Nordic immunohistochemical Quality Control

NordiQC

Workshop in Diagnostic Immunohistochemistry

Aalborg Hospital, 19th – 21th September 2016

Optimization of antibodies, protocols and controls

Hematolymphoid markers

Michael Bzorek

Histotechnologist

Department of Pathology

Næstved Hospital, Denmark

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

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

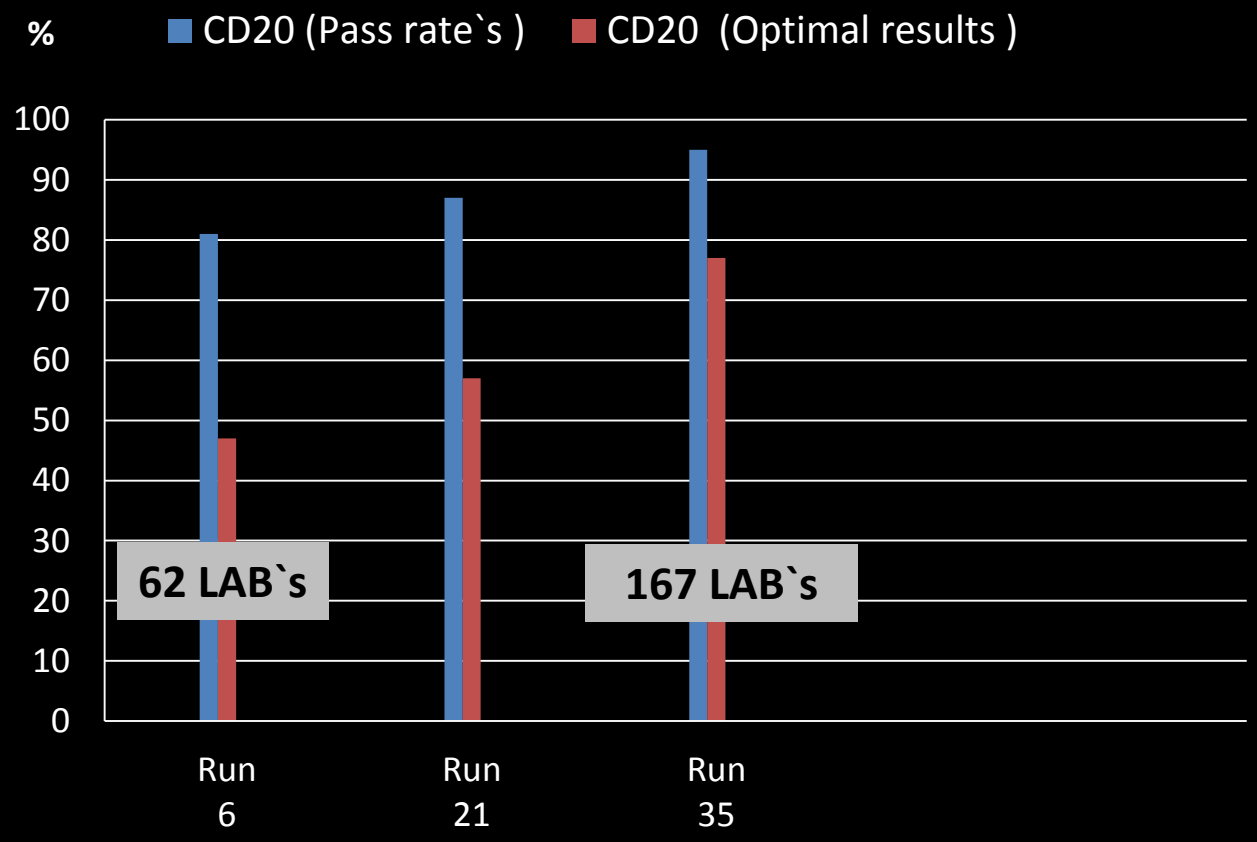
Courtesy: Steve Hamilton-Dutoit

Basic IHC panel for lymphoma diagnosis (NordiQC results)

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 41	84	54
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
Sox11	√	Run 47	66	27
Kappa	√	Run 18	41	14
Lambda	√	Run 15	34	15
CD43	-	-	-	-

Protocols that could be optimized depending on the marker

CD20



CD20/ Run 31 (2012):

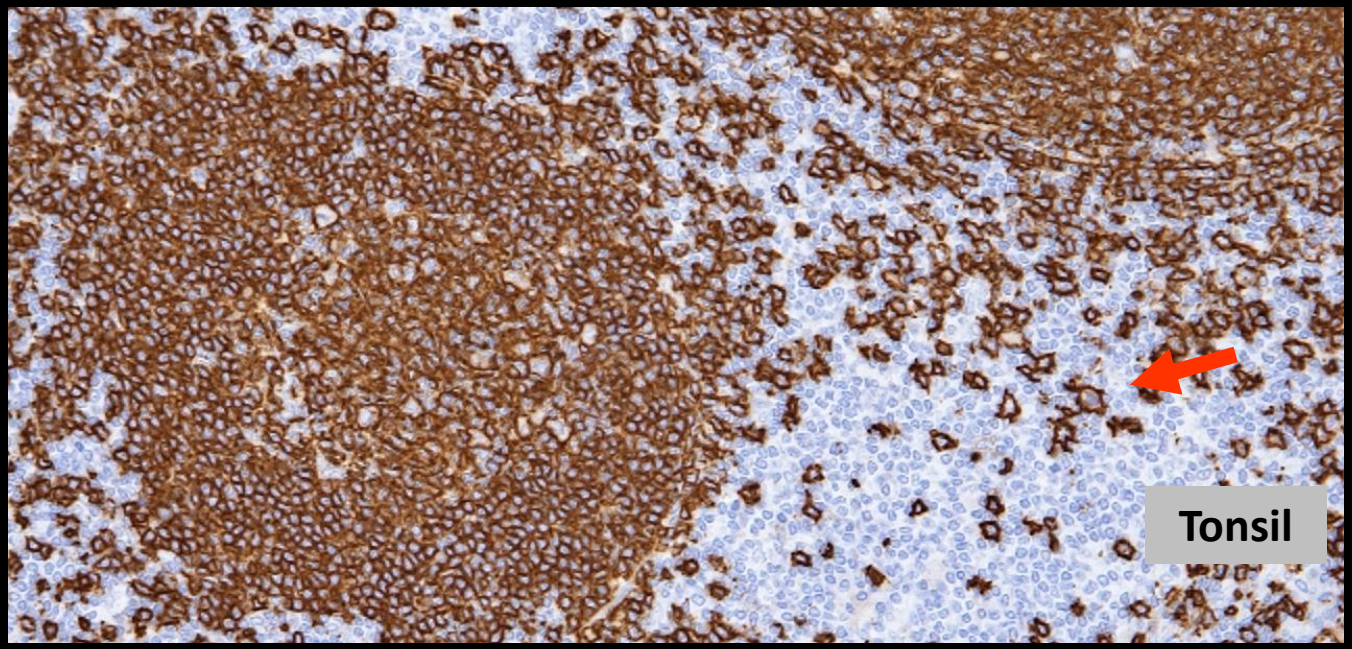
Sufficient: 95%

Optimal: 77%

Success due to very robust Abs

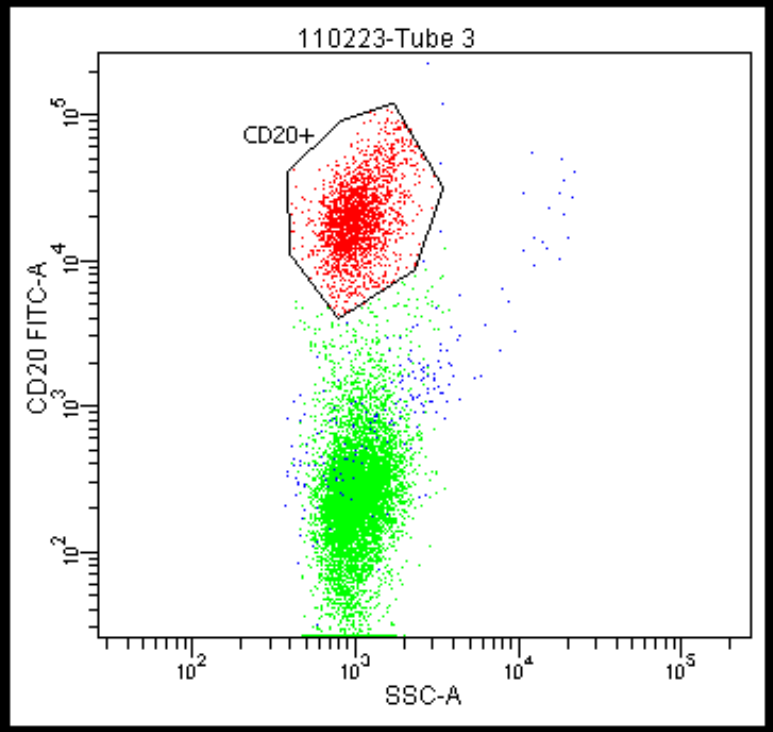
Clone L26 used by 97% of the LAB`s

CD20



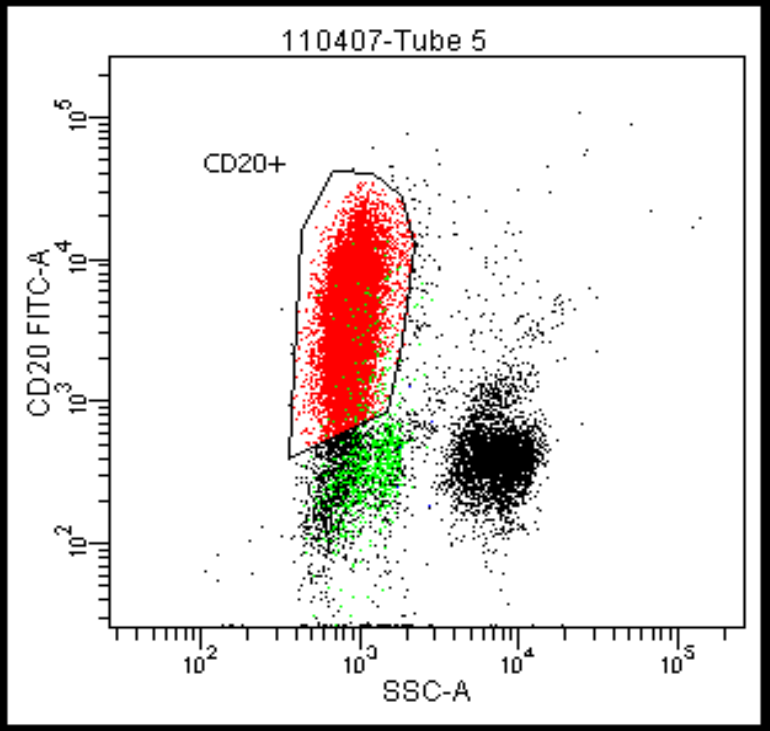
A strong, predominantly membranous staining of all B-cells in the mantle zone B-cells, the germinal centre B-cells and the interfollicular B-cells in the tonsil.

No staining in other cells.



Normal Lymph node

CD20 strong positive

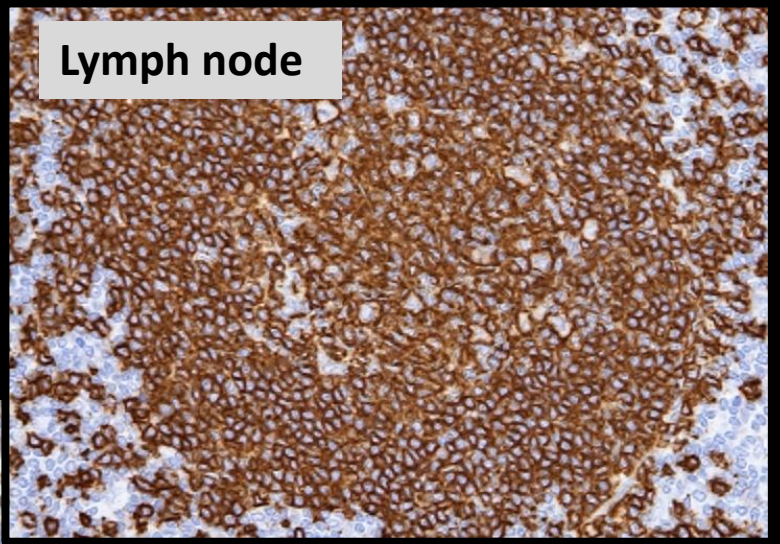


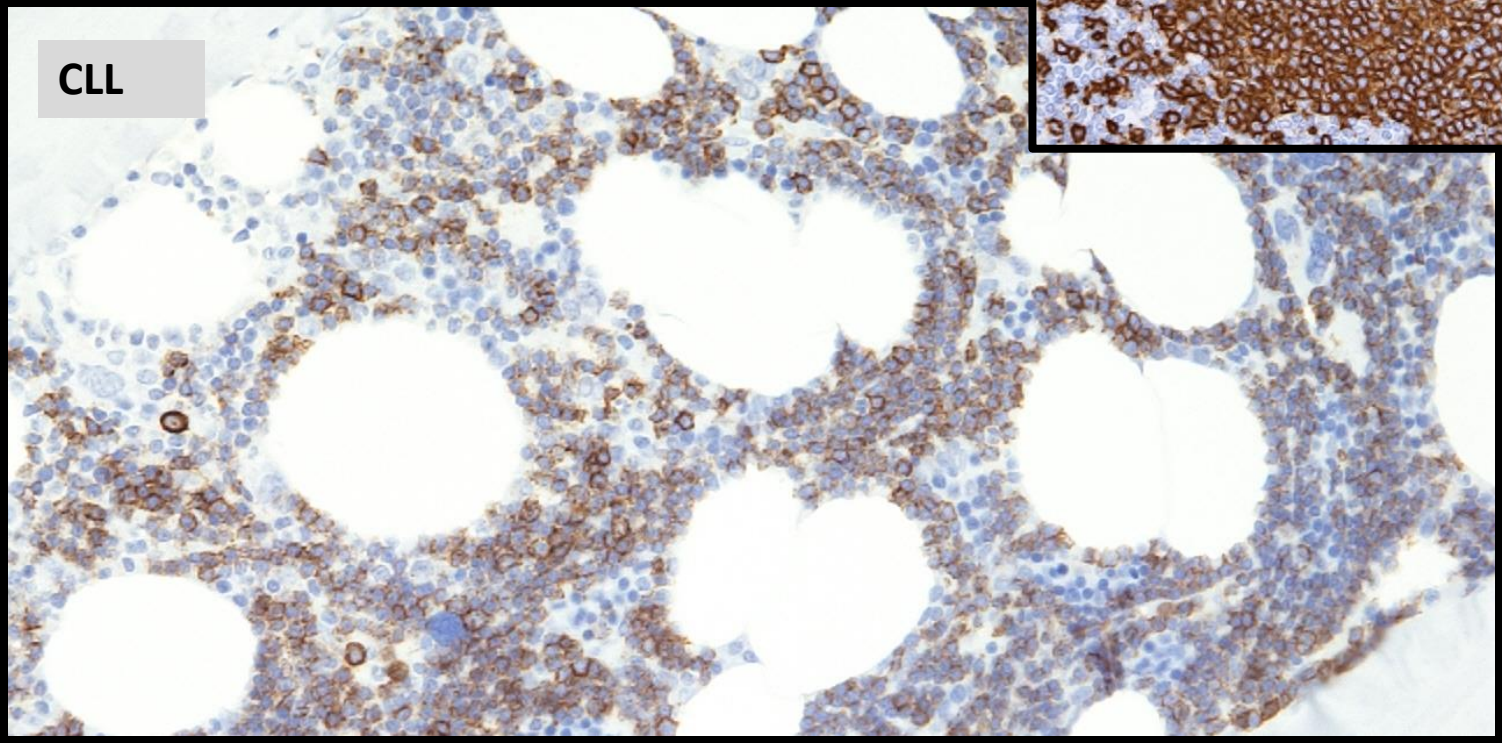
Bone Marrow Aspirate / CLL patient

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





Comment: It is importen to calibrate the staining reaction on low expressors e.g. B-CLL`s in bone marrow aspirate`s displaying weak/dim reaction by flowcytometric investigation. A weak to moderate, predominantly membranous staining of neoplastic B-cells should be seen.

CD20 / Run 35 2012

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	-	-
mAb 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)

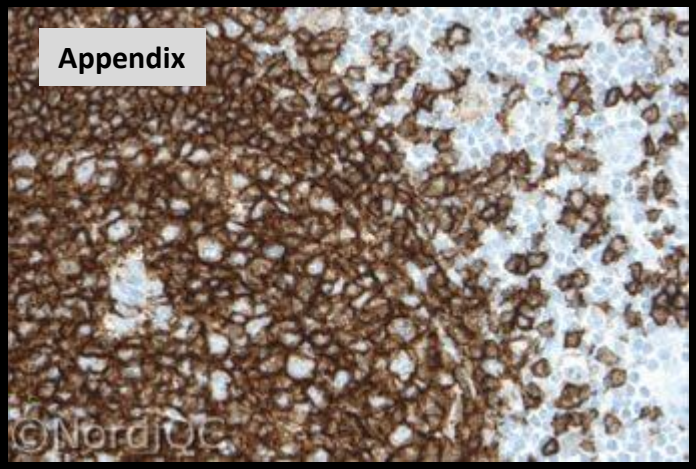


Fig. 1a. Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. **HIER was performed** using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the B-cells.

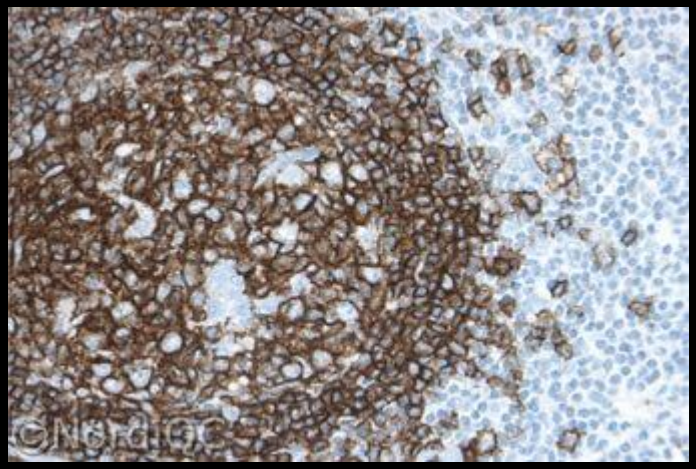


Fig. 1b. Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. **No HIER was performed**. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors 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).

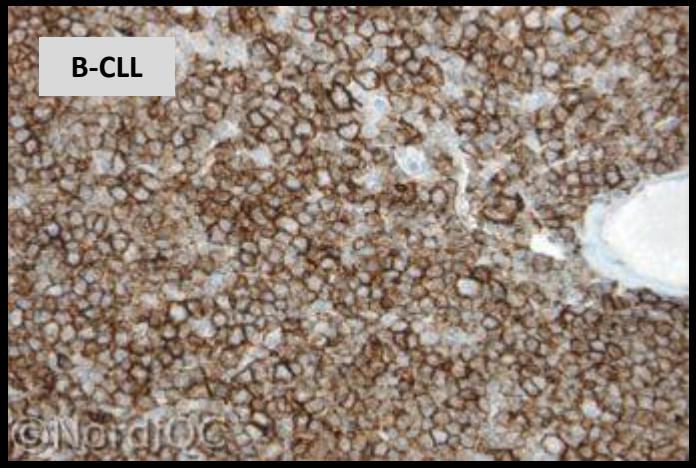


Fig. 2a. B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

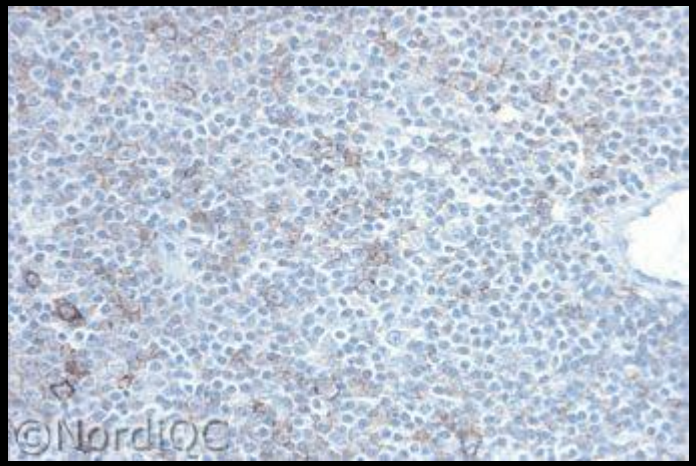
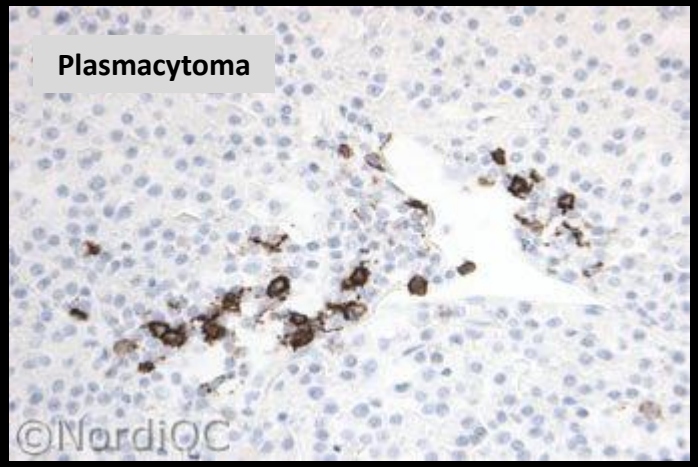
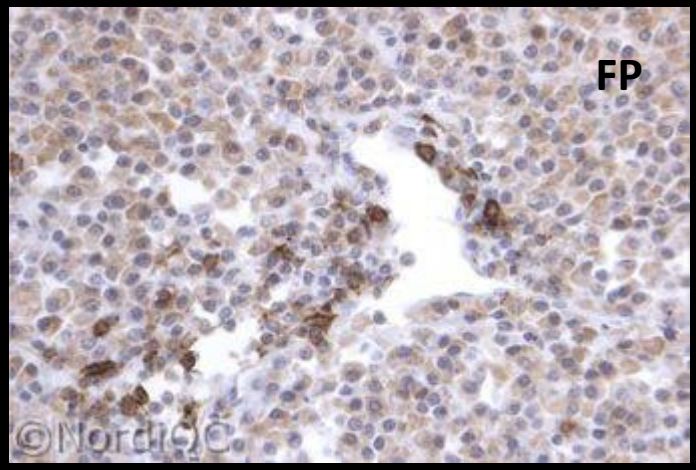
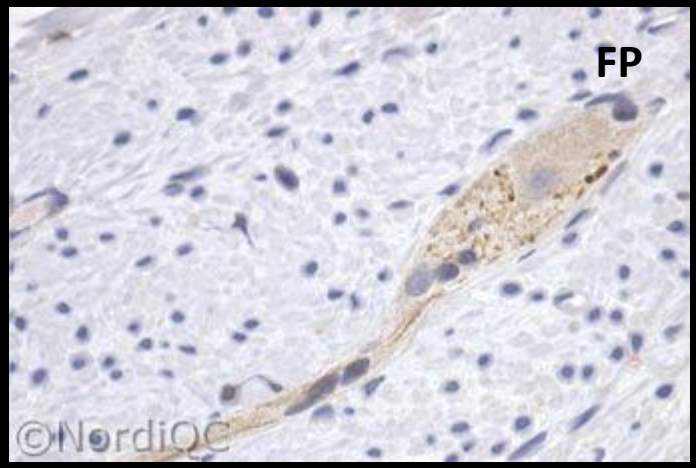


Fig. 2b. B-CLL. Insufficient staining for CD20 using the same protocol as in Fig. 1b. Omitting HIER, only scattered cells are positive. The majority of the neoplastic cells are negative. Compare with the optimal result in Fig. 2a, same field.

CD20 / Run 35 2012







Optimal staining reaction for CD20.

Insufficient staining reaction for CD20 using the the pAb RB-9013-P giving a false positive staining reaction in the peripheral nerves and the neoplastic cells of the plasmacytoma.

CD20 / Run 35 2012

Lymphoma panel: CD20
Optimal protocol settings (NQC)

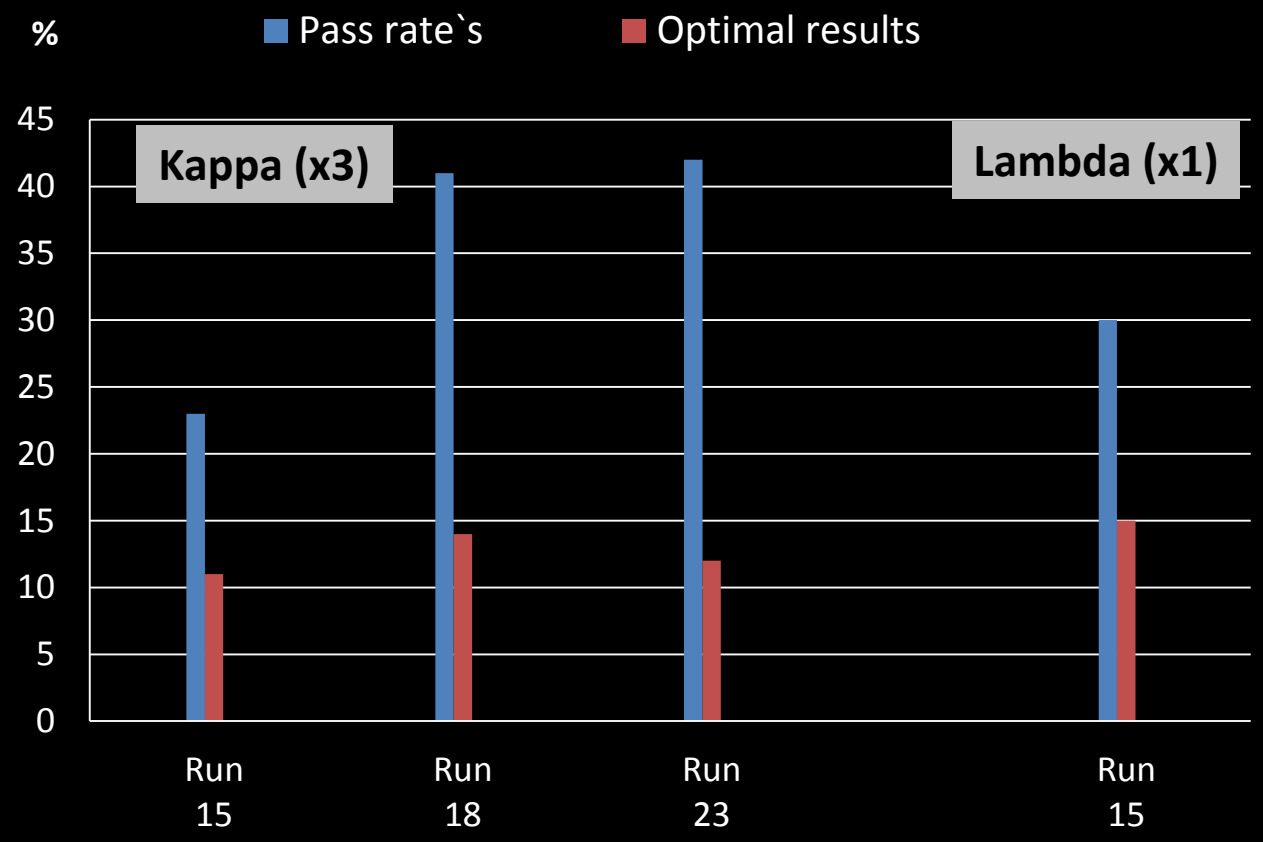
CD20	Retrieval buffers	Titer	Detection systems	RTU	Detection
mmAb **L26**	**HIER High pH or Low pH buffer**	**1:75-1:2000**	**2 & 3-step**	**Dako (IR604)**	**Flex** **Flex+**
	CC1	**-**	**-**	**Ventana (760-2531)**	**iView** **UltraView** **OptiView**
mmAb **7D1**	**HIER Low pH buffer (BERS1)**	**1:200**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

Kappa & Lambda Ig light chains



Kappa & Lambda

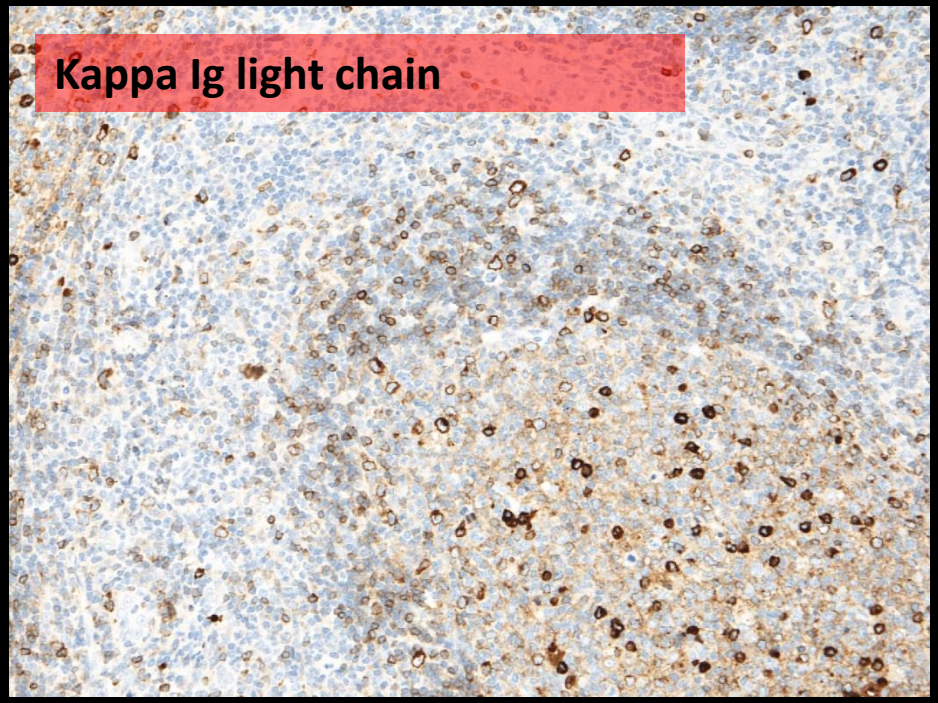
Very low pass rate

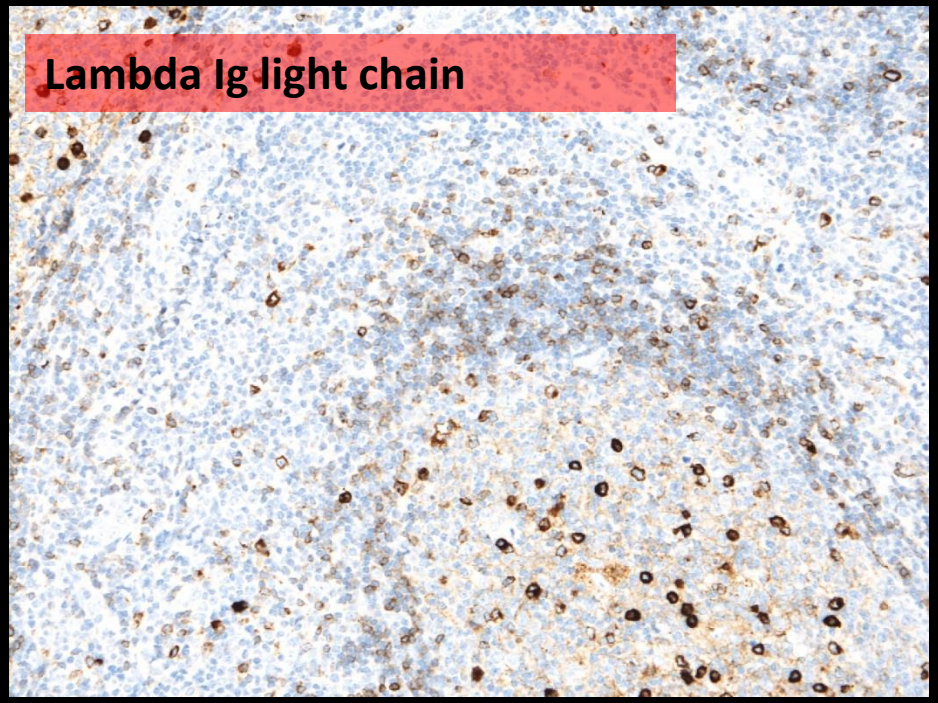
Very low optimal score rate

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 (Kappa or Lambda)

No staining of T-cells (Kappa or Lambda)

"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"

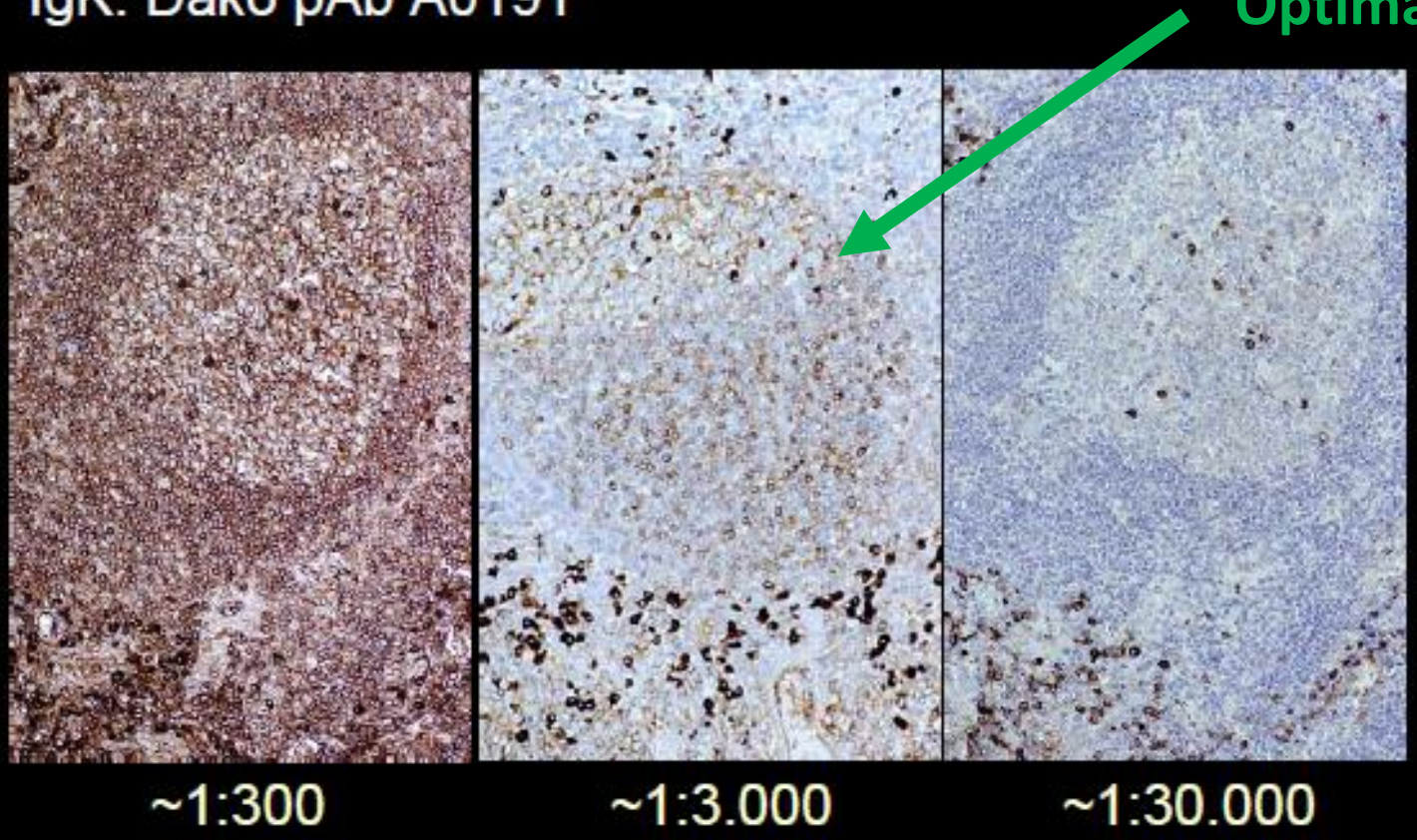
- **Careful calibration of the primary Ab**

  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

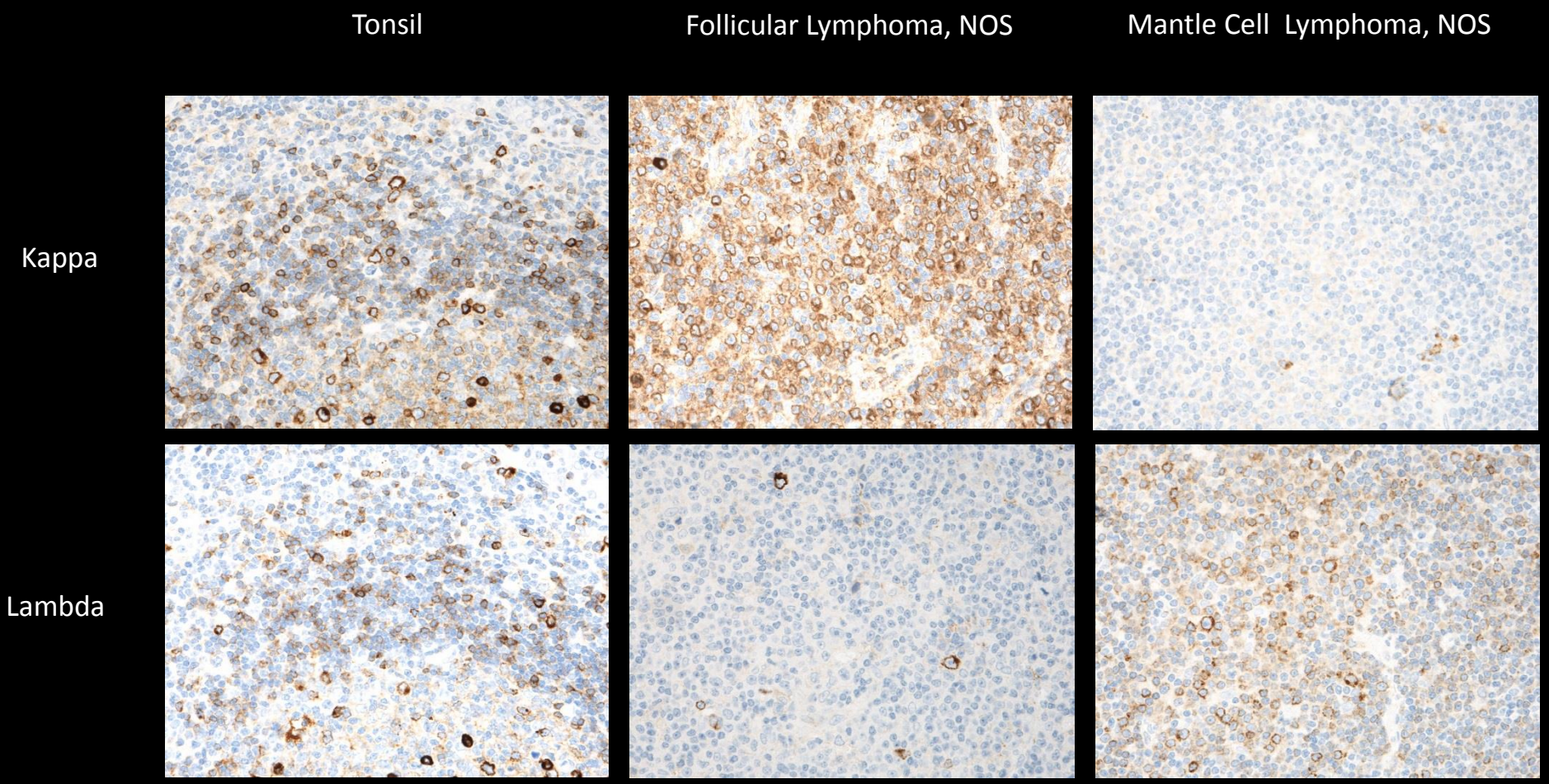
Inappropriate antibody dilution – Ig light chains

IgK: Dako pAb A0191

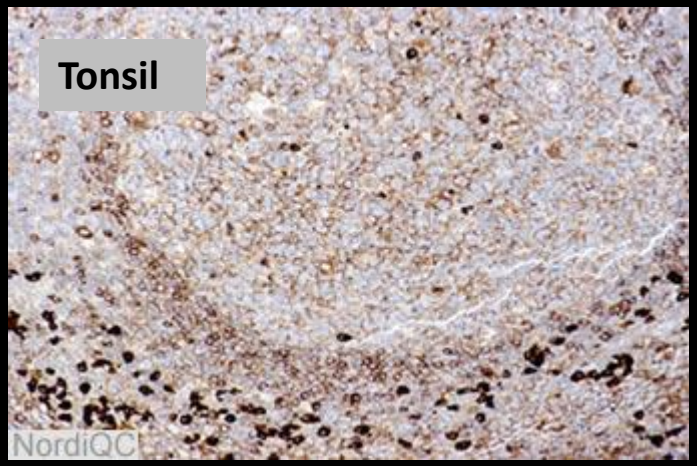


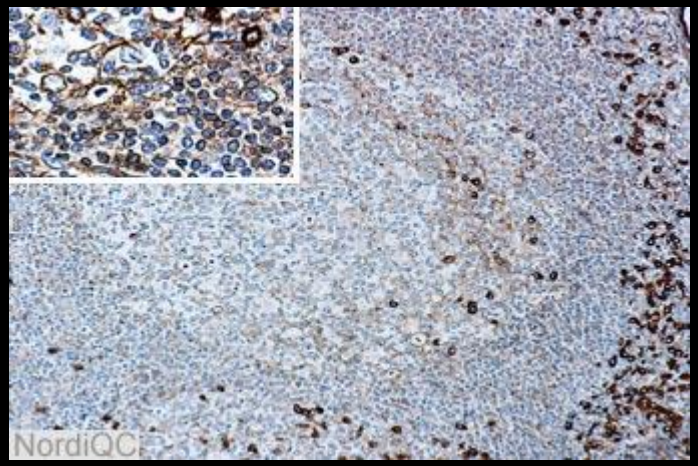
Courtesy: Søren Nielsen

Kappa & Lambda light chain restriction



Kappa & Lambda Ig light chains

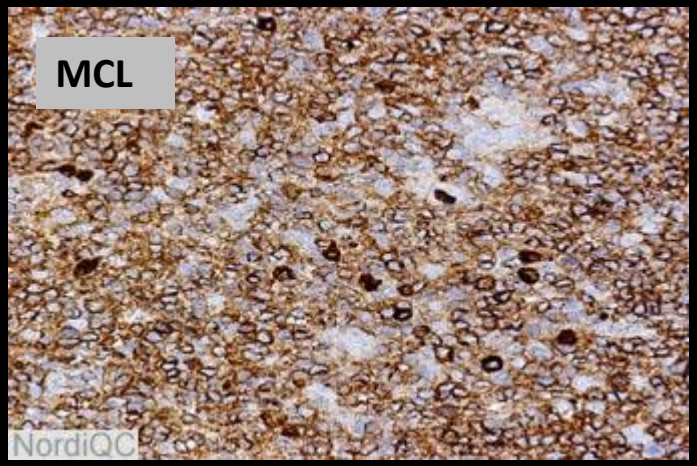


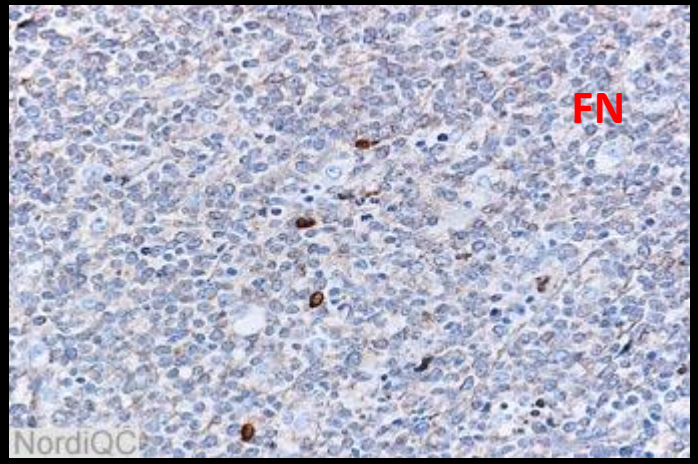


Problem:

Proteolysis

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



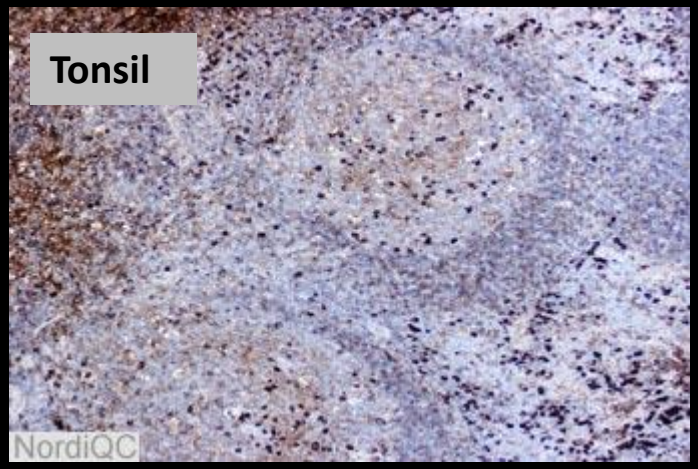


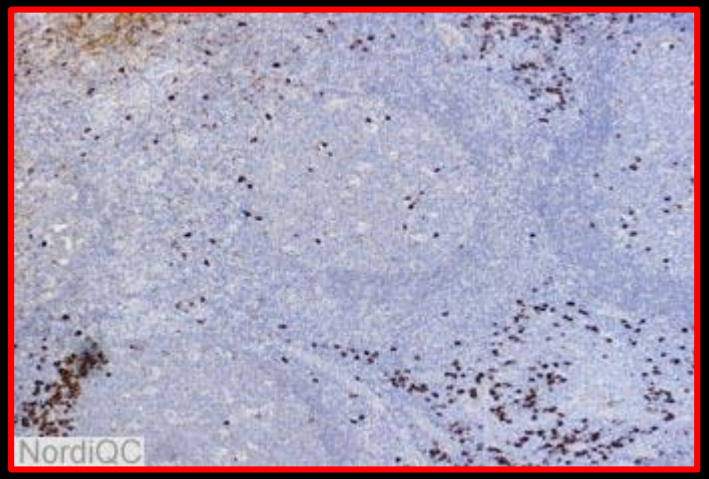
False negative staining for IgL of the MCL using the same protocol as above (right side)
The cell membranes are over digested.

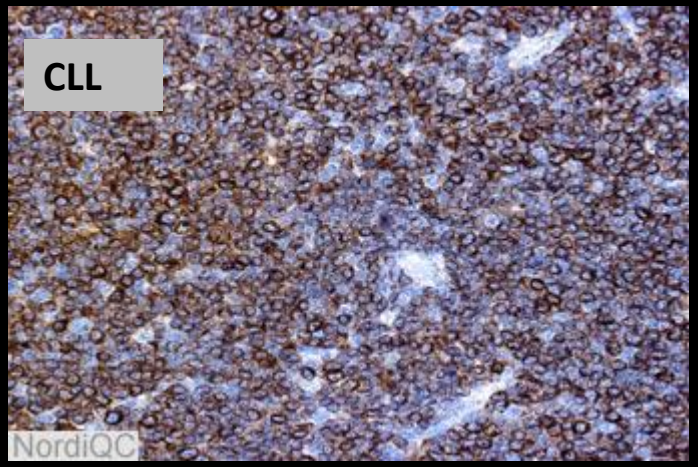
Optimal

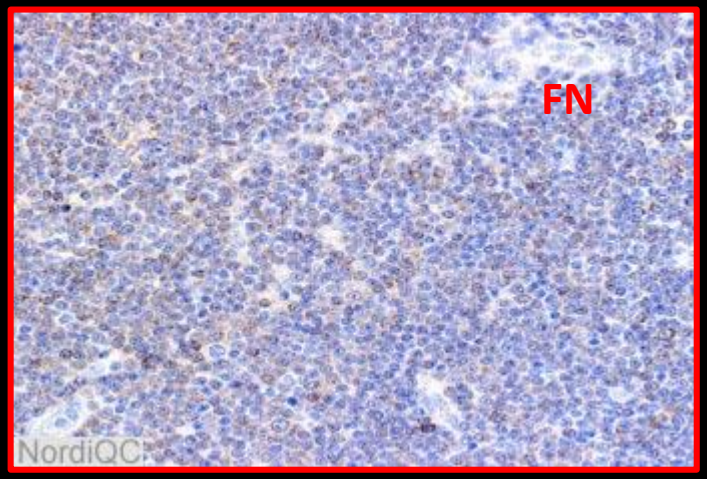
Insufficient

Kappa & Lambda Ig light chains









Optimal

Insufficient

Problem:

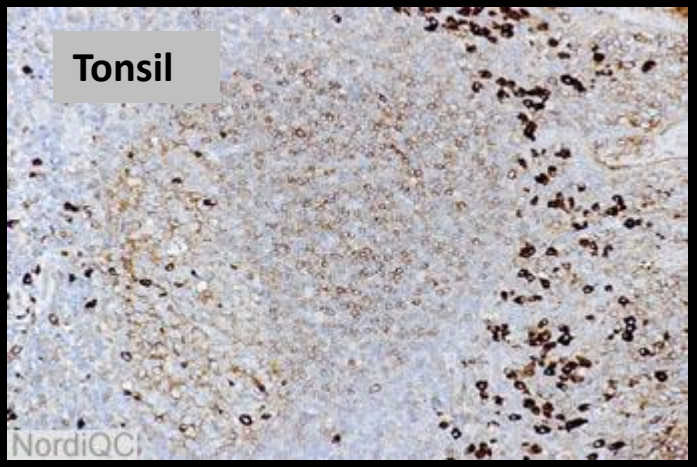
Too low conc. of the primary Ab

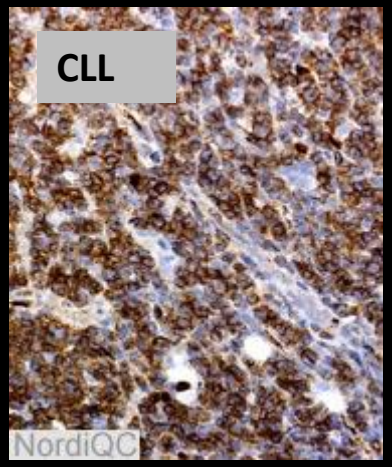
Only plasma cells are stained

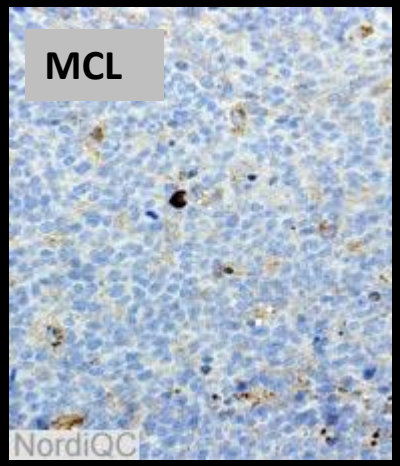
False negative staining for IgK of the CCL using the same protocol as above (right side)

Kappa & Lambda Ig light chains

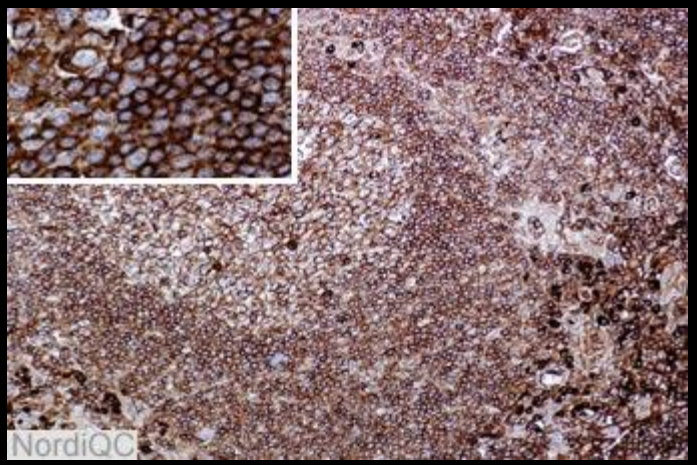
Optimal

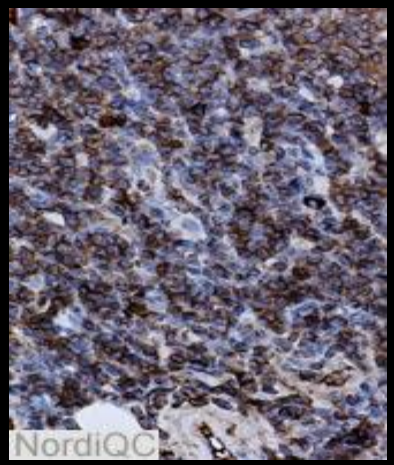


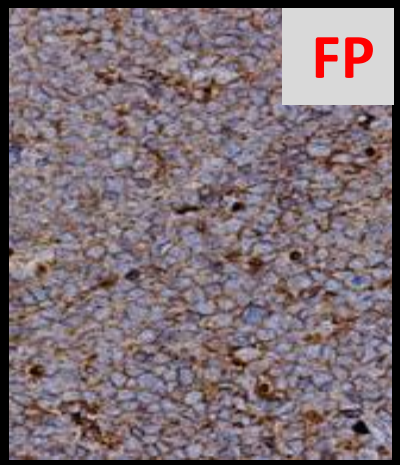




Insufficient







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:

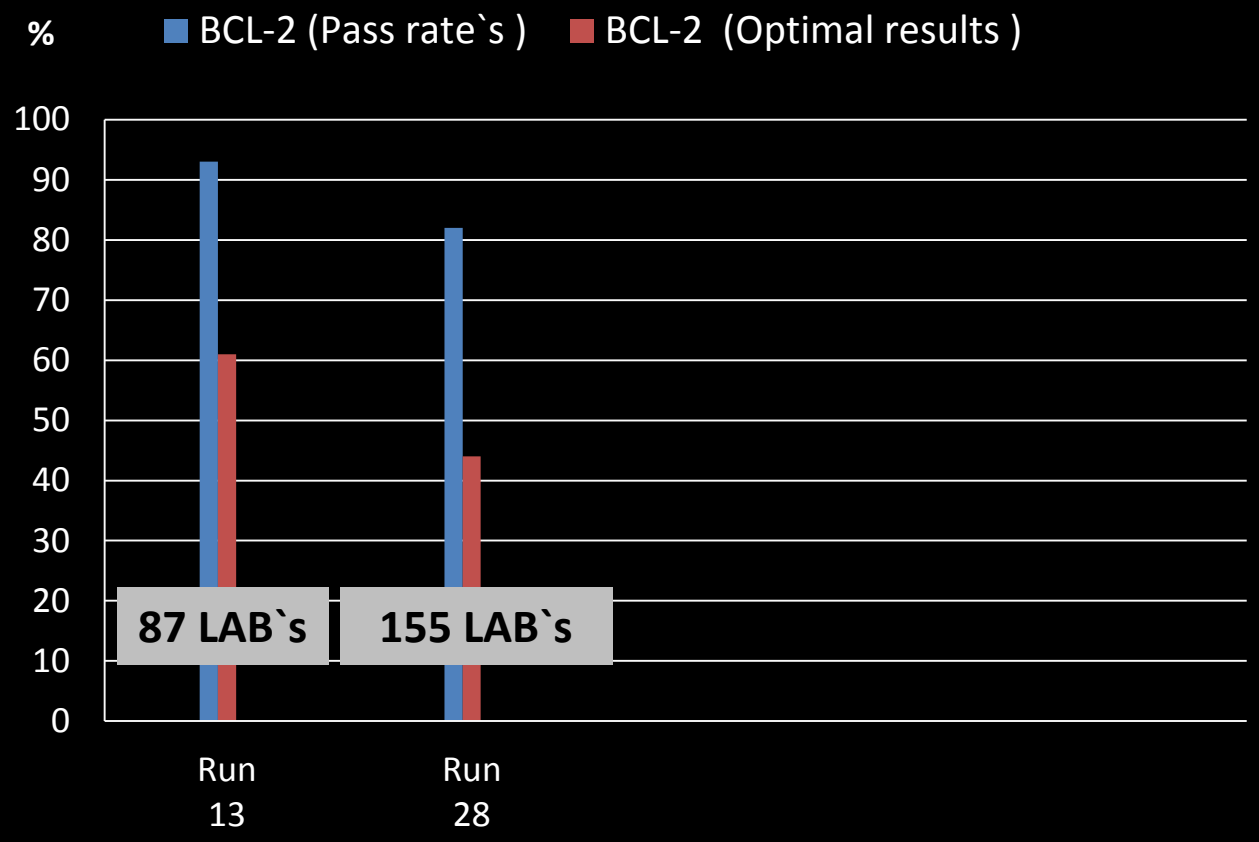
A moderate to strong, distinct membranous staining reaction of approximately half of the B-cells in the mantle zone of the follicles in the tonsil (Kappa or Lambda)

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

BCL-2



BCL-2/ Run 28 (2010):

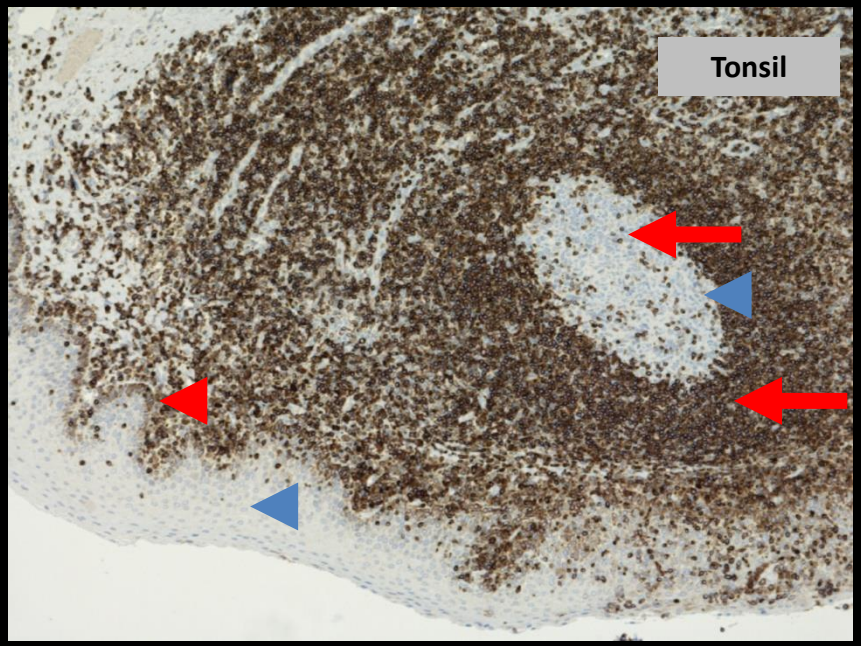
Sufficient: 82%

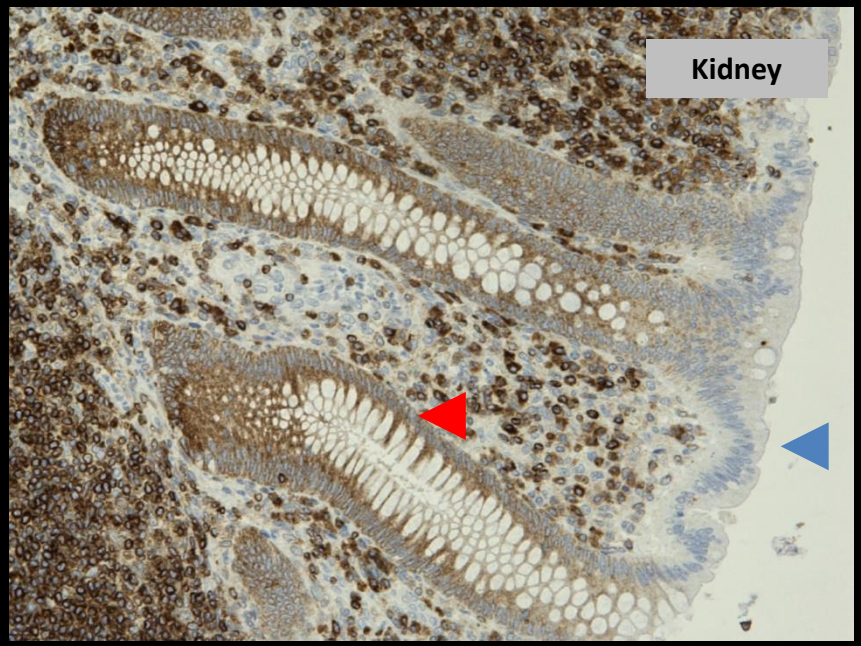
Optimal: 44%

Robust primary Abs:

mAb clone 124
mAb clone 100/D5
mAb clone 100
mAb clone BCL-2/100/D5

BCL-2





A strong, predominantly cytoplasmic staining of virtually all the mantle zone B- cells and of the T-cells (including intra germinal centre T-cells) in the tonsils and appendix.

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

No staining reaction in the germinal centre B-cells.

BCL-2/ Run 28 2010

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 in high or low pH buffers

Careful calibration of primary Ab

3-step detection systems

Insufficient results

Low concentration of the primary Ab

Platform dependent mAb clone 124

BCL-2/ Run 28 2010

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 clone (1:10 – 1:20), efficient HIER by Standard CC1, and UltraView + amplification as the detection system.

No optimal results were obtained when the clone was applied as a RTU format (Ventana/Cell Marque).

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.

BCL-2 (Ventana/Roche package inserts)

Ventana, rmAb SP66 (790-4604)

?

Ventana, mmAb 124 (790-4464) (760-4240 ?)

?

UltraView

OptiView

Recommendations for mmAb 124:

Low sensitive protocol settings

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT/BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-bcl-2 (124) with *ultra*View Universal DAB Detection Kit on a BenchMark XT/BenchMark ULTRA instrument

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[5]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are B cells of the mantle zone and interfollicular T cells found in tonsil.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1, Table 2, and Table 3 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-bcl-2 (SP66) with *ultra*View Universal DAB Detection Kit on a BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 24 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for anti-bcl-2 (SP66) with VIEW DAB Detection Kit on a BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Antibody (Primary)	BenchMark ULTRA instrument 32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 3. Recommended Staining Protocol for anti-bcl-2 (SP66) with OptiView DAB IHC Detection Kit on a BenchMark ULTRA instrument.

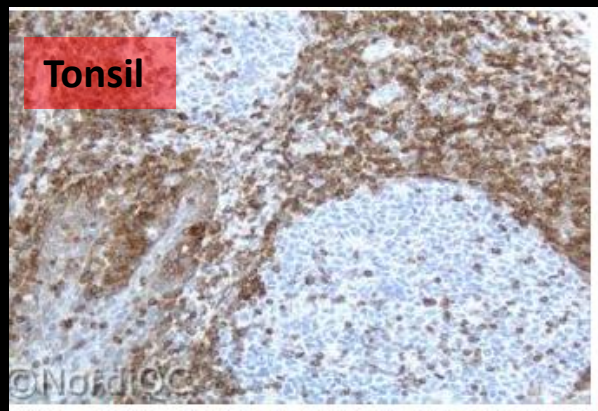
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 48 minutes
Pre Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	BenchMark ULTRA instrument 16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[7]

POSITIVE TISSUE CONTROL

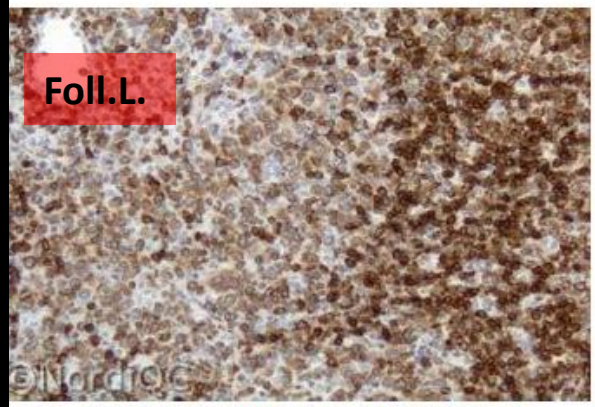
Examples of positive control tissues for this antibody are B cells of the mantle zone and interfollicular T cells in tonsil.

BCL-2/ Run 28 2010



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.

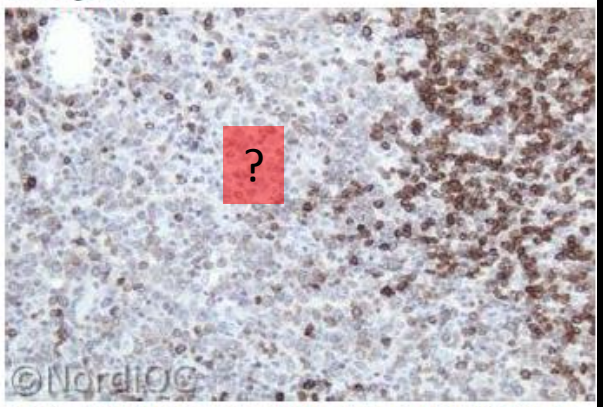


Fig. 3b. Insufficient Bcl-2 staining of the follicular lymphoma grade III using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. The normal lymphocytes show a moderate staining, while the neoplastic cells only show a weak, equivoca staining.

Problem:

Protocol with too low sensitivity

mAb clone 124

Too low conc of the primary Ab

BCL-2 / Run 28 2010

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:

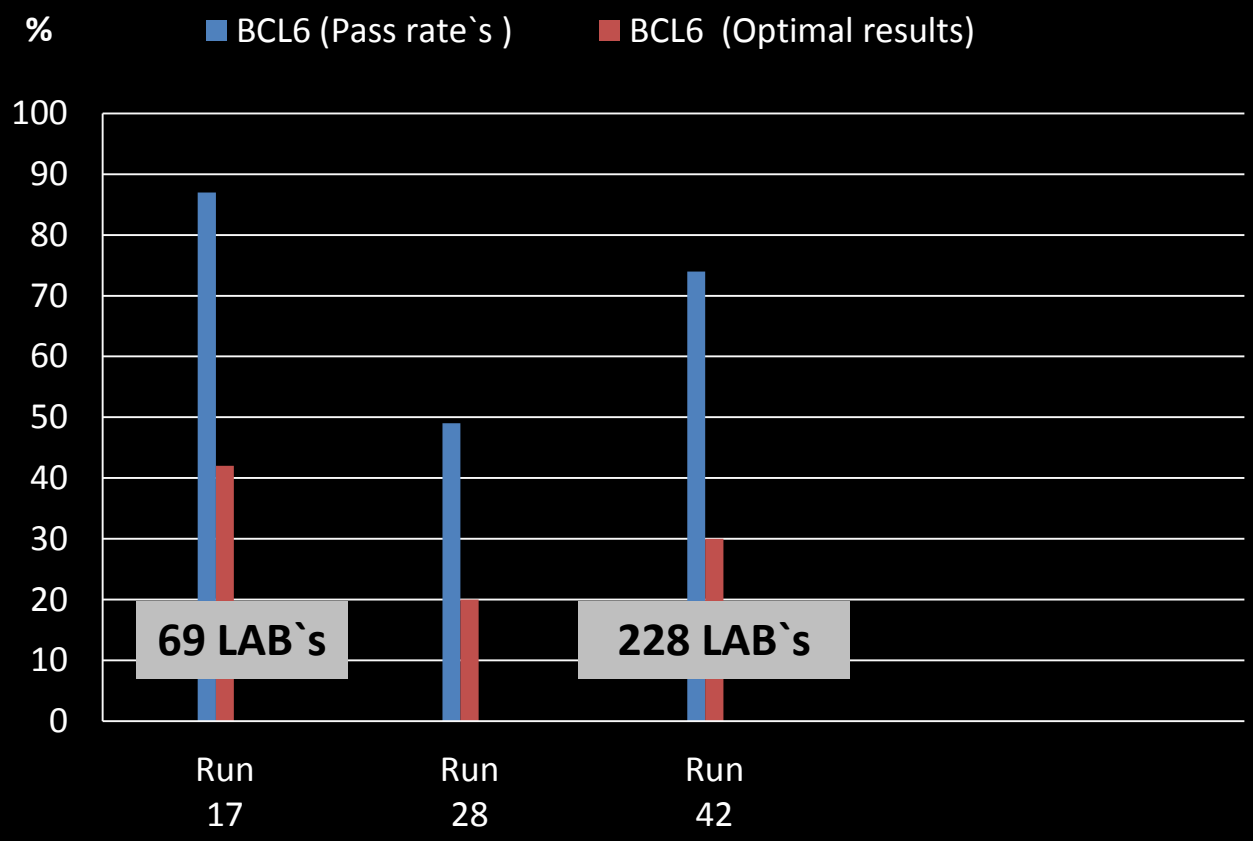
A moderate to strong predominantly cytoplasmic staining of virtually all the peripheral B- and T-cells in the tonsils.

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

No staining reaction in the germinal centre B-cells.

BCL6

Pass & Optimal score rate`s



BCL6 / Run 42:

Sufficient: 74%

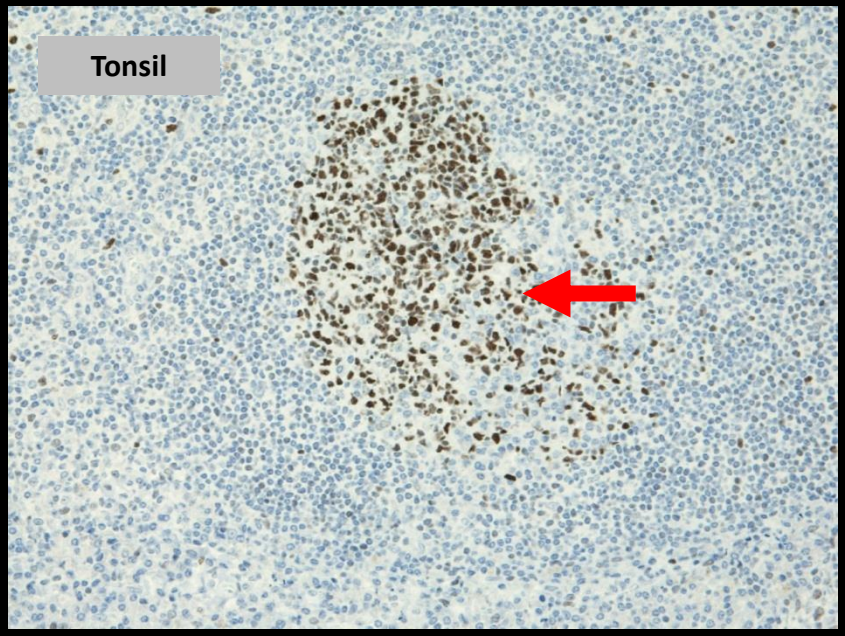
Optimal: 30%

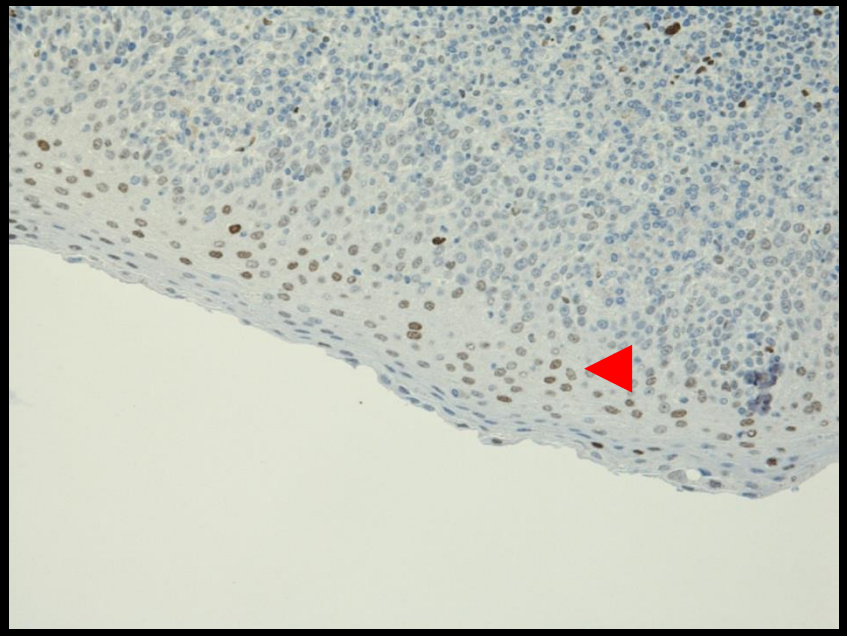
A challenging marker

Robust primary Abs:

mmAb clone LN22
mmAb clone GI191E/A8
"mmAb clone PG-B6p"

BCL-6





An at least weak to moderate distinct nuclear staining reaction of the majority of the squamous epithelial cells in the tonsil (arrow-head).

A moderate to strong distinct nuclear staining reaction of virtually all normal germinal centre B -cells in the tonsil (arrow).

BCL-6 / Run 42 2014

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

Concentrated antibodies	n	Vendor	Optim	40%	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		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		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.

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

Optimal protocol settings

HIER in CC1, dil. range 1:50-1:200

HIER in alkaline buffer, dil. range 1:25 -1:100

HIER in alkaline buffer, dil. range 1:10 -1:50

CC1 (30-90`), Ab Inc (12-52`), UV+/- amp. or OV+/-

BERS2 (20-30`), Ab Inc (15-30`), BOND Refine

TRS pH9 (10`), Ab Inc (20-30`), Flex+

TRS pH9 (30`), Ab Inc (12.5`), Flex+

Tendency:

1) HIER in High pH buffers
2) 3-step polymer/multimer system

Observations influencing the final 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)

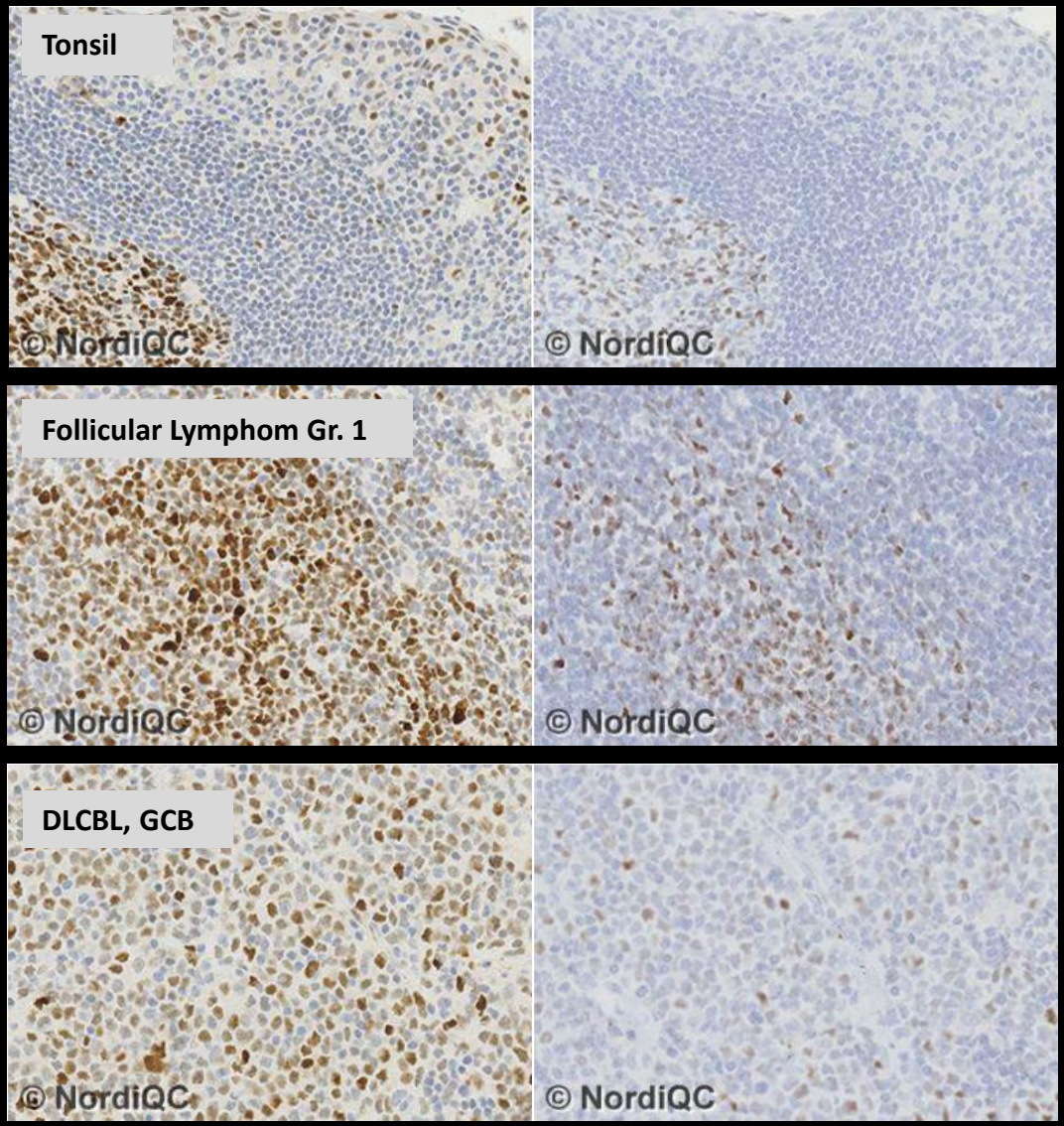
- Too low concentration of the primary Ab

BCL-6 / Run 42 2014

LN22
Optimally calibrated

HIER in alkaline buffer (BERS2)

3-step polymer system (BOND refine)



LN22
Too diluted

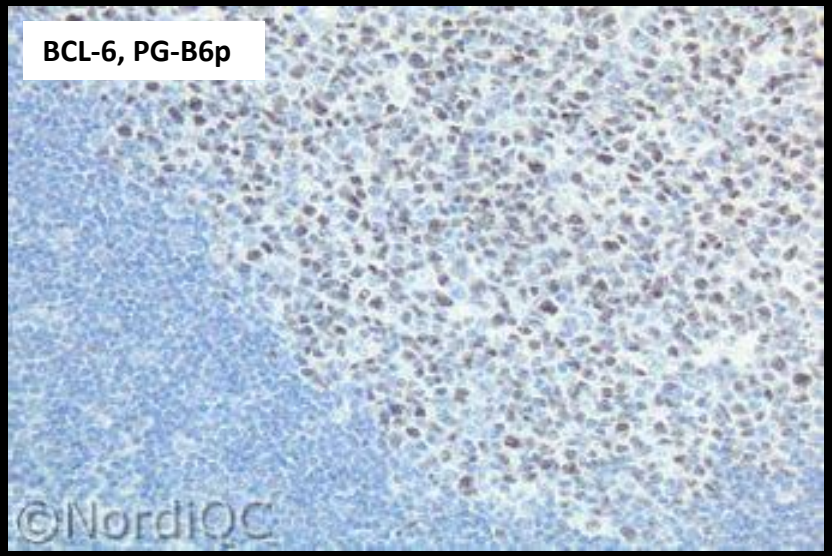
HIER in alkaline buffer (TRS pH9)

2-step polymer system (Flex)

BCL-6 (Run 28)

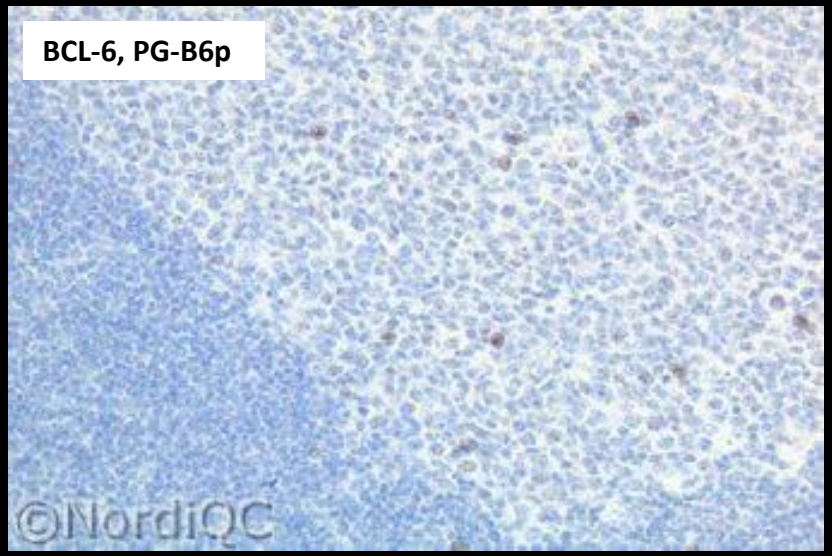
The mAb PG-B6p is sensitive to peroxidase blocking with H_2O_2

Tonsil



Endogenous peroxidase blocking was performed with a 0.03% H_2O_2solution for 10 min after HIER in an alkaline buffer.

Compare with Fig. on the right using same protocol settings except for peroxidase blocking performed with 3 % H_2O_2.



Peroxidase blocking was performed with 3 % H_2O_2

This effect was most marked when the blocking in 3% H_2O_2 was performed after HIER, but also seen if performed before HIER.

BCL-6 / Run 42 2014

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

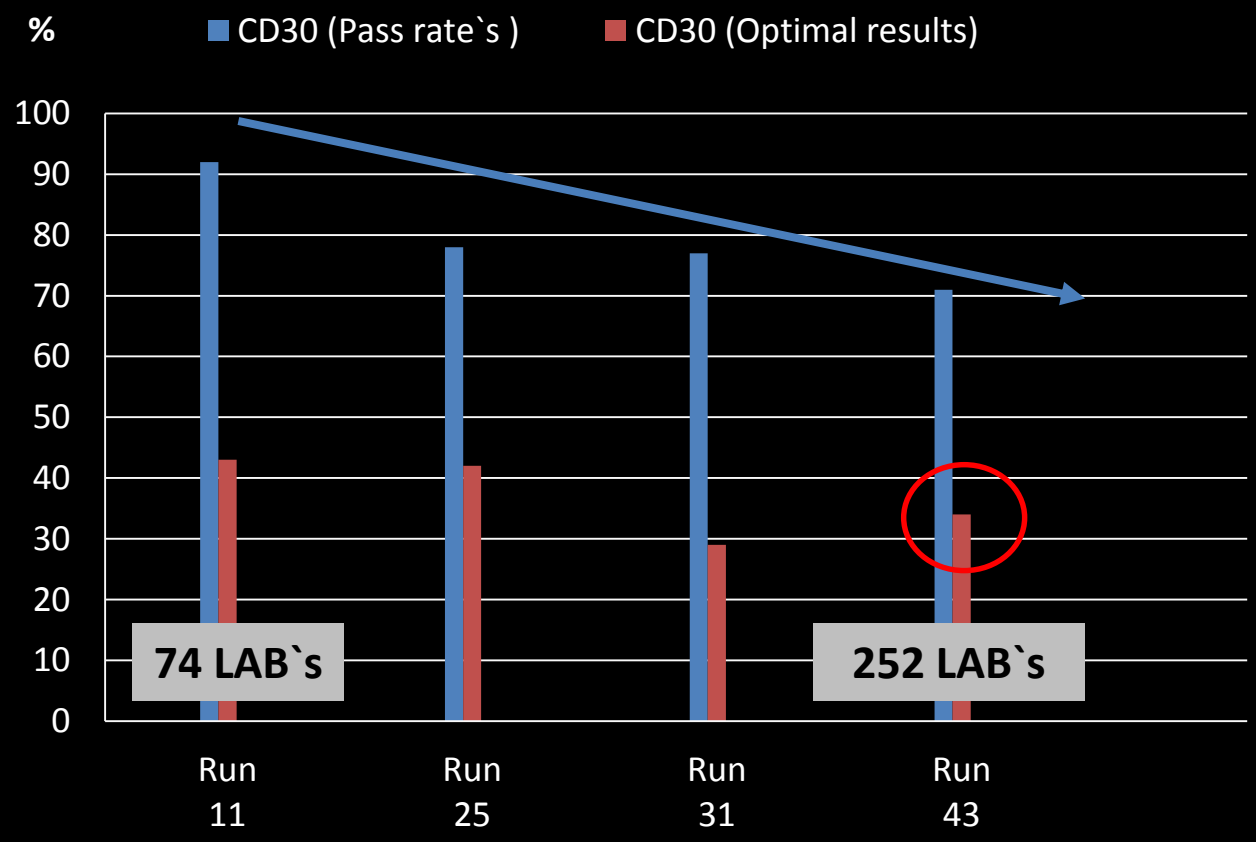
Control material / Tonsil:

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

CD30

Pass & Optimal score rate`s



CD30 / Run 43:

Sufficient: 71%

Optimal: 34%

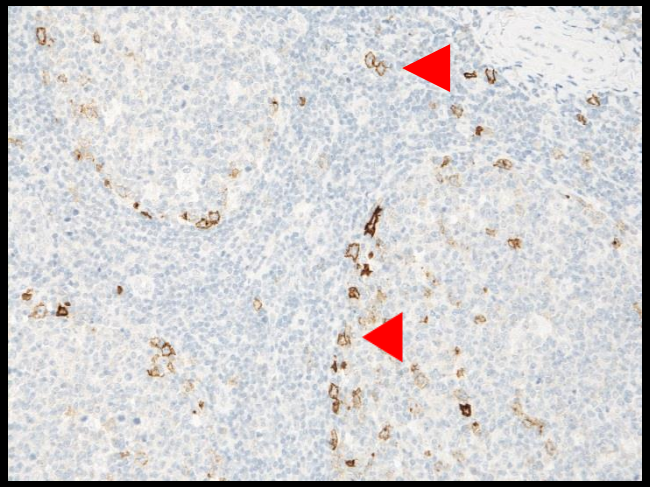
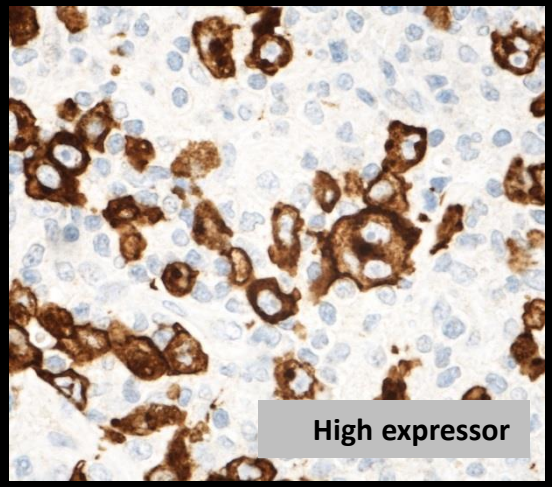
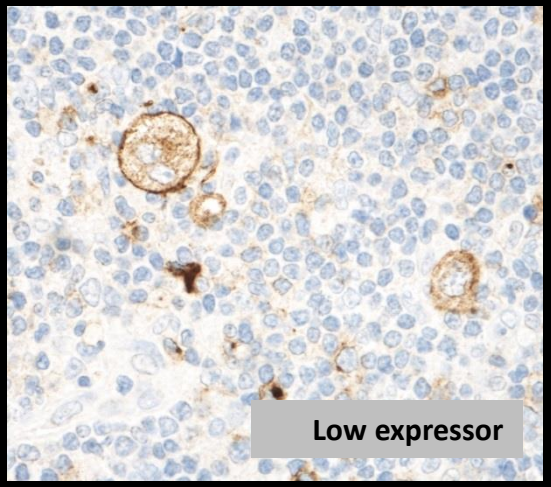
A challenging marker

Robust primary Abs:

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

mmAb clone CON6D/5
rmAb clone EP154

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.

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 ……

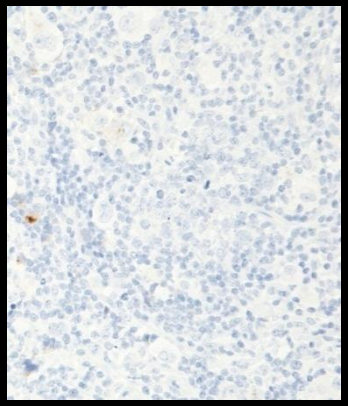
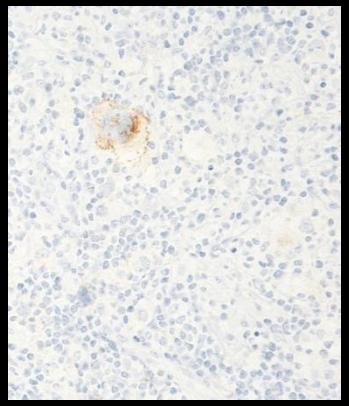
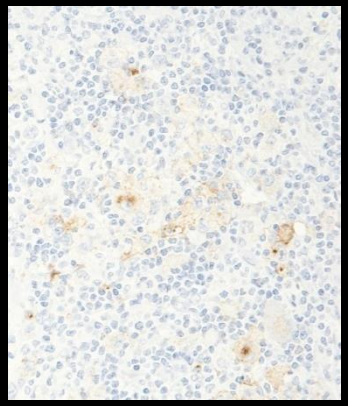
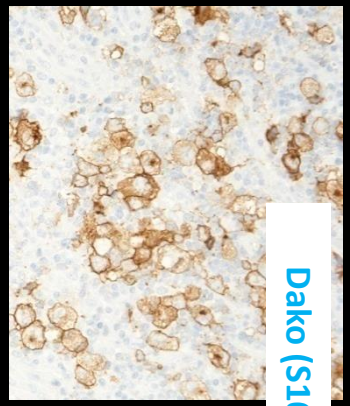
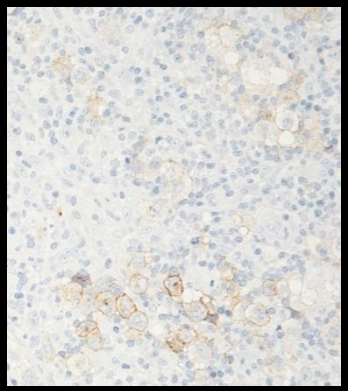
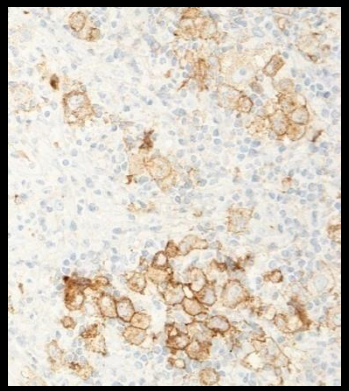
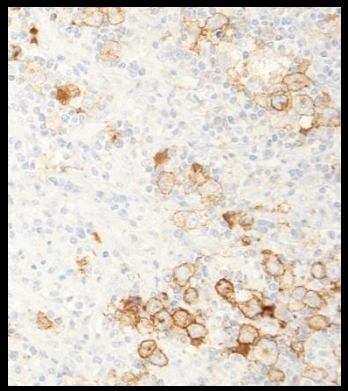
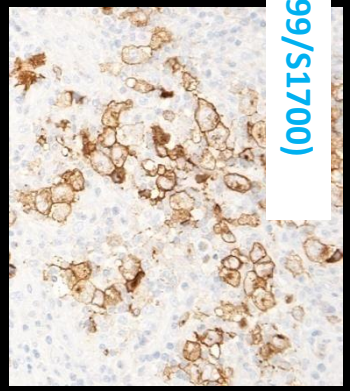
HIER buffer	Pass Rate`s (%)	Optimal (%)
Alkaline buffer as TRS pH9 or TRS pH9 (3-1), Dako	79 (22 of 28 protocols)	25
TRS pH6.1, Dako (modified low pH buffer)	80 (7 of 8 protocols)	75
mAb BER-H2 as concentrate (any dil. range) and Flex or Flex+ as the detection system:		

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

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

Important questions: 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)

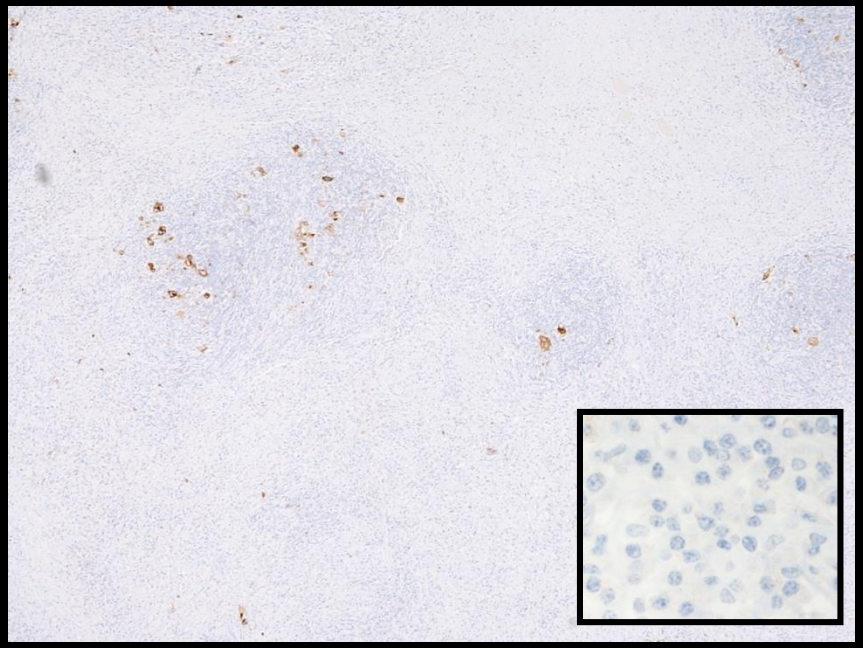
Hodgkin Lymphoma

HIER (modified low pH buffer)

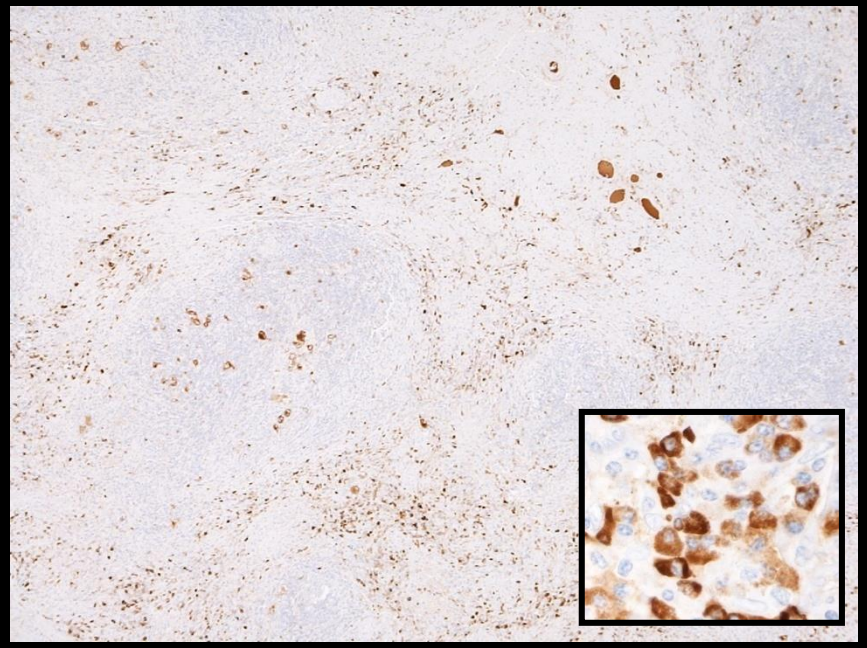
CD30

Hodgkin Lymphoma

Clone ConD6/B5



Clone Ber-H2



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

CD30 (Run 43 2015) : Influence of the chosen “RTU formats (Ventana)”

mAb 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

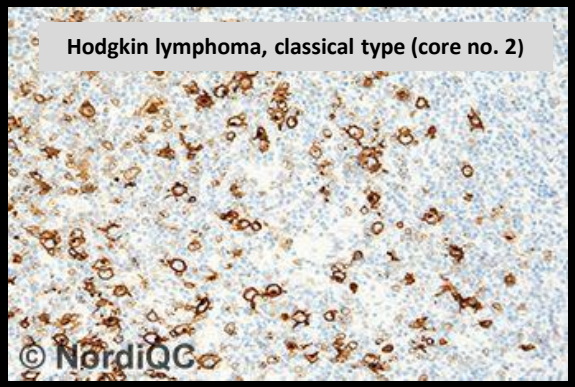
CD30 / Run 43 2015

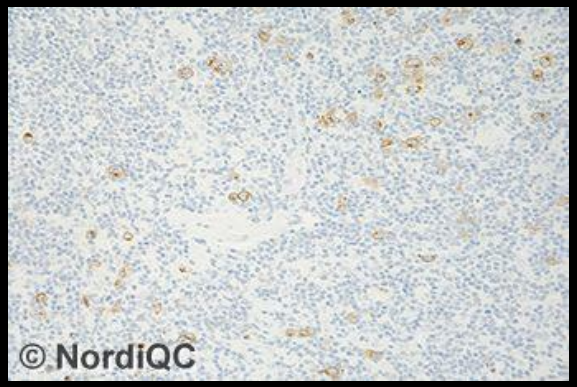
Optimal

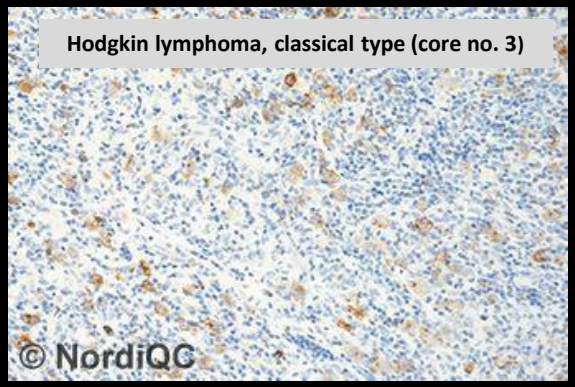
CON6D/5 (1:50)

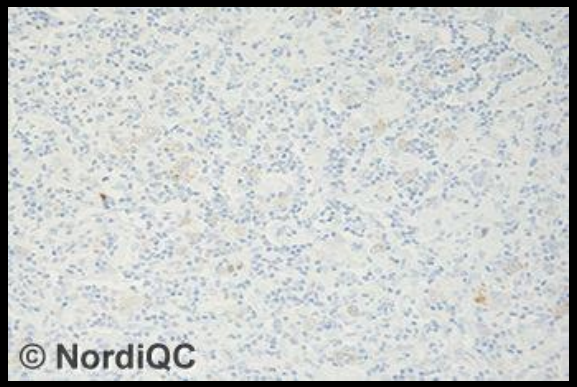
TRS pH6.1 buffer

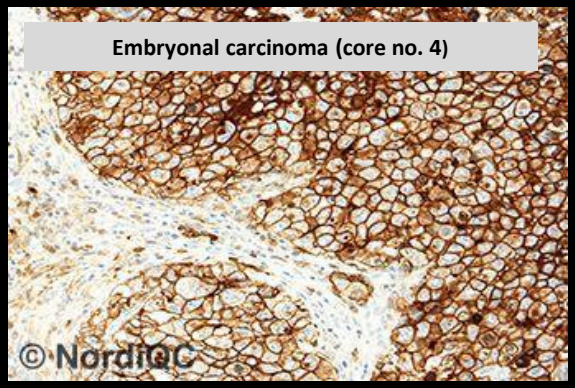
Flex+

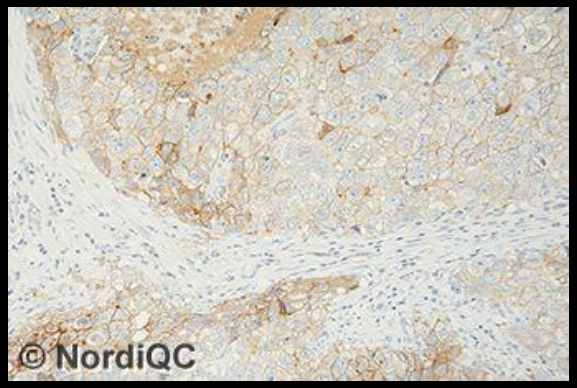












Insufficient

Ber-H2 (concentrate)
Too low concentration

Inefficient HIER
TE pH9 (too short time)

2-step polymer system (GTVsion)
Too low sensitivity

CD30 / Run 43 2015

Lymphoma panel: CD30
Optimal protocol settings (NQC)

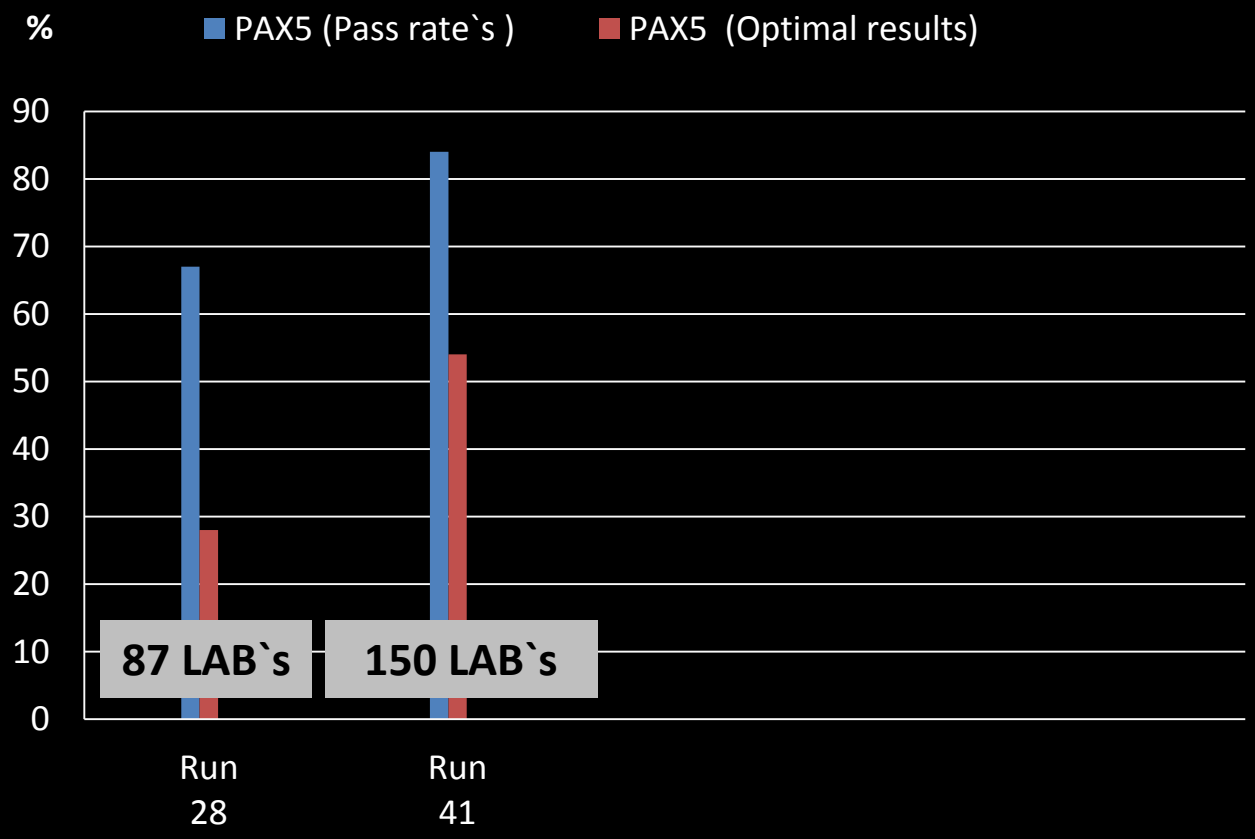
CD30	Retrieval buffers	Titre	Detection	RTU	Detection
mAb **BER-H2**	**HIER High pH & mod. Low pH**	**1:20-1:75**	**3-step**	**Dako (IS602/IR602)**	**Flex/ Flex+**
				Ventana (790-4858)	**UltraView + Amp OptiView .**
mAb clone **1G12**	**HIER High pH**	**1:10-1:25**	**3-step**	**Leica (PA0153)**	**BOND Refine**
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**	**1:50**	**3-step (Flex+)**		
rmAb clone **EP154:**	**HIER High pH**	**1:200**	**3-step (BOND refine)**		

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.

BSAP (PAX5)

Pass & Optimal score rate`s



PAX5 / Run 41:

Sufficient: 84%

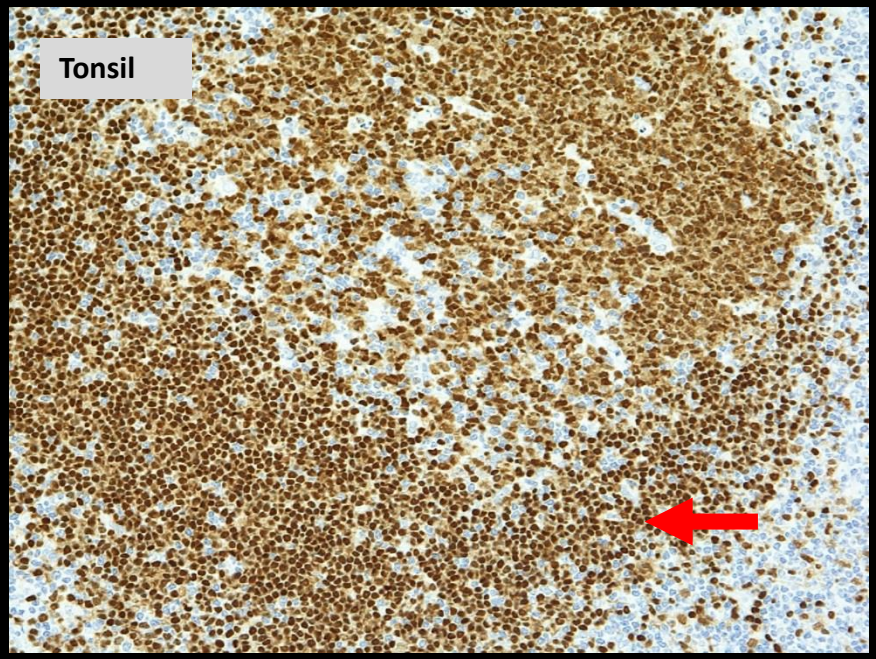
Optimal: 54%

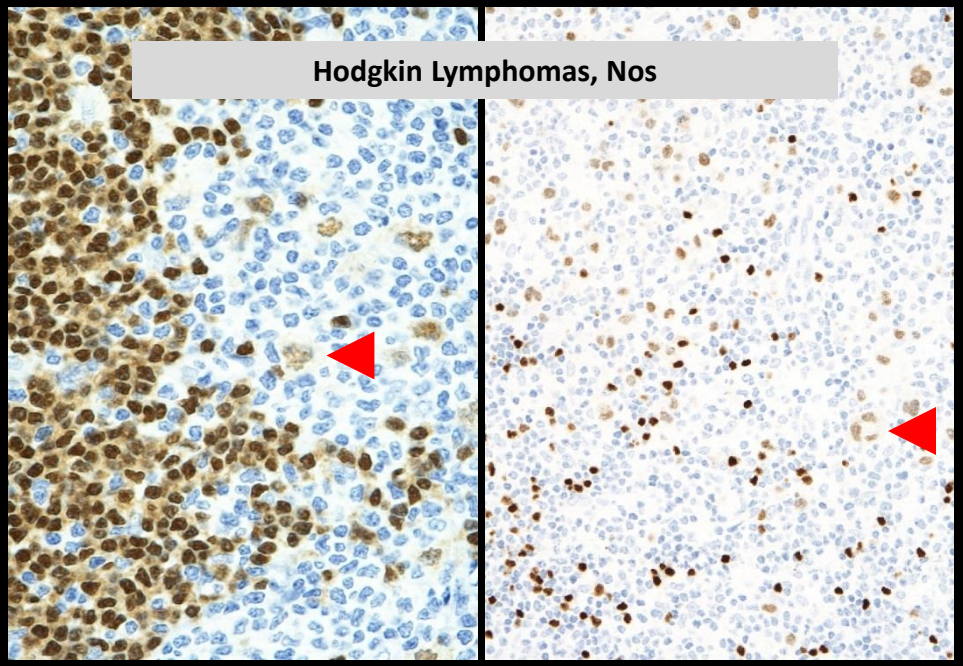
Robust primary Abs:

mmAb clone DAK-PAX5
mmAb clone 1EW
rmAb clone SP34

mmAb clone 24

PAX-5





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

<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.

Table 1. **Antibodies and assessment marks for BSAP, run 41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1EW**	9	Leica/Novocastra	4	3	2	0	78%	100%
mAb clone **24**	20	BD Biosciences	8	7	3	2	75%	89%
mAb clone **BC/24**	4	Biocare	1	2	1	0	-	-
mAb clone **DAK-Pax5**	23	Dako	11	8	4	0	83%	84%
rmAb clone **3852-1**	1	Abcam	1	0	0	0	-	-
rmAb clone **SP34**	9 4 2	Cell Marque Spring Biosciences Thermo/NeoMarkers	4	6	5	0	71%	86%
pAb **ILP46318**	1	Immunologic	0	1	0	0	-	-
pAb **RB-9406**	5	Thermo/NeoMarkers	0	2	3	0	-	-
pAb **RBK008**	1	Zytomed	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **1EW PA0552**	4	Leica/Novocastra	3	1	0	0	-	-
mAb clone **BC/24 PM207**	1	Biocare	0	1	0	0	-	-
mAb clone **DAK-Pax5 IS/IR650**	21	Dako	20	0	1	0	95%	95%
mAb clone **DAK-Pax5 GA650**	5	Dako	5	0	0	0	100%	100%
rmAb clone **BV6 RMPD027**	1	Diagnostic Biosystems	0	0	1	0	-	-
rmAb clone **SP34 790-4420**	37	Ventana	23	12	2	0	95%	94%
rmAb clone **SP34 312R-18**	1	Cell Marque	0	1	0	0	-	-
pAb **MAD-005661QD**	1	Master Diagnostica	1	0	0	0		
Total	150		81	45	22	2	-	
Proportion			54%	30%	15%	1%	84%	

25/26 protocols ~ optimal

HIER in alkaline buffers; dil. range 1:25-1:40

Best performance:

RTU format mAb 1EW (PA0552)

RTU format mAb DAK-Pax5 (IS/IR650 or GA650,Dako)

RTU format rmAb SP34 (790-4420, Ventana)

HIER in TRS pH9 or TRS pH 6.1 (10-30 ` at 97-99C), primary Ab Inc (20`), Flex/Flex+

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

In this run 44% (66 of 150) of the participants used a RTU system from one of these three vendors and grouped together a pass rate of 97% was obtained.

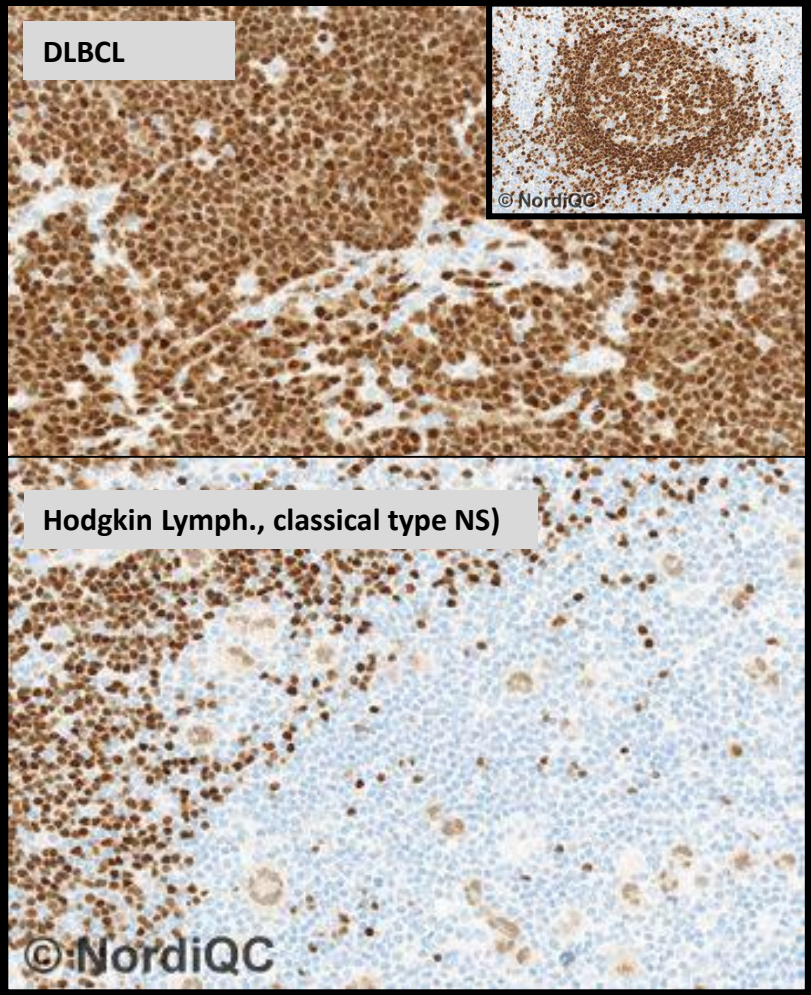
PAX5 (Run 41 2014) : Observations influencingon the final result

- The mAb clone 24 consistently gives inferior results on the Benchmark XT/ Ultra (Ventana) compared to the Autostainer Link/Classic (Dako)
  - Change to the rmAb SP34 / RTU system
- Performance of the mAb clone 1EW is affected by endogenous peroxidase blocking just prior to incubation of the primary Ab (NordiQC internal study and Leica / Abcam datasheet)
  - Blocking step must be performed after incubation of the primary Ab
- Contamination of the rmAb SP34 (concentrate from the vendors Cell Marque, Spring Bioscience and NeoMarkers/Thermo)
  - Most likely contaminated with CK20
  - Observed in 5/15 protocols (lot dependent ?)
  - Followed up by correspondence to the respective vendors by NordiQC.

PAX5 / Run 41 2014

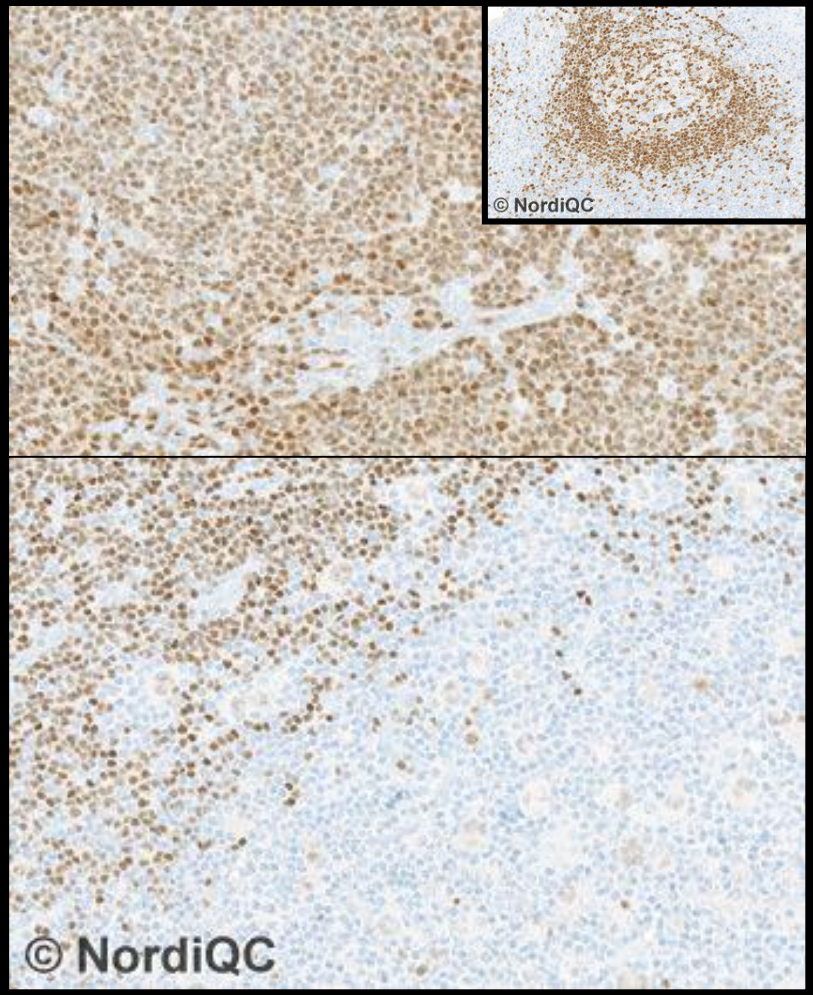
Optimal

rmAb SP34 , HIER CC1, pH 8.5 , OV (3-step multimer)



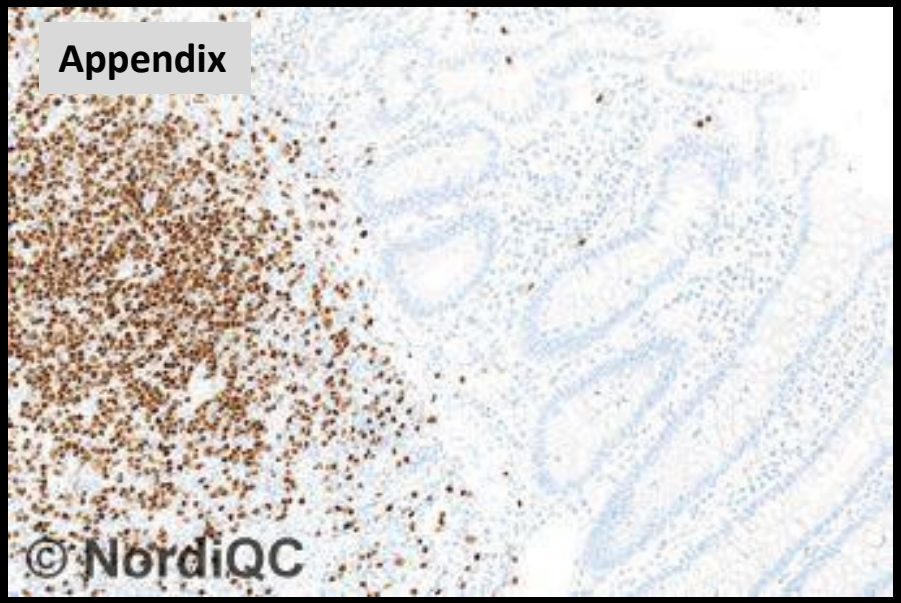
Insufficient

DAK-PAX5: (too low titre), UV (2-step multimer)

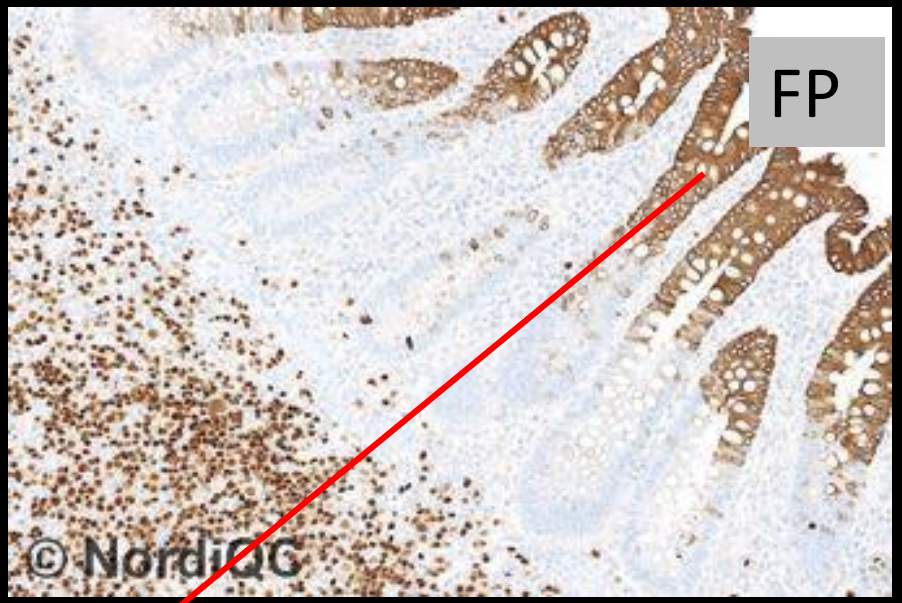


PAX5 / Run 41 2014

rmAb SP34 / Optimal



rmAb SP34 / Insufficient



Aberrant BSAP staining of the appendix
(most likely contamination with CK20)

PAX5 / Run 41 2014

Lymphoma panel: PAX5
Optimal protocol settings (NQC)

PAX5	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **DAK-PAX5**	**HIER High pH, mod. & standard low pH**	**1:10-1:150**	**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**	**1:25-1:40**	**2 & 3-step**	**Leica (PA0552)**	**BOND Refine**
mmAb **24**	**HIER High pH & standard low pH**	**1:10-1:100**	**2 & 3-step**	**-**	**-**

Control material / Tonsil or Appendix:

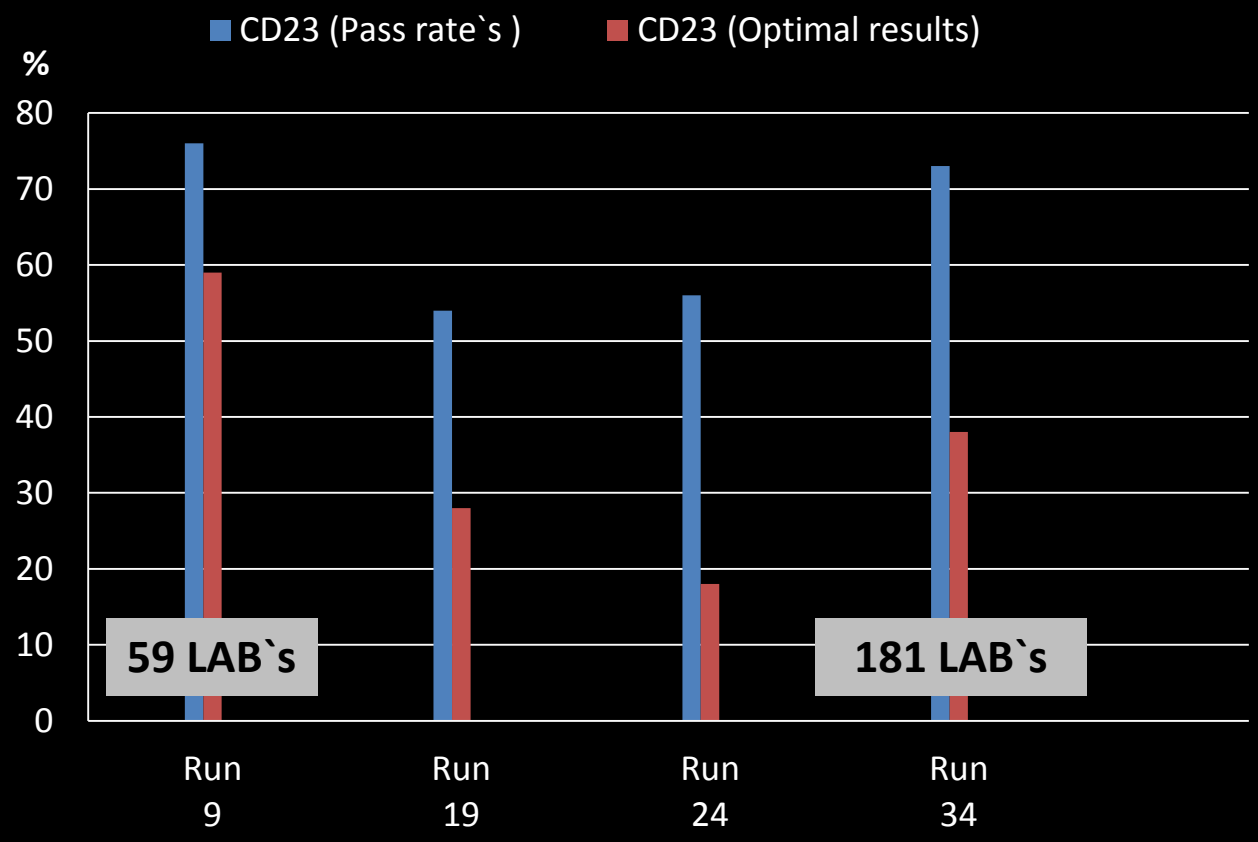
A distinct moderate to strong nuclear staining reaction of virtually all mantle zone B-cells, germinal centre B-cells and interfollicular peripheral B-cells in the tonsils and appendix.

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 ?

CD23

Pass & Optimal score rate`s



CD23 / Run 34:

Sufficient: 73%

Optimal: 38%

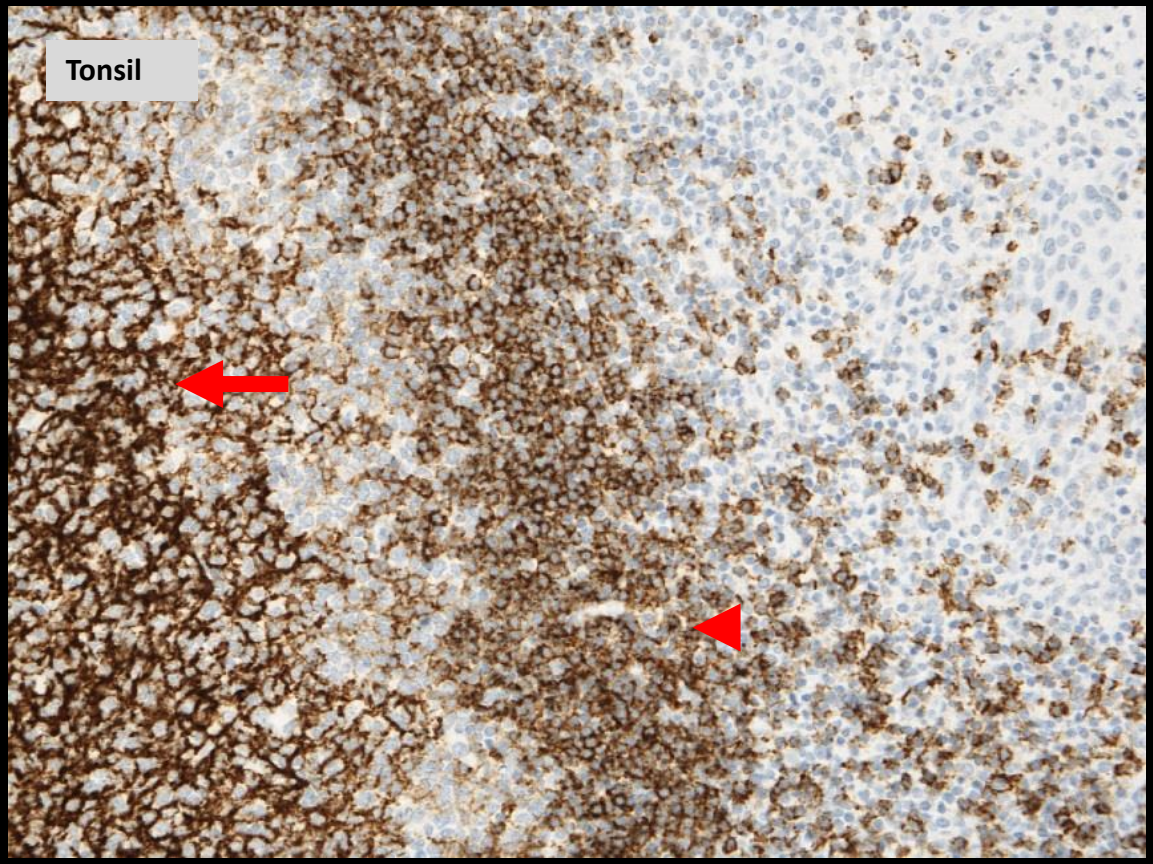
A challenging marker

Robust primary Abs:

mmAb clone 1B12
rmAb clone SP23

mmAb clone DAK-CD23

CD23



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

A strong, distinct staining of the follicular dendritic cells in the germinal centres in the tonsil.

No reaction in other cells

CD23 / Run 34 2012

Table 1. Abs and assessment marks for CD23, run 34

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B12**	61 6 5 1 1	Leica/Novocastra Thermo/NeoMarkers Monosan Biocare Cell Marque	21	28	24	1	66 %	86 %
mAb clone **DAK-CD23**	1	Dako	1	0	0	0	-	-
mAb clone **MHM6***	8	Dako	0	1	5	2	12 %	
rmAb clone **EP73**	1	Epitomics	0	1	0	0	-	-
rmAb **SP23**	19 8 2 1 1	Thermo/NeoMarkers Dako* Spring Bioscience DBS Master Diagnostics	16	9	6	0	81 %	85 %
Ready-To-Use Abs								
mAb clone **1B12 PA0169**	6	Leica	5	1	0	0	100 %	100 %
mAb clone **1B12 PM100**	1	Biocare	0	0	1	0	-	-
mAb clone **1B12 MONX10379**	1	Monosan	0					
mAb clone **1B12 MS-729-R7**	1	Thermo/NeoMarkers	0	0	1	0	-	-
mAb clone **DAK-CD23 IS/IR781**	3	Dako	2	0	1	0	-	-
rmAb clone **SP23 790-4408**	29	Ventana	13	13	3	0	90 %	95 %
rmAb clone **SP23 IR800***	22	Dako	11	9	1	1	91 %	100 %
rmAb clone **SP23 123R-17**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **SP23 760-2616***	1	Ventana/Cell Marque	0	1	0	0	-	-
rmAb clone **SP23 RMA-0504**	1	Maixin	0	0	0	1	-	-
Total	181		69	63	44	5	-	
Proportion			38 %	35 %	24 %	3 %	73 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Product has been discontinued by the vendor

80%

Optimal protocol settings results

HIER in High pH or mod low pH, dil . 1:20-1:100

Product has been discontinued by the vendor

HIER in BERS2 pH 9, Inc. time 10-25 min, BOND Refine (DS9800)

HIER CC1; primary Ab (8-44`); iView, UltraView+/- amp or OptiView

Optimal results could be obtained with the mAb clones 1B12, DAK-CD23 and the rmAB clone SP23

Best performance:

RTU clone 1B12 (PA0169, Leica)

RTU format SP23 (IR800,Dako*)

RTU format SP23 (790-4408, Ventana)

* Product has been discontinued by the vendor

CD23(Run 34 2012) : Observations with impact on the final result

- **Less successful primary Ab**
  - **CD23 clone MHM6 : Run 19, 24 & 34 ~ only 2 / 24 protocols were assessed as sufficient (none were optimal)**

- **Less successful performance of the mAb clone 1B12 on the BenchMark IHC platform, Ventana**
  - **Only 6 out of 19 (32 %) protocols were assessed as sufficient, none were optimal**

CD23 (Run 34 2012) : Observations influencing the final result

- Use of detection systems with a too low sensitivity

LD assay (mmAb clone 1B12) Optimal dil. Range (1:20- 1:100)	Detection system	Pass Rate`s (%)	Optimal (%)
2-step polymer/multimer system	e.g. Flex (Dako) or UltraView (Ventana)	69 (18 of 26)	12 (3 of 26)
3-step polymer/multimer system	e.g Flex+ (Dako), UltraView + amp (Ventana) or BOND Refine (Leica)	87 (20 of 23)	65 (15 of 23)

LD assay (rmAb clone SP23) Optimal dil. Range (1:20-1:100)	Detection system	Pass Rate`s (%)	Optimal (%)
2-step polymer/multimer system	e.g. Flex (Dako) or UltraView (Ventana)	74	57
3-step polymer/multimer system	e.g Flex+ (Dako), UltraView + amp (Ventana) or BOND Refine (Leica)	100	80

It is highly recommended to use a 3-step polymer/multimer system for optimal staining of CD23

CD23 (Run 34)

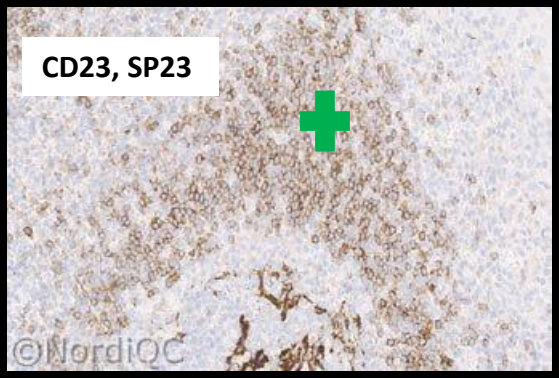
Optimal result

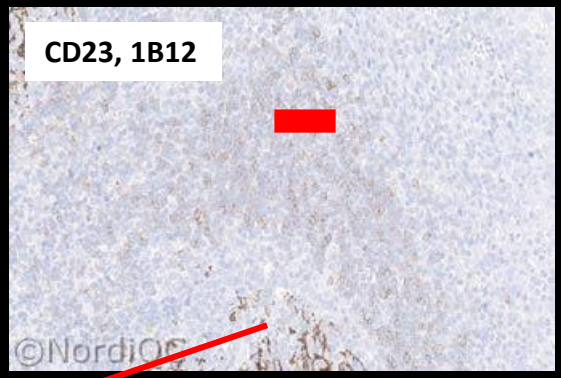
Protocol optimal calibrated, HIER in an alkaline buffer and a 3-step multimer based detection system.

Insufficient result

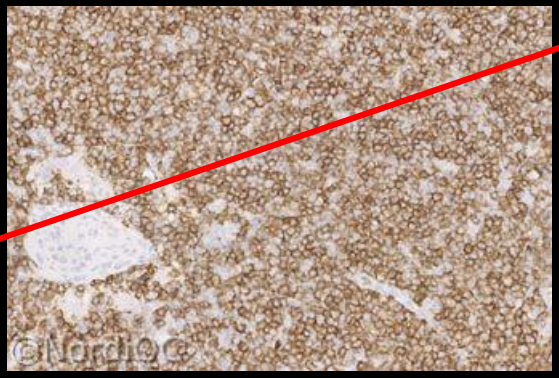
Protocol with too low sensitivity (too low. conc. of the primary Ab and a 2-step multimer conjugate)

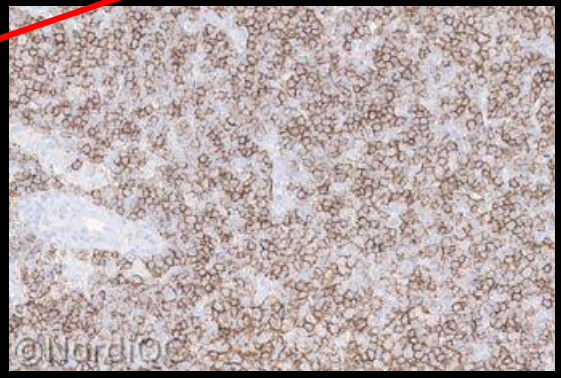
Tonsil





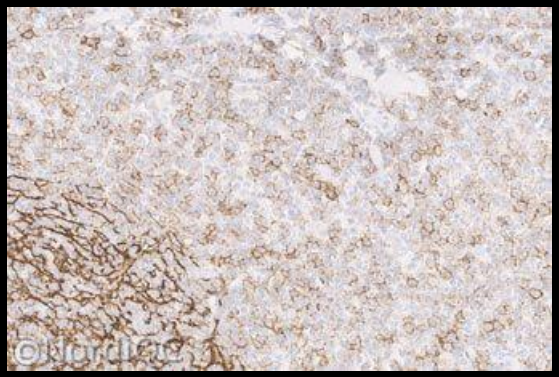
B-CLL (no. 4)

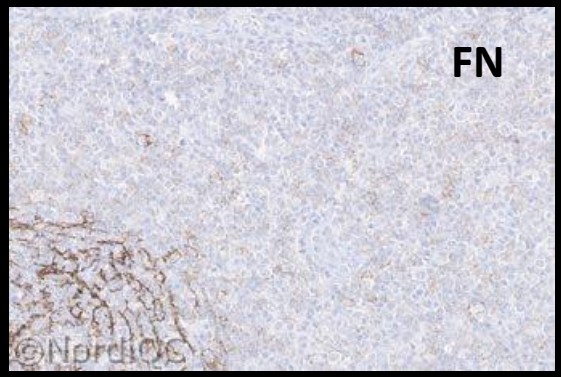




Only FDC`s are positive

B-CLL (no.5)





CD23 / Run 34 2012

Lymphoma panel: CD23
Optimal protocol settings (NQC)

CD23	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb 1B12	HIER High pH or mod. Low pH	1:20-1:100	3-step	Leica (PA0169)	BOND refine
rmAb SP23	HIER High pH or standard Low pH	1:20-1:100	3-step	Ventana (790-4408)	UltraView +/- Amp* OptiView
				Dako (IR800)	Discontinued
DAK-CD23	HIER High pH or mod. Low pH	1:200	3-step	Dako (IS/IR781)	Flex+

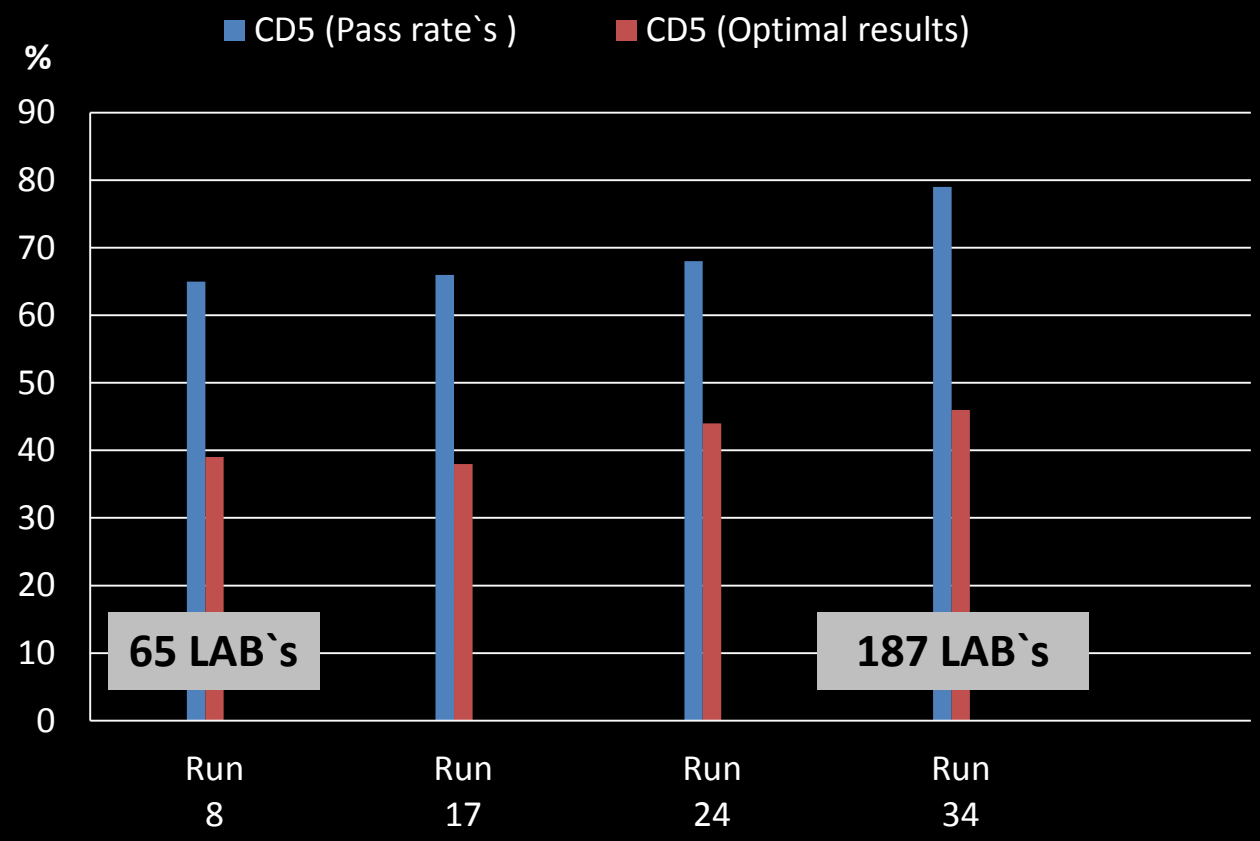
* Optimal results could also be obtained with the detection system UltraView without amplification but at overall lower frequency compared to laboratories using UltraView with amplification

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.

CD5

Pass & Optimal score rate`s



CD5 / Run 34:

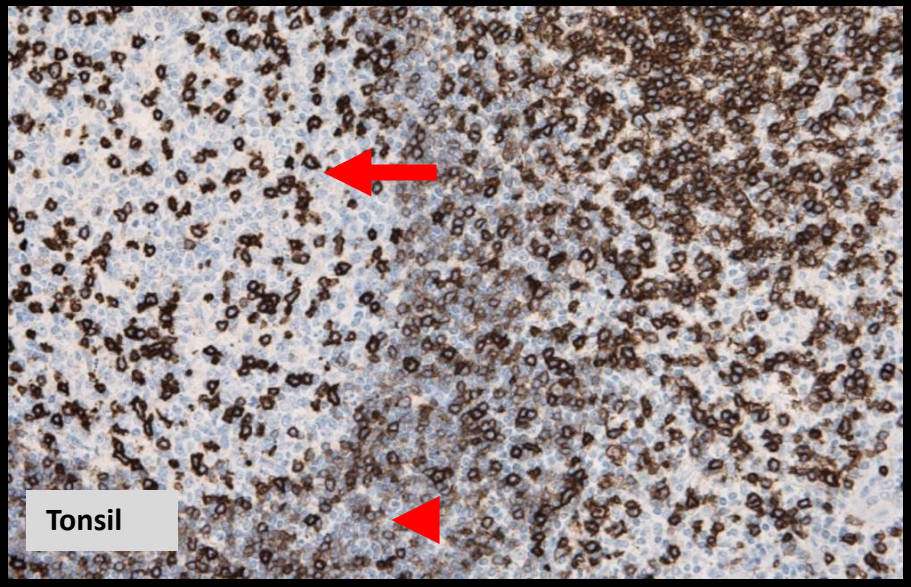
Sufficient: 79%

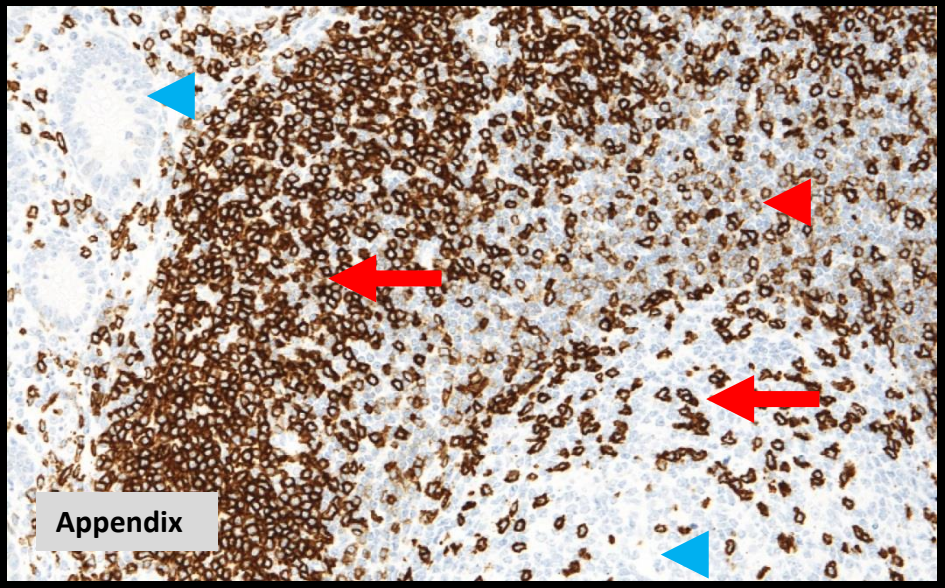
Optimal: 46%

Robust primary Abs:

mmAb clone 4C7
rmAb clone SP19

CD5





A strong and distinct, predominantly membranous staining reaction of virtually all the T-cells in both the T-zones and within the germinal centres in the tonsils.

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.

No reaction in other cells

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone 4C7	62 9 6 5 2	Leica/Novocastra Dako Thermo/NeoMarkers Monosan Biocare	35	31	16	2	79 %	83 %
mAb cloneCD5/54/F6	5	Dako*	0	0	1	4	0 %	-
rmAb clone A25-G	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone EP77	1	Epitomics	0	1	0	0	-	-
rmAb clone RBT-CD5	1	Bio SB	0	1	0	0	-	-
rmAb SP19	14 3 2 1 1 1	Thermo/NeoMarkers Spring Bioscience Dako* Cell Marque Zeta Corporation Zytomed	7	9	5	1	73 %	77 %
pAb E2474	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use Abs				43 %				
mAb clone 4C7 IS/IR082	14	Dako	6	6	2	0	86 %	90 %
mAb clone 4C7 PA0168	6	Leica	4	2	0	0	100 %	100 %
mAb clone 4C7 PM099	1	Biocare	0	80 %	0	0	-	-
mAb clone 4C7 CD5-4C7-R-7	2	Novocastra	0	0				
mAb clone 4C7 MS-393-R7	1	Thermo/NeoMarkers	0	79 %		0	-	-
rmAb clone SP19 790-4451	33	Ventana	26	6	1	0	97 %	97 %
rmAb clone SP19 IS/IR081	10	Dako	7	1	1	1	80 %	100 %
rmAb clone SP19 760-4280	3	Ventana/Cell Marque*	1	1	1	0	-	-
rmAb clone SP19 205R-17	1	Cell Marque	1	0	0	0	-	-
rmAb clone SP19 RMA-0593	1	Maixin	0	0	0	1	-	-
Total	187		87	61	30	9	-	
Proportion			46 %	33 %	16 %	5 %	79 %	

NordiQC

Optimal protocol settings

CD5, mmAb 4C7

HIER in High pH or mod low pH, dil . 1:50-1:200

CD5, rmAb SP19

HIER in High pH, dil . 1:25-1:100

HIER in TRS pH9; Flex

HIER in BERS2; BOND refine

HIER CC1; iView, UltraView +/- amp or OptiView

All protocols were modified compared to recommended protocol settings giving by the vendor, typically prolonging primary Ab inc. & use of a 3-step multimer detection system

Best performance:

RTU CD5, 4C7 (PA0168,Leica)

RTU CD5, SP19 (790-4451, Ventana)

CD5 (Run 34 2012) : Observations influencing the final result

❑ Use of detection systems with a too low sensitivity

LD assay (mmAb clone 4C7 & rmAb SP19)	Detection system	Pass Rate`s (%)	Optimal (%)
2-step polymer/multimer system	e.g. Flex (Dako) or UltraView (Ventana)	67 (51 of 76)	24 (18 of 76
3-step polymer/multimer system	e.g Flex+ (Dako), UltraView + amp (Ventana) or BOND Refine (Leica)	89 (33 of 37)	60 (22 of 37)

❑ Unsuccessful performance using the mmAb CD5/54/F6

- In this run, all 5 protocols were assessed insufficient
- In the last 3 runs, only 1 out of 33 (3 %) stainings were assessed as sufficient (assessed as good)

CD5 / Run 34 2012

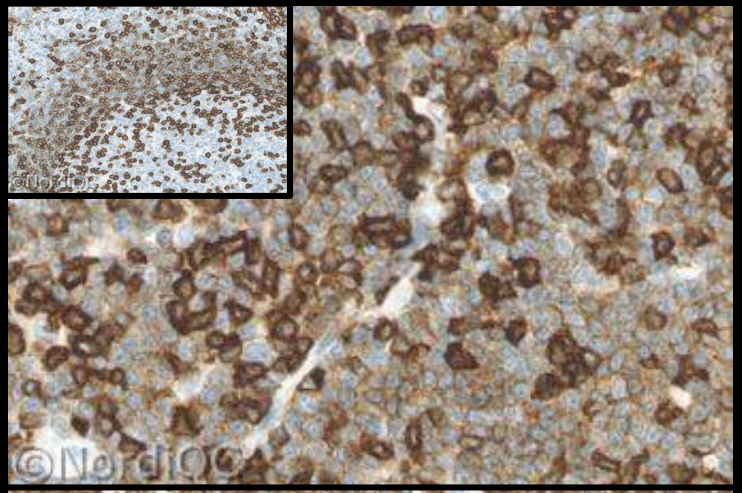
Optimal

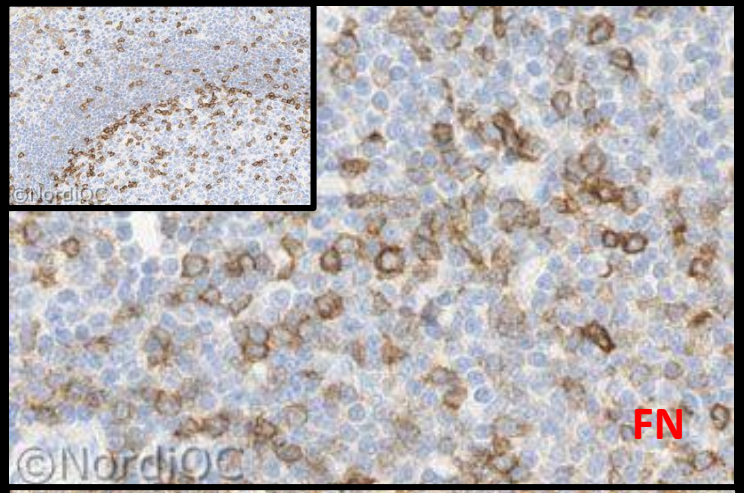
rmAb SP19 , HIER CC1, pH 8.5 3-step multimer system

Insufficient

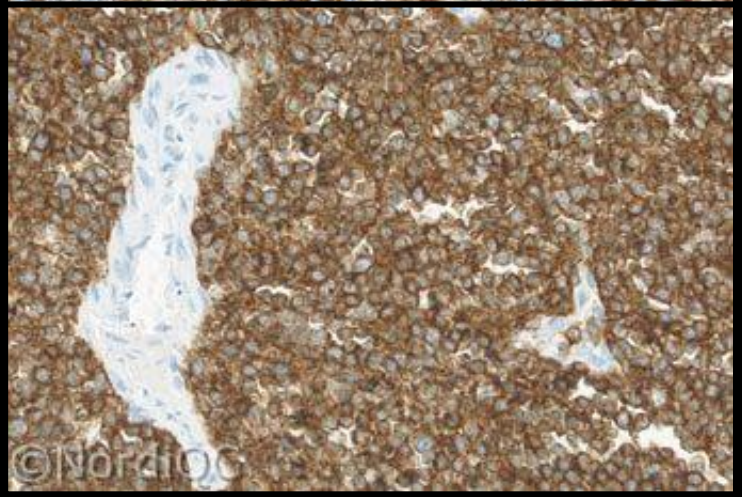
mmAb 4C7 (too low titre), 2-step polymer system

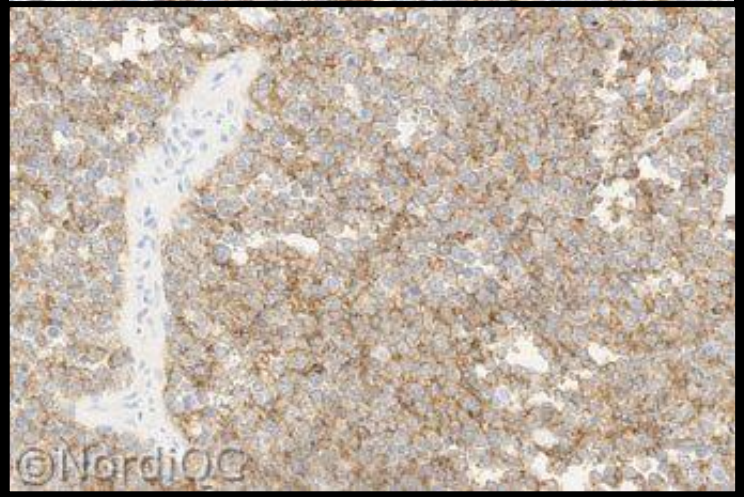
B-CLL





Mantle cell lymphoma

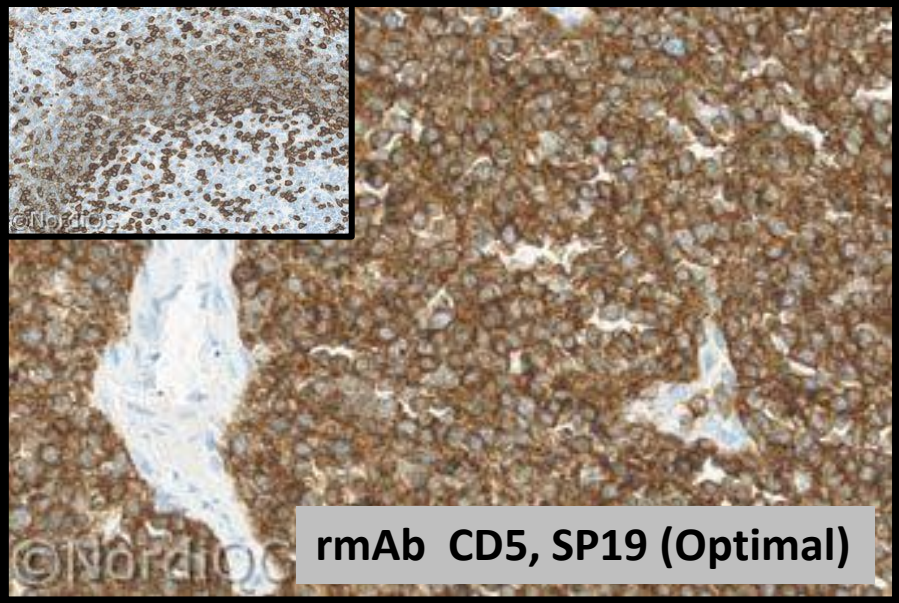


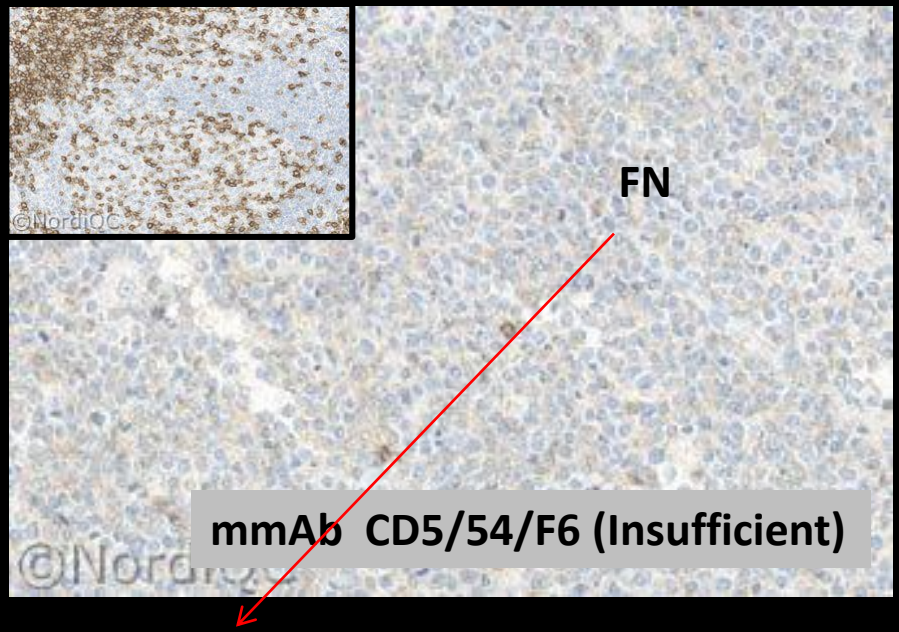


CD5 / Run 34 2012

Unsuccessful primary Ab mm CD5/54/F6

Mantle cell lymphoma





mmAb CD5/54/F6: Despite a high titre of the primary Ab, efficient HIER in an alkaline buffer and a 3-step polymer conjugate is used, only the T-cells are demonstrated. No staining reaction is seen in the mantle zone B-cells /Tonsil (insert).

CD5 / Run 34 2012

Lymphoma panel: CD5
Optimal protocol settings (NQC)

CD5	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb 4C7	HIER High pH or mod. Low pH	1:50-1:200	3-step	Leica (PA0168)	BOND refine
				Dako (IS/IR082)	Flex
rmAb SP19	HIER High pH	1:25-1:100	3-step	Ventana (790-4451)	iView UltraView +/- Amp* OptiView

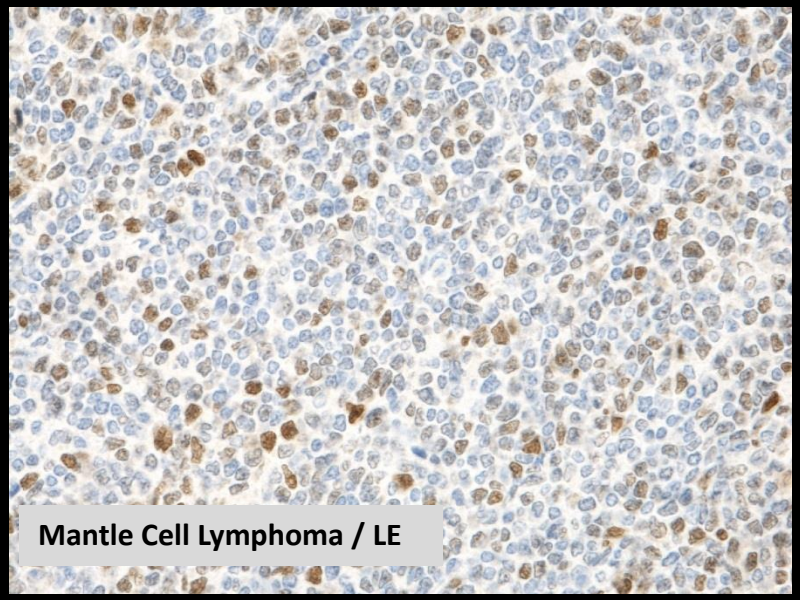
* Optimal results could also be obtained with the detection system UltraView without amplification but at overall lower frequency compared to laboratories using UltraView with amplification

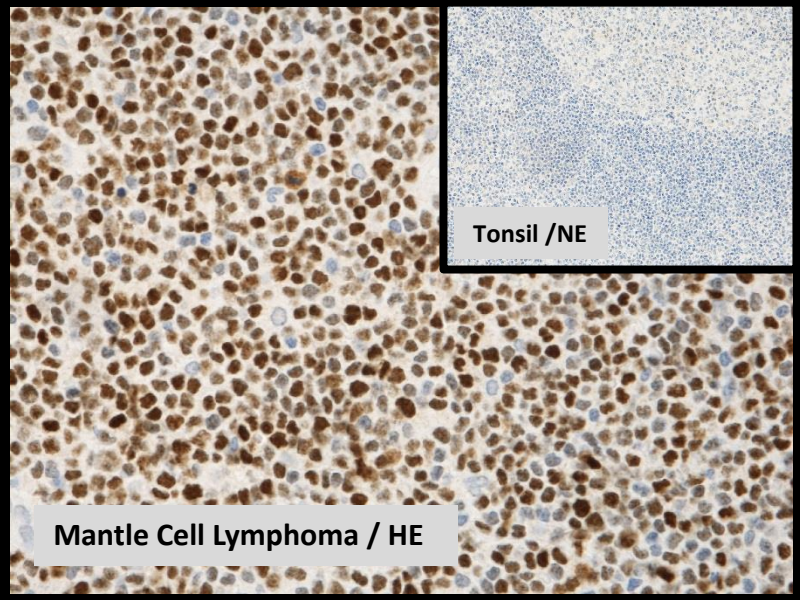
Control material / Tonsil:

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

Sox11





No normal tissue components express Sox11

Control material:

Include 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 expected

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.

The prevalent features of an Insufficient staining results were:

Too weak staining reaction of cells expected to be demonstrated

Poor signal-to-noise ratio compromising the interpretation.

Sox11/ Run 47 (2016):

First assessment of this new 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

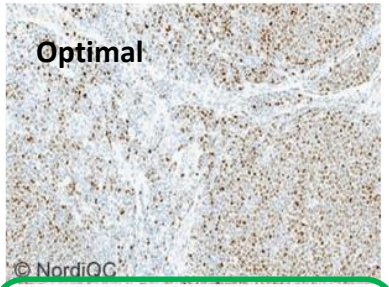


Fig. 1a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 4, using the mAb clone SOX-C11 diluted 1:25, HIER in CC1, a 3-step multimer based detection kit (OptiView) and performed on BenchMark Ultra, Ventana. The vast majority of neoplastic cells show a moderate, distinct, nuclear staining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

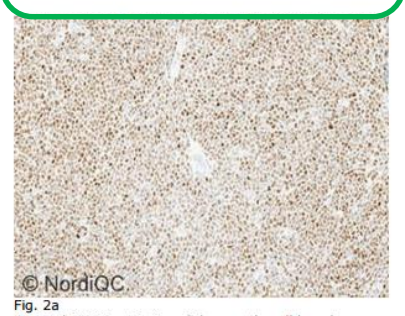


Fig. 2a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background reaction is seen.

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.

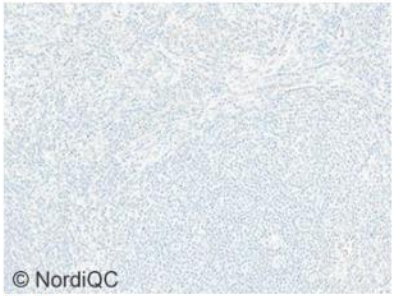


Fig. 3a
Optimal SOX11 staining of the B-CLL using same protocol as in Figs. 1a and 2a. No staining is seen.

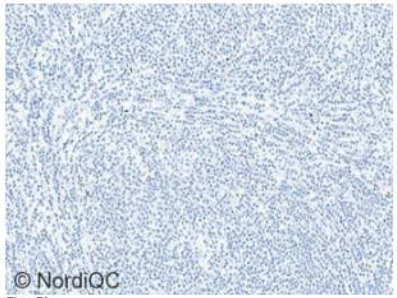


Fig. 3b
SOX11 staining of the B-CLL using same protocol as in Figs. 1b and 2b. No staining is seen.

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.

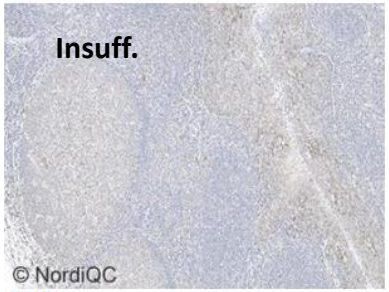


Fig. 4b
Insufficient SOX11 staining of the tonsil using a pAb providing an insufficient result characterized by a poor signal-to-noise ratio. In the tonsil a general background staining is seen and in e.g. plasma cells and squamous epithelial cells a moderate aberrant cytoplasmic staining reaction is 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 (also seen with mm Ab´s)

Sox11 / Run 47 2016

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

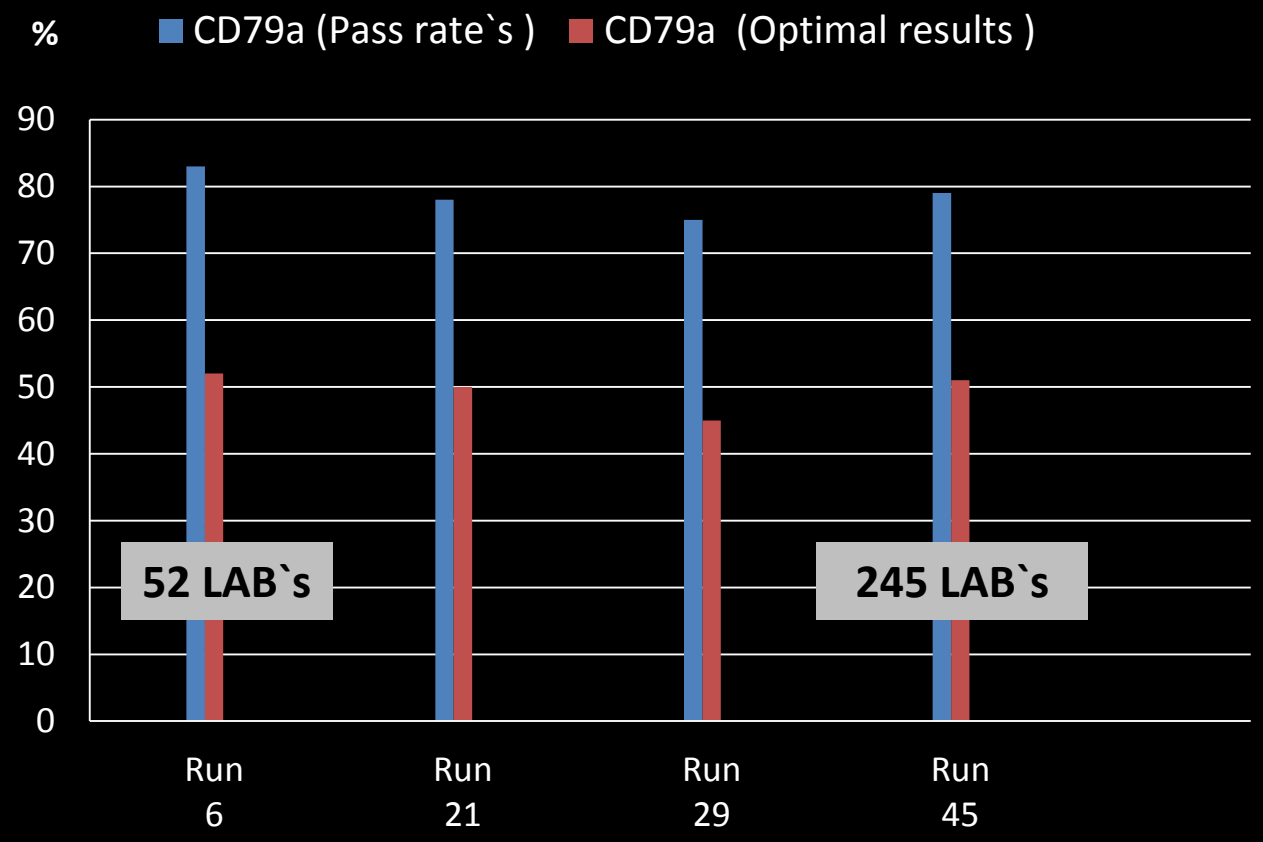
Lymphoma´s (Basic panel): Antibodies

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: SP34	pAb: RB-9406 , mmAb: 24[#] & 1EW (PO blocking)*
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, rmAb: SP23	mmAb: MHM6 & 1B12[#]
CD30	mmAb: BER-H2 & 1G12 & JCM182 & "CON6D/5"	-
Sox11	mmAb: MRQ-58 & SOX11-C1	-
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: MIB1 & MM1, 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

CD79a



CD79a/ Run 45 (2015):

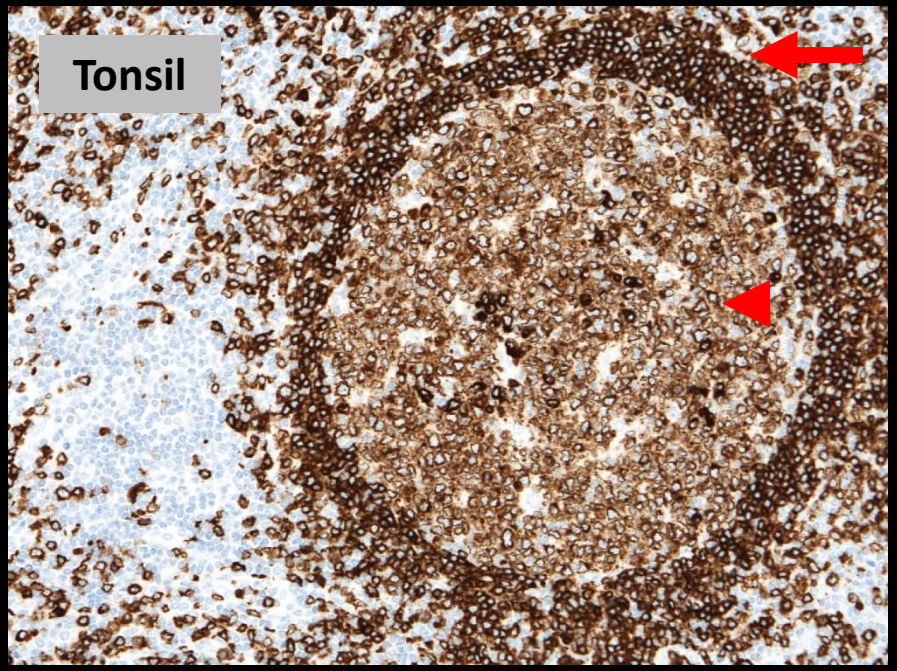
Sufficient: 79%

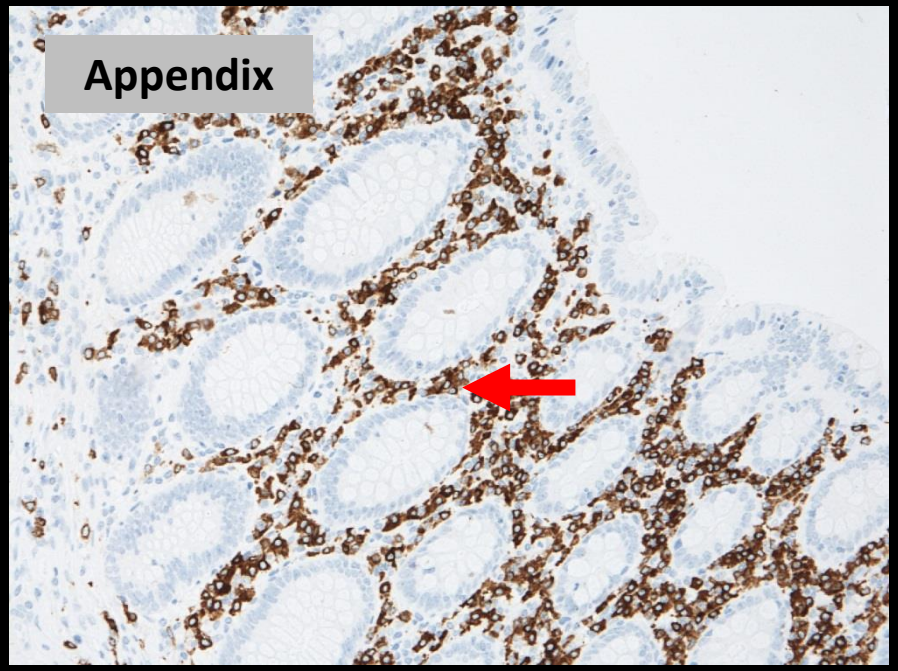
Optimal: 51%

Clone JCB117 & SP18 robust clones and used by 95% of the LAB`s

CD79a

Note strong staining of plasma cells in lamina propria of the appendix





Note strong staining of plasma cells in lamina propria of the appendix

Virtually all mantle zone B-cells must show a strong and distinct membranous staining reaction (HE)

An at least moderate staining reaction of the germinal centre B-cells (LE).

Plasma cells must show a moderate to strong cytoplasmic staining reaction

No staining of other cell types including epithelial cells of the appendix.

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	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	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.

25%, only Ventana users

86%

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)

Using similar protocol settings on other platforms gave a false positive reaction of epithelial cells (Colon)

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 frequency 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.

Alternative: rmAb SP18 on the Ventana Benchmark platforms

Table 2: Performance of the four most commonly used Abs in four CD79a runs

CD79	Run 6 2002		Run 21 2007		Run 29 2010		Run 45 2015		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone JCB117	48	43	97	80	124	92	151	118	420	333 (79%)
mAb clone HM47	3	0	6	0	8	0	2	0	19	0 (0%)
mAb clone 11E3	0	0	2	0	2	0	9	0	13	0 (0%)
rmAb clone SP18	0	0	6	6	11	10	81	76	98	92 (94%)

Leica or Dako/Agilent IHC instruments: Use mAb clone JCB117

Ventana/Roche IHC instruments: Use rmAb clone SP18

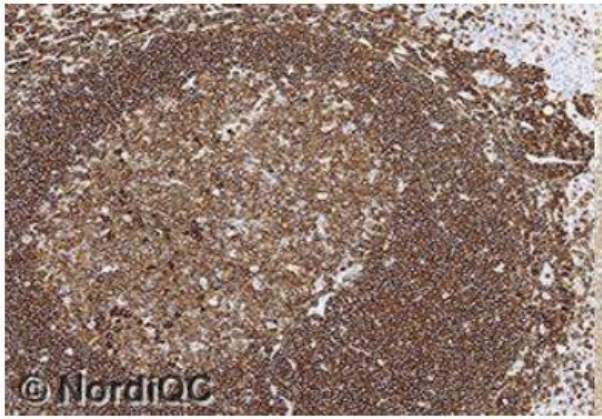


Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone JCB117 as Ready-To-Use format (GA621, Dako), with HIER in TRS High pH 9 for 30 min., a 3-step polymer based detection kit and performed on Omnis, Dako.
Mantle zone B-cells show an intense membranous staining reaction, while the germinal 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.

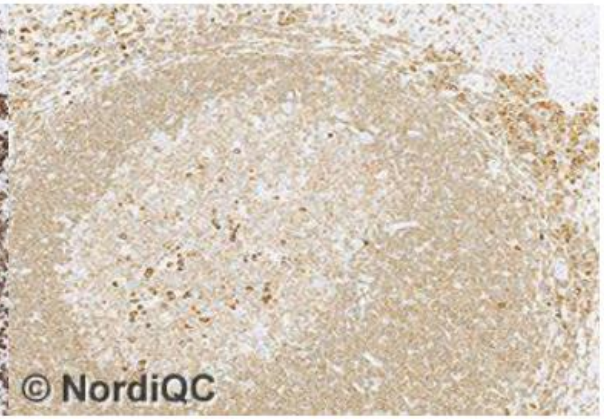


Fig. 1b
CD79a staining of the tonsil using the mAb clone JCB117 with an insufficient protocol – same field as in Fig. 1a.
The primary Ab was used at a titre of 1:500 and a 2-step multimer based detection system providing a too low sensitivity.
The mantle zone B-cells and the late stage germinal centre 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.

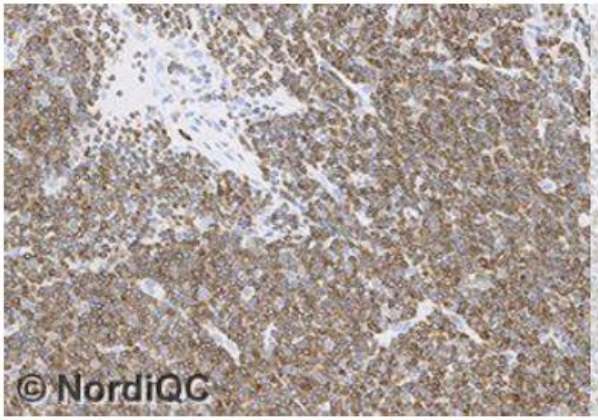


Fig. 2a
Optimal CD79a staining of the B-CLL using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate and distinct membranous staining reaction.
No background reaction is seen.

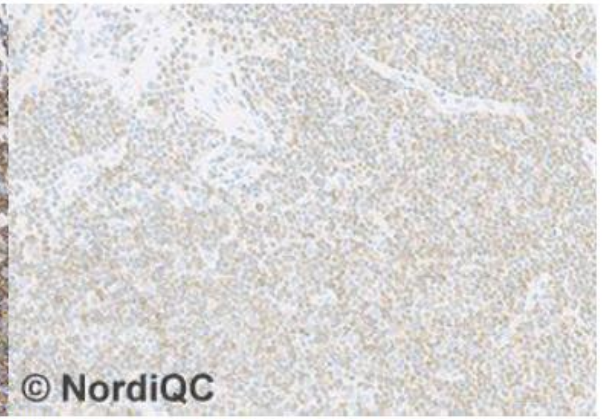


Fig. 2b
Insufficient CD79a staining of the B-CLL using same protocol as in Fig. 1b - same field as in Fig. 2a.
The neoplastic cells only show a weak and equivocal staining reaction.
Also compare with Fig. 3b – same protocol.

Problem:

Too low sensitivity

Low concentration of primary
Low sensitive detection system

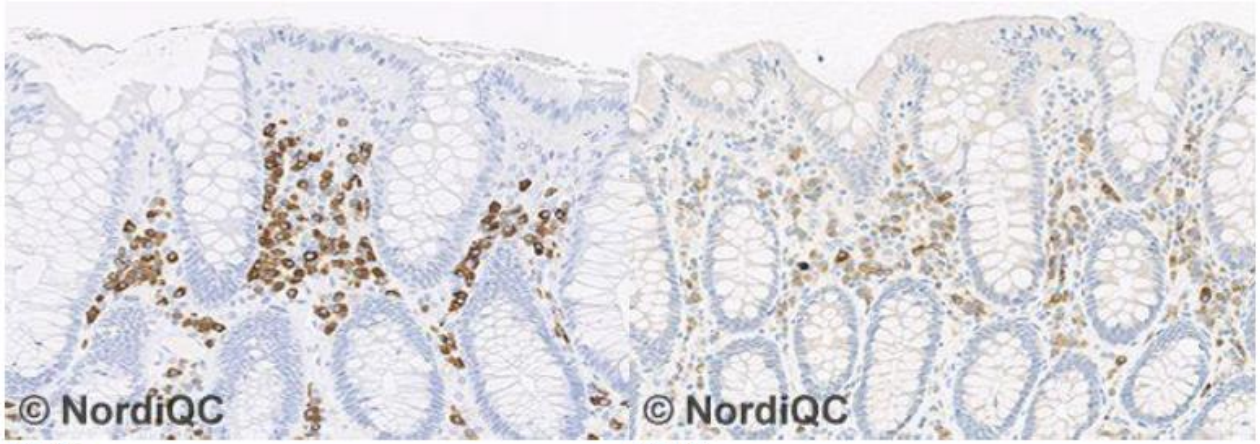


Fig. 4a
Optimal CD79a staining of colon using same protocol as in Figs. 1a - 3a.
Plasma cells show a moderate to strong cytoplasmic staining reaction.
No background reaction is seen.

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

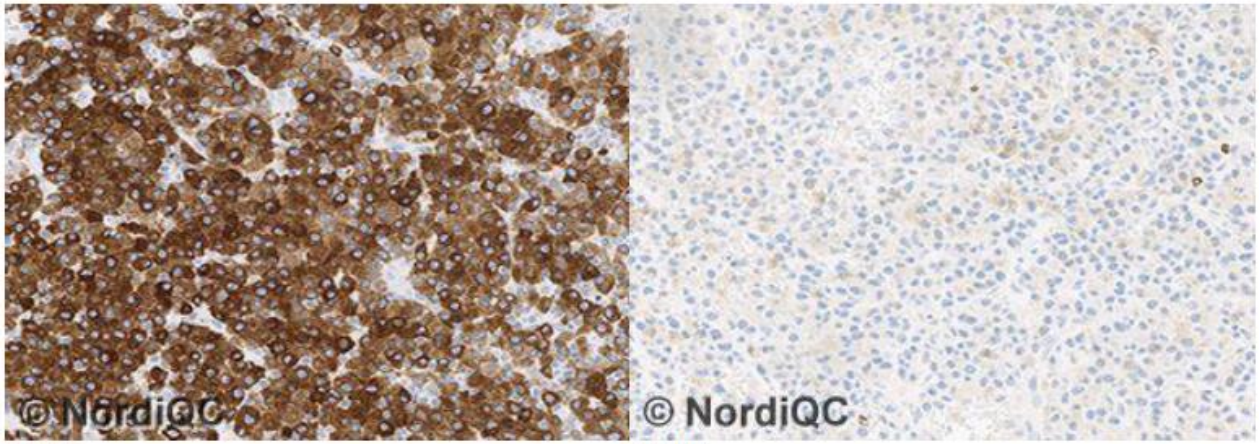


Fig. 5a
Optimal CD79a staining of the plasmacytoma using same protocol as in Figs. 1a - 4a.
Virtually all neoplastic cells show a moderate cytoplasmic staining reaction.

mAb JCB117 - optimal

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

CD79a / Run 45 2015

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:

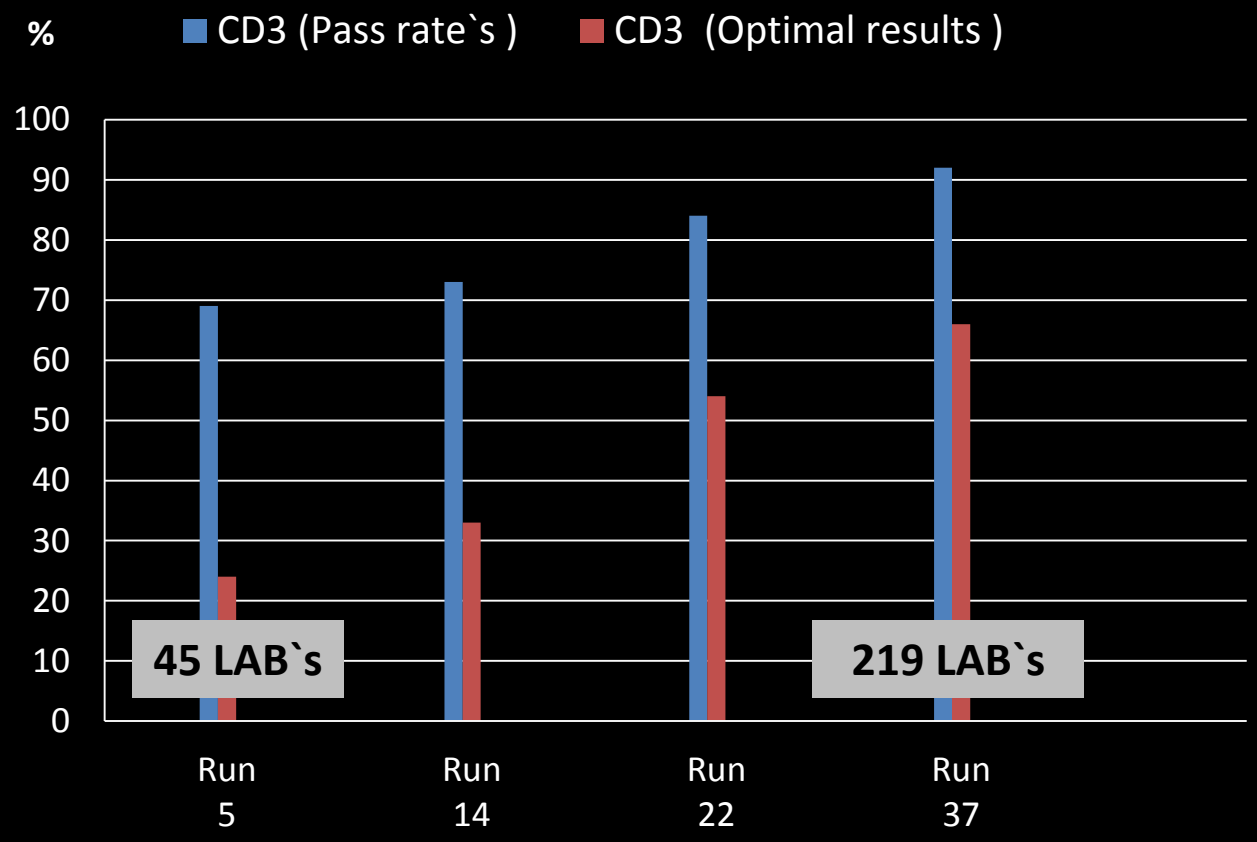
A strong, distinct membranous staining reaction of B-cells in the mantle zone in the tonsil

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

CD3



CD3/ Run 37 (2013):

Sufficient: 92%

Optimal: 66%

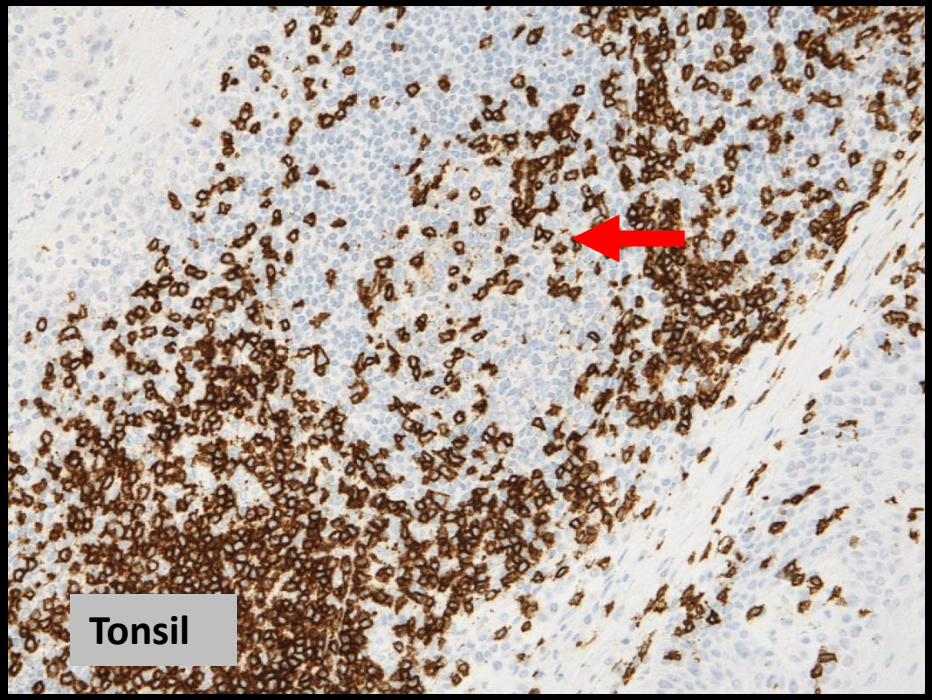
Robust primary Abs:

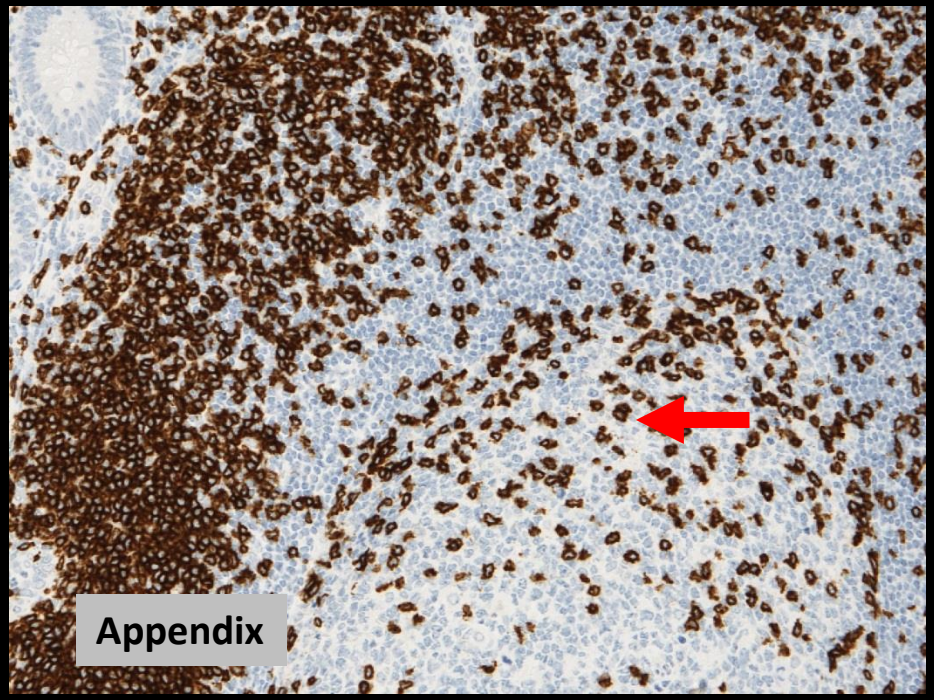
mAb`s: F7.2.38, LN10 & PS1

rmAb`s: EP449E, SP7, MRQ-39, & 2GV6

pAb A0542

CD3





A moderate to strong, distinct, predominantly membranous staining reaction of all T-cells both in the interfollicular T-zones and in the germinal centres of the tonsil.

Comment: As strong as possible without staining of other cellular structures

CD3 / Run 37 2013

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&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

RTU`s - High quality performance

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

Optimal

Insuffcient

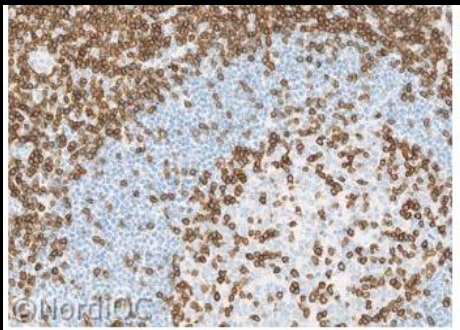


Fig. 1a. Optimal CD3 staining of the tonsil using the rmAb clone 2GV6, Ready-To-Use, Ventana. Virtually all the T-lymphocytes in the T-zone and within the germinal centre show a strong and distinct membranous staining reaction. No background staining or staining of the B-cells is seen. Also compare with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

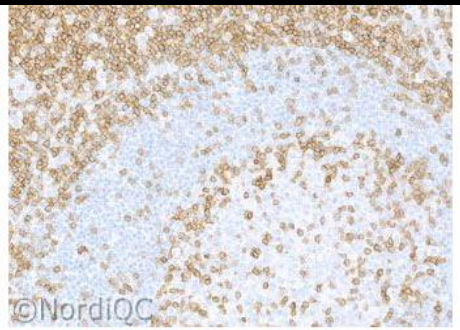


Fig. 1b. CD3 staining of the tonsil using the mAb clone F7.2.38 by protocol settings giving a too low sensitivity - same field as in Fig. 1a. The vast majority of the T-lymphocytes are demonstrated. A slightly weaker and less intense staining reaction is seen. However also compare with Figs. 2b – 3b,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

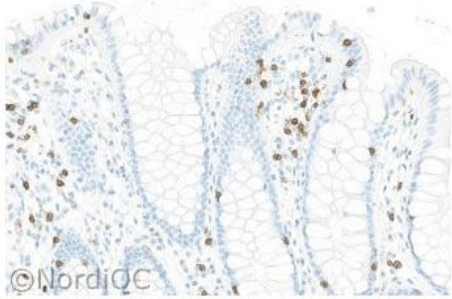


Fig. 2a. Optimal CD3 staining of the colon using same protocol as in Fig. 1a. The dispersed intraepithelial T-lymphocytes show a distinct staining reaction. The columnar epithelial cells are negative and no background staining is seen.

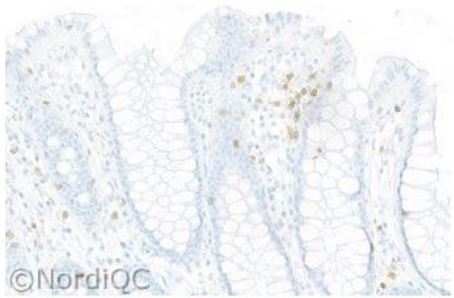


Fig. 2b. Insufficient CD3 staining of the colon using same protocol as in Fig. 1b – same field as in Fig. 2a. The intraepithelial T-lymphocytes are virtually negative. Also compare with Fig. 3b, same protocol.

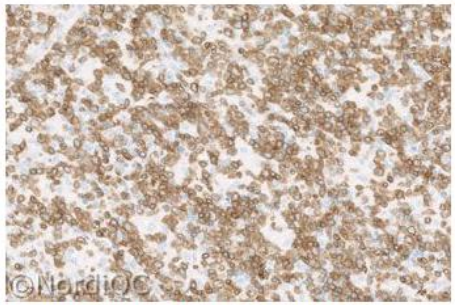


Fig. 3a. Optimal CD3 staining of the peripheral T-cell lymphoma, NOS, using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a moderate to strong and distinct predominantly membranous staining reaction. No background staining is seen.

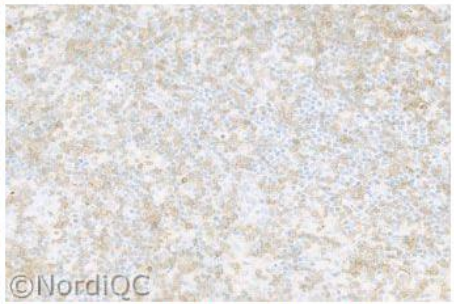


Fig. 3b. Insufficient CD3 staining of the peripheral T-cell lymphoma, NOS, using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a.

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

CD3 / Run 37 2013

Lymphoma panel: CD3
Optimal protocol settings – most common primary Abs for CD3 (NQC)

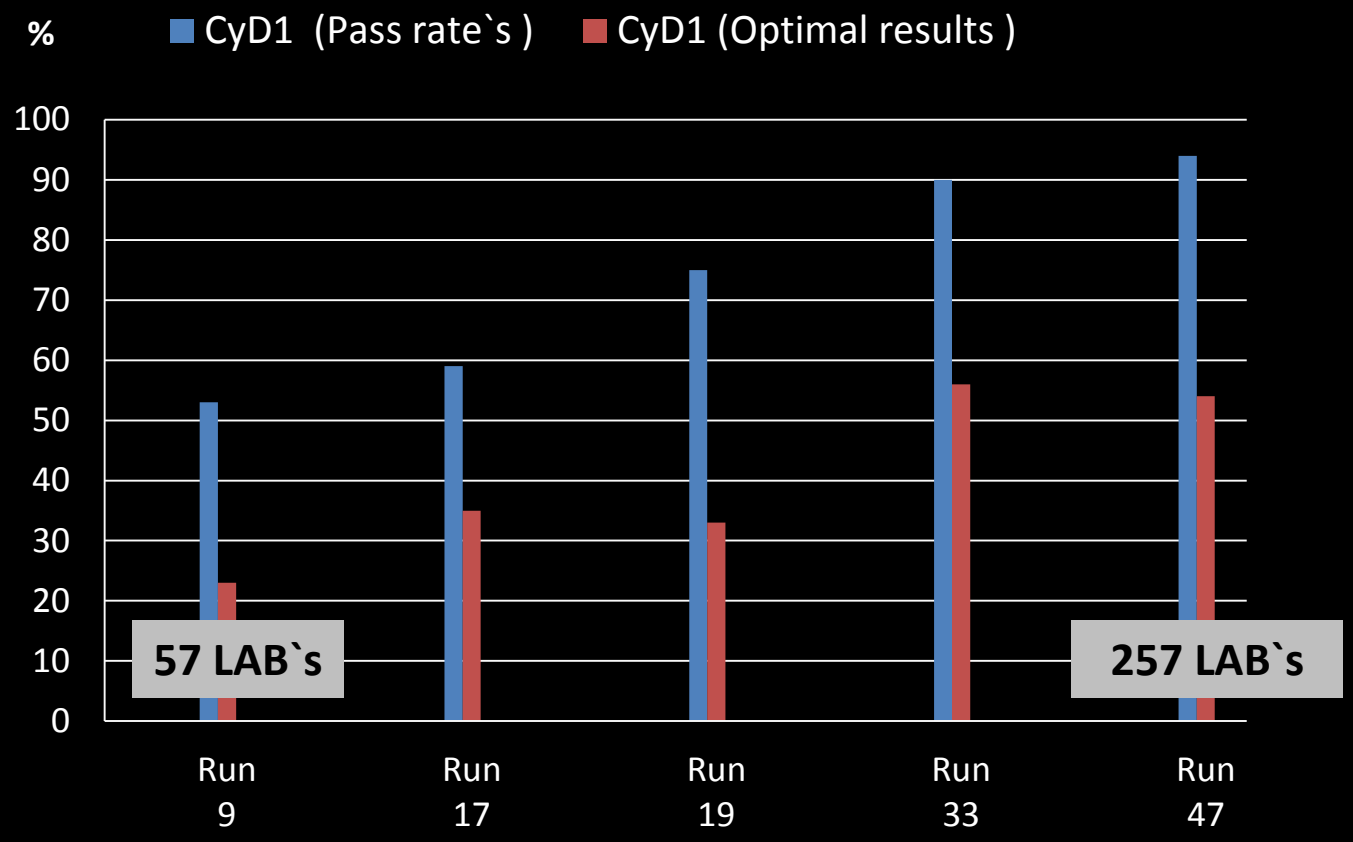
CD3	Retrieval buffers	Titre	Detection	RTU	Detection
mmAb **F7.2.38**	**HIER High pH**	**1:50-1:200**	**2 & 3-step**	**-**	**-**
pAb **A0452**	**HIER High pH**	**1:50-1:300**	**2 & 3-step**	**Dako (IS503/IR503)**	**Flex/ Flex+**
mmAb **LN10**	**HIER High pH & Low pH**	**1:50-1:140**	**2 & 3-step**	**Leica (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

CYCLIN D1



CyD1/ Run 47 (2016):

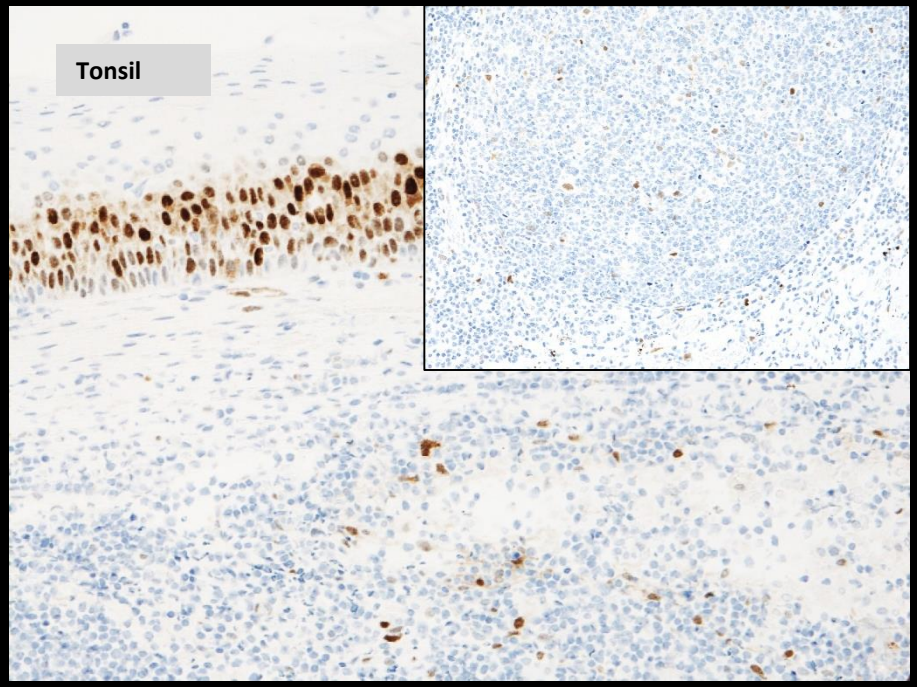
Sufficient: 94%

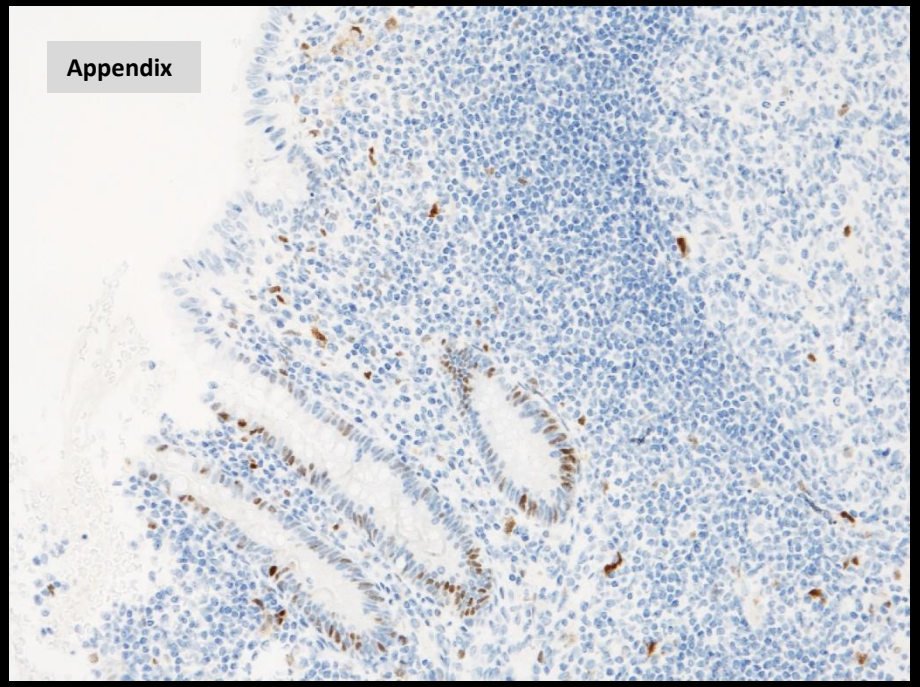
Optimal: 54%

Robust primary Abs:

rmAb`s: SP4 & EP12

Cyclin D1





Tonsil is recommendable as positive and negative tissue control:

Virtually all suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells must show a moderate to strong distinct nuclear staining reaction, whereas an at least weak but distinct staining reaction of germinal centre macrophages should be seen.

Mantle zone B-cells and germinal centre B-cells should be negative. The positive staining of endothelial cells is a valuable internal positive tissue control for CyD1.

Crypts of appendix - basal and middle part (LE) / surface epithelium (NE)

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)

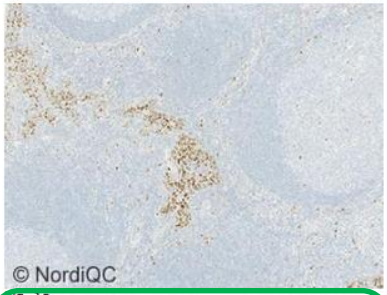


Fig. 1a
Optimal staining for Cyclin D1 of the tonsil, tissue core no. 1, using the rmAb clone SP4-R as Ready-To-Use format (Ventana prod. no. 790-4508) using HIER in CC1 for 64 min. and UltraView as detection system.
Even at low power field squamous epithelial cells, dispersed endothelial cells and germinal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

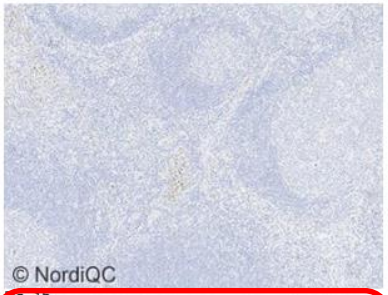


Fig. 1b
Insufficient staining for Cyclin D1 of the tonsil, tissue no. 1, using the rmAb clone SP4 by a laboratory developed assay giving a too low sensitivity (too low. conc. of the primary Ab) - same field as in Fig. 1a.
The proportion of positive cells and the intensity of the staining reaction are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

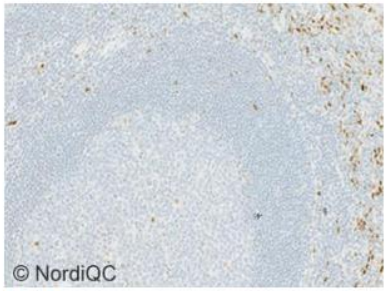


Fig. 2a
Optimal staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1a. High power field x200.
Virtually all squamous epithelial cells, dispersed endothelial cells and germinal centre macrophages show a moderate to strong nuclear staining reaction. The vast majority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

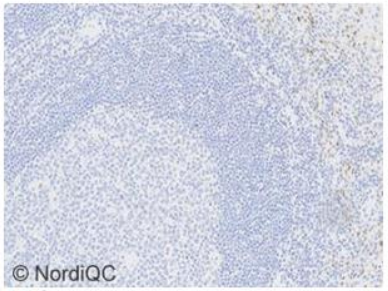


Fig. 2b
Insufficient staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1b - same field as in Fig. 2a.
Only scattered squamous epithelial cells show a weak and equivocal staining reaction, while endothelial cells and germinal centre macrophages are negative.
Also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

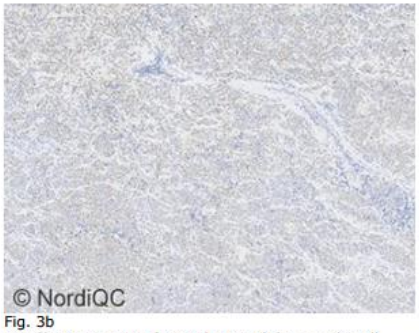


Fig. 3b
Insufficient staining for Cyclin D1 of the mantle cell lymphoma, tissue core no. 4, using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result expected and obtained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

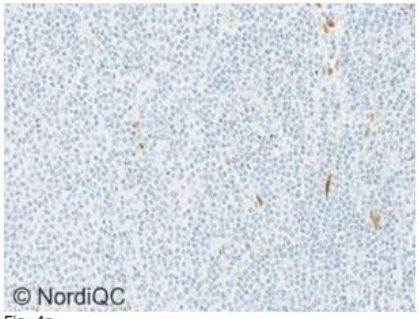


Fig. 4a
Optimal staining for Cyclin D1 of the B-CLL using same protocol as in Figs. 1a - 3a.
The neoplastic cells are negative, while scattered endothelial cells show a moderate nuclear staining reaction serving as internal positive tissue control.

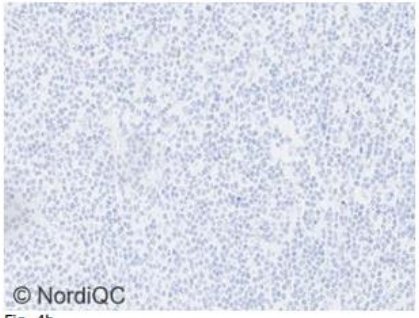


Fig. 4b
Staining for Cyclin D1 of the B-CLL using same insufficient protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
No staining is seen.

CyD1 / Run 47 2016

Lymphoma panel: CyD1
Optimal protocol settings (NQC)

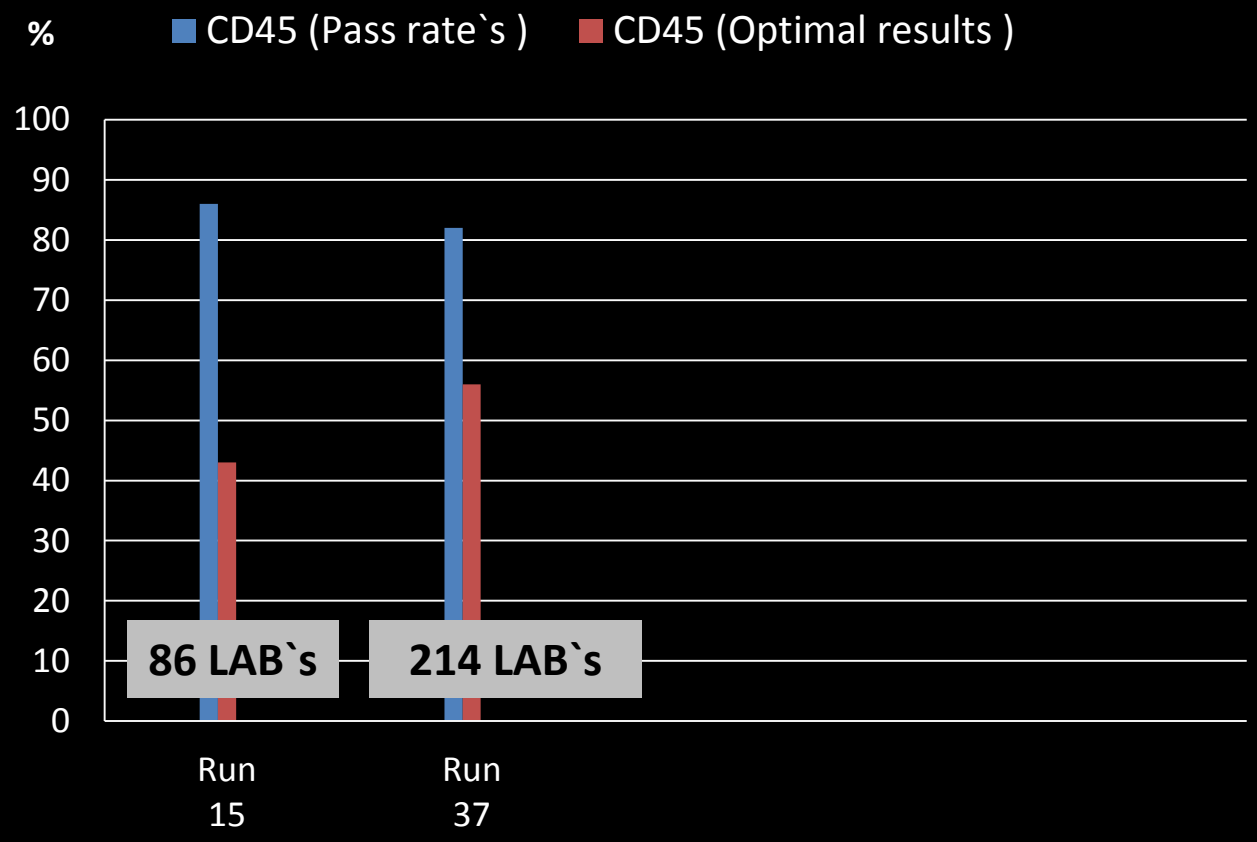
CyD1	Retrieval buffers	Titre	Detection	RTU	Detection
rmAb EP12	HIER High pH	1:20-1:200	2 & 3-step	Dako (IS/IR083)	Flex/Flex+
				Biocare (PME432)	MACH4
rmAb SP4	HIER High pH	1:20-1:150	2 & 3-step	Ventana (790-4508)	UltraView +/- Amp OptiView

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

CD45, LCA



CD45 / Run 37 (2013):

Sufficient: 82%

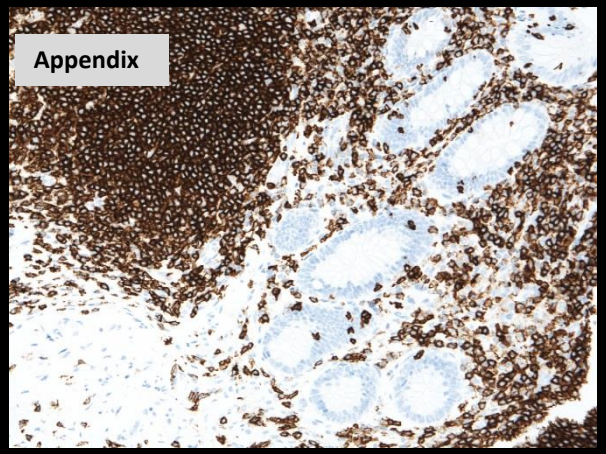
Optimal: 56%

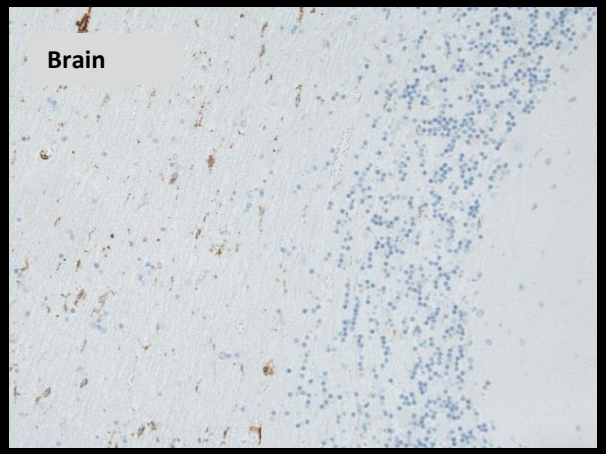
Robust primary Abs:

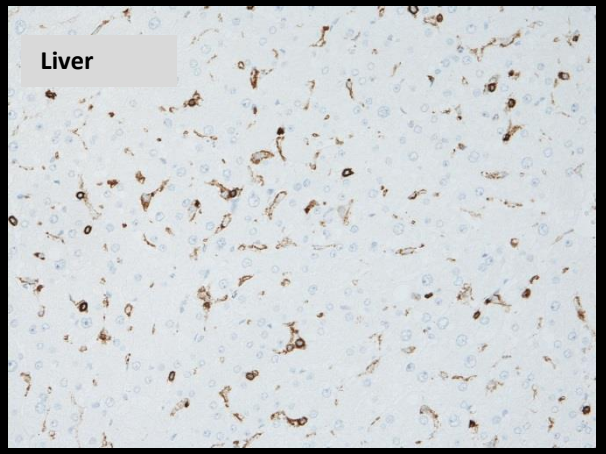
mmAb: 2B11+PD7/26

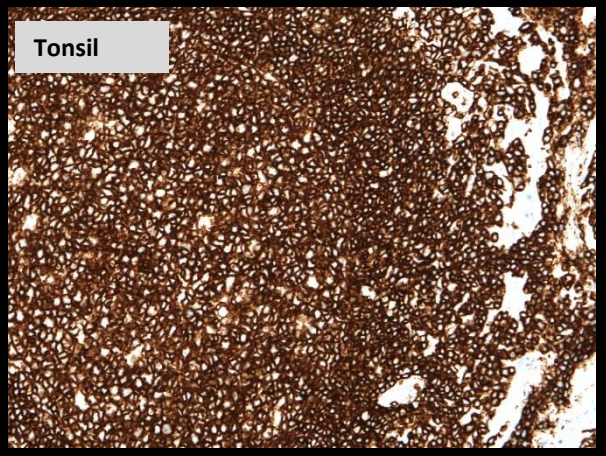
mmAb: X16/99

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

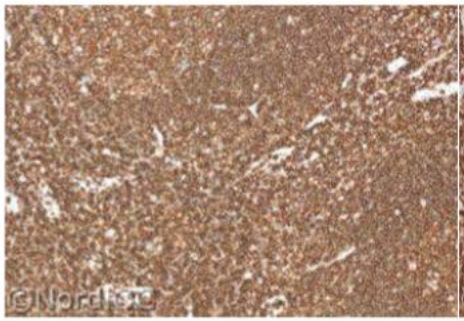


Fig 1a
Optimal CD45, LCA staining of the tonsil using the mAb clones 2B11+PD7/26 optimally calibrated and with HIER. Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.
Also compare with Figs. 2a – 4a, same protocol.

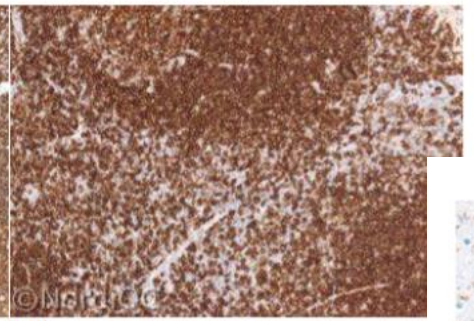


Fig 1b
Staining for CD45, LCA of the tonsil using the mAb clone 2B11+PD7/26 by protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a.
The vast majority of the B- and T-lymphocytes are demonstrated. However also compare with Figs. 2b – 4b same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

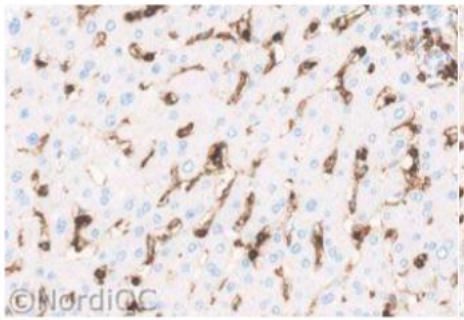


Fig 2a
Optimal CD45, LCA staining of the liver using same protocol as in Fig. 1a.
The lymphocytes show a strong staining reaction, while the Kupffer cells display a weak to moderate staining reaction. The liver cells are negative and no background staining is seen.

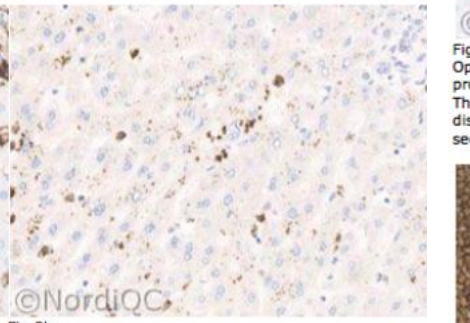


Fig 2b
Insufficient CD45, LCA staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only lymphocytes are demonstrated and the Kupffer cell with a low CD45 expression are false negative.

Optimal

Insuff.

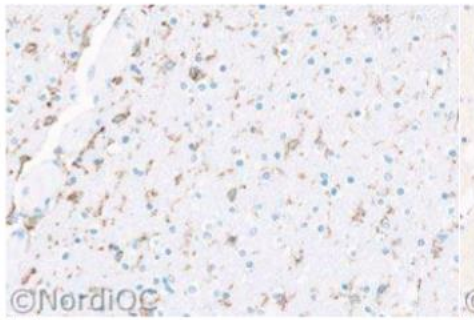


Fig 3a
Optimal CD45, LCA staining of the brain using same protocol as in Figs. 1a & 2a.
The microglial with a low CD45 expression are distinctively demonstrated and no background staining is seen.

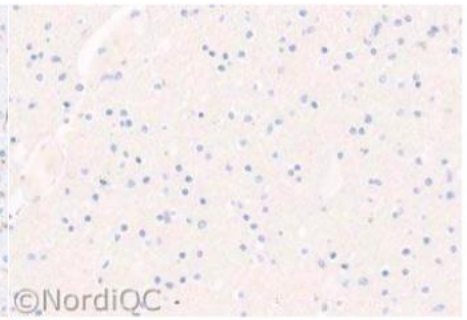


Fig 3b
Insufficient CD45, LCA staining of the brain using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The microglial cells are false negative.

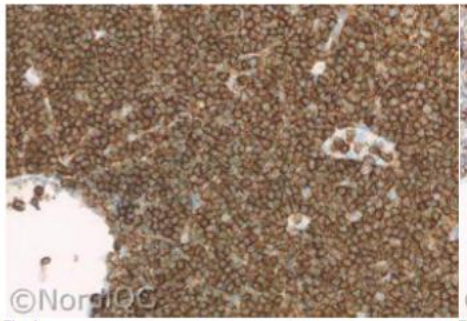


Fig 4a
Optimal CD45, LCA staining of the B-CLL using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong and distinct membranous staining reaction.
No background staining is seen.

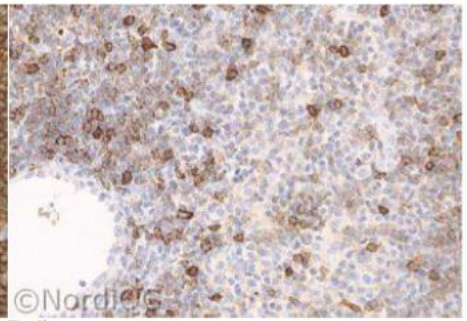


Fig 4b
Insufficient CD45, LCA staining of the B-CLL using same protocol as in Figs. 1b - 3b. – same field as in Fig. 4a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

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:

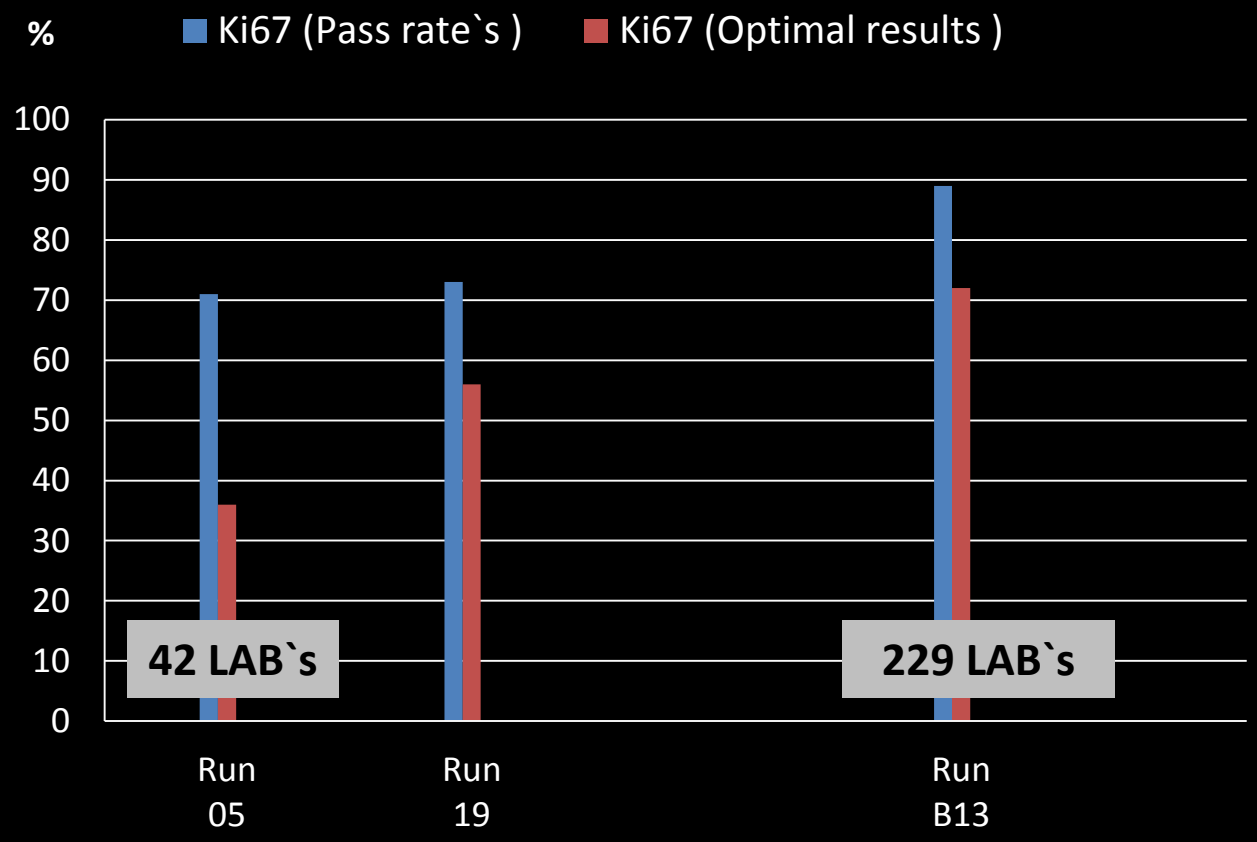
In tonsillar tissue, all B- and T-cells must show strong and distinct membranous staining reaction

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.

Ki67



Ki67 / Run B13 (2012):

Sufficient: 89%

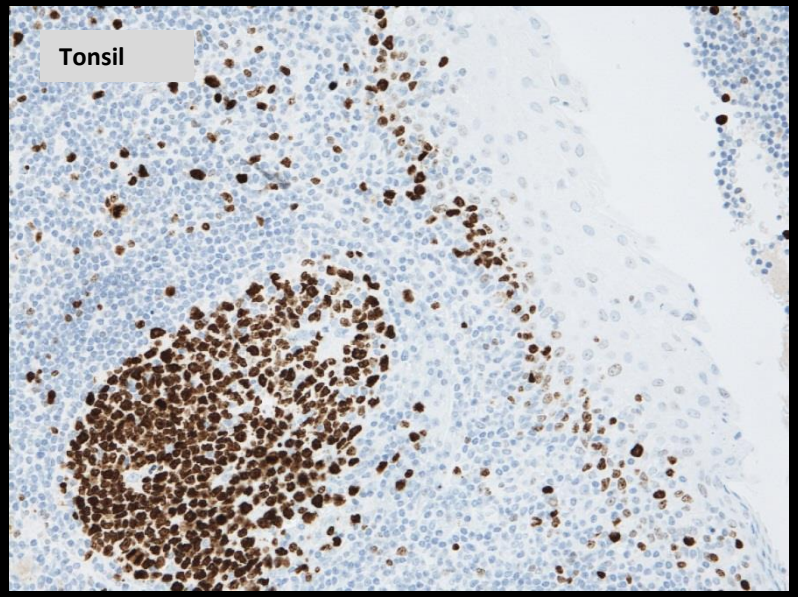
Optimal: 72%

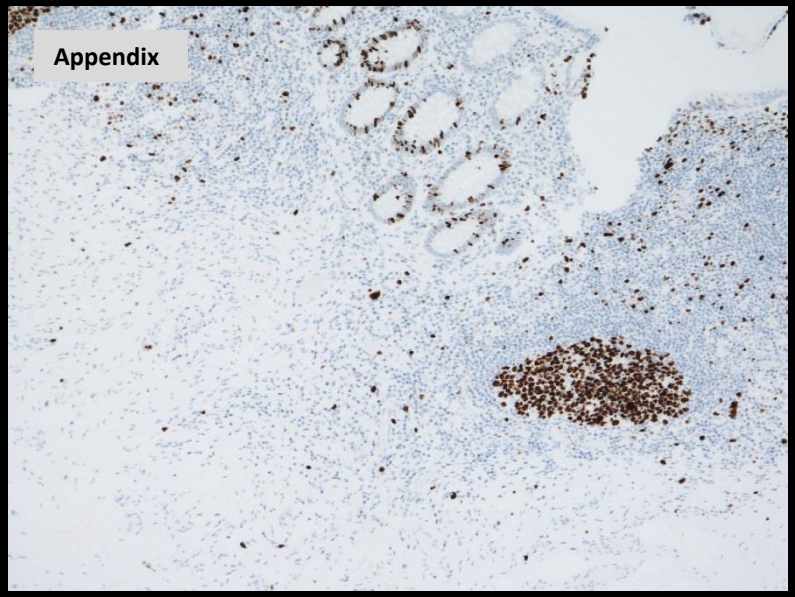
Robust primary Abs:

mmAb: MIB-1 & MM1

rmAb: SP6 & 30-9 (RTU,Ventana)

Ki67





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. **Abs and assessment marks for Ki67, run B13**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **7B11**	2	Invitrogen	1	0	1	0	-	-
mAb clone **BGX-297**	1	Biogenex	0	1	0	0	-	-
mAb clone **K2**	1	Immunologic	1	0	0	0	-	-
mAb clone **MIB-1**	101 1	Dako DBS	69	20	10	3	87 %	87 %
mAb clone **MM1**	9	Leica/Novocastra	5	2	1	1	78 %	100 %
rmAb clone **EP5**	1	Epitomics	0	0	1	0	-	-
rmAb **SP6**	8 7 1 1 1	Thermo/NeoMarkers Cell Marque Biocare Master Diagnostica Spring	10	5	2	1	83 %	92 %
pAb **A0047**	1	Dako*	1	0	0	0	-	-
pAb **RB-1510**	1	Thermo/NeoMarkers	1	0	0	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use Abs:								
mAb clone **MIB-1 IR/IS626**	38	Dako	32	3	2	1	92 %	97 %
mAb clone **MM1 PA0118**	2	Leica/Novocastra	1	1	0	0	-	-
mAb clone **MM1 RTU-Ki67-MM1**	2	Leica/Novocastra	1	1	0	0	-	-
mAb clone **MM1 PM375**	1	Biocare	0	1	0	0	-	-
rmAb clone **30-9**	48	Ventana	43	4	1	0	98 %	98 %
790-4286								
rmAb clone **EP5 ZA-0502**	1	Zhongshan	0	1	0	0	-	-
Total	229		166	39	18	6	-	
Proportion			72 %	17 %	8 %	3 %	89 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below
* Product has been discontinued by the vendor

In addition to the primary Abs highlighted in the table, several antibodies can be used to produce an optimal result e.g. mmAb`s 7B11 & K2, pAb`s A0047 & RB-1510

Optimal (mmAb MIB-1 & rmAb SP6)

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

1:50-1:600 (MIB-1)

1:30-1:300 (SP6)

2 & 3 step detection systems

Optimal (mmAb MM1)

Efficient HIER in High / BERS2 (20 min)

1:50-1:200 (MM1)

3 step detection systems

Best performance:

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

RTU Ki67, MIB-1 (IS/IR626, Dako)

“RTU Ki67, MM1, (PA0118,Leica)”

Ki67

The frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody

- Insufficient HIER (too short heating time)

- Excessive HIER

- Inadequate dewaxing

A too weak staining reaction was seen in 50 % of the insufficient staining results

Ki67

Optimal **Insufficient**

Fig. 1a
Optimal staining for Ki67 of the tonsil fixed for 24 hours in NBF 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 & 3a – same protocol.

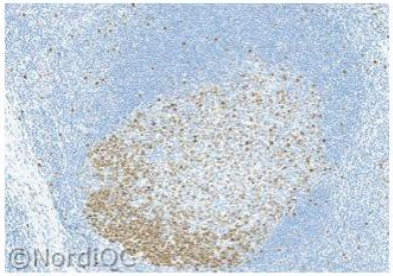


Fig. 1b
Insufficient staining for Ki67 of the tonsil fixed for 24 hours in NBF using the mAb clone MIB1 with a protocol providing a too low sensitivity, most likely due to a too

The majority of the germinal centre B-cells are demonstrated, but especially the B-cells in the light zone only show a weak and diffuse nuclear staining reaction – same field as in Fig. 1a.
Also compare with Figs. 2b & 3b - same protocol.

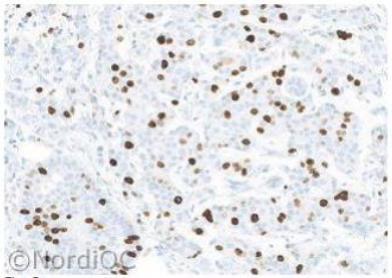


Fig. 2a
Optimal staining for Ki67 of the breast carcinoma no. 4 using same protocol as in Fig. 1a.
≥ 10 and < 24 % of the neoplastic cells show a moderate to strong and distinct nuclear staining reaction. The nuclear staining reaction for Ki67 is easily interpreted.

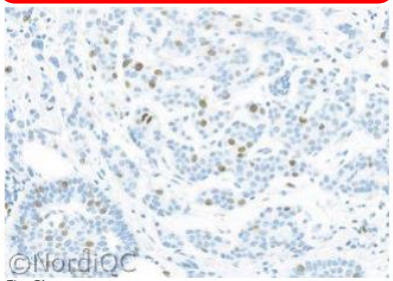


Fig. 2b
Insufficient staining for Ki67 of the breast carcinoma no. 4 using same protocol as in Fig. 1b. - same field as in Fig. 2a.

The intensity and proportion of the positive cells is significantly reduced compared to the result in Fig. 2a.
Also compare with Fig. 3b – same protocol.

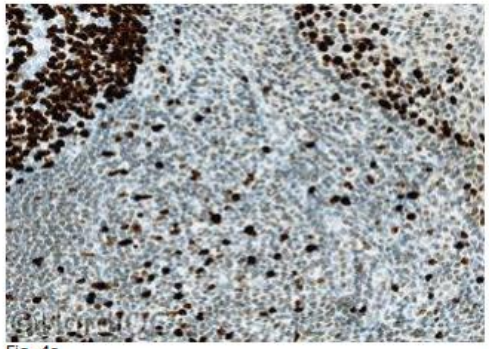


Fig. 4a
Insufficient staining for Ki67 of the tonsil as a false positive staining reaction is seen. Virtually all cells show a positive nuclear staining reaction. The germinal centre B-cells, top left, show an intense nuclear staining reaction, but also the vast majority of the mantle zone B-cells and interfollicular lymphocytes show a positive nuclear staining reaction. A too high concentration of the primary Ab and/or inadequate wash in buffers may be the cause for this aberrant staining pattern.

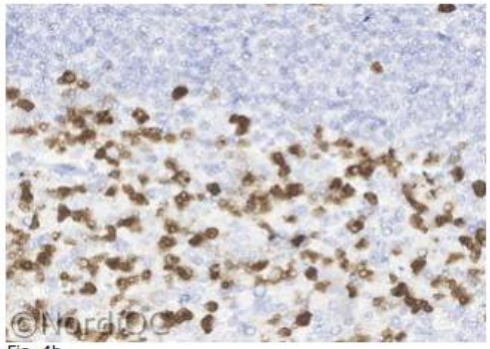


Fig. 4b
Insufficient staining for Ki67 of the tonsil as an impaired morphology is observed
The germinal centre B-cells are demonstrated, but the interpretation is compromised by an extensive nuclear bubbling in all cells. This artefact most likey was caused by inadequate deparaffination occasionally seen by the use of 3-in-1 (deparaffination-dehydration-HIER) protocols.

Insufficient:

All interfollicular lymphocytes are stained – false positive

Insufficient:

Inadequate dewaxing or air drying after IHC staining

Protocol with too low sensitivity:

Low concentrations of the primary Ab ?

Ki67 / Run B13 2012

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/IR626)	Flex Flex+
mmAb **MM1**	HIER High pH buffer (BERS2)	1:50-1:200	3-step	Leica (PA0118)	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.

Haematolymphoid 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 and stain according to the recommended intensity

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

For Ig Kappa/Lambda (Dako), HIER in standard acidic buffers or mod. low pH buffers is preferable
For CD30 clone CON6D/5A, HIER in mod. low pH buffer`s is mandatory

Don´t use enzymatic pretreatment - All the haematolymphoid markers ever assessed by NordiQC require efficient HIER to get an optimal result

Use a sensitive polymer/multimer detection system (preferable a 3 –step system)

Don`t use of a biotin based detection system (problems with endogenous biotin and low sensitivity)

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

Platform dependent primary Abs
Epitops sensitive to H_2O_2 blocking
Lot - to - lot variations
Too much counterstain
???????

Thank you