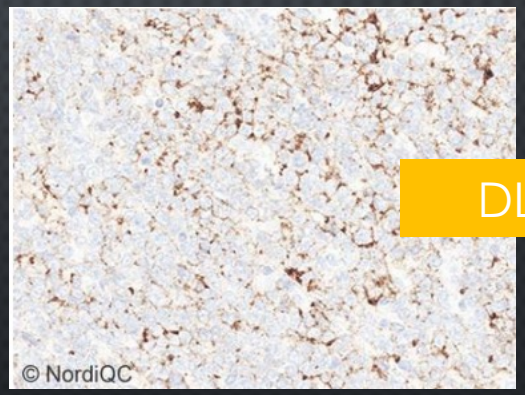
Ventana, clone SP67 RTU, HIER 64 min + amp 4+4

Tonsil

With great power and tyramide comes great responsibility

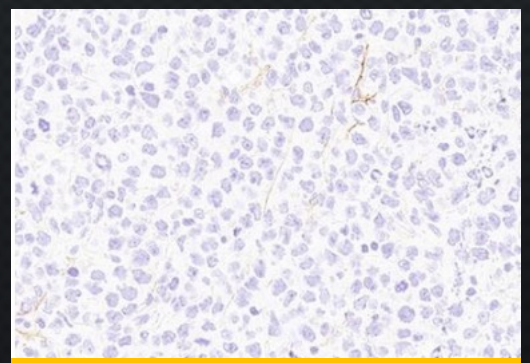




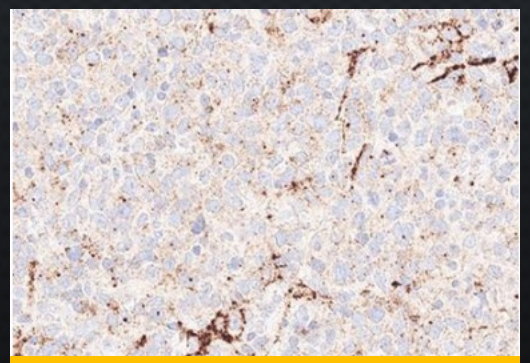


DLBCL - GCB

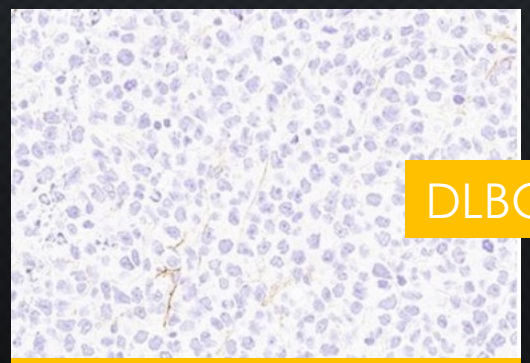
GCB= germinal center B-cell subtype



Optimal



Insufficient



Optimal

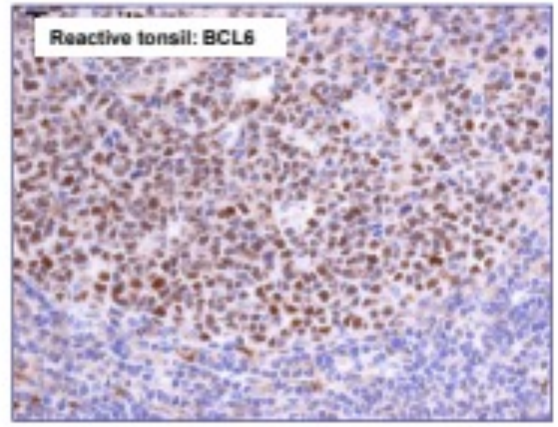
DLBCL – non-GCB

BCL6

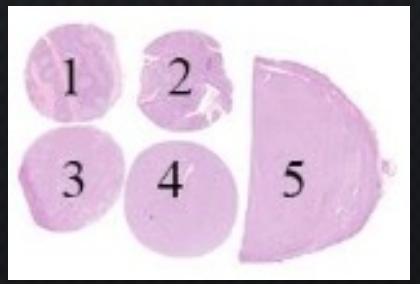
Purpose:
primarily used for subclassification of B-lymphomas and to discriminate Diffuse Large B-Cell Lymphoma (DLBCL) of germinal center B-cell like (GCB) from nongerminal center/activated B-cell (non-GCB/ABC) subtype.

Basic stain: Bcl-6

- Nuclear protooncogene product
- Normal:
  - germinal centre cells
- In lymphomas:
  - follicular lymphoma
  - most BL
  - variable DLBCL
    - 'cell of origin' staining in DLBCL
  - HL-LP (not classical)
  - SLL, MCL, MZL, HCL: negative



From Stephen Hamilton NordiQC workshop 2023

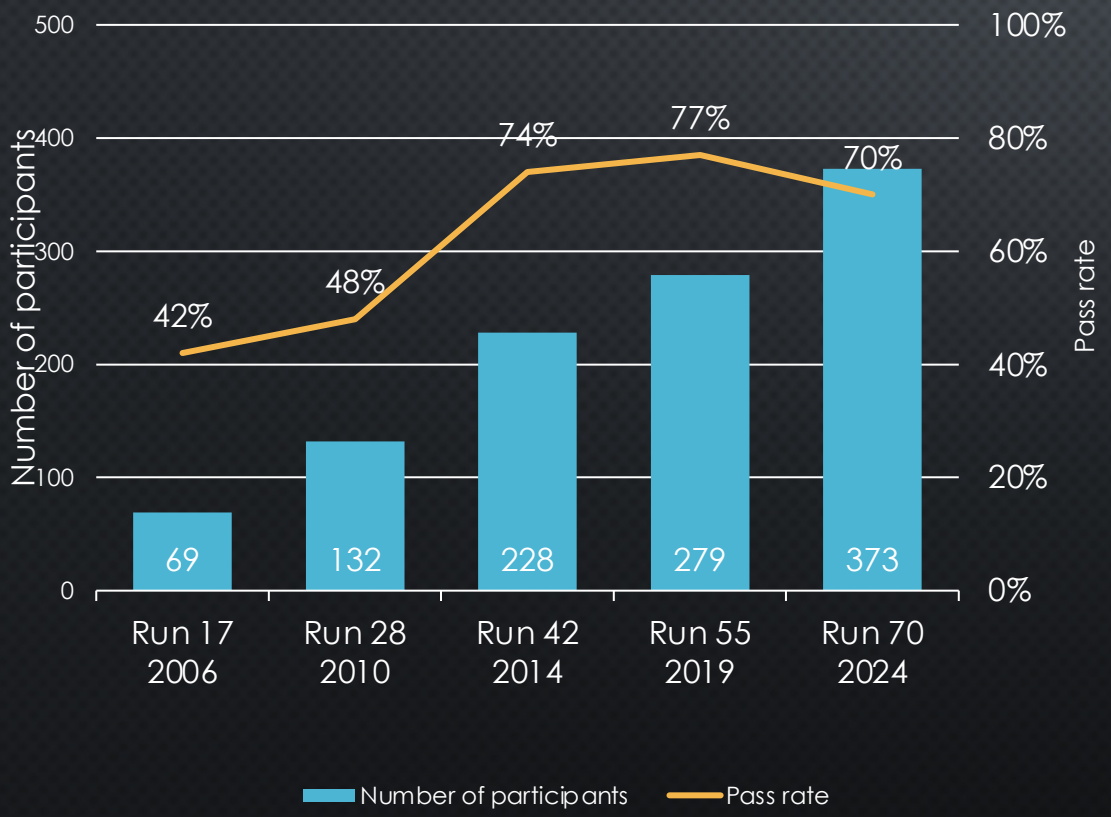


1-2. Tonsils, 3. DLBCL (GCB subtype), 4. DLBCL (non-GCB/ABC subtype), 5. Follicular lymphoma (FL)

BCL6

Table 1a. **Overall results for BCL6, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	87	41	29	15	2	80%	47%
Ready-To-Use antibodies	286	89	102	86	9	67%	31%
Total	373	130	131	101	11		
Proportion		35%	35%	27%	3%	70%	



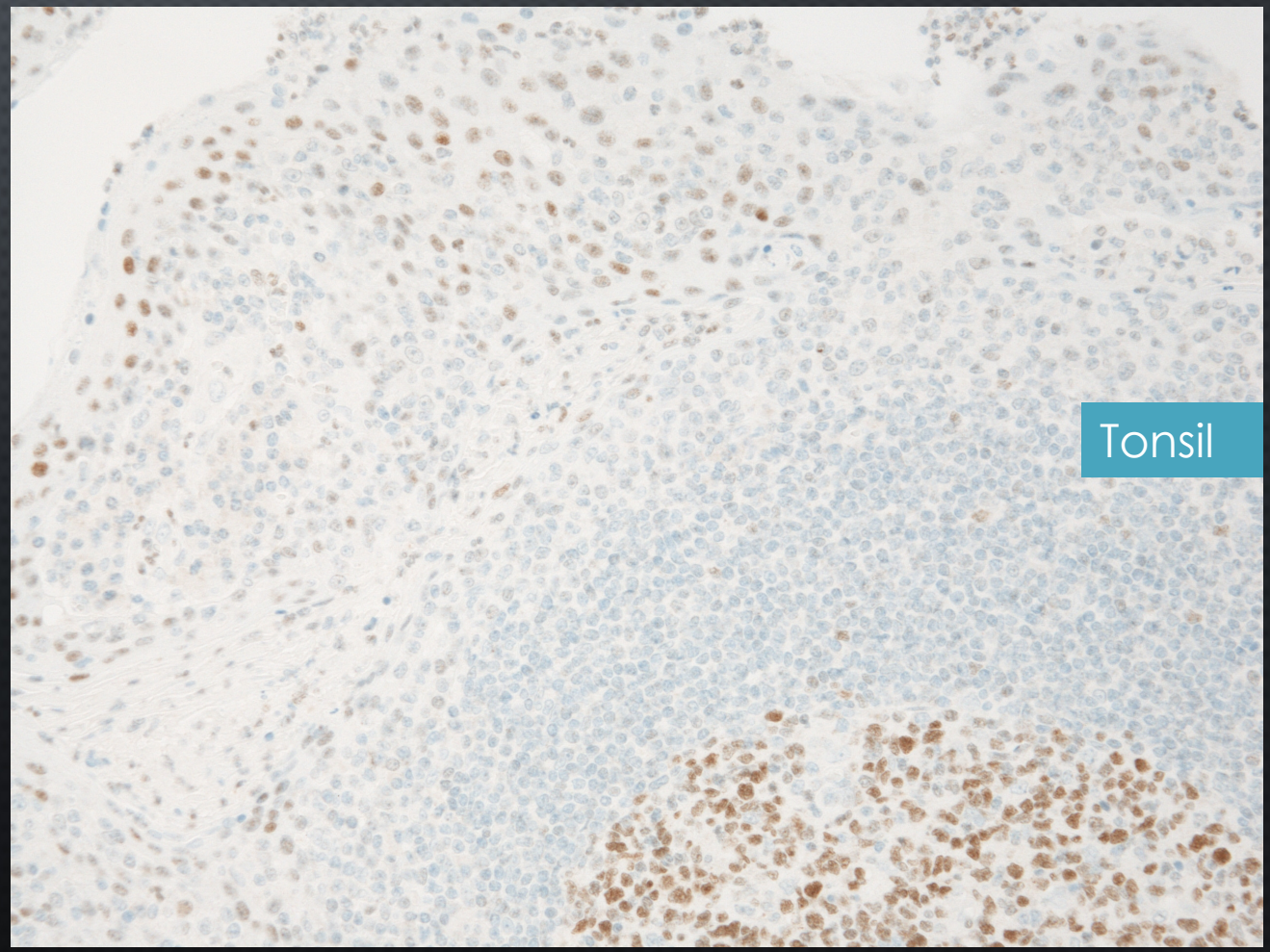


Table 1c. **Ready-To-Use antibodies and assessment marks for BCL6, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **LN22 PA0204**[3]	23	Leica Biosystems	10	7	6	0	74%	43%
mAb clone L**N22 PA0204**[4]	16	Leica Biosystems	10	2	3	1	75%	63%
mAb clone **PG-B6p IR625**[3]	8	Dako/Agilent	3	2	3	0	63%	38%
mAb clone **PG-B6p IR625**[4]	16	Dako/Agilent	6	1	8	1	44%	38%
mAb clone **PG-B6p GA625**[3]	36	Dako/Agilent	20	11	4	1	86%	56%
mAb clone **PG-B6p GA625**[4]	37	Dako/Agilent	24	10	3	0	92%	65%
mAb clone **GI191E/A8 760-4241**[3]	21	Ventana/Roche	0	16	5	0	76%	0%
mAb clone **GI191E/A8 760-4241**[4]	98	Ventana/Roche	10	42	42	4	53%	10%
mAb clone **GI191E/A8 227M-9x**	21	Cell Marque	3	10	7	1	62%	14%
Total	286		89	102	86	9		
Proportion			31%	36%	30%	3%	67%	

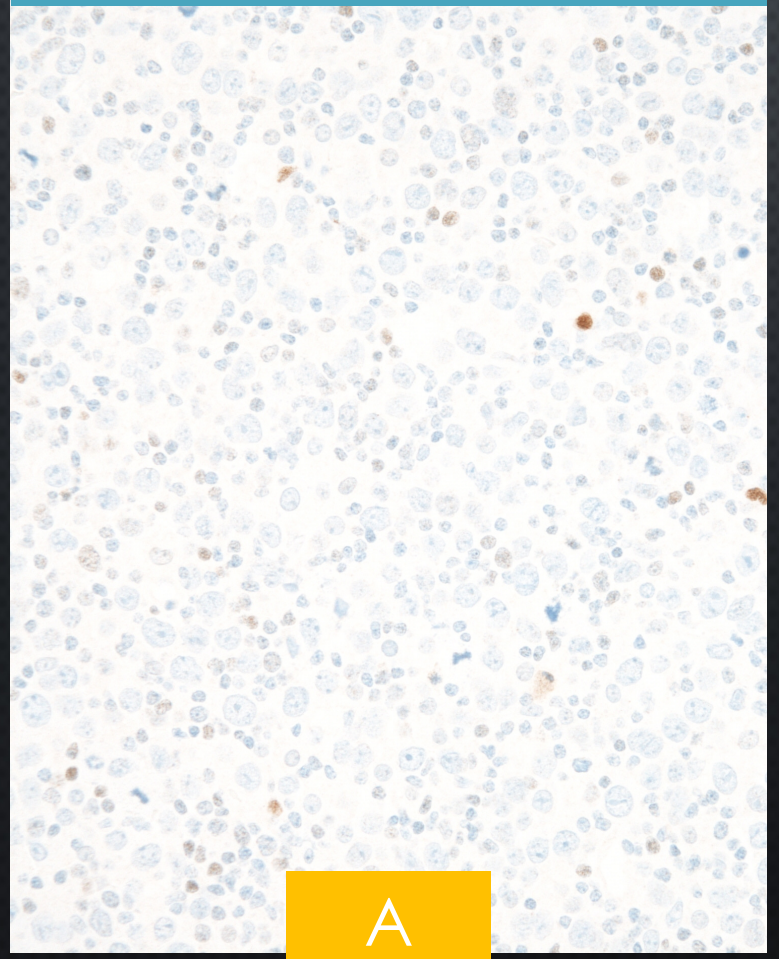
Lowest passrate of the RTU products.

Highest passrate of the RTU products.
LMPS: Prolonging Ab incubation

UltraView protocols did not work.
OptiView protocols only gained optimal in 10%, with a total passrate of 60%

CHOOSE THE OPTIMAL NON-GCB DLBCL = A

LN22 mAb clone LN22 as concentrate, optimally calibrated with HIER in CC1 (32 min. at 100°C) and OptiView as detection system

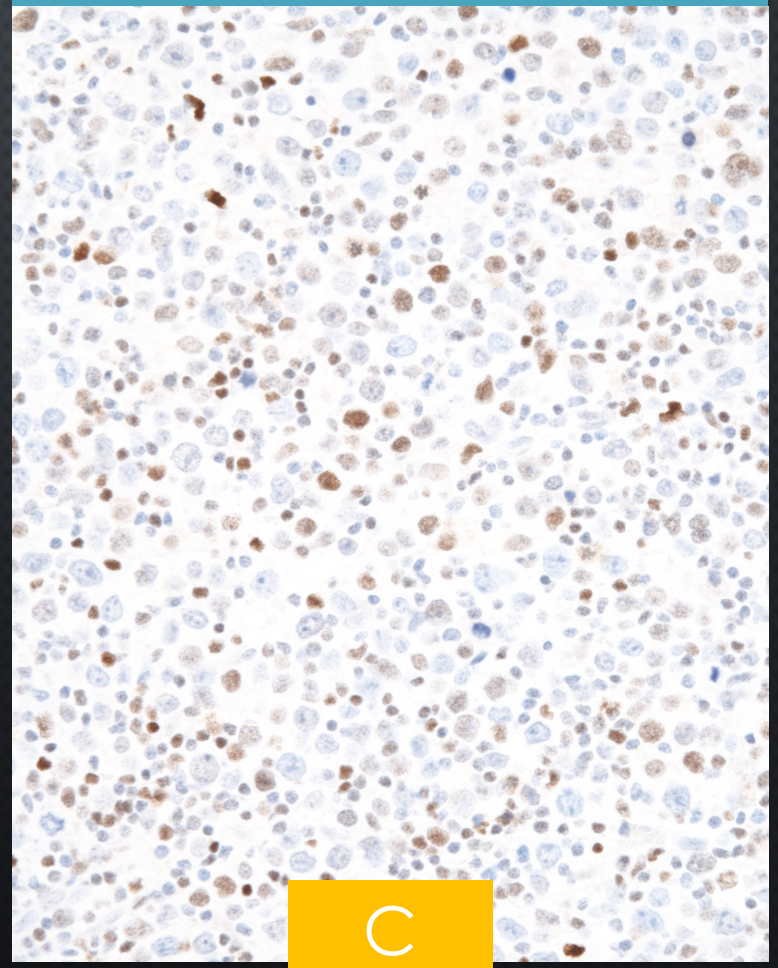


A

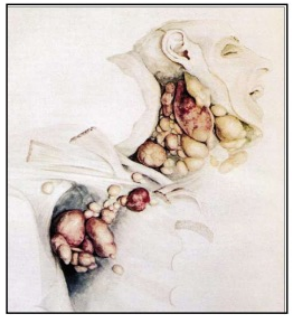
RTU product 760-4241 (Ventana/Roche) based the mAb clone GI191/A8, HIER in CC1 (64 min.) and OptiView as detection system

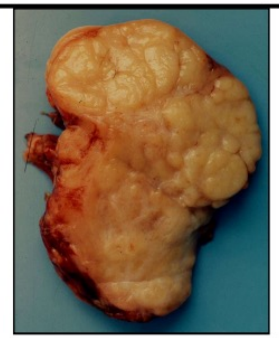
B

Protocol using the RTU product MAB-0746 (Fuzhou Maixin) based the mAb clone MX042.



C





Lymphoma

Hodgkins lymphoma

- HL, LP
- HL, NS
- HL, MC
- HL, LR
- HL, LD

Non-Hodgkins lymphoma

B-cell

- precursor
- peripheral
  - ~32 subtypes

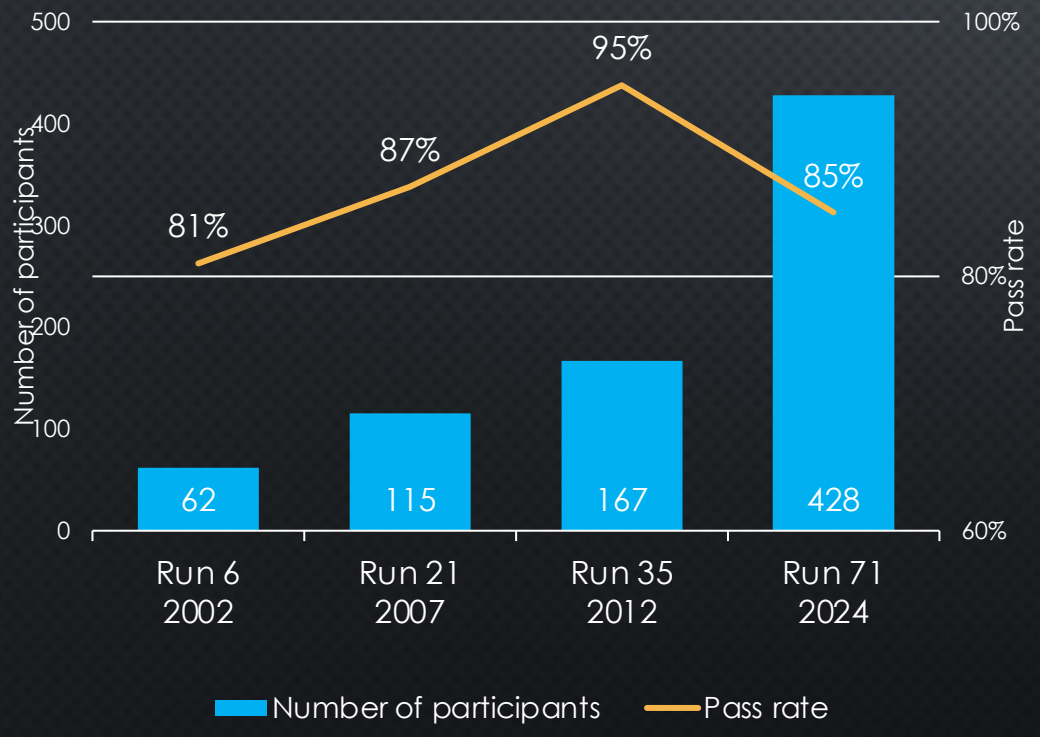
T/NK-cell

- precursor
- peripheral
  - ~20 subtypes

From Stephen Hamilton NordiQC workshop 2023

CD20

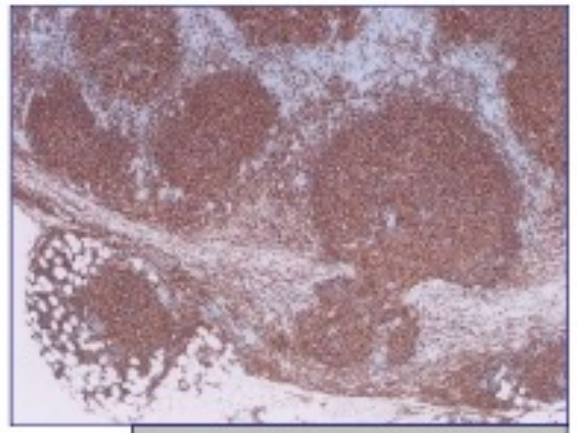
CD20 performance in NordiQC assessments



From Stephen Hamilton NordiQC workshop 2023

Basic stains: CD20

- Many B-cell neoplasms
- Negative in:
  - early precursor B-LB
  - plasma cell neoplasms
- Negative in T-cell lymphomas
  - rare cases positive
- Hodgkins lymphoma
  - HL-LP: 90% positive
  - Other types – variably positive (10% - 30%; not all HRS cells)
- Predictive marker for Rituximab therapy
  - may be aberrantly lost after treatment with Rituximab



Follicular lymphoma: CD20

KEY POINTS FOR CD20 IMMUNOASSAYS

- The mAb clone **L26** was used by 97% of all participants.
- RTUs developed for the Autostainer, BOND and Benchmark platforms gave superior results applying vendor recommended protocol settings
- The performance of the mAb clone L26, both as concentrate and RTU, was less successful on the Omnis platform
- Tonsil and appendix are not reliable tissue controls to monitor the accuracy and precision of CD20 IHC assays.

1. Appendix, 2. Tonsil, 3. Malignant melanoma, 4. Diffuse Large B-Cell Lymphoma (DLBCL), 5-6. B-Chronic Lymphatic leukemia (B-CLL)

Table 1a. **Overall results for CD20, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	113	77	19	16	1	85%	68%
Ready-To-Use antibodies	315	236	30	47	2	84%	75%
Total	428	313	49	63	3		
Proportion		73%	12%	15%	1%	85%	

Table 1b. **Concentrated antibodies and assessment marks for CD20, run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L26**	89 8 5 2 1 1 1	Dako/Agilent Leica Biosystems Cell Marque ZytoMed Systems Biocare Medical Diagnostic Biosystems Epredia	73	17	16	1	84%	68%
mAb clone **IHC532**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **EP459Y**	1	Abcam	0	1	0	0	-	-
rmAb clone **QR094**	1	Quartett	1	0	0	0	-	-
rmAb clone **SP32**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **ZR243**	1	Zeta Corporation	1	0	0	0	-	-
pAb clone **PA5-16701**	1	Invitrogen	0	1	0	0	-	-
Total	113		77	19	16	1	-	
Proportion			68%	17%	14%	1%	85%	

Table 2. Proportion of optimal results for CD20 for the most commonly used antibody concentrate on the 4 main IHC systems

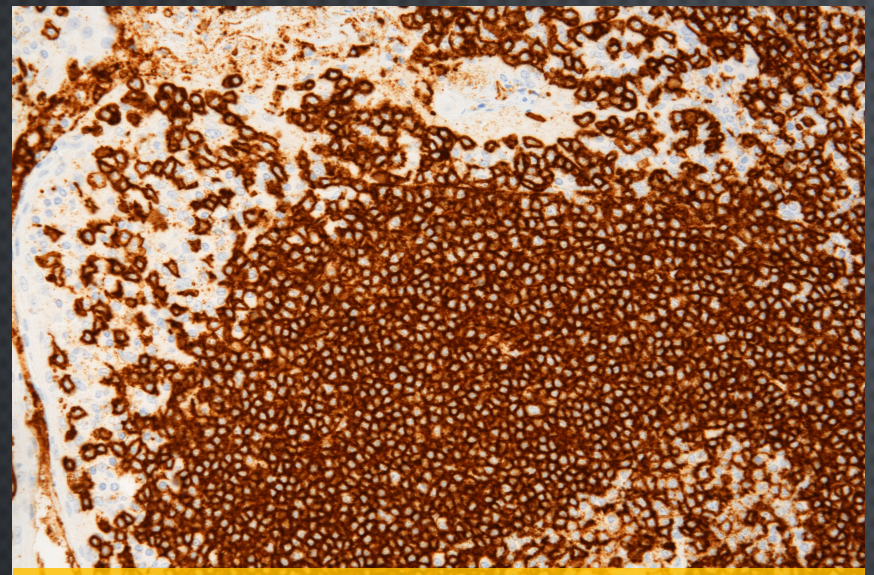
Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1
mAb clone **L26**	3/6** (50%)	-	0/8 (0%)	1/2

Concentrated antibodies	**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **L26**	34/38 (89%)	1/1	10/10 (100%)	11/13 (85%)

Table 1c. **Ready-To-Use antibodies and assessment marks for CD20, run 71**

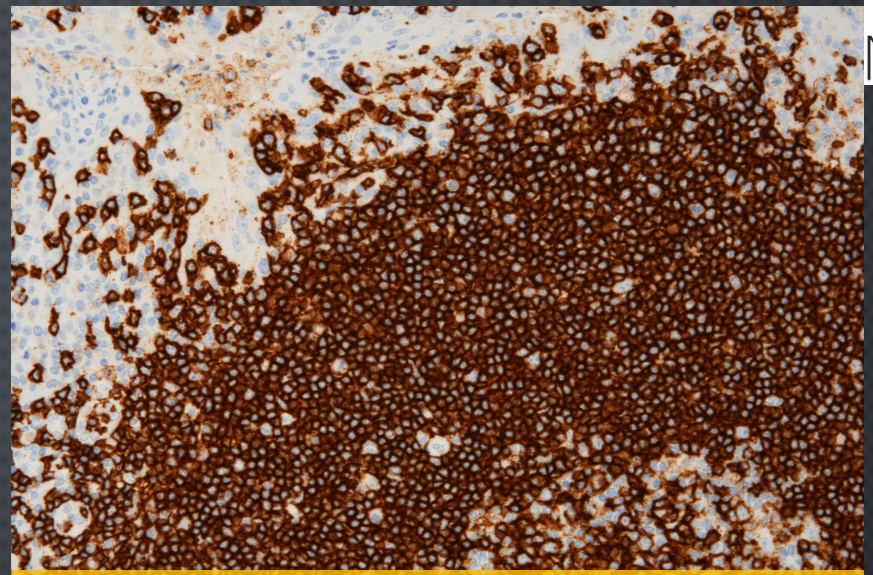
Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L26 PA0200/PA0359**[3]	19	Leica Biosystems	19	0	0	0	100%	100%
mAb clone L**26 PA0200/PA0359**[4]	11	Leica Biosystems	11	0	0	0	100%	100%
mAb clone **L26 760-2531**[3]	58	Ventana/Roche	58	0	0	0	100%	100%
mAb clone **L26 760-2531**[4]	110	Ventana/Roche	103	6	1	0	99%	94%
mAb clone **L26 IR604**[3]	13	Dako/Agilent	12	1	0	0	100%	92%
mAb clone **L26 IR604**[4]	13	Dako/Agilent	7	3	3	0	77%	54%
mAb clone **L26 GA604**[3]	44	Dako/Agilent	8	12	23	1	45%	18%
mAb clone **L26 GA604**[4]	32	Dako/Agilent	4	8	20	0	38%	13%
Total	315		236	30	47	2		
Proportion			75%	10%	15%	1%	85%	

Conclusion:
Not the clone
Not the AB titer
Not the sensitivity of detection system
But maybe…. Problems with the HIER buffer and temp on the Omnis

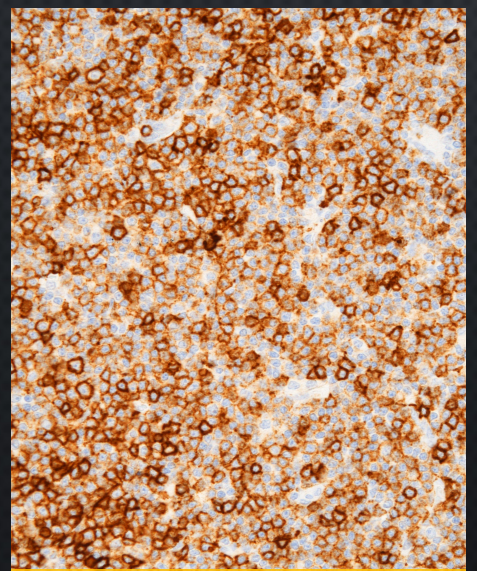


Tonsil - optimal

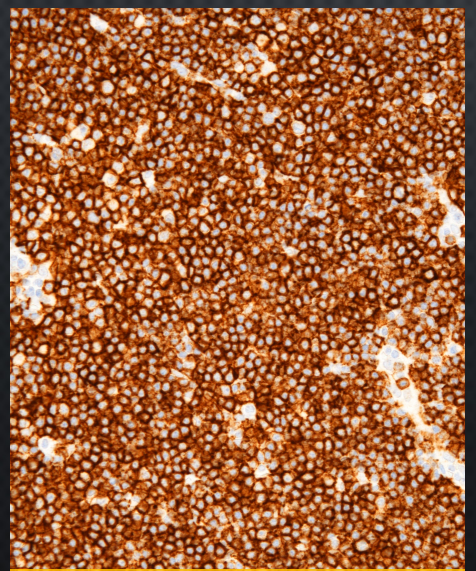
GA604
RTU
protocol



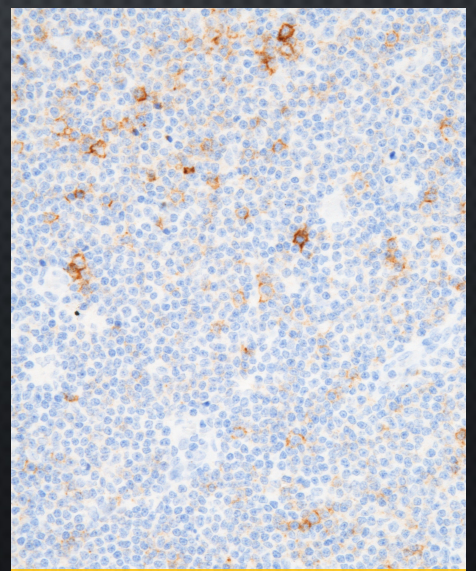
Tonsil - Insufficient



Core 5 - optimal



Core 6 - optimal



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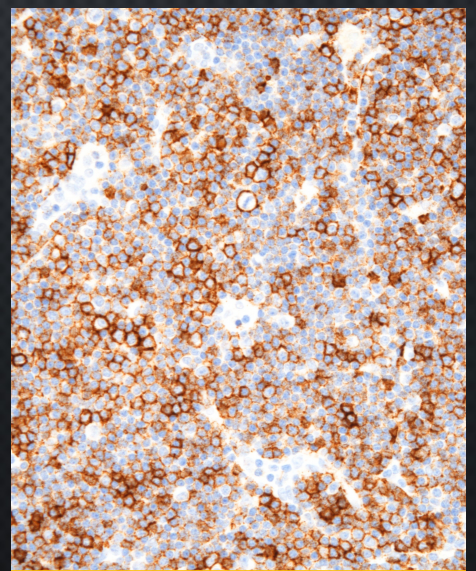


Core 6

New CD20 alternative splice variants: molecular identification and differential expression within hematological B ce malignancies

Clémentine Gamonet[1], Elodie Bole-Richard[1], Aurélia Delherme[1], François Aubin[2], Eri
Francine Garnache-Ottou[1,2], Yann Godet[1,2], Loïc Ysebaert[5], Olivier Tournilhac[6], Carolir
Fabrice Larosa[1,8], Eric Deconinck[1,2,8], Philippe Saas[1,2], Christophe Borg[1,2], Marina Desc
and Christophe Ferrand[1,9*]

Discrepancy of CD20 Protein Expression In IHC and FCM Analyses In Primary B-Cell Lymphoma: Relationship Between FCM-Negative Phenotype and Rituximab Binding with Lymphoma Cells

Takashi Tokunaga, MD,[*,1] Akihiro Tomita, MD, PhD,[*,1] Kazuyuki Shimada, MD, PhD,[*,1]
Junji Hiraga, MD, PhD,[*,2] Takumi Sugimoto, MD, PhD,[*,3] Naoe Goto, MD, PhD,[*,4]
Tsuyoshi Takami, MD, PhD,[*,5] Tomohiro Kinoshita, MD, PhD,[1] Tomoki Naoe, MD, PhD[1]

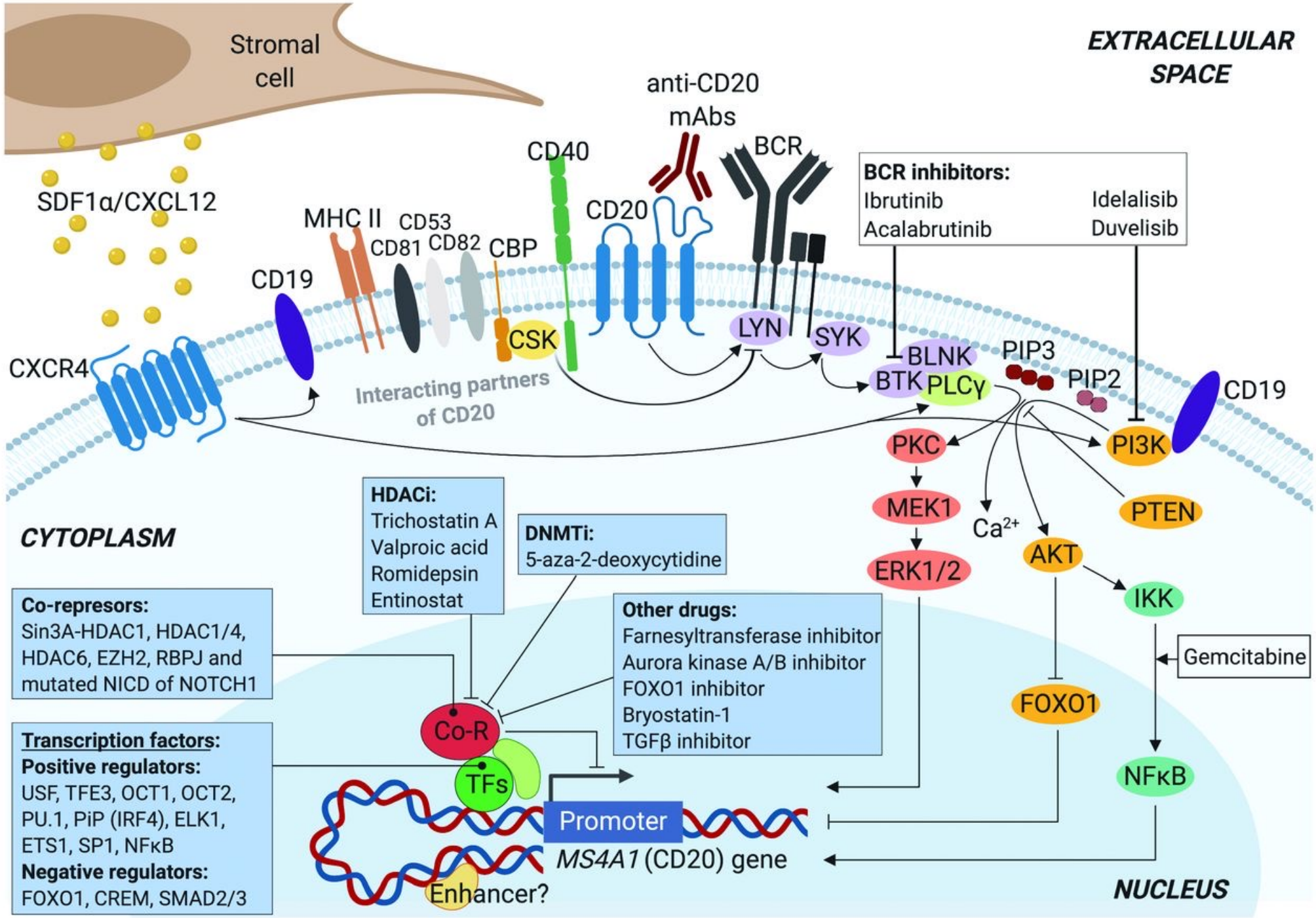
The regulation and function of CD20: an "enigma" of B-cell biology and targeted therapy

Gabriela Pavlasova, Marek Mraz

Vol. 105 No. 6 (2020): June, 2020 https://doi.org/10.3324/haematol.2019.243543

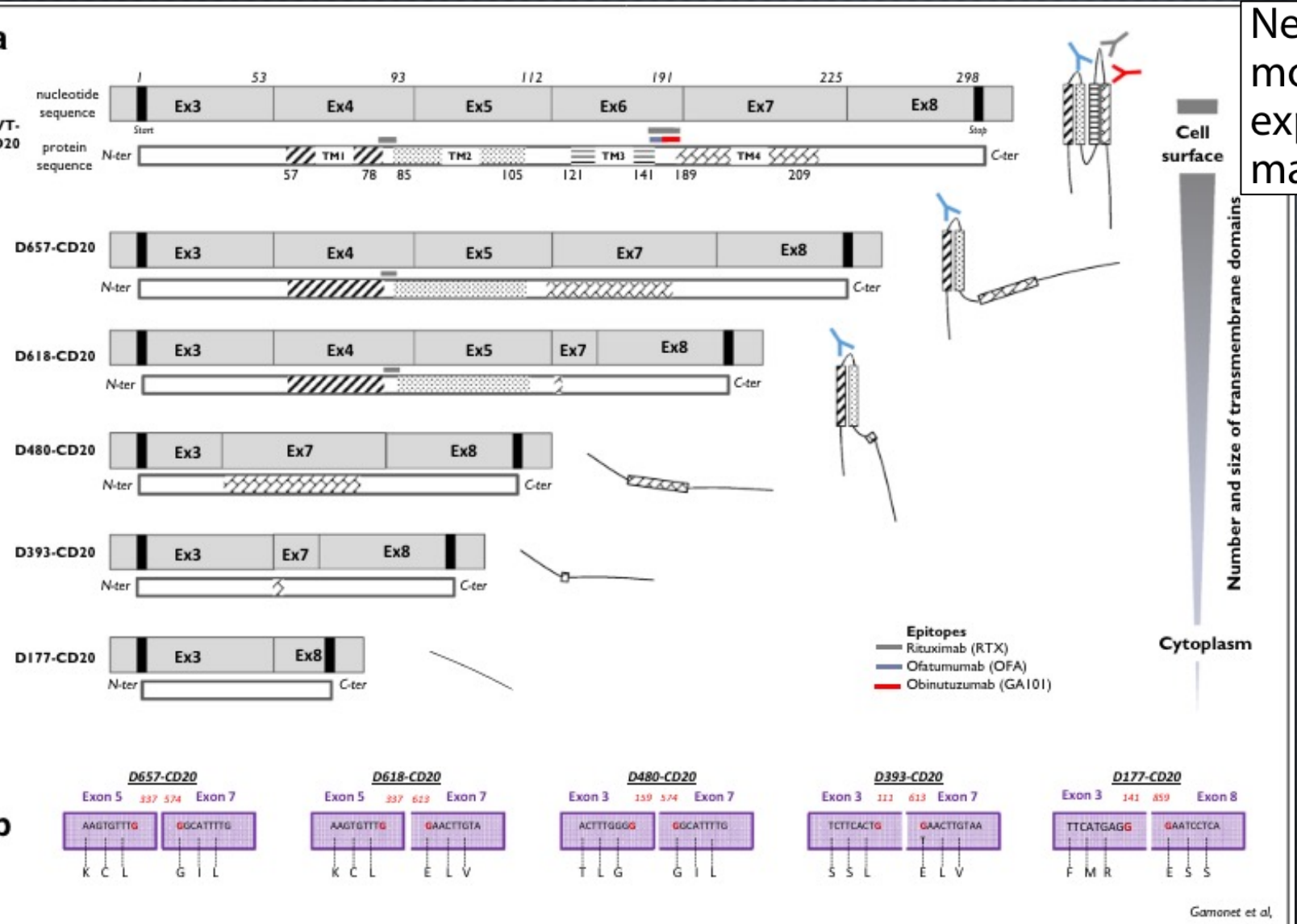
The Effectiveness of Dual-Staining Immunohistochemistry in the Detection of Mantle Cell Lymphoma in the Bone Marrow

Ifeyinwa E. Obiorah, MD, PhD,[1,2] Hao-Wei Wang, MD, PhD,[1,3]
David Ma, HT(ASCP),[2] Eddie Martin, HTL, QIHC(ASCP),[2]
Wyndham H. Wilson, MD, PhD,[4] and Raul Braylan, MD[2]


Stromal cell
EXTRACELLULAR SPACE
anti-CD20 mAbs
CD40
BCR
SDF1α/CXCL12
MHC II
CD53
CD81
CD82
CBP
CD20
CD19
BCR inhibitors:
Ibrutinib
Acalabrutinib
Idelalisib
Duvelisib
CXCR4
CSK
LYN
SYK
BLNK
BTK
PLCγ
PIP3
PIP2
CD19
Interacting partners of CD20
PKC
PI3K
MEK1
Ca2+
PTEN
CYTOPLASM
HDACi:
Trichostatin A
Valproic acid
Romidepsin
Entinostat
DNMTi:
5-aza-2-deoxycytidine
ERK1/2
AKT
IKK
Co-represors:
Sin3A-HDAC1, HDAC1/4, HDAC6, EZH2, RBPJ and mutated NICD of NOTCH1
Other drugs:
Farnesyltransferase inhibitor
Aurora kinase A/B inhibitor
FOXO1 inhibitor
Bryostatin-1
TGFβ inhibitor
Gemcitabine
FOXO1
Co-R
Transcription factors:
Positive regulators:
USF, TFE3, OCT1, OCT2, PU.1, PiP (IRF4), ELK1, ETS1, SP1, NFκB
Negative regulators:
FOXO1, CREM, SMAD2/3
TFs
NFκB
Promoter
MS4A1 (CD20) gene
Enhancer?
NUCLEUS

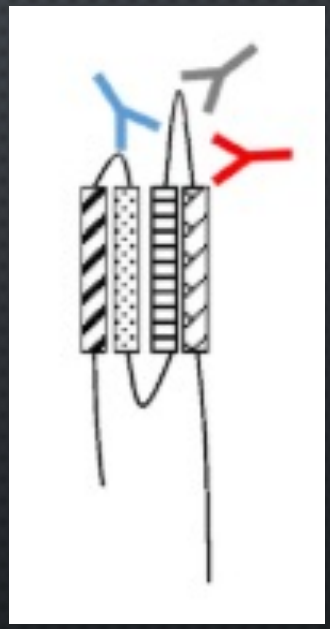
New CD20 alternative splice variants: molecular identification and differential expression within hematological B cell malignancies

Gamonet *et al. Exp Hematol Oncol (2016) 5:7*
DOI 10.1186/s40164-016-0036-3



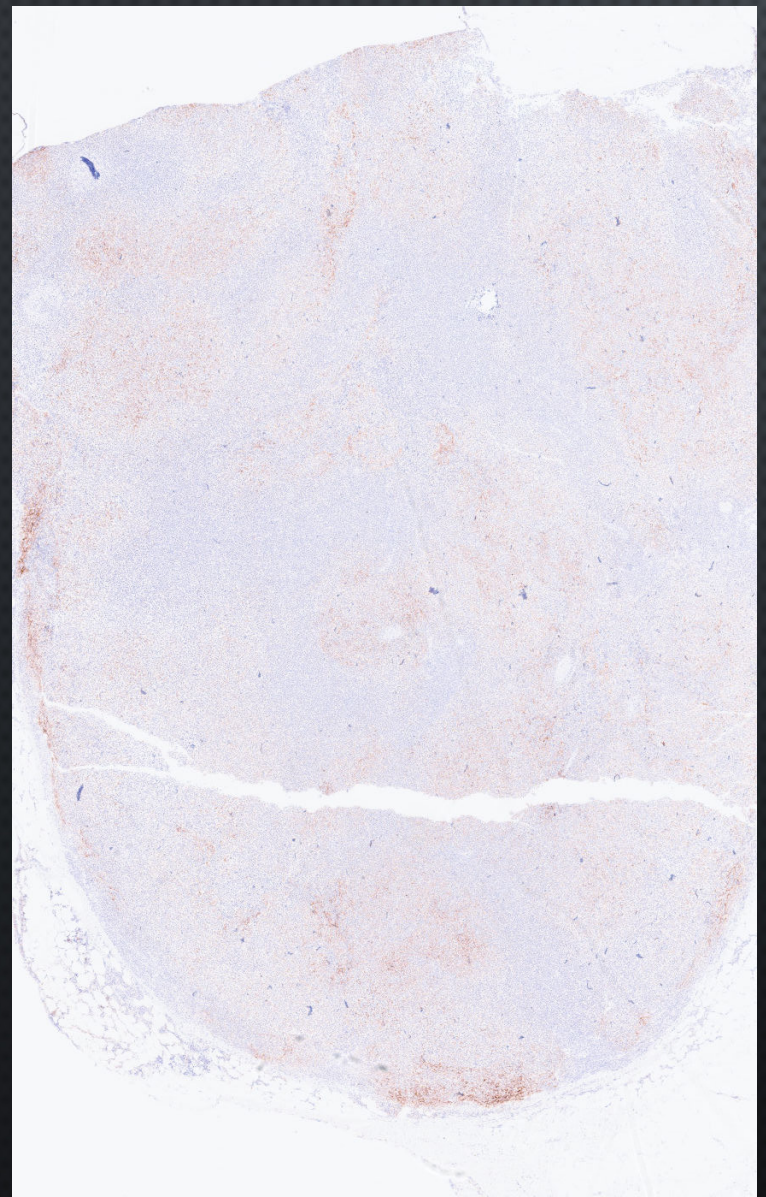
Gamonet et al.

Fig. 3 Alternative CD20-transcripts, putative proteins and junction characterization. **a** Schematic representation of CD20 variant coding transcripts. Transmembrane domains (TM) are positioned on the linear N-ter/C-ter protein, as well as position of the main clinical anti-CD20 antibody epitopes. Rituximab (*gray line*), Ofatumumab (*blue line*) and Obinutuzumab (*red line*). Schematic representation of antibody recognition on the putative CD20 variant-proteins. AA position is provided and numbered from the first ATG (Met) start codon. **b** Schematic reconstitution of amino acidic (AA) junction area after splicing involving canonical (*closed boxes*) or cryptic (*open boxes*) splice. AA sequence flanking junction is provided as well as AA position

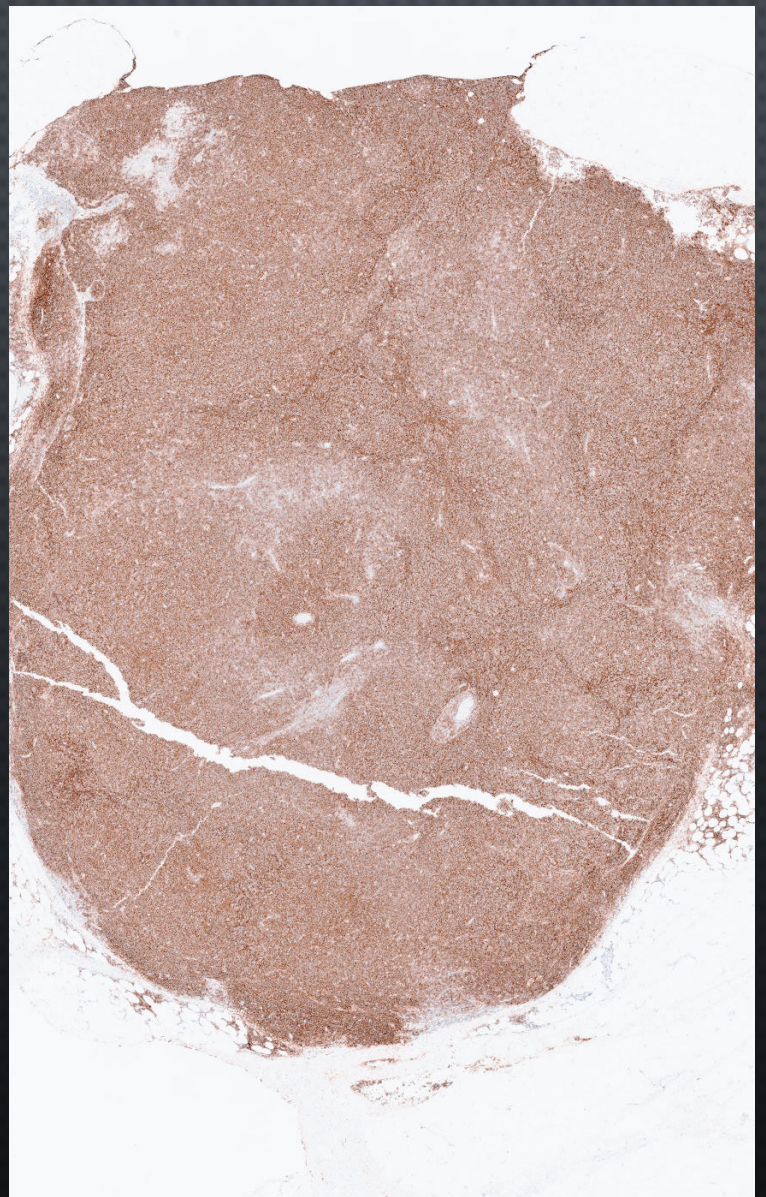


Epitopes
Rituximab (RTX)
Ofatumumab (OFA)
Obinutuzumab (GA101)

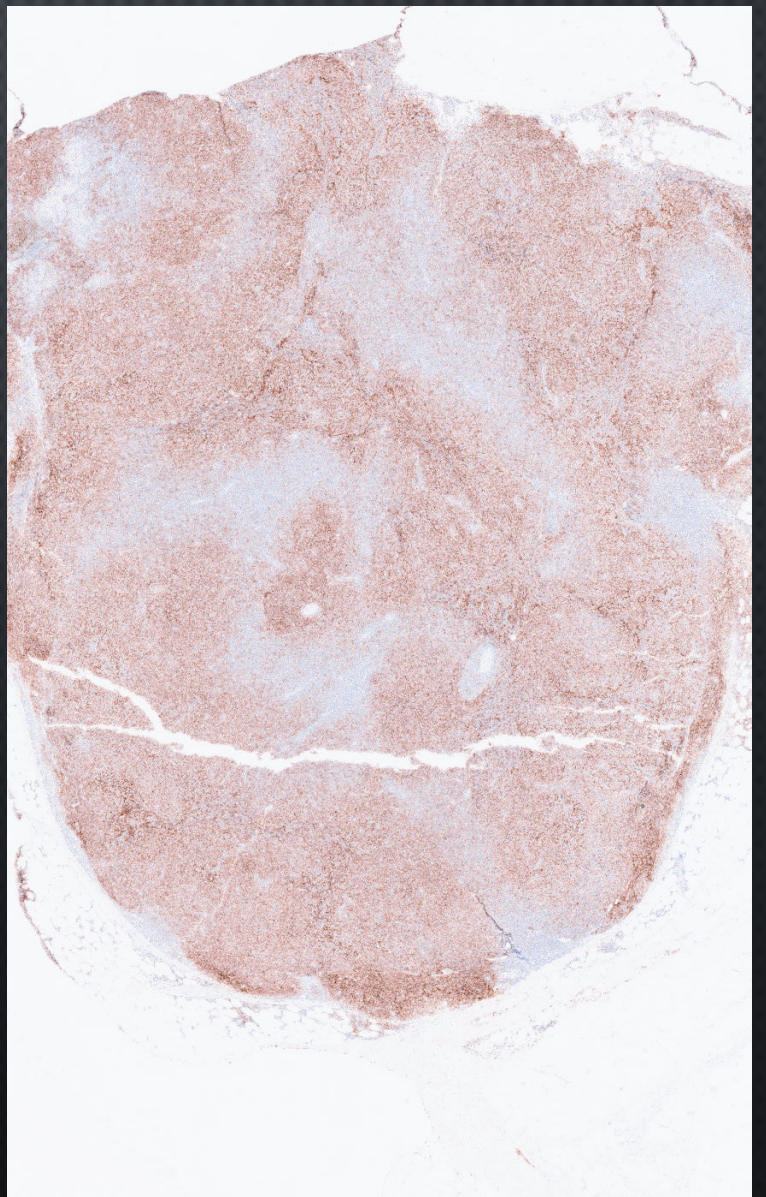
CD20 lot: 41582271
Dako Omnis VPRS



CD20
Ventana Ultra VPRS



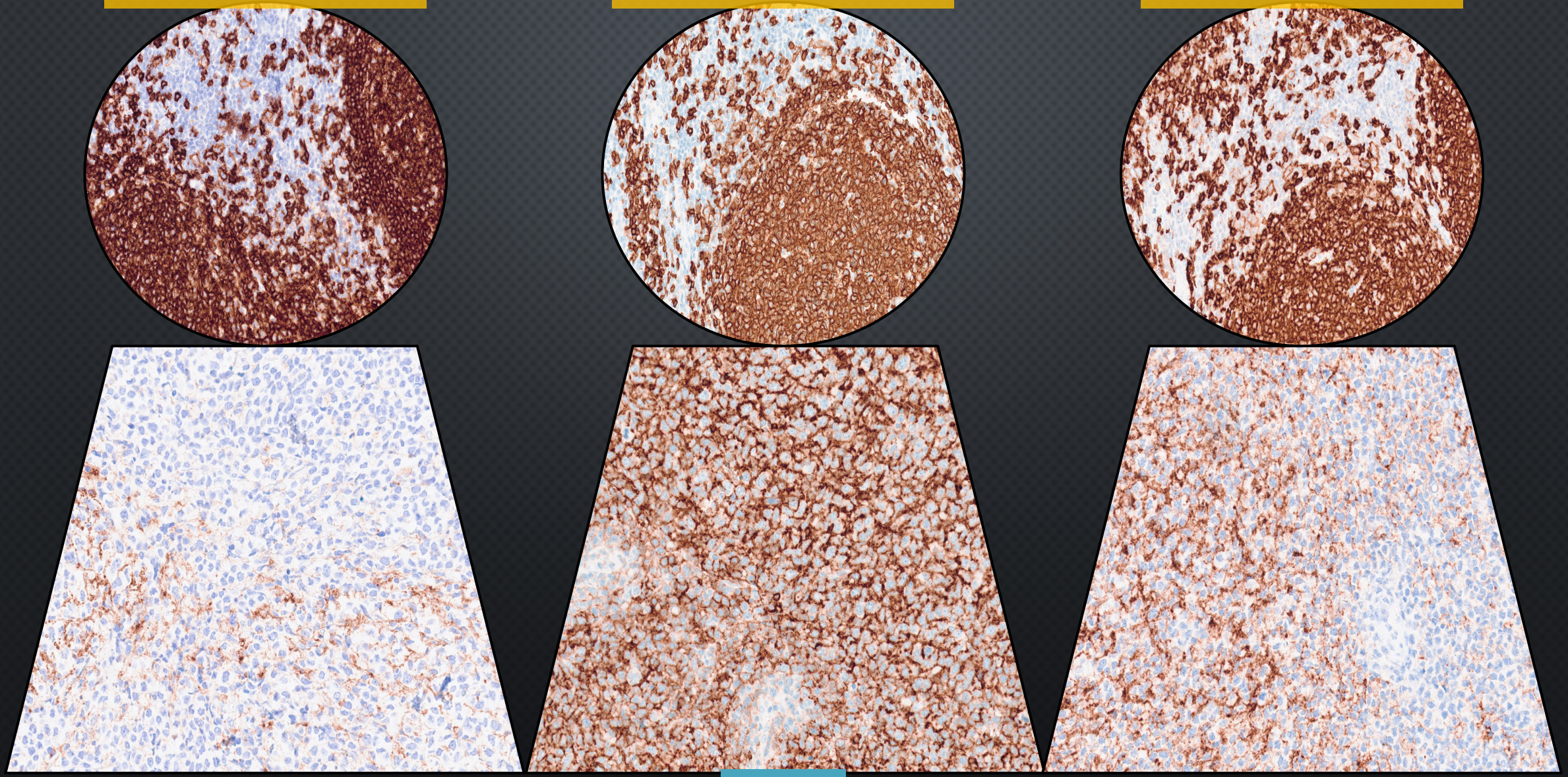
CD20 lot: 41700704
Dako Omnis VPRS



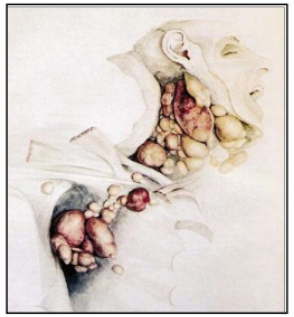
CD20 lot: 41582271
Dako Omnis VPRS

CD20
Ventana Ultra VPRS

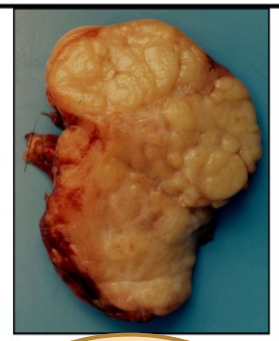
CD20 lot: 41700704
Dako Omnis VPRS



X200



Lymphoma



Hodgkins lymphoma

- HL, LP
- HL, NS
- HL, MC
- HL, LR
- HL, LD

Non-Hodgkins lymphoma

B-cell

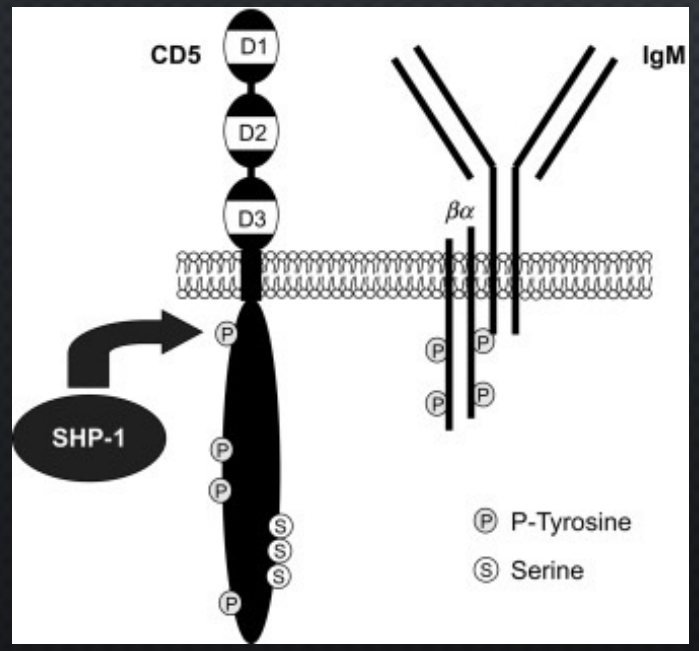
- precursor
- peripheral
  - ~32 subtypes

T/NK-cell

- precursor
- peripheral
  - ~20 subtypes

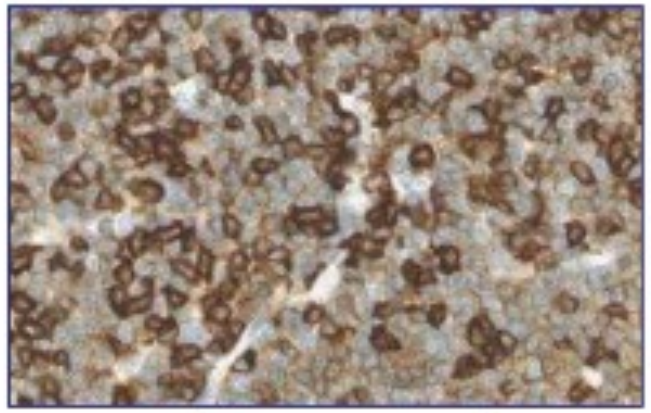
From Stephen Hamilton NordiQC workshop 2023

CD5



Basic stains: CD5

- Modulates T & B cell signalling
- Pan-T cell marker
  - 95% thymocytes
  - 100% post-thymic T-cells
  - ↑ expression with maturity
- Minor population normal B-cells:
  - ca. 10%+ peripheral B-cells
  - ↑ in autoimmunity
- Lymphomas:
  - 90% T-cell neoplasias
  - B-cell NHL
    - B-CLL / SLL (90%)
    - Mantle cell NHL (90%)
    - 10%+ DLBCL

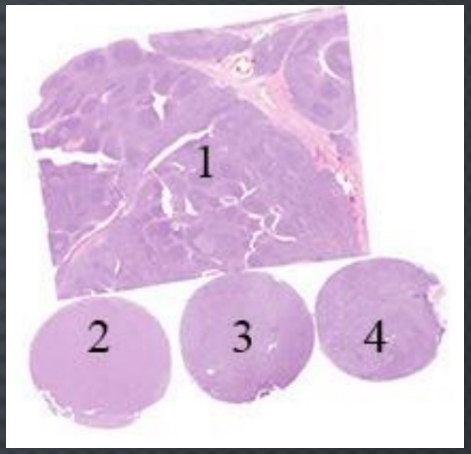


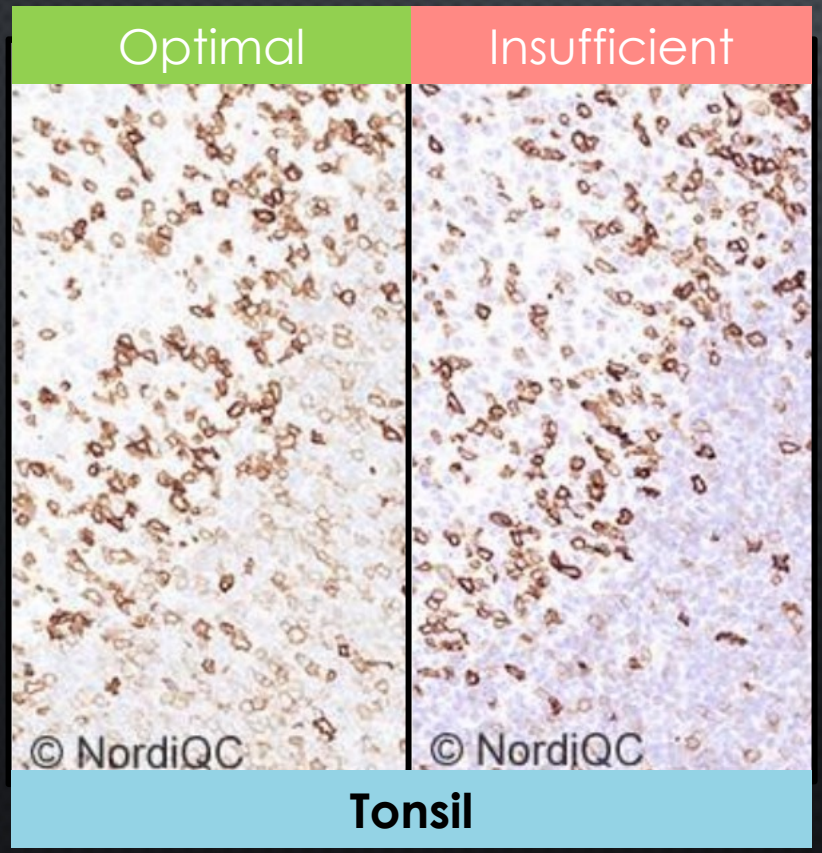
- B-CLL
- B-cells 'dim'
- reactive T-cells 'strong'

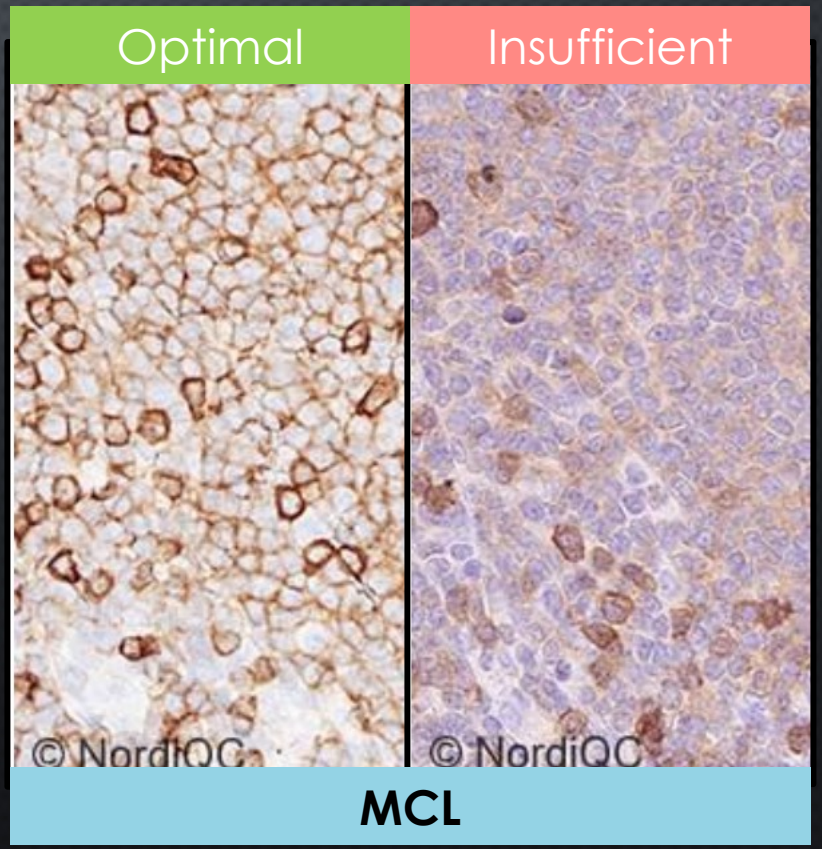
From Stephen Hamilton NordiQC workshop 2023

CD5

1. Tonsil, 2. Diffust storcellet B lymfom (DLBCL), 3. Mantle celle lymfom (MCL), 4. B-celle kronisk lymfatisk leukæmi (B-CLL)







CD5

Run 69	No. of Labs	Passrate	Development
CD5	379	72% , 54% optimale	↓

Table 2. **Proportion of optimal results for CD5 for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark Ultra/GX		Leica Biosystems Bond III , Max, PRIME	
	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 **4C7**	1/3	-	5/14 (36%)	-	9/18 (50%)	-	6/17 (35%)	0/5 (0%)
rmAb clone **SP19**	1/1	-	4/4	-	2/3	0/1	0/1	-

Clone 4C7 IR/IS082 caused 50% of the insufficient results – Do not apply on the Omnis

Table 3. **Proportion of sufficient and optimal results for CD5 for the most commonly used RTU IHC systems**

RTU systems	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb 4C7 **IR/IS082**	36% (4/11)	18% (2/11)	53% (8/15)	40% (6/15)
Leica BOND III mAb 4C7 **PA0168**	100% (13/13)	38% (5/13)	67% (10/15)	7% (1/15)
VMS XT/Ultra/Ultra Plus rmAb SP19 **790-4451**	91% (20/22)	73% (16/22)	97% (144/149)	83% (124/149)

VRPS:
Use UltraView CC1 64 min, Ab 16 min.
Modification:
increase sensitivity

THANK YOU FOR LISTENING