Nordic immunohistochemical Quality Control

NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, 19-21 September 2018

Optimization of antibodies, protocols and controls
Hematolymphoid markers

Michael Bzorek

Histotechnologist

Department of Surgical Pathology

University Hospital, Region Zealand, Denmark

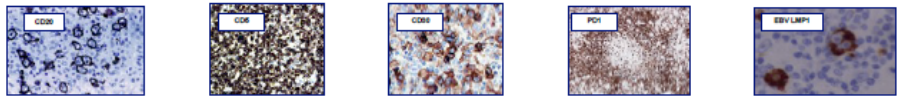
Courtesy: Steve Hamilton-Dutoit

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

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



Mission impossible

The challenge

Basic IHC panel for lymphoma diagnosis

- CD45
- CD20
- CD79α
- (PAX-5)
- kappa/lambda
- CD3
- CD5
- CD30
- CD43
- Bcl-2
- Bcl-6
- CD23 (CD21)
- Cyclin-D1
- Ki-67

Courtesy: Steve Hamilton-Dutoit

Relative frequency of lymphoid malignancies

10	3	1
B-Cell	Hodgkin	T-Cell

Focus on the basic lymphoid markers/panel

+ Update on additional markers assessed by NordiQC during the period 2017-2018

Antigen	NQC assessments	Latest Run	Pass rate (%)	Optimal (%)
CD20	√	Run 35	95	77
CyclinD1	√	Run 47	94	54
CD3	√	Run 37	92	66
Ki67	√	Run B13	89	72
Pax5	√	Run 53	86	40
CD45	√	Run 37	82	56
BCL2	√	Run 28	82	44
CD79a	√	Run 45	79	51
CD5	√	Run 34	79	46
BCL6	√	Run 42	74	30
CD23	√	Run 34	73	38
CD30	√	Run 43	71	34
Kappa	√	Run 18	41	14
Lambda	√	Run 15	34	15
CD43	-	-	-	-

23%

86%

B-Cell lymphoma markers - lineage "specific" (1):

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD19 (membr.) LE-CD19, BT51E	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells	Plasma cells	No staining of other cell types including T-cells and epithelial cells of the appendix.
CD20 (membr.). L26, 7D1, EP7	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells	None	No staining of other cell types including T-cells and epithelial cells of the appendix.
CD79a (membr. + cytopl) JCB117, SP18	Tonsil/Appendix	Mantle zone B-cells and plasma cells	Germinal centre B-cells	No staining of other cell types including T-cells and epithelial cells of the appendix.
BSAP (PAX5) (nuclear) 1EW, 24, DAK-PAX5, MXo17, SP34, EP156, BSR59, BV6	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells*	None	No staining of other cell types including T-cells and epithelial cells of the appendix.
IgK (membr. + cytopl). pAb A0191	Tonsil	Plasma cells (App. 50%)	Mantle zone B-cells (App. 50 %)	No staining of other cell types including T-cells (weak background staining my be seen)
IgL (membr. + cytopl) pAb A0193	Tonsil	Plasma cells (App. 50%)	Mantle zone B-cells (App. 50 %)	No staining of other cell types including T-cells (weak background staining may be seen)
IgM (membr. + cytopl) pAb A0425, 760-2654	Tonsil	Plasma cells (app. 35%)	Virtually all mantle zone B-cells	No staining of other cell types including T-cells (weak background staining may be seen)
OCT-2 & BOB.1	See Hodgkin Lymphoma panel			

* A weak cytoplasmic staining reaction in B-cells must be accepted. In the technical calibration phase, it is recommended to verify the protocol on Hodgkin lymphoma, classical subtype.

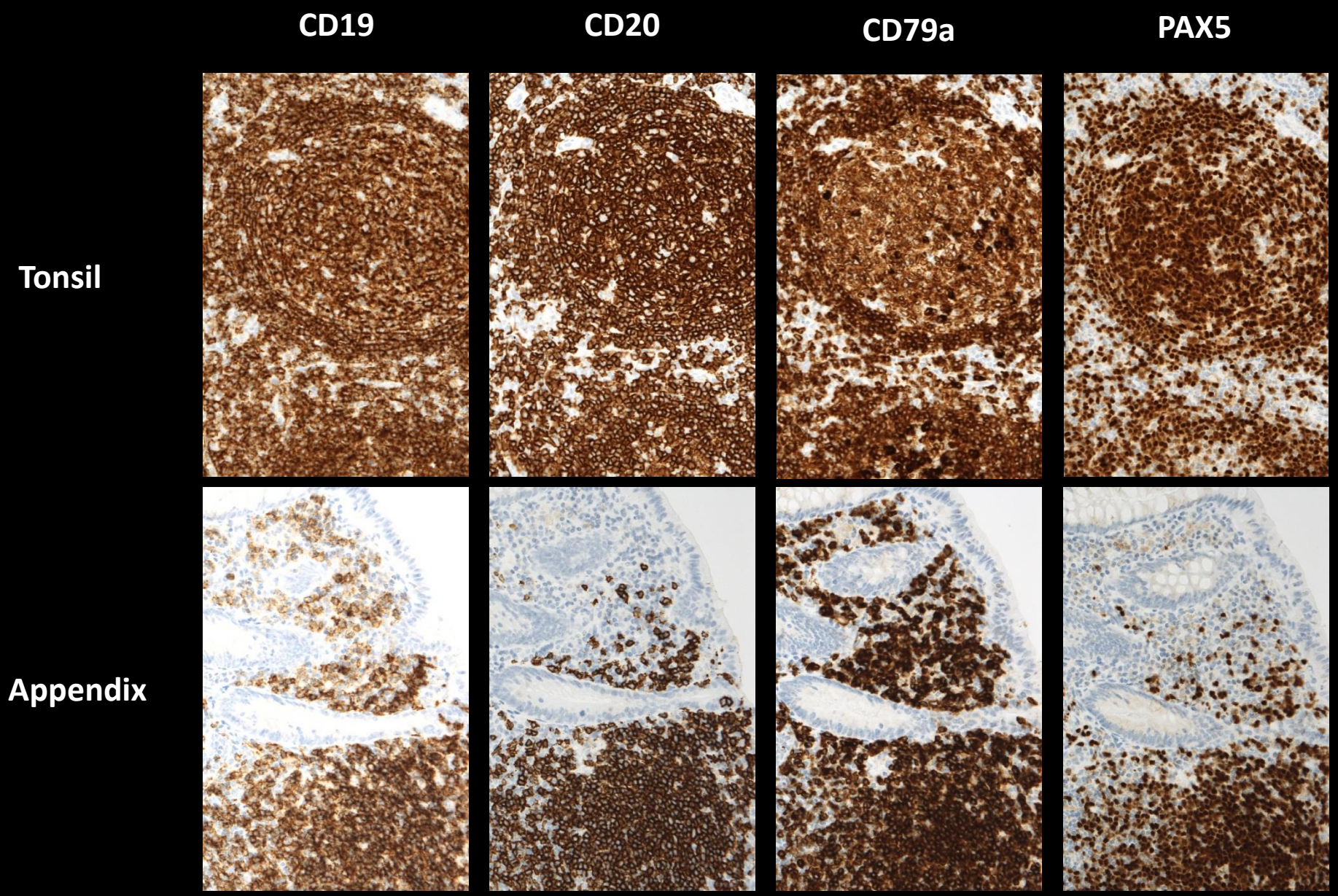
Clones (mAbs, rmAbs & pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

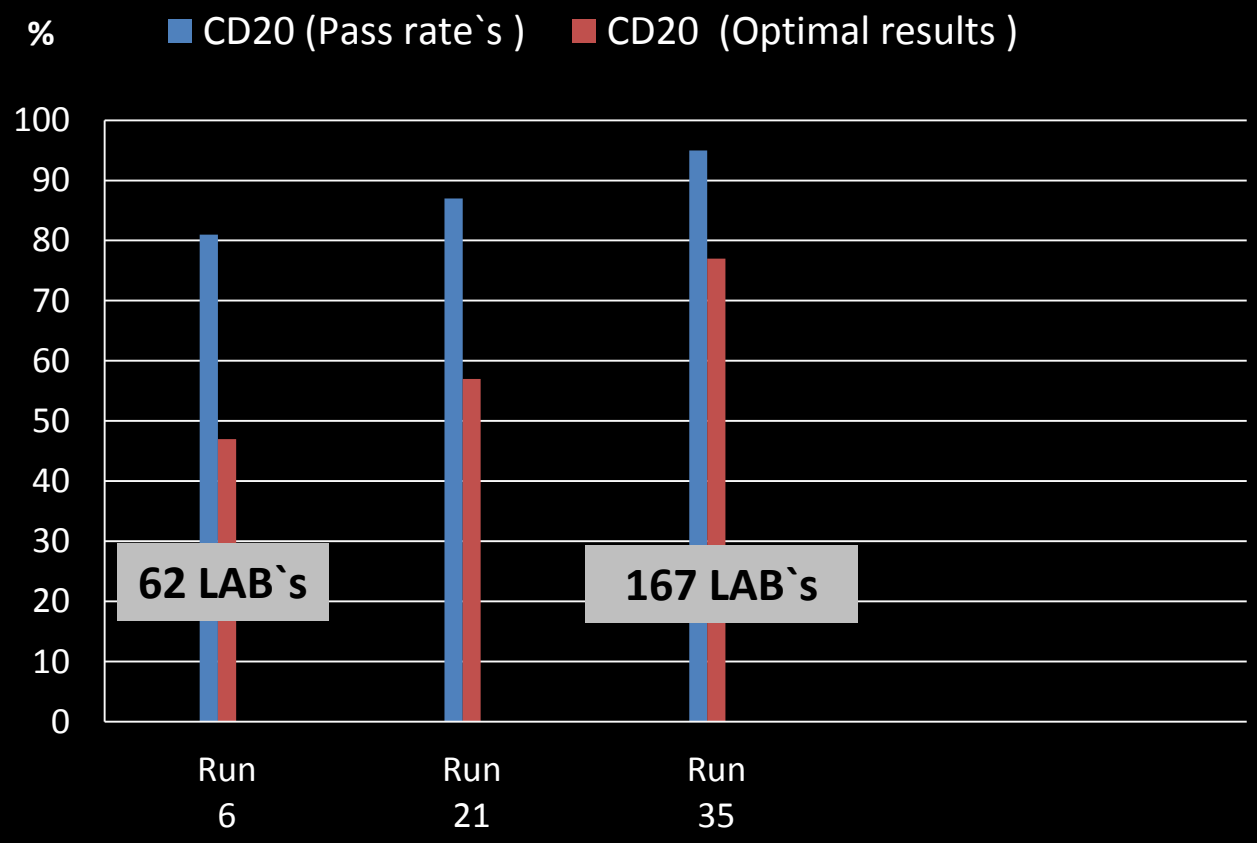
iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

B-Cell lymphoma markers - lineage “specific” (1):



CD20



CD20/ Run 31 (2012):

Sufficient: 95%

Optimal: 77%

Success due to very robust Abs

Clone L26 used by 97% of the LAB`s

J Clin Pathol 1998;51:364–369

Levels of expression of CD19 and CD20 in chronic B cell leukaemias

Lia Ginaldi, Massimo De Martinis, Estella Matutes, Nahla Farahat, Ricardo Morilla, Daniel Catovsky

Table 1 Mean ABC (antibody binding capacity) values × 10^3 in normal peripheral blood B lymphocytes and B lineage leukaemias

Antigen	Normal B cells	CLL	PLL	MCL	SLVL	HCL
CD19	22 (7)	13 (7)	16 (9)	10 (7)	15 (11)	38 (16)
(p value)*		<0.001	<0.05	<0.001	<0.05	<0.001
CD20	94 (16)	65 (11)	129 (47)	123 (51)	167 (72)	312 (110)
(p value)*		<0.001	<0.01	<0.05	<0.001	<0.001

Values are mean (SD); *comparison with normal peripheral blood B lymphocytes. CLL, chronic lymphatic leukaemia; HCL, hairy cell leukaemia; MCL, mantle cell lymphoma; PLL, prolymphocytic leukaemia; SLVL, splenic lymphoma with villous lymphocytes.

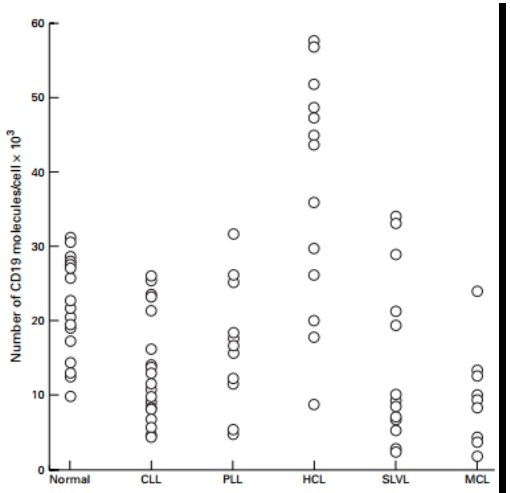


Figure 2 Distribution of individual ABC values for CD19 in normal peripheral blood B lymphocytes and B cell leukaemias.

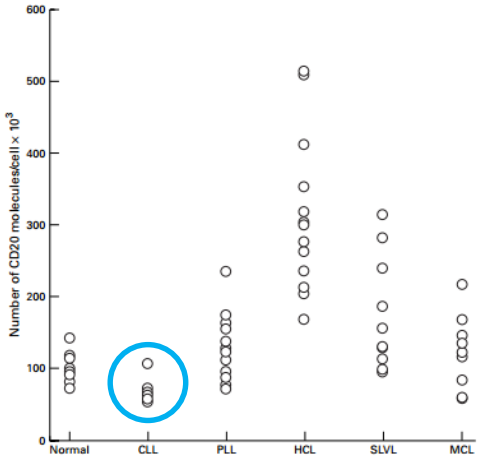


Figure 4 Individual ABC values for CD20 in normal peripheral blood B cells and B lineage leukaemias.

Prevodnik et al. Diagnostic Pathology 2011, 6:33
http://www.diagnosticpathology.org/content/6/1/33

DIAGNOSTIC PATHOLOGY

RESEARCH Open Access

The predictive significance of CD20 expression in B-cell lymphomas

Veronika Kloboves Prevodnik[1*], Jaka Lavrenčak[1], Mateja Horvat[2] and Barbara Jezeršek Novaković[3]

Abstract

Background: In our recent study, we determined the cut-off value of CD20 expression at the level of 25 000 molecules of equivalent soluble fluorochrome (MESF) to be the predictor of response to rituximab containing treatment in patients with B-cell lymphomas. In 17.5% of patients, who had the level of CD20 expression below the cut-off value, the response to rituximab containing treatment was significantly worse than in the rest of the patients with the level of CD20 expression above the cut-off value. The proportion of patients with low CD20 expression who might not benefit from rituximab containing treatment was not necessarily representative. Therefore the aim of this study was to quantify the CD20 expression in a larger series of patients with B-cell lymphomas which might allow us to determine more reliably the proportion of patients with the CD20 expression below the cut-off.

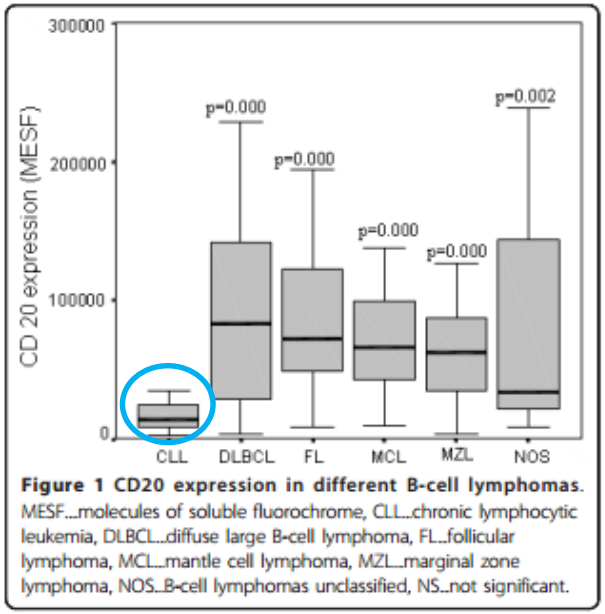
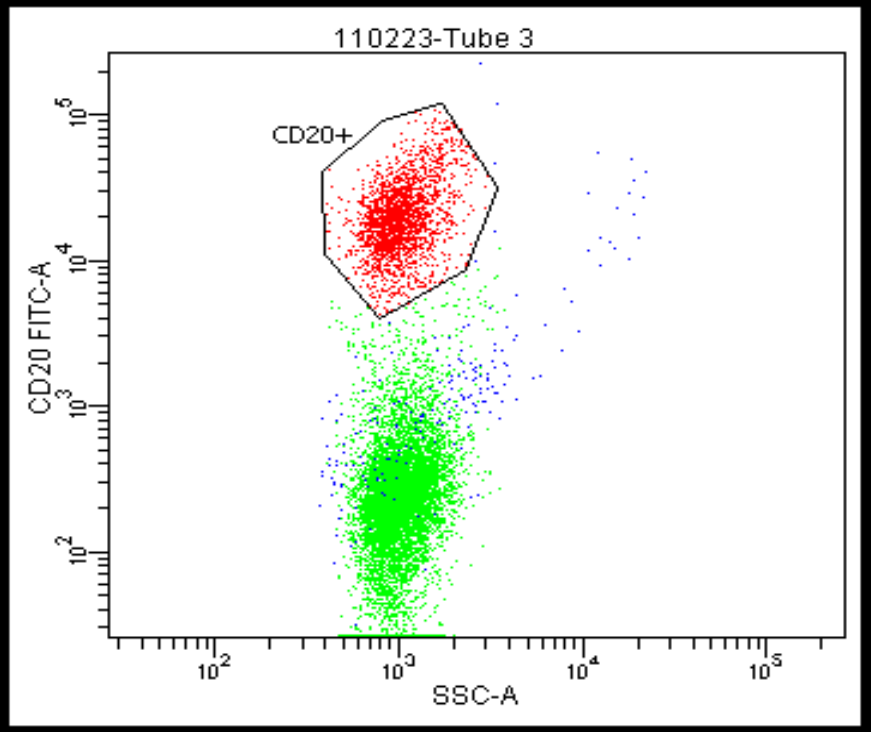


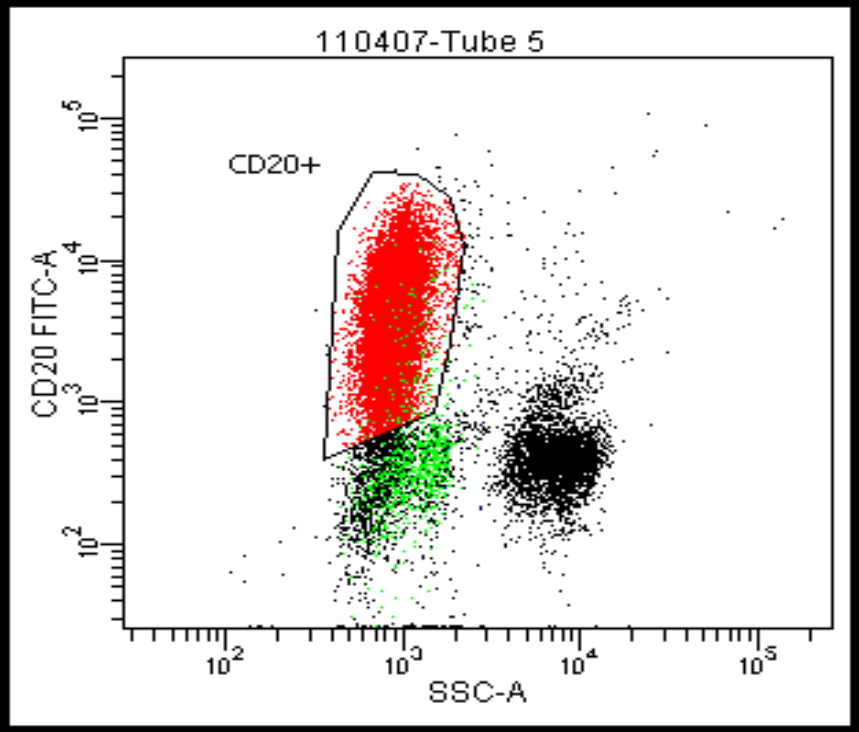
Figure 1 CD20 expression in different B-cell lymphomas. MESF...molecules of soluble fluorochrome, CLL...chronic lymphocytic leukemia, DLBCL...diffuse large B-cell lymphoma, FL...follicular lymphoma, MCL...mantle cell lymphoma, MZL...marginal zone lymphoma, NOS...B-cell lymphomas unclassified, NS...not significant.

In the calibration phase of CD20 – test on tissue material diagnosed with CLL (10-20 cases) as most of these cases express CD20 at lower level compared to normal lymphoid tissue or other lymphoid malignancies



Normal Lymph node

CD20 strong positive

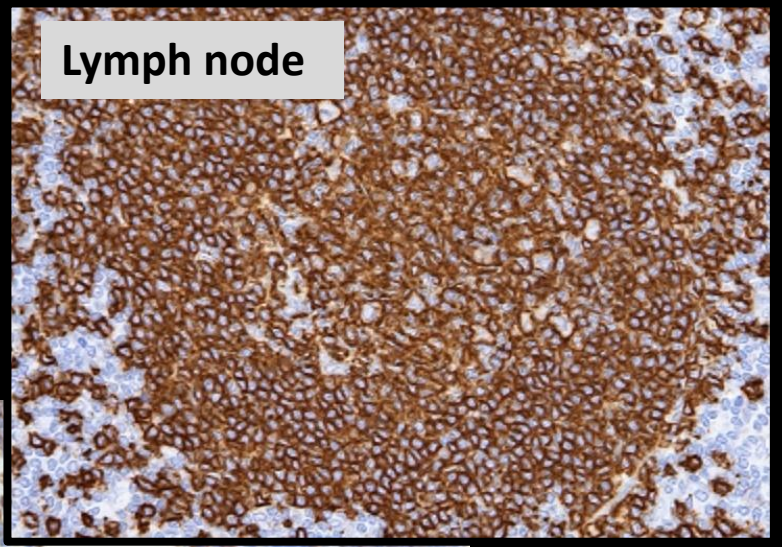


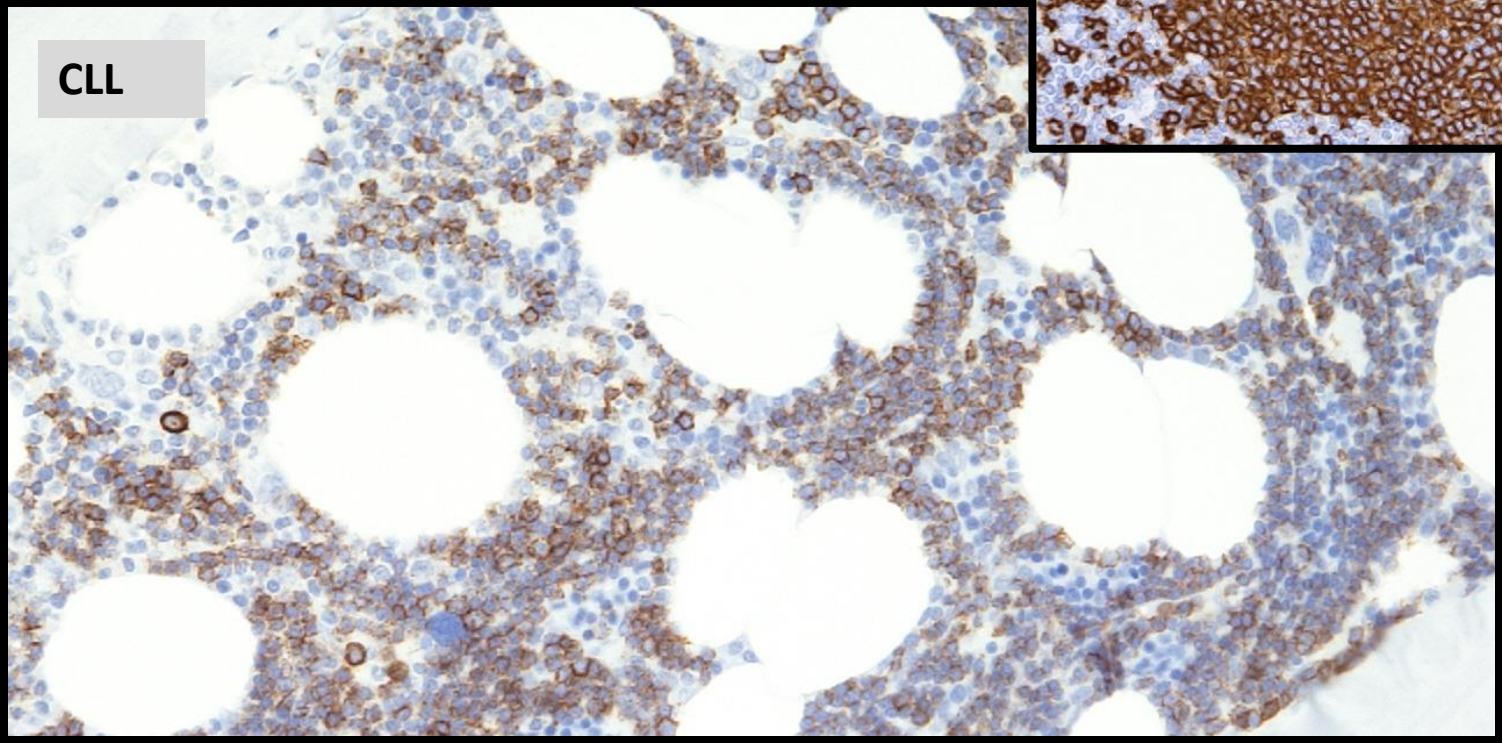
Bone Marrow Aspirate / CLL patient

Marker profile: CD19+, CD5+, CD10-neg, CD20-dim, CD38-neg, CD23+, Kappa+

CD20-dim reaction in the vast majority of the neoplastic B-cells (CLL)

CD20





B-CLL`s in bone marrow specimens often display weak/dim reaction (flowcytometry). A weak to moderate, predominantly membranous staining of the majority of the neoplastic B-cells should be seen.

CD20

Table 1. Abs and assessment marks for CD20, run 35

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **L26**	104	Biocare Cell Marque Dako Master Diagnóstica Leica/Novocastra Scytek Thermo/NeoMarkers Zymed Zytomed Systems	73	25	5	1	94 %	94 %
mAb clone **7D1**	1	Leica/Novocastra	1	0	0	0	-	-
rmAb clone **EP7**	1	Epitomics	1	0	0	0	-	-
pAb **RB-9013-P**	1	Thermo/NeoMarkers	0	0	1	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use Abs								
mAb clone **L26, 760-4380**	38	Ventana	35	1	2	0	95 %	100 %
mAb clone **L26, IR604/N1502**	17	Dako	15	2	0	0	100 %	100 %
mAb clone **L26, PM004**	1	Biocare	1	0	0	0	-	-
mAb clone **L26, CD20-L26-R-7-CE**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **MJ1, PA0906**	2	Leica/Novocastra	0	2	0	0	-	-
Total	167		128	30	8	1	-	
Proportion			77 %	18 %	4 %	<1%	95 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

Suff. (clone L26)

HIER (preferable in alkaline buffer`s)

1:75-1:2000

All detection systems

Insuff. (clone L26)

Omission of HIER

Too low conc. of primary Ab

Provided optimal results on the 3 main platforms (Ventana Benchmark, Dako Autostainer and Leica BOND)

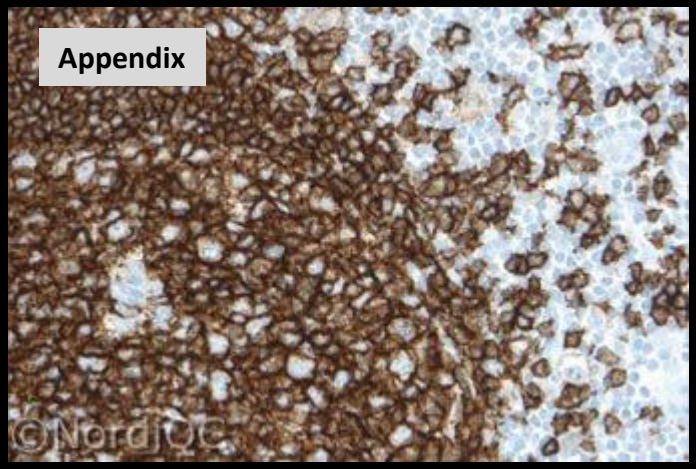


Fig. 1a. Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. **HIER was performed** using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the B-cells.

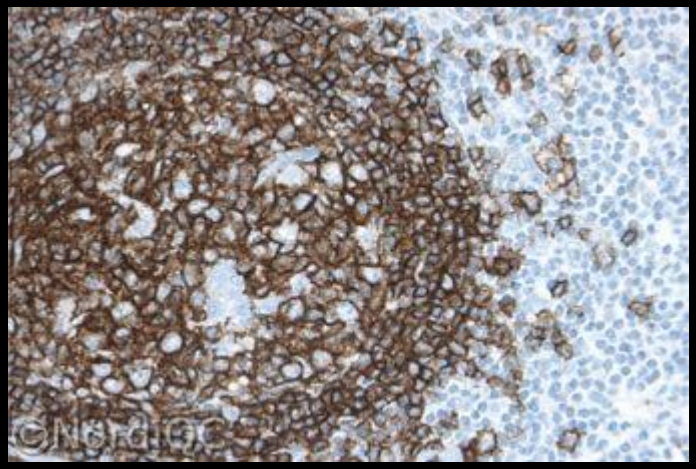


Fig. 1b. Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. **No HIER was performed**. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors of CD20, hence the relatively strong reaction. Even so, the staining intensity should be improved in order to detect low expressors of CD20 (e.g. B-CLL in Fig. 2a and 2b).

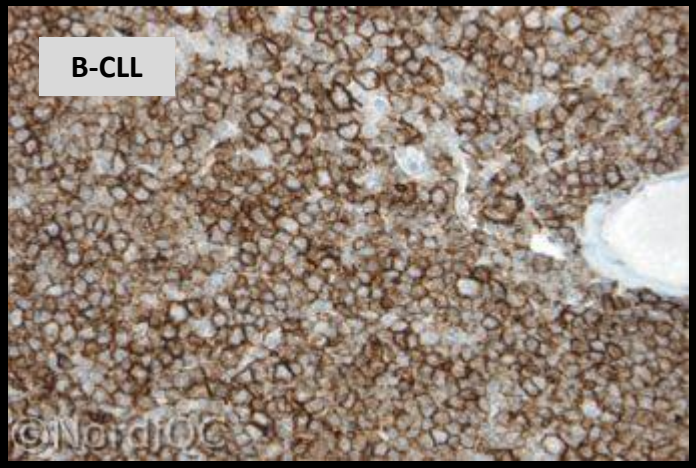


Fig. 2a. B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

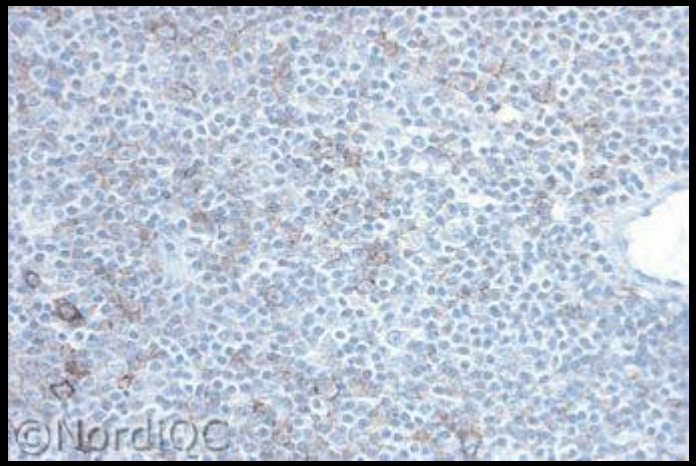


Fig. 2b. B-CLL. Insufficient staining for CD20 using the same protocol as in Fig. 1b. Omitting HIER, only scattered cells are positive. The majority of the neoplastic cells are negative. Compare with the optimal result in Fig. 2a, same field.

Lymphoma panel: CD20

Optimal protocol settings (NQC)

CD20	Retrieval buffers	Titer	Detection systems	RTU	Detection
mmAb **L26**	**HIER High pH or Low pH buffer**	**1:75-1:2000**	**2 & 3-step**	**Dako (IR604)**	**Flex** **Flex+**
	CC1	**-**	**-**	**Ventana (760-2531)**	**iView** **UltraView** **OptiView**
mmAb **7D1**	**HIER Low pH buffer (BERS1)**	**1:200**	**3-step**		**BOND Refine**
rmAb **EP7**	**HIER Low pH buffer (Citrate buffer pH6)**	**1:100**	**-**	**-**	**-**

Control material / Tonsil:

An strong, distinct membranous staining reaction of all B-cells in the tonsil.

No staining of other cellular structures

CD79a

Table 1. **Antibodies and assessment marks for CD79a, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **11D10**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **11E3**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **HM57**	2	Dako	0	0	0	2	-	-
mAb clone **JCB117**	94 3	Dako Thermo/NeoMarkers	37	35	19	6	74%	74%
rmAb clone **SP18**	12 3 2 1 1	Thermo/NeoMarkers Spring Bioscience Cell Marque Nordic Biosite Zytomed	21% 4	14	0	1	95%	83%
Ready-To-Use antibodies								
mAb clone **11E3 PA0192**	6	Leica/Novocastra	0	0	3	3	-	-
mAb clone **HM46/A9 PM067**	1	Biocarea	0	0	0	1	-	-
mAb clone **JCB117 IR/IS621**	40	Dako	23	11	5	1	85%	89%
mAb **JCB117 GA621**	11	Dako	9	2	0	0	100%	100%
mAb **JCB117 760-2639***	2	Ventana/Cell Marque	0	1	1	0	-	-
mAb clone **JCB117 PA0599**	1	Leica/Novocastra	0	0	0	1	-	-
rmAb clone **SP18 790-4432**	58	Ventana	86% 50	6	0	2	97%	96%
rmAb clone **SP18 MAD-00032QD**	2	Master Diagnostica	0	0	2	0	-	-
rmAb clone **SP18 179R-18**	1	Cell Marque	0	1	0	0	-	-
rmAb clone **SP18 RMA-0552**	1	Maixin	1	0	0	0	-	-
Total	245		124	70	30	21	-	
Proportion			51%	28%	12%	9%	79%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Discontinued product.

Optimal (clone JCB117)

HIER (preferable alkaline buffer)

1:25-1:600

2 & 3 step detection systems

Optimal (clone SP18)

HIER (CC1)

1:300-1:500

OptiView (Ventana Benchmark)

Insufficient results

Too short inefficient HIER

Too low conc. of primary Ab

Less successful primary Abs

Table 3: **Proportion of optimal results for CD79a for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **JCB117**	9/16** (56%)	0/1	11/31 (36%)	-	6/8 (75%)	2/2
rmAb clone **SP18**	0/2	-	4/6 (67%)	-	0/2	-

* 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).

mAb clone JCB117 provided optimal results on the 3 main platforms but……

The proportion of optimal results were lower on the Ventana Benchmark instruments compared to other platforms

<u>In concordance with Run 29, 2010 (mAb JCB117):</u>

Dako Autostainer /BOND platforms, 36 out of 39 of the protocols (92%) gave a sufficient result (77% optimal)

Ventana BenchMark instruments, 17 out of 25 protocols (68%) gave a sufficient staining (12 % optimal)

High Ab concentration (1:25 – 1:100) gave optimal results.

Use rmAb SP18 on the Ventana Benchmark platforms

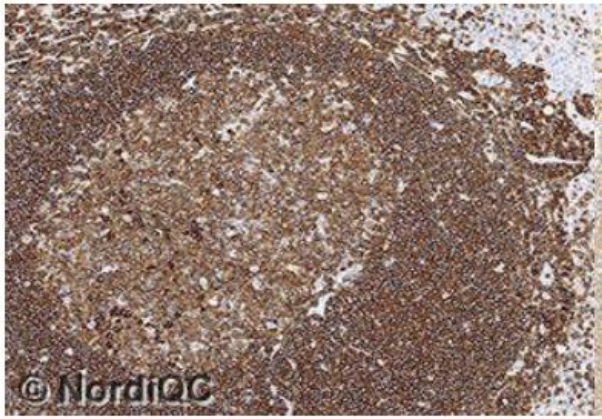


Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone JCB117 as Ready-To-Use format (GA621, Dako), with HIER in TRS High pH 9 for 30 min., a 3-step polymer based detection kit and performed on Omnis, Dako.
Mantle zone B-cells show an intense membranous staining reaction, while the germinal centre B-cells show a moderate staining reaction. Plasma cells and late stage germinal centre B-cells show a strong cytoplasmic staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

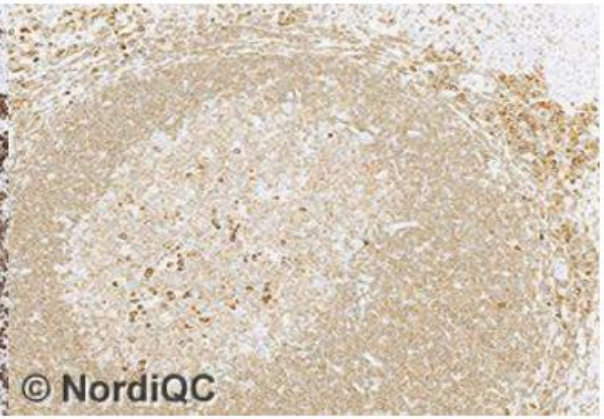


Fig. 1b
CD79a staining of the tonsil using the mAb clone JCB117 with an insufficient protocol – same field as in Fig. 1a.
The primary Ab was used at a titre of 1:500 and a 2-step multimer based detection system providing a too low sensitivity.
The mantle zone B-cells and the late stage germinal centre B-cells are demonstrated, while the germinal centre B-cells only show a weak and diffuse staining reaction.
Also compare with Figs. 2b & 3b – same protocol.

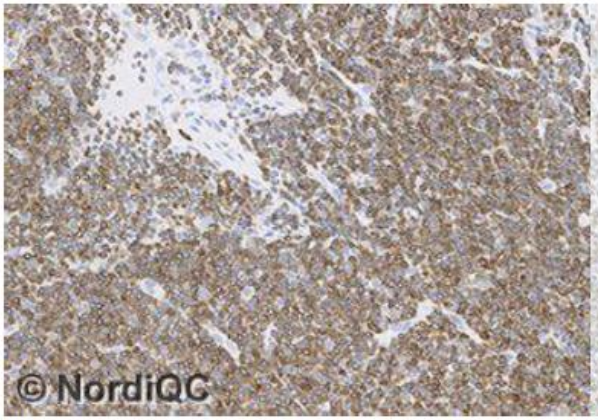


Fig. 2a
Optimal CD79a staining of the B-CLL using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate and distinct membranous staining reaction.
No background reaction is seen.

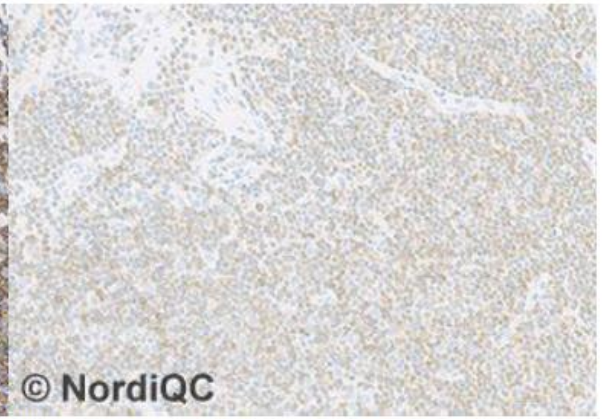


Fig. 2b
Insufficient CD79a staining of the B-CLL using same protocol as in Fig. 1b - same field as in Fig. 2a.
The neoplastic cells only show a weak and equivocal staining reaction.
Also compare with Fig. 3b – same protocol.

Problem:

Protocol with too low sensitivity

- Low concentration of primary

- Low sensitive detection system

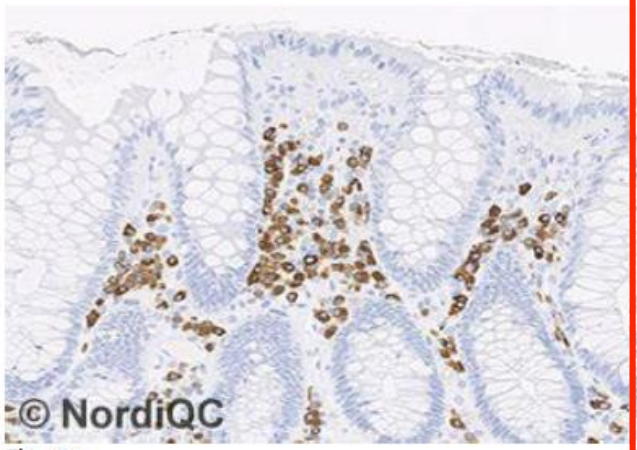


Fig. 4a
Optimal CD79a staining of colon using same protocol as in Figs. 1a - 3a.
Plasma cells show a moderate to strong cytoplasmic staining reaction.
No background reaction is seen.

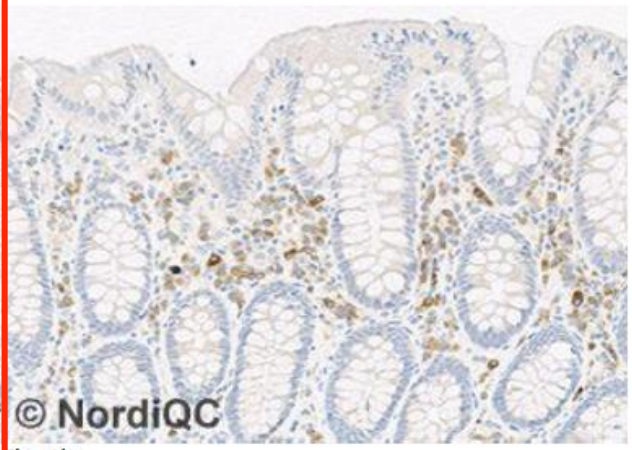


Fig. 4b
CD79a staining of the colon using an insufficient protocol based on the mAb clone 11E3.
The intensity and proportion of plasma cells demonstrated is reduced compared to the level expected.
However also compare with Fig. 5b – same protocol

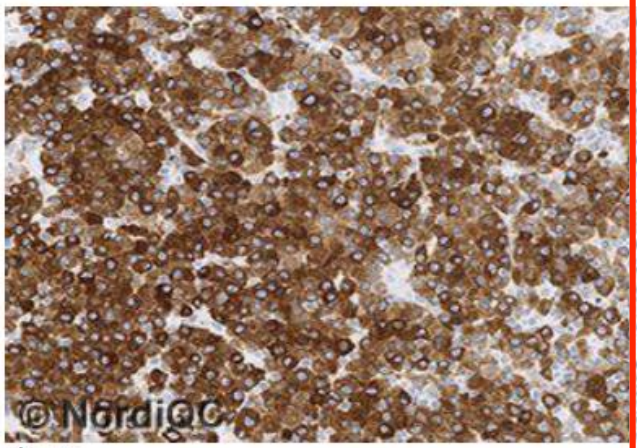


Fig. 5a
Optimal CD79a staining of the plasmacytoma using same protocol as in Figs. 1a - 4a.
Virtually all neoplastic cells show a moderate cytoplasmic staining reaction.

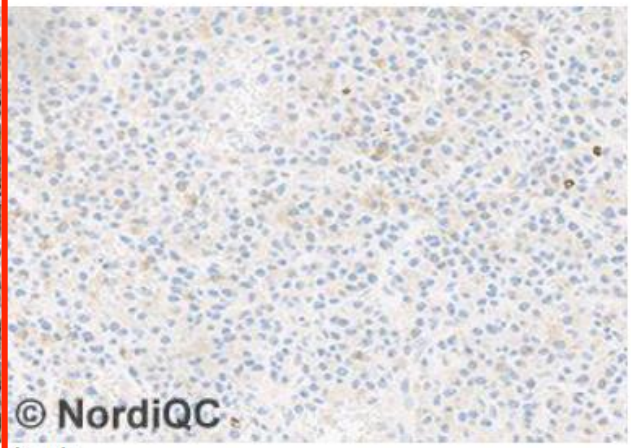


Fig. 5b
Insufficient CD79a staining of the plasmacytoma using same protocol as in Fig. 4b.
Only scattered normal B-cells are demonstrated, while the neoplastic cells are negative.
9 of 9 protocols based on mAb clone 11E3 provided an insufficient result due to a too weak or completely false negative staining reaction in both the plasmacytoma and the precursor B-ALL.

Problem:

Less successful primary Ab

mAb clone 11E3

Lymphoma panel: CD79a

Optimal protocol settings (NQC)

CD79a	Retrieval buffers	Titer	Detection systems	RTU	Detection
mmAb **JCB117**	**HIER High pH or Low pH buffer**	**1:25-1:600**	**2&3-step**	**Dako/Agilent (IR621)** **Dako/Agilent (GA621)**	**Flex** **Flex+**
rmAb **SP18**	**CC1**	**1:300-1:500**	**2&3-step**	**Ventana (790-4432)**	**UltraView** **OptiView**

Tonsil and Appendix/Colon is recommended as positive and negative control:

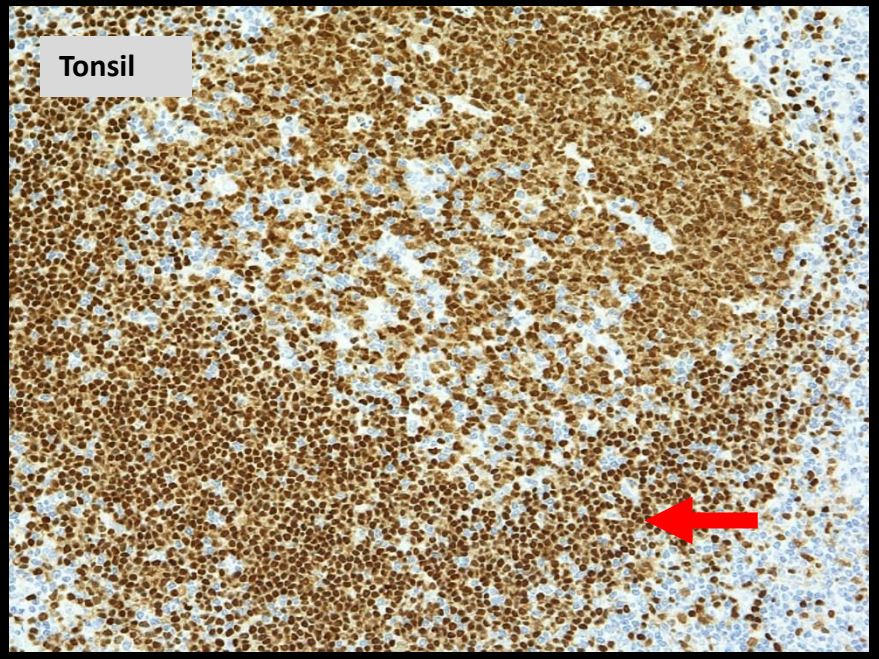
A strong, distinct membranous staining reaction of B-cells in the mantle zone in the tonsil

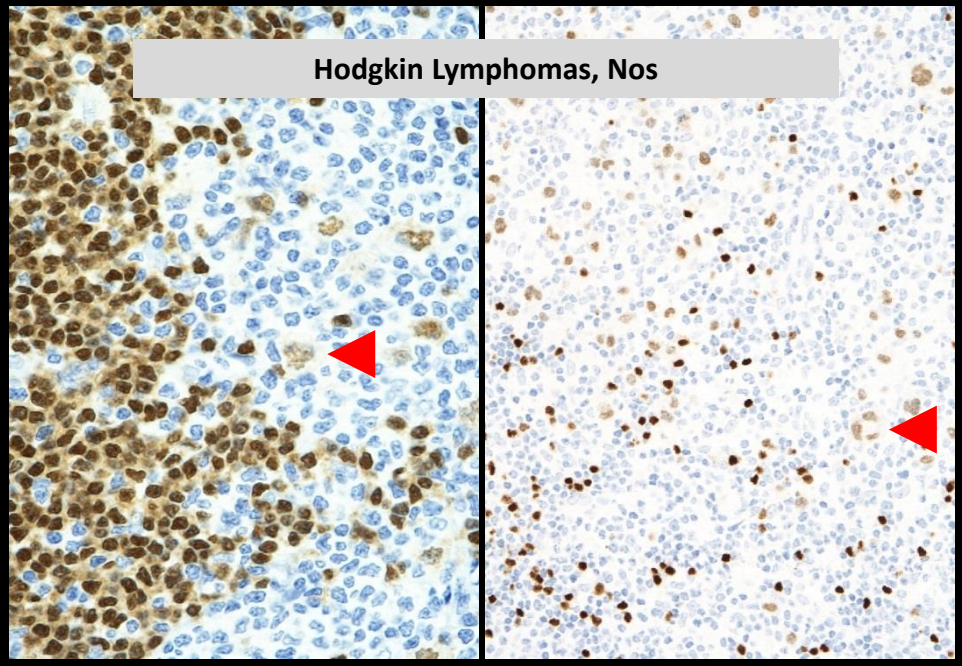
A moderate staining reaction of germinal centre B-cells

Plasma cells should show a strong cytoplasmic staining reaction

Epithelial cells in the appendix/colon should be negative

PAX-5





A moderate to strong, nuclear staining of virtually all the mantle zone B-cells, the germinal centre B-cells and the interfollicular B-cells in the tonsil.

<u>In addition:</u>

The majority of the Hodgkin and Reed-Sternberg cells in Hodgkin lymphomas often displays a weak nuclear reaction in the neoplastic cells.

PAX5 (Run 53)

Table 1. **Antibodies and assessment marks for BSAP, run 53**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1EW**	9	Leica/Novocastra	7	2	0	0	-	-
mAb clone **24**	6 2	BD Biosciences Immunologic	3	2	1	2	-	-
mAb clone **BC/24**	2	Biocare Medical	0	2	0	0	-	-
mAb clone **MX017**	1	Immunologic	1	0	0	0	-	-
~~mAb clone **ZR007**~~	~~1~~	~~Biogenex~~	~~0~~	~~1~~	~~0~~	~~0~~		
mAb clone **DAK-Pax5**	23	Agilent/Dako	15	7	0	1	96%	100%
rmAb clone **BSR59**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **BV6**	1	Diagnostic Biosystems	1	0	0	0	-	-
rmAb clone **EP156**	1	Cell marque	1	0	0	0	-	-
rmAb clone **SP34**	12 3 2	Cell Marque Thermo Scientific Spring Biosciences	4	11	2	0	88%	100%
pAb **RB-9406**	3	Thermo Scientific	0	0	1	2	-	-
Ready-To-Use antibodies								
mAb clone **1EW PA0552**	5	Leica/Novocastra	2	2	1	0	-	-
mAb clone **BC/24 PM207**	1	Biocare Medical	1	0	0	0	-	-
mAb clone **24 312M-18**	1	Cell marque	0	1	0	0	-	-
mAb clone **MX017 MAB-0706**	1	Maixin	1	0	0	0	-	-
mAb clone **MX017 MAD-000694QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **DAK-Pax5 IS/IR650**	23	Agilent/Dako	19	3	1	0	96%	100%
mAb clone **DAK-Pax5 IS/IR650**[3]	3	Agilent/Dako	3	0	0	0	-	-
mAb clone **DAK-Pax5 GA650**	24	Agilent/Dako	24	0	0	0	100%	100%
mAb clone **DAK-Pax5 GA650**[3]	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **EP156 8500-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **RBT-PAX5 BSB 5862**	1	BioSB	0	0	0	1	-	-
rmAb clone **SP34 790-4420**	33	Ventana	3	23	7	0	79%	75%
rmAb clone **SP34 312R-18**	35	Cell Marque	2	25	8	0	77%	100%
Total	198		92	79	21	6	-	
Proportion			46%	40%	11%	3%	86%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

Most common primary Abs
mAb DAK-Pax5 and rmAb SP34

rmAb SP34 as LD or RTU assays:

Low proportion of optimal results
- **Poor signal to noise ratio**

AS or Omnis (optimal results):
HIER in TRS pH9 or TRS pH 6.1 (10-20 ` at 95-99C), primary Ab Inc (15-30`), Flex/Flex+

Benchmark Ultra/XT/GX (optimal results):
HIER in CC1 (32-90`), primary Ab Inc (16-44`), UV+ amp or OV

Optimal (DAK-Pax5)

Insufficient (DAK-Pax5)

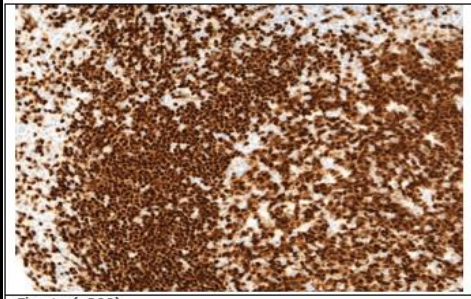
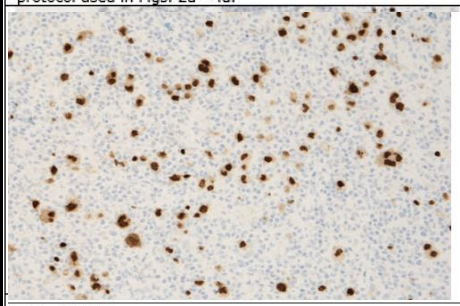


Fig. 1a.(x200)
Optimal BSAP staining reaction of the tonsil using the mAb clone DAK-Pax5, optimally calibrated, HIER in TRS (3-1) pH 9 (Dako) and a 3-step polymer based detection system (Flex+/Dako).
All mantle zone and germinal centre B-cells show a strong and distinct nuclear staining reaction. Cytoplasmic staining reaction in positive B-cells must be accepted. No staining reaction is observed in other cellular structures including T-cells. Same protocol used in Figs. 2a - 4a.

Fig. 1b.(x200)
Insufficient staining of BSAP in the tonsil using the mAb clone DAK-Pax5, too diluted, HIER in TRS (3-1) pH 6 (Dako) and the less sensitive detection system Flex (Dako) – same field as in Fig. 1a.
B-cells of the mantle zone and germinal centres only display weak to moderate staining intensity of the nuclei(compare with Fig. 1a). Same protocol used in Figs. 2b - 4b.



ig. 3a. (x200)
)ptimal BSAP staining of the Hodgkin Lymphoma (classical ype) using same protocol as in Figs. 1a and 2a. The vast najority of Hodgkin and Reed-Sternberg cells, intermingling etween B- and T-cells, show a moderate to strong but distinct uclear staining reaction.

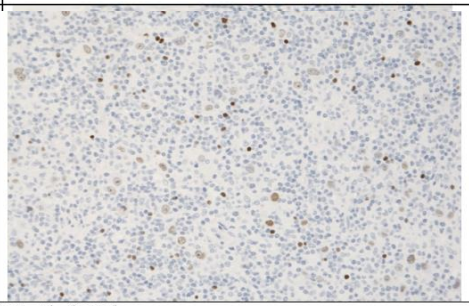
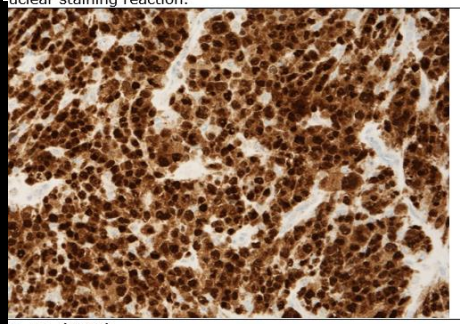


Fig. 3b. (x200)
Insufficient BSAP staining of the Hodgkin Lymphoma (classical type) using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The neoplastic cells are only faintly demonstrated and a proportion of Hodgkin and Reed-Sternberg cells are false negative.



ig. 4a. (x200)
)ptimal BSAP staining of the DLBCL using same protocol as in igs. 1a – 3a. All the neoplastic cells display a strong and istinct nuclear staining reaction. Cytoplasmic staining reaction f the neoplastic cells must be accepted.

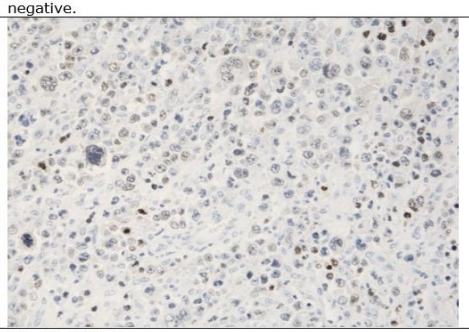


Fig. 4b. (x200)
Insufficient BSAP staining of the DLBCL using same protocol as in Figs. 1b and 3b - same field as in Fig. 4a. The staining intensity of the nuclei´s are barely visible and a significant proportion of the neoplastic cells are false negative.

The most frequent causes of insufficient staining reactions were:

- **Too low concentration of the primary antibody**
- **Use of low sensitivity detection systems**
- **False positive staining reaction or poor signal-to-noise ratio of assays based on the rmAb SP34**

Insufficient (rmAbSP34)

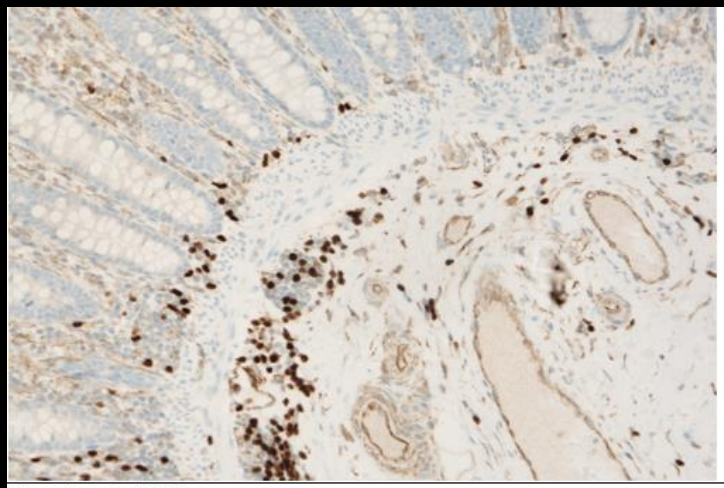


Fig. 5a. (x200)
Insufficient BSAP staining of the colon. The protocol was based on the rmAb clone SP34 as RTU format (790-4420, lot. no. Y18596, Ventana), HIER in CC1 and OptiView (Ventana) as the detection – same protocol used in Fig. 5b, but with a different lot. no. (both slides stained in a NQC reference lab.). Typical reaction pattern seen with the rmAb 34. The B-cells show the expected nuclear staining reaction, but vast majority of strom cells (e.g. endothelia cells) displays an unacceptable aberrant cytoplasmic staining reaction providing a poor signal-to-noise ratio.

Sufficient (rmAbSP34)

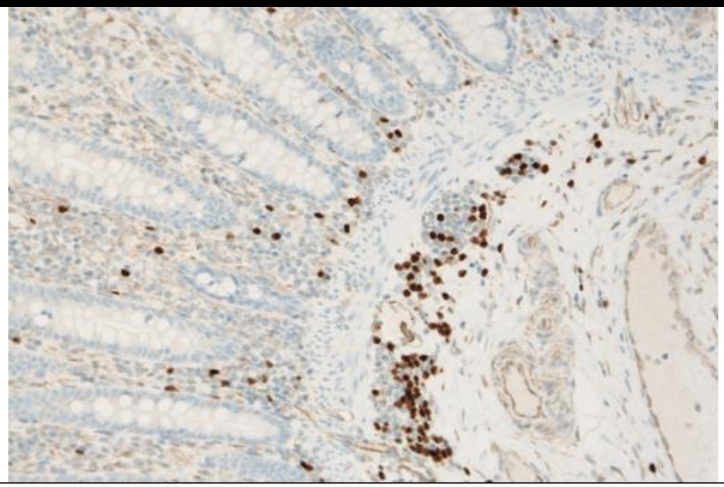


Fig. 5b.(x200)
Sufficient BSAP staining (good) of the colon using the same protocol as in Fig. 5a, but with lot.no. Y05958 (primary Ab). It has been observe from NQC reference labs, but also seen in this assessment, that there are lot-to-lot variation of

NordiQC ref. Lab:

Lot to lot variations ?

The most frequent causes of insufficient staining reactions were:

- **Too low concentration of the primary antibody**
- **Use of low sensitivity detection systems**
- **False positive staining reaction or poor signal-to-noise ratio of assays based on the rmAb SP34**

Lymphoma panel: PAX5 (most common markers)
Optimal protocol settings (NQC)

PAX5	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **DAK-PAX5**	**HIER High pH, mod. & standard low pH**	**1:20-1:100**	**2 & 3-step**	**Dako (IS/IR/GA650)**	**Flex/ Flex+**
rmAb **SP34**	**HIER High pH**	**1:50-1:100**	**2 & 3-step**	**Ventana (790-4420)**	**UltraView + Amp OptiView**
mmAb **1EW**	**HIER High pH & standard low pH**	**1:25-1:50**	**2 & 3-step**	**Leica (PA0552)**	**BOND Refine**
mmAb **24**	**HIER High pH**	**1:20-1:50**	**2 & 3-step**	**-**	**-**

Control material / Tonsil or Appendix:

A distinct moderate to strong nuclear staining reaction of virtually all mantle zone B-cells, germinal centre B-cells and interfollicular peripheral B-cells in the tonsils and appendix.

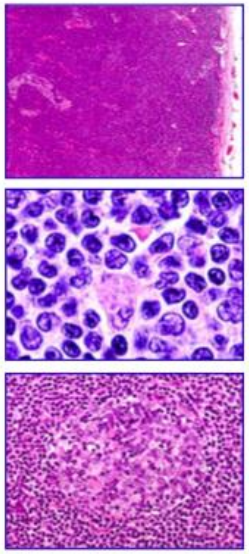
No staining reaction of other cells, including T-cells, squamous epithelial cells of the tonsils and columnar epithelial cells of the appendix.

Tech tip: Use Hodgkin Lymphoma`s in the calibration phase

Immunophenotype: Small B-Cell Lymphomas

	CD20	CD79A	CD10	CD23	CD5	CD43	bcl-2	CyclinD1	TdT
CLL	+	+	-	+	+	+	+	-	-
FL	+	+	+	-	-	-	+	-	-
MCL	+	+	-	-	+	+	+	+	-
LPL	+	+	-	-	-	-/+	+	-	-
MZL	+	+	-	-	-	-/+	+	-	-
SMZ	+	+	-	-	-	-/+	+	-	-
MALT	+	+	-	-	-	-/+	+	-	-
HCL	+	+	-	-	-	-	+	-	-
BLB	-/+	+	+/-	+/-	-	-	+	-	+

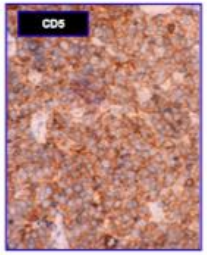
Mantle Cell Lymphoma

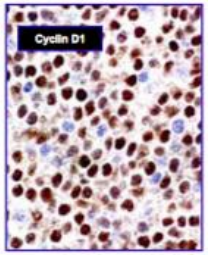


Morphology
- small-medium lymphocytes
- cleaved / irregular
- blastoid variant
- nodular / mantle / diffuse

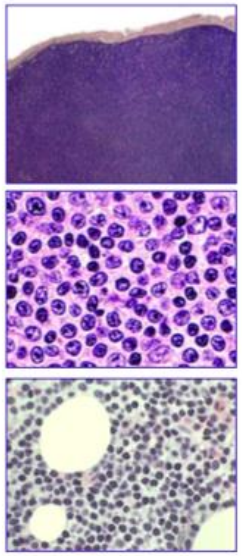
Immunology

surface Ig	+
CD19, 20, 22, 79a	+
CD5	+
CD23	-
Cyclin D1	+
CD10	-





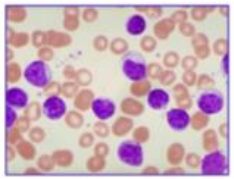
B-cell Small Lymphocytic Lymphoma (CLL)

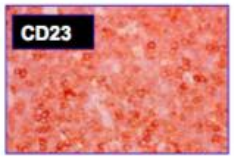


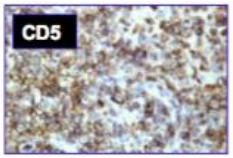
Morphology
- small lymphocytes
- proliferation centres

Immunology

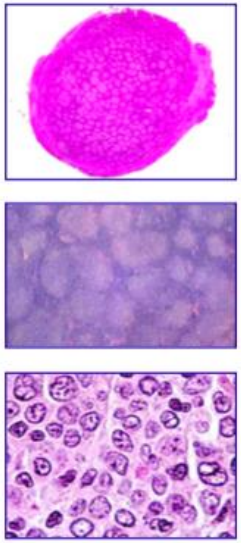
surface IgMD weak	
CD19, 20, 79a	+
CD5	+
CD23	+
CD10, CycD1	-







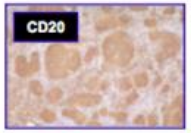
Follicular Lymphoma

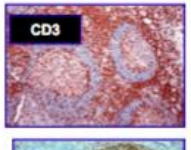


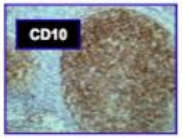
Morphology
- germinal centre cells
- CBs & CCs
- follicular

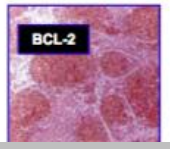
Immunology

surface Ig	+
CD19, 20, 22, 79a	+
BCL-2	+
CD10	+/-
Bcl-6	+
CD5	-









Courtesy: Steve Hamilton-Dutoit

B-Cell lymphoma markers (2)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
BCL2 (cytopl. + nuclear) 124, 100/D5, BCL/100/D5, 100	Tonsil/Appendix	Mantle zone B-cells & T-cells (including intra germinal centre T-cells)	Basal cells (squamous epithelium) in surface epithelium of the tonsil & columnar cells lining basal compartment of the crypts (appendix)	Germinal centre B-cells (tonsil)
CD10 (cytopl. + membr.) 56C6, GI191E/A8	Tonsil/Kidney	Germinal centre B-cells (Tonsil, moderate to strong intensity). Proximale tubuli (Kidney)	Scattered neutrophil granulocytes	Mantle zone B-cells and squamous epithelial cells (tonsil)
CD23 (membr.) 1B12, DAK-CD23, BS20, SP23	Tonsil	Follicular dendritic cells in the germinal centres	Mantle zone B-cells and scattered interfollicular B-cells	No staining of T-cells
CyclinD1 (nuclear) SP4, EP12	Tonsil	Suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells	Germinal centre macrophages	Mantle zone B-cells and germinal centre B-cells
SOX11 (nuclear) SOX11-C1, MRQ-58	MCL`s /Tonsil	MCL	MCL	Tonsil (all cells)
CD43 (membr.) DF-T1	Tonsil/Appendix	T-cells in the T-zone (tonsil)	Intra germinal centre T-cells (an at least moderate expression) , macrophages (tonsil, germinal centres) and activated B-cells (Ig pos)	Mantle zone B-cells of germinal centres (tonsil) and epithelium (app.)
CD5 (see T-cells) & TdT (see blasts/bonus material)				

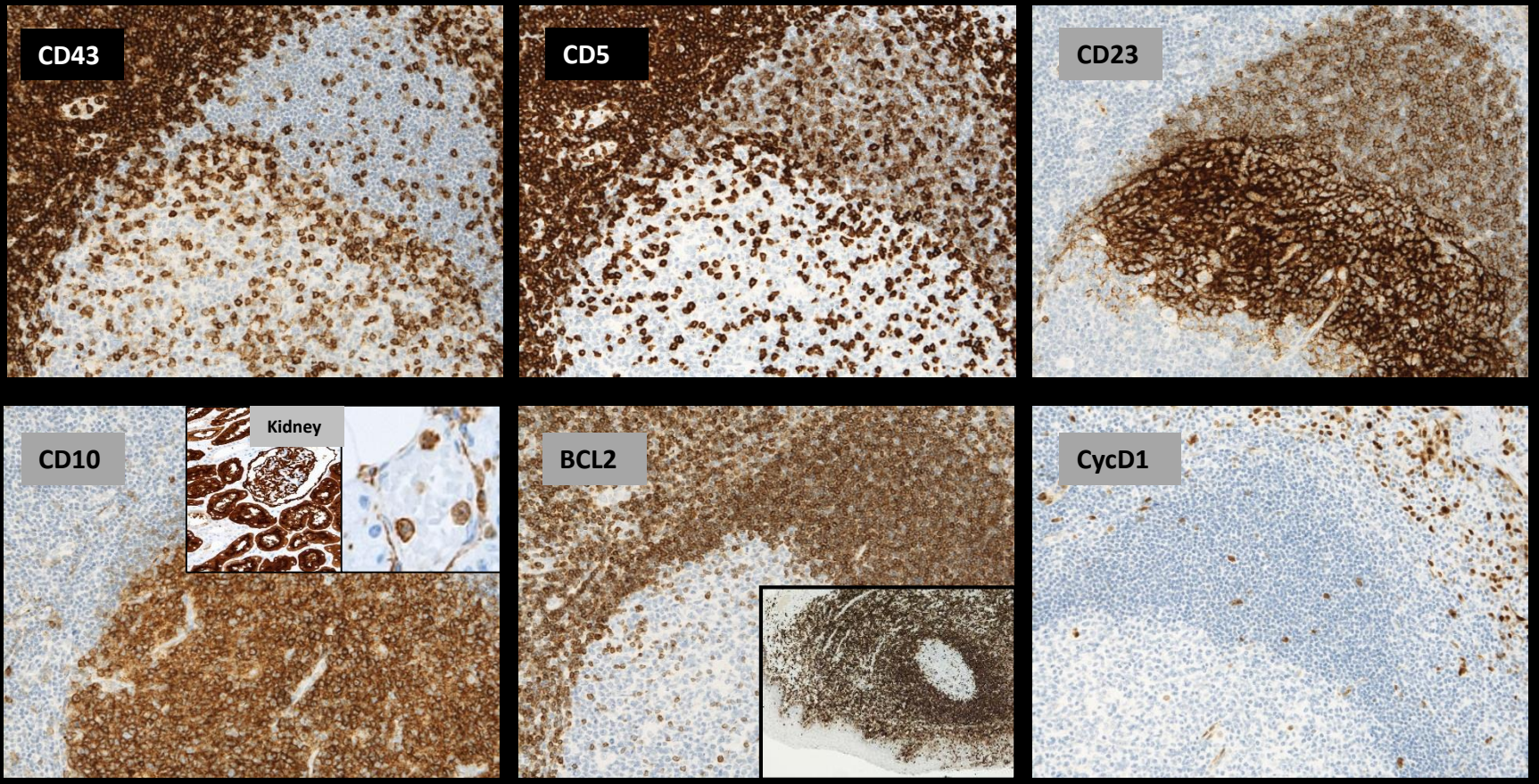
Clones (mAbs, rmAbs & pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

B-Cell lymphoma markers (2)



Tonsil

CD5 and CD43 are in principal T-cell markers, but very helpful in classification of small B-cell lymphomas (low grade)

BCL2

Table 1. Abs and assessment marks for Bcl-2, run 28

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **124**	98 1	Dako Cell Marque	49	35	15	0	85 %	86 %
mAb clone **100/D5**	5 1 1 1	NeoMarkers Biocare Immunologic Master Diagnostica	2	5	1	0	89 %	100 %
mAb clone **bcl-2/100/D5**	5	Novocastra	3	1	0	1	80 %	-
mAb clone **100**	2	BioGenex	2	0	0	0	-	-
mAb clone **3.1**	2	Novocastra	0	2	0	0	-	-
mAb clone **Bcl-2-100**	1	Zymed	0	0	1	0	-	-
mAb clone **8C8**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **124, IR614**	14	Dako	10	4	0	0	100 %	100 %
mAb clone **124, 760-4240**	18	Ventana/Cell Marque	0	8	9	1	44 %	-
mAb clone **124, MON-RTU1011**	1	Monosan	0	0	1	0	-	-
mAb clone **bcl-2/100/D5, PA0117**	2	Leica	2	0	0	0	-	-
mAb clone **100/D5, PM003**	1	Biocare	0	1	0	0	-	-
mAb clone **100/D5, 760-2693**	1	Ventana	0	1	0	0	-	-
Total	155		68	58	27	2	-	-
Proportion			44 %	38 %	17 %	1 %	82 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal Protocols

HIER preferable in alkaline buffer (high pH)

Careful calibration of primary Ab

3-step detection systems

Insufficient results

Low concentration of the primary Ab

Platform dependent mAb clone 124

BCL-2

mAb clone 124: The staining result was influenced by the platform used for the staining.

LD assay (mAb clone 124)	Pass Rate`s (%)
Ventana Benchmark	50% (21 of 42)
Dako Autostainer	97% (59 of 61)

Only 10% (4 of 42) were assessed as optimal on the Ventana Benchmark platform and optimal protocols were based on high concentration of the Ab (1:10 – 1:20), efficient HIER by Standard CC1, and UltraView + amplification as the detection system.

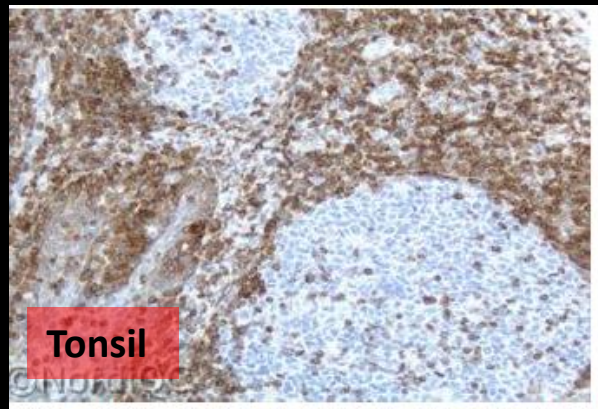
RTU assay (mAb clone 124)	Pass Rate`s (%)	Optimal (%)
Ventana Benchmark (760-4240)	44% (8 of 18)	0% (0 of 18)
Dako Autostainer (IR614)	100% (14of 14)	71% (10 of 14)

HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system.

RTU format (Ventana/Cell Marque) - No optimal results

Vendor protocol recommendations: HIER in CC1 (Standard), 16 min inc in primary Av and UltraView as the detection system.

BCL-2



ig. 2a. High magnification of the optimal Bcl-2 staining of the onsil shown in Fig. 1a. The scattered T-cells within the erminal centre show a distinct staining and also the basal quamous epithelial cells (left) show a weak to moderate taining. Same protocol as in Fig. 1a.

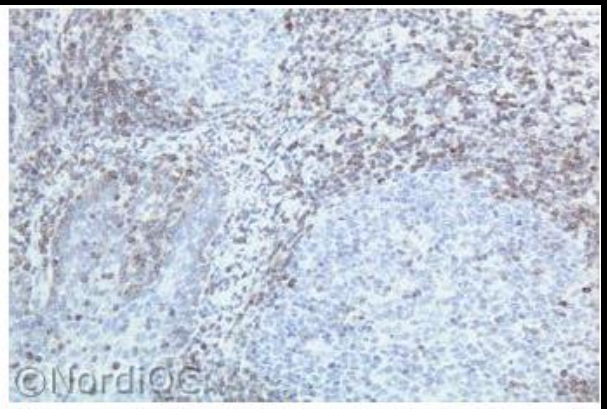
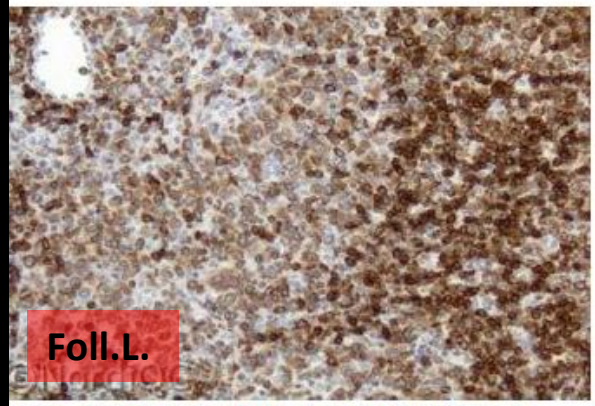


Fig. 2b. High magnification of the insufficient Bcl-2 staining of the tonsil shown in Fig. 1b – same field as in Fig. 2a. Only the grouped peripheral lymphocytes show a distinct staining, while the germinal centre T-cells and the basal squamous epithelial cells virtually are negative. Same protocol as in Fig. 1b.



ig. 3a. Optimal Bcl-2 staining of the follicular lymphoma rade III using same protocol as in Figs. 1a & 2a. Virtually all he neoplastic show a moderate staining, while the remnants f the normal lymphocytes (right) show a strong staining.

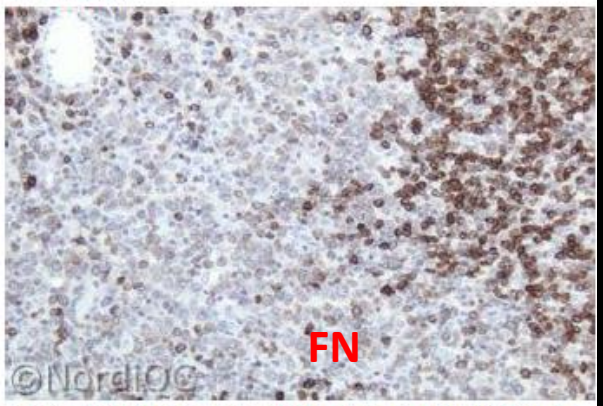


Fig. 3b. Insufficient Bcl-2 staining of the follicular lymphoma grade III using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. The normal lymphocytes show a moderate staining, while the neoplastic cells only show a weak, equivoca staining.

Problem:

Protocol with too low sensitivity

mAb clone 124

Too low conc of the primary Ab

Lymphoma panel: BCL-2

Optimal protocol settings (NQC)

BCL-2	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **124**	**HIER High pH & mod. Low pH**	**1:10-1:400**	**2 & 3-step**	**Dako (IS503/IR503)**	**Flex/ Flex+**
mmAb **100/D5**	**HIER High pH**	**1:20-1:40**	**3-step**	**Leica (PA0117)**	**BOND Refine**
mmAb **BCL2/100/D5**	**HIER High pH & mod. Low pH**	**1:50-1:140**	**2 & 3-step**	**-**	**-**
mmAb **100**	**HIER High pH**	**1:200-1:1200**	**2 & 3-step**	**-**	**-**

Control material / Tonsil:

A moderate to strong predominantly cytoplasmic staining of virtually all the peripheral B- and T-cells in the tonsils.

An at least weak cytoplasmic staining of the basal squamous epithelial cells of the tonsil.

No staining reaction in the germinal centre B-cells.

CD23

Table 1. **Antibodies and assessment marks for CD23, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B12**	51 3 2 2 2	Leica/Novocastra Cell Marque Biocare Thermo F. Scientific Monosan	22	27	8	3	82%	87%
mAb clone **DAK-CD23**	12	Agilent/Dako	5	4	2	1	75%	100%
mAb clone **BS20**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **MRQ-57**	1		0	0	1	0	-	-
mAb clone **MHM6***	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **SP23**	25 3 3 1 1	Thermo S./ Neomarkers Spring Bioscience Cell Marque Immunologic Diagnostic Biosystems	20	9	4	0	88%	90%
Ready-To-Use antibodies								
mAb clone **1B12 PA0169**	9	Leica/Novocastra	8	0	1	0	89%	100%
mAb clone **1B12* PA0169**	3	Leica/Novocastra	0	2	1	0	-	-
mAb clone **1B12 123M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **1B12 PM100**	1	Biocare	0	1	0	0	-	-
mAb clone **1B12 PDM143**	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone **DAK-CD23 IR781**	31	Agilent/Dako	24	5	1	1	94%	92%
mAb clone **DAK-CD23 IR781***	7	Agilent/Dako	3	4	0	0	100%	-
mAb clone **DAK-CD23 GA781**	15	Agilent/Dako	14	1	0	0	100%	100%
mAb clone **DAK-CD23 GA781***	1	Agilent/Dako	0	1	0	0	-	-
rmAb clone **SP23 790-4408**	78	Roche/Ventana	43	34	1	0	99%	99%
rmAb clone **SP23 123R-17/18**	5	Cell Marque	3	1	1	0	80%	100%
rmAb clone **SP23 MAD-00333QD**	3	Master Diagnostica	2	0	0	1	-	-
rmAb clone **SP23 M3231**	2	Spring Bioscience	0	2	0	0	-	-
rmAb clone **SP23 RMA-0504**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP23 IR800***	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **EP75 123R-27/28**	1	Cell Marque	1	0	0	0	-	-
pAb **AR460-5/10R**	1	Biogenex	0	0	0	1	-	-
Total	269		148	92	22	7	-	
Proportion			55%	34%	8%	3%	89%	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below. 3) RTU system developed for the Leica/Novocastra full-automatic system (BOND III/MAX) but used by laboratories on e.g. a Ventana Benchmark Ultra (Roche/Ventana), 4) RTU system developed for the Agilent/Dako semi-automated systems (Autostainer) but used by laboratories on the Omnis (Agilent/Dako). 5) RTU used in a manual assay.
*Product has been discontinued by the vendor.

Optimal protocols:

HIER in alkaline buffer

HIER in mod. Low pH buffer (Dako) /DAK-CD23

3-step pol./mul. Detection systems.

Careful calibration of the primary Ab.

HIER in BERS2/1 (10-20 min/95-100℃), BOND refine

HIER in TRS pH 6.1 (30 min/97℃), Flex/Flex+

HIER in CC1 (24- 98 min/95-100 ℃), Ultra/OptiView with or without amp.

Best performance:

RTU clone 1B12 (PA0169, Leica)

RTU clone DAK-CD23 (IR/GA781, Dako)

RTU format SP23 (790-4408, Ventana)

Table 3. **Proportion of optimal results for CD23 for the most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **1B12**	4/6** (67%)	-	2/4	-	7/19 (37%)	-	8/10 (80%)	0/2
mAb clone **DAK-CD23**	0/3	3/3	-	-	0/1	-	2/3	-
rmAb clone **SP23**	1/1	-	0/1	1/1	10/17 (59%)	0/1	3/3	1/1

* 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)

Table 4. **Proportion of sufficient and optimal results for CD23 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb **IR781**	100% (7/7)	100% (7/7)	92% (22/24)	71% (17/24)
Dako Omnis mAb **GA781**	100% (7/7)	100% (7/7)	100% (4/4)	75% (3/4)
Leica BOND MAX/III mAb **PA0169**	100% (4/4)	100% (4/4)	80% (4/5)	80% (4/5)
VMS Ultra/XT rmAb **790-4408**	100% (3/3)	0% (0/3)	99% (71/72)	59% (43/72)

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

UltraView

mAb clone 1B12 challenging on the Ventana Benckmark

Optimal results:

Efficient HIER in CC1 , high conc. of the primary Ab (1:10-20), 3-step mul. detection system

Alternative: Use SP23

Optimal results:

Efficient HIER in CC1 and the use of a 3-step mul. detection system (UltraView with amp. or OptiView)

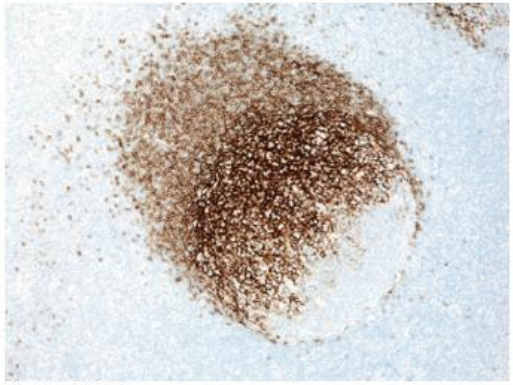


Fig. 1a (x100)
Optimal staining reaction for CD23 of the tonsil using the mAb clone 1B12 as concentrate, careful calibrated (1:10), HIER in an alkaline buffer (CC1, Ventana) and a 3-step multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 3a. The majority of B-cells in the mantle zone show a moderate but distinct membranous staining reaction. The follicular dendritic cells of the germinal centres display a strong staining reaction - compare with Fig.1b.

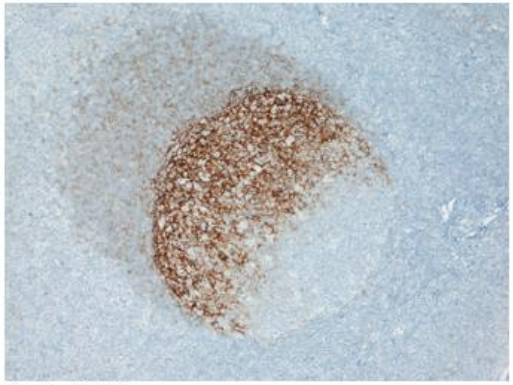


Fig. 1b (x100)
Insufficient staining reaction for CD23 of the tonsil using the mAb clone 1B12 as concentrate (too diluted, 1:50), HIER in CC1 and with a too low sensitive detection system (UltraView, Ventana) - same protocol used in Figs. 2b - 3b. The intensity of the staining reaction is significantly reduced and the majority of B-cells in the mantle zone show an equivocal staining reaction - compared with Fig. 1a (same field).

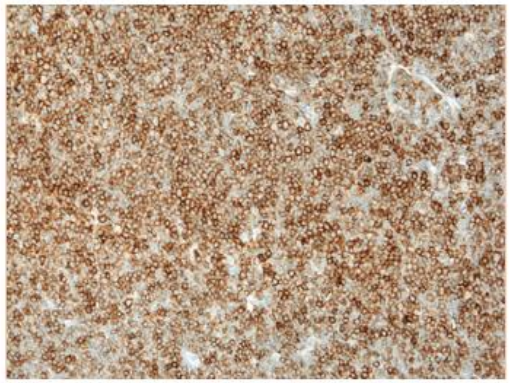


Fig. 3a (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1a and 2a. The vast majority of the neoplastic cells show a strong membranous staining reaction – compare with Fig. 3b.

Fig. 3b (x200)
Insufficient staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.
The majority of the neoplastic cells displays reduced staining intensity and a significant proportion of neoplastic cells are false negative.

mAb clone 1B12 (Ventana Benchmark Ultra)

Problem

Too diluted

Too low sensitive detection system

Fig. 2a (x100)
Optimal staining reaction for CD23 of the mantle cell lymphoma using the same protocol as in Fig. 1a. The neoplastic cells are negative and only remnants of the follicular dendritic cell meshwork show a strong staining intensity - compare with Fig. 2b.

Fig. 2b (x100)
Insufficient staining reaction for CD23 of the mantle cell lymphoma using same protocol as in Fig. 1b - same field as in Fig. 2a. The intensity of the staining reaction is significant reduced. The follicular dendritic cell meshwork is barely visible.

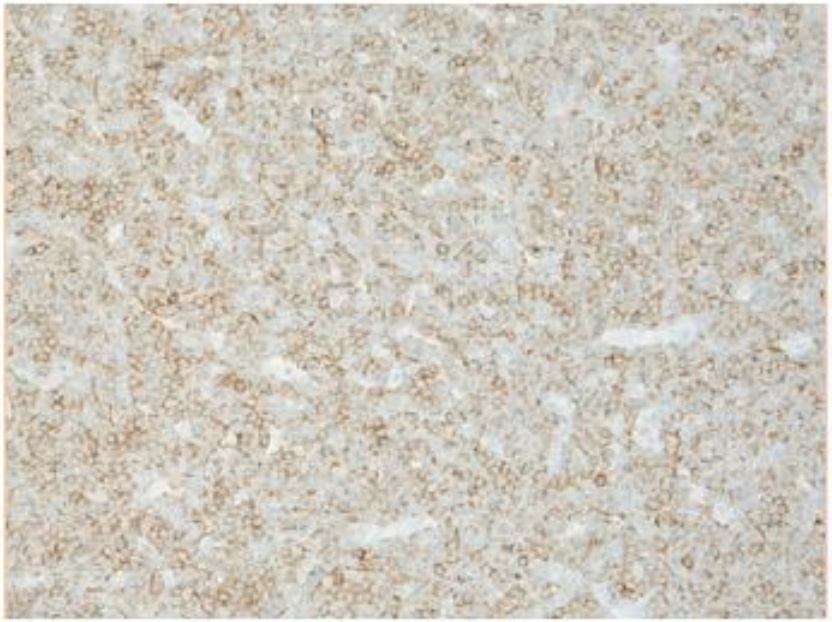


Fig. 4a (x200)
Good staining reaction for CD23 of the B-CLL, tissue core no. 5, using the rmAb clone SP23 in a RTU format (790-4408, Benchmark, Ventana), HIER in CC1 and with a 2-step multimer detection system (UltraView). Although the majority of the neoplastic cells show a weak to moderate distinct membranous staining reaction, the system can be optimized – see Fig. 4b.

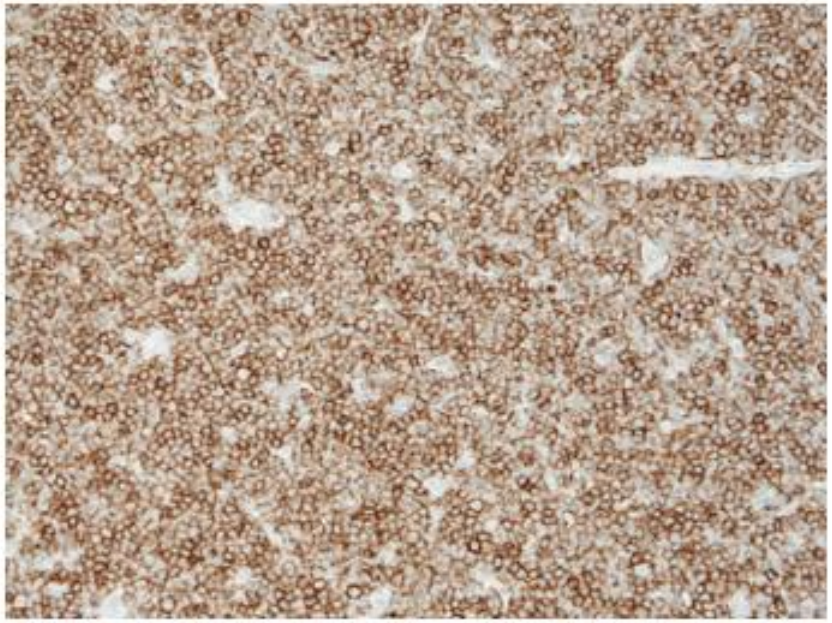


Fig. 4b (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 5, using the same system as in Fig. 4a but with a 3-step multimer detection system (OptiView) – same field as in Fig. 4a.
Virtually all neoplastic cells show a strong membranous staining reaction. For this RTU system, the use of OptiView or UltraView with amplification significantly increased the proportion of optimal results.

UltraView versus OptiView

Lymphoma panel: CD23

Optimal protocol settings (NQC)

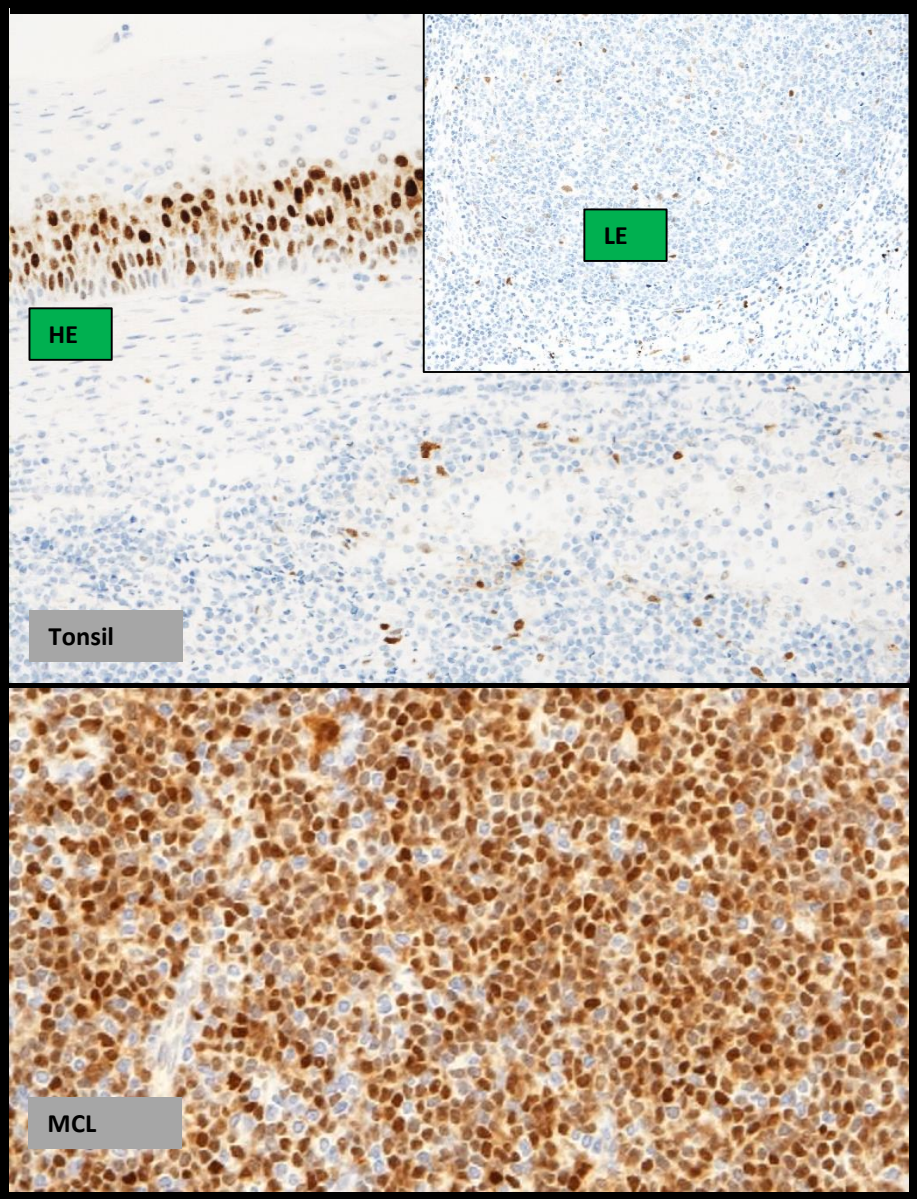
CD23	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb 1B12	HIER High pH or Low pH	1:10-1:50	3-step	Leica (PA0169)	BOND refine
rmAb SP23	HIER High pH or Low pH	1:20-1:100	3-step	Ventana (790-4408)	UltraView + Amp* OptiView
DAK-CD23	HIER mod. Low pH or High pH	1:25-1:100	3-step	Dako (IR/GA781)	Flex/Flex+

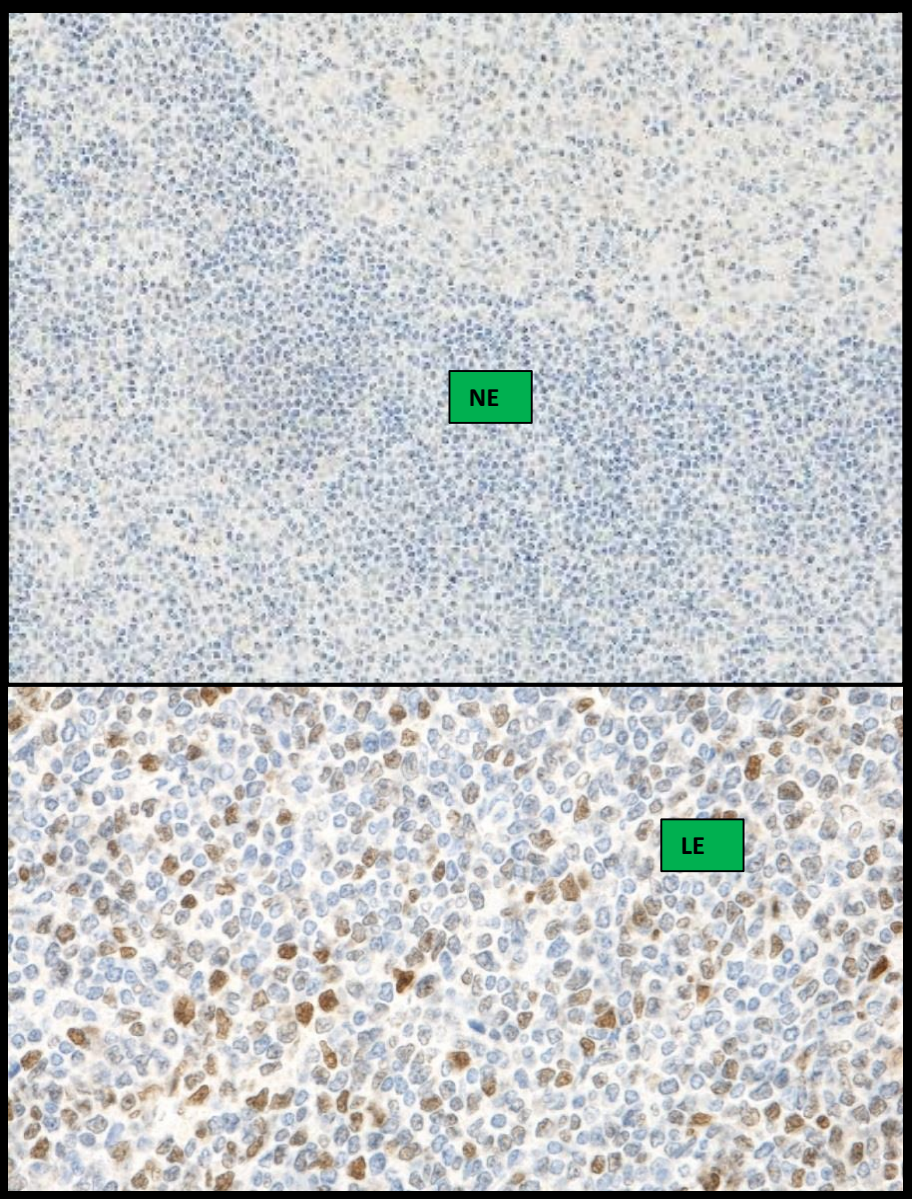
* Optimal results could also be obtained with the detection system UltraView without amplification but at overall lower frequency compared to laboratories using UltraView with amplification

Control material / Tonsil:

An at least weak to moderate, distinct membranous staining of the activated B-cells in the mantle zone of the germinal centres in the tonsils.

CyclinD1 & SOX11





Cyclin D1

Performance history

This was the fifth NordiQC assessment of CyD1. The pass rate was comparable to the previous run and maintained at a high and satisfactory level, as shown in table 2.

Table 2. **Proportion of sufficient results for CyD1 in the five NordiQC runs performed**

	Run 9 2003	Run 17 2006	Run 19 2007	Run 33 2011	Run 47 2016
Participants, n=	57	87	92	179	257
Sufficient results	53%	59%	75%	90%	94%

Increased pass rate & optimal performance

Primarily poor clones

mAb DCS6
mAb P2D11F11
pAbs

Primarily robust rabbit monoclonal Abs

rmAb EP12
rmAb SP4

CycD1

Table 1. **Antibodies and assessment marks for CyD1, run 47**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **P2D11F11**	4	Leica/Novocastra	0	2	2	0	-	-
rmAb clone **EP12**	13 1 1	Dako/Agilent Cell Marque Epitomics	8	6	1	0	93%	98%
rmAb clone **SP4**	69 6 5 4 2 1 1 1 1	Thermo/Neomarkers Cell Marque Biocare Spring Bioscience Zytomed Immunologic Maixin Nordic Biosite Thermo/Pierce	36	45	6	3	90%	92%
Unknown	1	Eptitomics	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **P2D11F11 RTU-CYCLIN D1-GM**	1	Leica/Novocastra	0	1	0	0	-	-
rmAb clone **EP12 IR/IS083**	57	Dako/Agilent	33	23	1	0	98%	100%
rmAb clone **EP12 MAD-000630QD**	3	Master Diagnostica	1	2	0	0	-	-
rmAb **EP12 PME432**	1	Biocare	1	0	0	0	-	-
rmAb **EP12 PA0046**	1	Leica/Novocastra	0	1	0	0	-	-
rmAb clone **EPR2241(IHC)-32 AN474**	1	Biogenex	0	1	0	0	-	-
rmAb clone **SP4 790-4508**	72	Ventana/Roche	54	17	1	0	99%	100%
rmAb clone **SP4 760-4282***	5	Cell Marque/Ventana	5	0	0	0	-	-
rmAb clone **SP4 IR152***	2	Dako	0	2	0	0	-	-
mAb clone **SP4 RM-9104-R7**	2	Thermo/Neomarkers	0	1	1	0	-	-
rmAb clone **SP4 241R-18**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP4 RMA-0541**	1	Maixin	1	0	0	0	-	-
Total	257		140	102	12	3	-	
Proportion			54%	40%	5%	1%	94%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
*discontinued products

Optimal (rmAb EP12 & SP4)

Efficient HIER in alkaline buffer (20 min)

1:20-1:200 (EP12)

1:20-1:150 (SP4)

2 & 3 step detection systems

Insufficient results

Too low concentration of the primary antibody

Less successful primary antibody

Unexplained technical issues

Table 3. **Proportion of optimal results for CyD1 for the most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer / Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
rmAb clone **EP12**	4/5** (80%)	-	3/5 (60%)	-	1/2	-
rmAb clone **SP4**	20/41** (64%)	0/1	11/31 (49%)	-	2/15 (13%)	0/1

* 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)

Bond™ Polymer Refine Detection
Catalog No: DS9800

Intended Use

This detection system is for *in vitro* diagnostic use.

Bond Polymer Refine Detection is a biotin-free, polymeric horseradish peroxidase (HRP)-linker antibody conjugate system for the detection of tissue-bound mouse and rabbit IgG and some mouse IgM primary antibodies. It is intended for staining sections of formalin-fixed, paraffin-embedded tissue on the Bond™ automated system.

The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls. They should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

The Bond Polymer Refine Detection Kit must be used with laboratory best practice in the use of tissue controls. For assurance, laboratories should stain each patient sample in conjunction with positive, negative, and other tissue specific controls as needed.

Summary and Explanation

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using Bond Reagents" in your Bond user documentation).

Bond Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates non-specific staining as a result of endogenous biotin.

Bond Polymer Refine Detection works as follows:

- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity.
- A user-supplied specific primary antibody is applied.
- Post Primary IgG linker reagent localizes mouse antibodies.
- Poly-HRP IgG reagent localizes rabbit antibodies.
- The substrate chromogen, 3,3'-Diaminobenzidine tetrahydrochloride hydrate (DAB), visualizes the complex via a brown precipitate.
- Hematoxylin (blue) counterstaining allows the visualization of cell nuclei.

Using Bond Polymer Refine Detection in combination with the Bond automated system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

The detection system Bond Refine acts by nature as a 2 step polymer system for detection of rabbit polyclonal or rabbit monoclonal primary antibodies

Only enhances mouse primary antibodies due to the Post Primary IgG linker (Rabbit antibody)

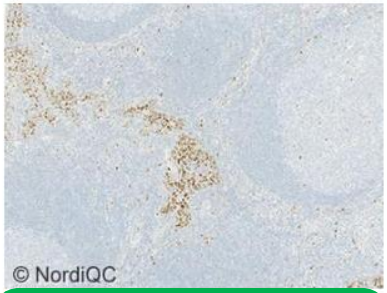


Fig. 1a
Optimal staining for Cyclin D1 of the tonsil, tissue core no. 1, using the rmAb clone SP4-R as Ready-To-Use format (Ventana prod. no. 790-4508) using HIER in CC1 for 64 min. and UltraView as detection system.
Even at low power field squamous epithelial cells, dispersed endothelial cells and germinal centre macrophages can be identified.
Also compare with Figs. 2a - 4a, same protocol.

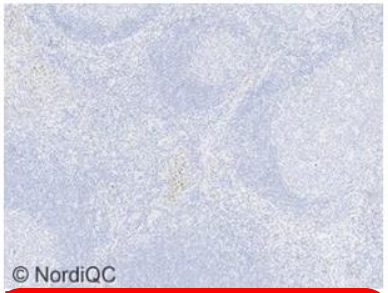


Fig. 1b
Insufficient staining for Cyclin D1 of the tonsil, tissue no. 1, using the rmAb clone SP4 by a laboratory developed assay giving a too low sensitivity (too low. conc. of the primary Ab) - same field as in Fig. 1a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 1a.
Also compare with Figs. 2b - 4b, same protocol.

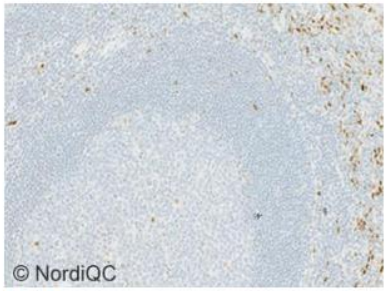


Fig. 2a
Optimal staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1a. High power field x200.
Virtually all squamous epithelial cells, dispersed endothelial cells and germinal centre macrophages show a moderate to strong nuclear staining reaction. The vast majority of lymphocytes are negative and no background staining is seen.

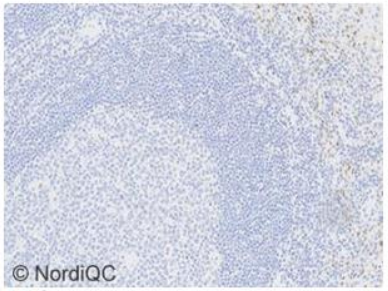


Fig. 2b
Insufficient staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1b - same field as in Fig. 2a.
Only scattered squamous epithelial cells show a weak and equivocal staining reaction, while endothelial cells and germinal centre macrophages are negative.
Also compare with Fig. 3b, same protocol.

Too low concentration of the primary Ab

Fig. 3a
Optimal staining for Cyclin D1 of the mantle cell lymphoma, tissue core no. 4, using same protocol as in Figs. 1a & 2a.
Virtually all the neoplastic cells show a distinct, moderate to strong nuclear staining reaction.

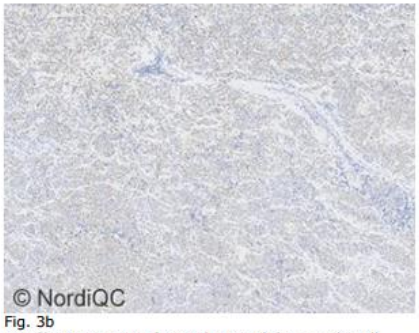


Fig. 3b
Insufficient staining for Cyclin D1 of the mantle cell lymphoma, tissue core no. 4, using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result expected and obtained in Fig. 3a.

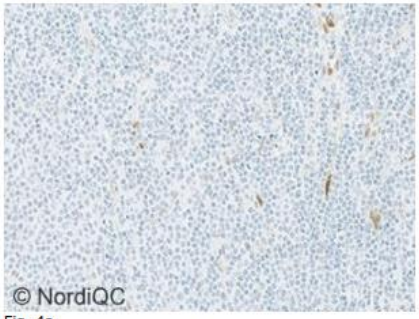


Fig. 4a
Optimal staining for Cyclin D1 of the B-CLL using same protocol as in Figs. 1a - 3a.
The neoplastic cells are negative, while scattered endothelial cells show a moderate nuclear staining reaction serving as internal positive tissue control.

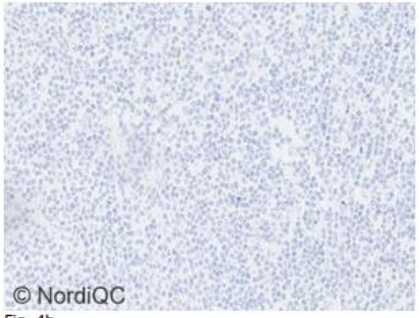


Fig. 4b
Staining for Cyclin D1 of the B-CLL using same insufficient protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
No staining is seen.

Lymphoma panel: CyD1

Optimal protocol settings (NQC)

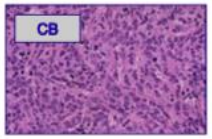
CyD1	Retrieval buffers	Titre	Detection	RTU	Detection
rmAb EP12	HIER High pH	1:20-1:200	2 & 3-step	Dako (IS/IR083)	Flex/Flex+
				Biocare (PME432)	MACH4
rmAb SP4	HIER High pH	1:20-1:150	2 & 3-step	Ventana (790-4508)	UltraView +/- Amp OptiView

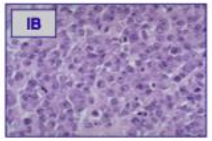
Control material / Tonsil:

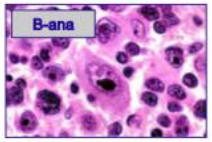
A moderate to strong, distinct nuclear staining reaction of virtually all suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells

An at least weak, distinct nuclear staining reaction of germinal centre macrophages

Diffuse Large B-cell Lymphoma



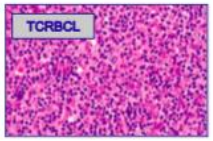


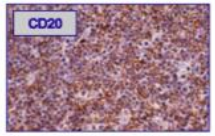


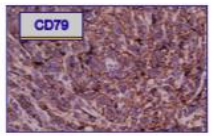
Morphology
- large cells
- nucleoli
- diffuse

Immunology

surface Ig	+/-
cytoplasmic Ig	-/+
CD19, 20, 22, 79a	+
CD30	-/+
CD38, CD138	pc
CD5	10%
CD10	40%
bcl6	79%
mum1	50%



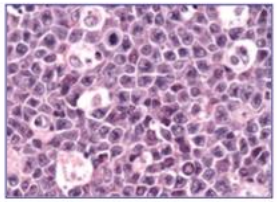


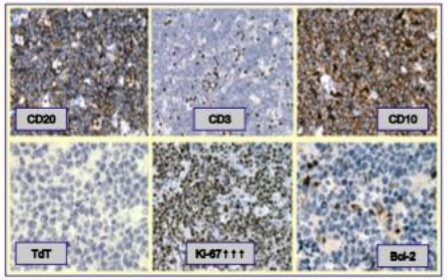


Courtesy: Steve Hamilton-Dutoit

Immunophenotyping in Gray zone B-NHL

	CD20	CD79a	CD5	CD10	CD23	Ki67	TdT	bcl-2	CyclinD1
Diffuse large B	+	+	-/+	-/+	-	<90%	-	+/-	-
Burkitt	+	+	-	+	-	>95%	-	-	-
Blastic mantle cell	+	+	+	-	-	<90%	-	+/-	+
B lymphoblastic	+	+	-	+	-	<90%	+	+/-	-
Blastic myeloma	-	+	-	-	-	<90%	-	+/-	-/+





- DLBCL-like morphology
- BL-like immunophenotype ($BCL2^{nuc}$)
- ↑↑ proportion of double-hit B-NHL (e.g. c-myc / bcl-2 rearranged

DLBCL - 'cell of origin':
Competing IHC classifiers

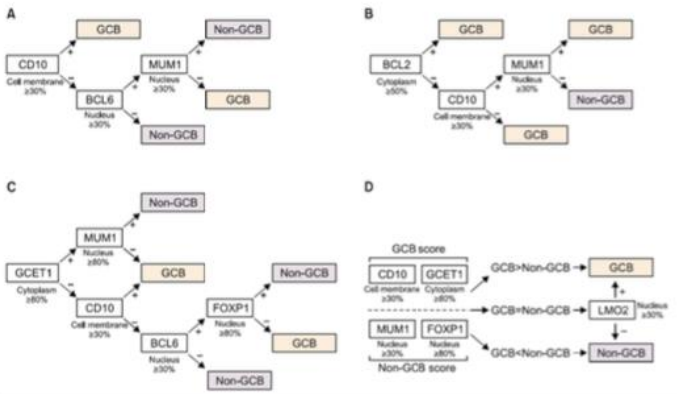


Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different from that of the other algorithms (more than 30%).

Diffuse Large B-cell Lymphoma (DLBCL)

- Differential diagnosis / Gray zone B-NHL

- IHC classification (subtypes/GC versus non-GC) and prognosis

BCL6
MUM1
CD138
Ki67

FOXP1
GCET1
CMYC

B-Cell lymphoma markers (3) - Diffuse Large B-Cell Lymphoma

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
BCL6 (nuclear) LN22, PG-B6p, SP18	Tonsil	Germinal centre B-cells	Squamous epithelial cells	The vast majority of cells in the mantle zones and interfollicular areas
MUM1 (nuclear). MUM1p, EAU32, EP190	Tonsil/Colon	Late stage germinal centre B-cells (tonsil) Plasma cells (tonsil & colon)	"Mantle zone B-lymphocytes (tonsil) "	Epithelia cells and smooth muscle cells (lamina muscularis propria) in the colon.
CD138 (membr.) B-A38, B-B4, MI15	Tonsil	Plasma cells and squamous epithelial cells	Activated germinal centre B-cells	Mantle zone B-cells and T-cells
Ki67 (nuclear) MIB-1, BS4, GM001, K2, UMAB107, 30-9, SP6	Tonsil/lLiver	All germinal centre B-cells (dark zone) in the tonsil	Most germinal centre B-cells (light zone) in the tonsil	99% of "normal" hepatocytes should be negative
FOXP1 (nuclear) EP137	Tonsil/Liver	Virtually all mantle zone B-cells T-cells are positive	App. 50% of germinal centre B-cells in the tonsil (moderate intensity) T-cells are positive	The vast majority of hepatocytes are negative
GCET1 (cytopl) RAM341	Tonsil	Intra germinal centre B-cells (centroblast) – moderate to strong intensity	None	All other cells including T-cells
CMYC (nuclear) EP121	Tonsil/appendix	Activated intragerminal centre B-lymphocytes and scattered lymphocytes in interfollicular zones	App. 10-50 % of the mantle zone B-cells. Suprabasal squamous epithelial cells in the tonsil often displays moderate intensity.	Luminal epithelia cells of the appendix. The basal crypt epithelia cells displays moderate intensity.
CD10, see B-cell lymphoma markers (2) & TdT, see blast`s/bonus material				

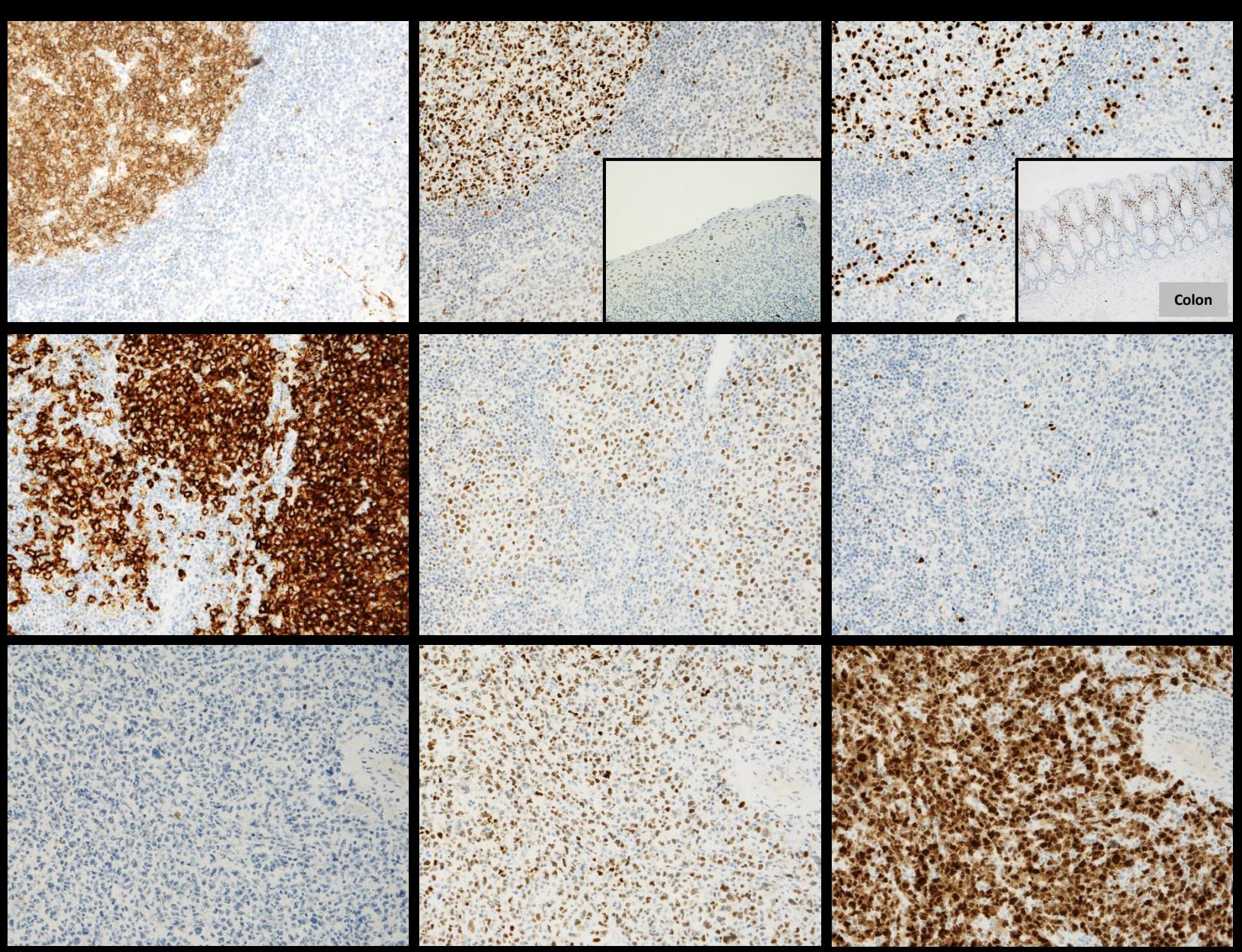
Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

DLBCL: HANS classification



BCL6

Table 1. **Antibodies and assessment marks for Bcl-6, run 42**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **GI191E/A8**	13 1 1	Cell Marque Immunologic Zytomed	6	8	0	1	93%	100%
mAb clone **LN22**	38 2 1 1 1	Leica/Novocastra DBS Biocare BioGenex Zeta Corporation	20	16	4	3	84%	100%
mAb clone **PG-B6p**	43 1 1	Dako DBS Thermo/Neomarkers	9	22	11	3	69%	86%
Ready-To-Use antibodies								
mAb clone **GI191E/A8 760-4241**	59	Ventana/Cell Marque	24	25	9	1	83%	84%
mAb clone **GI191E/A8 227M-9x**	1	Cell Marque	0	0	1	0	-	-
mAb clone **LN22 PA0204**	10	Leica/Novocastra	3	7	0	0	100%	100%
mAb clone **LN22 PM410**	1	Biocare	1	0	0	0	-	-
mAb clone **LN22 MAD-00638QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **PG-B6p IR/IS625**	44	Dako	4	17	21	2	48%	75%
mAb clone **PG-B6p GA625**	7	Dako	2	2	3	0	57%	75%
mAb **PG-B6p MAD-004023QD**	2	Master Diagnostica	0	1	1	0	-	-
Total	228		69	99	50	10	-	
Proportion			30%	44%	22%	4%	74%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal results
1) HIER in High pH buffers
2) 3-step pol./mul. Detec. systems

<u>Insufficient results</u>

Too low concentration of the primary antibody

Less successful performance of the mAb clone PG-B6p

Use of low sensitivity detection systems

LAB`s using HIER in acidic/ low pH buffers couldn`t produce an optimal result

-Less successful performance of the mAb clone PG-B6p

Table 3. **Proportion of optimal results for Bcl-6 for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **PG-B6p**	4/12** (33%)	-	1/11 (9%)	-	0/4	-
mAb clone **LN22**	2/2	-	9/16 (56%)	-	8/8 (100%)	-

* 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)

Sensitive to 3% peroxidase blocking before application of the primary Ab

- Use of a too low sensitive detection system

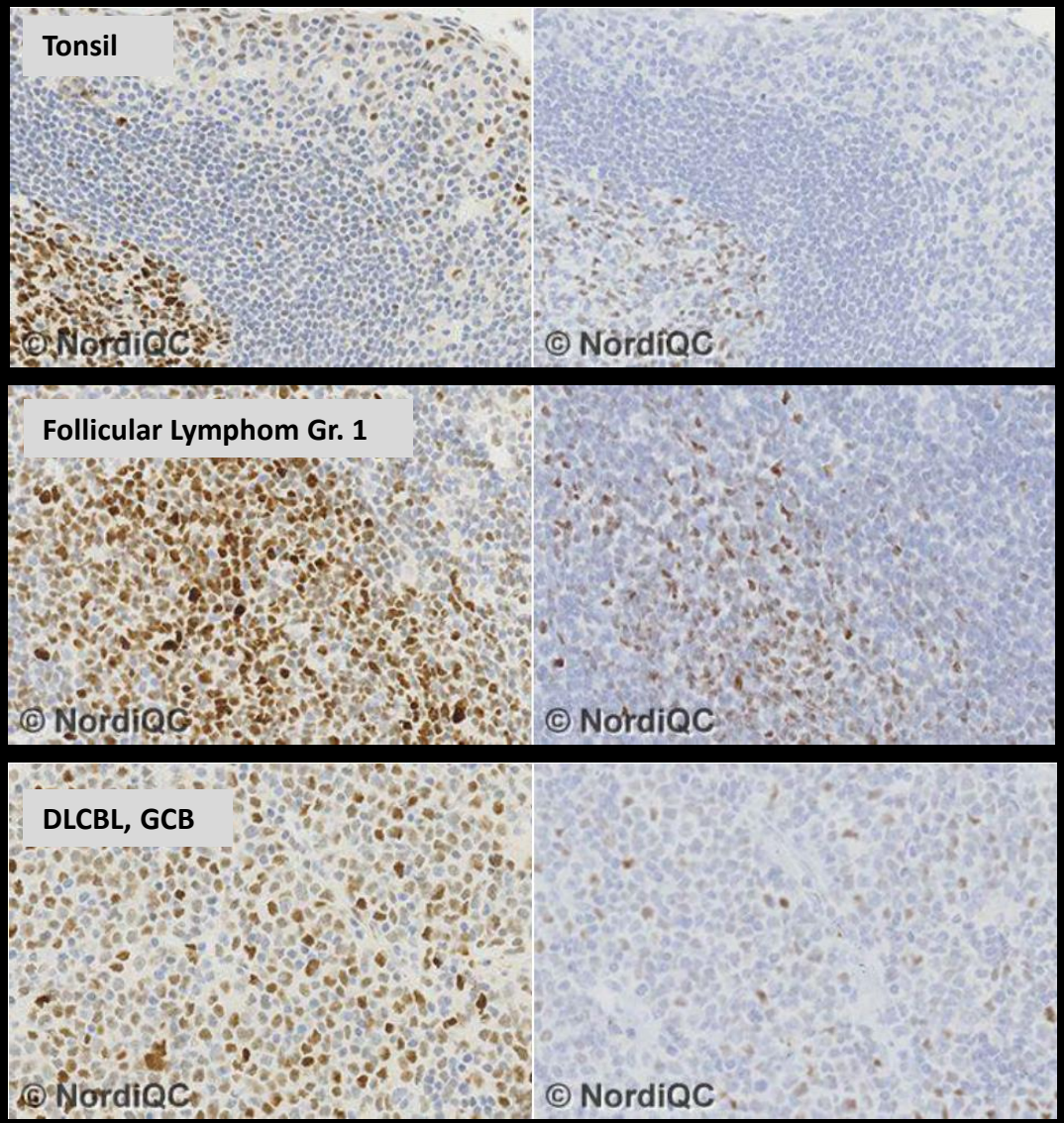
LD assay (PG-B6p, LN22 & GI191E/A8) HIER in alkaline buffer and optimal dil. range	Detection system	Pass Rate`s (%)	Optimal (%)
2-step polymer/multimer system	Flex (Dako) or UltraView (Ventana)	68 (27 of 40)	15 (6 of 40)
3-step polymer/multimer system	Flex+ (Dako), OptiView (Ventana) or BOND Refine (Leica)	93 (39 of 42)	62 (26 of 42)

BCL-6

LN22
Optimally calibrated

HIER in alkaline buffer (BERS2)

3-step polymer system
(BOND refine)



LN22
Too diluted

HIER in alkaline buffer
(TRS pH9)

2-step polymer system
(Flex)

Lymphoma panel: BCL6

Optimal protocol settings (NQC)

BCL6	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb GI191E/A8	HIER High pH	1:50-1:200	3-step	Ventana 760-4241	UltraView +/-Amp.* OptiView +/- Amp.
mmAb LN22	HIER High pH	1:25-200	3-step	Leica PA0204	BOND Refine
mmAb PG-B6p	HIER High pH	1:10-1:50	3-step	Dako IR/IS/GA 625	Flex+

* Optimal results could also be obtained with the detection system UltraView without amplification but at overall lower frequency compared to laboratories using UltraView with amplification

Control material / Tonsil:

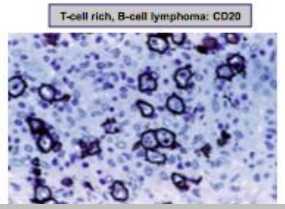
An at least weak to moderate distinct nuclear staining reaction of the majority of the squamous epithelial cells in the tonsil.

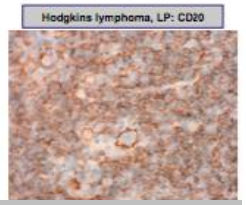
Strong nuclear staining of germinal centre B-cells

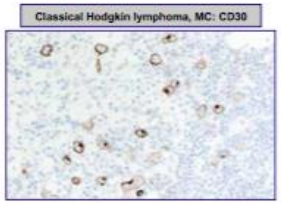
Hodgkins lymphoma: differential diagnosis

	CD20	CD79a	T-cell antigen	CD4 CD8	CD30	CD15	EMA
Nodular lymphocyte predominant HL	+	+	–	–	–/+	–	+
Classical HL	–/+	–/+	–	–	+	+	+
T-cell rich large B-cell lymphoma	+	+	–	–	–	–	–
Anaplastic large cell lymphoma	–	–	+/–	CD8>CD4> CD4&8 -ve	+	–	+

Key
+/– The lymphoma cells are commonly but not always positive
–/+ The lymphoma cells are usually but not always negative







Courtesy: Steve Hamilton-Dutoit

HL *vs* ALCL: Immunophenotype

	HL	ALK - pos T/null - ALC	ALK - neg T/null - ALC
ALK	-	+	-
EBV	> 40 %	-	-
CD30	+	+	+
CD15	ca. 90 %	< 5 %	- / +
EMA	-	ca. 50 %	ca. 50 %
PAX5	> 80 %	-	-
CD20	ca. 25 %	-	-
CD3	ca. 2 %	+ / -	+ / -
CD45	-	ca. 50 %	ca. 50 %
CD43	-	most +	most +
Granzyme/ perforin	10 – 20 %	ca. 90 %	ca. 70 %
TCR genes	G	R	R
Ig genes	R (single cell)	G	G

Marker / Neoplasm	Classical Hodgkin Lymphoma Hodgkin/Reed-Sternberg cells	Nodular lymphocytic predominantly Hodgkin lymphoma L & H (popcorn cells)
CD30	+	-/+
CD15	+/-	-
PAX5	+ (weak)	+ (strong)
BCL6	-/+	+
OCT2/BOB.1	- (both or one)	+ (both)
CD57	- (no rosettes)	+ (rosettes surrounding L & H)
EBV-EBER	+/-	-
EBV-LMP1	-/+	-

+ > 90% positive; +/- 50-90% positive; -/+ 10-50% positive; - < 10% positive.

Hodgkin Lymphoma

- Differential diagnosis
- IHC classification (subtypes) / classical HL vs N-LPHL

CD30
CD15

OCT2
BOB.1
CD57

EBV-EBER/EBV-LMP1/EBV-EBNA-2
ALK

Hodgkin lymphoma markers

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD30 (membr. + Golgi) Ber-H2, CON6D/5, 1G12, JCM182, rmAb EP154	Tonsil	None	Interfollicular activated B- and T-cells and perifollicular germinal centre B-cells (moderate intensity)	All other cells
CD15 (membr. + cytopl.) Carb-3, MMA and HI98	Tonsil/Kidney	Epithelial cells of the renal proximal tubules (predominantly membr.) Neutrophils	Follicular dendritic cells in the germinal centres (Tonsil)	All other cells
BOB.1 (nuclear + cytopl.) SP92	Tonsil	Germinal centre B-cells & plasma cells	Mantle zone B-cells	T-cells
OCT2 (nuclear) EP284	Tonsil	Germinal centre B-cells & plasma cells	Mantle zone B-cells ("moderate intensity")	"T-cells"
CD57 (membr.) TB01	Tonsil/Appendix	Intragerminal centre activated T-cells and NK-cells in the T-zone (Tonsil)	Schwann cells of peripheral nerves (ganglionic neurons) in the appendix	Epithelia cells of the Appendix. Neuroendocrine cells displays a distinct staining reaction
EBV-EBER/EBV-LMP1/EBV-EBNA2 **ALK (See markers for the Lung panel / Ole Nielsen)**				

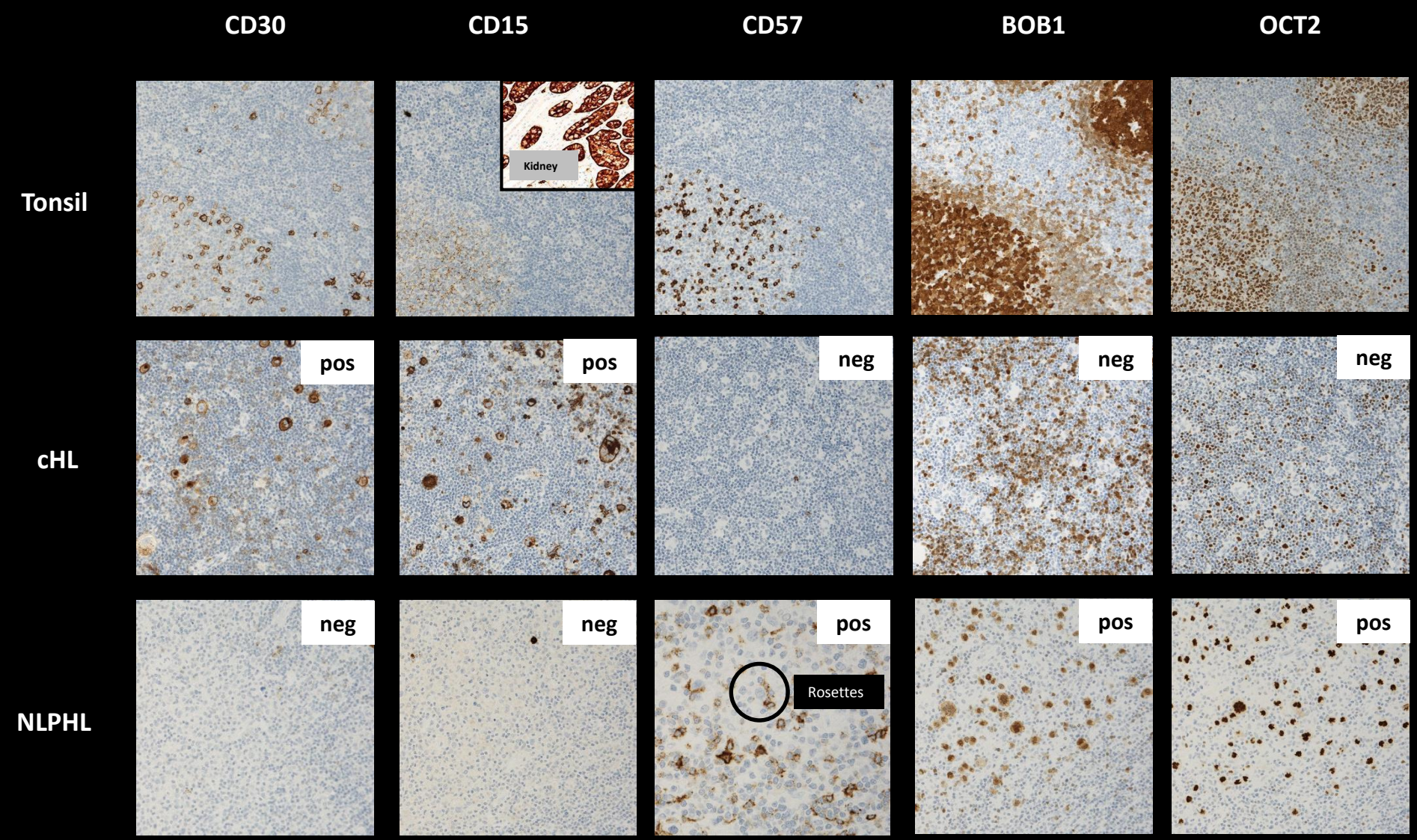
Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

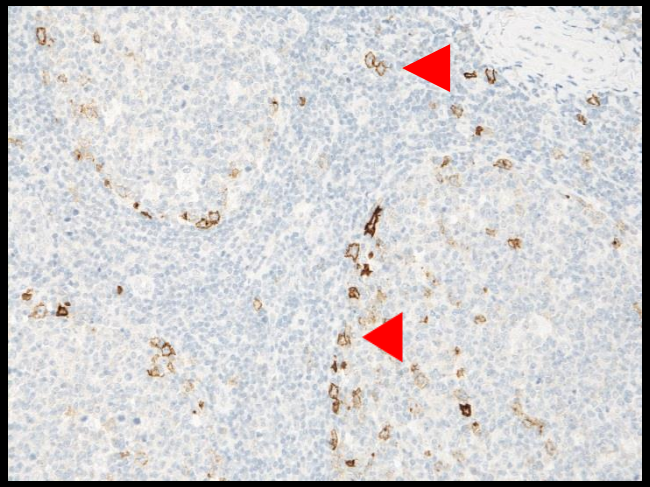
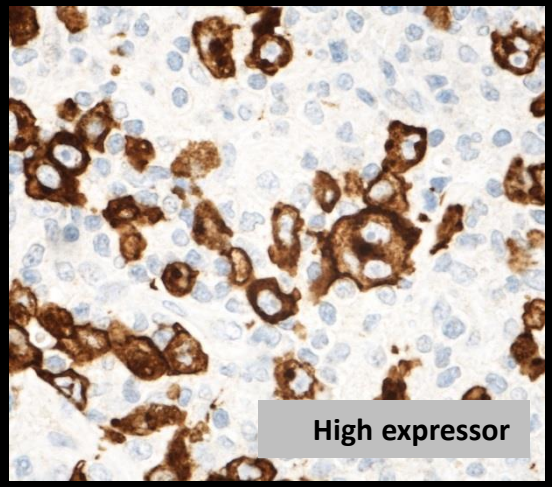
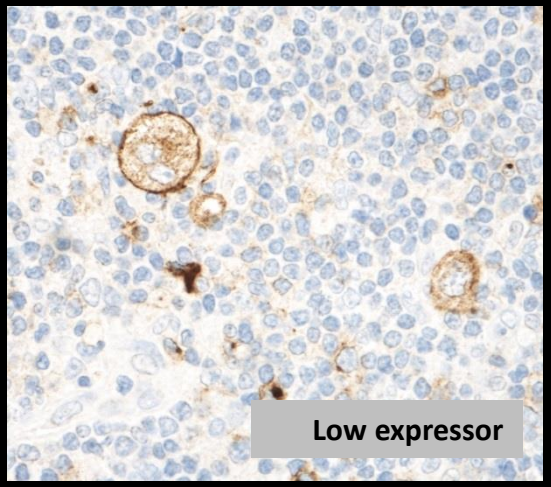
iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

Hodgkin lymphoma markers



CD30

Tonsil	Hodgkin Lymphomas, Nos	
	 High expressor	 Low expressor

An at least weak to moderate and distinct membranous staining reaction of interfollicular activated B- and T-cells and perifollicular germinal centre B-cells in the tonsil.

<u>In addition:</u>

Calibrate the assay using classical Hodgkin Lymphomas with “known” weak expression for CD30 (membranous or Golgi reaction) of the neoplastic cells.

CD30

Performance history
This was the fifth NordiQC assessment of CD30. The overall pass rate increased compared to run 43, 2015 (see Table 2).

Table 2. **Proportion of sufficient results for CD30 in the five NordiQC runs performed**

	Run 11 2004	Run 25 2009	Run 31 2011	Run 43 2015	Run 51 2017
Participants, n=	74	126	172	252	282
Sufficient results	92%	78%	77%	71%	83%

Table 1. **Antibodies and assessment marks for CD30, run 51**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	94 10 2 2 2 2 1	Agilent/Dako Cell Marque Thermo S./Neomarkers Diagnostic Biosystems Immunologic Zytomed Systems Nordic Biosite	53	41	13	6	83%	84%
mAb clone **JCM182**	10	Leica/Novocastra	6	2	1	1	80%	100%
mAb clone **1G12**	6	Leica/Novocastra	0	4	1	1	67%	-
mAb clone **CON6D/5**	5	Biocare Medical	4	0	1	0	80%	100%
mAb clone **HRS4**	1	Thermo Scientific	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **Ber-H2 IS/IR602**	30	Agilent/Dako	18	11	1	0	97%	96%
mAb clone **Ber-H2 IS/IR602**[3]	21	Agilent/Dako	15	4	1	1	90%	-
mAb clone **Ber-H2 790-4858**	75	Roche/Ventana	34	27	8	6	81%	87%
mAb **Ber-H2 MAD-002045QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb **Ber-H2 130M-XX**	2	Cell Marque	0	0	0	2	-	-
mAb clone **Ber-H2 MS-361-R7**	1	Thermo S. /Neomarkers	1	0	0	0	-	-
mAb clone **Ber-H2 MAB-0023**	1	Maxin	0	1	0	0	-	-
mAb clone **JCM182 PA0790**	10	Leica/Novocastra	7	2	1	0	90%	90%
mAb clone **1G12 PA0153**	3	Leica/Novocastra	0	1	2	0	-	-
mAb clone **HRS4 AM351-5/10**	1	BioGenex	0	1	0	0	-	-
mAb clone **unknown 8265-C010**	1	Sakura Finetek USA	0	0	1	0	-	-
Total	282		140	94	31	17	-	
Proportion			50%	33%	11%	6%	83%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Agilent/Dako semi-automated systems (Autostainer) but used by laboratories on the Omnis (Agilent/Dako), Ventana Benchmark XT/Ultra or manually.

Robust primary Abs:

mAb clone BER-H2
mAb clone JCM182
mAb clone CON6D/5

Optimal protocol settings

HIER in alkaline buffer
HiER in mod. Low pH buffers (TRS or Diva)

mAb clone BER-H2 (conc, dil. 1:20-1:100):

6/8 opt. (75%)~ Mod. Low pH buffers

47/94opt. (50%)~ Alkaline pH buffers

Detection System: 3-step mul./pol.

CD30 – Detection systems

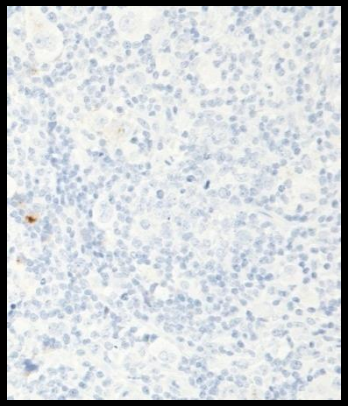
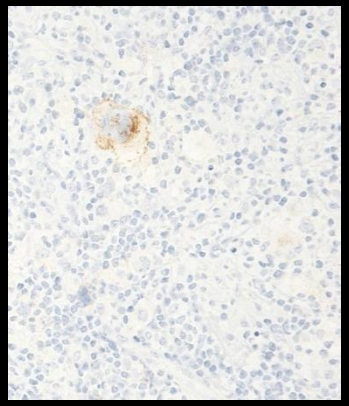
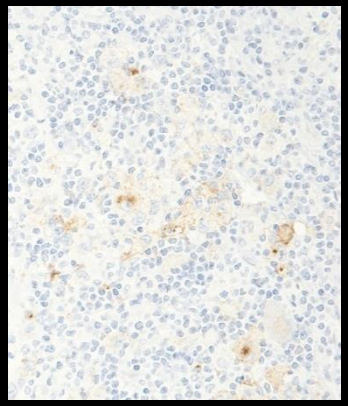
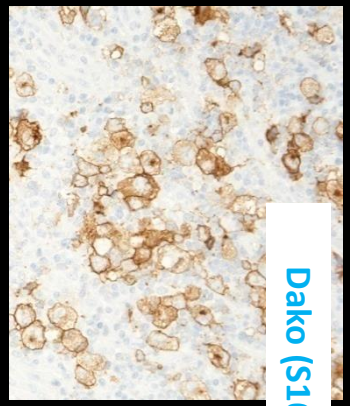
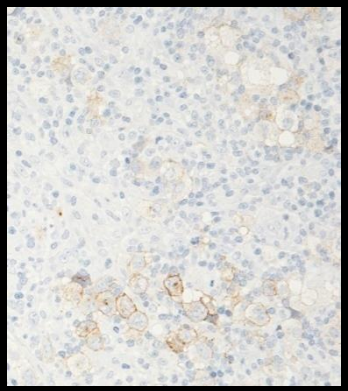
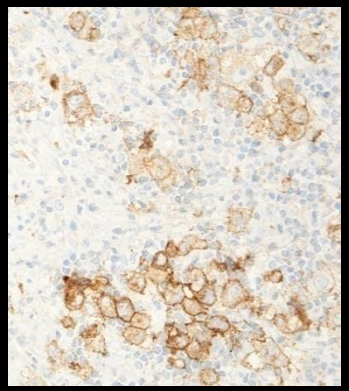
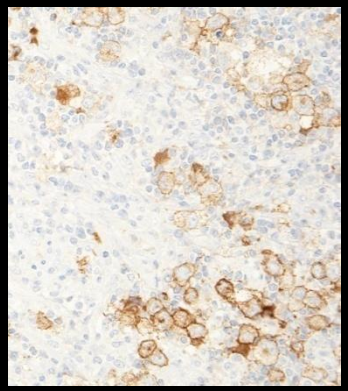
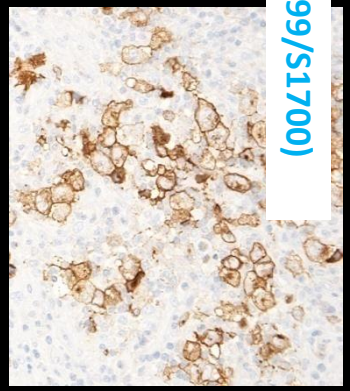
mAb BER-H2 (LD-assay):

The overall pass rate for participants using a 3-step polymer/multimer based detection system (e.g. Bond Refine (Leica), Envision Flex+ (Dako) and OptiView (Ventana)) was 87% (78 of 88) of which 53% (47 of 88) were assessed as optimal.

In comparison and for laboratories using a 2-step polymer/multimer based detection system (e.g. Envision Flex (Dako) and UltraView (Ventana)), the overall pass rate was only 59% (13 of 22) of which 18% (4 of 22) were assessed as optimal.

Modified HIER buffers (low pH) with high impact on the final result

Whish antibody - Whish antigen retrieval procedure – To whish platform

	Ci pH 6 MWO / 20 min	EDTA pH 8 MWO / 20 min	TE pH 9 MWO / 20 min	TRS pH 6.1 MWO / 20 min
CD30 Clone ConD6/B5				
CD30 Clone Ber-H2				

Dako (S1699/S1700)

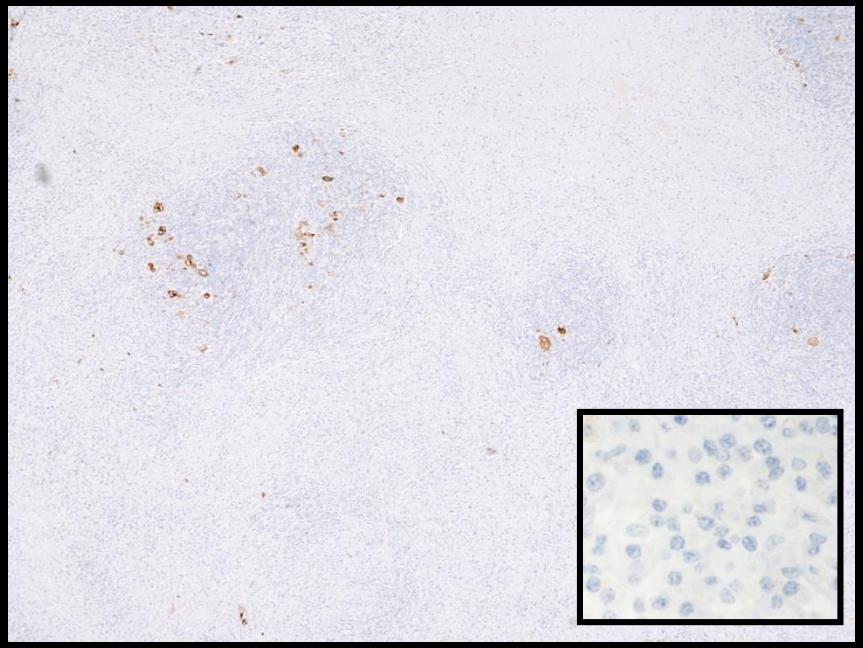
Hodgkin Lymphoma

HIER (modified low pH buffer)

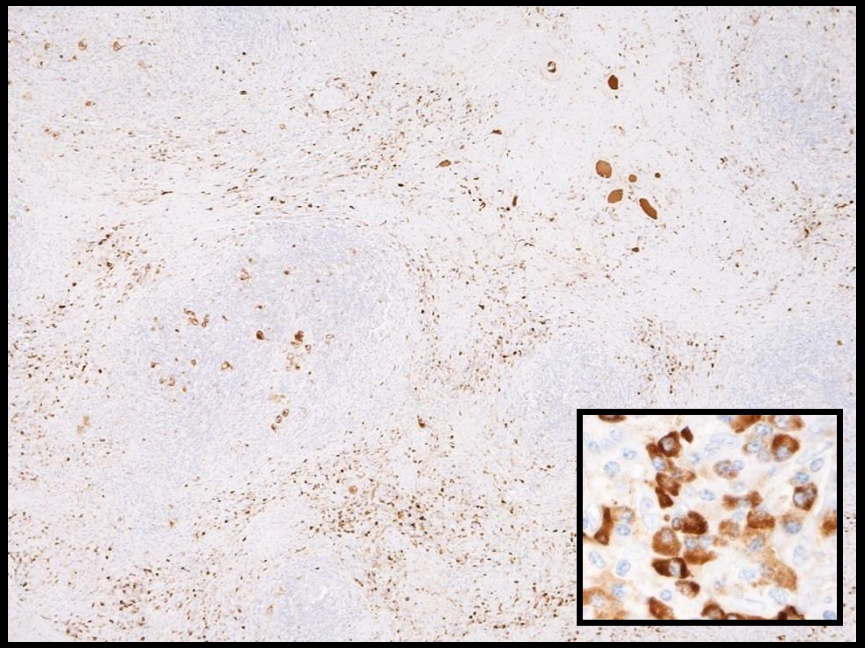
CD30

Hodgkin Lymphoma

Clone ConD6/B5



Clone Ber-H2



Note: No un-specific staining of plasma cells using the clone ConD6/B5

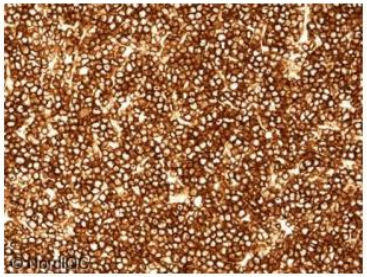


Fig. 1a (x200)
Optimal CD30 staining of the ALCL using the mAb clone CON6D/5 as concentrate, HIER in an modified low pH buffer (TRS pH 6.1, Dako) and a 3-step polymer based detection system (Flex+, Dako Omnis). Same protocol used in Figs. 2a - 5a. All neoplastic cells show a strong predominantly membranous staining reaction - compare with Fig. 1b.

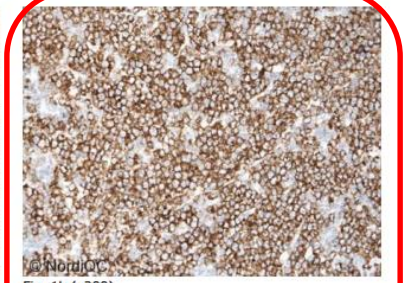


Fig. 1b (x200)
Insufficient staining for CD30 of the ALCL using the mAb clone CON6D/5 as concentrate (too diluted), HIER in Diva Decloaker solution pH 6.2 (excessive) and MACH1 (Biocare) as detection system – same protocol used in Figs. 2b – 6b. Staining intensity of the neoplastic cells are reduced - compare with Fig. 1a (same field), but also with Fig. 2a-5b.

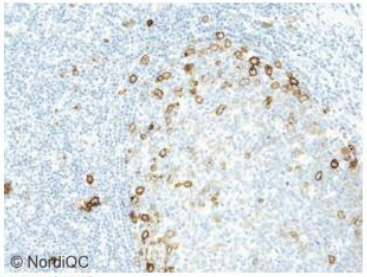


Fig. 2a (x200)
Optimal staining for CD30 in the tonsil, tissue core no 2, using same protocol as in Fig. 1a. The activated B- and T-cells, particularly B-cells located at the rim of the germinal centres, show a moderate to strong predominantly membranous staining reaction.

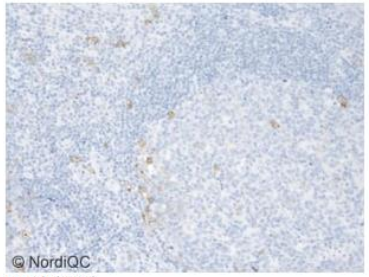


Fig. 2b (x200)
Insufficient staining for CD30 in the tonsil, tissue core no 2, using same protocol as in Fig 1b. The proportion of activated B- and T-cells is significantly reduced and staining intensity is too weak - compare with Fig. 2a (same field).

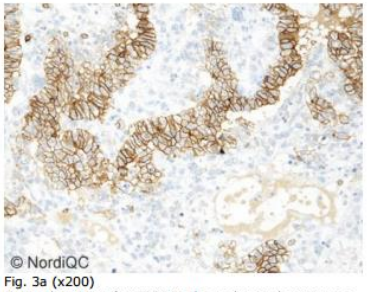


Fig. 3a (x200)
Optimal staining for CD30 in the embryonal carcinoma using same protocol as in Fig. 1a. All the neoplastic cells displays a strong continuous membranous staining reaction.

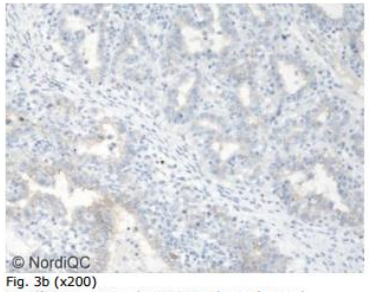


Fig. 3b (x200)
Insufficient staining for CD30 in the embryonal carcinoma using same protocol as in Fig. 1b. The neoplastic cells are false negative or only display a faint inconsistent membranous staining reaction - compare with Fig. 3a (same field).

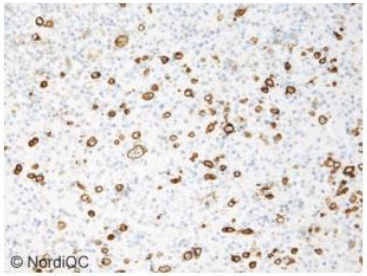


Fig. 4a (x200)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 5, using same protocol as in Fig. 1a - 3a. Virtually all the neoplastic cells show a strong predominately membranous staining reaction.

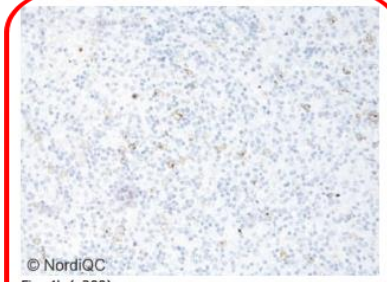


Fig. 4b (x200)
Insufficient staining for CD30 of the Hodgkin lymphoma, tissue core no 5, using same protocol as in Fig. 1b -3b. Staining intensity of the neoplastic cells is too weak or false negative - compare with Fig. 4a (same field).

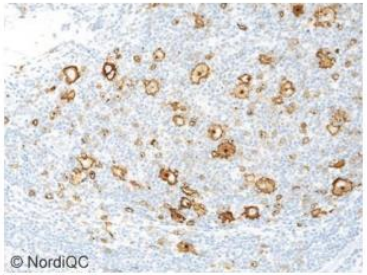


Fig. 5a (x200)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using same protocol as in Fig. 1a - 4a. Virtually all Reed-Sternberg and Hodgkin cells show a moderate to strong, distinct membranous and cytoplasmic dot-like staining pattern.

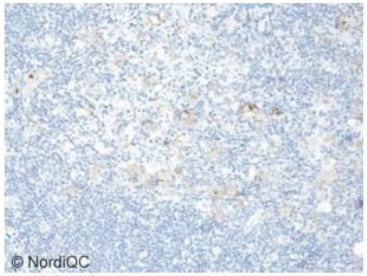


Fig. 5b (x200)
Insufficient staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using same protocol as in Fig. 1b - 4b. The Reed-Sternberg and Hodgkin cells only display weak, inconsistent membranous staining reaction. In addition, the cytoplasmic dot-like staining reaction of the Reed-Sternberg and Hodgkin cells is weak and proportion of positive cells is significantly reduced - compare with Fig. 5a (same field).

CD30 clone CON6D/5 (HIER mod. Low pH buffers)

Protocol providing too low sensitivity (right and in red frame) - Too diluted and the use of a low sensitive detection system)

Lymphoma panel: CD30

Optimal protocol settings (NQC)

CD30	Retrieval buffers	Titre	Detection	RTU	Detection
mAb **BER-H2**	**HIER High pH & mod. Low pH**	**1:20-1:100**	**3-step**	**Dako (IS602/IR602)**	**Flex/ Flex+**
				Ventana (790-4858)	**UltraView + Amp OptiView .**
mAb clone **JCM182**	**HIER High pH & Low pH**	**1:25-1:100**	**3-step**	**Leica (PA0790)**	**BOND Refine**
mAb **CON6D/5**	**HIER mod. Low pH**	**1:25-1:100**	**3-step (Flex+)**		

Control material / Tonsil:

An at least weak to moderate and distinct membranous staining reaction of interfollicular activated B- and T-cells and perifollicular germinal centre B-cells in the tonsil.

T-Cell lymphoma markers (1)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD3 (membr.) F7.2.38, LN10, PS1, JCM182, EP449E, SP7, 2GV6, pAb A0542	Tonsil / Appendix	T-cells in the T-zone	T-cells in the mantle zones and within the germinal centres (moderate to strong intensity)	All other cells including B-cells and epithelia cells of the appendix
CD5 (membr.) 4C7, SP19	Tonsil / Appendix	T-cells	Dispersed mantle zone B-cells	All other cells including B-cells and epithelia cells of the appendix
CD4 (membr.) 4B12, 1F6, SP34, EP204, EPR6855	Tonsil / Appendix	Helper/inducer T-cells	Germinal centre macrophages	All other cells including B-cells and epithelia cells of the appendix
CD8 (membr.) C8/144B, 4B11, 1A5	Tonsil / Appendix	T-cytotoxic/suppressor cells & NK cells	None	All other cells including B-cells and epithelia cells of the appendix
CD1a (membr.) O10, EP3622	Tonsil/Skin/Thymus	The Langerhans' cells in the squamous epithelium (tonsil & skin) and cortical thymocytes (Thymus)	None	All other cells including epitheliums
CD2 (membr) AB75, SP304, BS60	Tonsil / Appendix	See CD3	See CD3	See CD3
CD7 (membr.) CBC.37, BSR9, BS8	Tonsil / Appendix	See CD3	See CD3	See CD3
In addition to the previous panels EBV-EBER/EBV-LMP1				

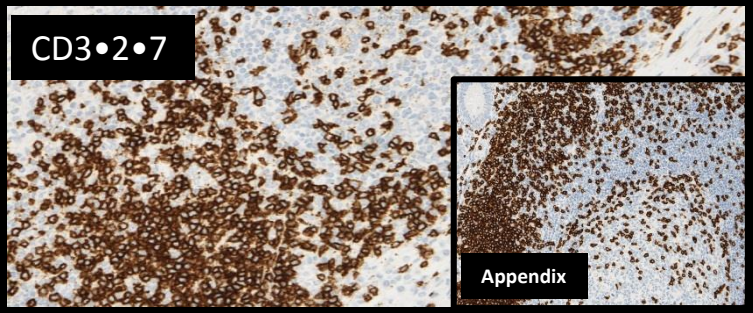
Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

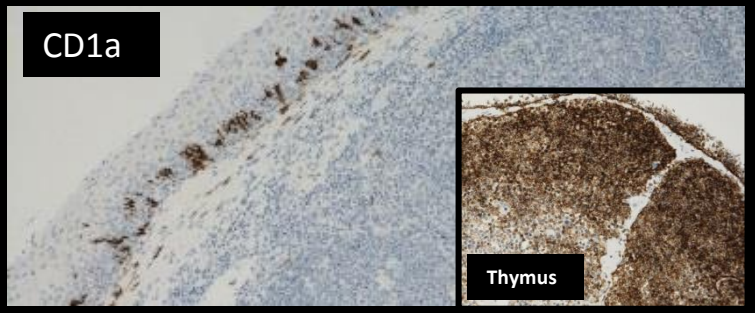
iCAPs (HE): Strong staining intensity/reactions should be expected

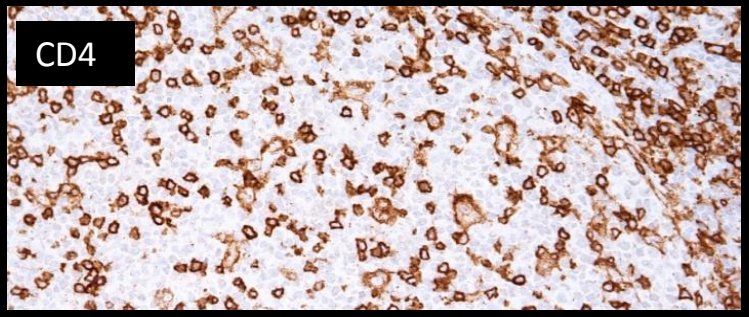
iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

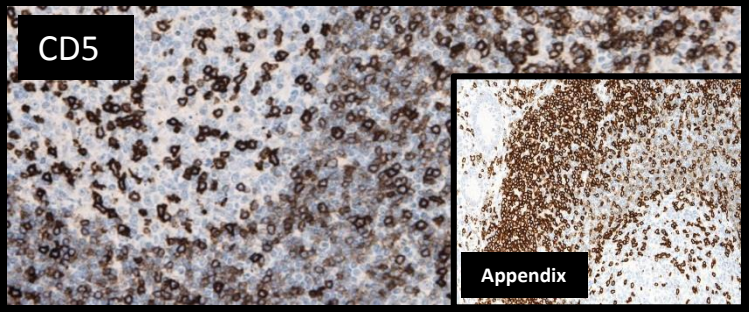
iCAPs (NE): No staining/reactions should be expected

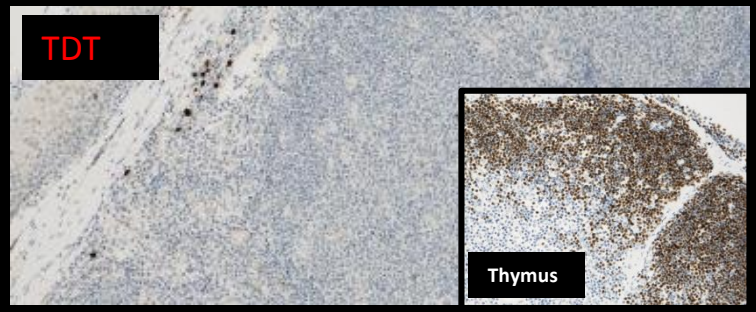
T-Cell lymphoma markers (1):





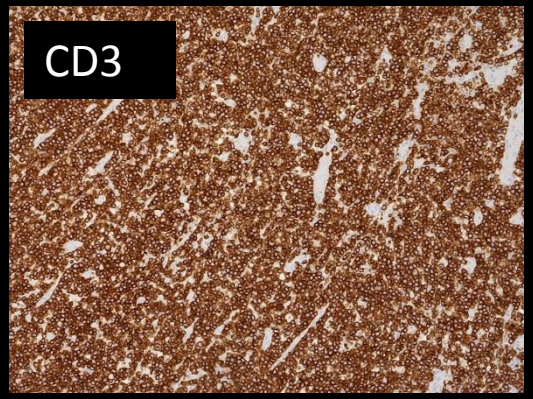


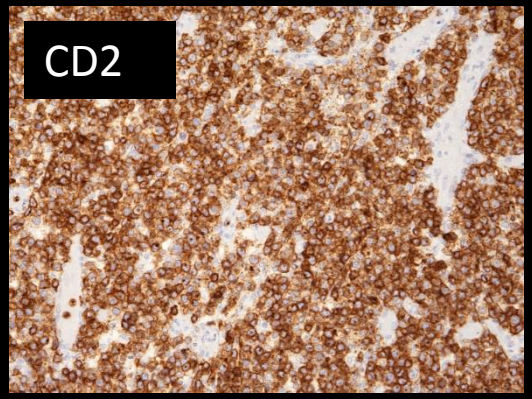


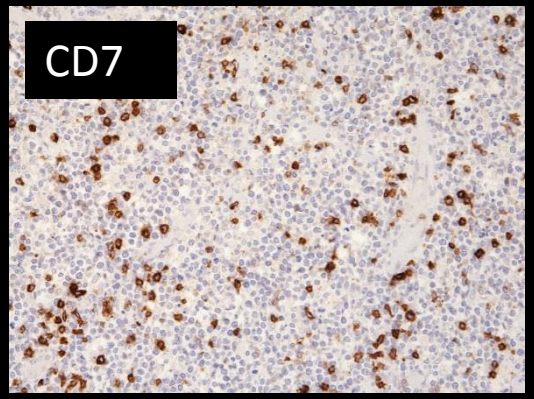


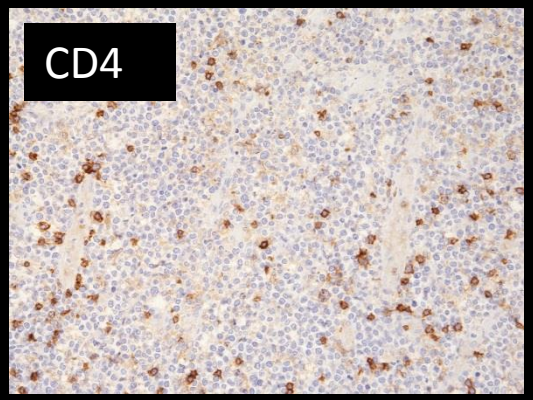
Tonsil

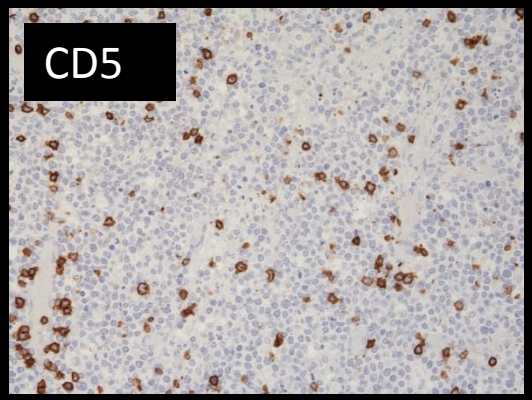
T-cell Lymphoma`s immunophenotype: Complex

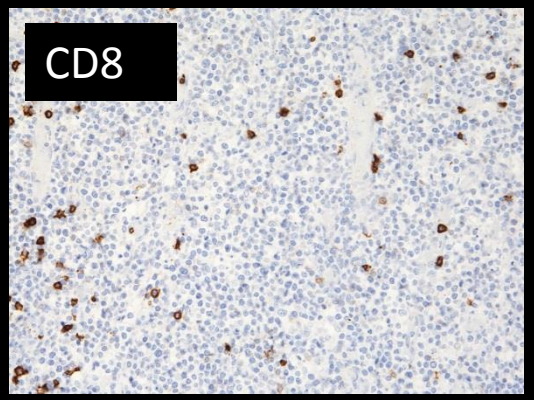












Note: Loss of T cell markers (CD7, CD4 and CD5)

CD3

Table 1. Abs and assessment marks for CD3, run 37

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **F7.2.38**	24	Dako	16	2	6	0	75 %	95 %
mAb clone **LN10**	12	Leica/Novocastra	5	5	2	0	83 %	100 %
mAb **PS1**	25 3 2 1 1	Leica/Novocastra Monosan Biocare Gene Tech Vector	18	10	4	0	88 %	92 %
rmAb **EP41**	1	Epitomics	0	1	0	0	-	-
rmAb **EP449E**	1	Epitomics	1	0	0	0	-	-
rmAb **SP7**	18 1 1	Thermo/NeoMarkers Cell Marque Zytomed	6	11	3	0	85 %	89 %
pAb **A0542**	29	Dako	14	13	2	0	93 %	96 %
Ready-To-Use Abs								
mAb clone **LN10 PA0553**	10	Leica/Novocastra	10	0	0	0	100 %	100 %
mAb clone **PS1 CD3-PS1-R-7**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **PS1 PM110**	1	Biocare	1	0	0	0	-	-
rmAb clone **2GV6 790-4341** ★	54	Ventana	51	3	0	0	100 %	100 %
rmAb clone **EP272 MAD-000325QD**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **MRQ-39 103R**	1	Cell Marque	1	0	0	0	-	-
pAb **IR503/IS503**	31	Dako	20	10	1	0	97 %	97 %
pAb clone **N1580**	1	Dako	0	1	0	0	-	-
Total	219		144	57	18	0	-	
Proportion			66 %	26 %	8 %	0 %	92 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

★ Not available as concentrate

Optimal Protocols

HIER preferable in alkaline buffer

Careful calibration of primary Ab

2 and 3-step detection systems

Insufficient results

Inefficient HIER (too low temp. or too short time)

Low concentration of the primary Ab

Platform dependent mAb F7.2.38

CD3

Table 2. Optimal results for CD3 using concentrated Abs on the 3 main IHC systems*

Table 2. **Optimal results for CD3 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
Buffer	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 **F7.2.38**	92 % 11/12**	-	0 % 0/4	0 % 0/1	-	-
mAb clone **PS1**	63 % 5/8	-	50 % 5/10	-	50 % 4/8	100 % 2/2
pAb **A0542**	64 % 9/14	-	18 % 2/11	-	100 % 1/1	-

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

mAb F7.2.38 performed less successful on the Ventana Benchmark platform compared to protocols with similar settings applied on Dako Autostainers

<u>Alternative : Use Ventana`s RTU system (790-4341) based on the rmAb 2GV2</u>

54 protocols (100% sufficient/94% optimal), HIER in CC1 and iView, UltraView or OptiView

CD3

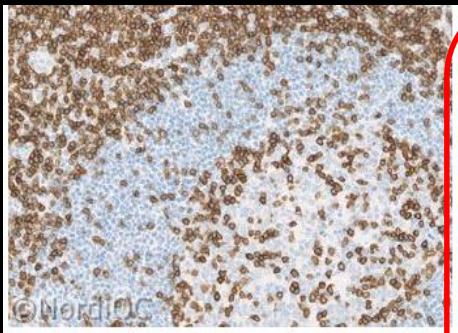


Fig. 1a. Optimal CD3 staining of the tonsil using the rmAb clone 2GV6, Ready-To-Use, Ventana. Virtually all the T-lymphocytes in the T-zone and within the germinal centre show a strong and distinct membranous staining reaction. No background staining or staining of the B-cells is seen. Also compare with Figs. 2a – 3a, same protocol.

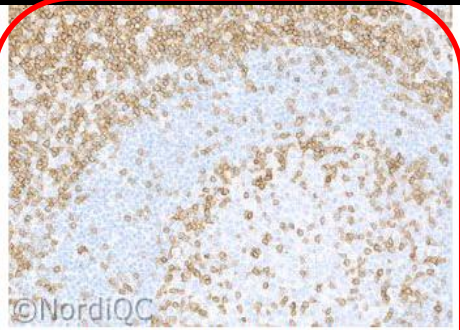


Fig. 1b. CD3 staining of the tonsil using the mAb clone F7.2.38 by protocol settings giving a too low sensitivity - same field as in Fig. 1a. The vast majority of the T-lymphocytes are demonstrated. A slightly weaker and less intense staining reaction is seen. However also compare with Figs. 2b – 3b, same protocol.

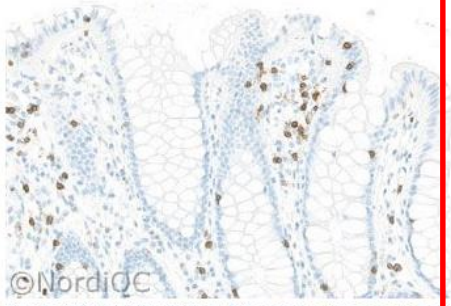


Fig. 2a. Optimal CD3 staining of the colon using same protocol as in Fig. 1a. The dispersed intraepithelial T-lymphocytes show a distinct staining reaction. The columnar epithelial cells are negative and no background staining is seen.

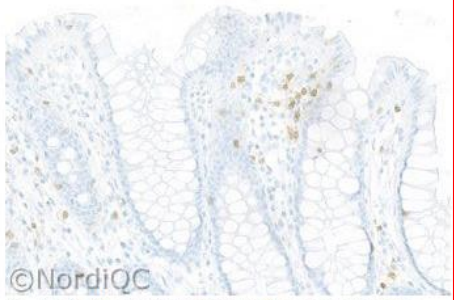


Fig. 2b. Insufficient CD3 staining of the colon using same protocol as in Fig. 1b – same field as in Fig. 2a. The intraepithelial T-lymphocytes are virtually negative. Also compare with Fig. 3b, same protocol.

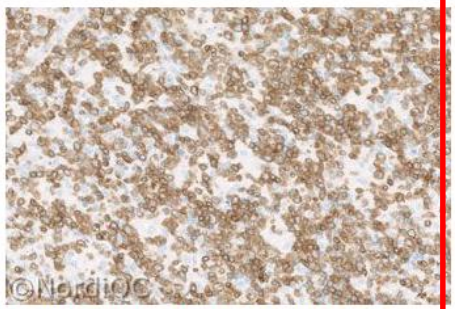


Fig. 3a. Optimal CD3 staining of the peripheral T-cell lymphoma, NOS, using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a moderate to strong and distinct predominantly membranous staining reaction. No background staining is seen.

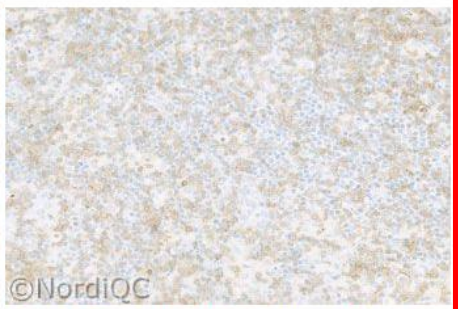


Fig. 3b. Insufficient CD3 staining of the peripheral T-cell lymphoma, NOS, using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a.

Problem:

Low sensitive protocols

Too low HIER temperature

Too short HIER time

Too low concentration of the primary Ab

Too low sensitivity of the detection system

All these parameters should be calibrated carefully to give optimal results = focus on critical staining indicators

Lymphoma panel: CD3

Optimal protocol settings (NQC)

CD3	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **F7.2.38**	**HIER High pH**	**1:50-1:200**	**2 & 3-step**	**-**	**-**
pAb **A0452**	**HIER High pH**	**1:50-1:300**	**2 & 3-step**	**Dako (IS503/IR503)**	**Flex/ Flex+**
mmAb **LN10**	**HIER High pH & Low pH**	**1:50-1:140**	**2 & 3-step**	**Leica (PA0553)**	**BOND Refine**
mAb clone **PS1**	**HIER High pH & Low pH**	**1:40-1:100**	**2 & 3-step**	**Biocare (PM110)**	**MACH4**
rmAb **2GV2**	**HIER High pH (CC1)**	**-**	**-**	**Ventana (790-4341)**	**iView UltraView OptiView**
rmAb **SP7**	**HIER High pH**	**1:100-1:200**	**2 & 3-step**	**-**	**-**

Control material / Tonsil:

A moderate to strong, distinct predominantly membranous staining reaction of all T-cells.

No staining of other cellular structures

CD5

Table 1. **Antibodies and assessment marks for CD5, run 49**

Concentrated	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C7**	55 11 6 4 2 1 1	Leica/Novocastra Dako/Agilent Thermo S./LabVision Biocare Medical Cell Marque BioGenex Monosan	43	28	9	0	89%	93%
rmAb clone **SP19**	9 7 6 2	Thermo S./LabVision Cell Marque Spring Bioscience Zytomed Systems	15	5	2	2	83%	83%
rmAb clone **EP77**	1 1	Cell Marque Zeta	0	0	2	0	-	-
pAb **E2474**	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use								
mAb clone **4C7 IR/IS082**	39	Dako/Agilent	27	10	1	1	95%	97 %
mAb clone **4C7 IR/IS082**[3]	13	Dako/Agilent	7	5	1	0	92%	-
mAb clone **4C7 PA0168**	12	Leica Biosystems	9	2	1	0	92%	90%
mAb clone **4C7 PA0168**[4]	7	Leica Biosystems	3	3	0	1	86%	-
mAb clone **4C7 205M-17/18**	1	Cell Marque	1	0	0	0	-	-
mAb clone **4C7 MS-393-R7**	1	Thermo S./LabVision	1	0	0	0	-	-
mAb clone **4C7 AM430-5/10**	1	BioGenex	1	0	0	0	-	-
mAb clone **4C7 PDM095**	1	Diagnostic BioSystems	1	0	0	0	-	-
mAb clone **4C7 PM099**	1	Biocare medical	0	1	0	0	-	-
rmAb clone **SP19 790-4451**	88	Ventana/Roche	76	11	1	0	99%	99%
rmAb clone **SP19 205R-17/18**	4	Cell Marque	4	0	0	0	-	-
rmAb clone **SP19 KIT-0033**	1	Maixin	1	0	0	0		
rmAb clone **EP77 MAD-000602QD**	2	Master Diagnostica	0	1	0	1	-	-
Total	278		189	67	17	5	-	
Proportion			68%	24%	6%	2%	92%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU system developed for the Dako/Agilent semi-automatic system (Autostainer) but used by laboratories on the Omnis platform (Dako/Agilent).
4) RTU system developed for the Leica Biosystem full-automated systems (BOND III/MAX) but used by laboratories on different platforms (e.g. Ventana Benchmark) or manually.

High Pass rate due to use of robust clones (mAb 4C7 & rmAb SP19) both as concentrates and RTU systems

Efficient HIER, preferable in alkaline buffer and careful calibration of the primary Ab titre

Insufficient protocols

Too low primary Ab concentration

ADF 1:142 (range 1:10-1:1200) / Opt. result

ADF 1:282 (range 1:20-1:1500) / Insuff. result

RTU systems gave higher pass rate compared to Laboratory developed assays

Best performance: rmAb clone SP19, 790-4451 (Ventana)

CD5, Run 49

Table 5. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

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

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

Optimal results could be obtained by using vendor recommended or laboratory modified protocol settings – all vendors (see table 5).

<u>RTU 790-4451 (rmAb SP19) / Ventana Benchmark (all protocol setting):</u>

Proportion of optimal results was influenced by the choice of detection system):

- **76% (26 of 34) produced an optimal result using UltraView as the detection system**
- **97% (31 of 32) produced an optimal result using OptiView as the detection system.**

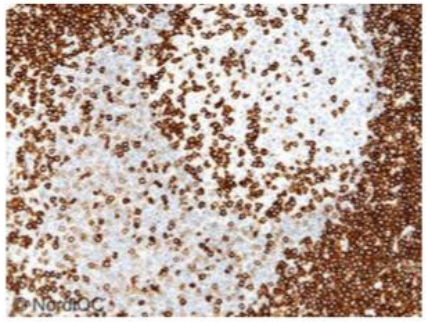


Fig. 1a (x200)
Optimal staining for CD5 of the tonsil, core 1, using the mAb 4C7 as a concentrate, HIER in an alkaline buffer (BERS2) and a polymer based detection system (BOND Refine, Leica) - same protocol used in Figs. 2a - 5a. The T-cells in the interfollicular T-zone and within the germinal centre show a strong distinct membranous staining reaction. Dispersed B-cells in the mantle zone show a weak to moderate but distinct membranous staining reaction.

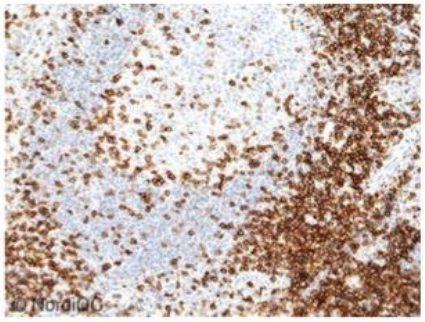


Fig. 1b (x200)
Insufficient staining for CD5 of the tonsil, core 1, using the mAb clone 4C7 as concentrate (too diluted), HIER in an alkaline buffer (BERS2, too short time) and BOND Refine (Leica) as the detection system - same protocol used in Figs. 2b – 5b. The intensity of the staining reaction, both of germinal centre T-cells and mantle zone B-cells, is reduced - compare with Fig. 1a (same field).

Problem: Too diluted primary Ab and inefficient HIER (too short time)

CD5, Run 49

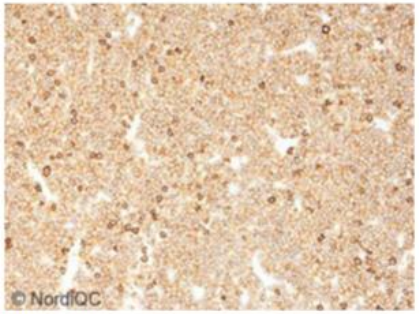


Fig. 4a (x200)
Optimal staining for CD5 of the MCL, core 4, using same protocol as in Figs. 1a - 3a. Virtual all the neoplastic cells show a weak to moderate, distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

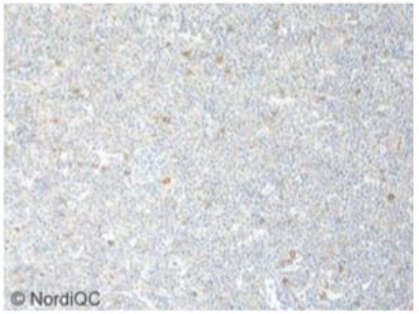


Fig. 4b (x200)
Insufficient staining for CD5 of the MCL, core 4, using same protocol as in Figs. 1b -3b. The neoplastic cells are false negative and only T-cells with reduced intensity are demonstrated - compare with Fig. 4a (same field).

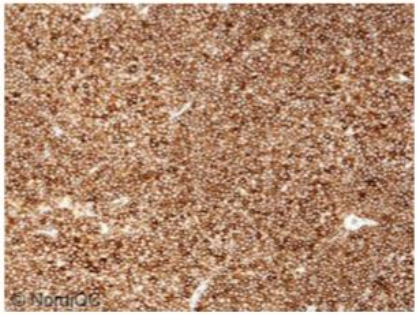


Fig. 5a (x200)
Optimal staining for CD5 of the B-CLL using same protocol as in Figs. 1a - 4a. All the neoplastic cells show a strong and distinct membranous staining reaction.

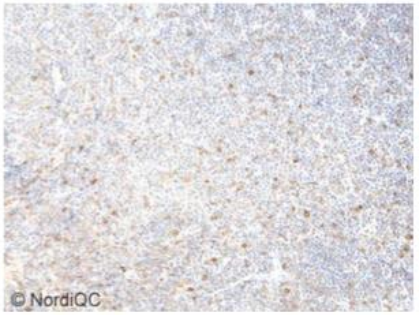


Fig. 5b (x200)
Insufficient staining for CD5 of B-CLL using same protocol as in Figs. 1b - 4b. The vast majority of neoplastic cells are false negative or shows reduced intensity. T-cells display a moderate staining intensity - compare with Fig. 5a (same field).

Lymphoma panel: CD5

Optimal protocol settings (NQC)

CD5	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb 4C7	HIER High pH or mod. Low pH	1:20-1:200	2 & 3-step	Leica (PA0168)	BOND refine
				Dako (IS/IR082)	Flex
rmAb SP19	HIER High pH	1:25-1:100	2 & 3-step	Ventana (790-4451)	iView UltraView +/- Amp* OptiView

* Optimal results could also be obtained with the detection system UltraView without amplification but at overall lower frequency compared to laboratories using UltraView with amplification

Control material / Tonsil:

An at least weak to moderate and distinct membranous staining reaction of dispersed B-cells in the mantle zone of the secondary follicles in the tonsils.

Strong membranous staining of T-cells

T-Cell lymphoma markers (2)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
PD-1 (membr.) **NAT105**	Tonsil/	Follicular centre T-cells (helper T-cells)	Scattered extrafollicular and mantle zone lymphocytes	All other cells
CXCL-13 (cytopl.) **53610**	Tonsil	Follicular centre T-cells (helper T-cells), scattered T-cells in the mantle zone and interfollicular areas	None	All other cells
Granzyme B (cytopl.) **GrB-7**	Tonsil	Activated cytotoxic T-cells & NK cells	None	All other cells including B-cells
TIA-1 (cytopl.) **TIA-1**	Tonsil	Cytotoxic T-cells & NK cells	Dispersed unstimulated T-cells, NK-cells and some myeloid cells	All other cells including B-cells

Blast marker(s)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
TdT (nuclear) **SEN28, EP266**	Thymus/Tonsil	Dispersed immature T-cells in the interfollicular zones of tonsils.	Cortical thymocytes (moderate intensity)	Mantle zone and germinal centre B-cells.

Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

TdT

Table 1. **Antibodies and assessment marks for TdT, run 52**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **SEN28**	66 3 2 1 1	Leica/Novocastra Diagnostic Biosystems Thermo/NeoMarkers Monosan Immunologic	20	29	19	5	67%	70%
rmAb clone **EP266**	13 2 1 1	Agilent/Dako Cell Marque Diagnostic Biosystems Epitomics	11	3	3	0	82%	87%
pAb **A3524**[3]	2	Agilent/Dako	0	1	1	0	-	-
pAb **ILP 0049**	3	Immunologic	0	1	2	0	-	-
pAb **338A-76**	2	Cell Marque	0	0	1	1	-	-
pAb **CP134**	1	Biocare Medical	0	1	0	0	-	-
pAb **44811**	1	Menarini Diagnostics	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **SEN28 PA0339**	11	Leica/Novocastra	6	5	0	0	100%	100%
mAb clone **SEN28 PA0339**[4]	5	Leica/Novocastra	2	1	1	1	-	-
mAb clone **SEN28 8243-C010**	1	Sakura FineTek	1	0	0	0	-	-
mAb clone **SEN28 MAB-0197**	1	Maixin	1	0	0	0	-	-
mAb clone **SEN28 MS-1105-R7**	1	Thermo/Neomarkers	0	1	0	0		
rmAb clone **EP266 IR093**	36	Agilent/Dako	26	8	2	0	94%	95%
rmAb clone **EP266 IR093**[4]	17	Agilent/Dako	17	0	0	0	100%	100%
rmAb clone **EP266 MAD-000659QD**	2	Master Diagnostica	1	1	0	0	-	-
rmAb clone **EP266 338R-28**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **EP266**	1	Unknown	0	1	0	0		
pAb **760-2670**	45	Ventana/Cell Marque	1	39	4	1	89%	100%
pAb **338A-78**	4	Cell Marque	0	4	0	0	-	-
pAb **IR001**[3]	1	Agilent/Dako	0	1	0	0	-	-
Total	225		87	97	33	8	-	
Proportion			39%	43%	15%	3%	82%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Product discontinued.
4) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

Robust antibodies

mAb clone SEN28
rmAb clone EP266

HIER in alkaline buffer

mAb clone SEN28
2- or 3-step mul./pol detection sys

rmAb clone EP266
3-step mul./pol detection sys.

Inappropriate platforms ?
88% (15 of 17) on the Omnis?

Proportion of optimal results ?

RTU better than LD assays

TdT

Table 3. **Proportion of optimal results for TdT for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana 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	ER2 pH 9.0	ER1 pH 6.0
mAb clone **SEN28**	3/3**	-	2/4	-	8/30 (27%)	-	2/5 (40%)	0/2
rmAb clone **EP266**	1/3	-	2/2	-	5/8 (63%)	-	1/1	-

* 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)

Low proportion of optimal results ?

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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/III mAb SEN28 **PA0339**	100% (3/3)	0% (0/3)	100% (8/8)	75% (6/8)
Dako AS mAb EP266 **IR093**	92% (11/12)	50% (6/12)	100% (20/20)	90% (18/20)
VMS Ultra/XT/GX pAb **760-2670**	0% (0/1)	0%(0/1)	89% (34/38)	3% (1/38)

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

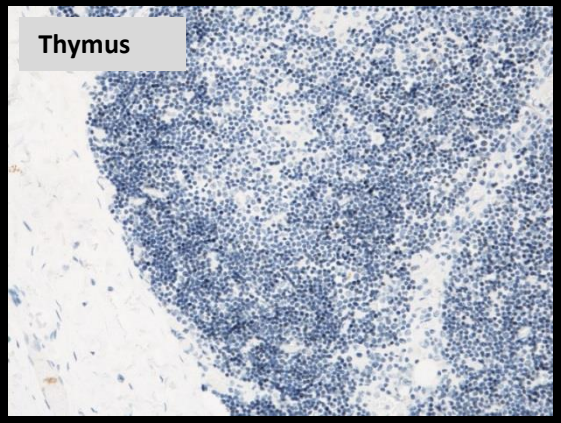
Prolonging inc. time

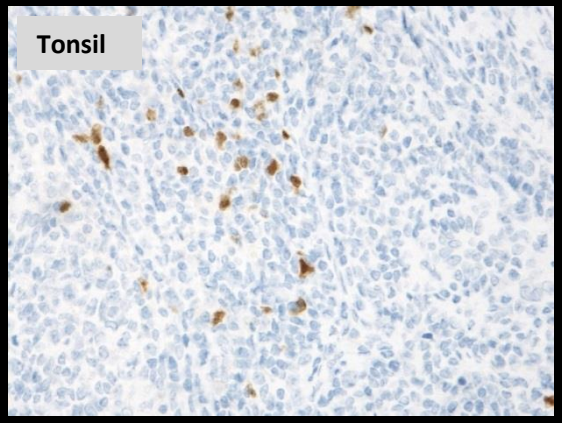
Substituting Flex with Flex+

Poor signal-to-noise ratio

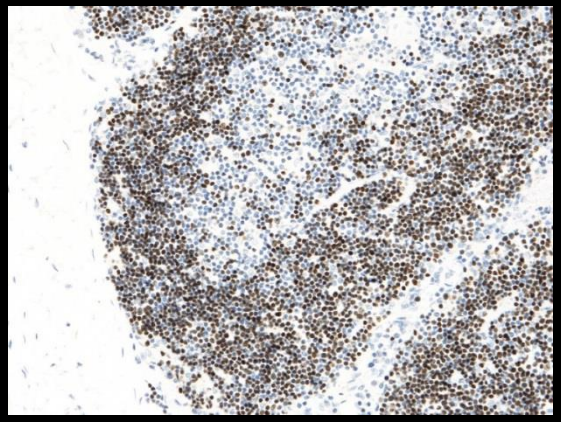
TdT: A marker sensitive to the choice of antibody diluent ?

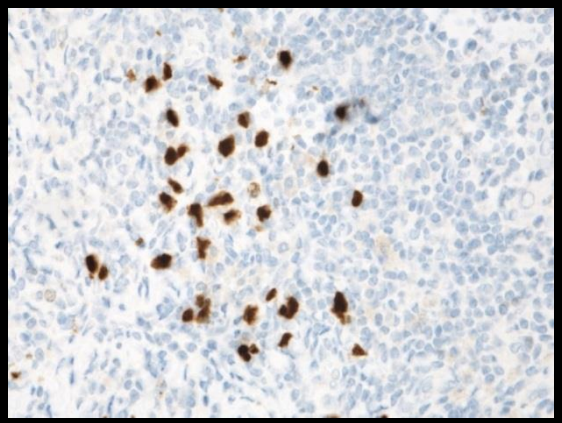
TdT, SEN28 1:50
Dako dil. pH 7.3





TdT, SEN28 1:50
Renoir Red pH 6.2

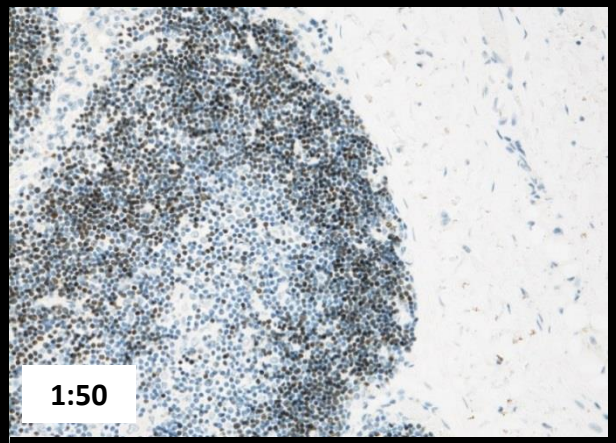


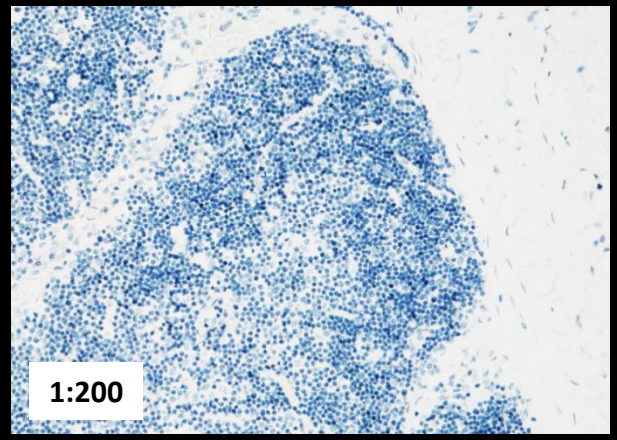


Omnis: HIER/HIGH pH 24`, Flex+ Mouse (10+20`)

TdT: A marker sensitive to the choice of antibody diluent ?

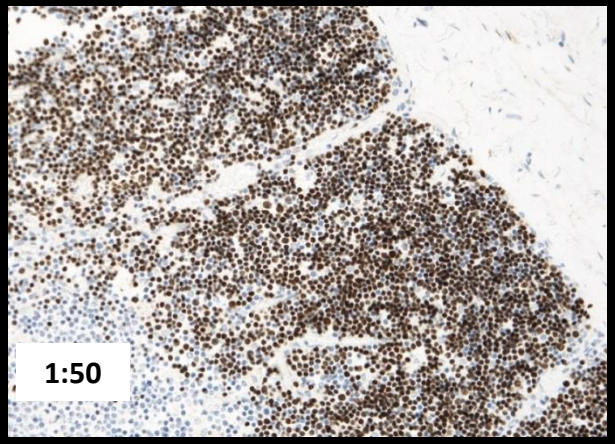
TdT, EP266
Dako dil. pH 7.3

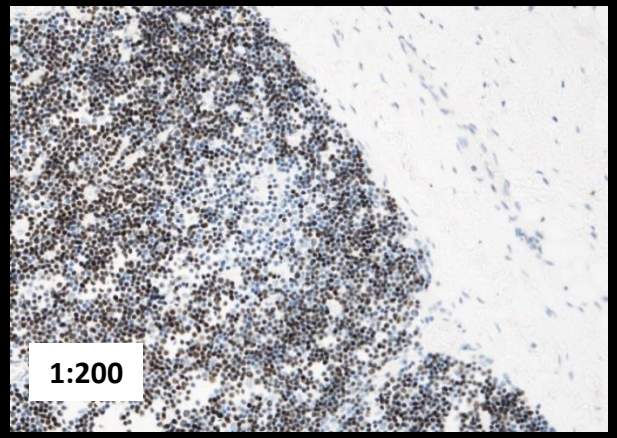




Omnis: HIER/HIGH pH 24`, Flex+ Rabbit (10+20`)

TdT, EP266
Renoir Red pH 6.2





Thymus

TdT

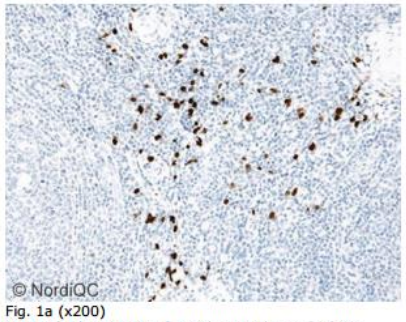


Fig. 1a (x200)
Optimal TdT staining of tonsil using the mAb clone SEN28, optimally calibrated, HIER in TRS (3-1) pH 9 (Dako) and a 3-step polymer based detection system (Flex+/Dako).
Dispersed pre-mature T-cells of the interfollicular zones show a strong and distinct nuclear staining reaction. Same protocol used in Figs. 2a - 4a.

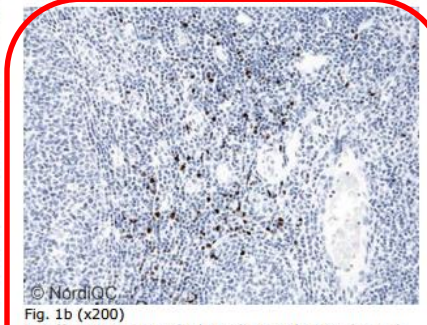


Fig. 1b (x200)
Insufficient staining of TdT in the tonsil using the mAb clone SEN28, too diluted and applying the less sensitive detection system Flex (Dako) – same field as in Fig. 1a. Although the pre-mature T-cells of the interfollicular zones display a relative strong nuclear staining intensity, the protocol provided too low sensitivity (compare Figs. 1a - 4b). Same protocol used in Figs. 2b - 4b.

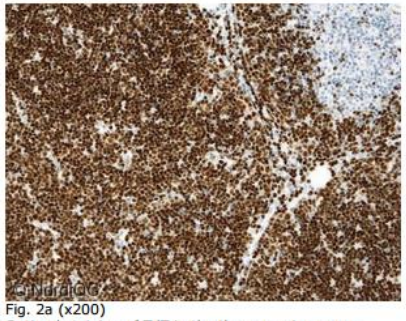


Fig. 2a (x200)
Optimal staining of TdT in the thymus using same protocol as in Fig. 1a. Immature cortical thymocytes and scattered pre-mature T-cells of medulla show a strong and distinct nuclear staining reaction.

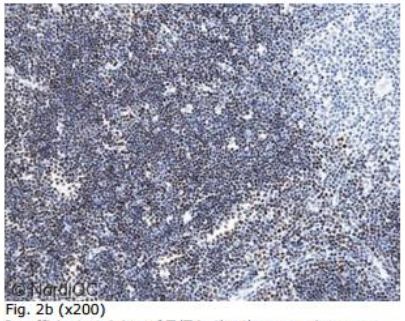


Fig. 2b (x200)
Insufficient staining of TdT in the thymus using same protocol as in Fig. 1b – same field as in Fig. 2a.
The staining intensity and proportion of positive cortical thymocytes is significantly reduced.

Optimal result

HIER (TRS pH9)/ Optimal calibrated primary Ab and use of Flex+ as the detection system

Insufficient result:

HIER (TRS pH9)/ Too diluted primary Ab and use of the less sensitive detection system Flex

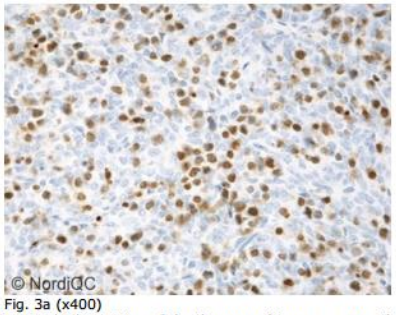


Fig. 3a (x400)
Optimal TdT staining of the thymoma (tissue core no. 4) using same protocol as in Figs. 1a and 2a. The vast majority of immature T-cells intermingling between the neoplastic cells show a weak to moderate but distinct nuclear staining reaction.

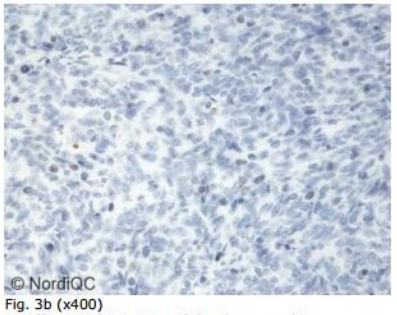


Fig. 3b (x400)
Insufficient TdT staining of the thymoma (tissue core no. 4) using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The immature T-cells intermingling between the neoplastic cells are false negative or only faintly demonstrated in a small fraction of the total population of T-cells.

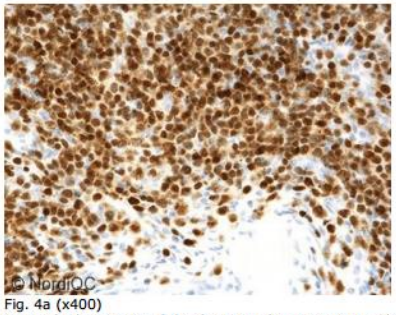


Fig. 4a (x400)
Optimal TdT staining of the thymoma (tissue core no. 5) using same protocol as in Figs. 1a – 3a. Virtually all the immature T-cells show a strong and distinct nuclear staining reaction.

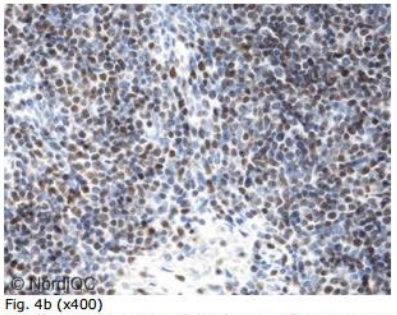


Fig. 4b (x400)
Insufficient TdT staining of the thymoma (tissue core no. 5) using same protocol as in Figs. 1b and 3b - same field as in Fig. 4a. The staining intensity of the immature T-cells is significantly reduced.

Blast panel: TdT

Optimal protocol settings (NQC)

TdT	Retrieval buffers	Titre	Detection	RTU	Detection
mAb SEN28	HIER High pH	1:20-1:50	2 & 3-step	Leica (PA0339)	BOND refine
rmAb EP266	HIER High pH	1:25-1:100	2 & 3-step	Dako (IR093)	Flex/Flex+

Control material / Thymus:

An at least moderate and distinct nuclear staining reaction of cortical thymocytes.

Lymphoma´s (Basic panel): Antibodies

Based on the result`s in NordiQC (> 5 protocols pr. clone assessed in the latest run)

Target	High scoring clones	Low scoring clones
CD20	mmAb: L26	-
Pax5 (BSAP)	mmAb: DAK-PAX5 & 24 & 1EW, rmAb: BV6 & BSR59 & EP156	pAb: RB-9406 , mmAb: 24# & 1EW (PO blocking)* & SP34◊
BCL2	mmAb: 124 & 100/D5 & BCL2/100/D5	mmAb: 124#
CD5	mmAb: 4C7, rmAb: SP19	mmAb: CD5/54/F6
BCL6	mmAb: GI181E/A8 & LN22 & PG-B6p	mmAb: PG-B6p (PO blocking) *
CD23	mmAb: 1B12 & DAK-CD23 & BS20 , rmAb: SP23	mmAb: 1B12#
CD30	mmAb: BER-H2 & JCM182 & "CON6D/5"	-
Kappa	pAb: A0191	All other pAbs and mmAbs
Lambda	pAb: A0193	All other pAbs and mmAbs
CD79a	mmAb: JCB118, rmAb: SP18	mmAb: 11E3 & "HM57" & JCB118# , rmAb: SP18‡
CD3	mmAb: F7.2.38 & LN10 & PS1, rmAb: SP7 & 2GV6, pAb: A0542	-
CyD1	rmAb : EP12 & SP4	mmAb: P2D11F11
CD45	mmAb: 2B11+PD7/26 & X16/99 & "RP2/18 (RTU, Ventana)"	-
Ki67	mmAb: MIB-1 & K2 & UMAB107, rmAb: SP6 & "30-9 (RTU, Ventana)"	-
CD43	mmAb: DF-T1 ?	?

#Platform issues (Ventana)
‡Platform issues (Autostainer / BOND)
*PO blocking before appl. of the primary Ab
◊ Lot variations

Hematolymphoid markers

Go for primary Abs with the highest optimal score rates and carefully calibrated the primary Abs

Go to the NordiQC website ~ look for recommended controls / iCAPs

Use efficient HIER in app. buffer`s (20-40 min at 97°C-100°C)

For CD30 clone CON6D/5A, HIER in mod. low pH buffer`s is mandatory

Use a sensitive 3-step polymer/multimer detection system

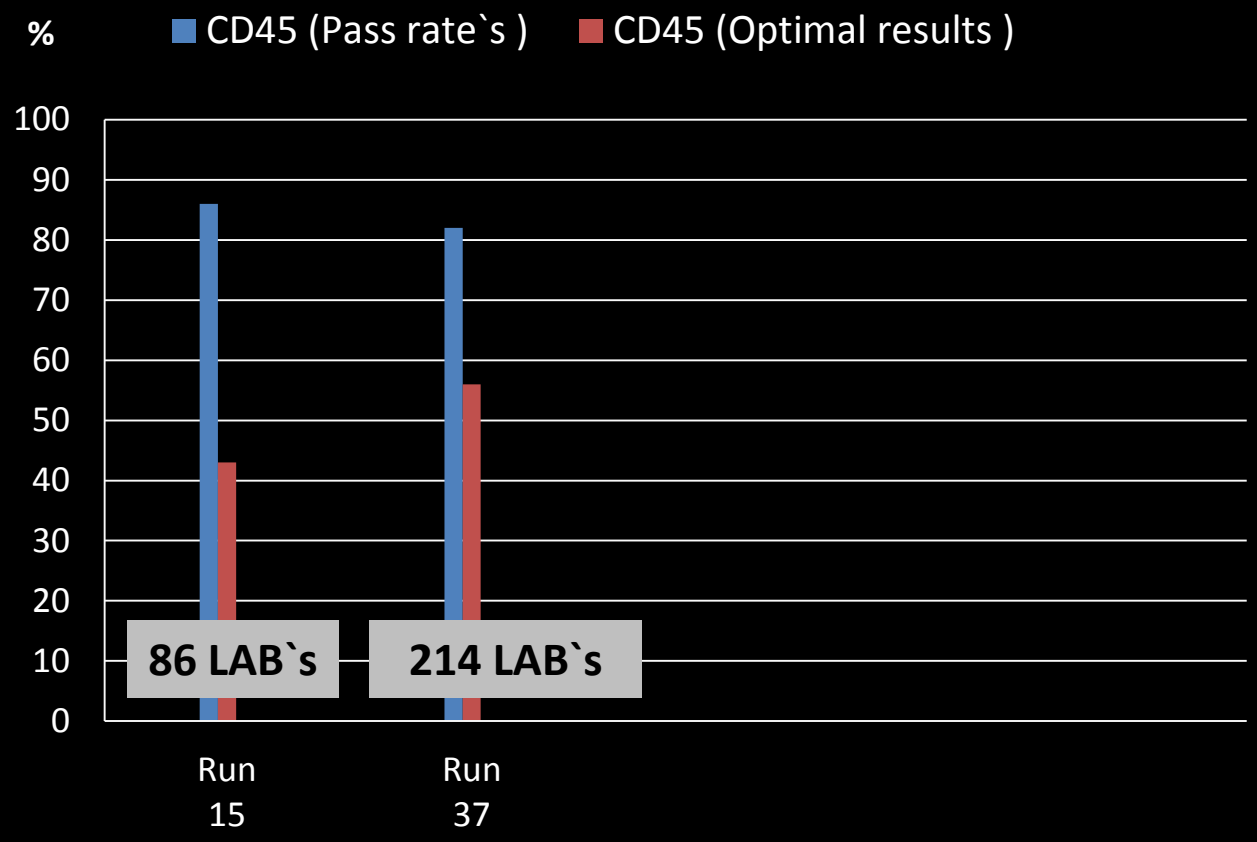
In addition, consider other parameters that may influence the quality of the IHC-staining

Platform dependent primary Abs
Epitopes sensitive to H_2O_2 blocking
Lot - to - lot variations
Too much counterstain
…….

Thank you

Bonus material

CD45, LCA



CD45 / Run 37 (2013):

Sufficient: 82%

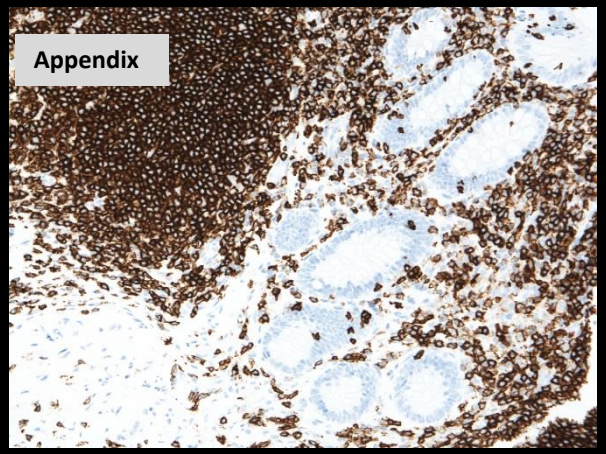
Optimal: 56%

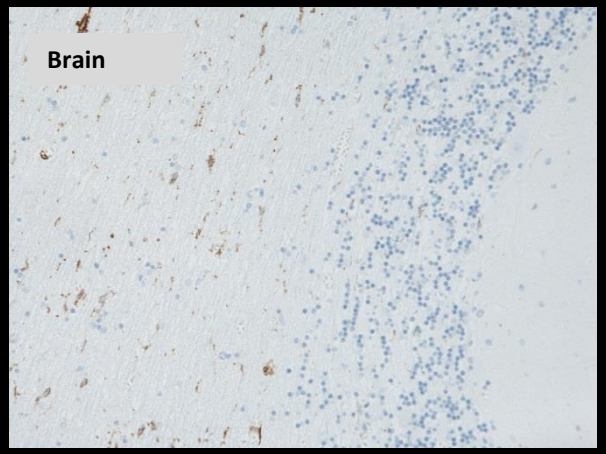
Robust primary Abs:

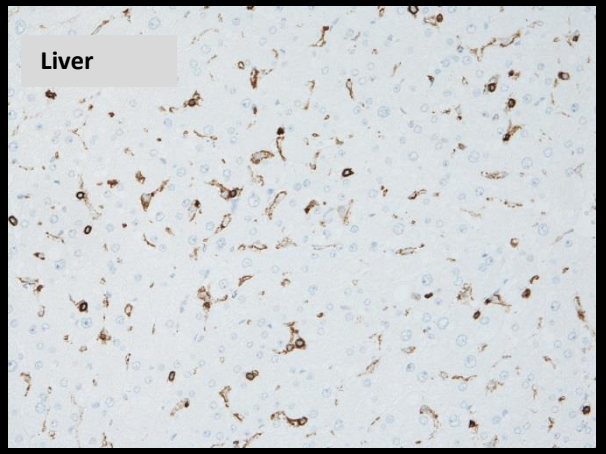
mmAb: 2B11+PD7/26

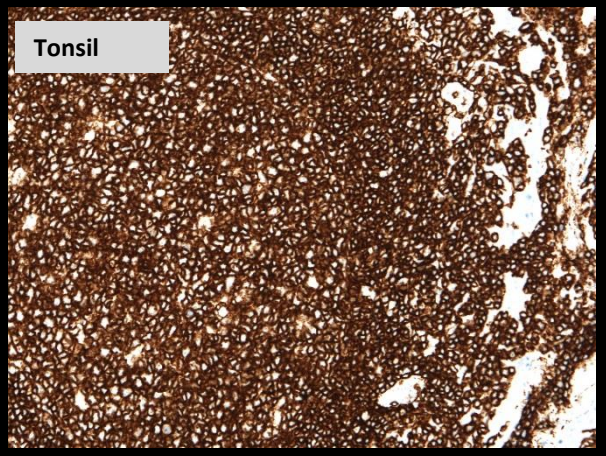
mmAb: X16/99

CD45, LCA









Tonsil in combination with liver is recommended as controls for CD45, LCA.

In tonsil all B- and T-cells must show strong and distinct membranous staining reaction, while Kupffer cells in liver or microglia in brain tissue must show an at least weak to moderate but distinct staining reaction.

No staining should be seen in the squamous epithelial cells and hepatocytes.

Table 1. **Antibodies and assessment marks for CD45, run 37**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clones **MEM28/MEM56 /MEM55**	1	Invitrogen	0	1	0	0	-	-
mAb clones **PD7/26/26+2B11**	3	Thermo/Neomarkers	0	1	2	0	-	-
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
rmAb clone **EP68**	1	Epitomics	0	0	0	1	-	-
Ready-To-Use Antibodies								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100%	100%
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clones **2B11+PD7/26 148M-98**	2	Cell Marque	2	0	0	0	-	-
mAb clones **2B11+PD7/26 N1514**	1	Dako	1	0	0	0	-	-
mAb clones **2B11+PD7/26 E005**	1	Linaris	0	0	1	0	-	-
mAb clones **2B11+PD7/26 MAD-004010QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11 PM-016**	1	Biocare	0	1	0	0	-	-
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	%
Total	205		115	54	30	6	-	
Proportion			56 %	26 %	15 %	3 %	82 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal (mmAb X16/99 & 2B11+PD7/26)

Efficient HIER in High or Low pH buffers (20 min)

1:100-1:1000 (2B11+PD7/26)

1:50-1:300 (X16/99)

2 & 3 step detection systems

Best performance:

RTU CD45, X16/99, (PA0042,Leica)

RTU CD45, 2B11+PD7/26 (IS/IR751, Dako)

Table 2. **Optimal results for CD45 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clones **2B11+PD7/26**	64 % 18/28**	100 % 3/3	48 % 21/44	33 % 1/3	90 % 9/10	100 % 1/1
mAb clone **X16/99**	-	100 % 1/1	100 % 2/2	-	50 % 1/2	100 % 2/2

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

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Omission of HIER

Misleading and imprecise guidelines regarding epitope retrieval and protocol set-up from many vendors still is a central issue and contributes to insufficient results.

Run37, 2013 and still not corrected in 2016 ?

Similar observations and inconsistent guidelines were seen for the mAb clones 2B11+PD7/26, Thermo/NeoMarkers. In the package insert omission of HIER is recommended if used with UltraVision LP (Thermo) but HIER is recommended if UltraVision Quanto (Thermo) is used.

Table 1. Recommended Staining Protocols for CONFIRM anti-CD45, LCA (RP2/18)

Procedure Type	Platform or Method	
	NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None required	None required
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37° C	Approximately 16 minutes, 37° C
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin II, 2 to 4 minutes	Hematoxylin II, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

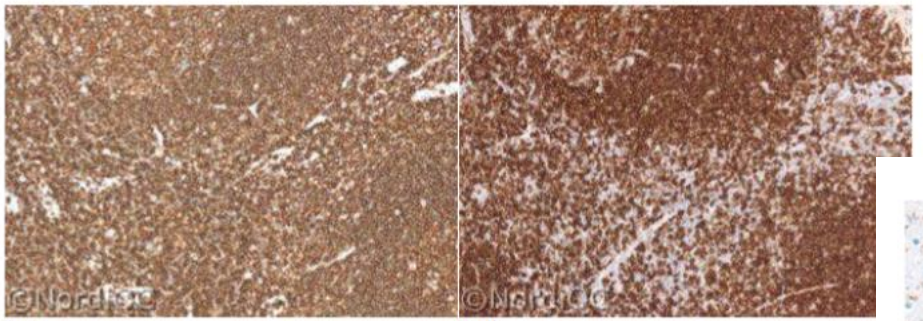


Fig 1a
Optimal CD45, LCA staining of the tonsil using the mAb clones 2B11+PD7/26 optimally calibrated and with HIER. Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.
Also compare with Figs. 2a – 4a, same protocol.

Fig 1b
Staining for CD45, LCA of the tonsil using the mAb clone 2B11+PD7/26 by protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a.
The vast majority of the B- and T-lymphocytes are demonstrated. However also compare with Figs. 2b – 4b same protocol.

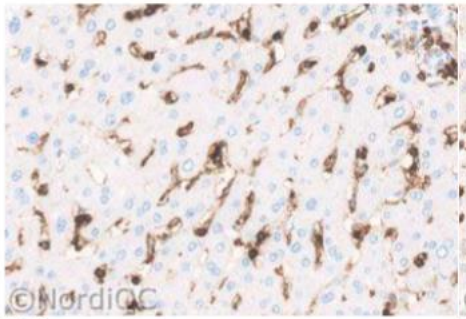


Fig 2a
Optimal CD45, LCA staining of the liver using same protocol as in Fig. 1a.
The lymphocytes show a strong staining reaction, while the Kupffer cells display a weak to moderate staining reaction. The liver cells are negative and no background staining is seen.

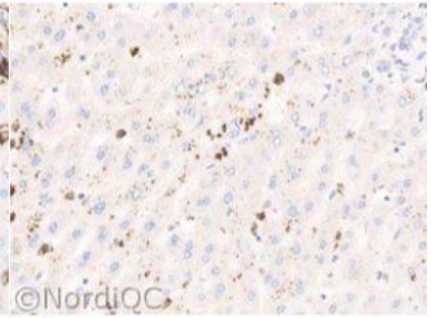


Fig 2b
Insufficient CD45, LCA staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only lymphocytes are demonstrated and the Kupffer cell with a low CD45 expression are false negative.

Optimal

Insuff.

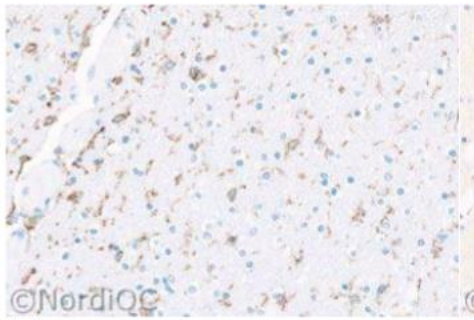


Fig 3a
Optimal CD45, LCA staining of the brain using same protocol as in Figs. 1a & 2a.
The microglial with a low CD45 expression are distinctively demonstrated and no background staining is seen.

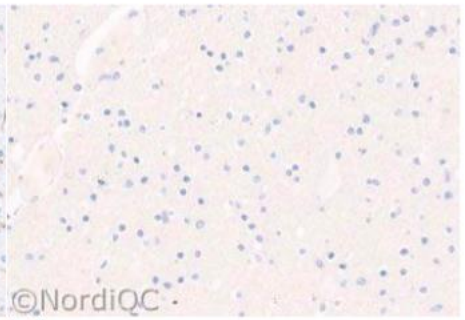


Fig 3b
Insufficient CD45, LCA staining of the brain using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The microglial cells are false negative.

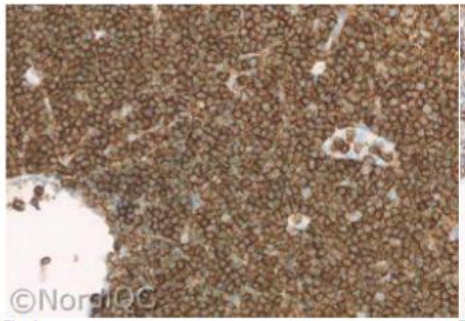


Fig 4a
Optimal CD45, LCA staining of the B-CLL using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong and distinct membranous staining reaction.
No background staining is seen.

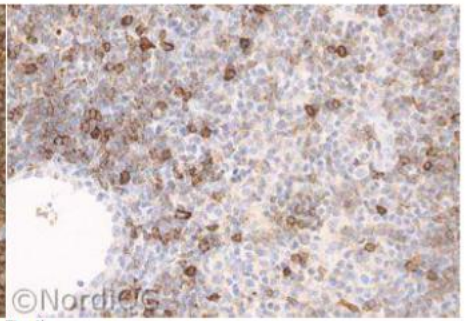


Fig 4b
Insufficient CD45, LCA staining of the B-CLL using same protocol as in Figs. 1b - 3b. – same field as in Fig. 4a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

Optimal

Insuff.

Problem:

Too low concentration of the primary Ab

CD45, LCA / Run 37 2013

Lymphoma panel: CD45, LCA
Optimal protocol settings (NQC)

CD45, LCA	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb 2B11+PD7/26	HIER High pH or Low pH buffers	1:100-1:1000	2 & 3-step	Dako (IS/IR751)	Flex/Flex+
mmAb X16/99	HIER High pH or Low pH buffers	1:50-1:300	2 & 3-step	Leica (PA0042)	BOND refine

Control material: Tonsil and/or Liver and/or Brain:

In tonsillar tissue, all B- and T-cells must show strong and distinct membranous staining reaction

In liver tissue , the Kupffer cells must show an at least weak to moderate but distinct staining reaction.

In brain tissue, the microglia cells must show an at least weak to moderate but distinct staining reaction

No staining should be seen in the squamous epithelial cells and hepatocytes.

B-Cell lymphoma markers (1):

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD19 (membranous). LE-CD19, BT51E	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells	Plasma cells	No staining of other cell types including T-cells and epithelial cells of the appendix.
CD20 (membraneous). L26, 7D1, EP7	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells	None	No staining of other cell types including T-cells and epithelial cells of the appendix.
CD79a (membr. + cytopl). JCB117, SP18	Tonsil/Appendix	Mantle zone B-cells and plasma cells	Germinal centre B-cells	No staining of other cell types including T-cells and epithelial cells of the appendix.
BSAP (PAX5) (nuclear) 1EW, 24, DAK-PAX5, SP34	Tonsil/Appendix	Mantle zone-, germinal centre- & interfollicular B-cells*	None	No staining of other cell types including T-cells and epithelial cells of the appendix.
IgK (membr. + cytopl). pAb A0191	Tonsil	Plasma cells (App. 50%)	Mantle zone B-cells (App. 50 %)	No staining of other cell types including T-cells (weak background staining my be seen)
IgL (membr. + cytopl). pAb A0193	Tonsil	Plasma cells (App. 50%)	Mantle zone B-cells (App. 50 %)	No staining of other cell types including T-cells (weak background staining may be seen)
IgM (membr. + cytopl). pAb A0425, 760-2654	Tonsil	All mantle zone B-cells and plasma cells (app. 35%)	None	No staining of other cell types including T-cells (weak background staining may be seen)

* A weak cytoplasmic staining reaction in B-cells must be accepted. In the technical calibration phase, it is recommended to verify the protocol on Hodgkin lymphoma, classical subtype.

Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

CD19

Table 1. **Abs and assessment marks for CD19, run 35.**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **LE-CD19**	11	BioCare BioSite Dako Serotec	5	1	5	0	55 %	75 %
mAb clone **BT51E**	1	Novocastra/Leica	0	0	1	0	-	-
Not specified	2		1	0	1	0	-	-
Ready-To-Use Abs:								
mAb clone **LE-CD19, IR656**	4	Dako	3	1	0	0	100 %	100 %
mAb clone **BT51E, PA0843**	1	Novocastra/Leica	1	0	0	0	-	-
mAb clone **MRQ-36, 119M-17**	1	Cell Marque	0	0	0	1	-	-
Total	20		10	2	7	1	-	
Proportion			50 %	10 %	35 %	5 %	60 %	-

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.

mAb clone LE-CD19 Dako most consistent

HIER in alk. pH

3-step polymer

mAb clone LE-CD19 (Serotec, Biocare …)

False positive (e.g. T-cells)

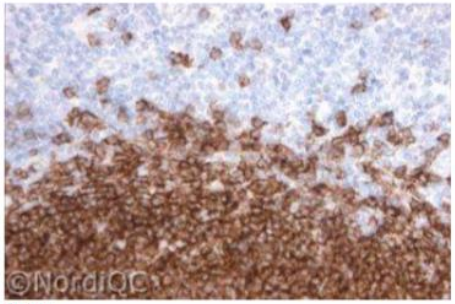


Fig. 1a
Normal tonsil showing an optimal staining for CD19 using the mAb clone LE-CD19 from Dako, diluted 1:50, on the Autostainer platform. HIER was performed using TRS pH 9 (3-in-1) (Dako). A strong and distinct membranous staining reaction is seen in virtually all B-cells. T-cells are negative.

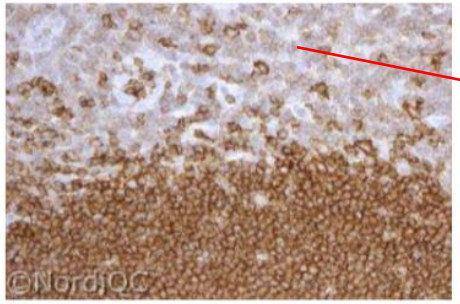


Fig. 1b
Normal tonsil showing an insufficient staining for CD19 using the mAb clone LE-CD19 from Serotec, diluted 1:500, on the Autostainer platform. HIER was performed using Citrate pH 6. In addition to a moderate to strong staining reaction in the normal B-cells (albeit weaker than that seen in Fig 1a), the majority of T-cells shows a false positive staining reaction.

False Positive (T-cells)

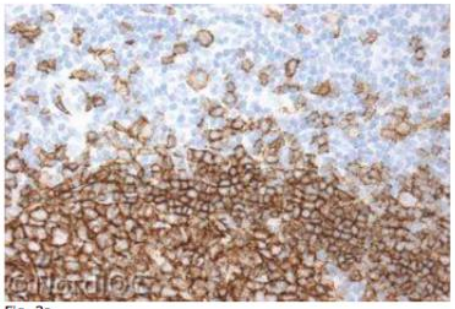


Fig. 2a
Lymphatic tissue in the appendix showing an optimal staining for CD19 using the mAb clone BT51E (RTU) on the BOND-III platform. HIER was performed using Bond Epitope Retrieval Solution 1. A strong and very distinct membranous staining is seen in virtually all B-cells, while the T-cells are negative.

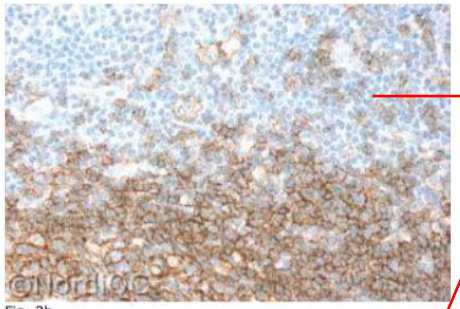


Fig. 2b
Lymphatic tissue in the appendix showing an insufficient staining for CD19 using the mAb clone BT51E, diluted 1:30, on the BenchMark platform. HIER was performed using Cell Conditioning 1. Only a weak to moderate staining is seen in the majority of B-cells. T-cells are negative. Also compare with Fig. 3b, same protocol.

Too weak

mAb clone BT51E applied by protocol settings with too low sensitivity

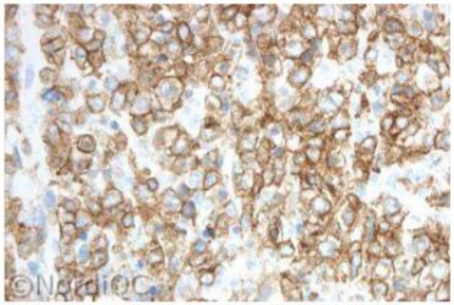


Fig. 3a. Optimal staining reaction for CD19 of the DLBCL. Same protocol used as in Fig. 2a based on the mAb clone BT51E. A moderate to strong membranous staining reaction is seen in virtually all the neoplastic cells.

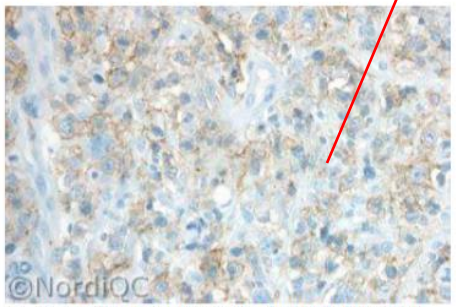


Fig. 3b. Insufficient staining reaction for CD19 of the DLBCL using same protocol as in Fig. 2b. Only a weak staining is seen in scattered neoplastic cells. The majority of the tumour cells are negative. Compare with the optimal protocol in Fig. 3a, same field.

Lymphoma panel: CD19

Optimal protocol settings (NQC)

CD19	Retrieval buffers	Titer	Detection systems	RTU	Detection
mmAb **LE-CD19**	**HIER High pH**	**1:25-1:200**	**3-step**	**Dako (IR656)**	**Flex+**
mmAb **BT51E**	**HIER Low pH buffer (BERS1)**	**RTU**	**3-step**		**BOND Refine**

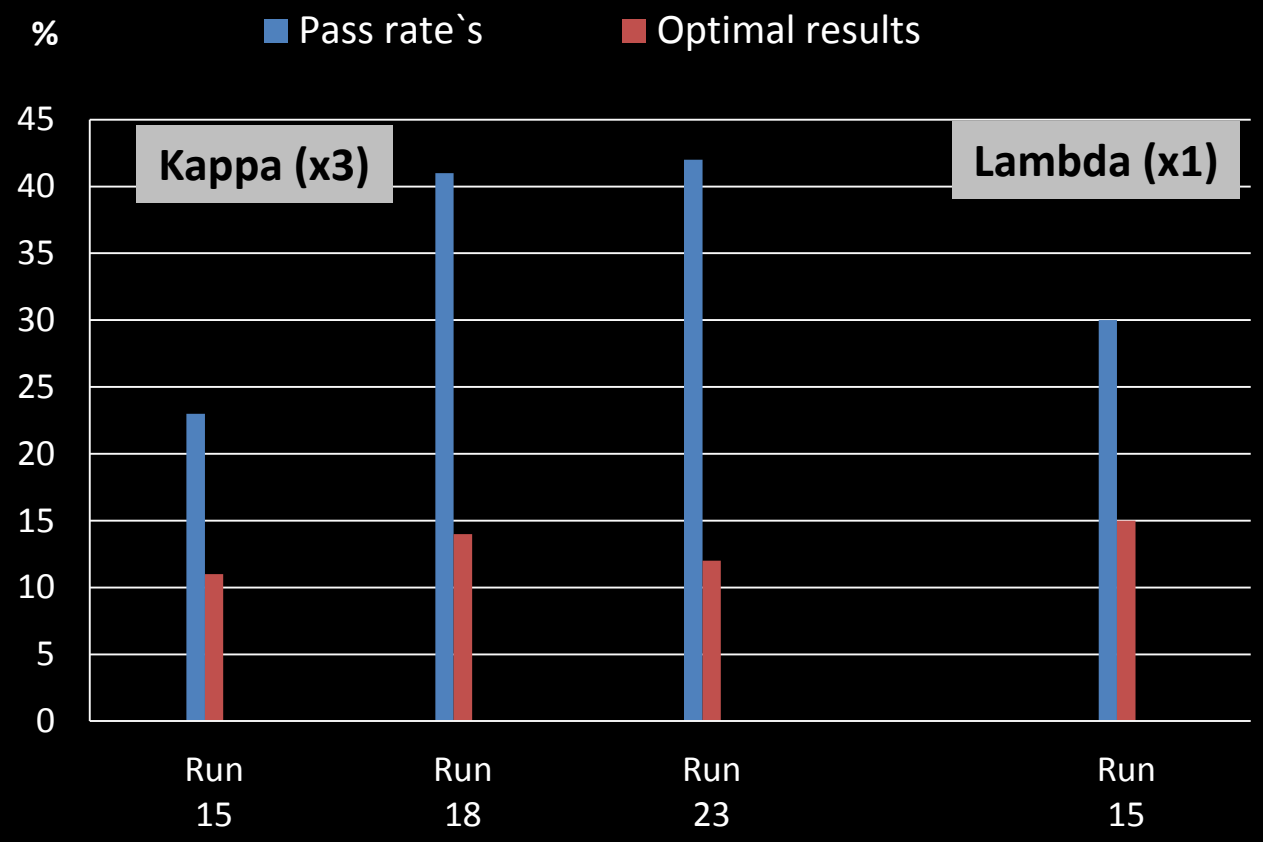
Control material / Tonsil:

An strong, distinct membranous staining reaction of all B-cells in the tonsil.

A weak staining of normal plasma cells in the tonsil and the appendix.

No staining of other cellular structures

Kappa & Lambda Ig light chains



Kappa & Lambda

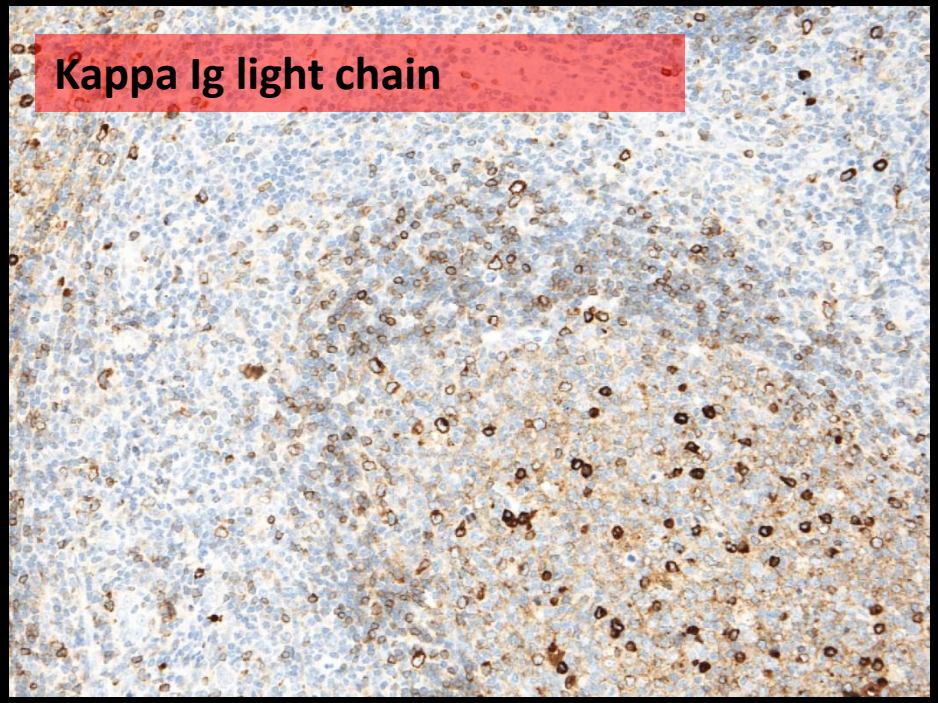
Very low pass rate

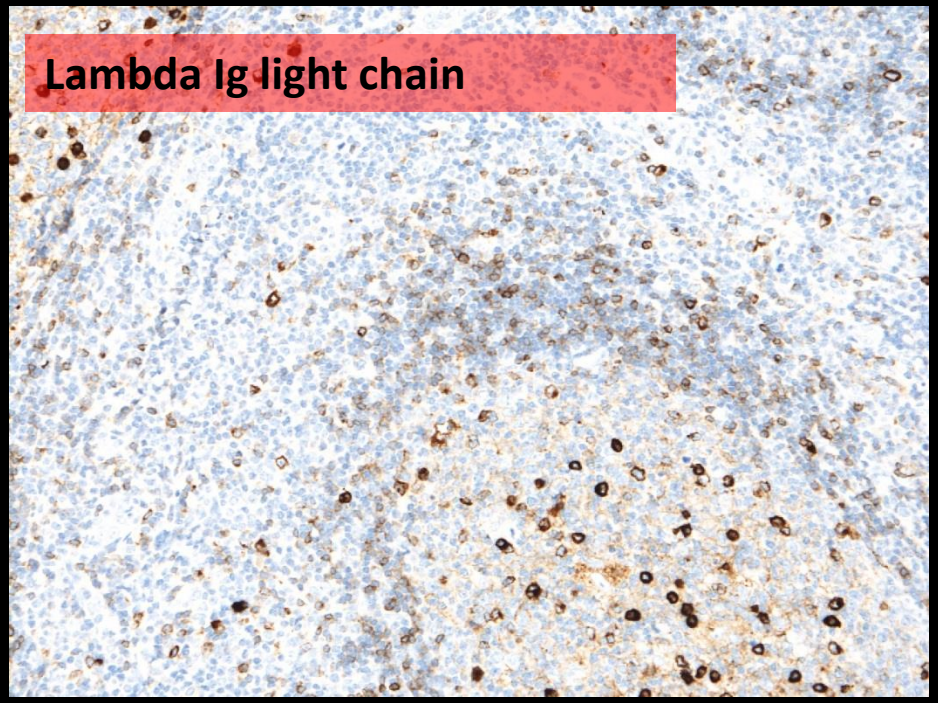
Very low optimal score rate

Challenging assays

App. 80-90 participants pr. Run

Tonsil





A moderate to strong, distinct membranous staining of approximately half of the normal B-cells in the mantle zone in the tonsils (Kappa or Lambda)

A strong cytoplasmic reaction of approximately half of the plasma cells / activated B-cells transforming to plasma cells (Kappa or Lambda)

No staining of T-cells

"Weak" background staining due to normal Ig`s circulating in plasma (Kappa or Lambda)

Kappa & Lambda Ig light chains

The most frequent causes of insufficient staining were:

- Less successful primary antibody
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Inappropriate epitope retrieval (proteolytic pre-treatment)
- No pretreatment.

Optimal results could only be obtained with the pAb`s from Dako:

Kappa: pAb`s A0191 & A0192 (A0192 discontinued)

Lambda: pAb`s A0193 & A0194

Kappa & Lambda Ig light chains

Table 2. Proportion of sufficient and optimal results with Abs used for membranous IgK in the three NordiQC assessments.

	Sufficient	Sufficient %	Optimal	Optimal %
mAb clone **A8B5*)**	0/9	0	0/9	0
mAb clone **HP6053**	0/3	0	0/3	0
mAb clone **KDB-1**	0/2	0	0/2	0
mAb clone **kp-53**	0/2	0	0/3	0
mAb clone **L1C1**	0/3	0	0/3	0
mAb clone **R-10-21F3**	1/9	11	0/9	0
pAb **760-2514**	2/12	17	0/12	0
pAb **A0191**	85/181	47	30/181	17
pAb **A0192**	7/13	54	1/13	8
pAb **N1510**	0/3	0	0/3	0
pAb **NCL-KAPp**	0/2	0	0/2	0

*) Removed from the Dako portfolio before 2005. (Note added 10.12.09 /mv)

Table 3. Proportion of sufficient results with HIER and proteolytic pre-treatment for the IgK pAb A0191 in the three NordiQC assessments:

	HIER		Proteolysis	
	Sufficient	Optimal	Sufficient	Optimal
pAb A0191	52% (84/161)	19% (30/161)	5% (1/20)	0% (0/20)

Table 4. Showing the difference in the proportion of sufficient results using pAb A0191 in its optimal protocol settings versus the general protocol settings.

	All protocols Runs 15, 18 & 23		Optimal protocol settings* Runs 15, 18 & 23	
	Sufficient	Optimal	Sufficient	Optimal
pAb A0191	47% (85/181)	17% (30/181)	72% (75/104)	29% (30/104)

* HIER in citrate pH 6.0 or Target Retrieval Solution pH 6.1 (TRS, Dako, S1699/1700) and a dilution of A0191 in the range of 1:2.000 – 16.000.

Kappa Ig light chain:

Summarized data for the three NordiQC asessements

Run 15
Run 18
Run 23

Kappa & Lambda Ig light chains

Condition for an optimal calibrated protocol:

- **HIER**

  Standard citrate buffer pH6

  Modified citrate buffer pH6.1 (TRS S1700, Dako)

  "Alkaline buffer"

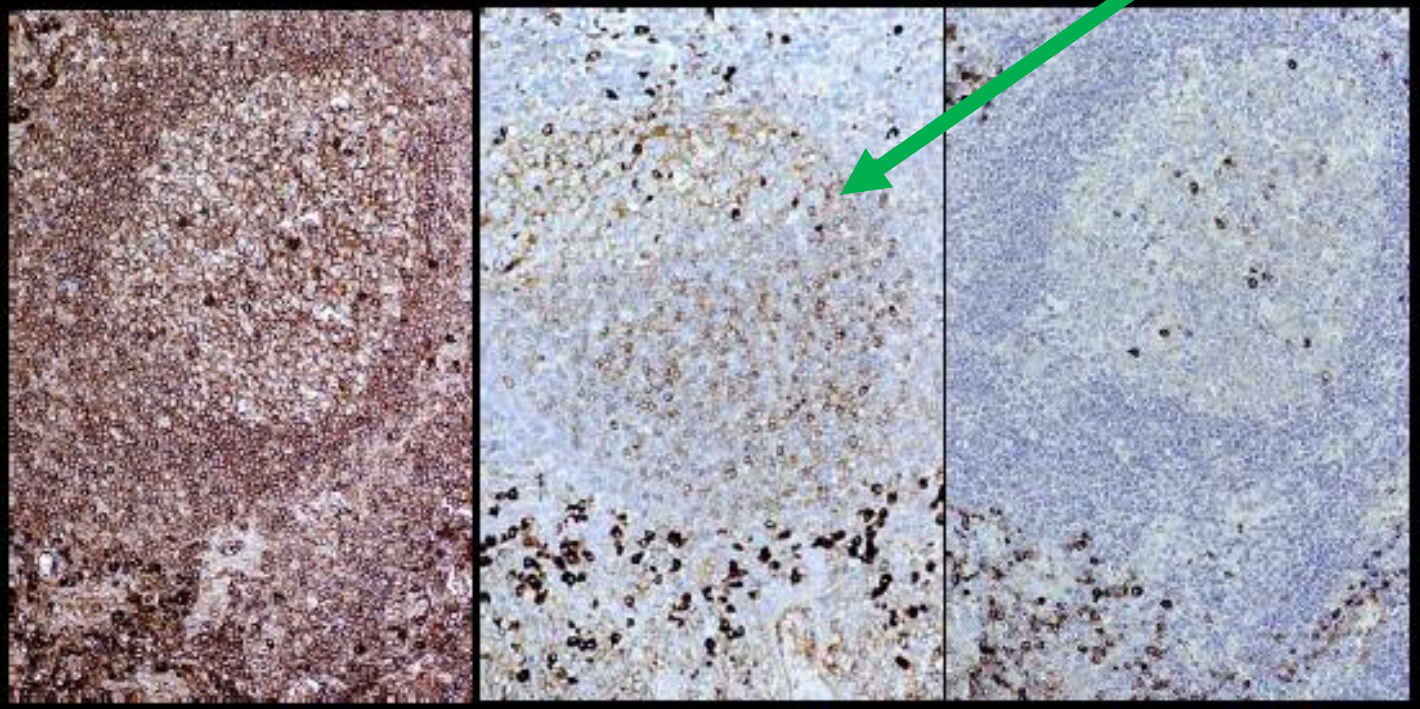
- **Careful calibration of the primary Ab**

  pAb A0191 Kappa (1:2000-8000) depending on the sensitivity of the IHC system

  pAb A0193 Lambda (1:2000-8000) depending on the sensitivity of the IHC system

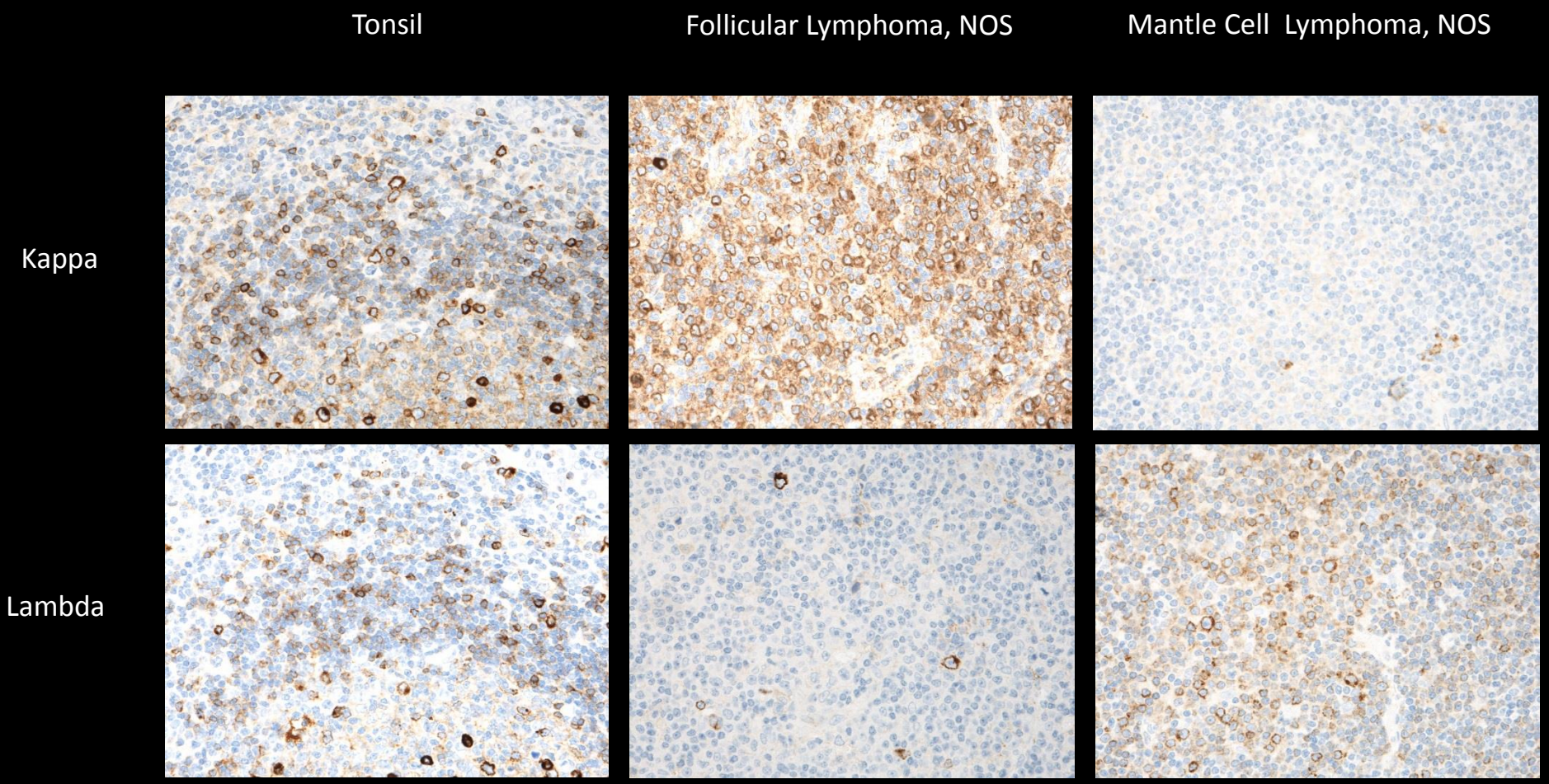
Inappropriate antibody dilution – Ig light chains

IgK: Dako pAb A0191

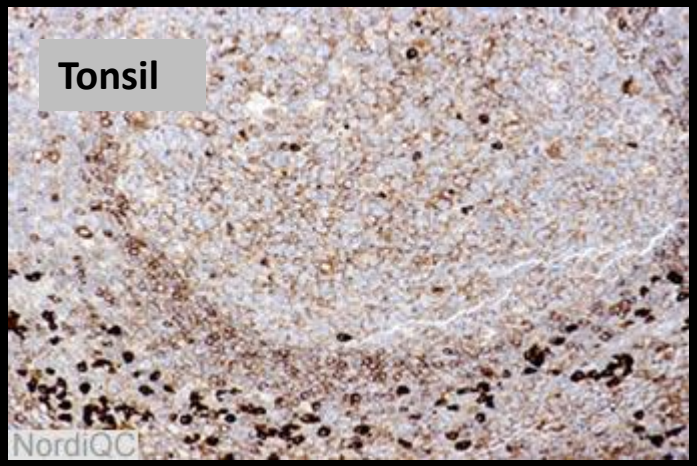


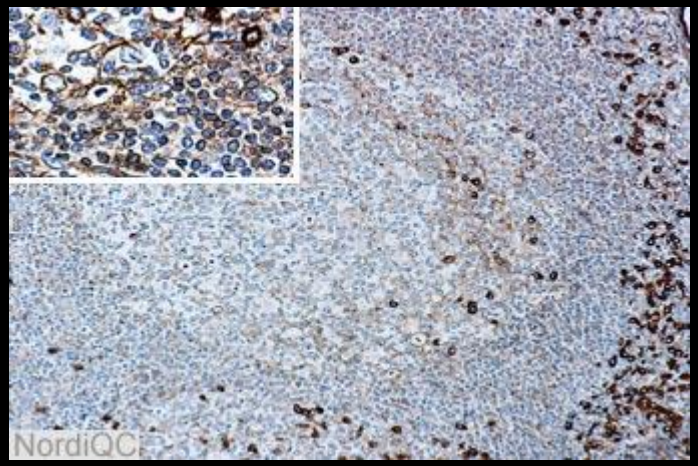
Courtesy: Søren Nielsen

Kappa & Lambda light chain restriction



Kappa & Lambda Ig light chains

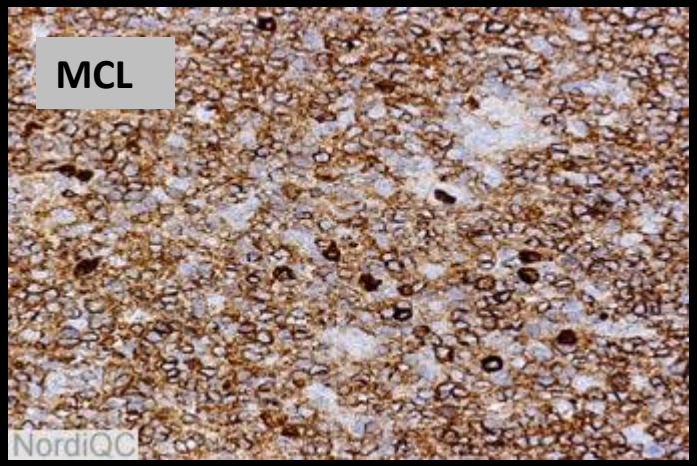


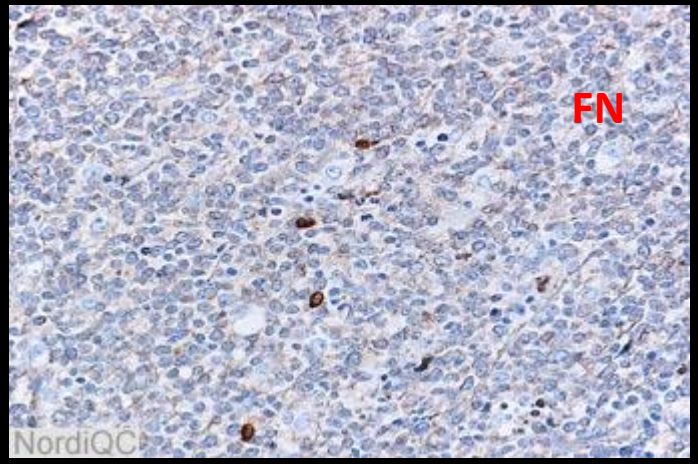


Problem:

Proteolysis

The cytoplasm of the B-cells is over digested causing a too weak staining of the mantle zone B-cells.



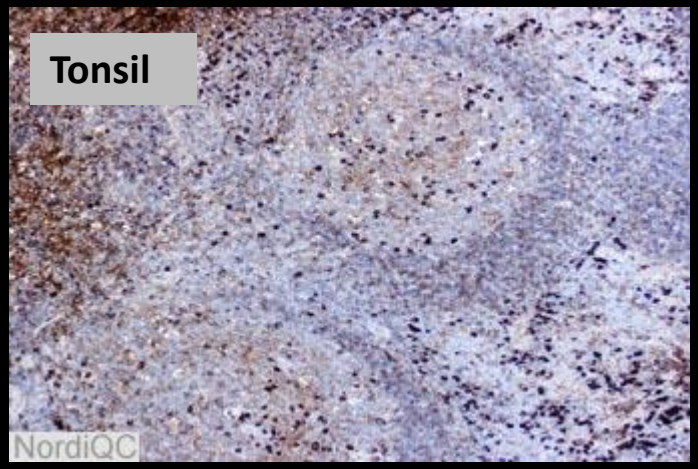


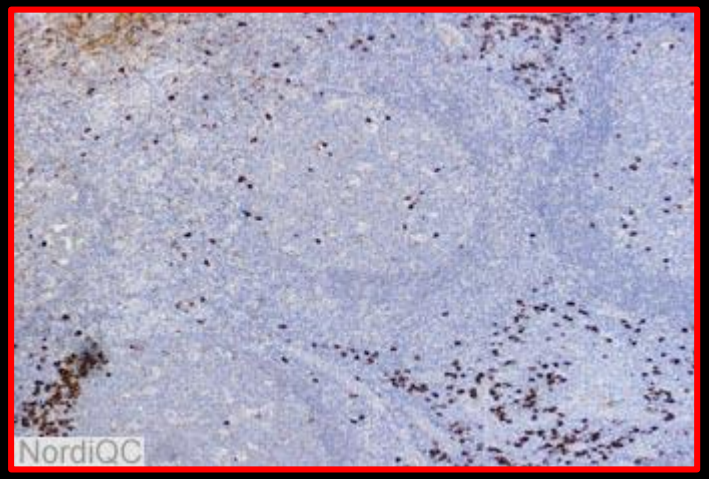
False negative staining for IgL of the MCL using the same protocol as above (right side)
The cell membranes are over digested.

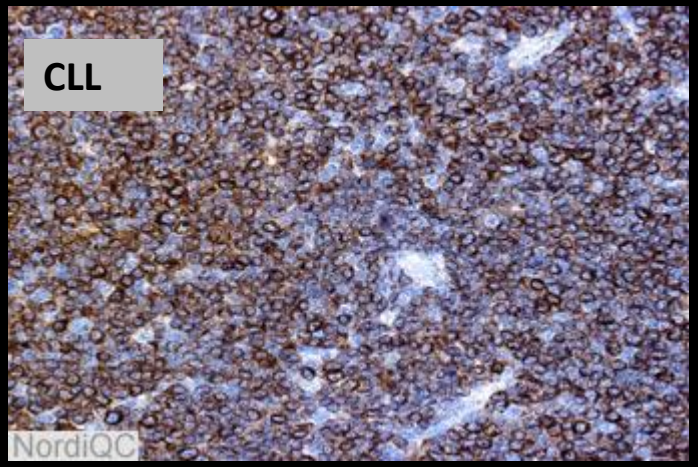
Optimal

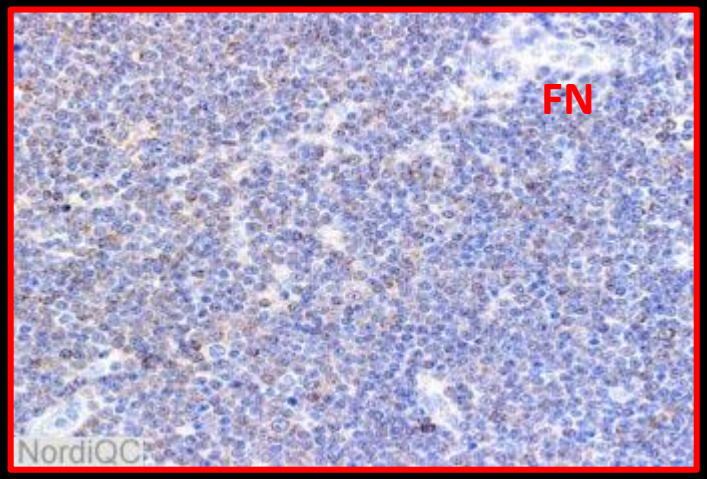
Insufficient

Kappa & Lambda Ig light chains









Optimal

Insufficient

Problem:

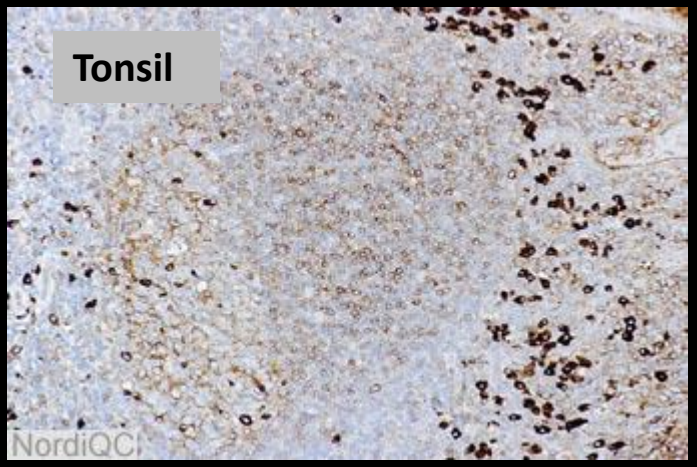
Too low conc. of the primary Ab

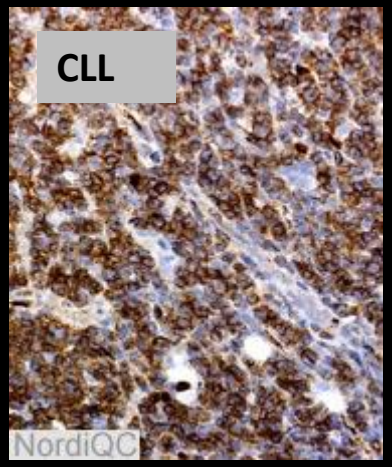
Only plasma cells are stained

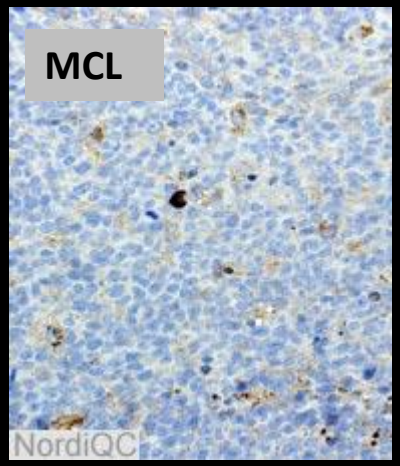
False negative staining for IgK of the CCL using the same protocol as above (right side)

Kappa & Lambda Ig light chains

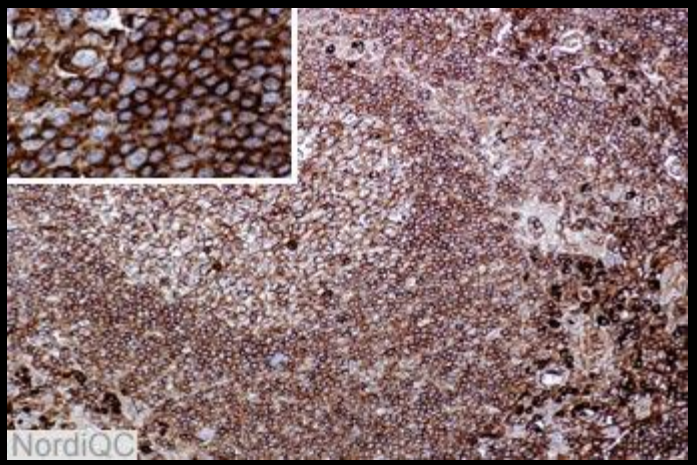
Optimal

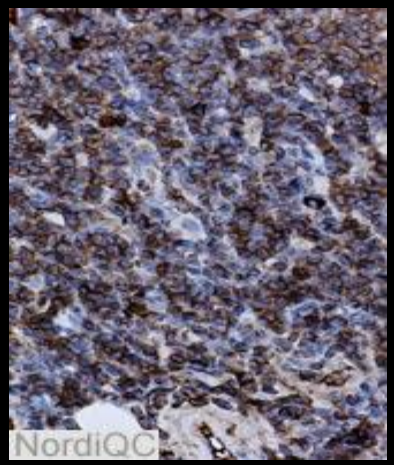


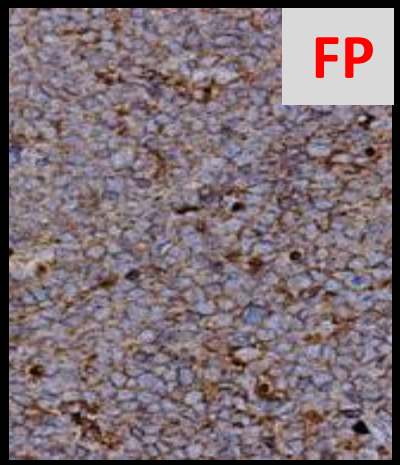




Insufficient







Problem: Too high conc. of the primary Ab

Kappa & Lambda Ig light chains

Lymphoma panel: Kappa and Lambda
Optimal protocol settings (NQC)

Kappa/Lambda	Retrieval buffers	Titer	Detection systems	RTU	Detection
pAb **A0191 (Kappa)**	**HIER Citrate based buffer pH 6**	**1:2000-8000**	**2-step**	**Dako/Agilent (IR/IS506)*** **Dako/Agilent (GA506)**	**Flex**
pAb **A0193 (Lambda)**	**HIER Citrate based buffer pH 6**	**1:2000-8000**	**2-step**	**Dako/Agilent (IR/IS507)*** **Dako/Agilent (GA507)**	**Flex**

*** Not available in run15/18**

Tonsil is recommended as positive and negative control:

A moderate to strong, distinct membranous staining reaction of approximately half of the B-cells in the mantle zone of the follicles in the tonsil (Kappa or Lambda)

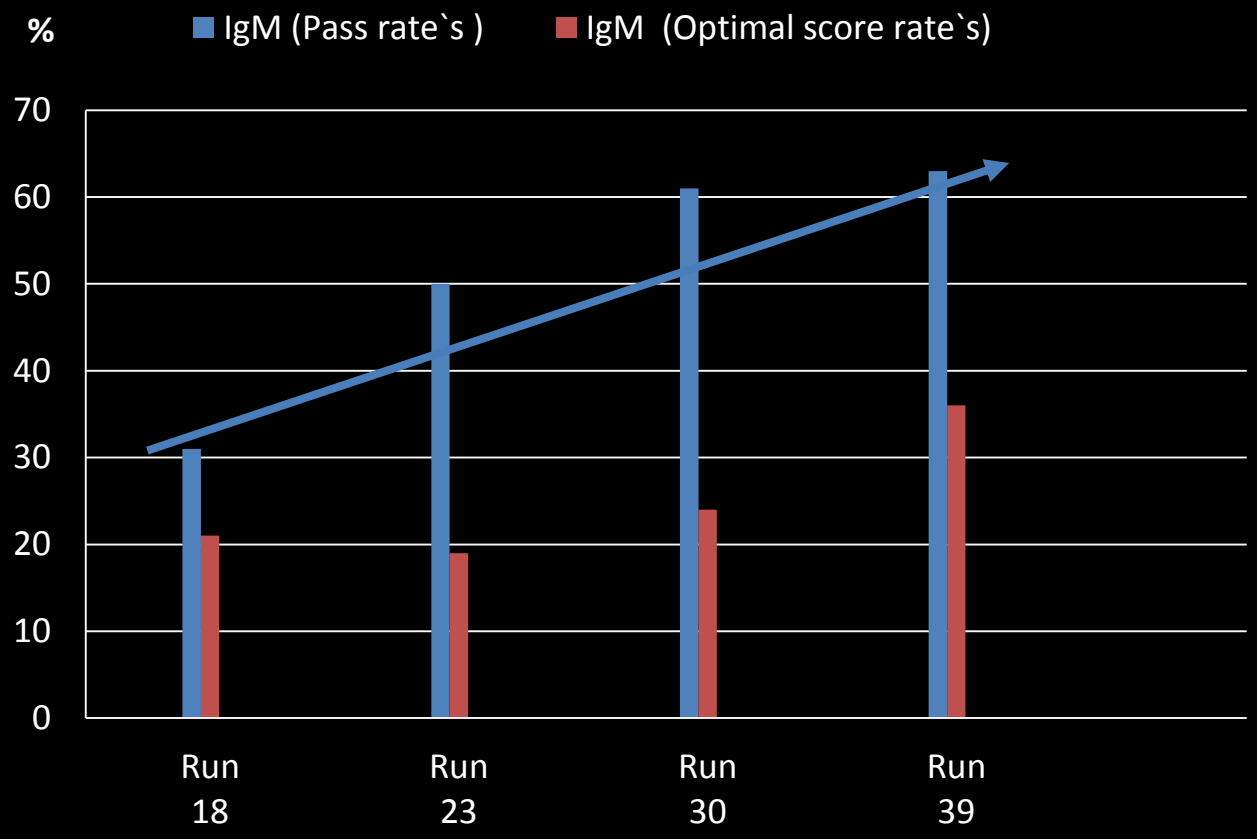
Strong cytoplasmic staining of approximately half of the plasma (Kappa or Lambda)

No staining og T-cells

"Weak" background is acceptable due to circulating Ig`s in plasma

IgM

Pass & Optimal score rate`s



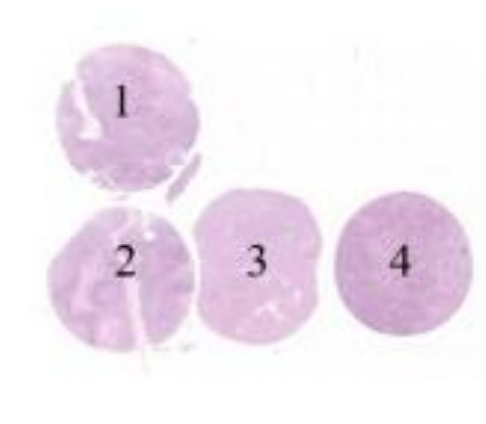
IgM/ Run 39:

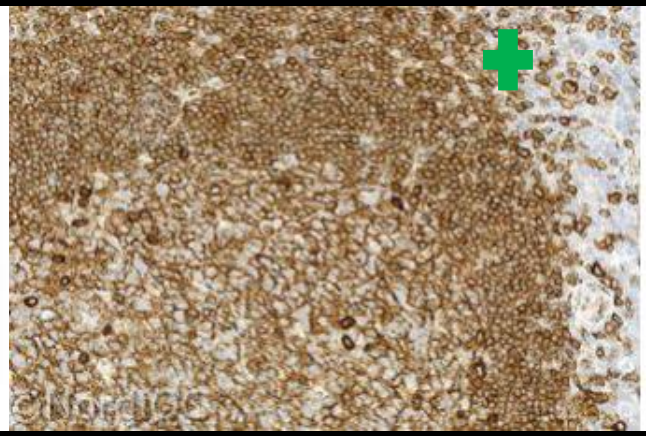
Sufficient: 63%

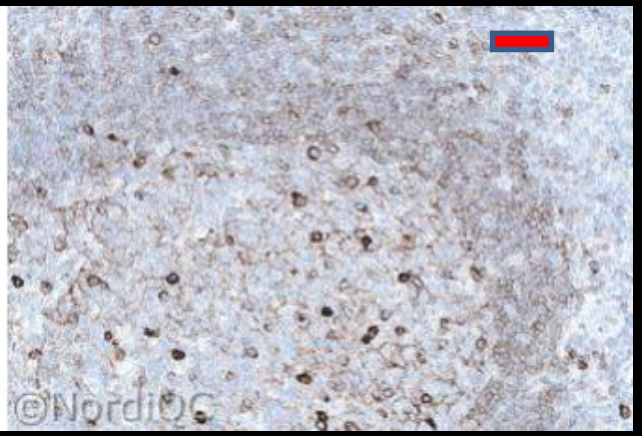
Optimal: 36%

A challenging marker

IgM / Run 39 2013







Criteria for assessing a IgM staining as optimal included:

Core	Membranous/ Cytoplasmic staining reaction
1. Tonsil (24h)	**+ Mantle zone B-cells of the germinal centres /Follicular dendritic network/Plasma cells**
2. Tonsil (48h)	**+ Mantle zone B-cells of the germinal centres /Follicular dendritic network/Plasma cells**
3. Mantle cell lymphom	(+)
4. Follicular lymphoma	(+)
No more than weak background . T- cells are negative.	

Tonsil is recommended as control material

IgM / Run 39 2013

Table 1. **Antibodies and assessment marks for mIgM, run 39**

Concentrated antibodies	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8H6**	6	Leica/Novocastra	0	0	3	3	0 %	-
mAb clone **IgM88**	1	BioGenex	0	0	0	1	-	-
pAb **A0425**	75	Dako	35	15	11	14	67 %	95 %
pAb **A0091***	2	Dako	0	0	2	0	-	-
pAb **NCL-IgMp***	1	Leica/Novocastra	0	0	1			
pAb **PU427-UP**	1	BioGenex	0	1	0			
pAb **RaHu/IgMFC**	1	Nordic MUbio	0	0	0			
pAb **RB-1434**	5	Thermo/NeoMarkers	0	1	1	3	20 %	-
Ready-To-Use antibodies	N							
pAb **270A-17/18**	2	Cell Marque	0	2	0	0	-	-
pAb **760-2654**	21	Ventana/Cell Marque	6	9	2	4	71 %	92 %
pAb **AR427-5R**	1	BioGenex	0	1	0	0	-	-
pAb **GA04250**	1	Gene Tech	0	0	0	1	-	-
pAb **IR/IS513**	21	Dako	7	9	4	1	76 %	93 %
pAb **MAD-005029QD**	1	Master Diagnostica	1	0	0	0		-
pAb **N1509***	1	Dako	1	0	0	0	-	-
Total	140		50	38	24	28		
Proportion			36 %	27 %	17 %	20 %		

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings onl
*discontinued Abs

Optimal results (%)

50%

0%

29%

33%

Optimal titre (1:500 – 1:2000) and appropriate epitope retrieval (HIER)

Protocol settings recommended by Dako
(HIER in TRS High pH (20`) at 95-97°C 20 min. inc. primary Ab EnVision FLEX

Optimal results could only be obtained with the pAb A0425 as concentrate and the pAb`s 760-2654 (Ventana), IR/IS513 (Dako), MAD-005029QD (Master Diagnostica) & N1509 (Dako – discontinued)

IgM (Run 39 2013) : Observations with impact on the final result

- **Inappropriate epitope retrieval (proteolytic pre-treatment or no pre-treatment)**
  - Insufficient result in 8 of 9 protocols (none were assessed as optimal)
  - Change to HIER (preferable acidic/standard or mod. Low pH buffer)

Table 2. **Optimal results for mIgM using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
pAb A0425 **Dako**	40 % 4/10**	56 % 5/9	55 % 12/22	100 % 1/1	0 % 0/7	86 % 6/7

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

  - A high proportion of sufficient results was seen provided that HIER (preferable in acidic buffer – see table) and an appropriate titre was applied

- **Less successful primary Ab**
  - Protocols based on the mAb clone 8H6 ~ 6 out of 6 protocols were assessed as insufficient (borderline or poor)

mIgM (Run 39)

Optimal result

mIgM staining optimally calibrated and with HIER.

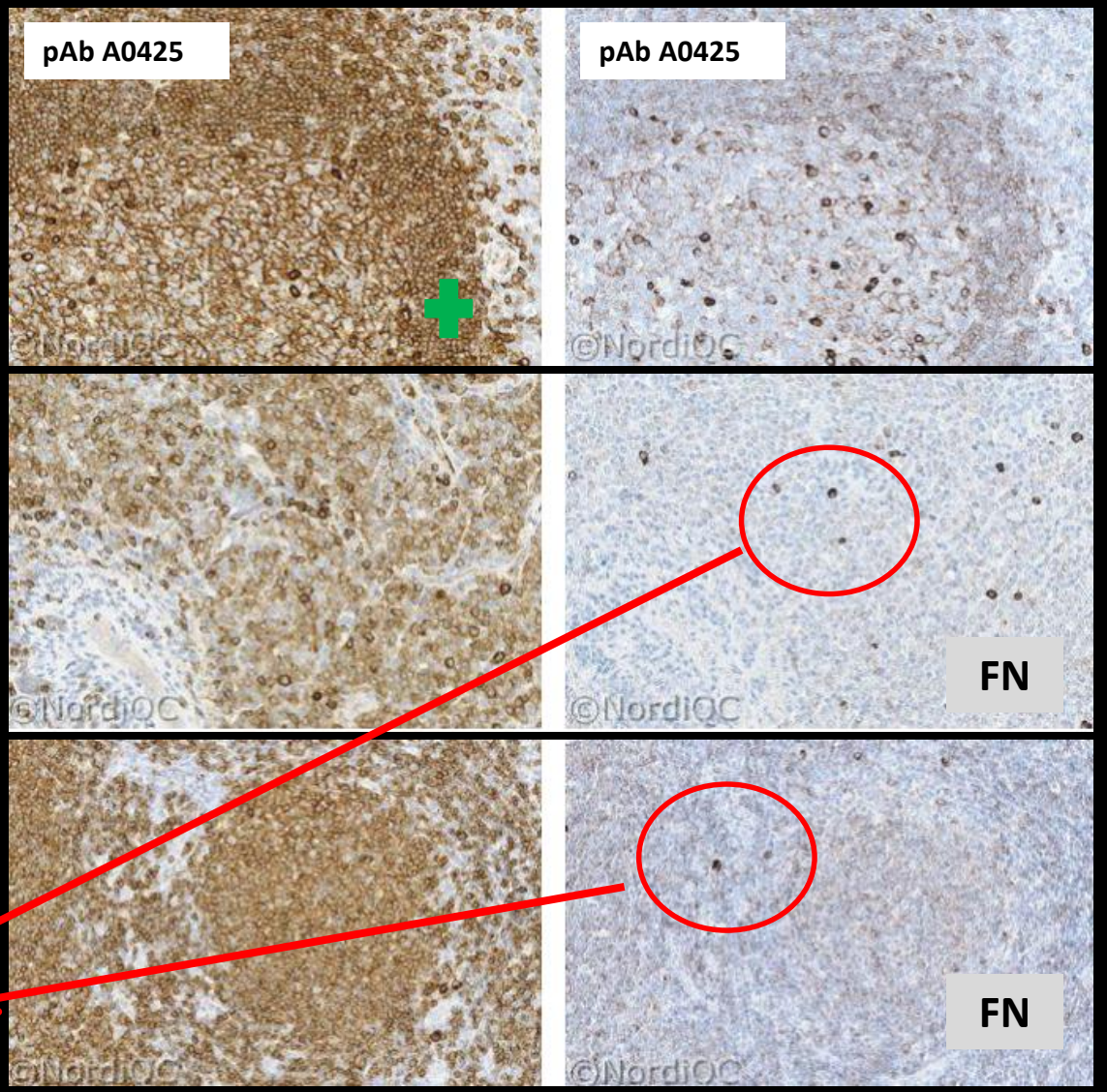
Insufficient result

Insufficient mIgM staining using HIER but with too low concentration of the primary Ab

Tonsil

Mantle cell lymphoma

Follicular lymphoma



Only plasma cells are stained

IgM/ Run 39 2013

Lymphoma panel: IgM
Optimal protocol settings (NQC)

IgM	Retrieval buffers	Titre	Detection	RTU	Detection
pAb **A0425**	**HIER , mod/standard low pH & High pH (RTU)**	**1:500-1:2000**	**-**	**Dako (IS/IR513)**	**Flex**
pAb 760-2654	**HIER, High pH (CC1)**	**-**	**-**	**Dako (IS/IR/GA648)**	**UltraView + amp OptiView**

Control material / Tonsil:

A strong, distinct membranous staining reaction of virtually all mantle zone B-cells of the germinal centres in the tonsils.

A strong cytoplasmic reaction in plasma cells, immunoblasts and follicular dendritic network in the germinal centres of the tonsils.

B-Cell lymphoma markers (2)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
BCL2 (cytopl. + nuclear) 124, 100/D5, BCL/100/D5, 100	Tonsil/Appendix	Mantle zone B-cells & T-cells (including intra germinal centre T-cells)	Basal cells (squamous epithelium) in surface epithelium of the tonsil & columnar cells lining basal compartment of the crypts (appendix)	Germinal centre B-cells (tonsil)
CD10 (cytopl. + membr.) 56C6, GI191E/A8	Tonsil/Kidney	Germinal centre B-cells (Tonsil, moderate to strong intensity). Proximale tubuli (Kidney)	Scattered neutrophil granulocytes	Mantle zone B-cells and squamous epithelial cells (tonsil)
CD23 (membr.) 1B12, DAK-CD23, BS20, SP23	Tonsil	Follicular dendritic cells in the germinal centres	Mantle zone B-cells and scattered interfollicular B-cells	No staining of T-cells
CyclinD1 (nuclear) SP4, EP12	Tonsil	Suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells	Germinal centre macrophages	Mantle zone B-cells and germinal centre B-cells
SOX11 (nuclear) SOX11-C1, MRQ-58	MCL`s /Tonsil	MCL	MCL	Tonsil (all cells)
CD43 (membr.) DF-T1	Tonsil/Appendix	T-cells in the T-zone (tonsil)	Intra germinal centre T-cells (an at least moderate expression) , macrophages (tonsil, germinal centres) and plasma cells	Mantle zone B-cells of germinal centres (tonsil) and epithelium (app.)
CD5 (see T-cells) & TdT (see blasts/bonus material)				

Clones (mAbs, rmAbs & pAbs) giving optimal results (NordiQC assessments)

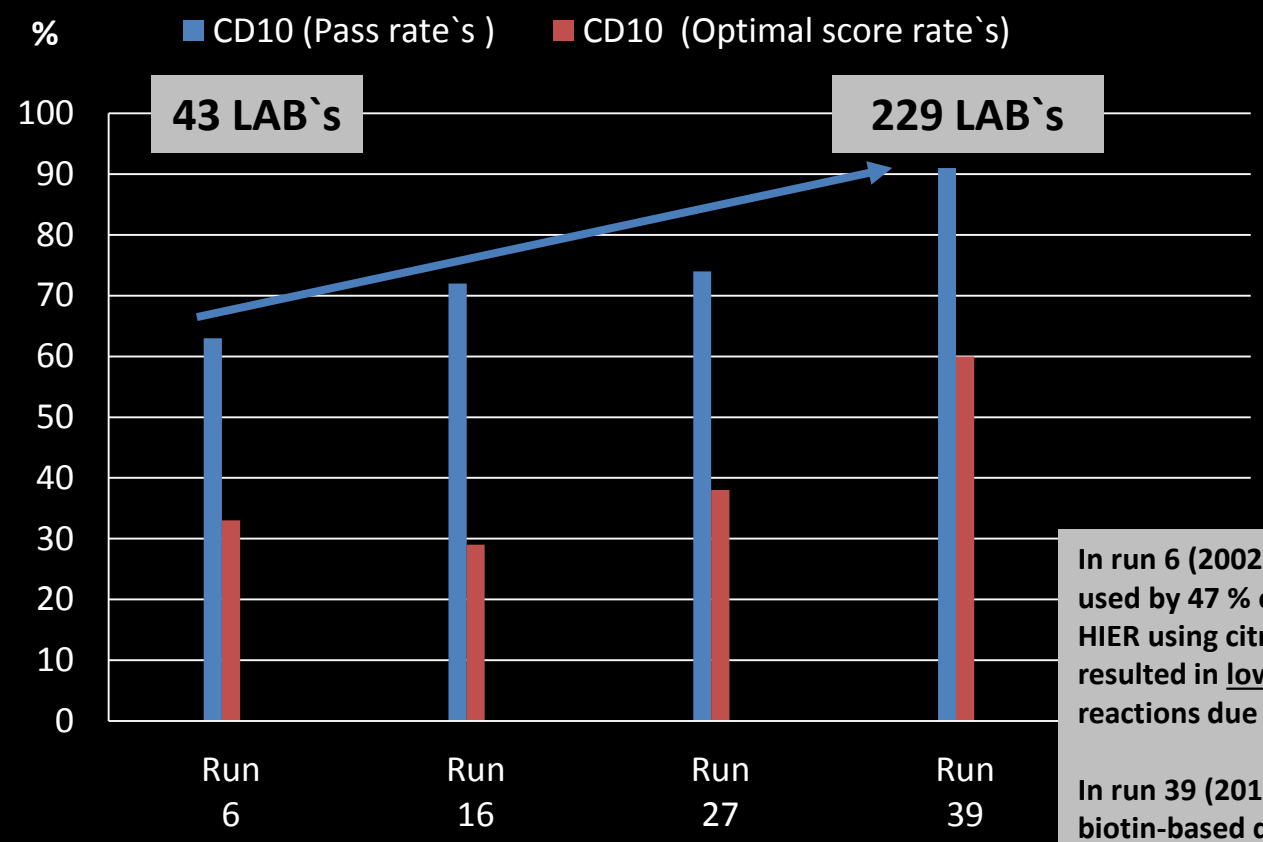
iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

CD10

Pass & Optimal score rate`s



CD10/ Run 39:

Sufficient: 91%

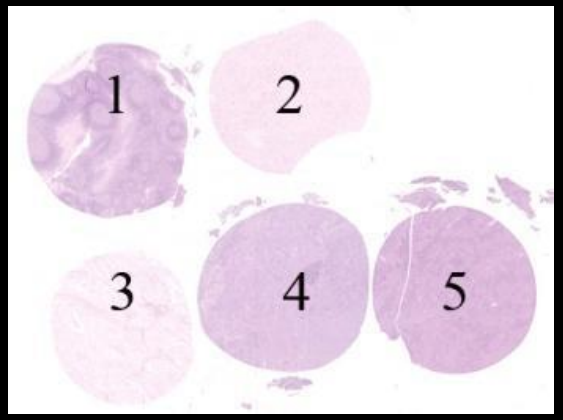
Optimal: 60%

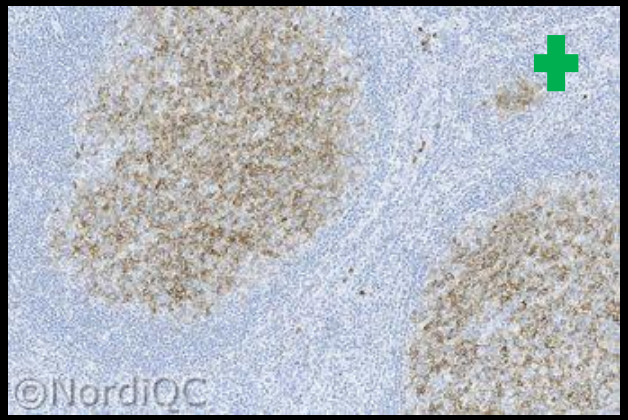
Success due to robust Abs and …..

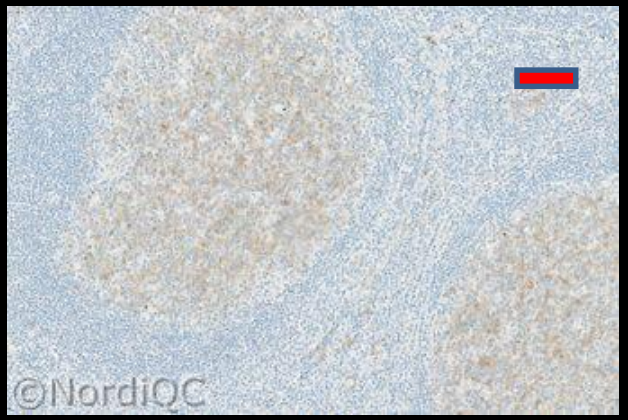
In run 6 (2002) biotin-based detection systems were used by 47 % of the participants and 23 % performed HIER using citrate pH 6. These protocol settings resulted in low sensitivity and false positive staining reactions due to endogenous biotin.

In run 39 (2013), 4 % of the participants used a biotin-based detection system and 2 % used HIER in a non-alkaline buffer as citrate pH 6.

CD10/ Run 39 2013







Criteria for assessing a CD10 staining as optimal included:

Core	Membranous/Cytoplasmic staining reaction
1. Tonsil (24h)	+ **germinal centre B-cells (moderate to strong membranous reaction)**
2. Kidney	+ Epithelial cells in the renal proximal tubules and the parietal layer of the Bowman's capsule (predominately strong membranous reaction).
3. Renal clear cell carcinoma	+ (moderate reaction)
4. Burkitt lymphoma	+ (moderate reaction)
5. Follicular lymphoma	(+)
An at least weak to moderate staining of neutrophil granulocytes in all the specimens.	

Tonsil is recommended as control material

Table 1. **Antibodies and assessment marks for CD10, run 39**

Concentrated antibodies	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **56C6**	80 14 9 6 4 4 1 1 1 1	Leica/Novocastra Dako Thermo/NeoMarkers Monosan Biocare Cell Marque Diagnostic Biosystems DCS Nordic Biosite Vector	81	32	6	2	93 %	95 %
				67%				
rmAb clone **EP195**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb clone **G27-P**	1	Biotech	0	0	0	1	-	-
Ready-To-Use antibodies				**77%**				
mAb clone **56C6 IS648/IR648**	47	Dako	36	10	1	0	98 %	98 %
mAb clone **56C6 GA648**	1	Dako	1		0	0	-	-
				86%				
mAb clone **56C6 PA0270**	7	Leica	6	1	0	0	100 %	100 %
mAb clone **56C6 110M-18**	3	Cell Marque	2	1	0	0	-	-
mAb clone **56C6 PM129**	1	Biocare	1	0	0	0	-	-
mAb clones **56C6 PDM107**	1	Diagnostic Biosystems	1	0	0	0	-	-
mAb clone **56C6 GT200402**	1	Gene Tech	0	0	1	0	-	-
rmAb clone **56C6 CD10-270-R-7**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **56C6 MAD-002022QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **56C6 MSG070**	1	Zytomed	1	**21%**	0	0	-	-
rmAb clone **SP67 790-4506**	43	Ventana	9	24	10	0	79 %	96 %
Total	230		138	71	18	3	-	
Proportion			60 %	31 %	8 %	1 %	91 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

2 robust clones:

mmAb 56C6 (conc. & RTU)

rmSP67 (RTU)

mmAb 56C6 (conc. & RTU)

HIER in alkaline buffer or mod. low pH buffer (Diva pH6.2), dil. range 1:10 -1:100

Flex/Flex+ (Dako)
BOND Refine (Leica)
MACH4 (Biocare)

rmAb SP67 (RTU 790-4506)

HIER in alkaline buffer (CC1 pH 8.5)

UltraView + amp (Ventana)
OptiView +/- amp (Ventana)

All 9 protocols with optimal results were using the protocol settings as described above

Recommended detection system giving by the vendor: UltraView

CD10 (Run 39 2013) : Observations with impact on the final result

Table 2. **Optimal results for CD10 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **56C6**	64 % 14/22**	0 % 0/1	67 % 35/52	-	95 % 19/20	0 % 0/1

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

HIER in BERS2 / BOND refine (3-step detection system)

- **Pass rate and optimal results was influenced by the choice of detection system**

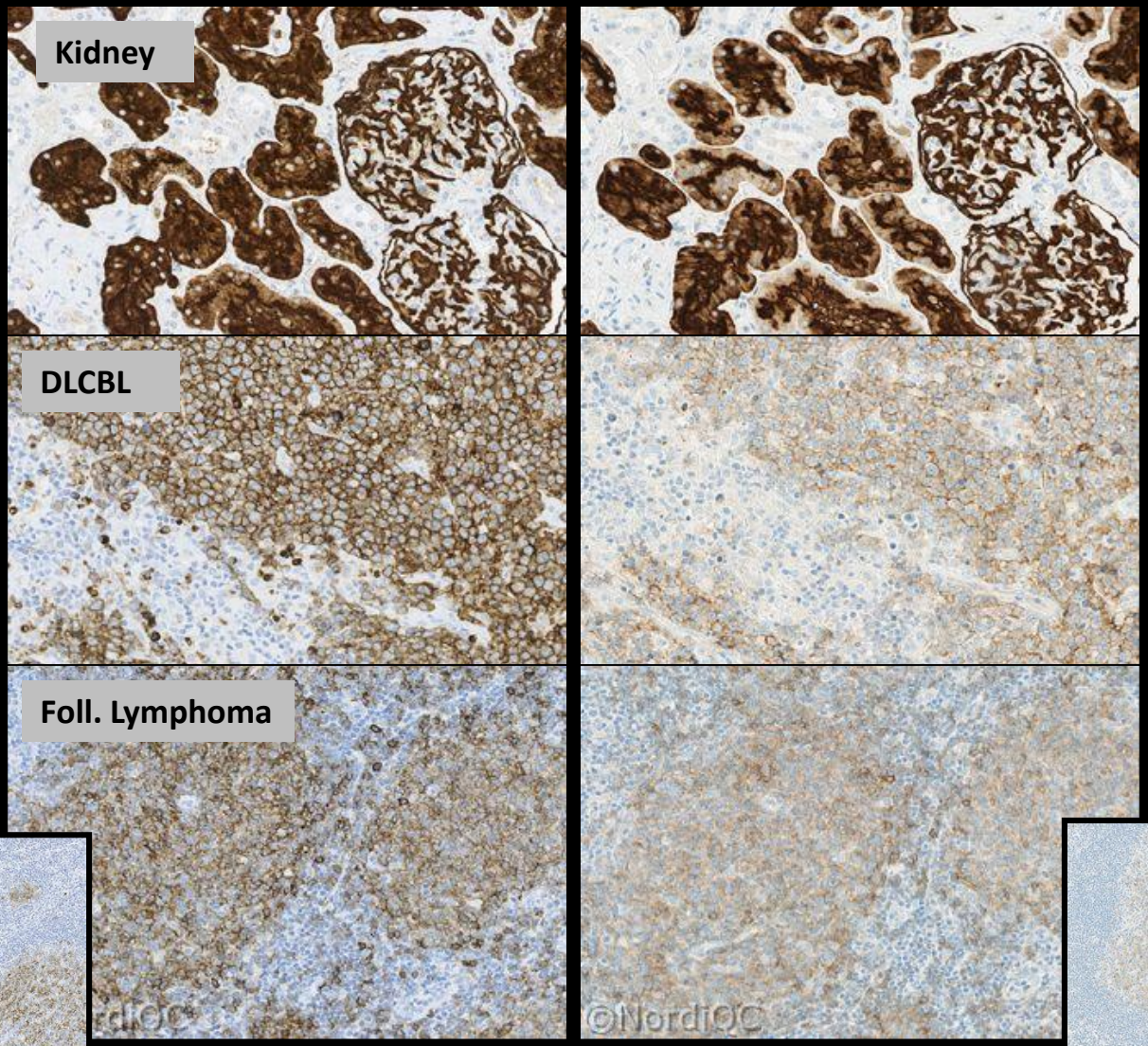
LD assay (mmAb clone 56C6) HIER in alkaline buffer and optimal dil. range	Detection system	Pass Rate`s (%)	Optimal (%)
2-step polymer/multimer system	Flex (Dako) or UltraView (Ventana)	91 (42 of 46)	52 (24 of 46)
3-step polymer/multimer system	Flex+ (Dako), OptiView (Ventana) or BOND Refine (Leica)	100 (58 of 58)	86 (50 of 58)

CD10/ Run 39 2013

Optimal

HIER in Alkaline buffer

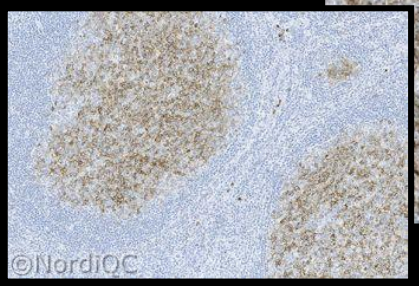
Correct calibrated

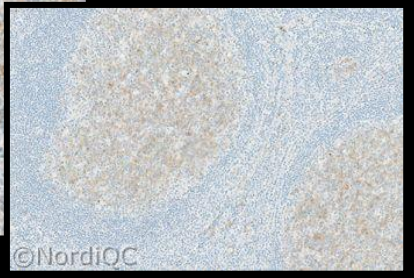


Insufficient

Too low sensitivity

Too weak staining





CD10 / Run 39 2013

Lymphoma panel: CD10
Optimal protocol settings (NQC)

CD10	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **56C6**	**HIER High pH or mod. Low pH buffer**	**1:10-1:100**	**3-step**	**Leica (PA0270)**	**BOND Refine**
		-	**-**	**Dako (IS/IR/GA648)**	**Flex/Flex+**
		-	**-**	**Biocare (PM129)**	**MACH4**
rmAb **SP67**	**HIER High pH buffer**	**-**	**-**	**Ventana (790-4506)**	**UltraView + amp OptiView +/- amp**

Control material / Tonsil:

An at least moderate, distinct membranous staining reaction of virtually all germinal centre B-cells in the tonsil.

An at least weak to moderate staining of neutrophil granulocytes

SOX11

Table 1. **Antibodies and assessment marks for SOX11, run 47**

Concentrated antibodies:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CL0142**	1	Abcam	0	0	1	0	-	-
mAb clone **CL0143**	1	Atlas	0	1	0	0	0	-
mAb clone **MRQ-58**	38 1 1	Cell Marque ImPath Zeta	13	17	7	3	75%	80%
mAb clone **SOX11-C1**	5 1	Affymetrix/eBioscience Biocare Medical	3	1	2	0	67%	100%
mAb clone **ZSX11**	1	Zytomed	0	0	1	0	-	-
Polyclonal	4 1	Sigma Atlas	0	1	1	3	20%	-
Ready-To-Use antibodies:								
mAb clone **MRQ-58 760-4888**	16	Ventana/Cell Marque	3	7	4	2	63%	100%
mAb clone **MRQ-58 382M-18**	5	Cell Marque	0	2	3	0	40%	-
mAb clone **MRQ-58 MAB-0699**	2	Maixin	1	1	0	0	-	-
mAb clone **MRQ-58 MAD-000581QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **SOX11-C1 API3120**	1	Biocare Medical	0	1	0	0	-	-
Total	79		21	31	19	8	-	
Proportion			27%	39%	24%	10%	66 %	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Insufficient staining results:

Too weak staining reaction of cells expected to be demonstrated

Poor signal-to-noise ratio compromising the interpretation.

Sox11/ Run 47 (2016):

A challenging marker

Optimal result as concentrates:

mAb MRQ-58 & SOX11-C1

Efficient HIER in alkaline buffer

1:25-1:200 (MRQ-58)

1:25-1:50 (SOX11-C1)

2 & 3 step detection systems

Protocols with optimal results:

HIER TRS High pH 24` & Flex+ (10+20`)
HIER CC1 & OptiView

Protocols with optimal results:

HIER CC1 64` & OptiView

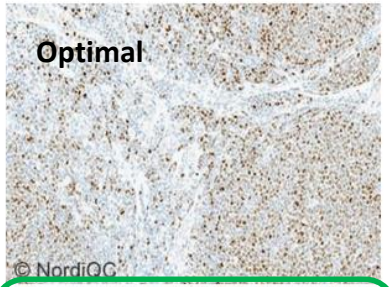
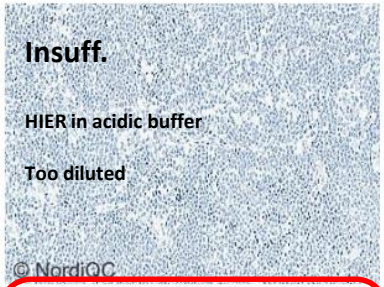


Fig. 1a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 4, using the mAb clone SOX-C11 diluted 1:25, HIER in CC1, a 3-step multimer based detection kit (OptiView) and performed on BenchMark Ultra, Ventana. The vast majority of neoplastic cells show a moderate, distinct, nuclear staining reaction. No background reaction is seen. Also compare with Figs. 2a – 4a, same protocol.



ig. 1b
Insufficient SOX11 staining of the mantle cell lymphoma, tissue core no. 4, using the mAb clone SOX-C11 with a protocol providing a too low sensitivity.
The Ab was used at 1:200, HIER in TRS pH 6,1, a 3-step polymer based detection system, FLEX+ (Dako) and performed on Autostainer Link 48, Dako. Only few cells show a faint nuclear staining reaction. Compare with Fig. 1a – same field.
Also compare with Figs. 2b – 3b – same protocol

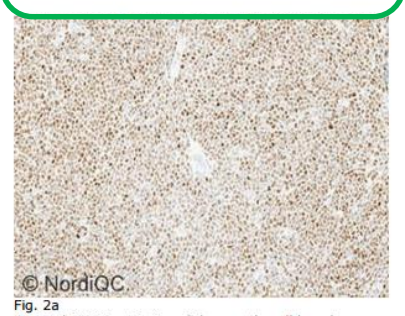


Fig. 2a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background reaction is seen.

Fig. 2b
SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 1b – same field as in Fig. 2b. The majority of neoplastic cells are demonstrated, but the proportion and intensity is reduced compared to the level expected.

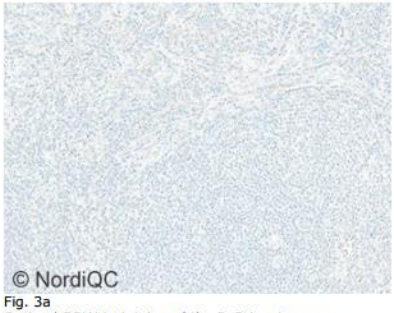


Fig. 3a
Optimal SOX11 staining of the B-CLL using same protocol as in Figs. 1a and 2a. No staining is seen.

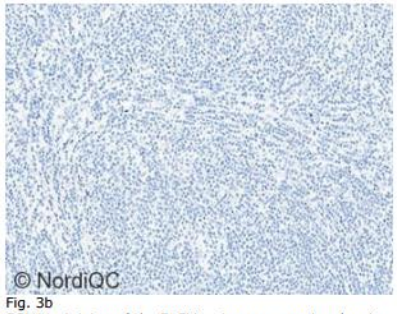


Fig. 3b
SOX11 staining of the B-CLL using same protocol as in Figs. 1b and 2b. No staining is seen.

Fig. 4a
Optimal SOX11 staining of the tonsil using same protocol as in Figs. 1a - 3a.
No staining is seen and the staining reaction of the tonsil confirms an adequate level of signal-to-noise ratio.
Compare with Fig. 4b.

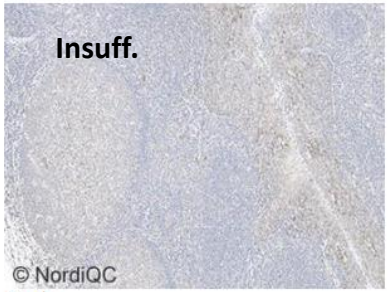


Fig. 4b
Insufficient SOX11 staining of the tonsil using a pAb providing an insufficient result characterized by a poor signal-to-noise ratio. In the tonsil a general background staining is seen and in e.g. plasma cells and squamous epithelial cells a moderate aberrant cytoplasmic staining reaction is seen. Also compare with Figs. 5a and 5b, same protocol.

Fig. 5a
SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 4b. Many neoplastic cells show a weak to moderate nuclear staining reaction, but simultaneously a general background staining is seen compromising the interpretation. The intensity and proportion of cells demonstrated is reduced compared to the level expected and obtained in Fig. 2a. Also compare with Fig. 5b, same protocol.

Fig. 5b
Insufficient SOX11 staining of the B-CLL. A poor signal-to-noise ratio is seen and the aberrant background staining complicates the interpretation of SOX11 in the neoplastic cells.

Problems:

Protocol providing to low sensitivity

Protocol providing poor signal-to-noise ratio (seen with all Ab´s)

Lymphoma panel: SOX11

Optimal protocol settings (NQC)

Sox11	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb MRQ-58	HIER High pH	1:25-1:200	2 & 3-step	Ventana (790-4888)	OptiView
mmAb SOX11-C1	HIER High pH	1:20-1:150	2 & 3-step	-	-

Control material:

Mantle cell lymphomas with varying levels of antigen density (low & high expressors) and non-expressor (Tonsil)

A nuclear staining reaction of the neoplastic cells in the mantle cell lymphoma`s should be observed

No staining should be observed in the tonsillar tissue

B-Cell lymphoma markers (3) - Diffuse Large B-Cell Lymphoma

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
BCL6 (nuclear) LN22, PG-B6p, SP18	Tonsil	Germinal centre B-cells	Squamous epithelial cells	The vast majority of cells in the mantle zones and interfollicular areas
MUM1 (nuclear). MUM1p, EAU32, EP190	Tonsil/Colon	Late stage germinal centre B-cells (tonsil) Plasma cells (tonsil & colon)	"Mantle zone B-lymphocytes (tonsil) "	Epithelia cells and smooth muscle cells (lamina muscularis propria) in the colon.
CD138 (membr.) B-A38, B-B4, MI15	Tonsil	Plasma cells and squamous epithelial cells	Activated germinal centre B-cells	Mantle zone B-cells and T-cells
Ki67 (nuclear) MIB-1, BS4, GM001, K2, UMAB107, 30-9, SP6	Tonsil/lLiver	All germinal centre B-cells (dark zone) in the tonsil	Most germinal centre B-cells (light zone) in the tonsil	99% of "normal" hepatocytes should be negative
FOXP1 (nuclear) EP137	Tonsil/Liver	Virtually all mantle zone B-cells T-cells are positive	App. 50% of germinal centre B-cells in the tonsil (moderate intensity) T-cells are positive	The vast majority of hepatocytes are negative
GCET1 (cytopl) RAM341	Tonsil	Intra germinal centre B-cells (centroblast) – moderate to strong intensity	None	All other cells including T-cells
CMYC (nuclear) EP121	Tonsil/appendix	Activated intragerminal centre B-lymphocytes and scattered lymphocytes in interfollicular zones	App. 10-50 % of the mantle zone B-cells. Suprabasal squamous epithelial cells in the tonsil often displays moderate intensity.	Luminal epithelia cells of the appendix. The basal crypt epithelia cells displays moderate intensity.
CD10, see B-cell lymphoma markers (2) & TdT, see blast`s/bonus material				

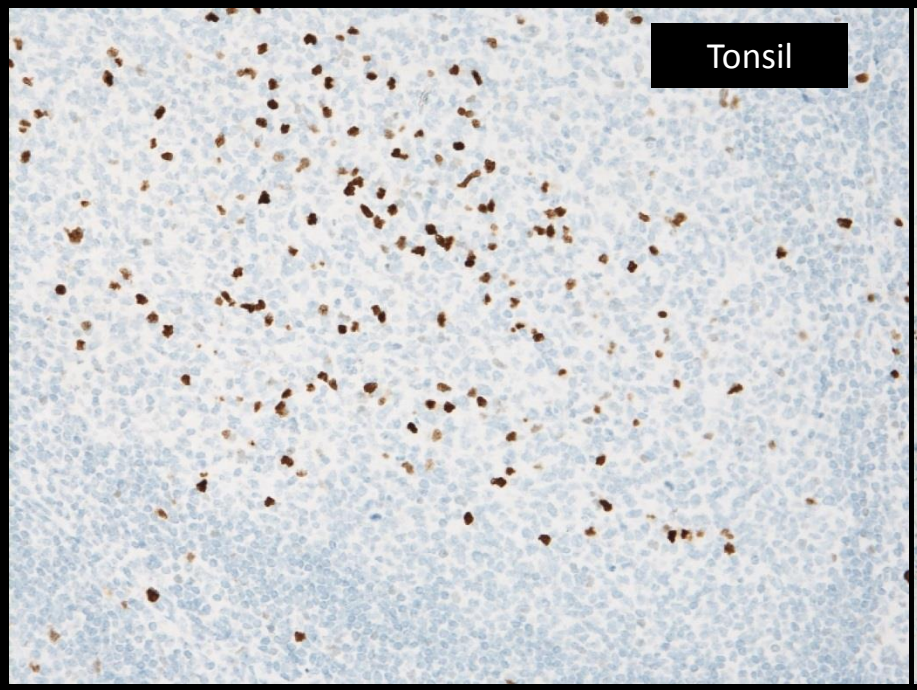
Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

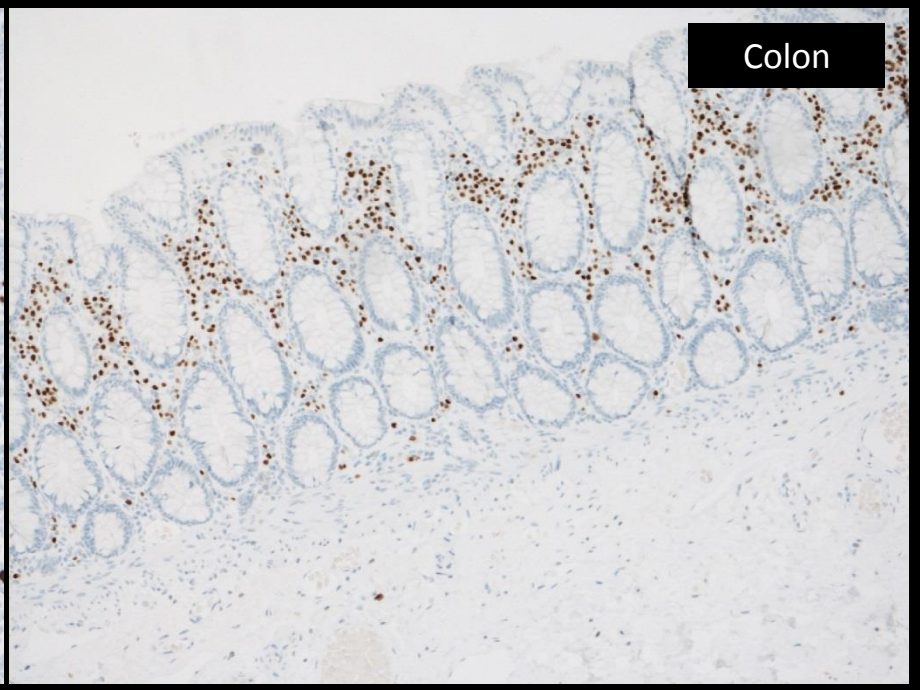
iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

Multiple myeloma oncogene 1 (MUM1)





A moderate to strong and distinct nuclear staining of late stage germinal centre B-cells and plasma cells in the tonsil.

A strong, distinct nuclear staining reaction of virtual all plasma cells in lamina propria of the colon.

No staining reaction in other cellular structures including epithelial cells and smooth muscle cells of lamina muscularis propria of the colon.

Table 1. **Antibodies and assessment marks for MUM1, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MUMp1**	84 1 1	Agilent/Dako Diagnostic Biosystem GeneMed	52	19	11	4	83%	86 %
mAb clone **MRQ-8**	3	Cell Marque	0	0	2	1	-	-
mAb clone **BC5**	3	Biocare Medical	0	0	3	0	-	-
mAb clone **EAU32**	3	Leica/Novocastra	0	2	1	0	-	-
rmAb clone **MRQ-43**	5 1 1	Cell Marque Menarini Zeta	0	0	3	4	-	-
rmAb clone **SP114**	1	Thermo S./ LabVision	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **MUMp1 GA644**	18	Agilent/Dako	8	7	2	1	83%	88 %
mAb clone **MUMp1 IR/IS644**	28	Agilent/Dako	13	12	3	0	89%	88 %
mAb clone **MUMp1 GA644, IR/IS644**[3]	5	Agilent/Dako	3	0	2	0	-	-
mAb clone **MUMp1 MAD-000470QD**	3	Master Diagnostica	1	1	1	0	-	-
mAb clone **MUMp1 MAD-0573**	1	Maixin	1	0	0	0	-	-
mAb clone **EAU32 PA0129**	6	Leica Biosystems	5	1	0	0	100%	100%
rmAb clone **MRQ-43 760-4529**	31	Ventana/Roche	0	0	25	6	0%	0%
rmAb clone **MRQ-43 358R-77/78**	15	Cell Marque	0	0	13	2	0%	0%
rmAb clone **EP190 358R-17/18**	1	Cell Marque	1	0	0	0	-	-
Total	211		84	43	66	18	-	
Proportion			40%	20%	31%	9%	60%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU systems developed for Agilent/Dako's automatic systems (Omnis/Autostainer) but used by laboratories off-label on the platforms Ventana Benchmark/Ultra or Leica BOND III.

mAb MUMp1 both as concentrate and RTU system performed well

The RTU system PA0129 based on the mAb clone EAU32 provided the highest pass rate and proportion of optimal results

The mAbs MRQ-8 & BC5 and rmAb MRQ-43 all gave false positive staining results

Efficient HIER preferable in alkaline buffer

3- step polymer/multimer detection system

Table 3. **Proportion of optimal results for MUM1 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic/ Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **MUMp1**	8/12 ** (67%)	1/1	24/39 (62%)	-	7/7 (100%)	-

* 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)

Best performance

Average dilution value (mAb clone MUMp1):

HIER in alkaline buffer/ 2 or 3 step polymer/multimer detection systems

Optimal results ➢ 1: 164 (range 1:20-1:1500)

Insufficient results ➢ 1: 496 (range 1:20-1:2000)

Choice of detection systems (mAb clone MUMp1):

HIER in alkaline buffer/ Optimal dil. Range 1:20-1:200

2-step polymer/multimer detection system

Suff. 71% (22 of 31) / Optimal 32% (10 of 31)

3-step polymer/multimer detection system

Suff. 100% (22 of 31) / Optimal 87% (32 of 37)

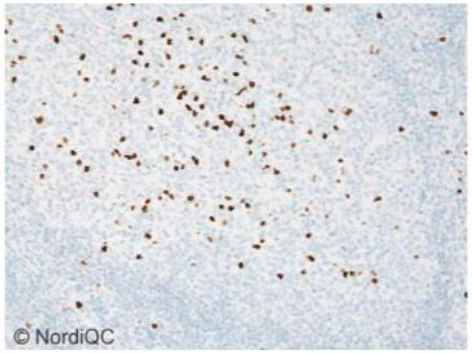


Fig. 1a (x200)
Optimal staining for MUM1 of the tonsil using the mAb MUMp1 as a concentrate, HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 5a. The late stage germinal centre B-cells show a distinct, moderate to strong nuclear staining reaction.

Fig. 1b (x200)
Insufficient staining for MUM1 of the tonsil using the mAb clone MUMp1 as concentrate (too diluted), HIER in an alkaline buffer (CC1) and a less sensitive multimer based detection system (Ultraview, Ventana) - same protocol used in Figs. 2b – 5b. The proportion of positive cells and the intensity of the staining reaction is significantly reduced - compare with Fig. 1a (same field).

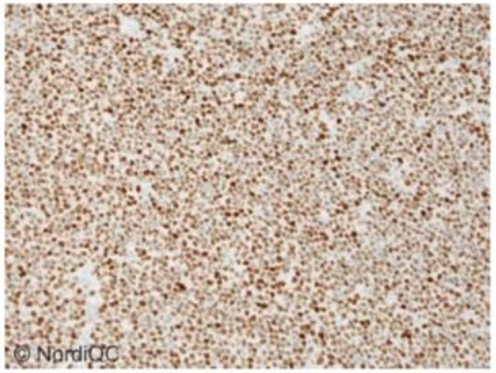


Fig. 4a (x200)
Optimal staining for MUM1 of the non-GCB DLBCL, tissue core 4 using same protocol as in Figs. 1a - 3a. Virtual all the neoplastic cells show a moderate to strong nuclear staining reaction.

Fig. 4b (x200)
Insufficient staining for MUM1 of the non-GCB DLBCL, tissue core 4 using same protocol as in Figs. 1b -3b. Intensity and proportion of stained neoplastic cells is significantly reduced - compare with Fig. 4a (same field).

Too weak

Protocol with to low sensitivity

Too diluted primary Ab (MUMp1) and 2-step multimer detection system

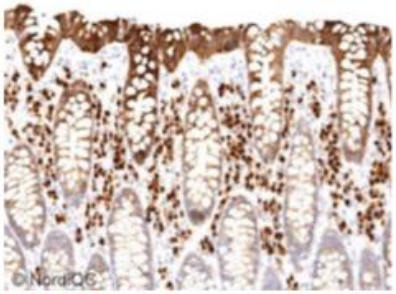


Fig. 6a (x200)
Insufficient staining for MUM1 using the rmAb MRQ-43 as Ready-To-Use format (760-4529, Ventana/Roche), with HIER in CC1 for 48 min. at 100°C and 3-step multimer OptiView, 760-700 (Ventana/Roche) as detection system. The epithelial cells in the colon are false positive displaying strong cytoplasmic reaction compromising the interpretation - compare with optimal protocol in Fig. 2a.

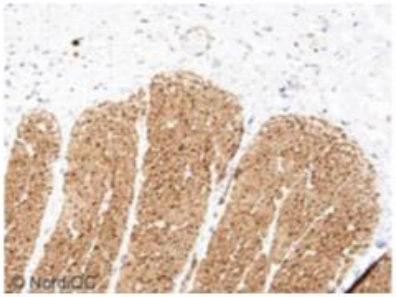


Fig. 6b (x200)
Insufficient and aberrant staining for MUM1 of the colon using the same protocol settings as in Fig. 6a. The smooth muscle cells in lamina muscularis propria are false positive displaying a distinct cytoplasmic but also strong nuclear staining reaction. In addition, smooth muscle cells surrounding the vessels are weakly labelled.

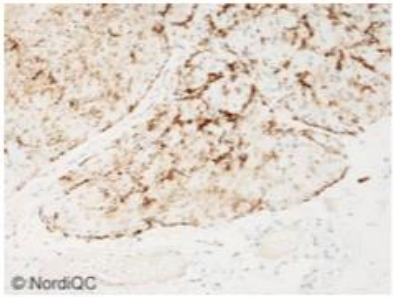


Fig. 6c (x200)
Insufficient staining for MUM1 of the colon using rmAb MRQ-43 as Ready-To-Use format (760-4529, Ventana/Roche), with HIER in CC1 for 60 min. at 100°C and 3-step multimer UltraView with amplification (Ventana/Roche) as detection system. The stellate cells (stromal fibroblast-like cells) intermingling with smooth muscle cells in lamina muscularis propria of the colon are aberrantly stained and displays a moderate to strong cytoplasmic reaction. The smooth muscle cells are only weakly labelled - compare with Fig. 6b.

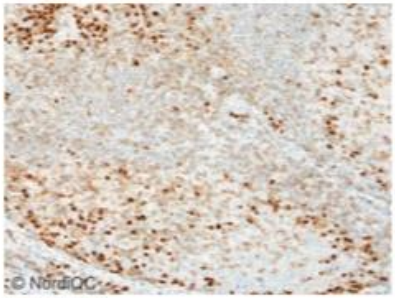


Fig. 6d (X200)
Insufficient staining for MUM1 of the tonsil using the rmAb MRQ-43 as concentrate, HIER in alkaline buffer (CC1) and a multimer based detection system (UltraView, Ventana/Roche). The late stage germinal centre B-cells show a moderate to strong nuclear staining reaction, but the lymphocytes (mostly T-cells) are aberrantly labelled displaying a weak to moderate membranous staning reaction – compare with optimal protocol in Fig. 1a.

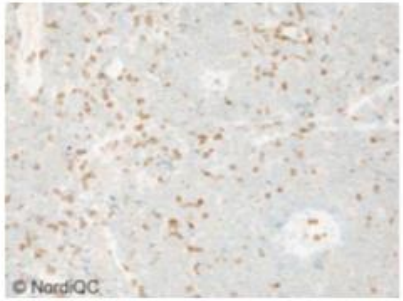


Fig. 6e (x200)
Insufficient staining for MUM1 of the GCB DLBCL, tissue core 3 using the same protocol as in Fig. 6d. T-cells are aberrantly stained compromising interpretation and it is difficult to identify normal plasma cells intermingling with the neoplastic cells of the DLBCL - compare with optimal protocol in Fig. 3a.

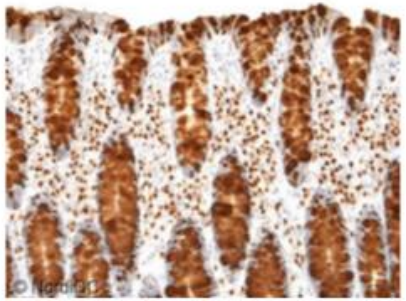


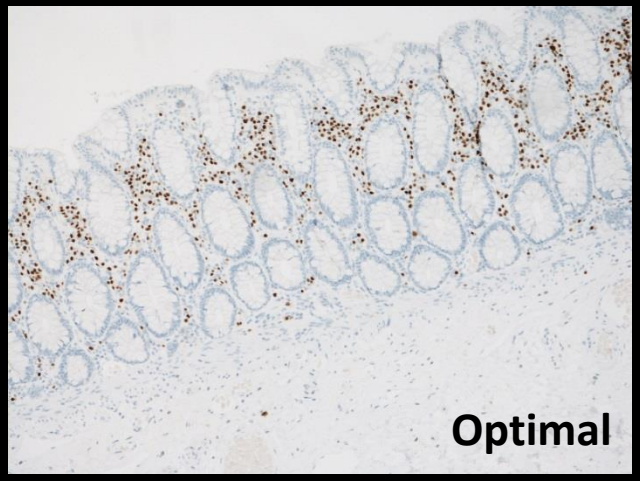
Fig. 6f (X200)
Insufficient staining for MUM1 of the colon using the rmAb BC5 as concentrate. Plasma cells show a distinct and strong nuclear staining, but goblet and luminal epithelial cells of the colon are aberrantly stained displaying strong cytoplasmic reaction – compare with optimal protocol in Fig. 2a.

False positive (MRQ-43,MRQ-8 & BC5)

Epithelium

Smooth muscles

T-cells



Optimal

MUM1

Lymphoma panel: MUM1
Optimal protocol settings (NQC)

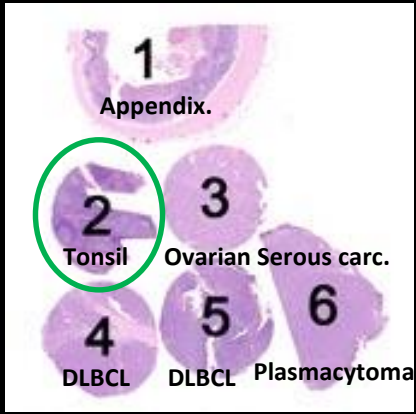
MUM1	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb MUM1p	HIER High pH , mod. or standard Low pH	1:25-1:400	3-step	Dako (IS/IR644)	Flex
mmAb EAU32	HIER High pH	-	-	Leica (PA0129)	Bond Refine
rmAb EP190	HIER High pH (CC1)			358R-17/18	UltraView

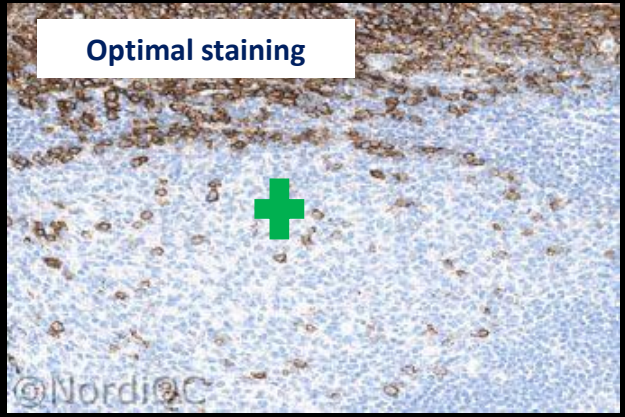
Control material / Tonsil:

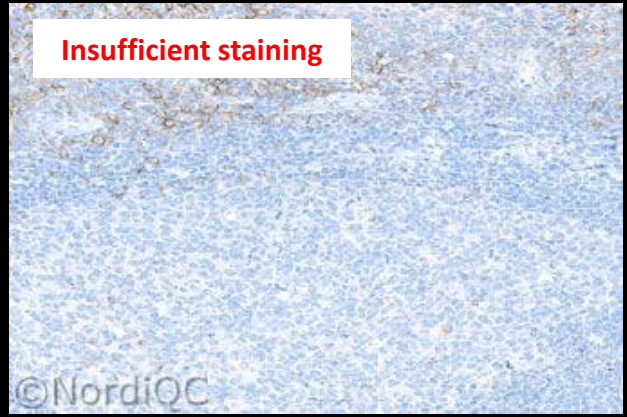
A moderate to strong and distinct nuclear staining of the plasma cells and the late stage germinal centre B-cells .

A weak cytoplasmic staining reaction is acceptable in the cells with a nuclear staining for MUM1

CD138 (Run 36)







Criteria for assessing a CD138staining as optimal included:

A moderate to strong, distinct predominantly membranous staining reaction of the activated late stage B-cells in the germinal centres and the plasma cells in the tonsil and appendix.

A strong, distinct membranous staining reaction of the majority of the squamous epithelial cells in the tonsil.

A moderate to strong membranous staining reaction of the majority of the neoplastic cells of the plasmacytoma (PC) and the DLBCL, core no. 6.

An at least weak to moderate predominantly membranous staining reaction of dispersed neoplastic cells of the ovarian serous carcinoma, core no.3.

No staining of the neoplastic cells of the DLBCL, core no. 5.

Critical Quality Staining Indicators and recommended control material

CD138 (Run 36)

Table 1. Abs and assessment marks for CD138, run 36

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5F7**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **B-A38**	8 6 4 2 1 1	Immunologic AbD Serotec Cell Marque Biocare Gen-Probe Zytomed	12	6	4	0	82 %	89 %
mAb clone **B-B4**	7 1	AbD Setotec IQ Products	4	4	0	0	100 %	100 %
mAb clone **CLB-1D4**	1	Biogenex	0	0	0			
mAb **MI15**	67 5 1	Dako Thermo/NeoMarkers Genemed	23	39	9	2	84 %	88 %
rmAb **EP201**	1	Epitomics	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **B-A38 760-4248**	41	Ventana/Cell Marque	12	25	4	0	90 %	91 %
mAb clone cocktail **B-A38 PM167AA**	1	Biocare	1	0	0	0	-	-
mAb clone **B-A38 138M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **MI15 IS/IR642**	26	Dako	9	13	4	0	85 %	85 %
mAb clone **MI15 PA0088**	1	Leica	1	0	0	0	-	-
mAb clone **MI15 MAD-000921QD**	1	Master Diagnostica	0	1	0	0	-	-
Total	179		62	89	22	6	-	-
Proportion			35 %	50 %	12 %	3 %	85 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal results (%)

55%

50%

HIER alkaline buffer and primary AB conc. 1:50-1:600

31%

29%

35%

Optimal results could be obtained the mAbs B-A38, B-B4 and MI15.

The proportion of optimal result was higher using B-A38 as concentrate (55%) compared to The RTU system from the Ventana (29%).

CD138

	Run 21 (2007)	Run 36 (2012)	
Participants, n=	77	179	
Sufficient results	74%	85%	
Optimal results	39%	35%	

CD138 (Run 36) ~ The most frequent causes of insufficient staining were :

Use of detection systems with a low to moderate sensitivity

Using the mAb clones B-A38, B-B4 or MI15 as concentrates:
Participants using a 2- step polymer system : 20/66 (20%) was able to produce an optimal result (pass rate 79%)
Participants using a 3- step polymer system : 16/36 (44%) was able to produce an optimal result (pass rate 94%)

Insufficient HIER

Too low concentration of the primary Ab

Less successful primary Abs

All 3 protocols based on the mAb clone 5F7 were assessed as insufficient (positive normal plasma cells but neoplastic plasma cells false negative)

The mAb clone 5F7 is consistently producing insufficient results as 11/11 protocols has been giving the mark poor (Run 21 & 36)

CD138 (Run 36)

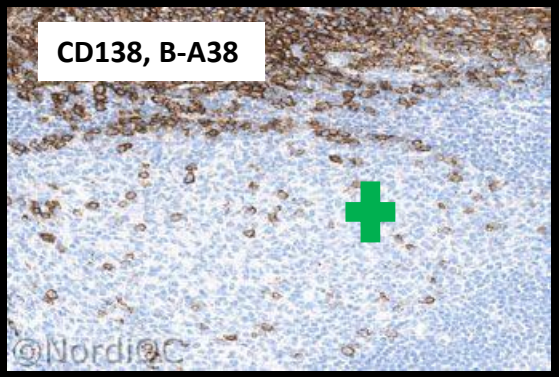
Optimal result

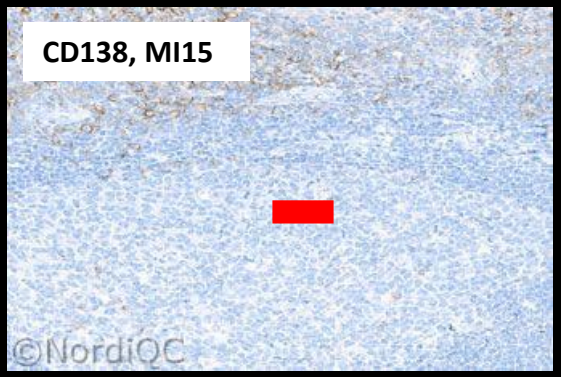
CD138staining optimally calibrated, HIER in Alkaline buffer and a 3-step multimer based detection system

Insufficient result

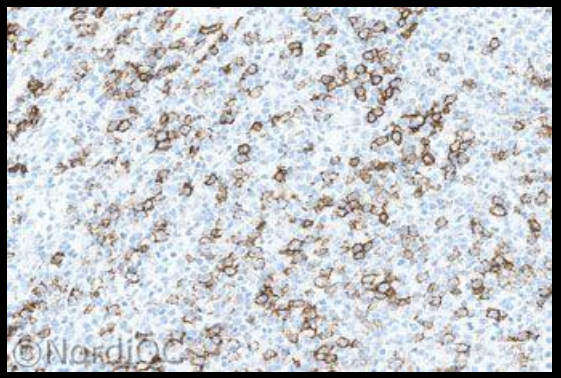
CD138 staining with too low sensitivty (too low concentration of the primary Ab and a 2-step polymer based detection system)

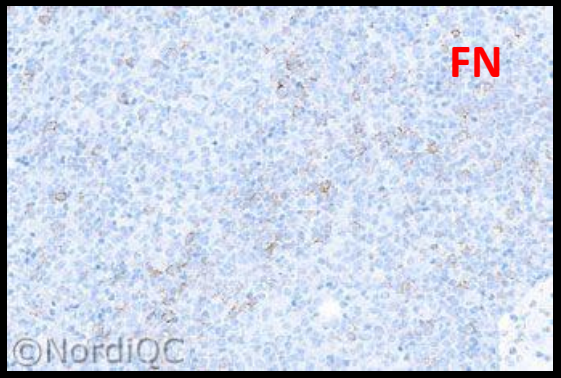
Tonsil



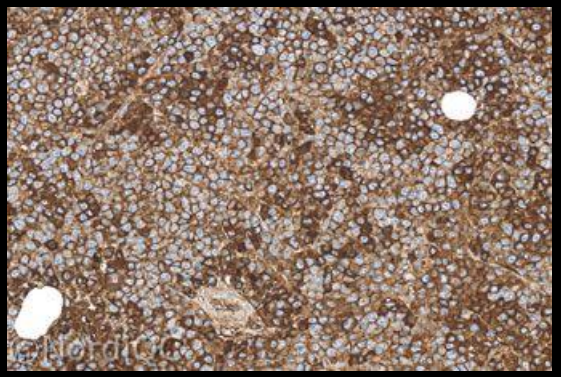


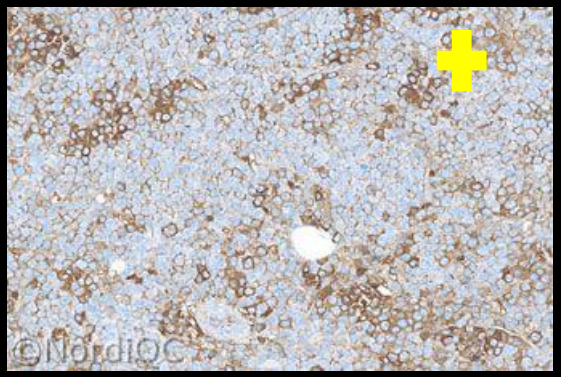
Diffuse large B-cell lymphoma





Plasmacytoma



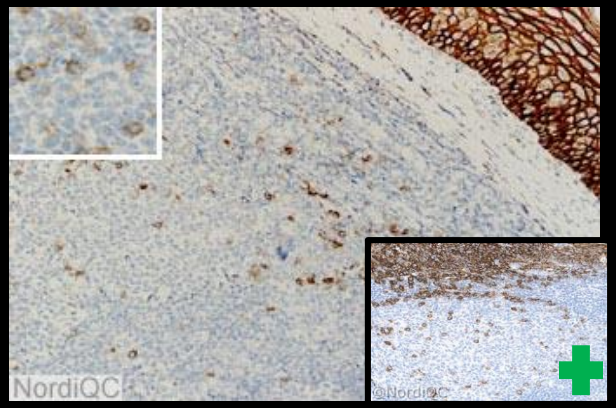


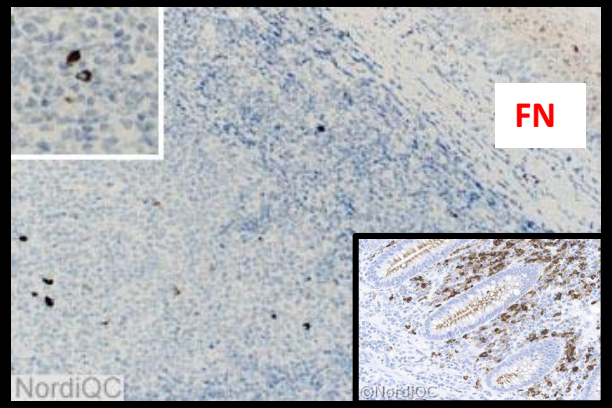
CD138 (Run 36)

CD138 clone MI15, B-A38 or B-B4

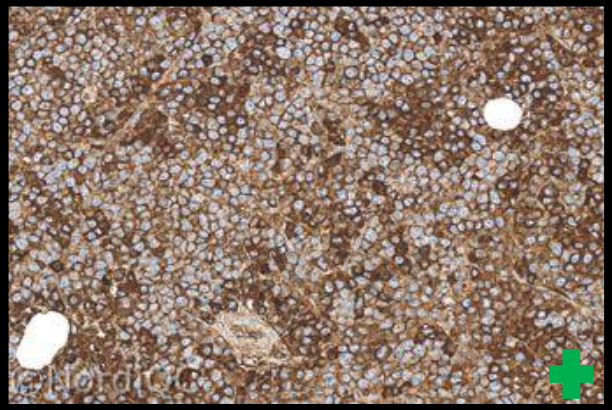
CD138 clone 5F7

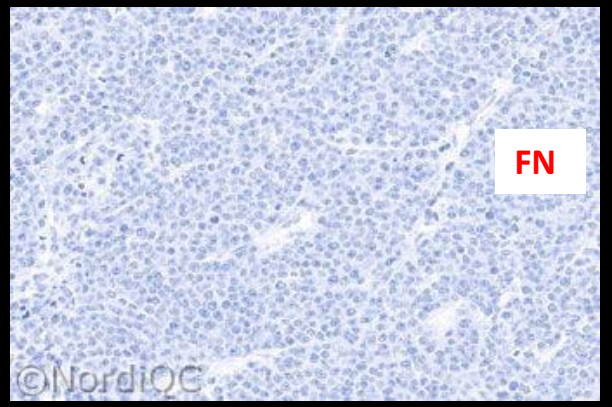
Tonsil





Plasmacytoma





Insufficient CD138 staining using the clone 5F7

The mab clone 5F7 will display false negative staining of activated late stage B-cells in the germinal centres and the squamous epithelium lining the surface of the tonsil.

Only normal plasma cell s will be stained with a cytoplasmic reaction pattern in contrast to the predominantly membranous pattern obtained with e.g., the mAb clone MI15.

Ki67

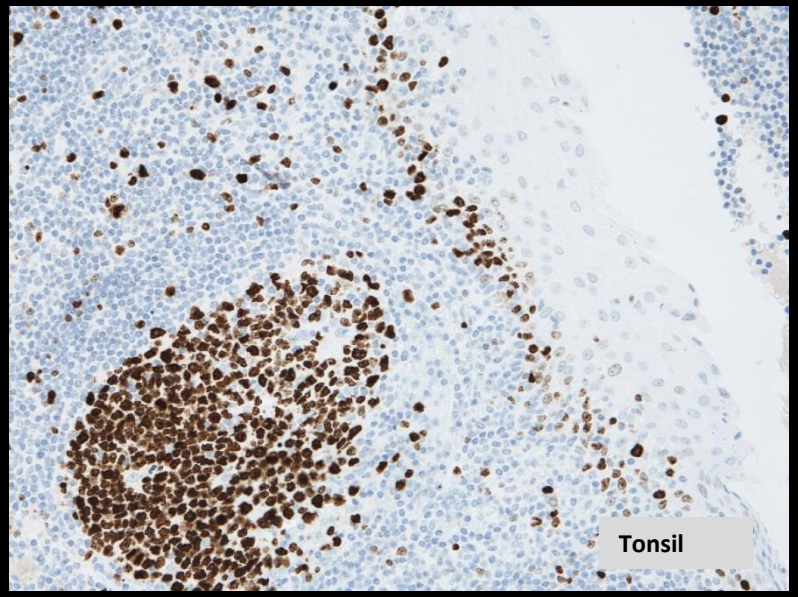
Material

The slide to be stained for Ki67 comprised the following 9 materials:

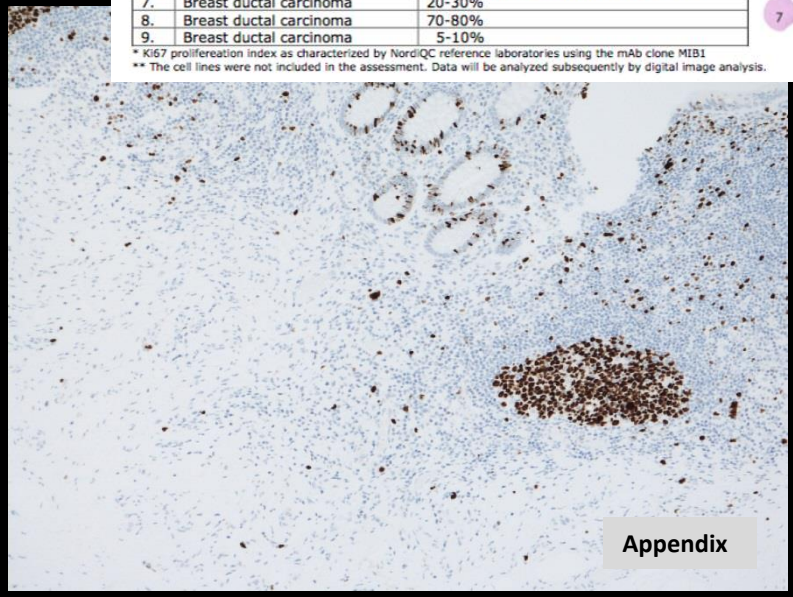
No.	Material	Ki67 proliferation index *
1.	Cell line 1. Horizon Discovery**	80-90%
2.	Cell line 2. Horizon Discovery**	70-80%
3.	Cell line 3. Horizon Discovery**	80-90%
4.	Pancreas	< 1% of the epithelial cells of the exocrine glands and ducts
5.	Liver	< 1% of the hepatocytes
6.	Tonsil	80-90% of the germinal centre B-cells
7.	Breast ductal carcinoma	20-30%
8.	Breast ductal carcinoma	70-80%
9.	Breast ductal carcinoma	5-10%

* Ki67 proliferation index as characterized by NordiQC reference laboratories using the mAb clone MIB1

** The cell lines were not included in the assessment. Data will be analyzed subsequently by digital image analysis.



Tonsil



Appendix

Tonsil is recommended as controls for Ki67.

In tonsil, 80-90 % of the germinal centre B-cells must show a moderate too strong and distinct nuclear staining reaction.

In the interfollicular areas dispersed lymphocytes also shows a moderate to strong nuclear staining reaction.

The vast majority of the mantle zone B-cells should be negative.

Table 1. **Antibodies and assessment marks for Ki67, run B22**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BS4**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **GM001**	1	Genemed	1	0	0	0	-	-
mAb clone **K2**	2 1	Zytomed Leica/Novocastra	2	1	0	0	-	-
mAb clone **MIB-1**	122 1	Agilent/Dako VWR/Immunologic	72	36	13	2	88%	90%
mAb clone **UMAB107**	7	ZSBio	2	4	1	0	86%	80%
rmAb clone **SP6**	7 5 3 3 3 1 1	Thermo/Neomarkers Cell Marque Biocare Spring Bioscience Zytomed Master Diagnostica Diagnostic Biosystems	17	5	1	0	96%	95%
pAb **RB-1510**	1	Thermo/Neomarkers	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **GM001 60-0040-7**	1	Genemed	1	0	0	0	-	-
mAb clone **K2 PA0230**	4	Leica/Novocastra	2	2	0	0	-	-
mAb clone **Ki88 AM370**	1	Biogenex	0	1	0	0	-	-
mAb **MIB-1 IR626/IS626**	65	Agilent/Dako	34	25	5	1	91%	94%
mAb **MIB-1 GA626**	31	Agilent/Dako	25	5	1	0	97%	100%
mAb clone **MIB-1 AM297**	1	Biogenex	1	0	0	0	-	-
mAb clone **MM1 PA0118**	9	Leica/Novocastra	0	8	1	0	-	-
mAb clone **MX006 MAB-0672**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP6 275R**	4	Cell Marque	2	1	1	0	-	-
rmAb clone **SP6 PRM 325**	1	Biocare	0	1	0	0	-	-
rmAb clone **SP6 MAD-000310QD**	1	Master Diagnostica	0	1	0	0		
rmAb clone **30.9 790-4286**	131	Roche/Ventana	121	9	1	0	99%	100%
Total	409		282	100	24	3	-	
Proportion			69%	24%	6%	1%	93%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Best performance:

RTU Ki67, 30-9, (790-4286,Ventana)

RTU Ki67, MIB-1 (IS/IR/GA626, Dako)

SP6 (concentrate)

Optimal (mmAb MIB-1 & rmAb SP6)

Efficient HIER in High or Low pH buffers (20 min)

1:50-1:400 (MIB-1)

1:50-1:200 (SP6)

2 & 3 step detection systems

Insufficient results

Too low conc. of primary Ab

Insuff. HIER

Platform issues (MIB-1) on the BOND III/MAX

Table 3. **Proportion of optimal results for Ki67 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer / Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **MIB-1**	16/20** (80%)	2/2	39/61 (64%)	-	5/16 (31%)	0/3

* 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)

For unexplained reasons, MIB-1 showed an inferior performance on the Leica, Bond IHC system compared to the other IHC systems despite using protocol settings similar in sensitive (HIER conditions, Ab titre and 3-step polymer based detection system) to other systems (e.g. Dako systems)

Ki67

Optimal

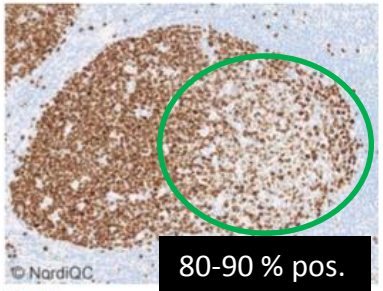


Fig. 1a
Optimal staining for Ki67 of the tonsil using the mAb clone MIB1 properly calibrated and with HIER in an alkaline buffer.
A moderate to strong, distinct nuclear staining reaction is seen in 80-90 % of the germinal centre B-cells in both the dark and the light zone.
Also compare with Figs. 2a - 5a – same protocol.

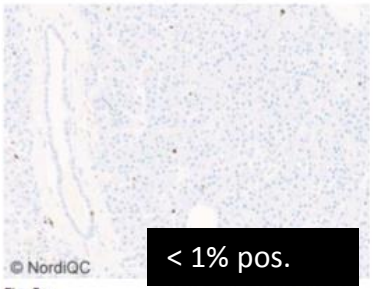


Fig. 2a
Optimal staining for Ki67 of the pancreas using same protocol as in Fig. 1a.
Dispersed epithelial cells of the exocrine glands and large ducts show a distinct nuclear staining reaction.
The nuclear staining reaction for Ki67 is easily identified even at a low magnification (x100).

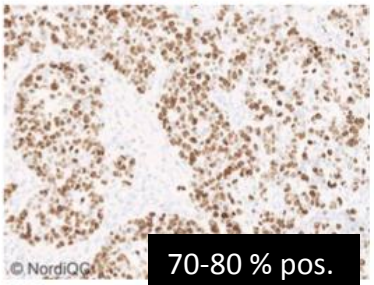


Fig. 3a
Optimal staining for Ki67 of the breast carcinoma, tissue core no. 8 using same protocol as in Figs. 1a and 2a.
>80% of the neoplastic cells show a distinct nuclear staining reaction and no background staining is seen.

Insufficient

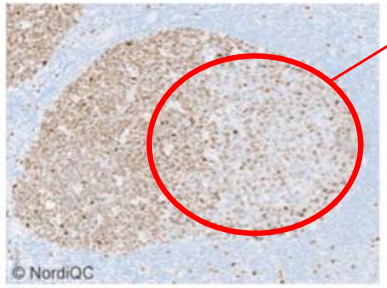


Fig. 1b
Insufficient staining for Ki67 of the tonsil using the mAb clone MIB1 with a protocol providing a too low sensitivity, most likely due to a too low concentration of the primary Ab.
The majority of the germinal centre B-cells are demonstrated, but especially the B-cells in the light zone only show a weak and equivocal nuclear staining reaction – same field as in Fig. 1a.
Also compare with Figs. 2b - 5b - same protocol.

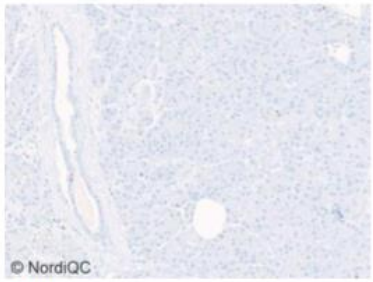


Fig. 2b
Insufficient staining for Ki67 of pancreas using same protocol as in Fig. 1b. - same field as in Fig. 2a.
The intensity and proportion of positive cells is significantly reduced compared to the result in Fig. 2a.
Also compare with Fig. 3b – same protocol.

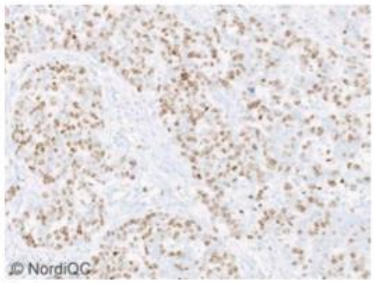


Fig. 3b
Insufficient staining for Ki67 of the breast carcinoma, tissue core no. 8 using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a.
The intensity and proportion of positive cells is significantly reduced compared to the result in Fig. 3a.

Too weak and few B-cells stained

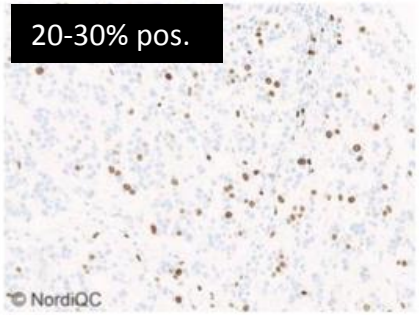


Fig. 4a
Optimal staining for Ki67 of the breast carcinoma, tissue core no. 7 using same protocol as in Figs. 1a - 3a.
20-30% of the neoplastic cells show a distinct nuclear staining reaction and no background staining is seen.

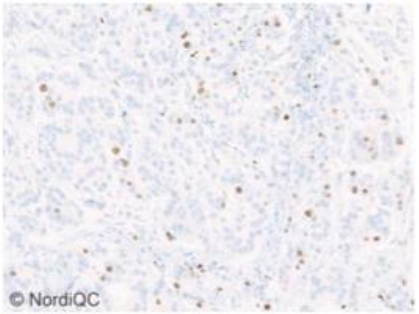


Fig. 4b
Insufficient staining for Ki67 of the breast carcinoma, tissue core no. 7 using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a.
The intensity and proportion of positive cells is significantly reduced compared to the result in Fig. 3a.

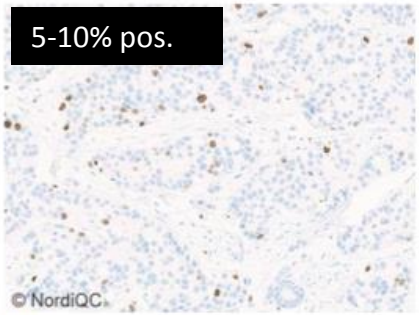


Fig. 5a
Optimal staining for Ki67 of the breast carcinoma, tissue core no. 9 using same protocol as in Figs. 1a - 4a.
5-10% of the neoplastic cells show a distinct nuclear staining reaction and no background staining is seen.

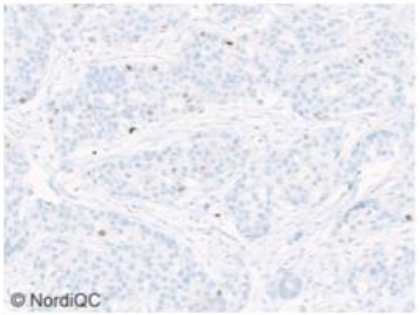


Fig. 5b
Insufficient staining for Ki67 of the breast carcinoma, tissue core no. 9 using same protocol as in Figs. 1b - 4b - same field as in Fig. 5a.
Only scattered cells show a distinct nuclear staining reaction.

Lymphoma panel: Ki67

Optimal protocol settings (NQC)

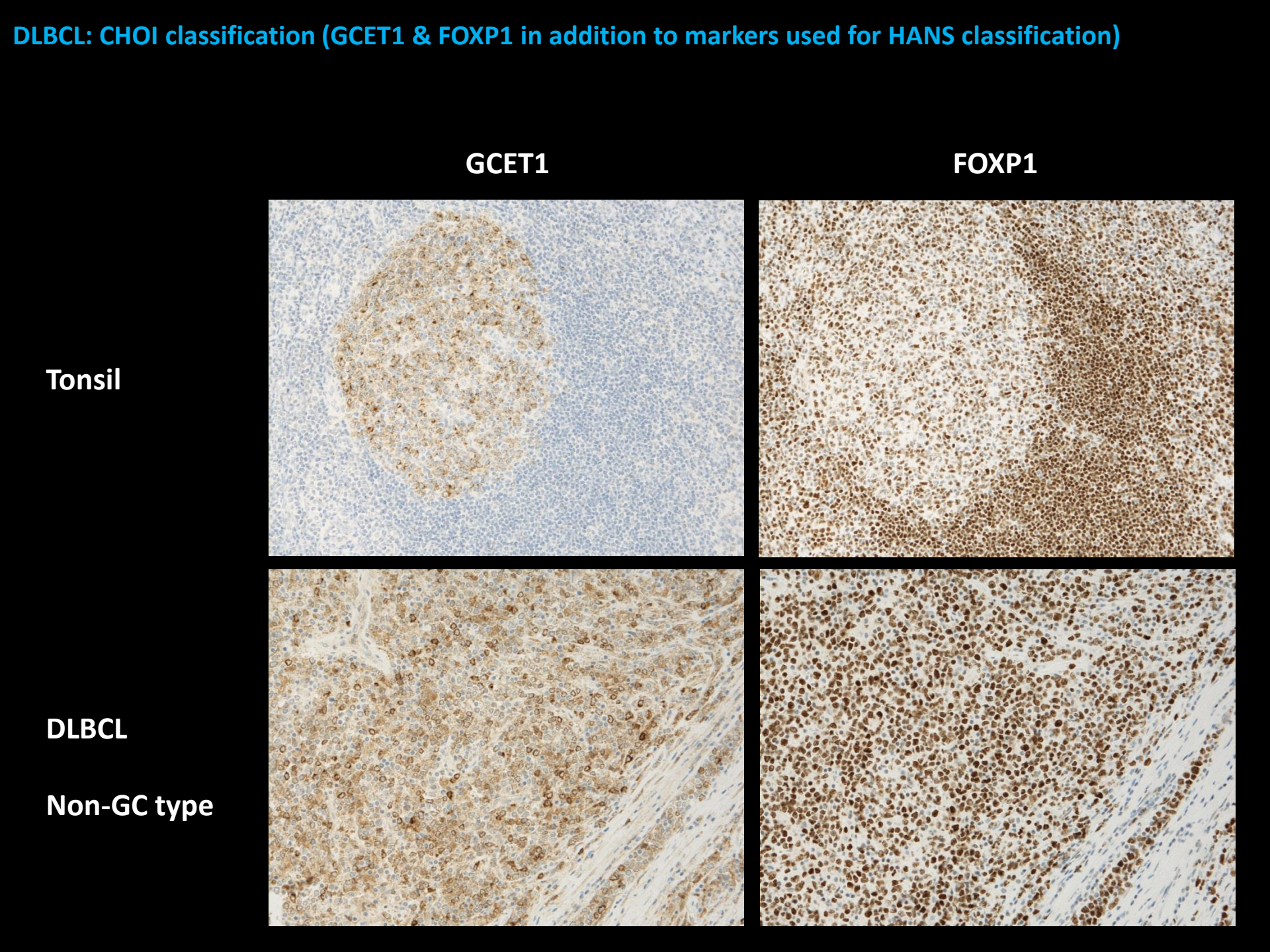
Ki67	Retrieval buffers	Titer	Detection systems	RTU	Detection
mmAb **MIB-1**	**HIER High pH or Low pH buffer**	**1:50-1:600**	**2 & 3-step**	**Dako (IS/IR/GA626)**	**Flex Flex+**
mmAb **K2**	**HIER High pH or low pH buffer**	**1:200-1:300**	**3-step**	**Leica (PA0230)**	**BOND Refine**
rmAb **SP6**	**HIER High pH or Low pH buffer**	**1:30-1:300**	**2 & 3-step**	**-**	**-**
rmAb **30-9**	**CC1 (mild or standard)**	**-**	**-**	**Ventana (790-4286)**	**iView UltraView OptiView**

Control material / Tonsil:

80-90 % of the germinal centre B-cells must show a moderate too strong and distinct nuclear staining reaction.

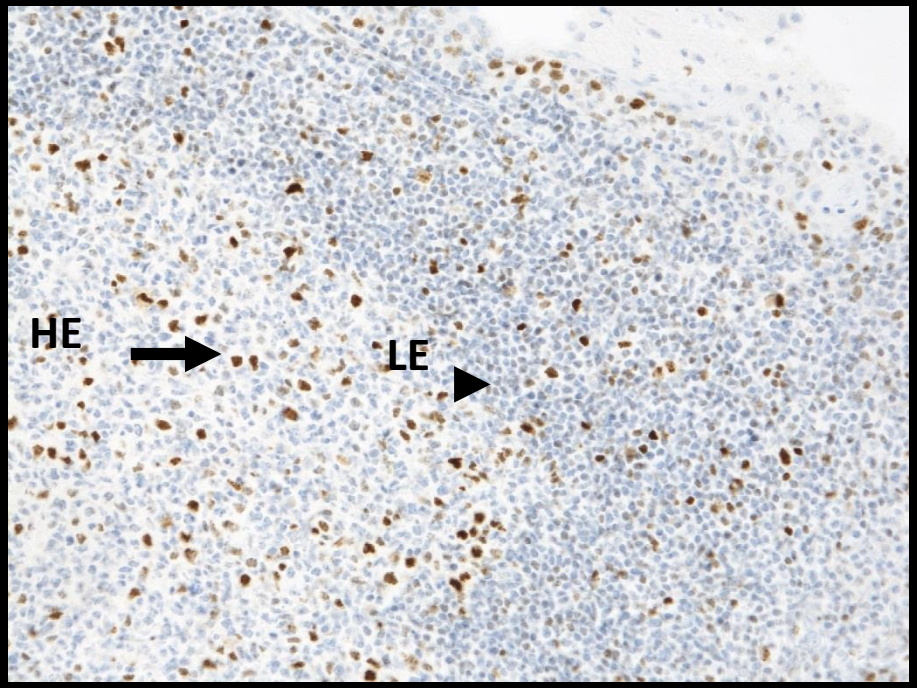
The vast majority of the mantle zone B-cells should be negative.

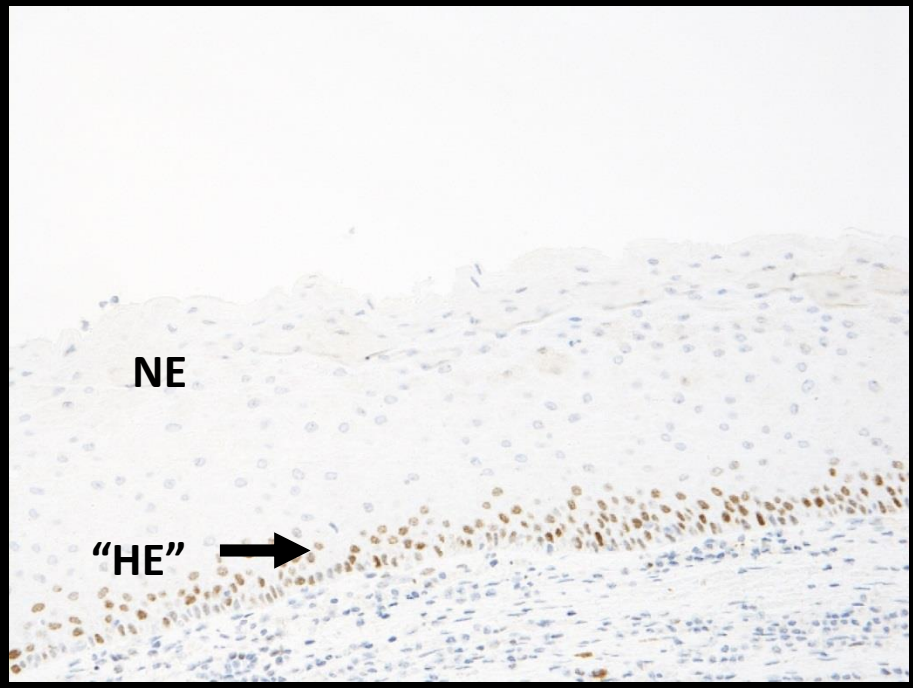
DLBCL: CHOI classification (GCET1 & FOXP1 in addition to markers used for HANS classification)



CMYC

Double/Triple hit DLBCL





Tonsil

Hodgkin lymphoma markers

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD30 (membr. + Golgi) Ber-H2, CON6D/5, 1G12, JCM182, rmAb EP154	Tonsil	None	Interfollicular activated B- and T-cells and perifollicular germinal centre B-cells (moderate intensity)	All other cells
CD15 (membr. + cytopl.) Carb-3, MMA and HI98	Tonsil/Kidney	Epithelial cells of the renal proximal tubules (predominantly membr.) Neutrophils	Follicular dendritic cells in the germinal centres (Tonsil)	All other cells
BOB.1 (nuclear + cytopl.) SP92	Tonsil	Germinal centre B-cells & plasma cells	Mantle zone B-cells	T-cells
OCT2 (nuclear) EP284	Tonsil	Germinal centre B-cells & plasma cells	Mantle zone B-cells ("moderate intensity")	"T-cells"
CD57 (membr.) TB01	Tonsil/Appendix	Intragerminal centre activated T-cells and NK-cells in the T-zone (Tonsil)	Schwann cells of peripheral nerves (ganglionic neurons) in the appendix	Epithelia cells of the Appendix. Neuroendocrine cells displays a distinct staining reaction
EBV-EBER/EBV-LMP1 **ALK (See markers for the Lung panel / Ole Nielsen)**				

Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

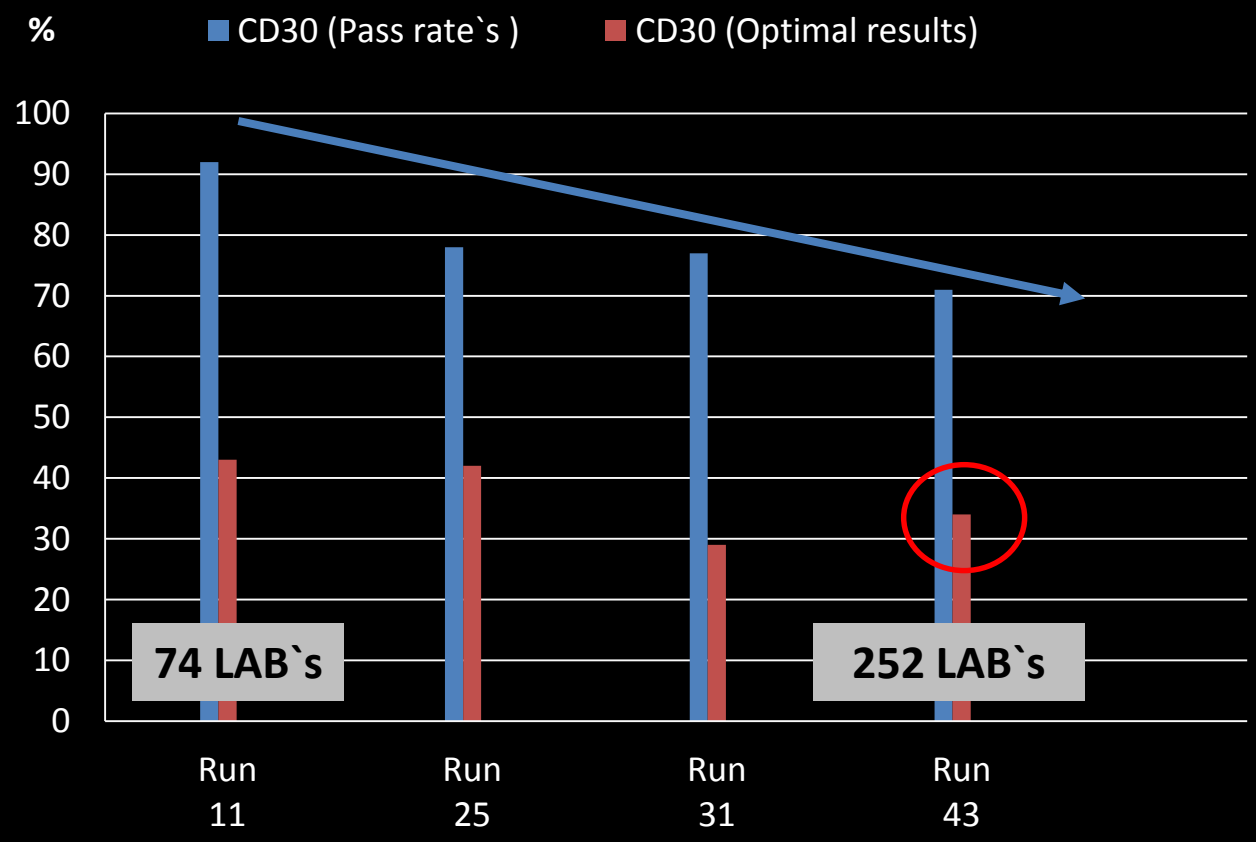
iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

CD30

Pass & Optimal score rate`s



CD30 / Run 43:

Sufficient: 71%

Optimal: 34%

A challenging marker

Robust primary Abs:

mmAb clone BER-H2
mmAb clone 1G12
mmAb clone JCM182

mmAb clone CON6D/5
rmAb clone EP154

Table 1. **Antibodies and assessment marks for CD30, run 43**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	102 9 2 1 1 1 1	Dako Cell Marque Thermo/Neomarkers Biosystems GeneMed Immunologic Zytomed Systems	38	46	27	6	72%	77%
mAb clone **1G12**	9	Leica/Novocastra	4	3	2	0	78%	100%
mAb clone **JCM182**	5	Leica/Novocastra	4	1	0	0	100%	100%
mAb clone **CON6D/5**	3	Biocare	3	0	0	0	-	-
mAb clone **15B3**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **HRS4**	1	Thermo/Neomarkers	0	0	1	0	-	-
rmAb **EP154**	1	Beijing Zhongsan	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **Ber-H2 IS/IR602**	47	Dako	17	21	8	1	81%	74%
mAb clone **Ber-H2 790-2926**	25	Roche/Ventana	6	11	7	1	68%	88%
mAb clone **Ber-H2 790-4858**	25	Roche/Ventana	6	3	8	8	36%	86%
mAb **Ber-H2 MAD-002045QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **Ber-H2 MAB-0023**	1	Maxin	1	0	0	0	-	-
mAb clone **Ber-H2 MS-361-R7**	1	Thermo/Neomarkers	0	1	0	0	-	-
mAb clone **Ber-H2 AM327-5M**	1	BioGenex	0	0	1	0	-	-
mAb clone **Ber-H2 130M**	1	Cell Marque	0	0	0	1	-	-
mAb clone **JCM182 PA0790**	5	Leica/Novocastra	4	0	1	0	80%	80%
mAb clone **1G12 PA0153**	3	Leica/Novocastra	1	2	0	0	-	-
mAb clone **1G12 CD30-R-7-CE**	2	Leica/Novocastra	0	2	0	0	-	
mAb clone **CON6D/5 PM346**	1	Biocare	0	0	1	0	-	
Total	252		86	93	56	17	-	
Proportion			34%	37%	22%	7%	71%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Ber-H2: HIER in alkaline or modified low pH buffer (Diva/TRS pH6.1), dil. range 1:20 -1:75

1G12: HIER in CC1 or BERS2, dil. range 1:10 -1:25

JCM182: HIER in BERS1 or BERS2, dil. range 1:25 - 1:100

HIER in modified low pH buffer (TRS pH6.1, Dako) dil. 1:50 and FLEX+

Pass Rate and proportion of optimal score results was highly influenced by the chosen detection system

mAb Ber-H2:

No significant difference in performance between the LD assays compared to the RTU formats

Optimal results could be obtained with the mAbs BER-H2,1G12, JCM182, CON6D/5 and the mrAb EP154.

CD30 (Run 43 2015) : Influence of the chosen HIER Buffer

mAb BER-H2 within a LD assay:

Optimal result could be obtained with both alkaline and modified low pH buffers (TRS pH 6.1, Dako or Diva Decloaker,Biocare) but ……

HIER buffer	Pass Rate`s (%)	Optimal (%)
Alkaline buffer as TRS pH9 or TRS pH9 (3-1), Dako	79 (22 of 28 protocols)	25
TRS pH6.1, Dako (modified low pH buffer)	80 (7 of 8 protocols)	75
mAb BER-H2 as concentrate (any dil. range) and Flex or Flex+ as the detection system:		

Also - 3 labs used the clone CON6D/5, Biocare (1:50) with optimal results , all performing HIER with the modified low pH buffer TRS pH6.1 (Dako) and Flex+ as the detection system

No protocol based on HIER in standard citrate buffer pH6 were assessed as optimal

“RTU formats (Ventana)” and influence of the chosen detection system

CD30 clone BER-H2 (Two available RTU systems /formats from Ventana):

790-2926 (UltraView /iView) ~ Optimal result could only be obtained by a laboratory modified protocol typically prolonging incubation time of the primary Ab or using an amplification step ~ It questions the definition of a true RTU system ?

790-4858 (OptiView)

Protocol settings	Optimal (%)
Protocol settings as recommended by the Vendor* (OptiView or UltraView + Amplification.)	86 (6 of 7 protocols)
UltraView	0 (0 of 8 protocols)
HIER in CC1 64 min., 32 min. incubation of the primary Ab and OptiView or UltraView +/- amplification as detection kit	

For laboratories using the RTU format 790-4858 (mAb BER-H2) from Ventana , it is strongly advisable to follow the recommendations giving by the vendors package insert for optimal performance

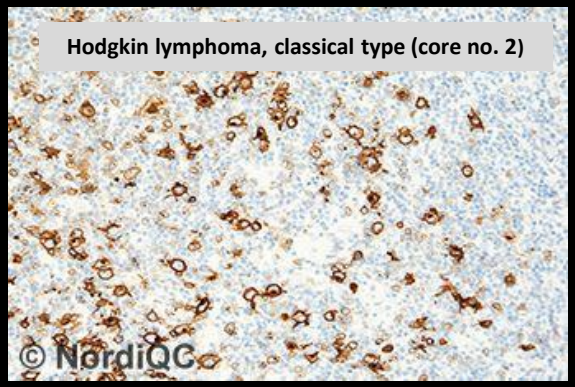
CD30 / Run 43 2015

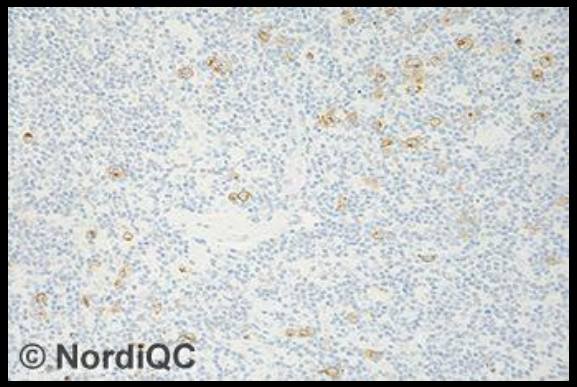
Optimal

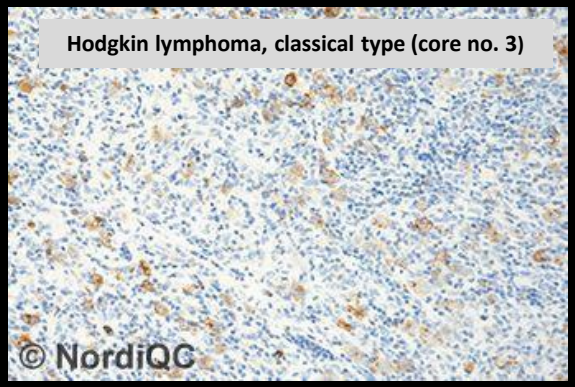
CON6D/5 (1:50)

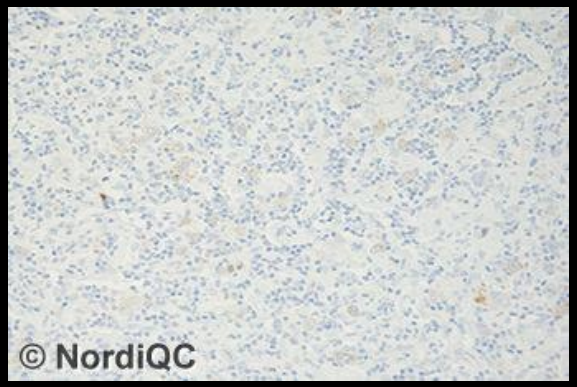
TRS pH6.1 buffer

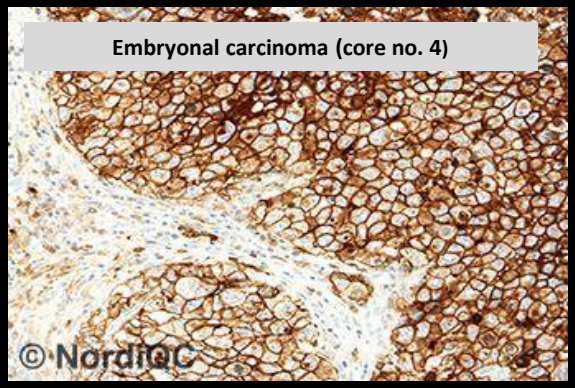
Flex+

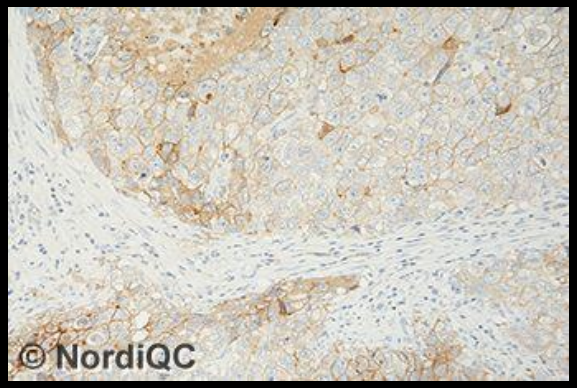












Insufficient

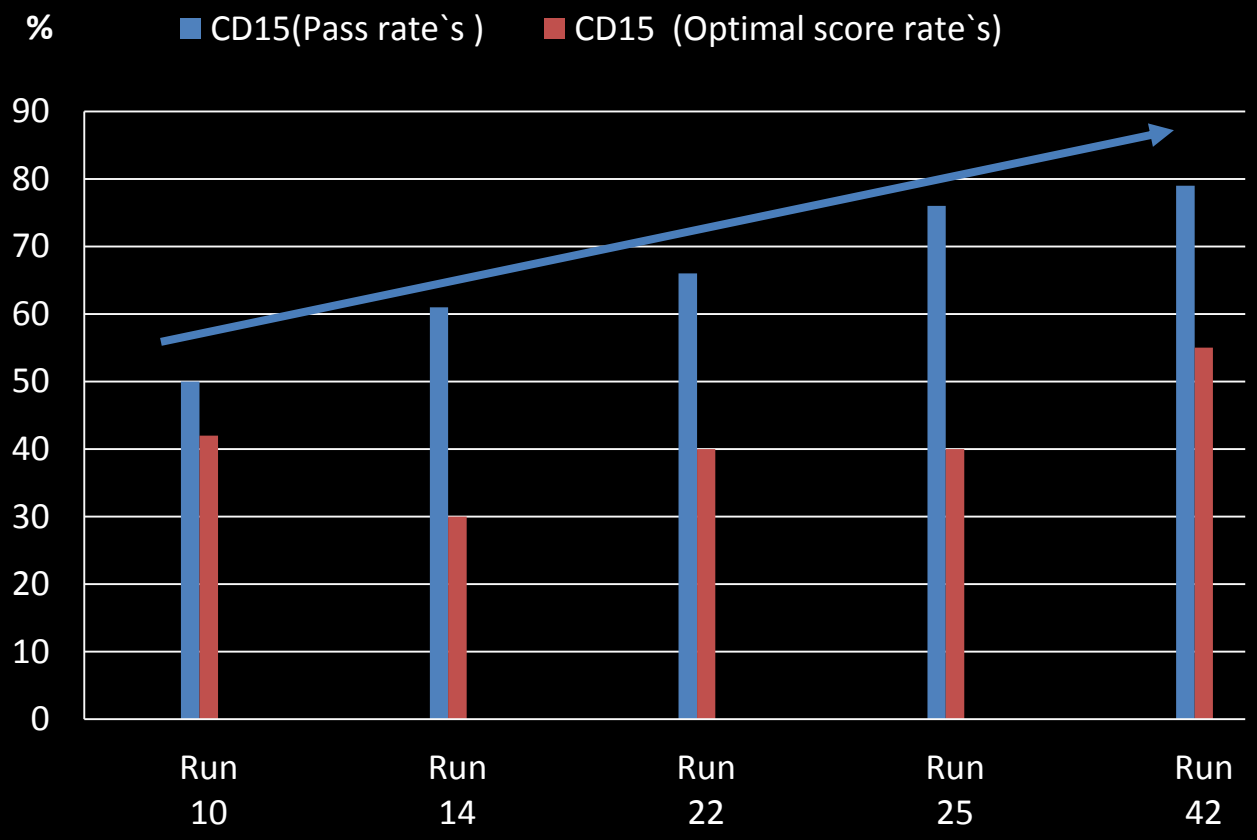
Ber-H2 (concentrate)
Too low concentration

Inefficient HIER
TE pH9 (too short time)

2-step polymer system (GTVsion)
Too low sensitivity

CD15

Pass & Optimal score rate`s

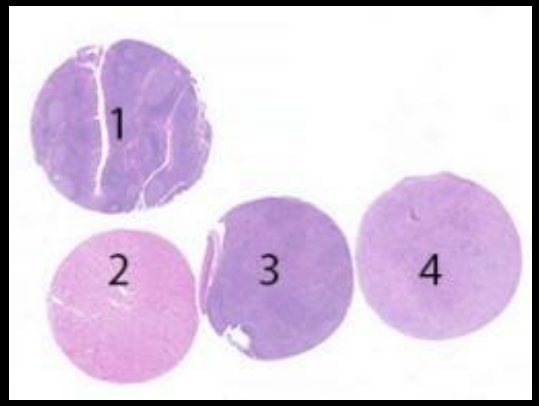


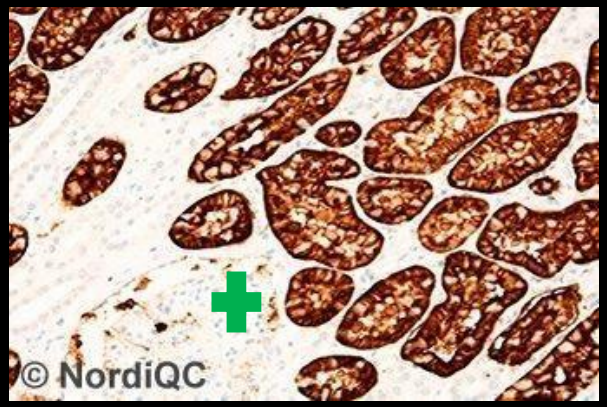
CD15 / Run 44:

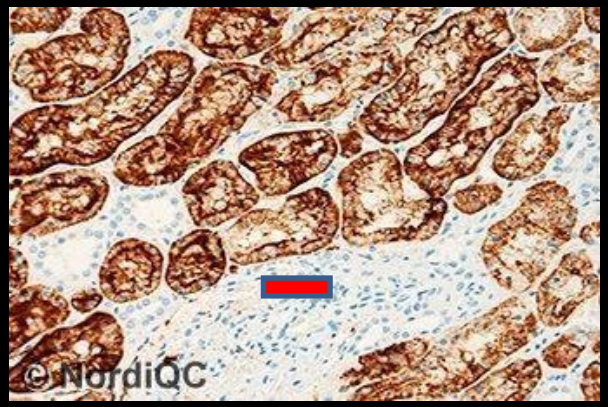
Sufficient: 79%

Optimal: 55%

CD15/ Run 42 2014







Criteria for assessing a CD15 staining as optimal included:

Core	Membranous / Golgi staining reaction
1. Tonsil	**(+) Follicular dendritic cells (germinal centres) – Membranous reaction**
2. Kidney	**+ Epithelial cells lining the renal proximal tubules – Membranous reaction.**
3.Hodgkin Lymphoma, classical type Lymphocyte rich	+ Hodgkin and Reed-Sternberg cells – Membranous & Golgi reaction
4.Hodgkin Lymphoma, classical type lymphocyte rich	+ Hodgkin and Reed-Sternberg cells – Membranous & Golgi reaction
Strong cytoplasmic staining reaction of neutrophil granulocytes in all four specimens	

Kidney is recommended as control material

Table 1. **Antibodies and assessment marks for CD15, run 42**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Carb-3**	53 1 1	Dako Zytomed Systems Nordic Biosite	31	**57%**	6	2	85%	89%
mAb clone **MMA**	24 7 3 2	BD Biosciences Cell Marque Thermo/NeoMarkers Immunologic	12	**33%**	8	7	60%	64%
mAb clone **BY87**	7	Leica/Novocastra	0	0	0	7	-	-
mAb clone **HI98**	2	BD Biosciences	1	1	0	0	-	-
mAb clone **MMA+BY87**	2	Biocare	0	0	2	0	-	-
mAb clone **C3D-1**	1	Dako	0	0	0	1	-	-
mAb **BRA4F1**	1	BioGenex	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **Carb-3 IS/IR062**	49	Dako	38	**77%**	1	1	96%	100%
mAb clone **Carb-3 GA062**	4	Dako	3	1	0	0	-	-
mAb clone **Carb-3 MSG005**	1	Zytomed Systems	0	0	0	1	-	-
mAb clone **MMA 760-2504**	70	Ventana	46	**66%**	6	1	90%	90 %
mAb **MMA MAD-005151QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **MMA 115M-18**	1	Cell Marque	0	1	0	0	-	-
mAb clone **MMA PDM 127**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone **Carb-1 PA0039**	4	Leica/Novocastra	0	1	1	2	-	-
mAb clone **MMA+BY87 PM073 AA**	2	Biocare	0	1	0	1	-	-
mAb **BRAF4F1 AM302-5M**	1	BioGenex	0	0	0	1		
Total	238		132	56	24	26	-	
Proportion			55%	24%	10%	11%	79%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal protocol settings

HIER in high, mod. low or standard low pH buffers; dil. range 1:10-1:100 ~ Robust Ab

HIER in alkaline buffers; dil. range 1:10-1:50

TRS pH9 (20- 30`), Ab Inc (20-30), Flex/Flex+

CC1 (32-64`), Ab Inc (16-64`), UV+/- amp or OV

Best performance:

Carb-3 as concentrate

RTU format Carb3 (IS/IR062,Dako)

RTU format MMA (760-2504, Ventana)

Optimal results could be obtained with the mAbs Carb-3, MMA, and HI98.

CD15 (Run 42 2015) : Observations with impact on the final result

Although the number of participants has increased considerably (97%) compared to the latest assessment (Run 25, 2009):

The substitution towards more robust clones and the use of robust RTU systems from the two major vendors (Dako & Ventana) accounts for the overall increase of sufficient results (good or optimal)

LD/RTU assays (C3D-1 versus Carb-3, Dako)	LAB`s using the clone	Pass Rate`s (%)	Optimal (%)
mAb C3D-1, Dako / Run 25 & 42 *	44	72 (31 of 44 protocols)	20 (9 of 44 protocols)
mAb Carb-3, Dako / Run 25 & 42	119	85 (102 of 119 protocols)	69 (82 of 119 protocols)

RTU assays (mAb Carb-3, Dako)	LAB`s using the clone	Pass Rate`s (%)	Optimal (%)
RTU mAb Carb-3, Dako Run 25 (2009)	5	100	100
RTU mAb Carb-3, Dako Run 42 (2014)	49	96 (47 of 49)	78 (38 of 49 protocols)

* Discontinued by the vendor

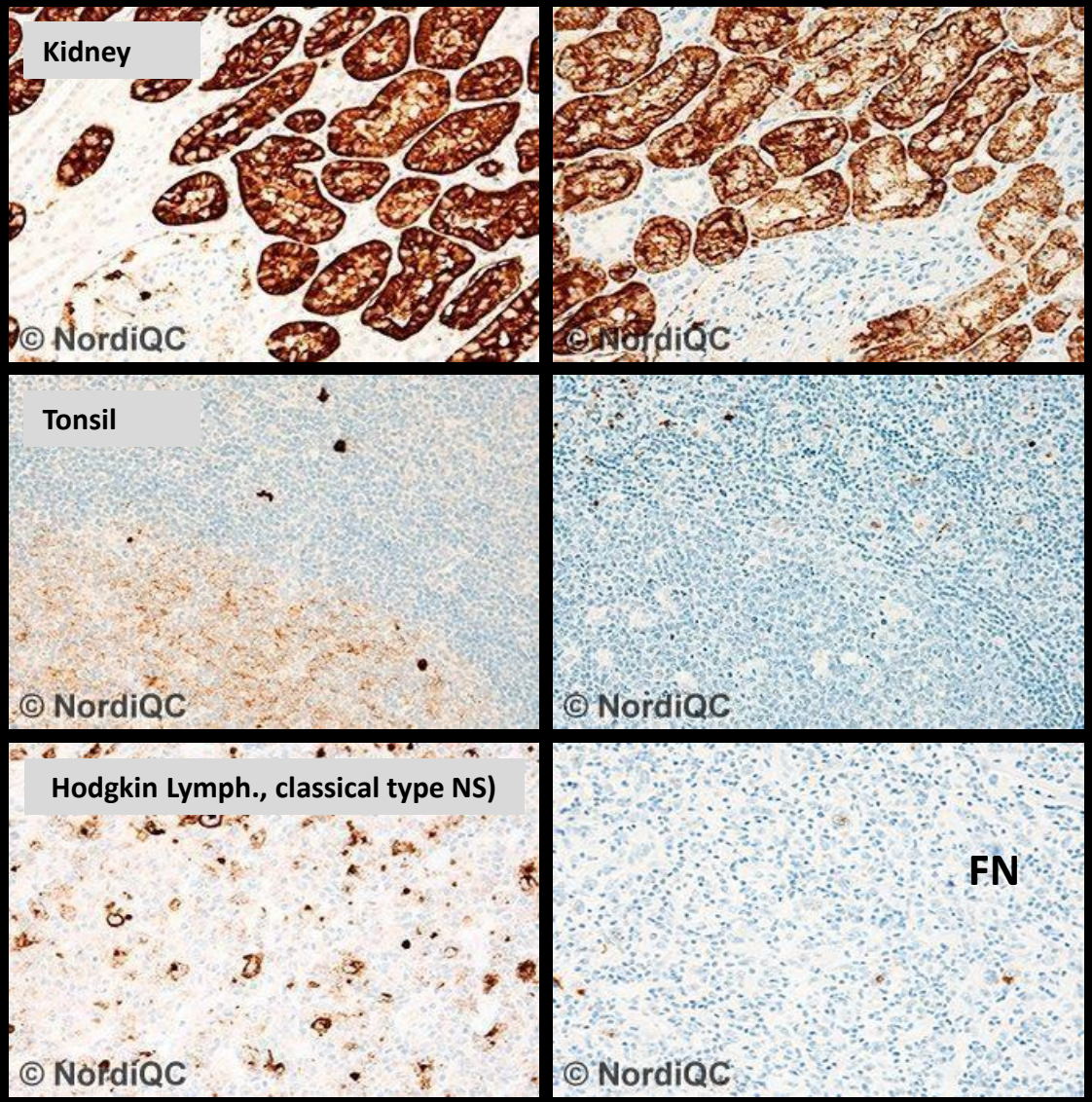
CD15 / Run 42 2014

Optimal

Carb-3 (1:100)

HIER CC1, pH 8.5 / 48`

OV (3-step multimer)



Insufficient

Carb-3 (1:100)

Inefficient HIER
HIER CC1, pH 8.5 / 16 `

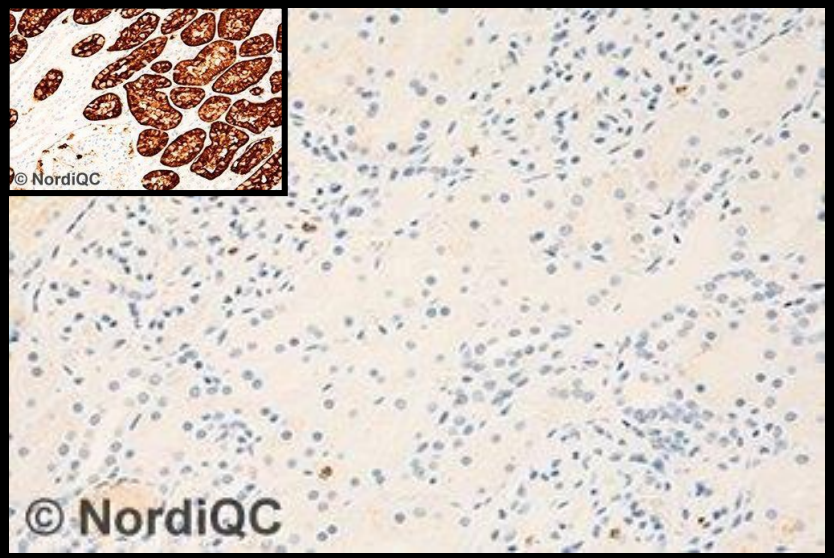
Too short HIER time

OV (3-step multimer)

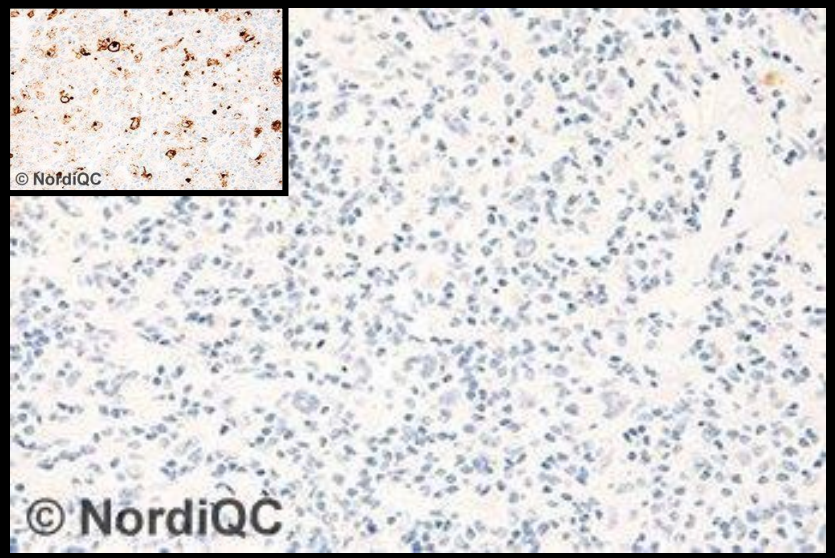
CD15 / Run 42 2014

Less successful performance of the primary Ab

All protocols (7 out of 7) using the mAb BY87 were assessed as poor



Kidney



Hodgkin Lymphoma, classic type (NS)

mAb clone BY87, HIER in an alkaline buffer (BERS2 pH 9, Leica) and a 3-step polymer based detection system (Bond Refine, Leica).

CD15 / Run 42 2014

Less successful performance of the chosen detection system (iView)

- provides low sensitivity
- provides false positive reaction due to endogenous biotin

10 of the participants (4 %) used a biotin based detection system (iView)

Kidney

The distal and collecting tubules are false positive (a typical granular reaction pattern) due to endogenous biotin

Also, too weak staining – compare with insert

CD15 / Run 42 2014

Lymphoma panel: CD15
Optimal protocol settings (NQC)

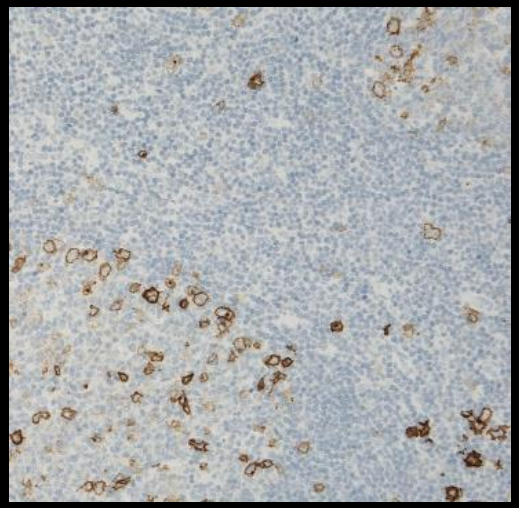
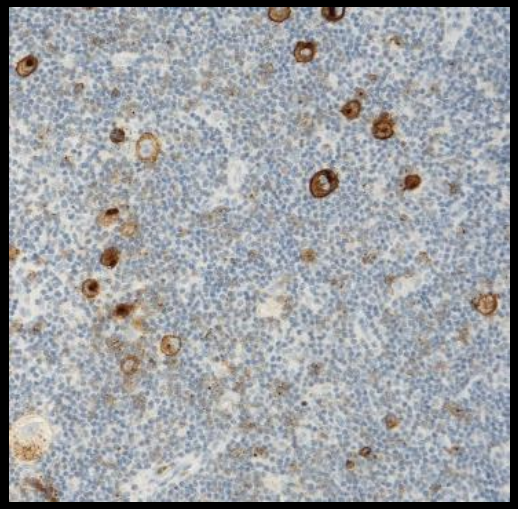
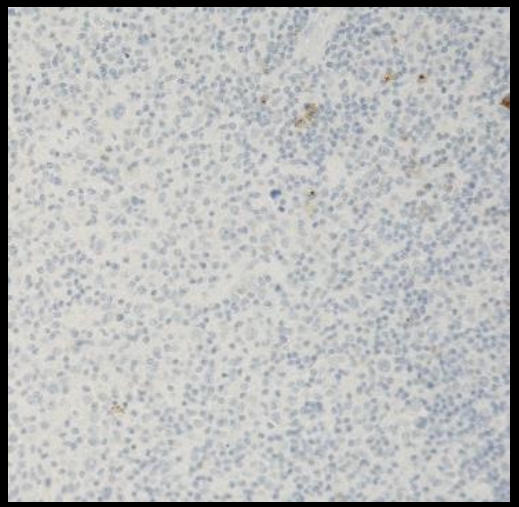
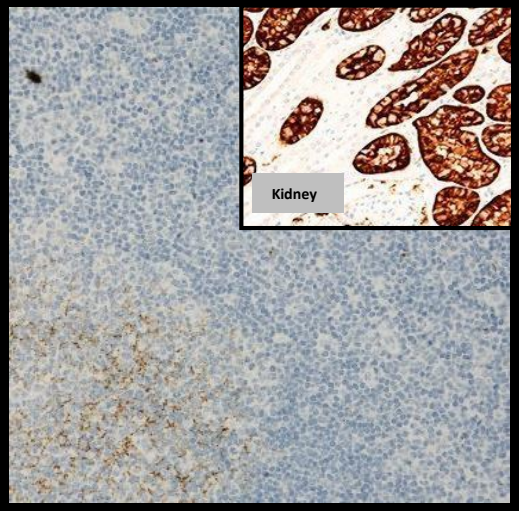
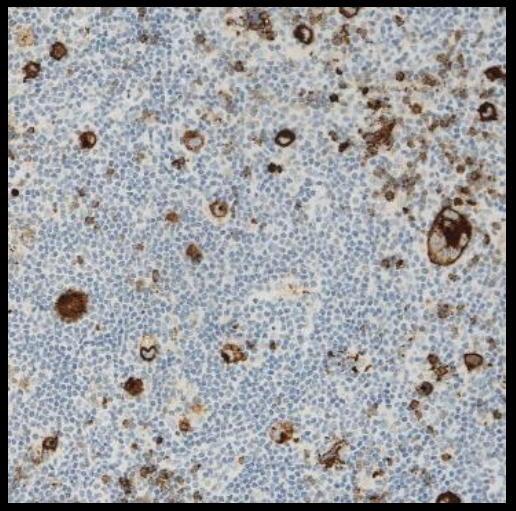
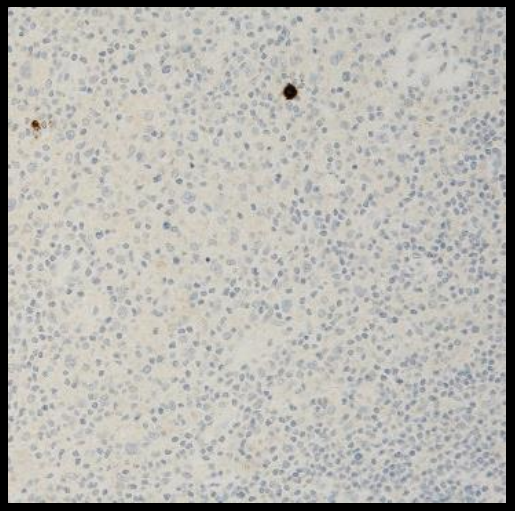
CD15	Retrieval buffers	Titre	Detection	RTU	Detection
mAb Carb-3	HIER High pH, mod. or standard Low pH	1:10-1:100	-	Dako (IS/IR062)	Flex/Flex+
mAb MMA	HIER High pH	1:10-1:50	-	Ventana (760-2504)	UltraView +/- Amp OptiView
mAb HI198	HIER High pH	1:20	-	-	-

Control material / Kidney:

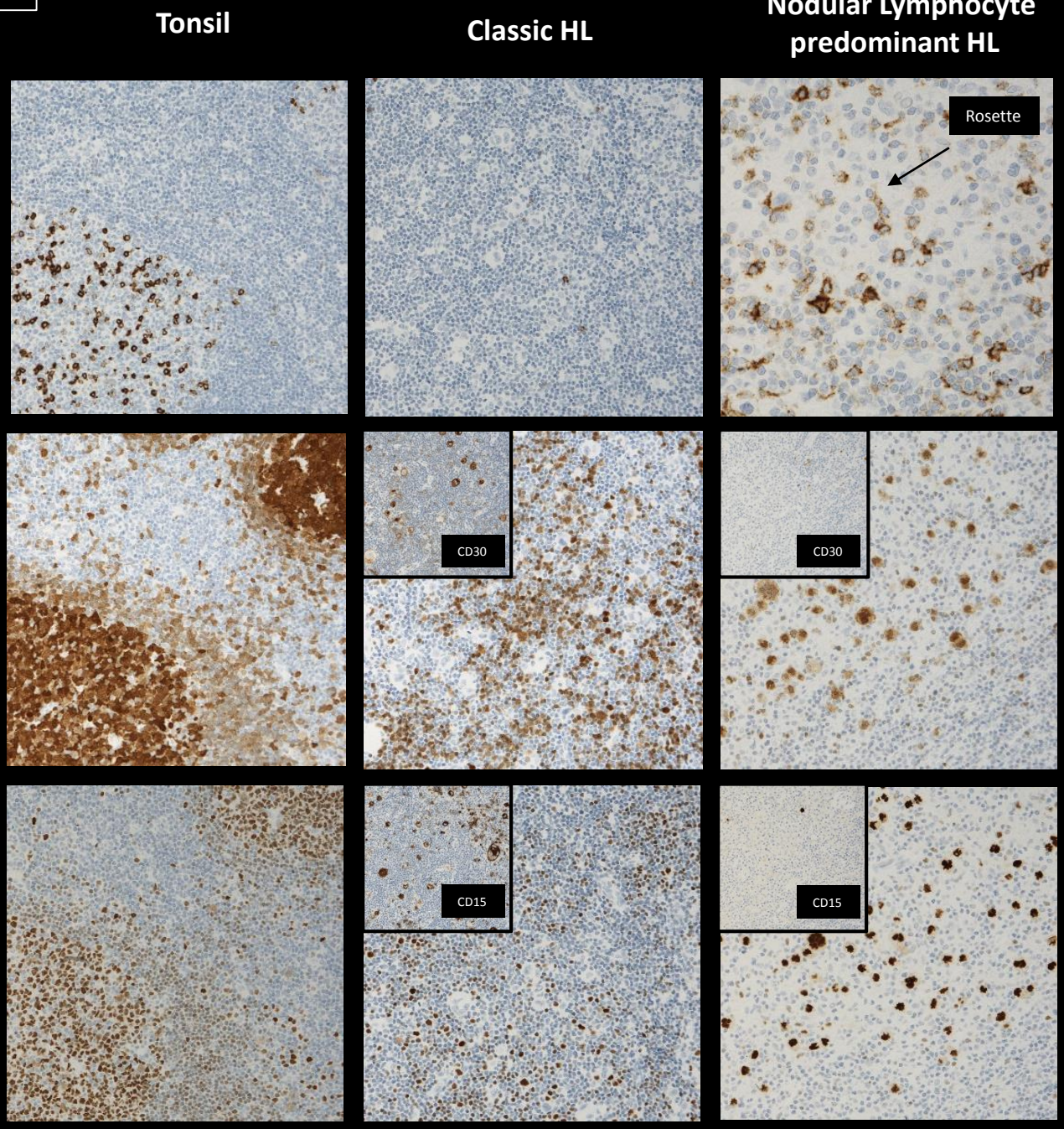
A moderate to strong predominantly membranous staining reaction of the epithelial cells lining the renal proximal tubules.

Tech tip: Look for weak reaction of follicular dendritic cells in the germinal centres of tonsillar tissue

Hodgkin lymphoma markers

	Tonsil	Classic HL	Nodular Lymphocyte predominant HL
CD30			
CD15			

Hodgkin lymphoma markers



T-Cell lymphoma markers (1)

Marker (localization)	Control	iCAPs (HE)	iCAPs (LE)	iCAPs (NE)
CD3 (membr.) F7.2.38, LN10, PS1, JCM182, EP449E, SP7, 2GV6, pAb A0542	Tonsil / Appendix	T-cells in the T-zone	T-cells in the mantle zones and within the germinal centres (moderate to strong intensity)	All other cells including B-cells and epithelia cells of the appendix
CD5 (membr.) 4C7, SP19	Tonsil / Appendix	T-cells	Dispersed mantle zone B-cells	All other cells including B-cells and epithelia cells of the appendix
CD4 (membr.) 4B12, 1F6, SP34, EP204, EPR6855	Tonsil / Appendix	Helper/inducer T-cells	Germinal centre macrophages	All other cells including B-cells and epithelia cells of the appendix
CD8 (membr.) C8/144B, 4B11, 1A5	Tonsil / Appendix	T-cytotoxic/suppressor cells & NK cells	None	All other cells including B-cells and epithelia cells of the appendix
CD1a (membr.) O10, EP3622	Tonsil/Skin/Thymus	The Langerhans' cells in the squamous epithelium (tonsil & skin) and cortical thymocytes (Thymus)	None	All other cells including epitheliums
CD2 (membr) AB75, SP304, BS60	Tonsil / Appendix	See CD3	See CD3	See CD3
CD7 (membr.) CBC.37, BSR9, BS8	Tonsil / Appendix	See CD3	See CD3	See CD3
In addition to the previous panels EBV-EBER/EBV-LMP1				

Clones (mAbs, rmAbs &pAbs) giving optimal results (NordiQC assessments)

iCAPs (HE): Strong staining intensity/reactions should be expected

iCAPs (LE): An at least weak to moderate staining intensity/reactions should be expected

iCAPs (NE): No staining/reactions should be expected

CD4, Run 44

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4B12**	23 13 8 1 1	Leica/Novocastra Dako Thermo/NeoMarkers Monosan Immunologic	5	22	10	9	59%	82%
mAb clone **1F6**	10	Leica/Novocastra	4	3	2	1	70%	75%
mAb clone **BC/1F6**	1	Biocare	0	0	0	0	-	-
rmAb clone **SP35**	17 7 2	Cell Marque Spring Biosciences Immunlogic	11	11	3	1	85%	86%
rmAb clone **EP204**	3	Nordic Biosite Zeta	2	1	0	0	-	-
rmAb clone **EPR6855**	1	Epitomics/Abcam	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **4B12 IS/IR649**	51	Dako	13	24	8	6	73%	81%
mAb clone **4B12 PA0368**	7	Leica/Novocastra	0	1	2	4	14%	-
mAb clone **4B12 PA0427**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **4B12 MS-1528-R7**	1	Thermo/NeoMarkers	0	0	0	1	-	-
mAb clone **1F6 MONX10330**	1	Monosan	0	1	0	0	-	-
rmAb clone **BC/1F6 PM153**	1	BioCare	0	1	0	0	-	-
rmAb clone **SP35 790-4423**	74	Ventana	63	10	0	1	99%	100%
rmAb clone **SP35 104R-17/104R-18**	4	Cell Marque	1	2	1	0	-	-
rmAb clone **SP35 RMA-0620**	2	Maixin	1	1	0	0	-	-
rmAb clone **EP204 MAD-000600QD**	3	Master Diagnostica	-	2	1	-	-	-
rmAb clone **EP204 AN722-5M**	1	BioGenex	1	-	-	-	-	-
rmAb clone **EP204 104R-28**	1	Cell Marque	-	1	-	-	-	-
Total	234		102	82	27	23	-	
Proportion			44%	35%	12%	9%	79%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

11%

42%

OPS: Concentrates

HIER in High pH buffer

mAb 4B12 ~ 1:40-1:150

mAb 1F6 ~ 1:20-1:50

rmAb SP35 ~ 1:10 -1:100

Best performance:

rmAb SP35 (concentrate)

RTU format rmAb SP35 (790-4423)

Ventana Benchmark CC1, UltraView+/- amp or OptiView

Used off- label (other system)

CD4 (Run 44 2015)

- The mAb clone 4B12 consistently gives inferior results on the Benchmark XT/ Ultra (Ventana)
  - Run 29 & 44: 15 out of 15 protocols were assessed as insufficient

- A decline in pass rate compared to the latest run was also observed with the mAb clone 4B12 on the BOND III/MAX (Leica)
  - Run 29: 91% (10 /11) protocols were assessed as sufficient / 18% optimal (2/11)
  - Run 44: 54% (7 /13) protocols were assessed as sufficient / 0% optimal (0/13)

- The RTU format of the rmAb SP35 (Ventana, 790-4423) was superior in performance compared to all other RTU systems
  - rmAb SP35 (Ventana) : 100% (73/73) protocols were assessed as sufficient / 86% optimal (63/73)
  - mAb 4B12 (Dako): 73% (37/51) protocols were assessed as sufficient / 25% optimal (13/51)

- Unexplained technical issues

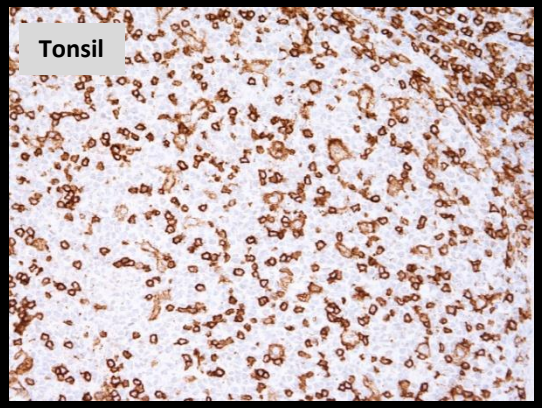
CD4/ Run 44 2015

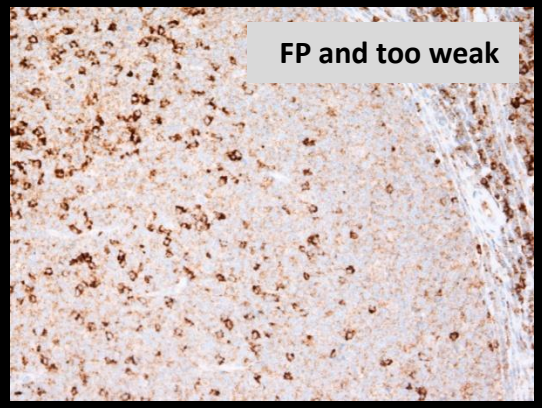
Optimal

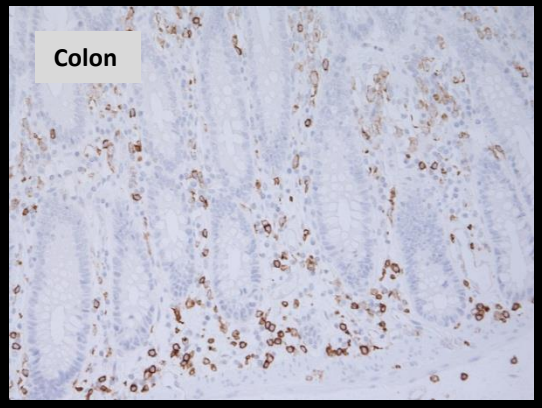
rmAb SP35 (concentrate)

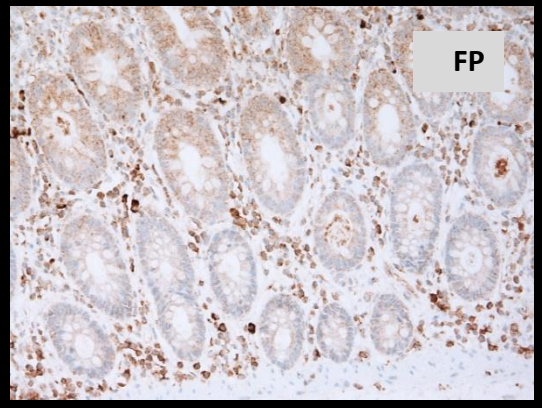
HIER CC1

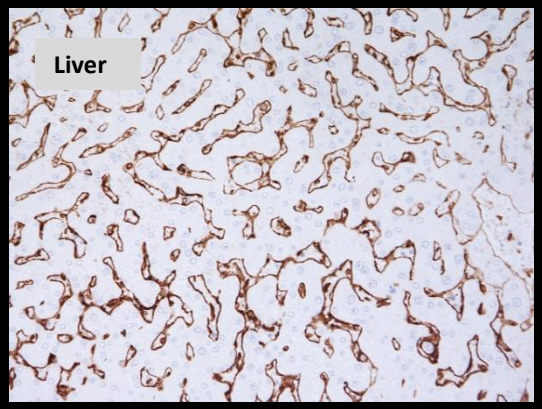
OptiView

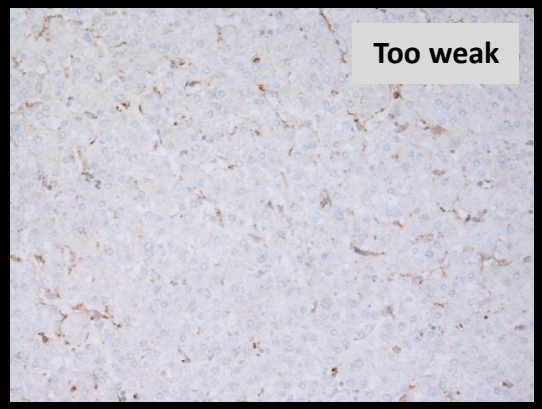












Insufficient

mAb 4B12 (concentrate)

HIER CC1

OptiView with Tyramide amp.

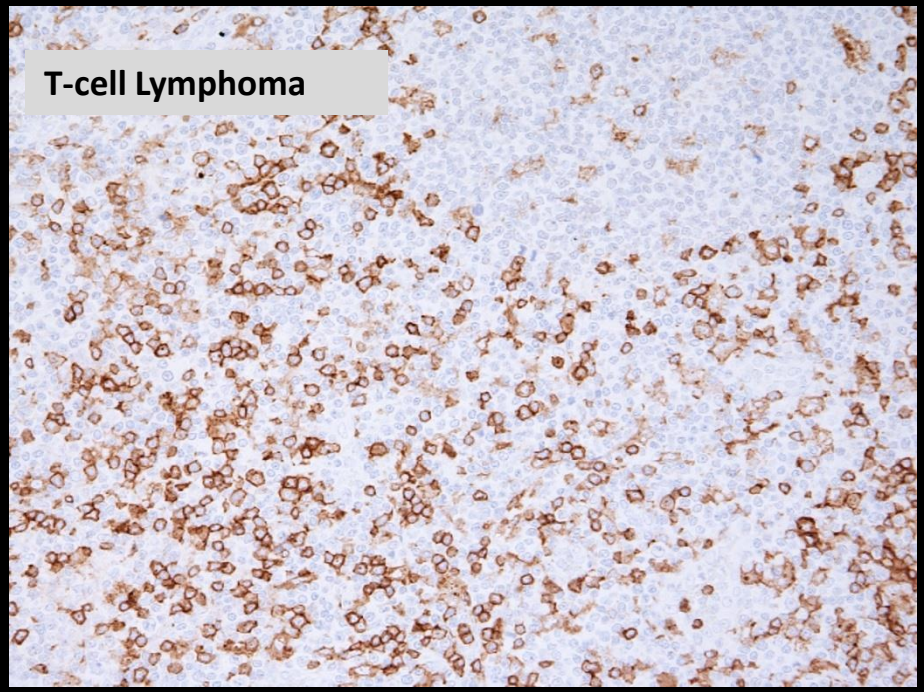
Inadequate balance of the staining reaction

The pattern of too weak staining reaction was observed with all protocols based on the mAb 4B12 on the Ventana BenchMark platform

CD4/ Run 44 2015

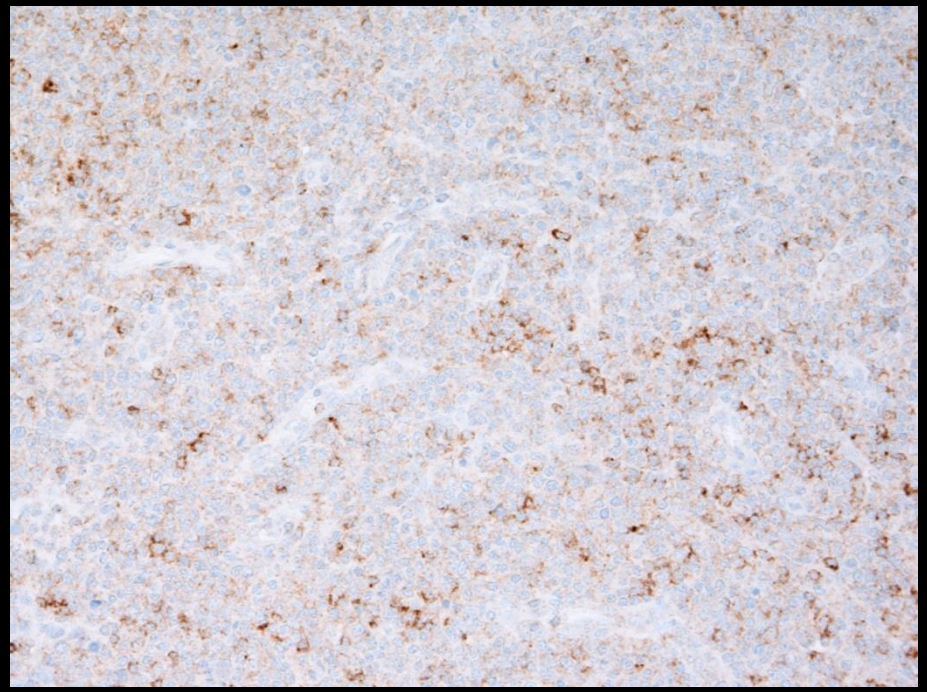
Optimal

rmAb SP35 (concentrate), HIER CC1, OptiView



Insufficient

mAb 4B12 (concentrate), HIER CC1, OptiView /TSA



Inadequate balance of the staining reaction

The pattern of too weak staining reaction was observed with all protocols based on the mAb 4B12 on the Ventana BenchMark platform

Lymphoma panel: CD4

Optimal protocol settings (NQC)

CD4	Retrieval buffers	Titre	Detection	RTU	Detection
mAb **4B12**	**HIER High pH**	**1:40-1:150**	**3-step**	**Dako (IS649/IR649)**	**Flex+**
mAb 1F6	**HIER High pH**	**1:20-1:50**	**3-step**		
rmAb **SP35**	**HIER High pH**	**1:10-1:100**	**2 & 3-step**	**Ventana (790-4423)**	**UltraView +/- Amp OptiView .**
rmAb **EP204/EPR6855**	**HIER High pH**	**1:25-1:100**	**3-step**		

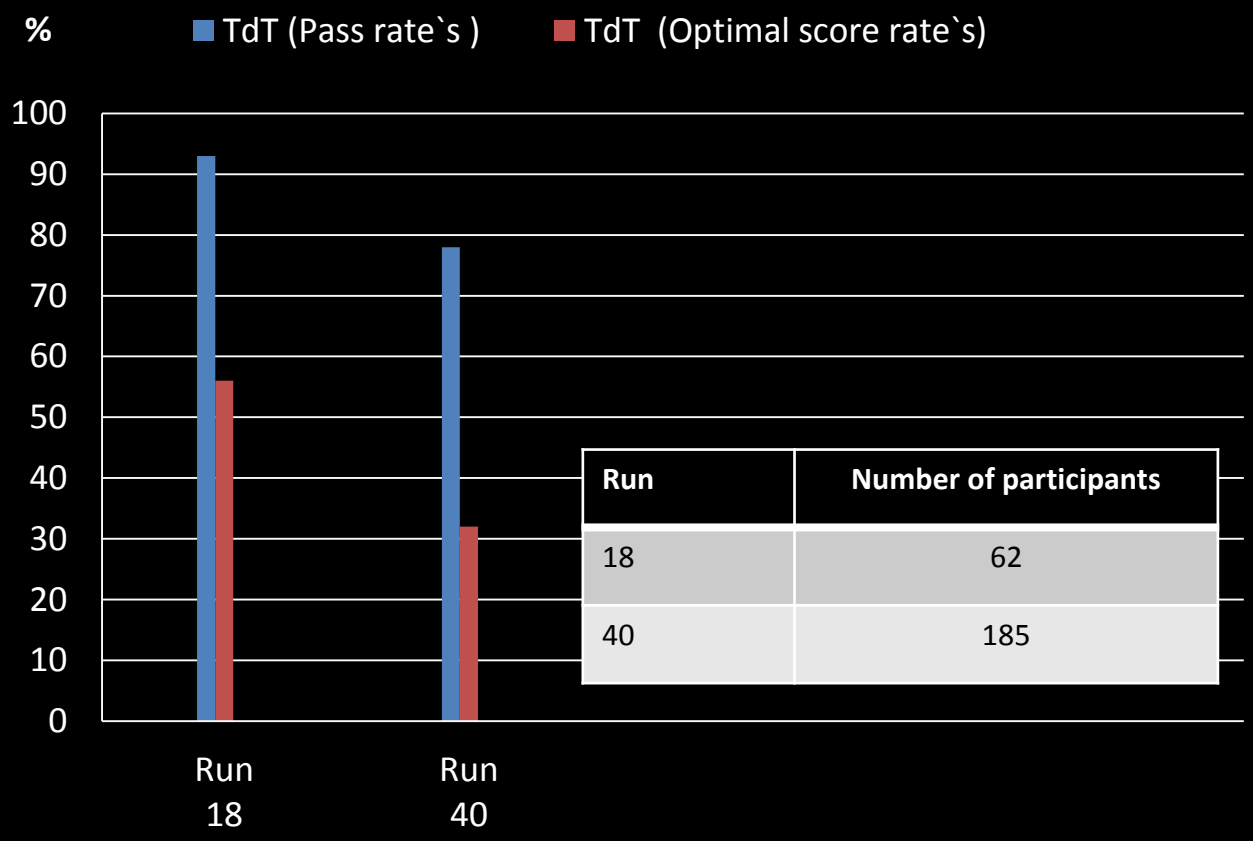
Control material / Tonsil:

A moderate and distinct membranous staining reaction of germinal centre macrophages in the tonsil. Inducer/helper T-cells should be strongly stained

Blasts

TdT

Pass & Optimal score rate`s



Run	Number of participants
18	62
40	185

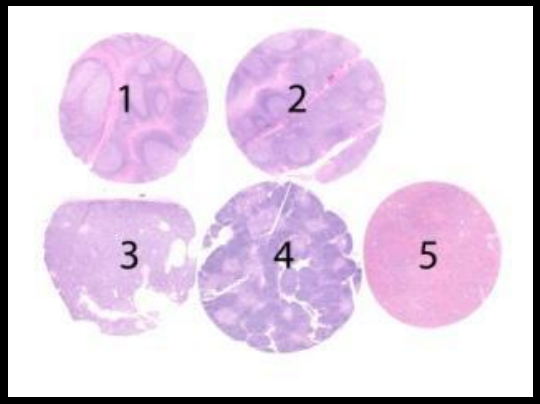
TdT / Run 40:

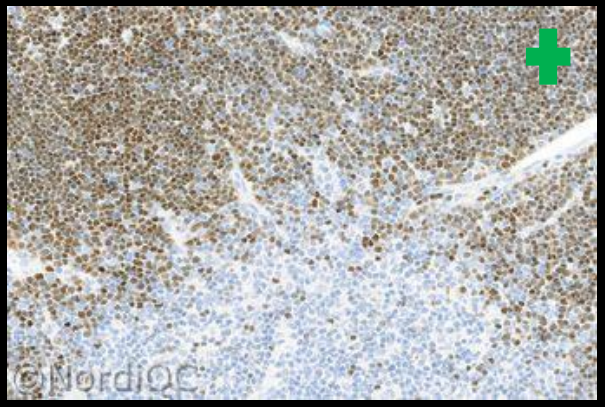
Sufficient: 78%

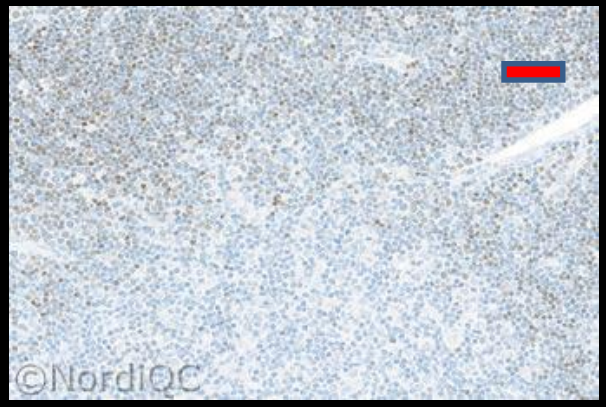
Optimal: 32%

A challenging marker

TdT / Run 40 2014







Criteria for assessing a TdT staining as optimal included:

Core	Nuclear staining reaction
1. Tonsil (24h)	+ Dispersed perisinusoidal cells in the interfollicular zones
2. Tonsil (48h)	+ Dispersed perisinusoidal cells in the interfollicular zones
3. Thymoma (NOS)	?
4. Thymus	**+ Cortical thymocytes (moderate to strong reaction)**
5 Precursor-B-acute lymphatic leukaemia (Pre-B-ALL).	+
No nuclear staining reaction of T- and B-cells in the tonsils and the vast majority of medulary thymocytes of the normal thymus.	

Thymus is recommended as control material

Table 1. **Antibodies and assessment marks for TdT, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **SEN28**	44 4 1 1 1	Leica/Novocastra Thermo/NeoMarkers Diagnostic Biosystems Gentech Vector	30	12	5	4	82%	84%
rmAb clone **EP266**	1	Abcam/Epitomics	0	1	0	0	-	-
pAb **A3524**	36	Dako	10	14	10	2	67%	81%
pAb **ILP 0049**	7	Immunologic	2	4	1	0	86%	100%
pAb **18-7237**	1	Life Tech/Invitrogen	0	1	0	0	-	-
pAb **61-0155-2**	1	Genemed	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **SEN28 PA0339**	6	Leica/Novocastra	3	1	1	1	67%	80%
mAb clone **SEN28 PDM 096**	1	Diagnostics Biosystems	0	0	1	0	-	-
mAb clone **SEN28 MAD-00909QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **SEN28 ZM-0358**	1	Zhonggshan	0	1	0	0		
pAb **338A-78**	2	Cell Marque	0		1			
pAb **760-2670**	37	Ventana/Cell Marque	1	24	10	2	68%	50%
pAb **IS001/IR001**	39	Dako	11	25	3	0	92%	92%
pAb **PP134**	1	Biocare	0	1	0	0	-	-
Total	185		58	86	32	9	-	
Proportion			32%	46%	17%	5%	78%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

59%

3%

HIER in high or standard low pH buffer (Ci pH 6); dil. range 1:25-1:40

HIER in high or standard low pH buffer (Ci pH 6); dil. range 1:25-1:40

FP ~10/12 protocols

In this assessment, participants using the mAb clone SEN28 produced significantly higher number of optimal scores compared to participants using other primary antibodies.

For all pAb TdT formats (RTU`s & Concentrates) except pAb ILP 0049:

An aberrant cytoplasmic staining was obseved e.g.

Optimal results could be obtained with the mAb SEN28 and the pAb ILP-0049, 760-2670.

TdT (Run 40 2014) : Observations with impact on the final result

Table 3. **Proportion of optimal results for TdT using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **SEN28**	5/8** (63%)	0/1	9/19 (47%)	-	11/13 (85%)	-
pAb **A3524**	4/10 (40%)	1/1	3/12 (25%)	0/1	0/4	-

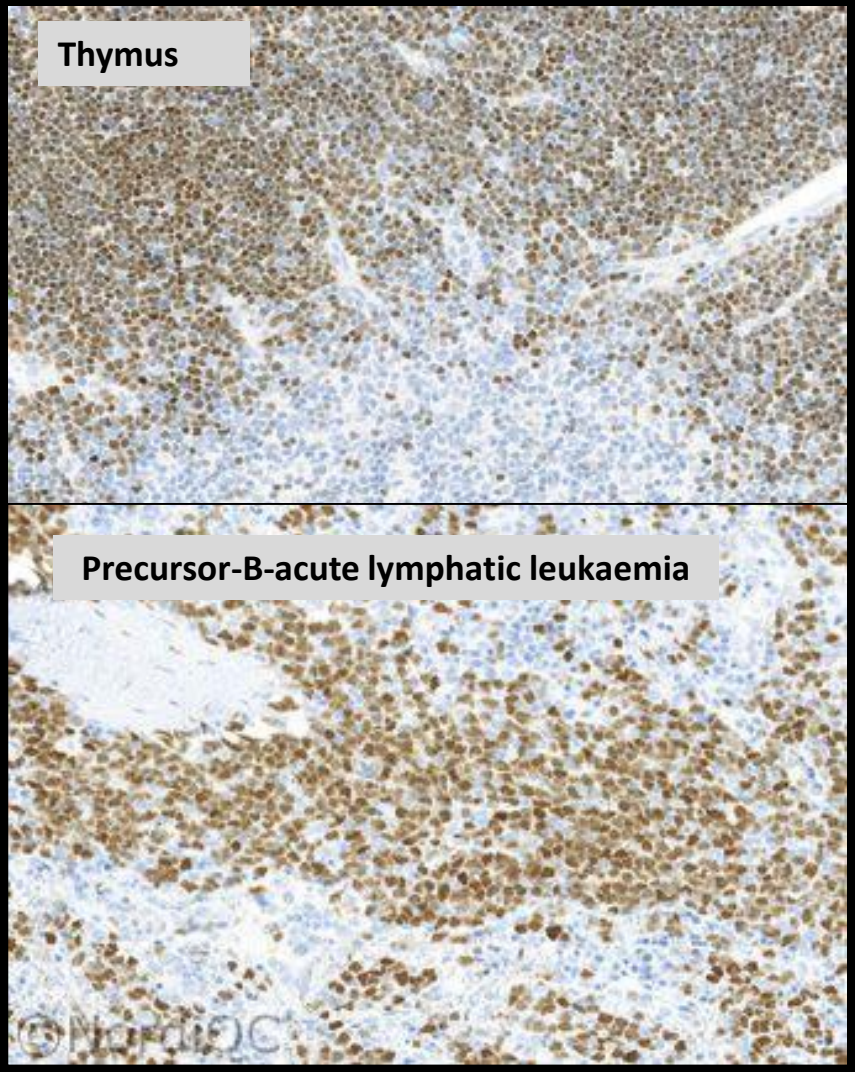
* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

- **The mAb clone SEN28 is robust and could produce optimal results at high frequency on the 3 main platforms**
- **Less successful antibodies**
  - **False positive staining reaction and a poor signal-to-noise was seen in 56% of the insufficient results (23 of 41)**
    - **pAb A3524 concentrate (Dako) ~ lot no. 10072158 versus lot no.1004890 ~ discontinued (new antibody)**
    - **RTU format IR/IS 001 (Dako) ~ discontinued (new antibody).**
    - **RTU format 760-2670 (Ventana/Cell M.)**

TdT / Run 40 2014

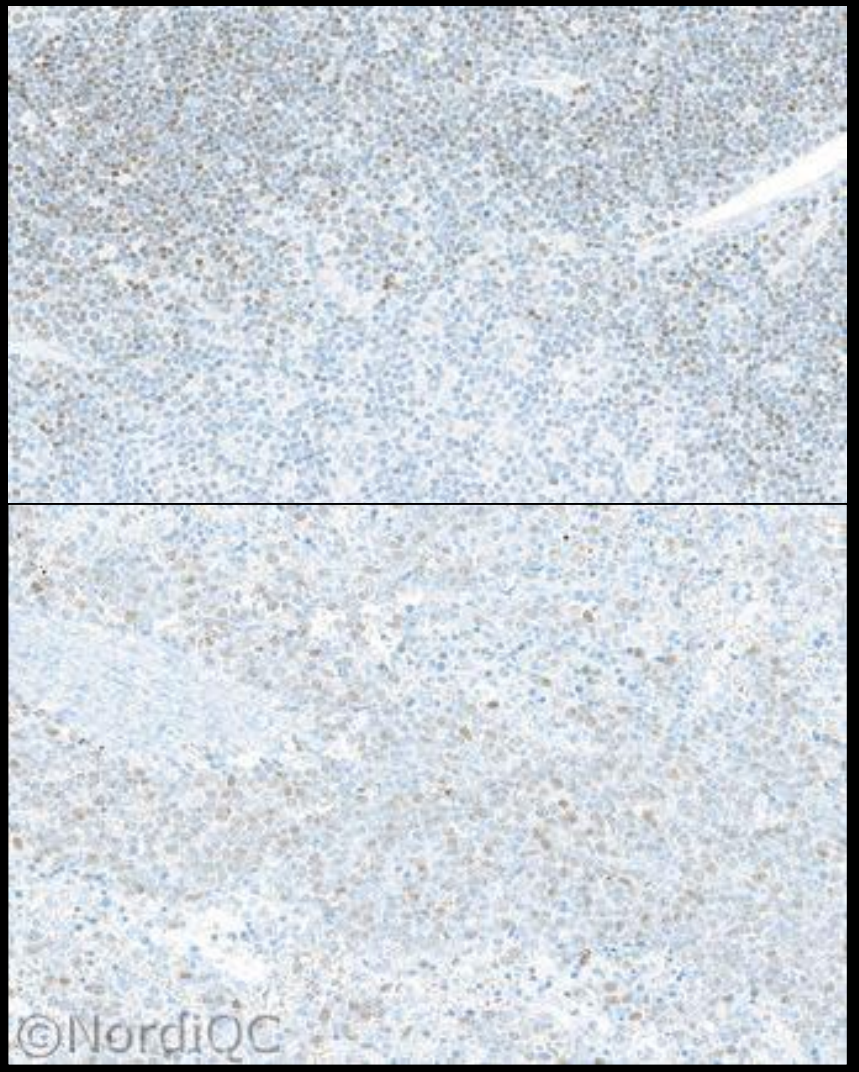
Optimal

TdT clone SEN28 , HIER CC1, pH 8.5 , OV (3-step multimer)



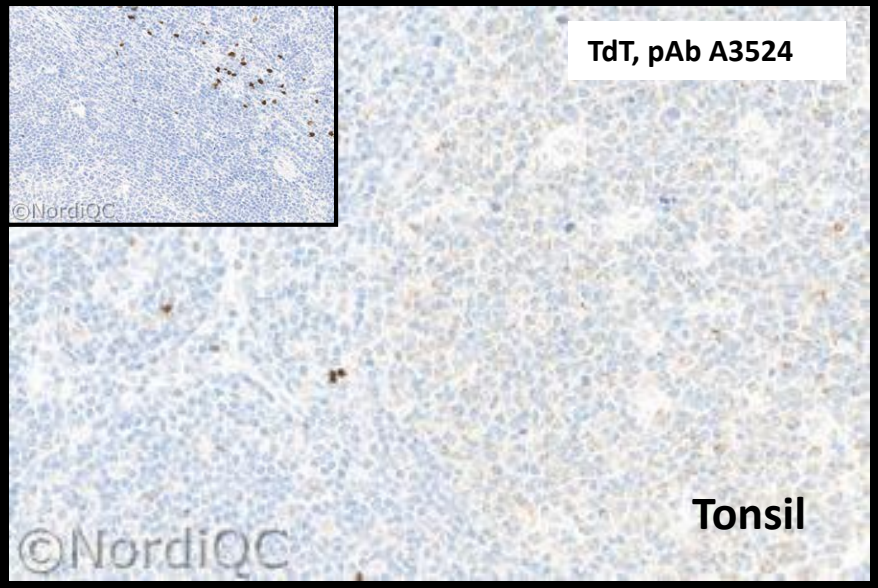
Insufficient

TdT clone SEN28 (too low titre), UV (2-step multimer)

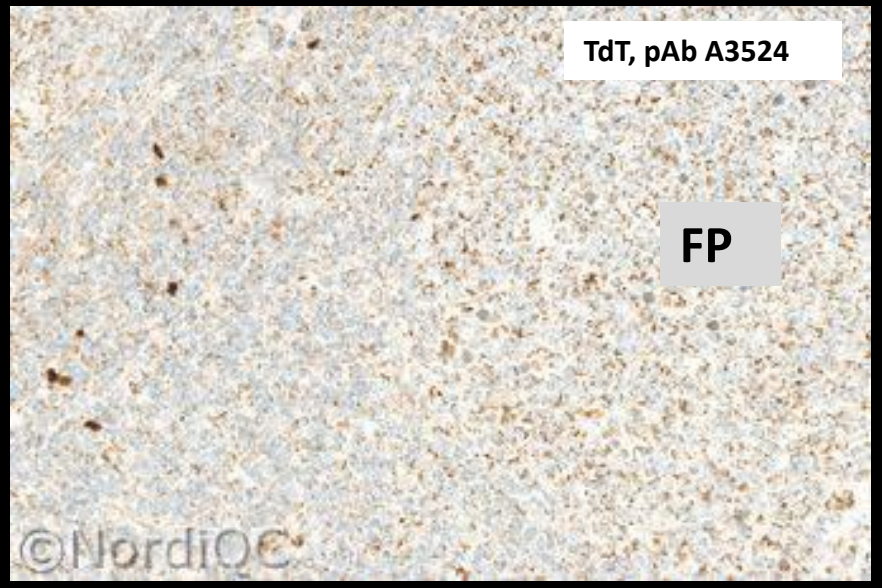


TdT / Run 40 2014

Optimal



Insufficient



The aberrant cytoplasmic staining reaction was typically seen for the pAb as concentrate A3524 (Dako).

The same pattern was also observed for the Ready-To-Use formats based on a pAb e.g. prod. no. IR/IS001 (Dako) and 760-2670 (Ventana/Cell Marque).

~ Lot to lot variations ?

The pAb ILP 0049, Immunologic, did not give any aberrant cytoplasmic staining reaction despite that similar protocol settings were applied. Same lot no. 1021, was used by all the participants using this product (n=7).

TdT / Run 40 2014

Lymphoma panel: TdT
Optimal protocol settings (NQC)

TdT	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **SEN28**	**HIER High pH & standard low pH**	**125-1:40**	**-**	**Leica (PA0339)**	**BOND Refine**
pAb **A3524***	**HIER High pH & mod. low pH**	**1:10-1:50**	**-**	**Dako (IS001/IR001)***	**Flex/Flex+**
pAb **ILP 0049**	**HIER High pH**	**1:50-1:200**	**-**	**-**	**-**
	HIER High pH	**-**	**-**	**Ventana (760-2670) (One protocol)**	**iView**
* **Discontinued by the vendor**					

Control material / Thymus:

An at least moderate distinct nuclear staining reaction of virtually all cortical thymocytes of the normal thymus.