The background of the slide features a microscopic image of tissue stained with hematoxylin and eosin (H&E). The tissue shows a dense population of cells with blue nuclei and pink cytoplasm/extracellular matrix. A prominent feature is a large, irregular area of brown staining, likely representing immunohistochemical (IHC) results for a specific marker, possibly a hematolymphoid antibody as mentioned in the title. This brown staining is concentrated in the center and right side of the image, contrasting with the blue-stained areas on the left and bottom.

NordiQC data: Hematolymphoid antibody selection, protocols and controls

TANYA JULIO

HISTOTECHNOLOGIST

PATHOLOGY DEPARTMENT

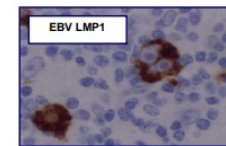
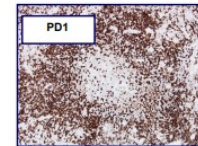
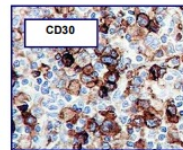
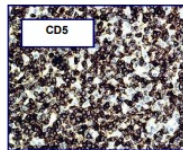
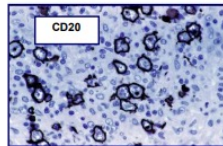
AARHUS UNIVERSITY HOSPITAL, DK

Useful antigens in haematopathology

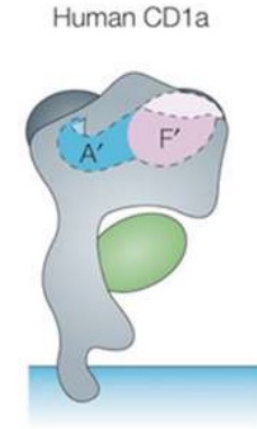
- **CD45**
- **B-cell 'specific'**
 - CD19
 - CD20
 - CD79 α
 - Pax-5
 - OCT-2 / BOB1
 - Ig
- **T-cell 'specific'**
 - CD3
 - CD5
 - CD2
 - CD7
 - CD1a
 - CD4
 - CD8
 - PD-1/CXCL-13 (TFH)

- **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

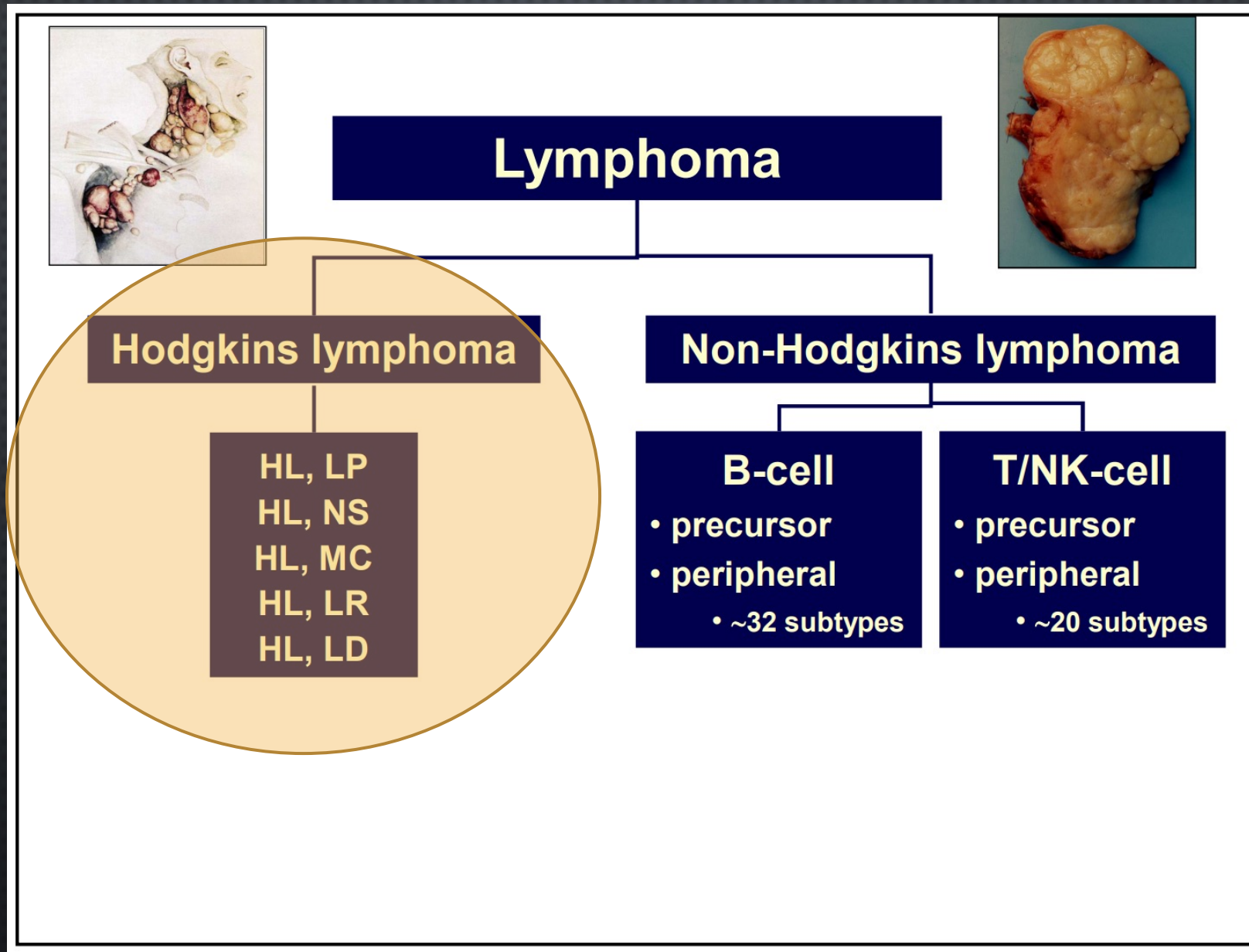


What are CD numbers?



- **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**





CD30

CD30 performance in NordiQC Assessments



Table 1. Antibodies and assessment marks for CD30, Run 65

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone Ber-H2	82 10 2 2 1 1	Dako/Agilent Cell Marque Diagnostic Biosystems Epredia Zytomed Systems Santa Cruz	59	26	9	4	87%	60%
mAb clone JCM182	6	Leica Biosystems	1	3	1	1	67%	17%
mAb clone IHC030	1	GenomeMe	1	0	0	0	-	-
mAb clone CON6D/B5	2	Biocare Medical	2	0	0	0	-	-
mAb clone HRS4	1	Thermo Scientific	1	0	0	0	-	-
rmAb clone ZR248	1	Zeta Corporation	0	0	0	1	-	-
Conc Total	109		64	29	10	6	85%	59%
Ready-To-Use antibodies								
mAb clone Ber-H2 IR602 ³	3	Dako/Agilent	1	1	1	0	-	-
mAb clone Ber-H2 IR602 ⁴	44	Dako/Agilent	23	18	3	0	93%	52%
mAb clone Ber-H2 GA602 ³	25	Dako/Agilent	13	9	3	0	88%	52%
mAb clone Ber-H2 GA602 ⁴	22	Dako/Agilent	10	7	4	1	77%	45%
mAb clone Ber-H2 790-4858 ³	14	Ventana/Roche	0	2	9	3	14%	0%
mAb clone Ber-H2 790-4858 ⁴	113	Ventana/Roche	41	26	38	8	59%	36%
mAb clone JCM182 PA0790 ³	13	Leica Biosystems	4	9	0	0	100%	31%
mAb clone JCM182 PA0790 ⁴	12	Leica Biosystems	5	7	0	0	100%	42%
RTU Total	256		97	85	59	15	71%	38%
Total	365		161	114	69	21	-	
Proportion			44%	31%	19%	6%	75%	

Use Link or Amp.

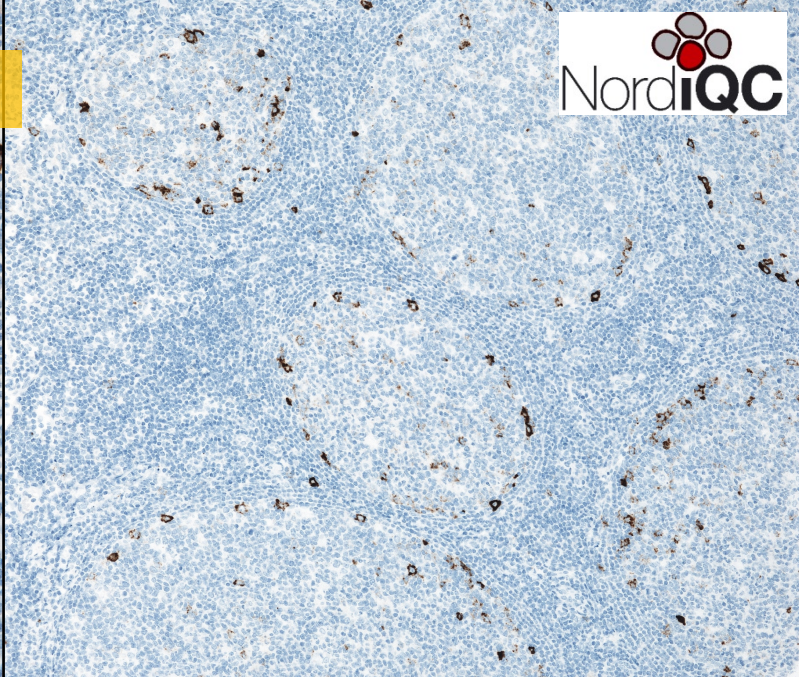
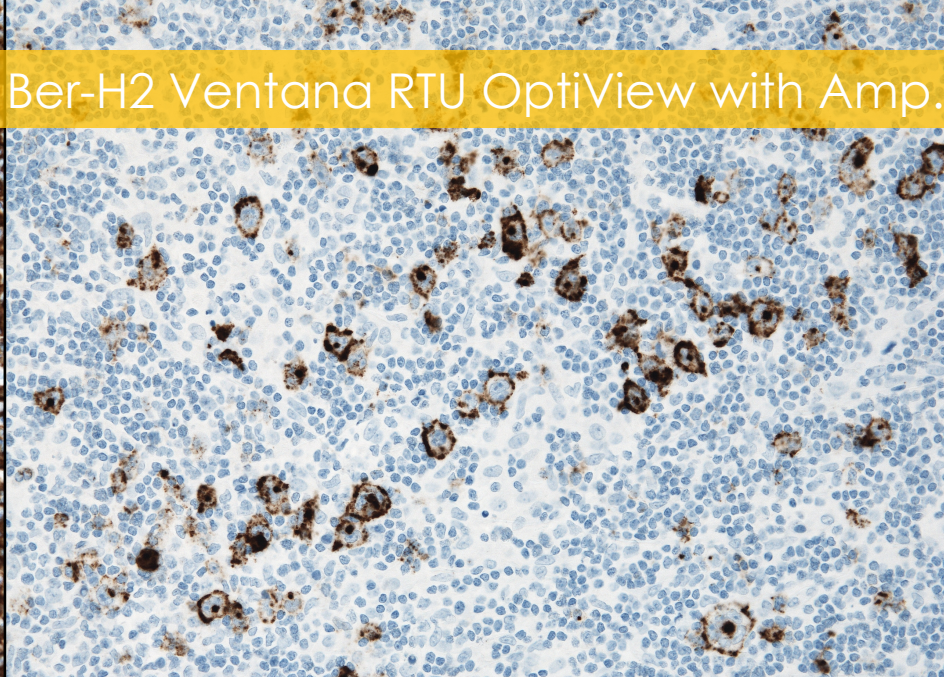
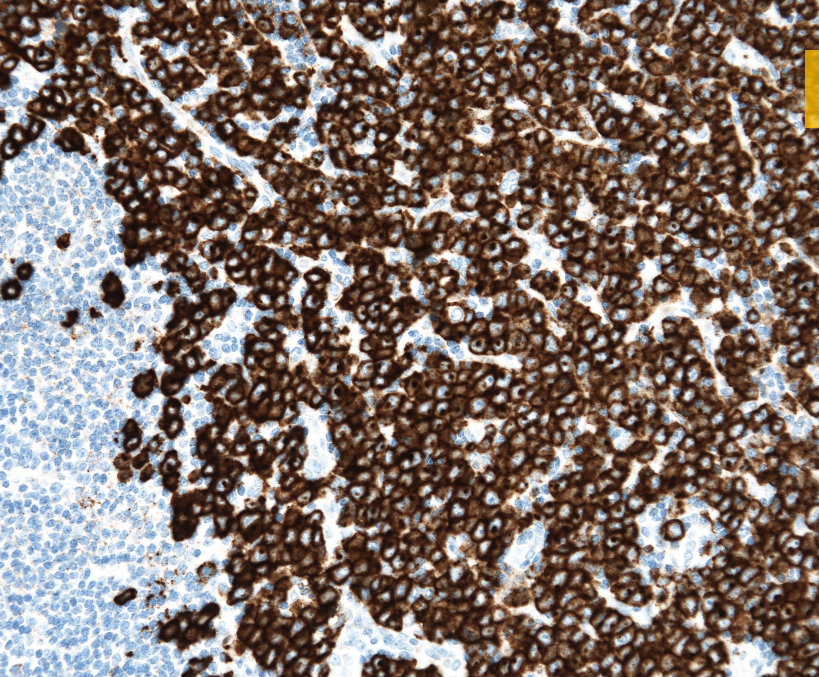
Flex+ = 100%
suff. 92% OR

+amp = 96% suff.
65% OR

Last run 70% OR,
now background



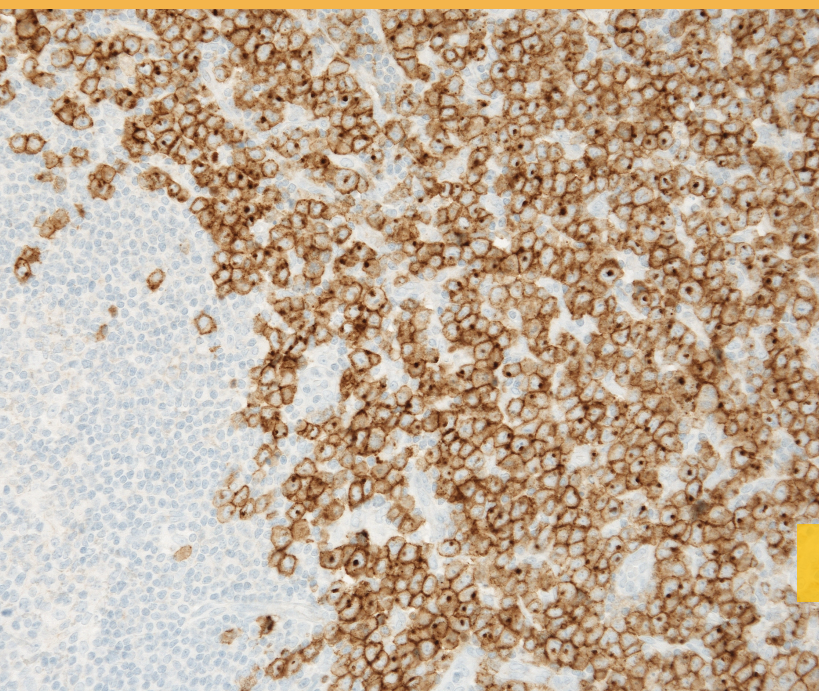
Ber-H2 Ventana RTU OptiView with Amp.



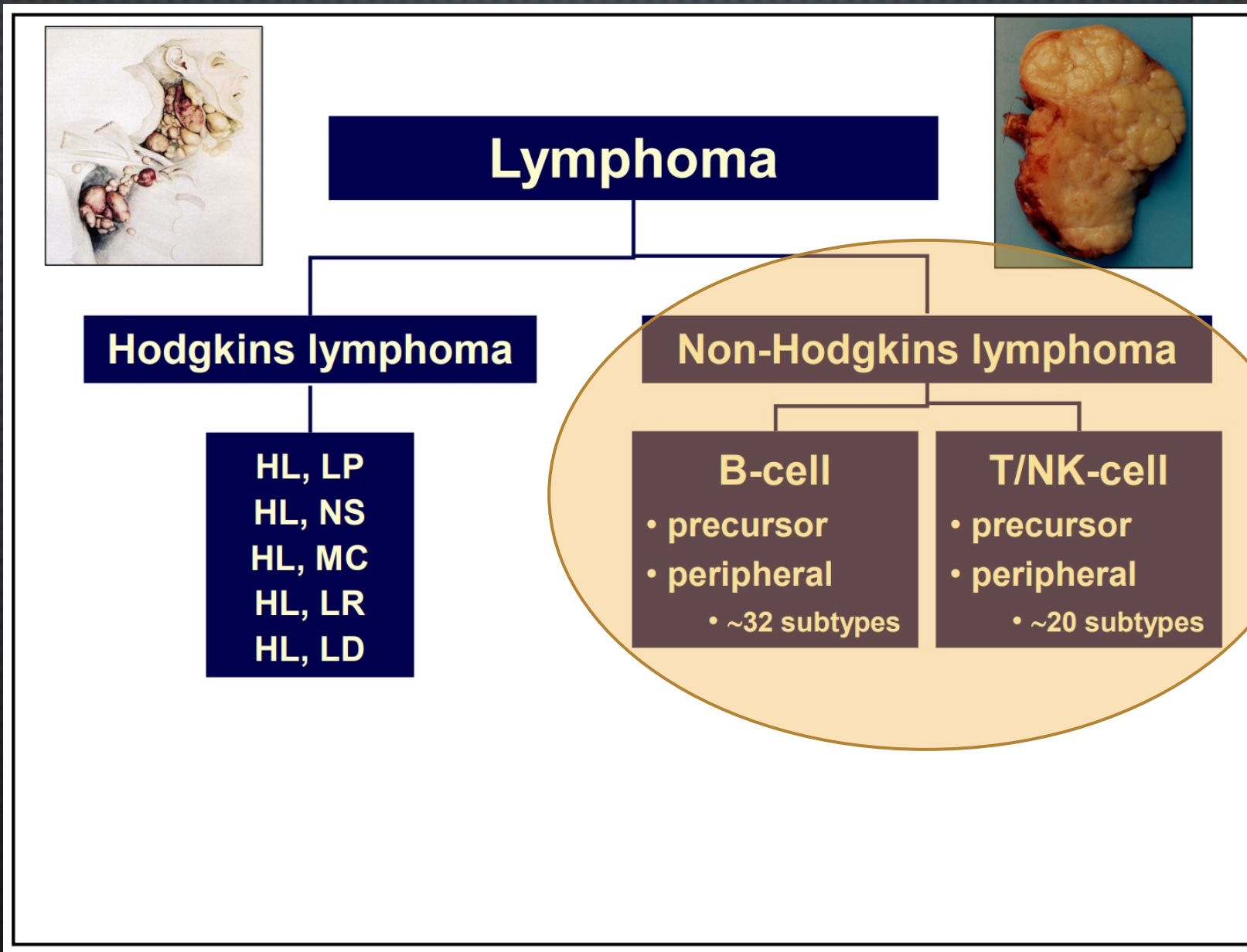
Anaplastic large cell lymphoma

Hodgkin' lymphoma

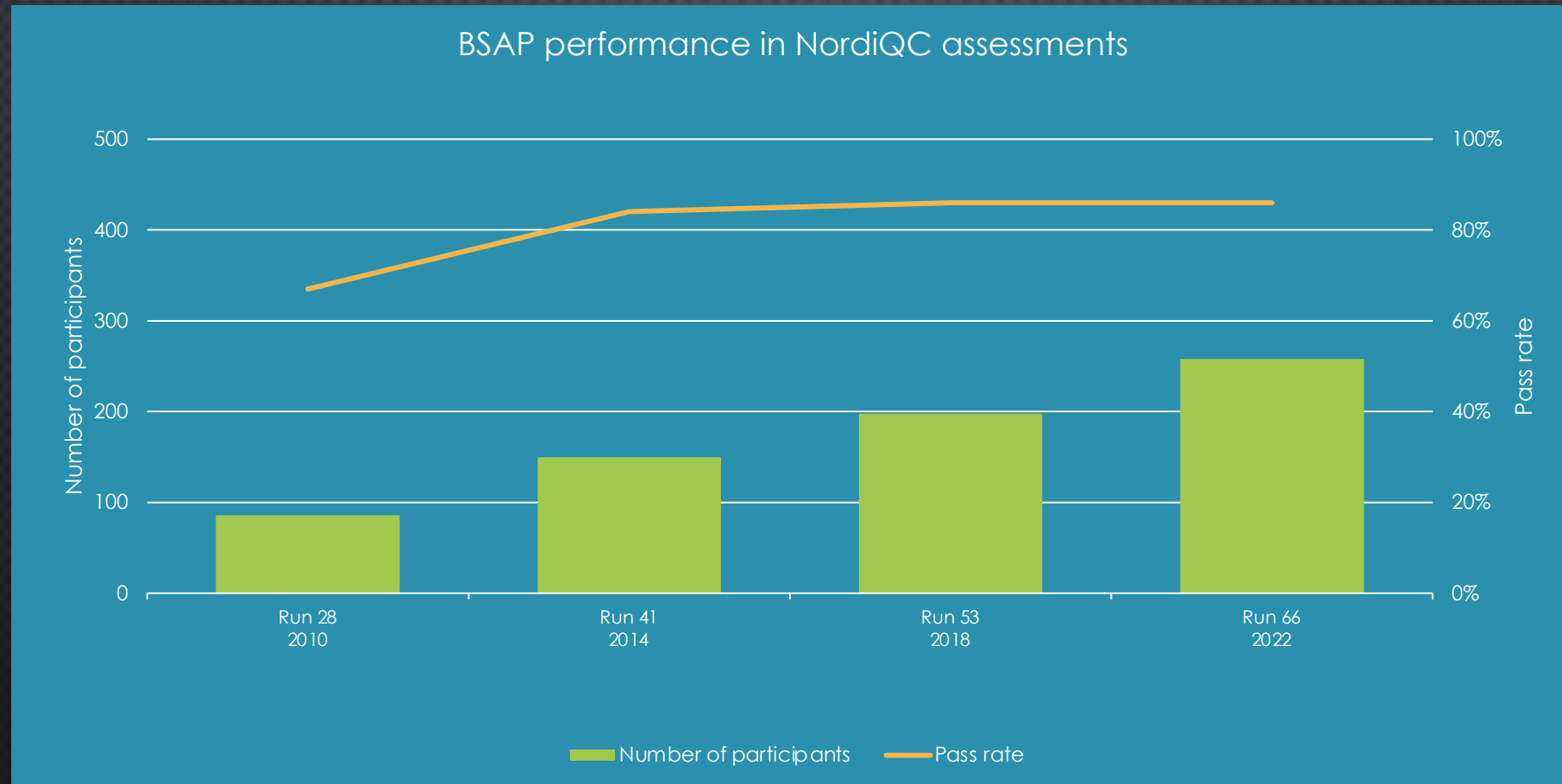
Tonsil



Ber-H2 Ventana RTU OptiView without Amp.



BSAP (PAX5) – RUN 66



PAX5 – A B-CELL MARKER AND TROUBLEMAKER

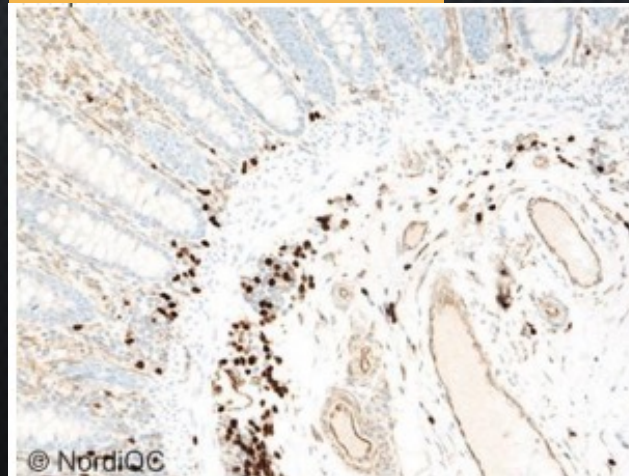
Table 1. Antibodies and assessment marks for BSAP, run 66

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 1EW	9	Leica Biosystems	2	5	1	1	78%	22%
mAb clone 24	5	BD Biosciences	1	4	2	1	63%	13%
	2	Biocare Medical						
mAb clone DAK-Pax5	31	Dako/Agilent	16	12	3	0	90%	52%
mAb clone BPM6172	1	Biolyntech	1	0	0	0	-	-
rmAb clone IHC115	1	GenomeMe	0	1	0	0	-	-
rmAb clone RBT-PAX5	1	Bio SB	1	0	0	0	-	-
rmAb clone D7H5X	1	Cell Signaling Tech.	0	1	0	0	-	-
rmAb clone SP34	9	Cell Marque	4	3	4	1	58%	33%
	1	Thermo Fisher						
	1	Zytomed Systems						
rmAb clone QR056	1	Quartett	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone 1EW PA0552³	8	Leica Biosystems	2	5	1	0	88%	25%
mAb clone 1EW PA0552⁴	5	Leica Biosystems	0	3	1	1	60%	0%
mAb clone BC/24 PM207	1	Biocare Medical	0	1	0	0	-	-
mAb clone 24/Pax-5 AM967	1	BioGenex	0	1	0	0	-	-
mAb clone MX017 MAB-0706	1	Malix	1	0	0	0	-	-
mAb clone DAK-Pax5 IR650³	10	Dako/Agilent		10	0	0	0	100%
mAb clone DAK-Pax5 IR650⁴	10	Dako/Agilent		7	1	2	0	80%
mAb clone DAK-Pax5 GA650³	40	Dako/Agilent		33	6	1	0	98%
mAb clone DAK-Pax5 GA650⁴	21	Dako/Agilent		17	2	2	0	90%
rmAb clone EP156 8500-C010	1	Sakura Finetek		0	0	1	0	-
rmAb clone SP34 790-4420³	17	Ventana/Roche		5	9	3	0	82%
rmAb clone SP34 790-4420⁴	69	Ventana/Roche		27	34	8	0	88%
rmAb clone GR001 GT2096	1	Gene Tech		1	0	0	0	-
rmAb clone C12A5 CPM-0244	1	Celovte		1	0	0	0	-
pAb BRB027	1	Zytomed Systems		0	0	0	1	-
Total	258			133	88	31	6	-
Proportion				52%	34%	12%	2%	86%

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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb DAK-Pax5 IR650	100% (10/10)	100% (10/10)	100% (7/7)	86% (6/7)
Dako Omnis mAb DAK-Pax5 GA650	98% (39/40)	83% (33/40)	89% (16/18)	78% (14/18)
Leica BOND III mAb 1EW PA0552	88% (7/8)	25% (2/8)	75% (3/4)	0% (0/4)
VMS Ultra/XT/GX mAb SP34 790-4420	82% (14/17)	29% (5/17)	88% (60/68)	40% (27/68)

Ventana, SP34



Dako, Dak-pax5

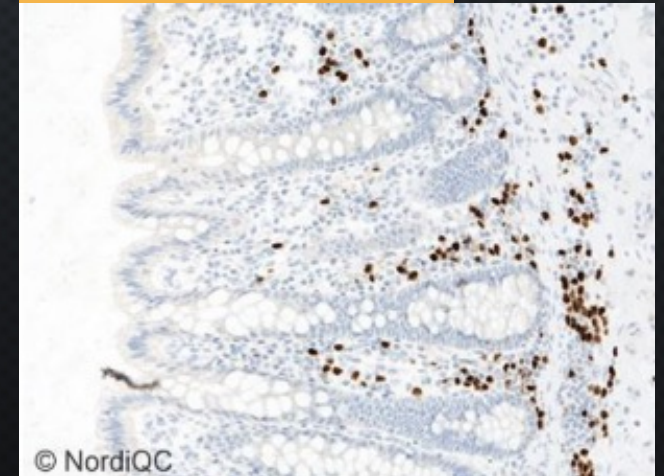


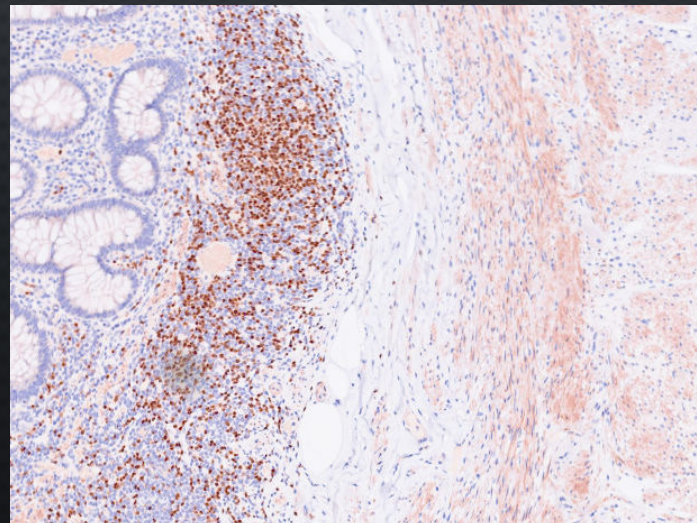
Table 1. Antibodies and assessment marks for BSAP, run 66

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 1EW	9	Leica Biosystems	2	5	1	1	78%	22%
mAb clone 24	5	BD Biosciences	1	4	2	1	63%	13%
mAb clone DAK-Pax5	31	Dako/Agilent	16	12	3	0	90%	52%
mAb clone BPM6172	1	Biolyx tech.	1	0	0	0	-	-
rmAb clone IHC115	1	GenomeMe	0	1	0	0	-	-
rmAb clone RBT-PAX5	1	Bio SB	1	0	0	0	-	-
rmAb clone D7H5X	1	Cell Signaling Tech.	0	1	0	0	-	-
rmAb clone SP34	9	Cell Marque	4	3	4	1	58%	33%
	1	Thermo Fisher						
	1	Zytomed Systems						
	1	DCS						
rmAb clone QR056	1	Quartett	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone 1EW PA0552³	8	Leica Biosystems	2	5	1	0	88%	25%
mAb clone 1EW PA0552⁴	5	Leica Biosystems	0	3	1	1	60%	0%
mAb clone BC/24 PM207	1	Biocare Medical	0	1	0	0	-	-
mAb clone 24/Pax-5 AM967	1	BioGenex	0	1	0	0	-	-
mAb clone MX017 MAB-0706	1	Malxin	1	0	0	0	-	-
mAb clone MX017 MAD-000694QD	2	Master Diagnostica	2	0	0	0	-	-
mAb clone DAK-Pax5 IR650³	10	Dako/Agilent	10	0	0	0	100%	100%
mAb clone DAK-Pax5 IR650⁴	10	Dako/Agilent	7	1	2	0	80%	70%
mAb clone DAK-Pax5 GA650³	40	Dako/Agilent	33	6	1	0	98%	83%
mAb clone DAK-Pax5 GA650⁴	21	Dako/Agilent	17	2	2	0	90%	81%
rmAb clone EP156 8500-C010	1	Sakura Finetek	0	0	1	0	-	-
rmAb clone SP34 790-4420³	17	Ventana/Roche	5	9	3	0	82%	29%
rmAb clone SP34 790-4420⁴	69	Ventana/Roche	27	34	8	0	88%	39%
rmAb clone SP34 312R-18	3	Cell Marque	1	0	2	0	-	-
rmAb clone 517B5E6 PA107	1	Abcarta	0	0	0	1	-	-
rmAb clone GR001 GT2096	1	Gene Tech	1	0	0	0	-	-
rmAb clone C12A5 CPM-0244	1	Celnovte	1	0	0	0	-	-
pAb BRB027	1	Zytomed Systems	0	0	0	1	-	-
Total	258		133	88	31	6	-	
Proportion			52%	34%	12%	2%	86%	

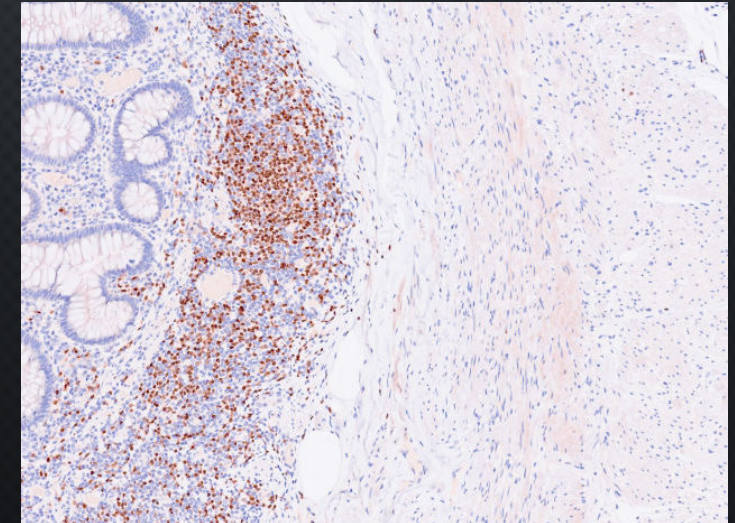
PAX5 – A B-CELL MARKER AND TROUBLEMAKER

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

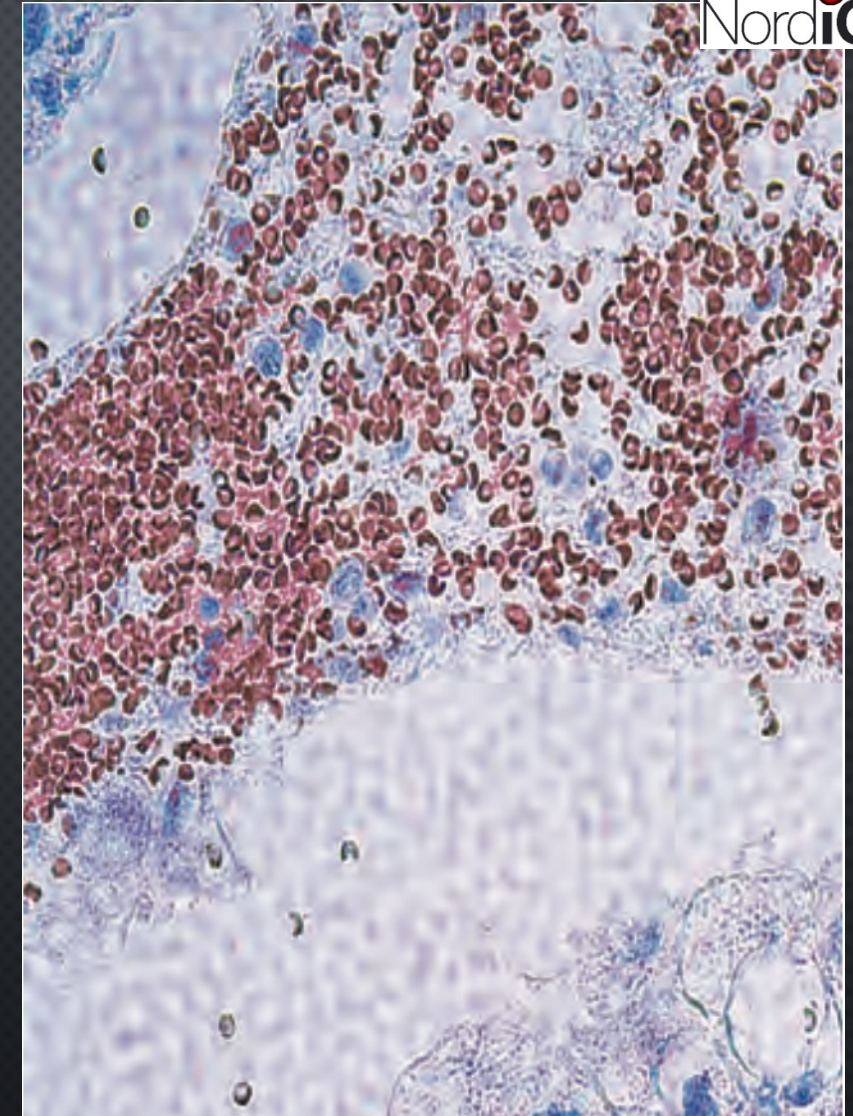
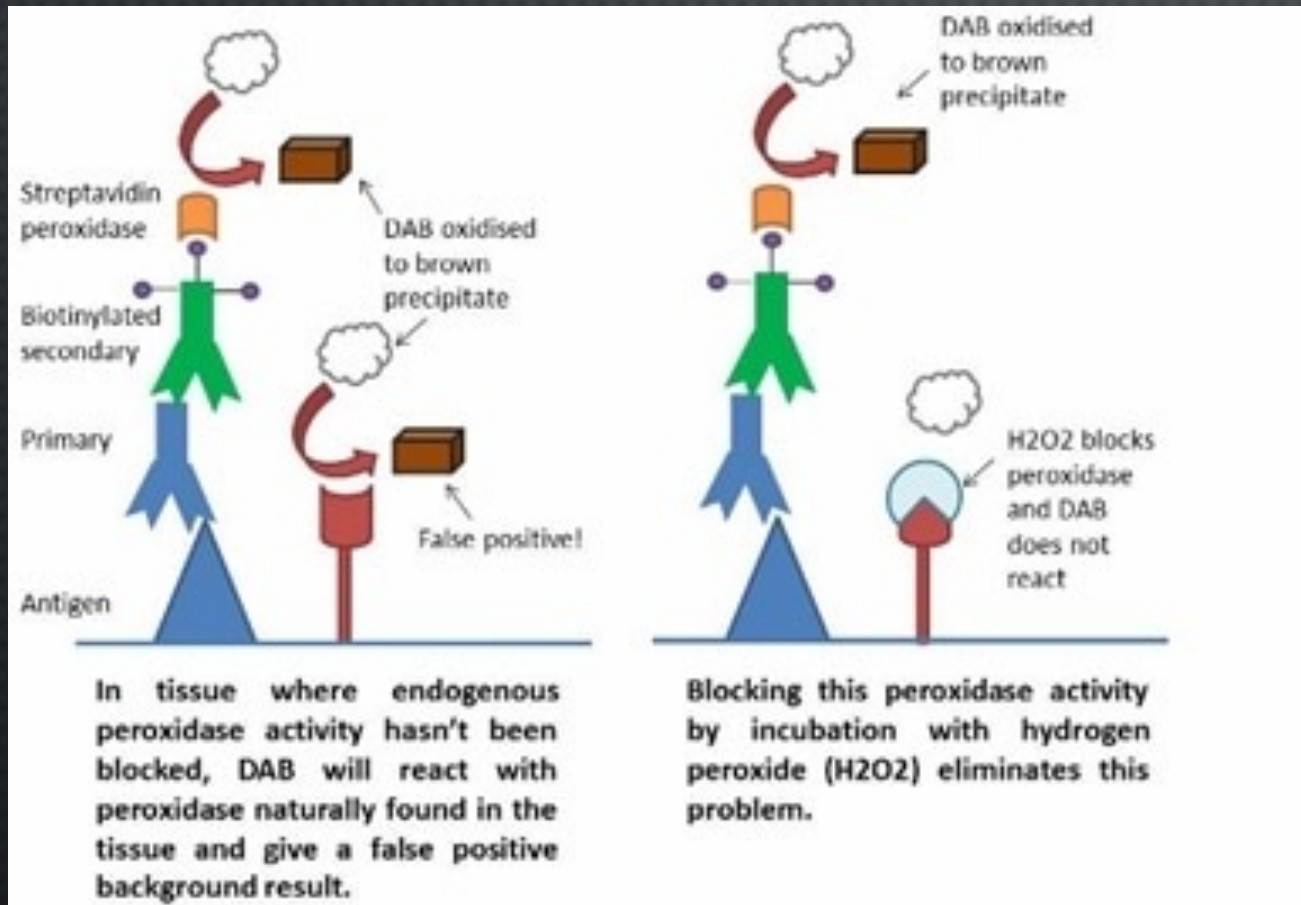
Protocol F	Protocol Q
Primary Ab	Primary Ab
Linker (post primary)	Peroxidase block
Peroxidase block	Linker (post primary)
Polymer	Polymer
DAB	DAB
Hematoxylin	Hematoxylin



Protocol F



Protocol Q



ENDOGENOUS PEROXIDASE

Protocol Summary

Procedure: U OptiView DAB IHC v6 (v1.00.0136)

BenchMark ULTRA IHC/ISH

Patologi Aarhus Univesitetshospital, Palle Juul-Jensens Boulevard 99 8200 Aarhus N

Validated: No		Active: Yes	
Protocol No	Protocol Name	Version	Creation Date
375	CD4	3	20-12-2022 12:03:22

- 1 Paraffin [Selected]
- 2 Deparaffinization [Selected]
- 3 Warmup Slide to [72 Deg C] from Medium Temperatures (Deparaffinization)
- 4 Cell Conditioning [Selected]
- 5 Ultra CC1 [Selected]
- 6 Warmup Slide to [100 Deg C], and Incubate for 4 Minutes (Cell Conditioner #1)
- 7 CC1 8 Min [Selected]
- 8 CC1 16 Min [Selected]
- 9 CC1 24 Min [Selected]
- 10 CC1 32 Min [Selected]
- 11 CC1 40 Min [Selected]
- 12 CC1 48 Min [Selected]
- 13 Pre Primary Peroxidase Inhibit. [Selected]
- 14 Primary Antibody [Selected]
- 15 Apply Coverslip, One Drop of [anti-CD4 (SP35)] (Antibody), and Incubate for [0 Hr 32 Min]
- 16 Counterstain [Selected]
- 17 Apply One Drop of [HEMATOXYLIN II] (Counterstain), Apply Coverslip, and Incubate for [8 Minutes]
- 18 Post Counterstain [Selected]
- 19 Apply One Drop of [BLUING REAGENT] (Post Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

Protokol - AE1/3

Fjern voks

Tofaset voksfjernelse IHC

Solvent	Transportvæske	Temperatur
Clearify Clearing Agent	DI Water	25 °C

Vask efter tofaset voksfjernelse IHC

Reagens	Inkubation	Cyklusser
DI Water	5 s	1

Epitop demaskering

Epitop demaskering IHC

Reagens	Temperatur	Inkubation
EnV FLEX TRS, High pH	97 °C	30 min

Vask uden target retrieval IHC

Farvning

Vask

Reagens	Inkubation	Cyklusser
Wash Buffer	2:40 min	2

Enzymatisk forbehandling

Vask

Proteinblokker

Primært antistof

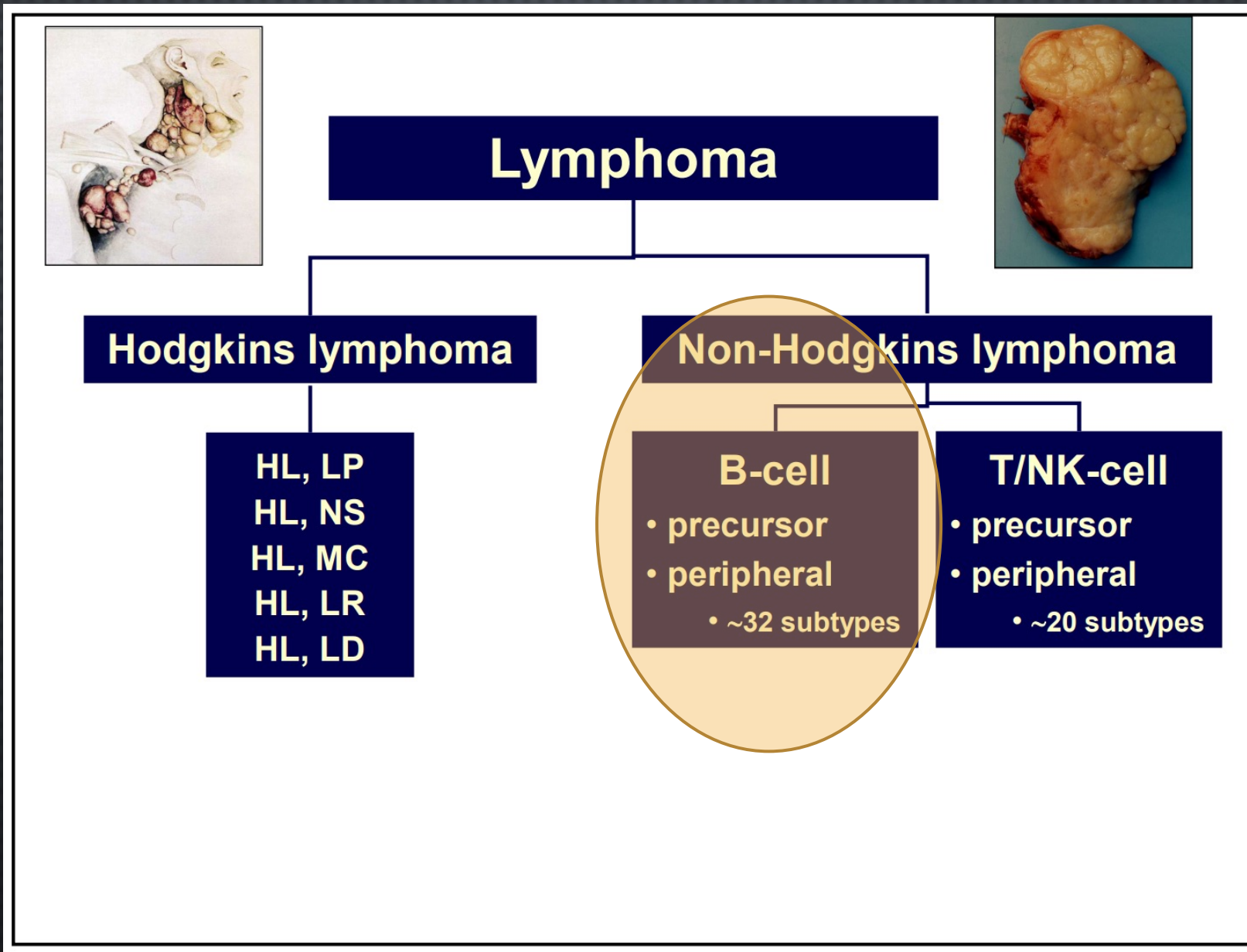
Reagens	Inkubation
Cytokeratin AE1/AE3	10 min

Vask

Reagens	Inkubation	Cyklusser
Wash Buffer	2 min	10

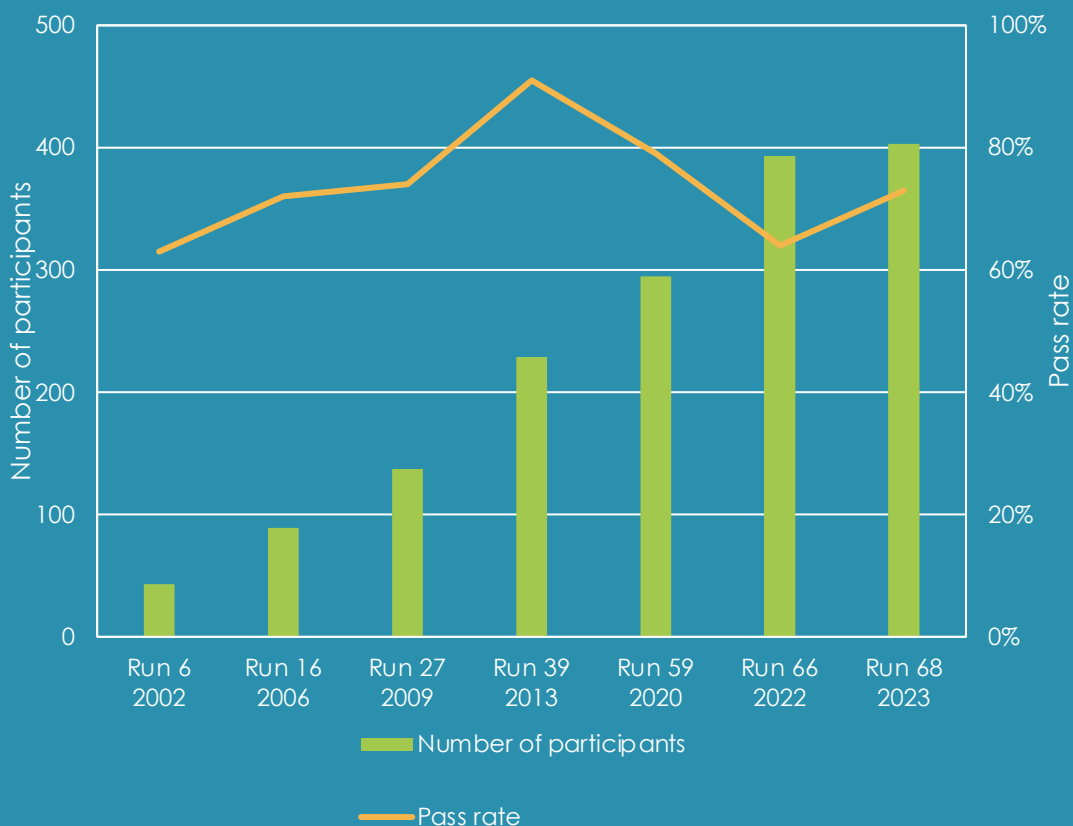
Endogen enzymblokker

Reagens	Inkubation
EnV FLEX Peroxidase-Blocking Reagent	3 min



CD10

CD10 performance in NordiQC assessments



Modified table 1

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 56C6	58	Leica Biosystems						
	7	Cell Marque						
	3	Monosan/Sanbio						
	2	Biocare Medical	46	7	17	4	72%	62%
	2	Thermo Scientific/Epredia						
	1	Immunologic						
	1	Zytomed						
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 PA370/0131	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

Primary antibody

Clone: SP67
 Producer: Ventana/Roche
 Product no. / lot no.: 790-4506 / F20122
 Format: Ready-To-Use (prediluted)
 Incubation time / temperature: 16 min. / 36°C

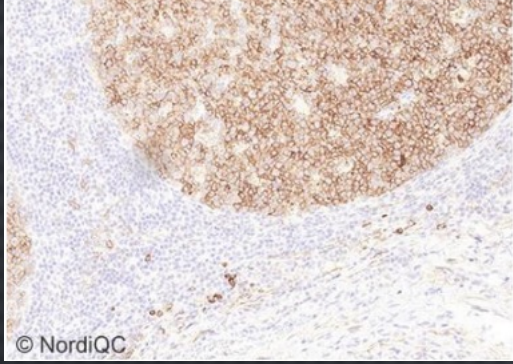
Epitope retrieval, HIER

Device: On Board / On Machine
 Buffer: Ventana CC1
 Heating time at max. temp.: 64 min.
 Maximum heating temp.: 100°C

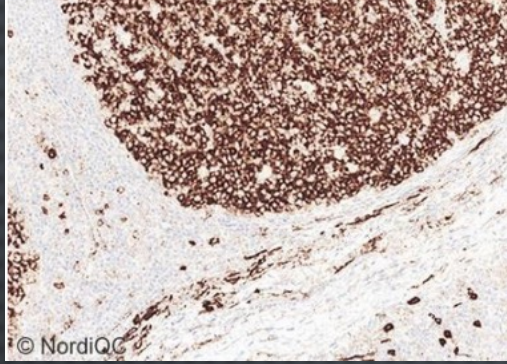
Visualization system

Producer: Ventana
 Product / no: OptiView DAB IHC Detection Kit / 760-700
 Incubation time linker: 8 min.
 Incubation time polymer: 8 min.
 Incubation temperature: 36°C

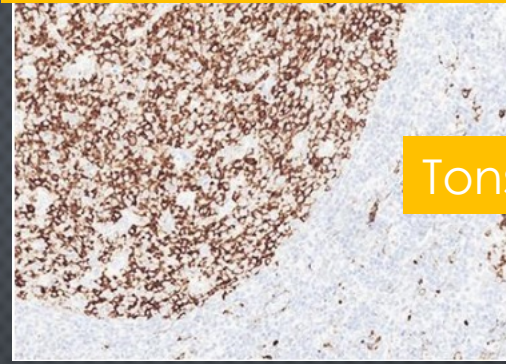
Bond, clone 56C6
RTU, VPRS



Ventana, clone
SP67 RTU, VPRS



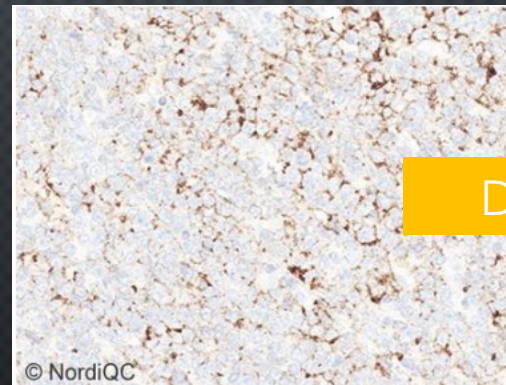
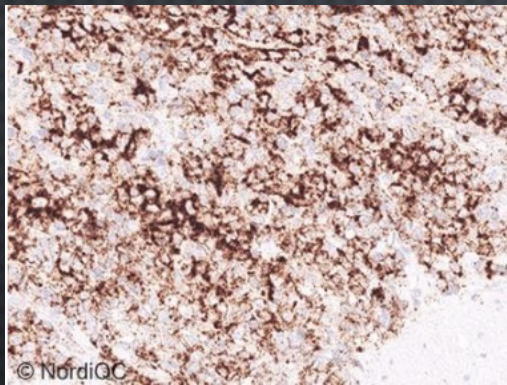
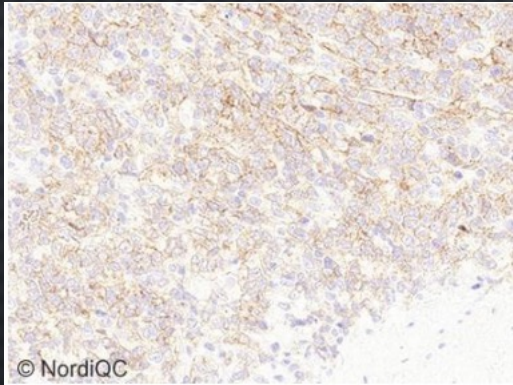
Ventana, clone SP67
RTU, HIER 64 min



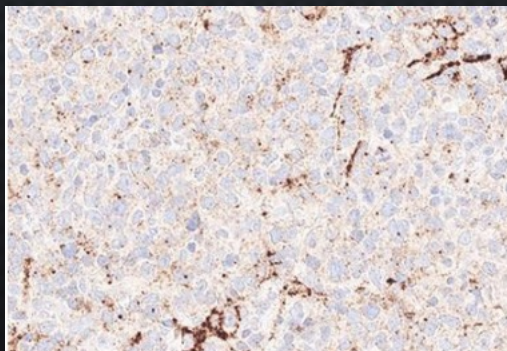
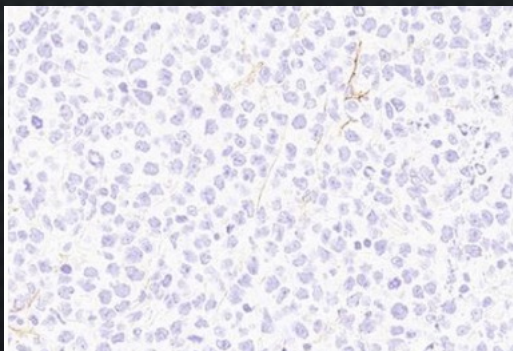
Tonsil



With great power
and tyramide
comes great
responsibility



DLBCL - GCB

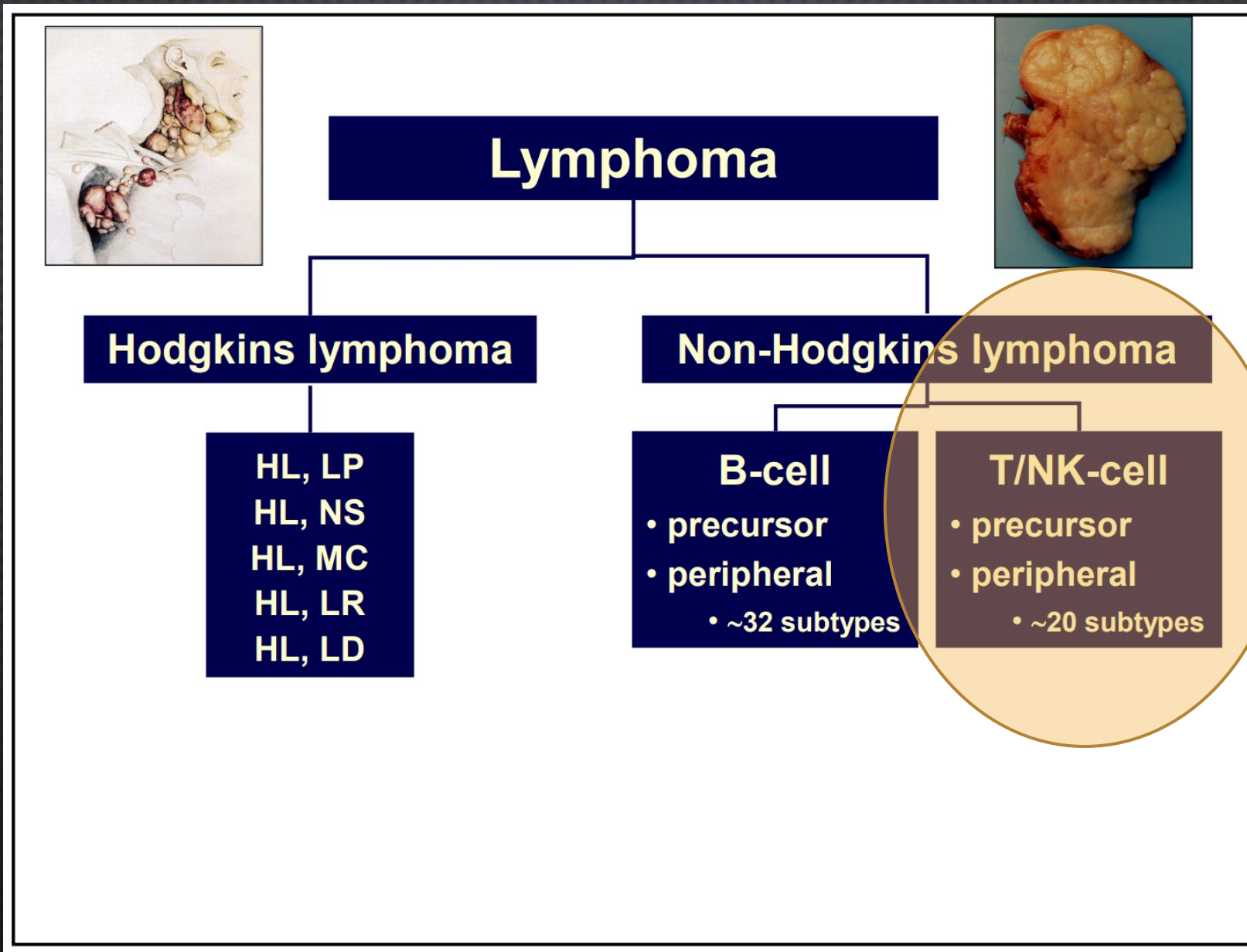


DLBCL – non-GCB

Optimal

Insufficient

Optimal



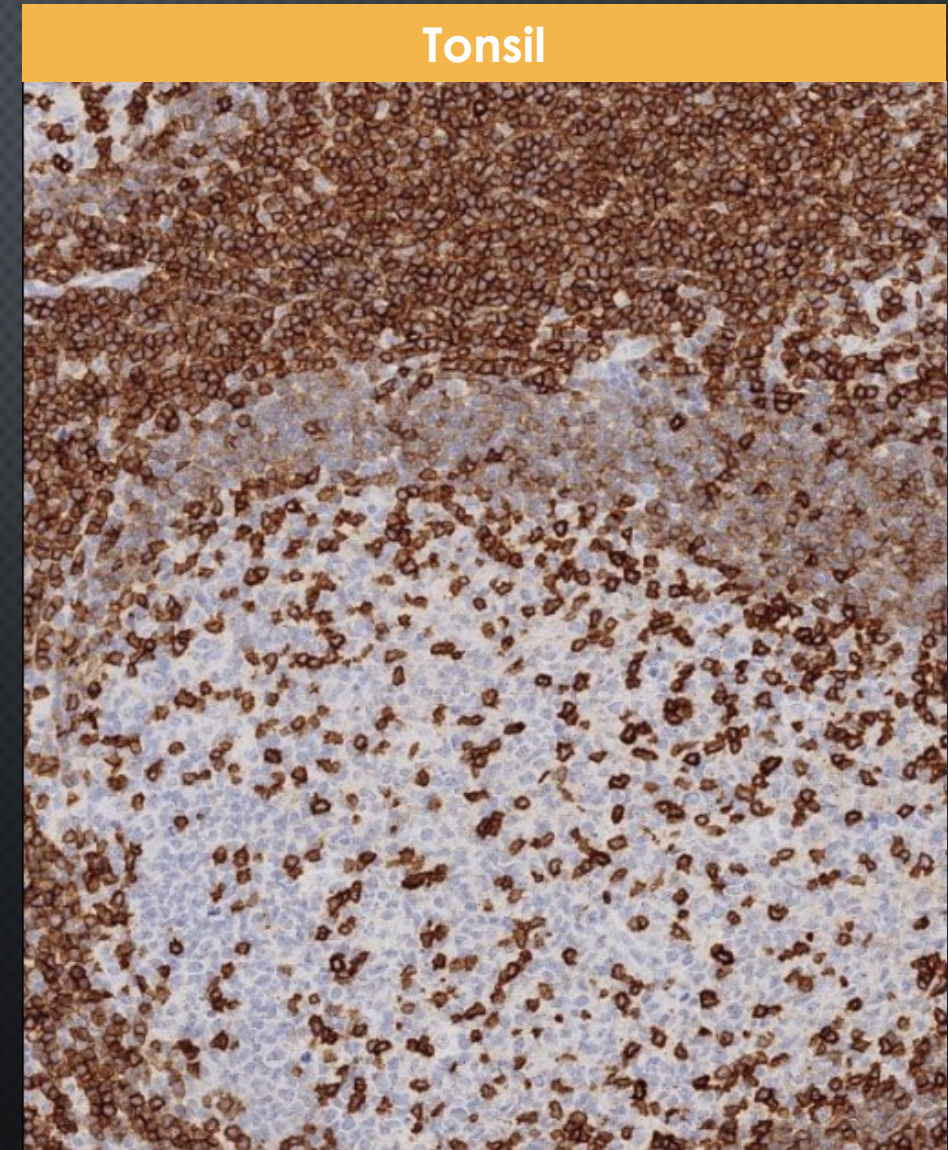
CAN YOU SWITCH A RTU TO ANOTHER
PLATFORM?



YES – CD5

Ready-To-Use antibodies								
mAb clone 4C7 IR/IS082	39	Dako/Agilent	27	10	1	1	95%	97 %
mAb clone 4C7 IR/IS082³	13	Dako/Agilent	7	5	1	0	92%	-

“In that context, the ideal RTU format of a primary Ab is used within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected. **Therefore, it is not advisable to use a RTU format of the primary Ab on a system/platform for which it has not been developed** and validated, although it might produce optimal results” Run 49 2017



NO - CD4

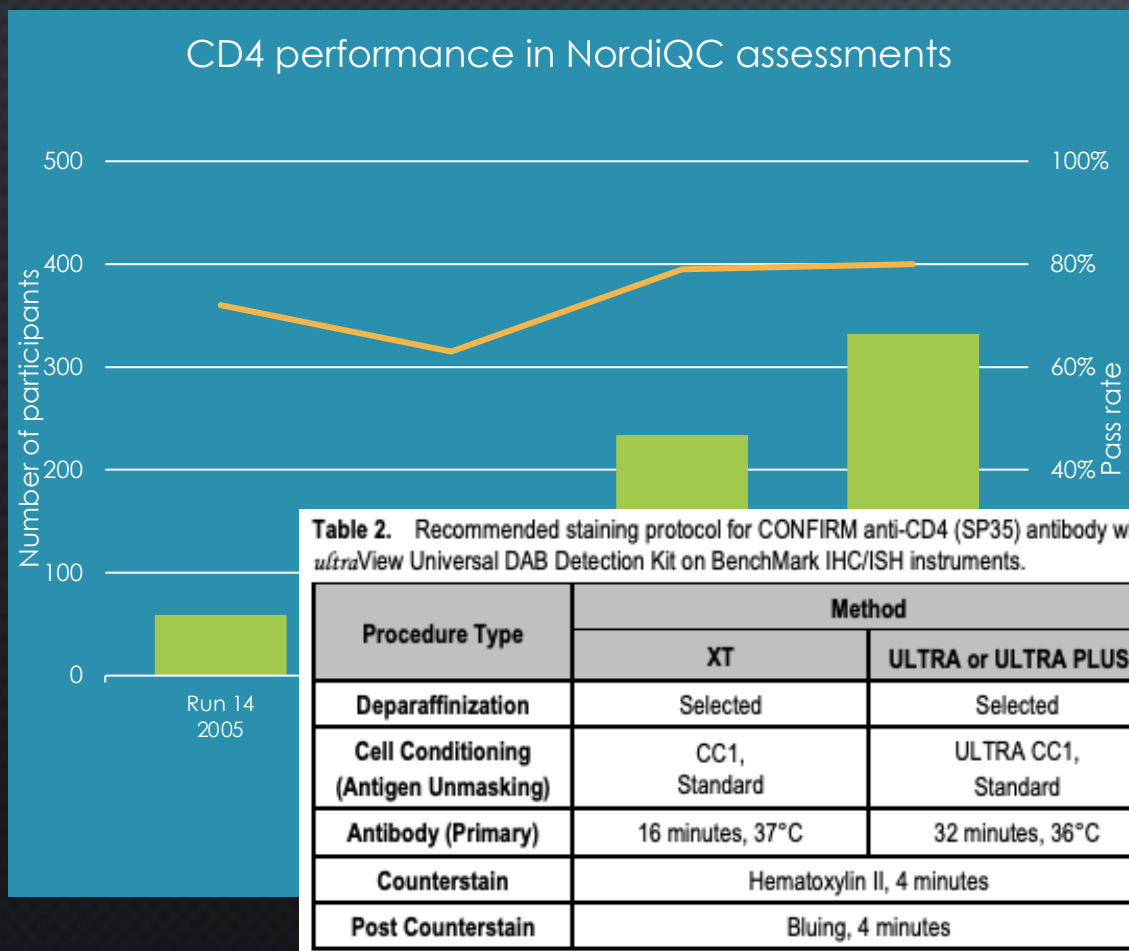


Table 2. Recommended staining protocol for CONFIRM anti-CD4 (SP35) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1, Standard
Antibody (Primary)	16 minutes, 37°C	32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

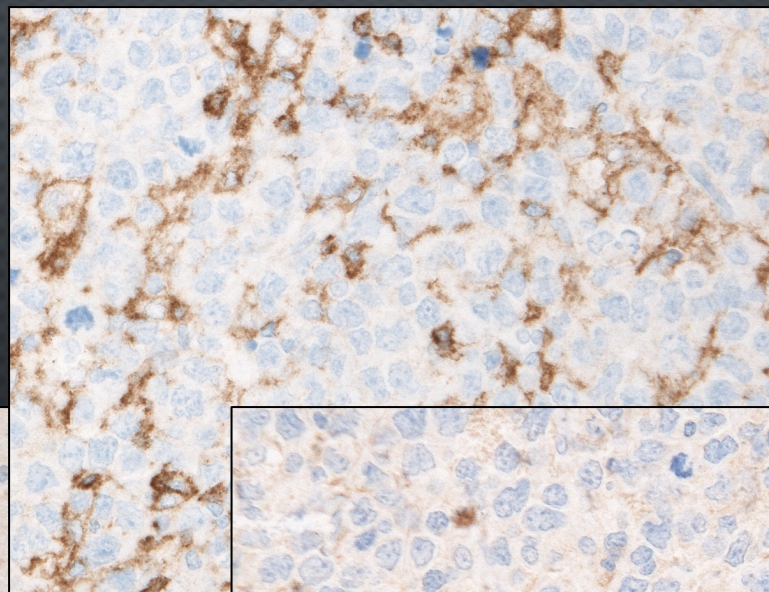
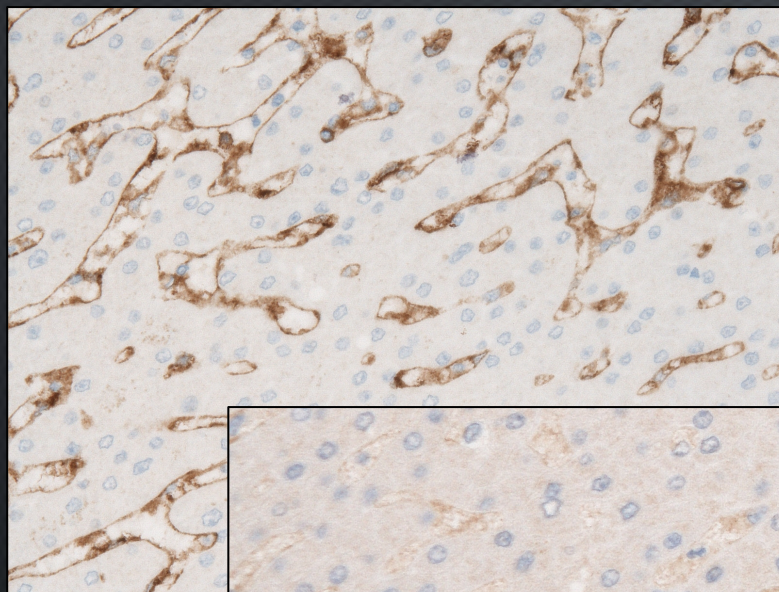
Modified table 1

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 4B12	14	Leica Biosystems						
	10	Dako/Agilent						
	1	Biocare medical						
	1	Epredia						
mAb clone 1F6	3	Leica Biosystems						
rmAb clone SP35	32	Cell Marque						
	2	Spring Biosciences						
	1	Abcam						
	1	SanBio						
rmAb clone EP204	3	Epitomics						
rmAb clone IHC535	1	GenomeMe						
rmAb clone ZR110	1	Zeta Corporation						
rmAb clone QR032	1	Quartett						
Conc total	71							
Ready-To-Use antibodies								
mAb clone 4B12	13	Leica Biosystems						
PA0427³								
mAb clone 4B12	9	Leica Biosystems						
PA0427⁴								
mAb clone 4B12	14	Dako/Agilent						
IR649³								
mAb clone 4B12	48	Dako/Agilent						
IR649⁴								
rmAb clone SP35	31	Ventana/Roche						
790-4423³								
rmAb clone SP35	116	Ventana/Roche						
790-4423⁴								
rmAb clone SP35	10	Cell Marque						
104R-17/18								
rmAb clone EP204	1	Sakura Finetek						
8226-C010								
RTU total	261							
Total	332							
Proportion								

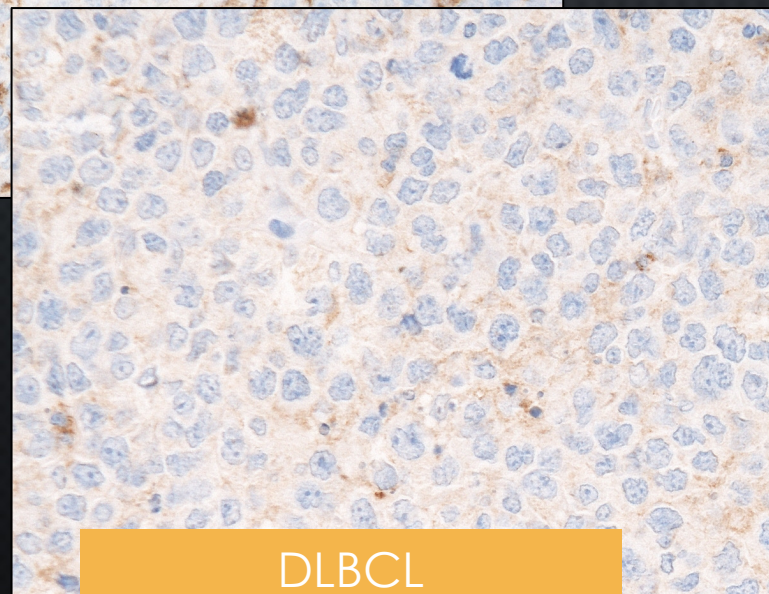
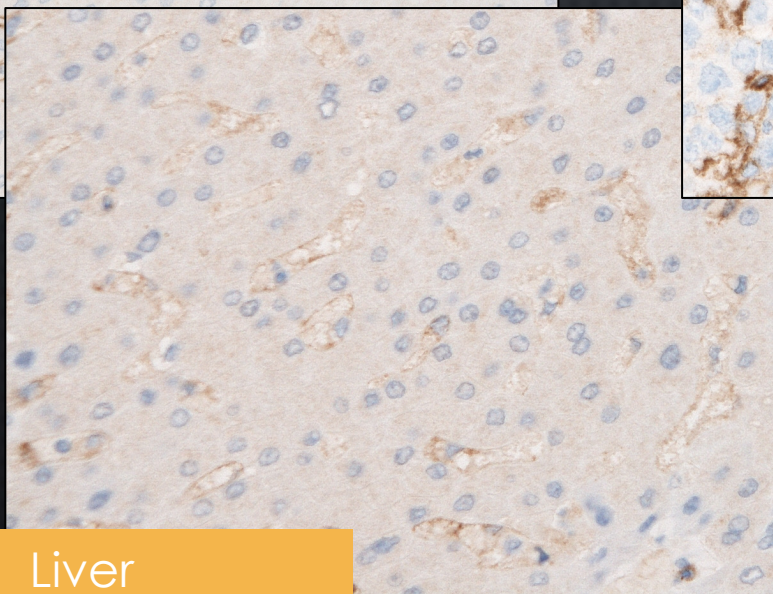
USE ROBUST CLONES

Table 2. Proportion of optimal results for CD4 for the two most commonly used antibody concentrates on 4 main IHC systems*

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark Ultra		Leica Biosystems Bond III	
	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 4B12	0/1**	1/1	0/2	-	-	-	1/7 (14%)	-
rmAb clone SP35	0/1	-	11/14 (79%)	-	8/11 (73%)	-	1/2	-



CD4 IR649 RTU
Autostainer VPRS



CD4 IR649 RTU
Omnis – same settings

Liver

DLBCL

COMING UP!

NordiQC assessment scheme 2023

Module	Winter	Spring	Autum
General	Run 67 <u>MLH1</u> <u>p53</u> <u>CD4</u> <u>CGA</u> <u>p40</u>	Run 68 <u>CD5</u> <u>TTF1</u> <u>PAX8</u> <u>PRAME</u> <u>MSH2</u> <u>URO II/III</u>	Run 69 <u>CD138</u> <u>EpCAM</u> <u>CK8/18</u> <u>PSA</u> <u>CD23</u>
Breast	Run B35 <u>PR</u> <u>HER2 IHC</u> <u>ER</u>		Run B36
HER2 ISH	Run H23 <u>HER2 ISH</u>		
Companion		Run C13 <u>PD-L1 (TPS/CPS)</u> <u>PD-L1 (IC)</u>	

Autumn

Run 66
CD10 SYP BSAP
SMH Napsin A

SO EXCITED

I CAN'T WAIT!!