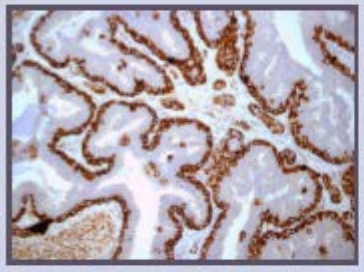
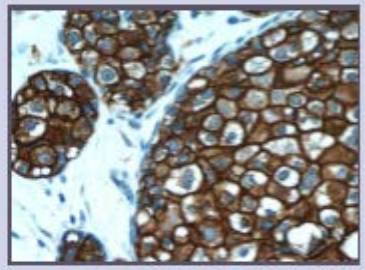




NordiQC TATA MEDICAL CENTER ISIMm International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

# QA of IHC Undifferentiated tumors and Malignant lymphomas

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

# QA Undifferented tumours and lymphomas

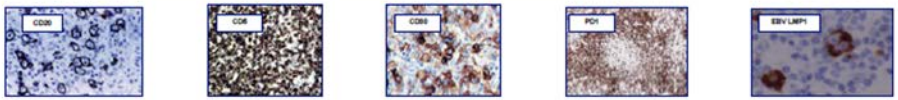
**Courtesy: Steve Hamilton-Dutoit**

## Useful antigens in haematopathology

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

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

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




CUP

Primary Panel (CK, S100, VIM, CD45)

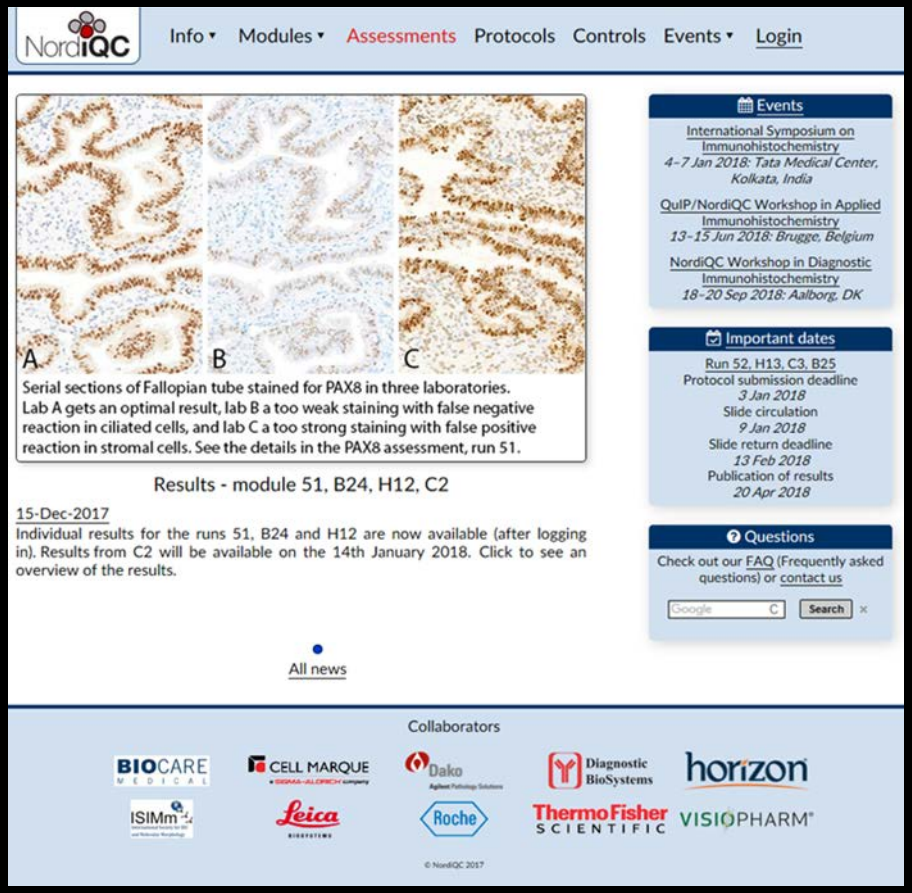
Secondary Panels (CKs, CDX2, TTF1, SOX10.....)

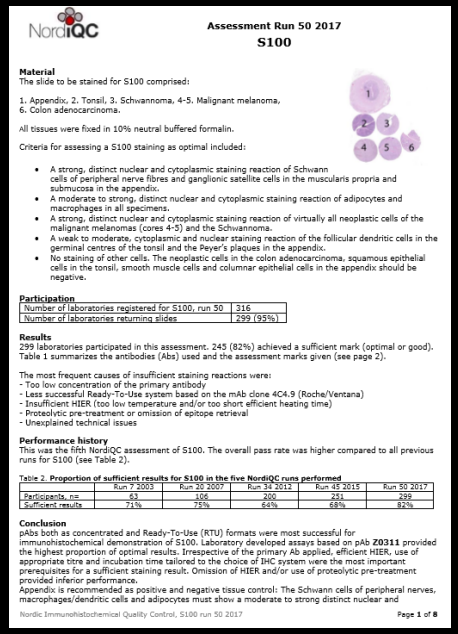
# QA Undifferented tumours and lymphomas

IHC assays to be used in diagnostics;

Best practice antibodies/clones
Best practice positive and negative tissue controls

**www.nordiqc.org**





NordiQC

**Assessment Run 50 2017**
**S100**

**Material**
The slide to be stained for S100 comprised:

1. Appendix, 2. Tonsil, 3. Schwannoma, 4-5. Malignant melanoma, 6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of Schwann cells of peripheral nerve fibres and ganglionic satellite cells in the muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of adipocytes and macrophages in all specimens.
- A strong, distinct nuclear and cytoplasmic staining reaction of virtually all neoplastic cells of the malignant melanomas (cores 4-5) and the Schwannoma.
- A weak to moderate, cytoplasmic and nuclear staining reaction of the follicular dendritic cells in the germinal centres of the tonsil and the Peyer's plaques in the appendix.
- No staining of other cells. The neoplastic cells in the colon adenocarcinoma, squamous epithelial cells in the tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative.

**Participation**

| | |
|---|---|
| Number of laboratories registered for S100, run 50 | 316 |
| Number of laboratories returning slides | 299 (95%) |

**Results**
299 laboratories participated in this assessment. 245 (82%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful Ready-To-Use system based on the mAb clone 4C4.9 (Roche/Ventana)
- Insufficient HIER (too low temperature and/or too short efficient heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Unexplained technical issues

**Performance history**
This was the fifth NordiQC assessment of S100. The overall pass rate was higher compared to all previous runs for S100 (see Table 2).

Table 2. **Proportion of sufficient results for S100 in the five NordiQC runs performed**

| | Run 7 2003 | Run 20 2007 | Run 34 2012 | Run 45 2015 | Run 50 2017 |
|---|---|---|---|---|---|
| Participants, n= | 63 | 106 | 200 | 231 | 299 |
| Sufficient results | 71% | 75% | 64% | 68% | 82% |

**Conclusion**
pAbs both as concentrated and Ready-To-Use (RTU) formats were most successful for immunohistochemical demonstration of S100. Laboratory developed assays based on pAb **Z0311** provided the highest proportion of optimal results. Irrespective of the primary Ab applied, efficient HIER, use of appropriate titre and incubation time tailored to the choice of IHC system were the most important prerequisites for a sufficient staining result. Omission of HIER and/or use of proteolytic pre-treatment provided inferior performance.
Appendix is recommended as positive and negative tissue control: The Schwann cells of peripheral nerves, macrophages/dendritic cells and adipocytes must show a moderate to strong distinct nuclear and



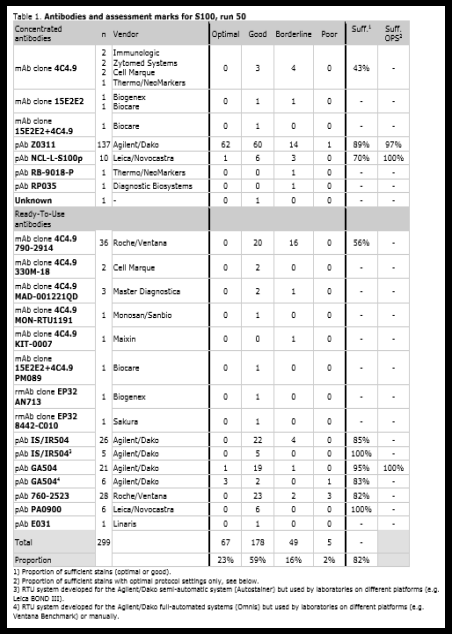


Table 1. **Antibodies and assessment marks for S100, run 50**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **4C4.9** | 2<br>2<br>2<br>1 | Immunologic<br>Zytomed Systems<br>Cell Marque<br>Thermo/NeoMarkers | 0 | 3 | 4 | 0 | 43% | - |
| mAb clone **1SE2E2** | 1<br>1 | Biogenex<br>Biocare | 0 | 1 | 1 | 0 | - | - |
| mAb clone **1SE2E2+4C4.9** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| pAb **Z0311** | 137 | Agilent/Dako | 62 | 60 | 14 | 1 | 89% | 97% |
| pAb **NCL-L-S100p** | 10 | Leica/Novocastra | 1 | 6 | 3 | 0 | 70% | 100% |
| pAb **RB-9018-P** | 1 | Thermo/NeoMarkers | 0 | 0 | 1 | 0 | - | - |
| pAb **RP035** | 1 | Diagnostic Biosystems | 0 | 0 | 1 | 0 | - | - |
| **Unknown** | 1 | - | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **4C4.9 790-2914** | 36 | Roche/Ventana | 0 | 20 | 16 | 0 | 56% | - |
| mAb clone **4C4.9 330M-18** | 2 | Cell Marque | 0 | 2 | 0 | 0 | - | - |
| mAb clone **4C4.9 MAD-001221QD** | 3 | Master Diagnostica | 0 | 2 | 1 | 0 | - | - |
| mAb clone **4C4.9 MON-RTU1191** | 1 | Monosan/Sanbio | 0 | 1 | 0 | 0 | - | - |
| mAb clone **4C4.9 KIT-0007** | 1 | Maixin | 0 | 0 | 1 | 0 | - | - |
| mAb clone **1SE2E2+4C4.9 PM089** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP32 AN713** | 1 | Biogenex | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP32 8442-C010** | 1 | Sakura | 0 | 1 | 0 | 0 | - | - |
| pAb **IS/IR504** | 26 | Agilent/Dako | 0 | 22 | 4 | 0 | 85% | - |
| pAb **IS/IR504**[3] | 5 | Agilent/Dako | 0 | 5 | 0 | 0 | 100% | - |
| pAb **GA504** | 21 | Agilent/Dako | 1 | 19 | 1 | 0 | 95% | 100% |
| pAb **GA504**[4] | 6 | Agilent/Dako | 3 | 2 | 0 | 1 | 83% | - |
| pAb **760-2523** | 28 | Roche/Ventana | 0 | 23 | 2 | 3 | 82% | - |
| pAb **PA0900** | 6 | Leica/Novocastra | 0 | 6 | 0 | 0 | 100% | - |
| pAb **E031** | 1 | Linaris | 0 | 1 | 0 | 0 | - | - |
| Total | 299 | | 67 | 178 | 49 | 5 | - | |
| Proportion | | | 23% | 59% | 16% | 2% | 82% | |

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

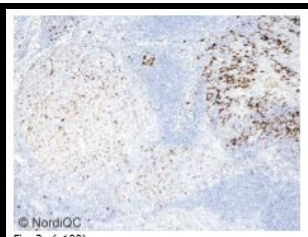


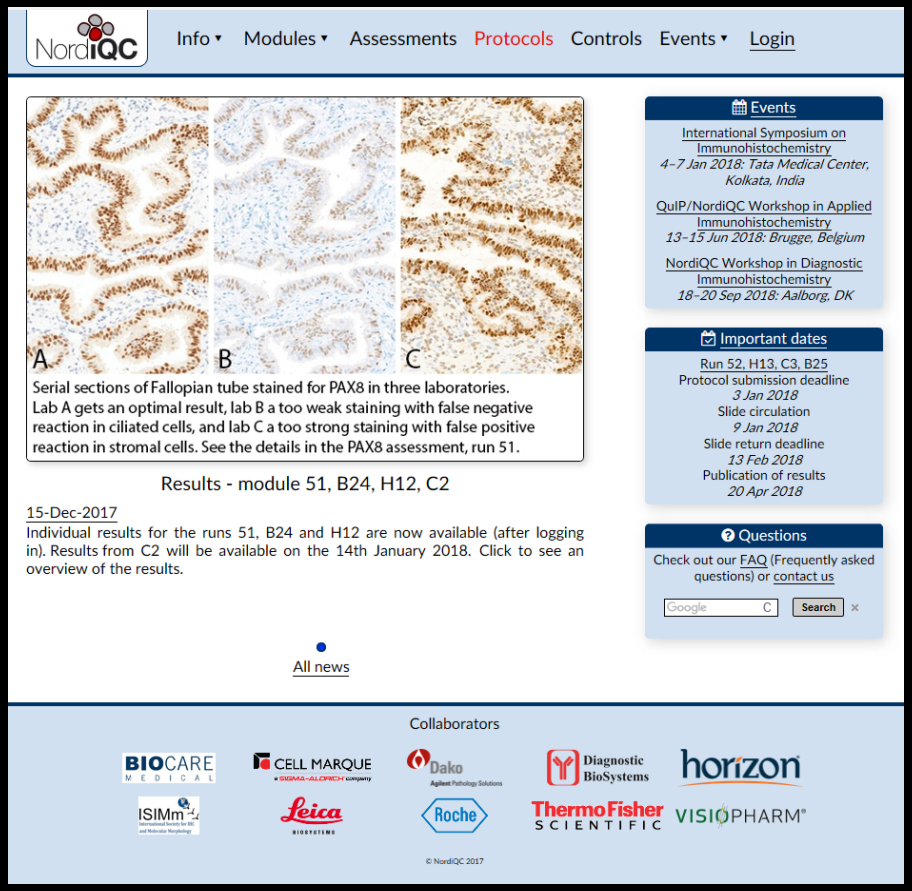
Fig. 2a (x100)
Optimal staining reaction for S100 of the tonsil using the same protocol as in Fig. 1a. The majority of the interfollicular dendritic and Langerhans cells in the squamous epithelium (crypts) display strong staining reaction, while the follicular dendritic cells in germinal centres show a weak to moderate but distinct nuclear and cytoplasmic staining reaction - compare with Fig. 2b.

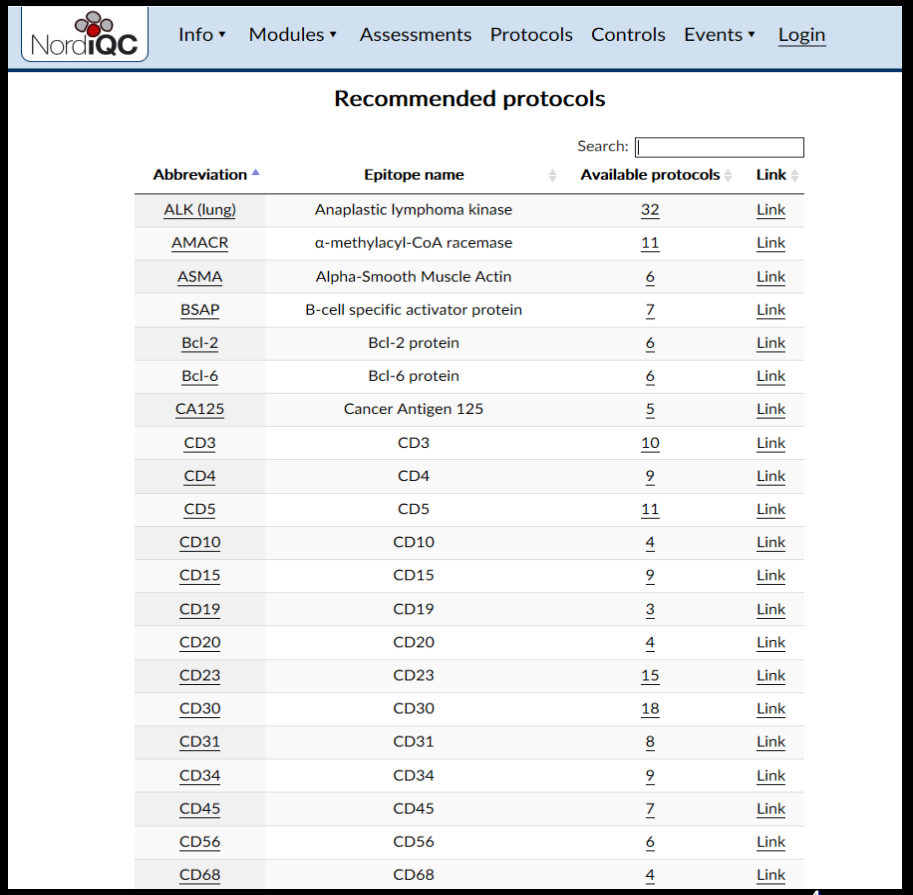
Fig. 2b (x100)
Insufficient staining reaction for S100 of the tonsil using same protocol as in Fig. 1b - same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced.

# QA Undifferented tumours and lymphomas

IHC assays to be used in diagnostics;

Best practice Antibodies/Clones
Best practice positive and negative tissue controls



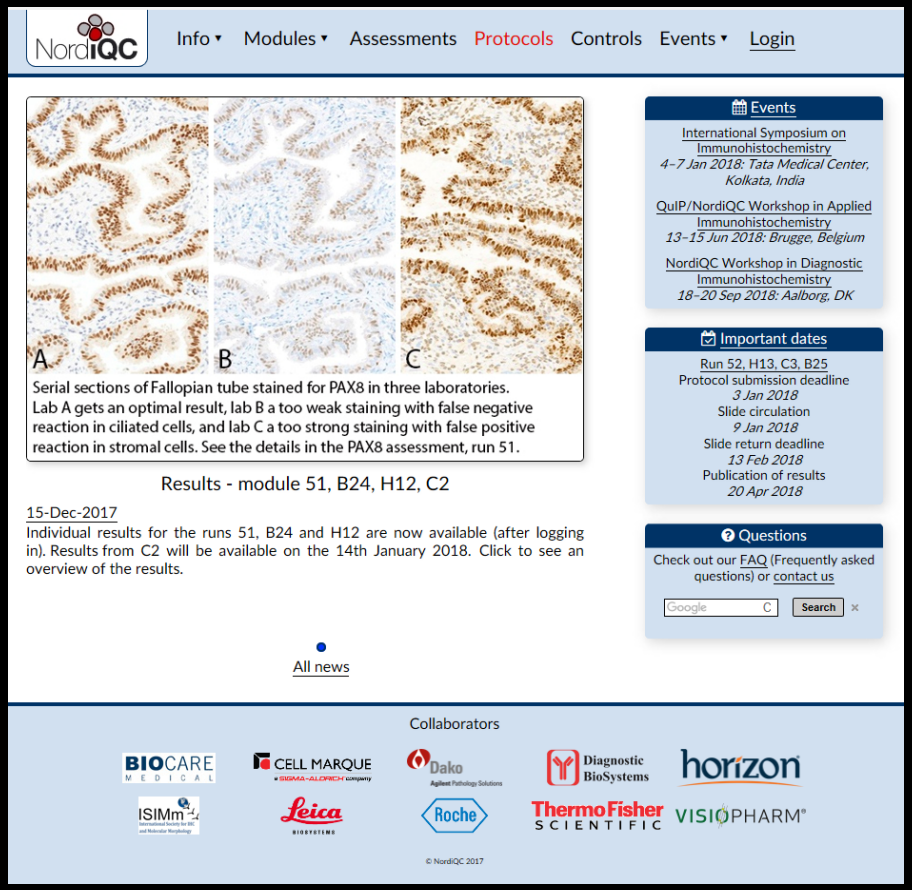



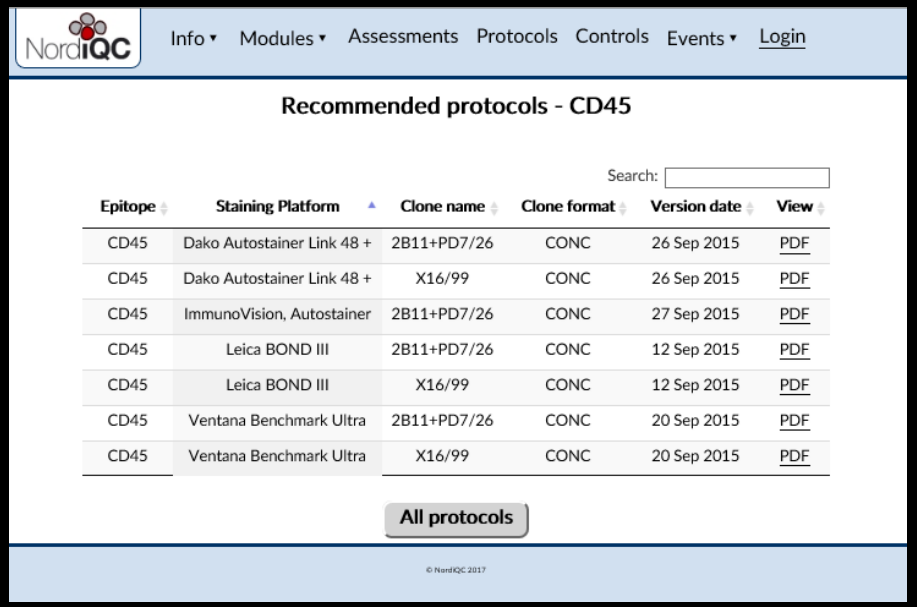

| Abbreviation | Epitope name | Available protocols | Link |
|---|---|---|---|
| ALK (lung) | Anaplastic lymphoma kinase | 32 | Link |
| AMACR | α-methylacyl-CoA racemase | 11 | Link |
| ASMA | Alpha-Smooth Muscle Actin | 6 | Link |
| BSAP | B-cell specific activator protein | 7 | Link |
| Bcl-2 | Bcl-2 protein | 6 | Link |
| Bcl-6 | Bcl-6 protein | 6 | Link |
| CA125 | Cancer Antigen 125 | 5 | Link |
| CD3 | CD3 | 10 | Link |
| CD4 | CD4 | 9 | Link |
| CD5 | CD5 | 11 | Link |
| CD10 | CD10 | 4 | Link |
| CD15 | CD15 | 9 | Link |
| CD19 | CD19 | 3 | Link |
| CD20 | CD20 | 4 | Link |
| CD23 | CD23 | 15 | Link |
| CD30 | CD30 | 18 | Link |
| CD31 | CD31 | 8 | Link |
| CD34 | CD34 | 9 | Link |
| CD45 | CD45 | 7 | Link |
| CD56 | CD56 | 6 | Link |
| CD68 | CD68 | 4 | Link |

# QA Undifferented tumours and lymphomas

IHC assays to be used in diagnostics;

Best practice Antibodies/Clones
Best practice positive and negative tissue controls



# QA Undifferented tumours and lymphomas

**Nordic Immunohistochemical Quality Control**
Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

## Recommended protocol for CD45

Obtained in run

26 Sep 2015

**Immunostainer**

| | |
|---|---|
| Type: | Dako Autostainer Link 48 + |

**Primary antibody**

| | |
|---|---|
| Clone: | 2B11+PD7/26 |
| Producer: | Dako |
| Product no. / lot no.: | M0701 / NS |
| Diluent: | Antibody Diluent |
| Dilution factor: | 1:200 |
| Incubation time / temperature: | 30 min. / 20°C |

**Epitope retrieval, HIER**

| | |
|---|---|
| Device: | PT-link / PT-module |
| Buffer: | Dako TRS High pH (3-1) |
| Heating time at max. temp.: | 20 min. |
| Maximum heating temp.: | 97°C |

**Visualization system**

| | |
|---|---|
| Producer: | Dako |
| Product / no: | EnVision FLEX / K8000/SM802 |
| Linker: | None |
| Incubation time polymer: | 20 min. |
| Incubation temperature: | 20°C |

**Chromogen**

| | |
|---|---|
| Producer: | Dako |
| Product / no: | DAB+ / DM827 / DM823 / K800 |
| Incubation time / temperature: | 10 min. / 20°C |
| Enhancement: | None |

**Disclaimer:**
NordiQC makes every attempt to provide accurate and up-to-date information, yet NordiQC does not make any claim or warranty regarding the accuracy of the provided information nor does it represent that the contents of the web site and protocols reflect the most recent developments in immunohistochemistry at any point in time.

**Nordic Immunohistochemical Quality Control**
Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

## Recommended protocol for CD45

Obtained in run

12 Sep 2015

**Immunostainer**

| | |
|---|---|
| Type: | Leica BOND III |

**Primary antibody**

| | |
|---|---|
| Clone: | 2B11+PD7/26 |
| Producer: | Dako |
| Product no. / lot no.: | M0701 / 00062209 |
| Diluent: | Bond Antibody Diluent |
| Dilution factor: | 1:350 |
| Incubation time / temperature: | 15 min. / 20°C |

**Epitope retrieval, HIER**

| | |
|---|---|
| Device: | On Board / On Machin |
| Buffer: | Leica Bond Epitope Retrieval Solution 2 |
| Heating time at max. temp.: | 20 min. |
| Maximum heating temp.: | 100°C |

**Visualization system**

| | |
|---|---|
| Producer: | Leica |
| Product / no: | Bond Refine / DS9800 |
| Incubation time linker: | 8 min. |
| Incubation time polymer: | 8 min. |
| Incubation temperature: | 20°C |

**Chromogen**

| | |
|---|---|
| Producer: | Leica |
| Product / no: | Bond Refine / DS9800 |
| Incubation time / temperature: | 10 min. / 20°C |
| Enhancement: | None |

**Disclaimer:**
NordiQC makes every attempt to provide accurate and up-to-date information, yet NordiQC does not make any claim or warranty regarding the accuracy of the provided information nor does it represent that the contents of the web site and protocols reflect the most recent developments in immunohistochemistry at any point in time.

# IHC – Protocols and controls for UPT I

| 1' panel | Recommendable clones (conc.) | Less successful clones (conc.) | RTU "plug and play" giving optimal result |
|---|---|---|---|
| CK-PAN | mAb AE1/AE3<br>mAb AE1/AE3/5D3<br>mAb BS5 | mAb C-11<br>mAb KL1*<br>mAb Lu-5<br>mAb MNF116<br>mAb "Oscar" | Dako: mAb AE1/AE3<br>VMS: mAb AE1/AE3/PCK26 |
| CD45 | mAb 2B11+PD7/26<br>mAb X1699 | | Dako: mAb 2B11+PD7/26<br>Leica: mAb X1699 |
| S100(B) | pAbs (e.g. Z0311) | mAb 15E2E2<br>mAb 4C7 | ……. |
| VIM | mAb V9<br>mAb 3B4<br>rmAb SP20 | | Dako: mAb V9<br>VMS: mAb V9 |

* Discontinued

# IHC – Protocols and controls for UPT I

| 1' panel | Positive tissue control HE* | Positive tissue control LE** | Negative tissue control NE*** |
|---|---|---|---|
| CK-PAN | Liver: Epithelial cells of bile ducts<br>Tonsil: Squamous epithelial cells | Liver: Hepatocytes<br>Tonsil: Squamous epithelial cells | Liver: Stroma<br>Tonsil: Lymphocytes |
| CD45 | Tonsil: T- and B-cells | Liver: Kupffer cells | Tonsil: Epithelial cells<br>Liver: Hepatocytes |
| S100(B) | Appendix: Nerves | Tonsil: Germinal centre dendritic cells# | Appendix: Epithelial cells |
| VIM | Appendix: Endothelial cells | Appendix: Intra-epithelial T-cells | Appendix: Epithelial cells |

* HE = High expression

** LE = Low expression

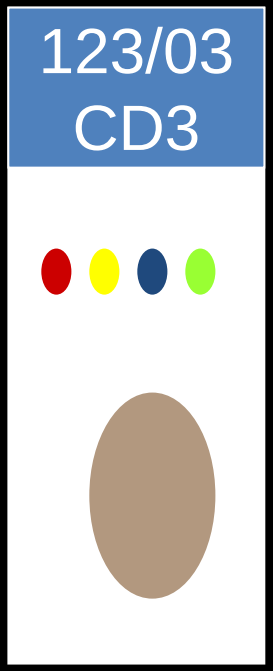
*** NE = No expression

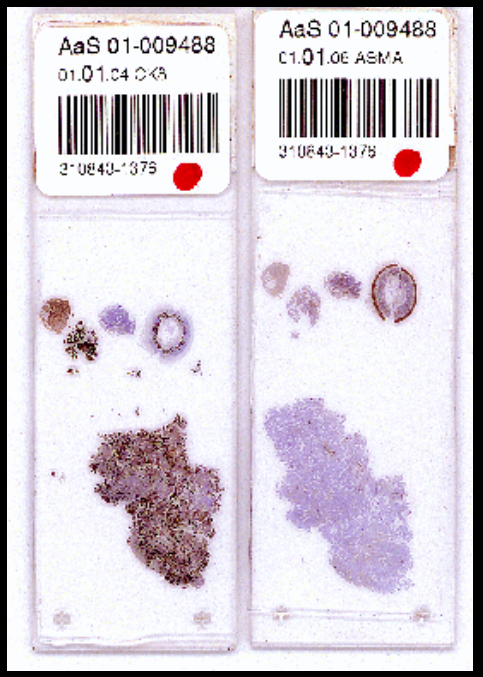
# pAb reacting with S100 A1, most likely

# IHC – The Technical Test Approach

"Ideal" daily <u>on-slide</u> control for the majority of routine markers:

Appendix
Liver
Pancreas
Tonsil



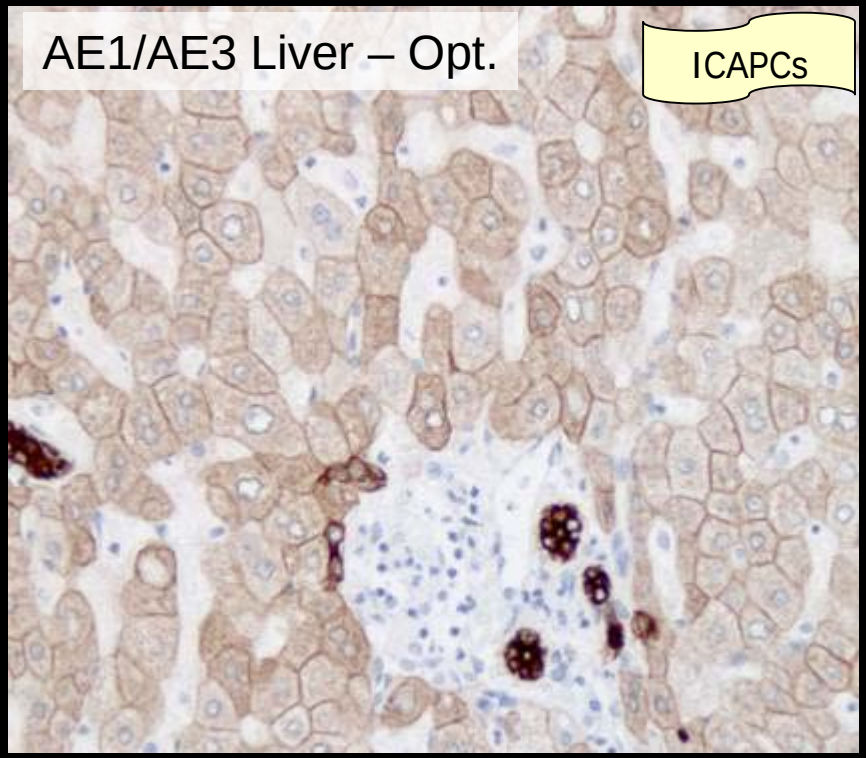




Each slide stained and evaluated has essential information of the obtained sensitivity and specificity

In contrast only using 1 external tissue run control, no information is available for the single slide evaluated

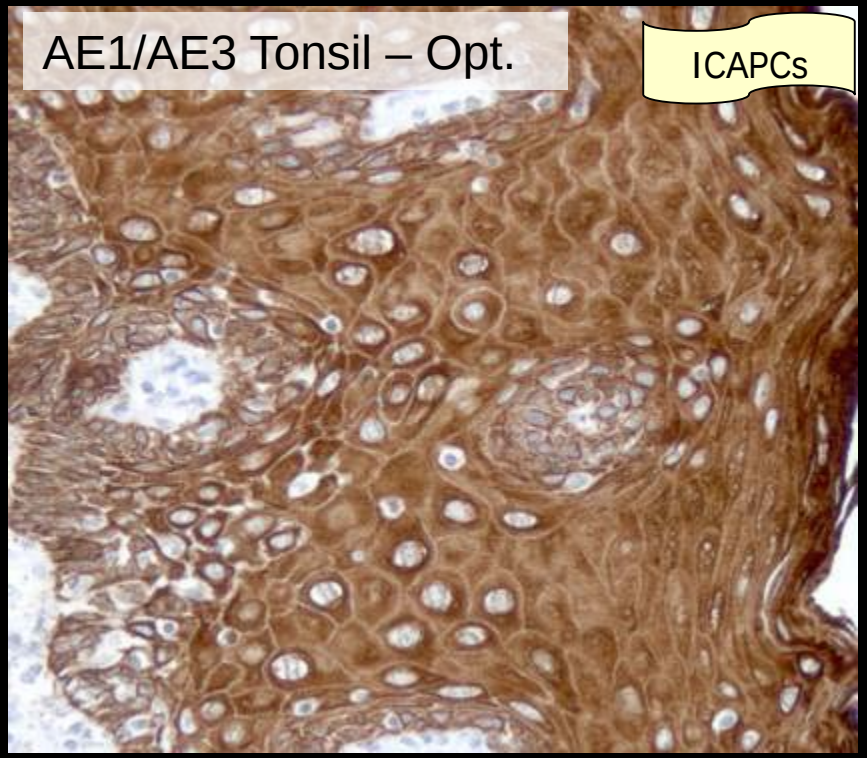
# IHC – Protocols and controls for UPT I

## CK LMW types
## AE1/AE3: 7, **8**, 19




A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and at least a moderate cytoplasmic staining reaction withmembrane accentuation of the vast majority of hepatocytes.

## CK HMW types
## AE1/AE3: 1, 4, **5**, 10, **14**



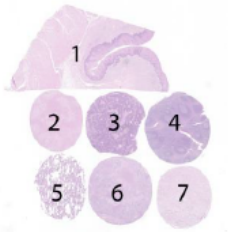

A strong, distinct cytoplasmic staining reaction of virtually all squamous epithelial cells throughout all cell layers.

# IHC – Protocols and controls for UPT I

**Assessment Run 47 2016**

## Pan Cytokeratin (CK-PAN)

**Material**
The slide to be stained for CK-PAN comprised:
1. Esophagus, 2. Liver, 3. Small cell lung carcinoma (SCLC), 4. Tonsil,
5. Lung adenocarcinoma, 6. Lung squamous cell carcinoma, 7. Renal clear cell carcinoma (RCC).




Criteria for assessing a CK-PAN staining as optimal were:

- A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and at least a moderate cytoplasmic staining reaction with membrane accentuation of the vast majority of hepatocytes.
- A strong, distinct cytoplasmic staining reaction of all squamous epithelial cells throughout all cell layers in the esophagus.
- A strong, distinct cytoplasmic staining reaction of the majority of neoplastic cells in the lung adenocarcinoma and squamous cell carcinoma.
- An at least moderate, distinct cytoplasmic, dot-like staining reaction of the majority of neoplastic cells in the SCLC.
- An at least weak to moderate, distinct cytoplasmic and membranous staining reaction of the majority of neoplastic cells in the RCC.

All tissues were fixed in 10% neutral buffered formalin.

**Participation**

| | |
|---|---|
| Number of laboratories registered for CK-PAN, run 47 | 298 |
| Number of laboratories returning slides | 276 (93%) |

**Results**
276 laboratories participated in this assessment. One laboratory used an inappropriate antibody (CK-HMW). Of the remaining 275 laboratories, 72% achieved a sufficient mark. Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Insufficient HIER – too short efficient heating time and/or use of non-alkaline HIER buffers
- Inappropriate epitope retrieval
- Less successful primary antibodies.

**Performance history**
This was the eighth NordiQC assessment of CK-PAN. The overall pass rate was slightly improved compared to previous runs performed, as shown in table 2.

Table 2. **Proportion of sufficient results for CK-PAN in the eight NordiQC runs performed**

| | Run 8 2003 | Run 15 2005 | Run 20 2008 | Run 24 2008 | Run 30 2010 | Run 36 2012 | Run 41 2014 | Run 47 2016 |
|---|---|---|---|---|---|---|---|---|
| Participants, n= | 72 | 85 | 103 | 123 | 168 | 202 | 233 | 275 |
| Sufficient results | 53% | 58% | 62% | 60% | 65% | 65% | 67% | 72% |

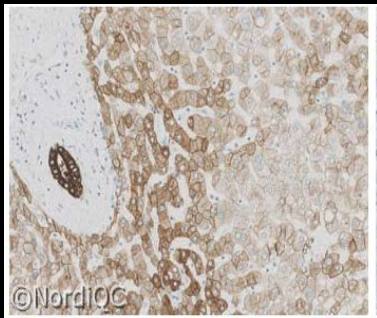



Fig. 1a. Optimal staining for CK-Pan of the liver based on HIER and the mAb clone cocktail AE1/AE3. The majority of the hepatocytes show a distinct, moderate to strong staining with membrane enhancement, while the columnar epithelial cells of the bile duct show a strong cytoplasmic staining. Compare with Figs. 2a-4a, same protocol.

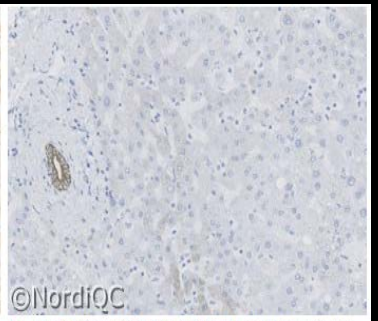



Fig. 1b. Insufficient CK-Pan staining of the liver, using an efficient HIER and Ab clone KL1 but applying the Ab in a too low concentration - same field as in Fig. 1a. Only the epithelial cells of the bile duct are demonstrated, while the hepatocytes are unstained. Compare with Figs. 2b-4b, same protocol.

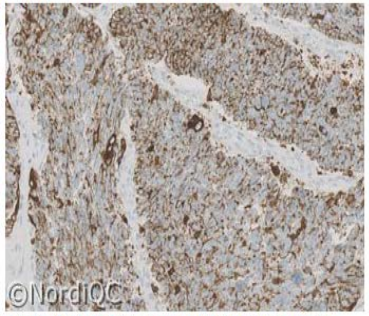



Fig. 2a. Optimal CK-pan staining of the small cell lung carcinoma using same protocol as in Figs. 1a, 3a and 4a. The majority of the neoplastic cells show a moderate, distinct dot-like cytoplasmic staining.

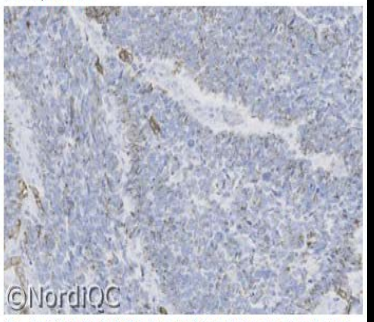



Fig. 2b. Insufficient CK-Pan staining of the small cell lung carcinoma using same protocol as in Figs. 1b, 3b and 4b - same field as in Fig. 2a. Only scattered neoplastic cells show a weak staining reaction. Also compare with Figs. 3b. & 4b., same protocol.

Too weak or false negative result is most commonly observed in the insufficient results.

# IHC – Protocols and controls for UPT I

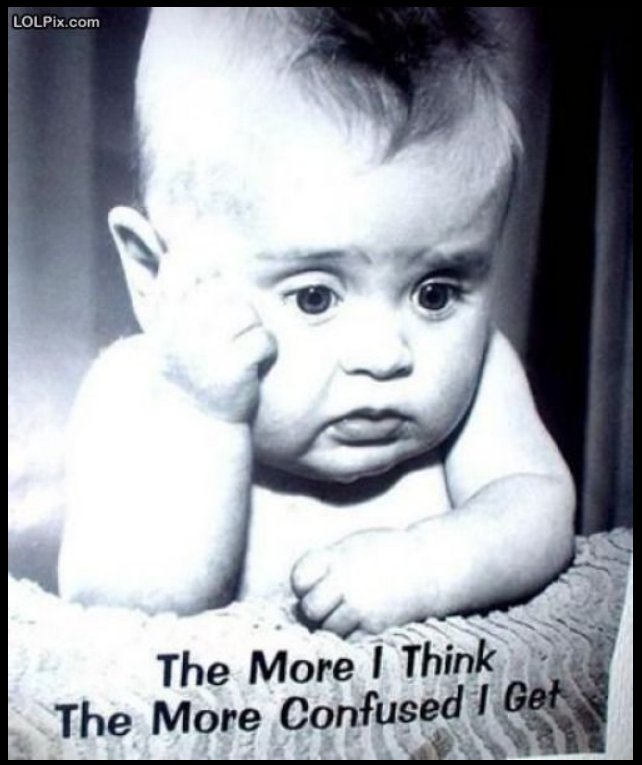
Table 1. **Antibodies and assessment marks for CK-PAN, run 47**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone cocktail **AE1/AE3** | 85 | Dako/Agilent | 33 | 28 | 17 | 7 | 72% | 83% |
| | 11 | Thermo/NeoMarkers | 2 | 3 | 4 | 2 | 45% | 100% |
| | 5 | Cell Marque | 0 | 3 | 0 | 2 | 60% | - |
| | 5 | Leica/Novocastra | 1 | 0 | 3 | 1 | 20% | 50% |
| | 3 | Biocare | 1 | 2 | 0 | 0 | - | - |
| | 2 | Zytomed | 0 | 2 | 0 | 0 | - | - |
| | 1 | Biosystems | 1 | 0 | 0 | 0 | - | - |
| | 1 | Genemed | 0 | 1 | 0 | 0 | - | - |
| | 1 | Gennova | 0 | 1 | 0 | 0 | - | - |
| | 1 | Immunologic | 0 | 0 | 1 | 0 | - | - |
| | 1 | Millipore | 1 | 0 | 0 | 0 | - | - |
| | 1 | Monosan | 0 | 0 | 0 | 0 | - | - |
| mAb clone cocktail **AE1/AE3/ks 13.2** | 1 | Linaris | 0 | 0 | 0 | 1 | - | - |
| mAb clone cocktail **AE1/AE3/5D3** | 2<br>1 | Biocare<br>Zytomed | 1 | 2 | 0 | 0 | - | - |
| mAb clone cocktail **PAN CK Ab-2** | 1 | Thermo/NeoMarkers | 0 | 1 | 0 | 0 | - | - |
| mAb clone **BS5** | 1<br>1 | Monosan<br>Nordic Biosite | 2 | 0 | 0 | 0 | - | - |
| mAb clone **C-11** | 1 | Leica/Novocastra | 0 | 0 | 0 | 1 | - | - |
| mAb clone **Lu-5** | 2 | Immunologic | 0 | 0 | 1 | 1 | - | - |
| mAb clone **MNF116** | 7 | Dako/Agilent | 0 | 0 | 4 | 3 | 0% | - |
| mAb clone **OSCAR** | 1<br>1 | Signet<br>"In-house" | 0 | 2 | 0 | 0 | - | - |
| Unknown | 3 | | 1 | 1 | 0 | 1 | - | - |
| "Laboratory made" antibody cocktails | | | | | | | | |
| mAb clone cocktail **AE1/AE3/5D3** | 2 | Leica/Novocastra & Milipore | 1 | 1 | 0 | 0 | - | - |
| mAb clone cocktail **AE1/AE3/5D3** | 1 | Leica/Novocastra | 1 | 0 | 0 | 0 | - | - |
| mAb clone cocktail **AE1AE3/CAM5.2** | 1 | Dako/Agilent & BD | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone cocktail **AE1/AE3 IR053** | 36 | Dako/Agilent | 28 | 5 | 2 | 1 | 92% | 95% |
| mAb clone cocktail **AE1/AE3 GA053** | 19 | Dako/Agilent | 18 | 0 | 1 | 0 | 95% | 100% |
| mAb clone cocktail **AE1/AE3 313M-18** | 3 | Cell Marque | 0 | 1 | 0 | 2 | - | - |
| mAb clone cocktail **AE1/AE3 MAD 001000QD** | 1 | Master Diagnostica | 1 | 0 | 0 | 0 | - | - |
| mAb clone cocktail **AE1/AE3 Kit-0009** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| mAb clone cocktail **AE1/AE3 PA0909** | 5 | Leica/Novocastra | 0 | 1 | 3 | 1 | 20% | - |
| mAb clone cocktail **AE1/AE3 RTU-AE1/AE3** | 2 | Leica/Novocastra | 0 | 0 | 2 | 0 | - | - |
| mAb clone cocktail **AE1/AE3/5D3 IP162** | 2 | Biocare | 1 | 1 | 0 | 0 | - | - |
| mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595** | 62 | Ventana/Roche | 37 | 8 | 5 | 12 | 73% | 96% |
| rmAb clone cocktail **EP24/EP67/B22.1/B23.1 MAD-000680QD** | 2 | Master Diagnostica | 0 | 2 | 0 | 0 | - | - |
| Total | 275 | | 132 | 65 | 43 | 35 | - | |
| Proportion | | | 48% | 24% | 16% | 12% | 72% | |

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

Clone/Retrieval/Titre/Control

Too many choices
Misleading datasheets

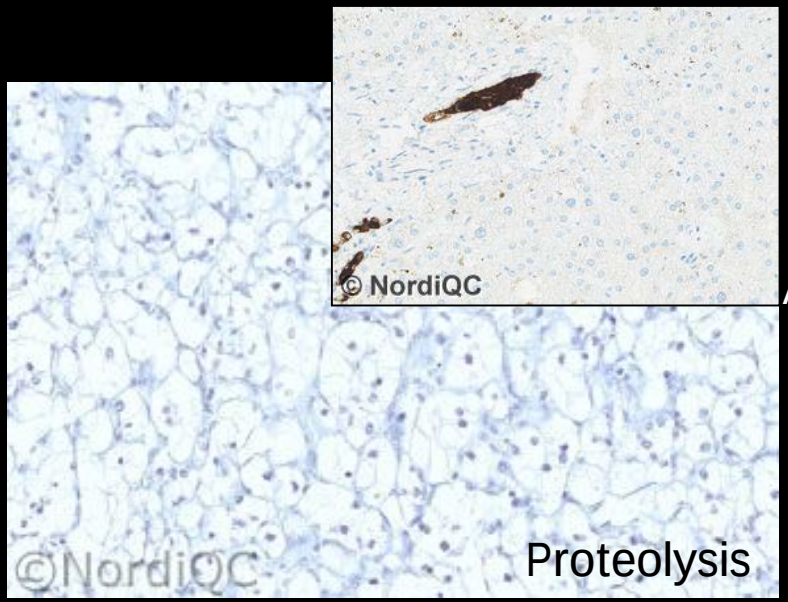


# IHC – Protocols and controls for UPT I

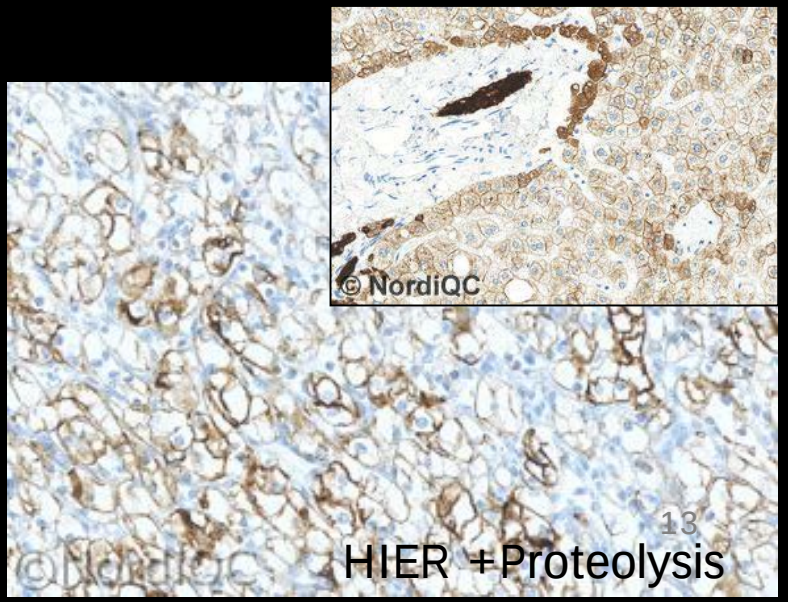
Clone/Retrieval/Titre/Control

Table 4. **Pass rates for antibody cocktails combined with epitope retrieval methods in seven NordiQC runs**

| | Pass rate for run 15, 20, 24, 30, 36, 41 & 47 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | | HIER | | Proteolysis | | HIER + proteolysis | |
| | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient |
| **mAb AE1/AE3** | 752 | 542 (72%) | 693 | 535 (77%) | 44 | 5 (11%) | 5 | 2 (40%) |
| **mAb AE1/AE3/5D3** | 37 | 34 (92%) | 36 | 34 (94%) | 1 | 0 | 0 | 0 |
| **mAb AE1/AE3/PCK26** | 176 | 105 (60%) | 25 | 13 (48%) | 34 | 0 | 117 | 92 (79%) |
| **mAb MNF116** | 91 | 30 (33%) | 40 | 9 (23%) | 47 | 21 (45%) | 4 | 2 (50%) |




RCC
AE1/AE3/PCK26



# IHC – Protocols and controls for UPT I

**Performance history**

This was the eighth NordiQC assessment of CK-PAN. The overall pass rate was slightly improved compared to previous runs performed, as shown in table 2.

Table 2. **Proportion of sufficient results for CK-PAN in the eight NordiQC runs performed**

| | Run 8 2003 | Run 15 2005 | Run 20 2008 | Run 24 2008 | Run 30 2010 | Run 36 2012 | Run 41 2014 | Run 47 2016 |
|---|---|---|---|---|---|---|---|---|
| Participants, n= | 72 | 85 | 103 | 123 | 168 | 202 | 233 | 275 |
| Sufficient results | 53% | 58% | 62% | 60% | 65% | 65% | 67% | 72% |

Too many choices

**Misleading data sheets**

Wrong control material used

AE1/AE3 : Optimal results only obtained by HIER in NordiQC runs

Dako: RTU – HIER
Leica: RTU – Proteolysis
Thermo:

Conc: Proteolysis or HIER
Conc: HIER
Conc: HIER Quanto – Proteolysis UltraVision

.....

AE1/AE3/PCK26: Optimal results mainly obtained by HIER+protelysis in NordiQC runs

VMS: RTU - Proteolysis

**Till 2015**

# IHC – Protocols and controls for UPT I

## STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on the VENTANA BenchMark ULTRA, BenchMark XT and BenchMark GX automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 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 instruments 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-Pan Keratin (AE1/AE3/PCK26) with *ultra*View Universal DAB Detection Kit on a BenchMark ULTRA instrument, BenchMark XT instrument or BenchMark GX instrument.

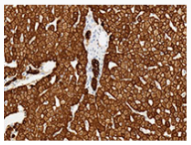
| Procedure Type | Method |
|---|---|
| **Deparaffinization** | Selected |
| **Cell Conditioning (Antigen Unmasking)** | Cell Conditioning 1, Mild |
| **Enzyme (Protease)** | Protease 3, 4 minutes |
| **Antibody (Primary)** | BenchMark ULTRA instrument 8 minutes, 36°C<br>BenchMark XT instrument 8 minutes, 37°C<br>BenchMark GX instrument 4 minutes, 37°C |
| **ultraBlock** | *VENTANA Antibody Diluent with Casein, 4 minutes |
| **Counterstain** | Hematoxylin II, 4 minutes |
| **Post Counterstain** | Bluing, 4 minutes |

*Use of VENTANA Antibody Diluent with Casein (Cat. No. 760-219/06440002001) at the ultraBlock step is recommended to reduce staining on smooth muscle.

Home > Products > Product Catalog > Primary Antibodies
> Pan Keratin (AE1/AE3/PCK26) Primary Antibody

Print Share

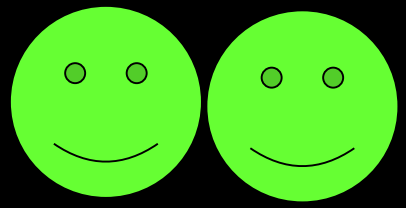
### *anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody*



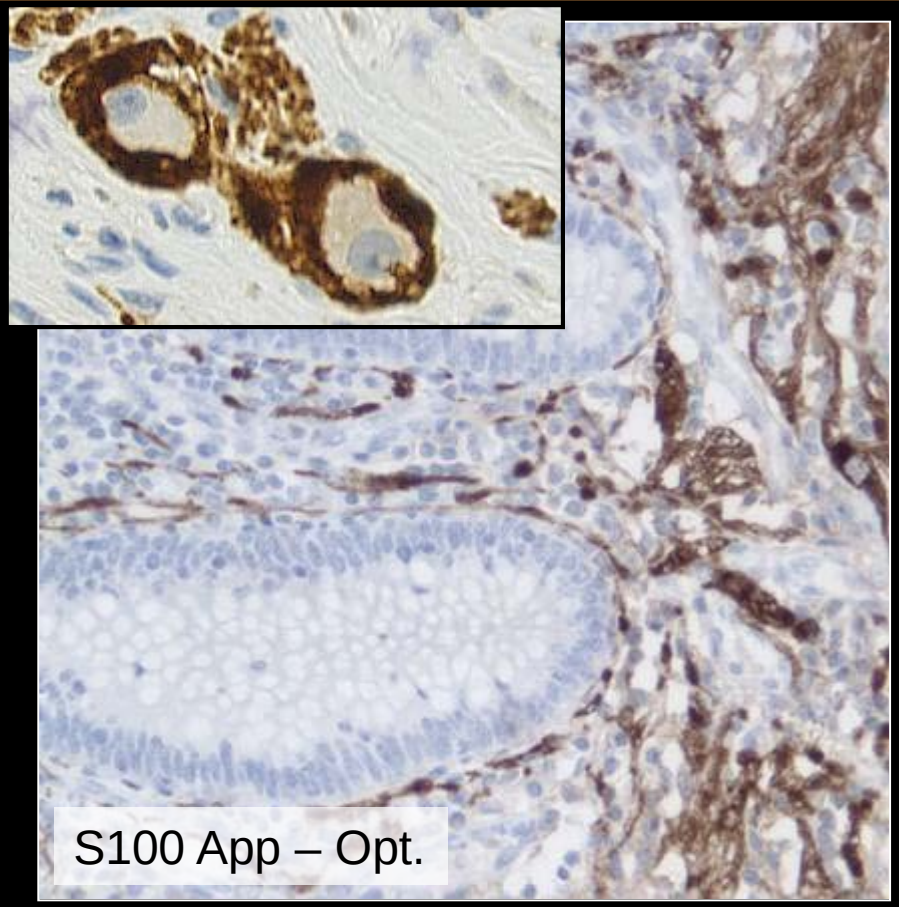
| | |
|---|---|
| **Catalog Number:** | 760-2135 |
| **Ordering Code:** | 05266840001 |
| **Quantity:** | 250 tests |
| **Controls:** | Intestine, Liver |
| **Isotypes:** | $IgG_1$ |
| **Clone Name:** | AE1/AE3 & PCK26 |
| **Species:** | Mouse |
| **Localization:** | Cytoplasmic |
| **Regulatory Status:** | IVD |

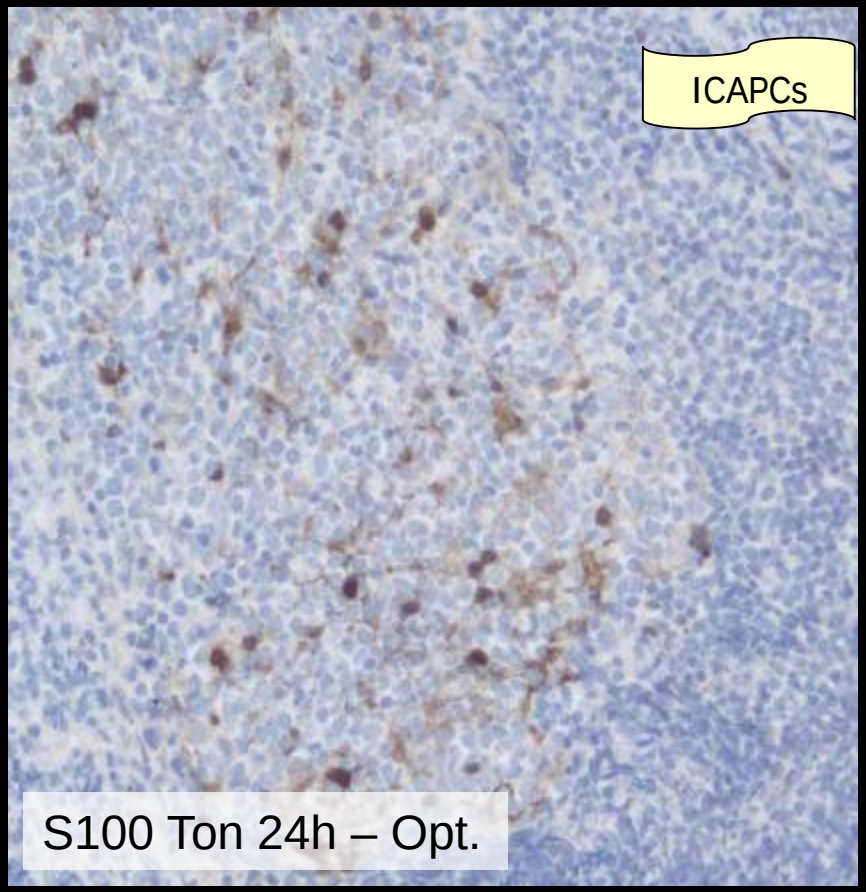
*Related Links*
Cytokeratin (Pan) MSDS/SDS
Package Inserts

This antibody is intended for in vitro diagnostic use. Ventana Medical Systems, Inc. (Ventana) anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody may be used to aid in the identification of normal and abnormal epithelial cells and to determine the lineage of poorly differentiated malignant tumors. The keratins are a group of intermediate filament proteins that occur in normal and neoplastic cells of epithelial origin. Nineteen human cytokeratins are known which are divided into acidic and basic subfamilies. They occur in pairs in epithelial tissues, the composition of pairs varying with the epithelial cell type, stage of differentiation, cellular growth environment, and disease state. This pan keratin cocktail recognizes most of the acidic and all of the basic cytokeratins, making it a useful stain for nearly all epithelial tissues and their tumors. Anti-Pan Keratin (AE1/AE3/PCK26) specifically binds to antigens located in the cytoplasm of simple and complex epithelial cells. The antibody is intended for laboratory use to qualitatively stain cytokeratins in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer. Anti-Pan Keratin (AE1/AE3/PCK26) contains a mouse monoclonal antibody cocktail raised against an epitope found on human epidermal keratins as reported by Woodcock-Mitchell, et al.1 This antibody cocktail reacts with the 56.5kD, 50kD, 50'kD, 48kD, and 40kD cytokeratins of the acidic subfamily and 65-67kD, 64kD, 59kD, 58kD, 56kD, and 52kD cytokeratins of the basic subfamily.1,2,3,4,5 Unexpected antigen expression or loss of expression may occur, especially in neoplasms. Occasionally stromal elements surrounding heavily stained tissue and or cells will show immunoreactivity. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Caution: U.S. Federal law restricts this device to sale by or on the order of a physician.



# IHC – Protocols and controls for UPT I



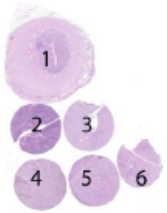

A strong, distinct nuclear and cytoplasmic staining reaction of the macrophages in lamina propria, the Schwann cells of the peripheral nerve fibres and the ganglionic satellite cells in the muscularis propria and submucosa in the appendix. The epithelial cells and muscle cells should be negative.

An at least weak but distinct nuclear and cytoplasmic staining reaction of the follicular dendritic cells in the germinal centres (most likely due to reaction to S100A and thus mainly seen for pAbs to S100).

# for UPT I

## Assessment Run 50 2017
## S100

**Material**
The slide to be stained for S100 comprised:




1. Appendix, 2. Tonsil, 3. Schwannoma, 4-5. Malignant melanoma, 6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of Schwann cells of peripheral nerve fibres and ganglionic satellite cells in the muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of adipocytes and macrophages in all specimens.
- A strong, distinct nuclear and cytoplasmic staining reaction of virtually all neoplastic cells of the malignant melanomas (cores 4-5) and the Schwannoma.
- A weak to moderate, cytoplasmic and nuclear staining reaction of the follicular dendritic cells in the germinal centres of the tonsil and the Peyer's plaques in the appendix.
- No staining of other cells. The neoplastic cells in the colon adenocarcinoma, squamous epithelial cells in the tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative.

**Participation**

| | |
|---|---|
| Number of laboratories registered for S100, run 50 | 316 |
| Number of laboratories returning slides | 299 (95%) |

**Results**
299 laboratories participated in this assessment. 245 (82%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful Ready-To-Use system based on the mAb clone 4C4.9 (Roche/Ventana)
- Insufficient HIER (too low temperature and/or too short efficient heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Unexplained technical issues

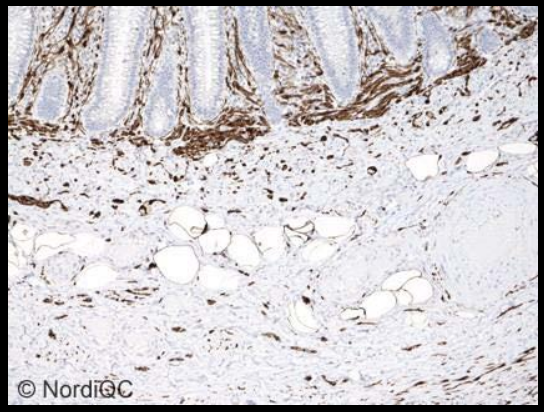
**Performance history**
This was the fifth NordiQC assessment of S100. The overall pass rate was higher compared to all previous runs for S100 (see Table 2).

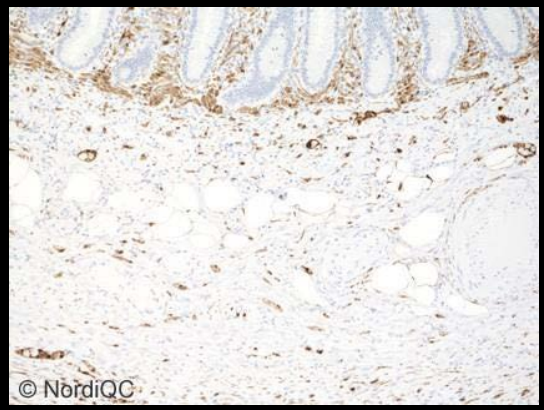
Table 2. **Proportion of sufficient results for S100 in the five NordiQC runs performed**

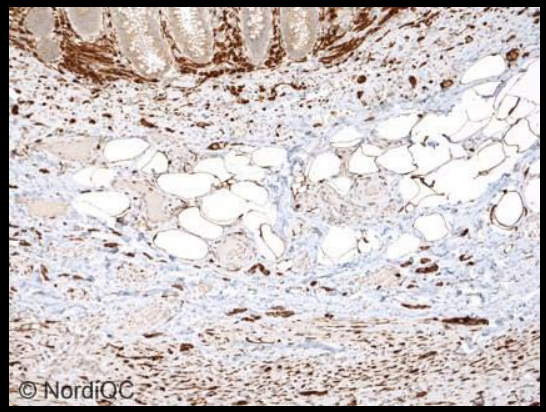
| | Run 7 2003 | Run 20 2007 | Run 34 2012 | Run 45 2015 | Run 50 2017 |
|---|---|---|---|---|---|
| Participants, n= | 63 | 106 | 200 | 251 | 299 |
| Sufficient results | 71% | 75% | 64% | 68% | 82% |

**Conclusion**
pAbs both as concentrated and Ready-To-Use (RTU) formats were most successful for immunohistochemical demonstration of S100. Laboratory developed assays based on pAb **Z0311** provided the highest proportion of optimal results. Irrespective of the primary Ab applied, efficient HIER, use of appropriate titre and incubation time tailored to the choice of IHC system were the most important prerequisites for a sufficient staining result. Omission of HIER and/or use of proteolytic pre-treatment provided inferior performance.
Appendix is recommended as positive and negative tissue control: The Schwann cells of peripheral nerves, macrophages/dendritic cells and adipocytes must show a moderate to strong distinct nuclear and





# IHC – Protocols and controls for UPT I

Table 1. **Antibodies and assessment marks for S100, run 50**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **4C4.9** | 2<br>2<br>2<br>1 | Immunologic<br>Zytomed Systems<br>Cell Marque<br>Thermo/NeoMarkers | 0 | 3 | 4 | 0 | 43% | - |
| mAb clone **15E2E2** | 1<br>1 | Biogenex<br>Biocare | 0 | 1 | 1 | 0 | - | - |
| mAb clone **15E2E2+4C4.9** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| pAb **Z0311** | 137 | Agilent/Dako | 62 | 60 | 14 | 1 | 89% | 97% |
| pAb **NCL-L-S100p** | 10 | Leica/Novocastra | 1 | 6 | 3 | 0 | 70% | 100% |
| pAb **RB-9018-P** | 1 | Thermo/NeoMarkers | 0 | 0 | 1 | 0 | - | - |
| pAb **RP035** | 1 | Diagnostic Biosystems | 0 | 0 | 1 | 0 | - | - |
| **Unknown** | 1 | - | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **4C4.9 790-2914** | 36 | Roche/Ventana | 0 | 20 | 16 | 0 | 56% | - |
| mAb clone **4C4.9 330M-18** | 2 | Cell Marque | 0 | 2 | 0 | 0 | - | - |
| mAb clone **4C4.9 MAD-001221QD** | 3 | Master Diagnostica | 0 | 2 | 1 | 0 | - | - |
| mAb clone **4C4.9 MON-RTU1191** | 1 | Monosan/Sanbio | 0 | 1 | 0 | 0 | - | - |
| mAb clone **4C4.9 KIT-0007** | 1 | Maixin | 0 | 0 | 1 | 0 | - | - |
| mAb clone **15E2E2+4C4.9 PM089** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP32 AN713** | 1 | Biogenex | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP32 8442-C010** | 1 | Sakura | 0 | 1 | 0 | 0 | - | - |
| pAb **IS/IR504** | 26 | Agilent/Dako | 0 | 22 | 4 | 0 | 85% | - |
| pAb **IS/IR504**[3] | 5 | Agilent/Dako | 0 | 5 | 0 | 0 | 100% | - |
| pAb **GA504** | 21 | Agilent/Dako | 1 | 19 | 1 | 0 | 95% | 100% |
| pAb **GA504**[4] | 6 | Agilent/Dako | 3 | 2 | 0 | 1 | 83% | - |
| pAb **760-2523** | 28 | Roche/Ventana | 0 | 23 | 2 | 3 | 82% | - |
| pAb **PA0900** | 6 | Leica/Novocastra | 0 | 6 | 0 | 0 | 100% | - |
| pAb **E031** | 1 | Linaris | 0 | 1 | 0 | 0 | - | - |
| Total | 299 | | 67 | 178 | 49 | 5 | - | |
| Proportion | | | 23% | 59% | 16% | 2% | 82% | |

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

82% sufficient

If using pAb Z0311 a titre of 1:1.000-4.000 & HIER:

97% sufficient
61% optimal

Prot. / omission:

75% sufficient.
8% optimal.

Typically false negative, too weak and/or impaired morphology

# IHC – Protocols and controls for UPT I

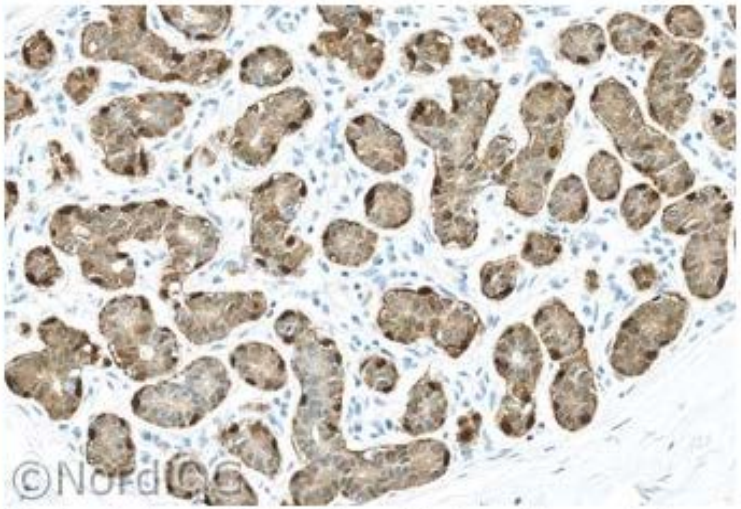



Fig. 3a. Optimal S100 staining of the breast hyperplasia using same protocol as in Figs. 1a & 2a. The myoepithelial cells show a moderate to strong cytoplasmic and nuclear staining reaction. A weaker staining of the secretory cells is seen, but no background staining is seen.

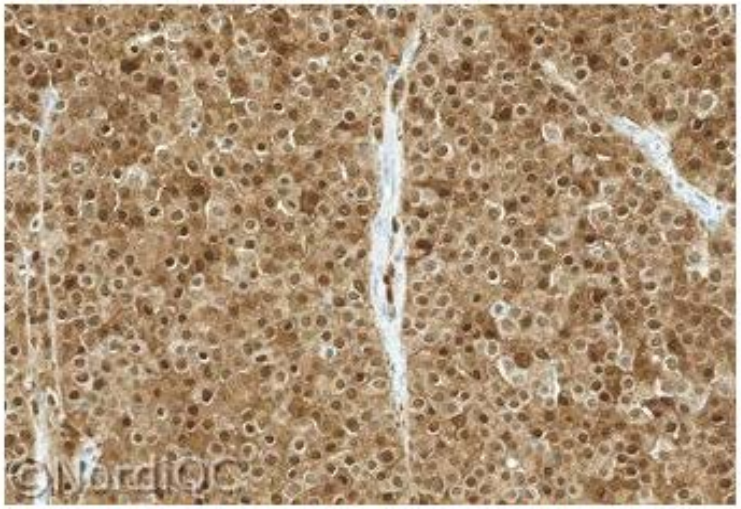



Fig. 4a. Optimal S100 staining of the malignant melanoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic and nuclear staining reaction.

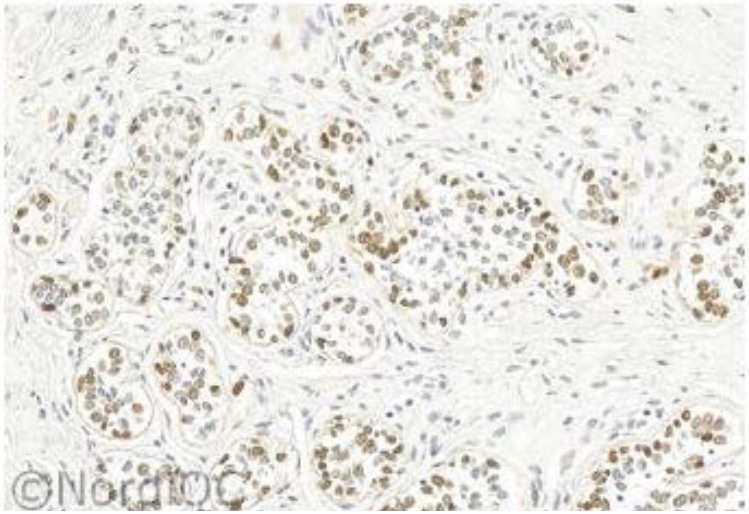



Fig. 5a. Insufficient S100 staining of the breast hyperplasia using proteolytic pre-treatment. The cytoplasmic compartment of both the myoepithelial cells and the glandular epithelial cells is digested and only the moderately stained nuclei are left. Also compare with Fig. 5b, same protocol.

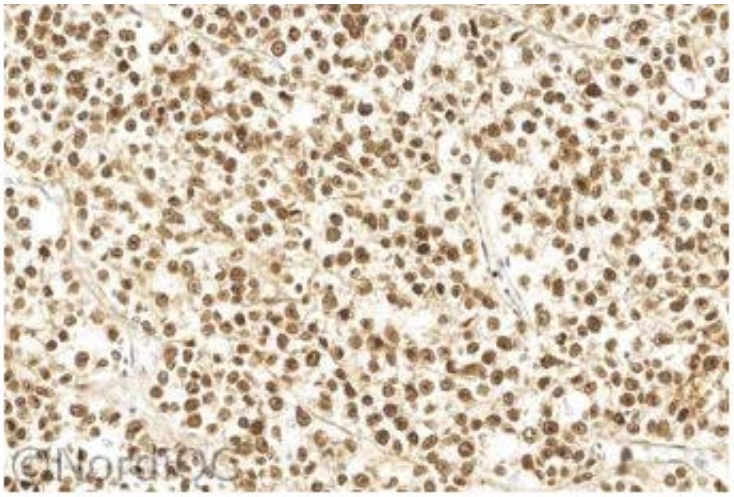
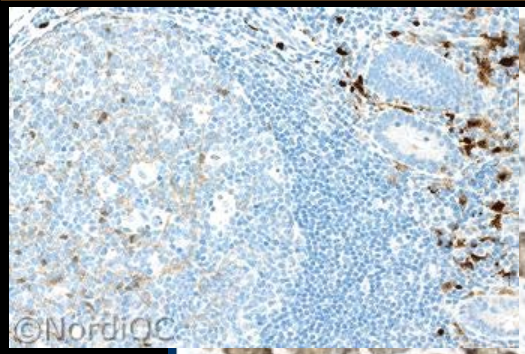



Fig. 5b. Insufficient S100 staining of the malignant melanoma using proteolytic pre-treatment, same protocol as in Fig. 5a. The cytoplasmic compartment is digested and only the moderately stained nuclei are left.

Proteolysis can provide impaired morphology

# IHC – Protocols and controls for UPT I



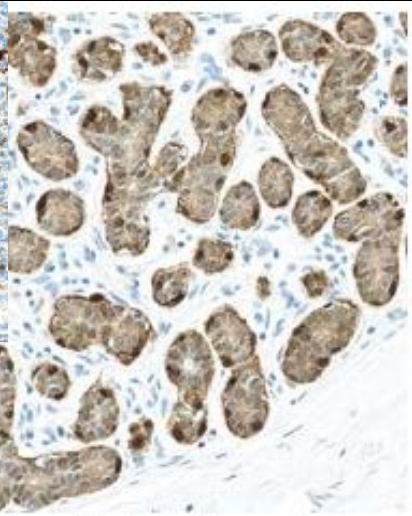
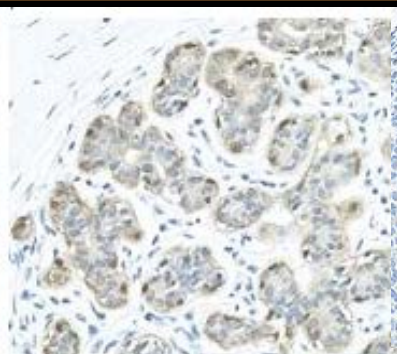




Fig. 3a. Optimal S100 staining of the breast hyperplasia using same protocol as in Figs. 1a & 2a. The myoepithelial cells show a moderate to strong cytoplasmic and nuclear staining reaction. A weaker staining of the secretory cells is seen, but no background staining is seen.



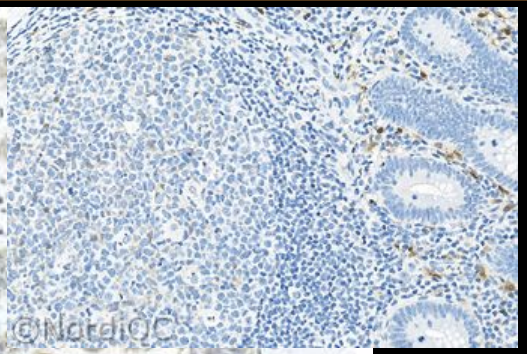




Fig. 3b. Insufficient S100 staining of the breast hyperplasia using same protocol as in Figs. 1b & 2b., same field as in Fig. 3a. The myoepithelial cells show a weak and equivocal staining reaction also compare with Fig. 4b, same protocol.

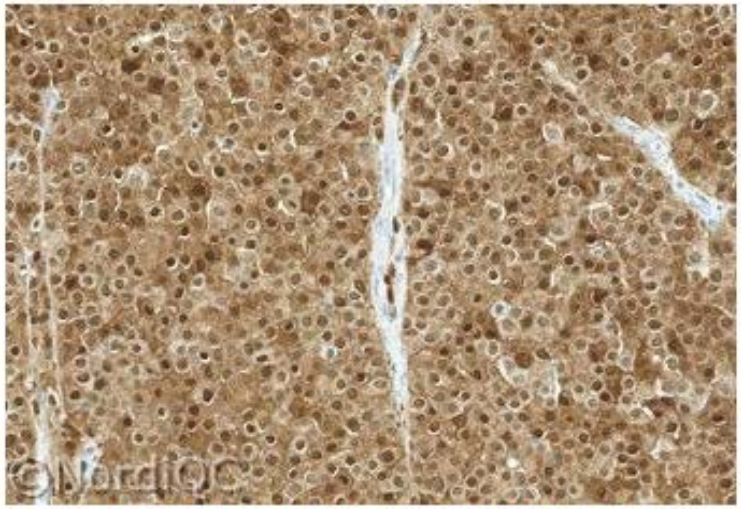



Fig. 4a. Optimal S100 staining of the malignant melanoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic and nuclear staining reaction.

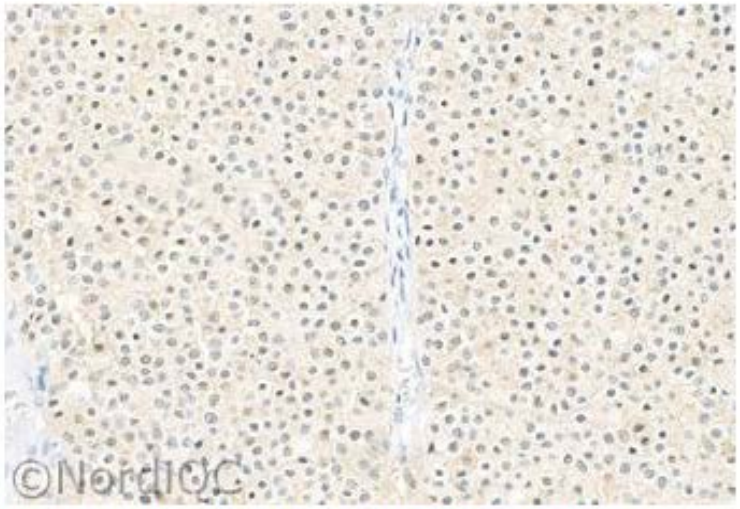



Fig. 4b. Insufficient S100 staining of the malignant melanoma using same protocol as in Figs. 1b – 3b., same field as in Fig. 4a. The neoplastic cells show a weak and equivocal staining reaction.

# IHC – Protocols and controls for UPT I

NordiQC

**Nordic immunohistochemical Quality Control**

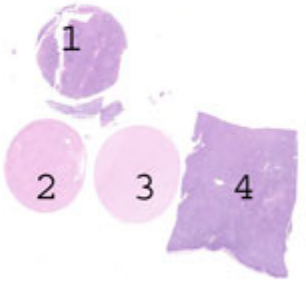
Home ■ Participation ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

Recommended CD45 protocols ■ Recommended CD45 control tissue

## Assessment Run 37 2013

## CD45 (Leucocyte Common Antigen, LCA)

The slide to be stained for CD45 comprised:




1. Tonsil, 2. Liver, 3. Brain, 4. B-CLL

All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD45 staining as optimal included:

- A moderate to strong, distinct, predominantly membranous staining reaction of all lymphocytes in all four tissues tested. In the tonsil both the B- and T-cells should be distinctively demonstrated.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the Kupffer cells in the liver and the microglial cells of the brain.
- An at least weak to moderate, predominantly membranous staining reaction of virtually all the neoplastic cells of the B-CLL
- No staining of squamous epithelial cells in the tonsil or hepatocytes in the liver.

214 laboratories participated in this assessment, but 9 participants used an inappropriate antibody (CD45R0 and CD45RA). Of the remaining 205 laboratories 82% achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks given are summarized in table 1.

**Table 3: Proportion of sufficient CD45 results in the two NordiQC runs performed**

| | Run 15 2005 | Run 37 2013 |
|---|---|---|
| Participants, n= | 80 | 205 |
| Sufficient results | 86 % | 82 % |

# IHC – Protocols and controls for UPT I

Table 1. Abs and assessment marks for CD45, run 37

| Concentrated Abs | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clones **2B11+PD7/26** | 111<br>1<br>1 | Dako<br>Diagnostic Biosystems<br>Zytomed | 64 | 29 | 16 | 4 | 82 % | 85 % |
| mAb clones **MEM28/ MEM56/MEM55** | 1 | Invitrogen | 0 | 1 | 0 | 0 | - | - |
| mAb clones **PD7/26/16+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 Abs** | | | | | | | | |
| 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 % | 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.

HIER

Ab Conc.

Control tissue

# IHC – Protocols and controls for UPT I

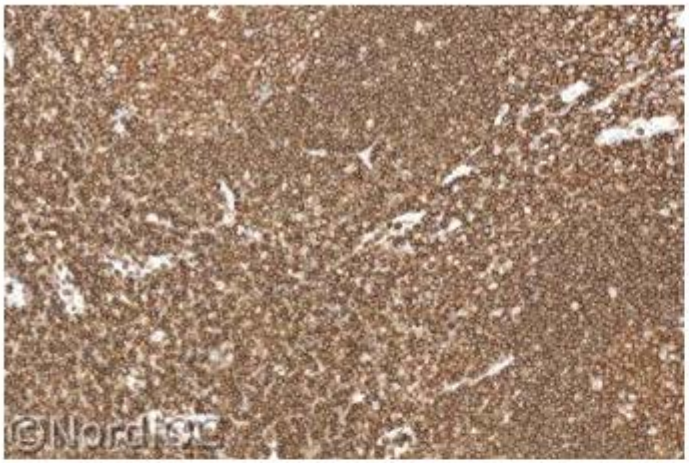



Fig. 1a. Optimal CD45 staining of the tonsil using the mAb clones 2B11+PD7/26 optimally calibrated and with HIER. Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.
Also compare with Figs. 2a – 4a, same protocol.

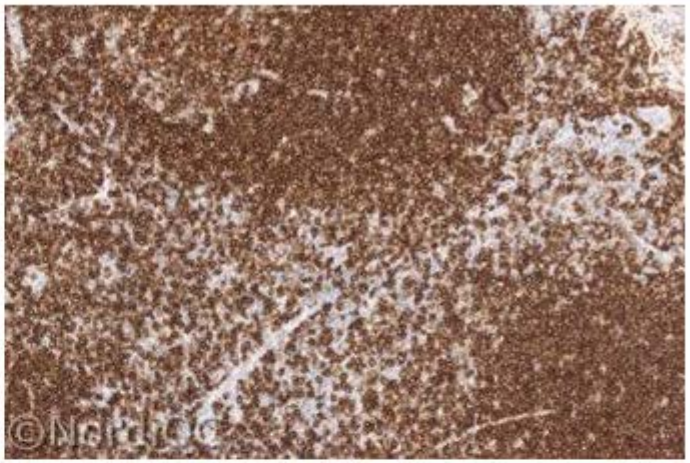



Fig. 1b. Staining for CD45 of the tonsil using the mAb clones 2B11+PD7/26 by protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a.
The vast majority of the B- and T-lymphocytes are demonstrated. However also compare with Figs. 2b – 4b, same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

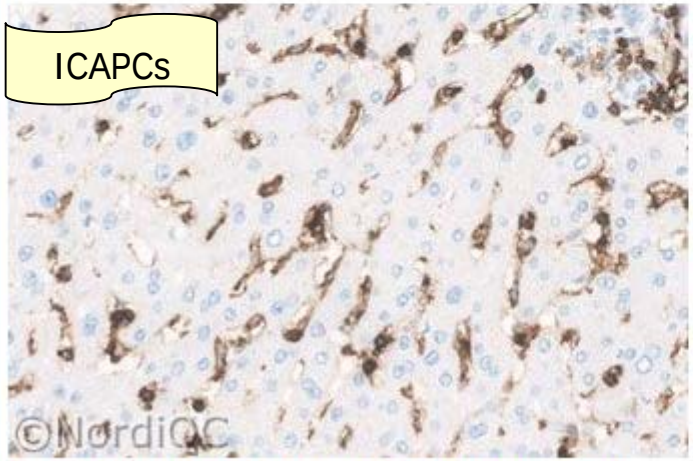



Fig. 2a. Optimal CD45 staining of the liver using same protocol as in Fig. 1a. The lymphocytes show a strong staining reaction, while the Kupffer cells display a weak to moderate staining reaction. The liver cells are negative and no background staining is seen.

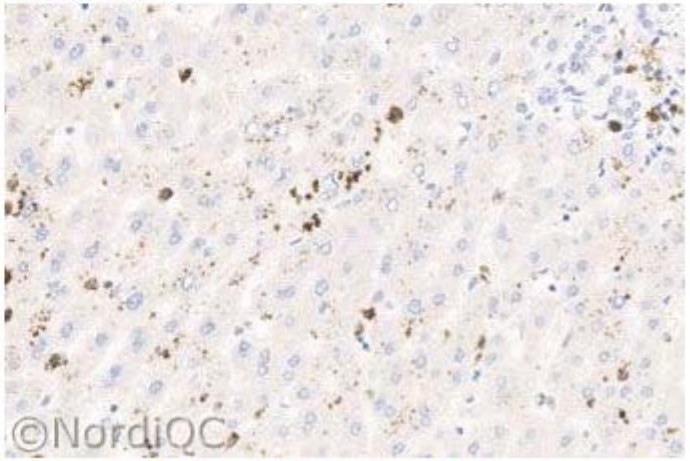



Fig. 2b. Insufficient CD45 staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a. Only lymphocytes are demonstrated and the Kupffer cells with a low CD45 expression are false negative.

# IHC – Protocols and controls for UPT I

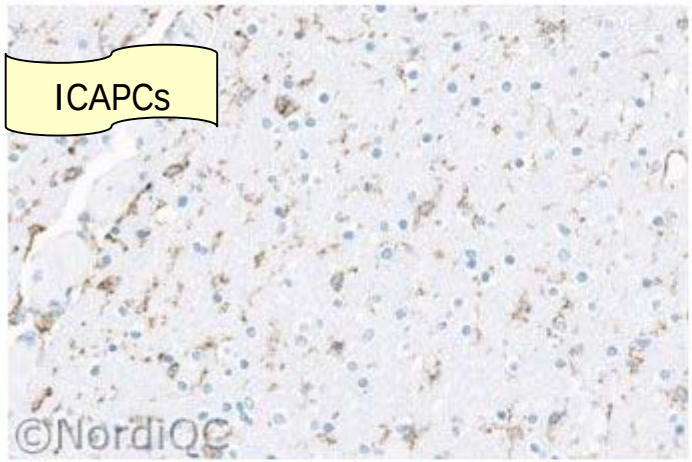



Fig. 3a. Optimal CD45 staining of the brain using same protocol as in Figs. 1a & 2a. The microglial cells with a low CD45 expression are distinctively demonstrated, no background staining is seen.

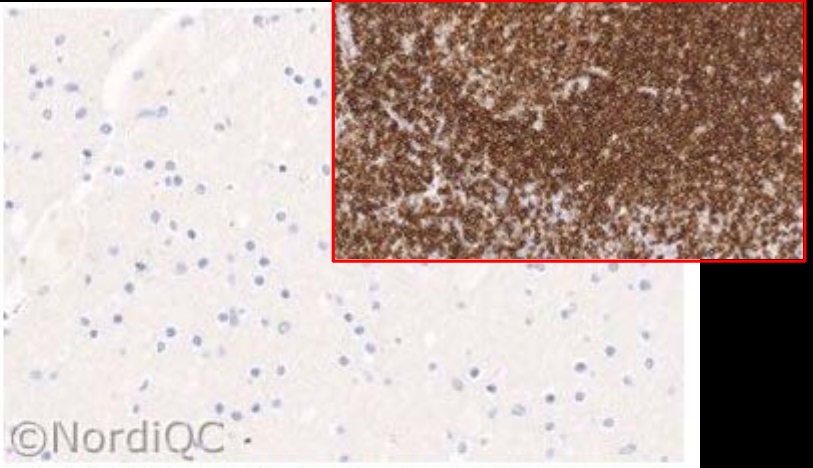



Fig. 3b. Insufficient CD45 staining of the brain using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. The microglial cells are false negative.

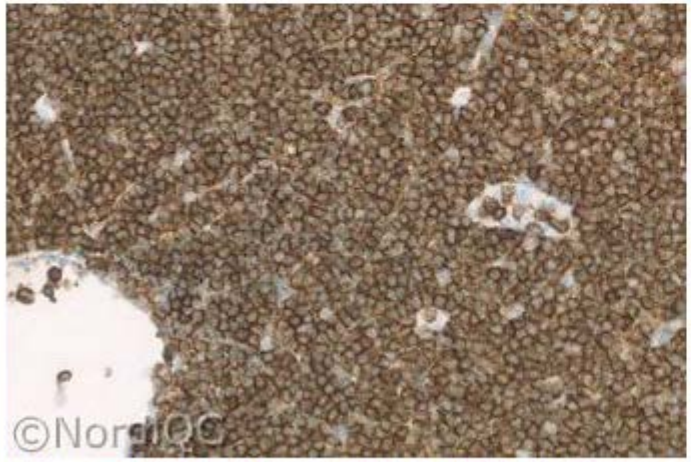



Fig. 4a. Optimal CD45 LCA staining of the B-CLL using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong and distinct membranous staining reaction. No background staining is seen.

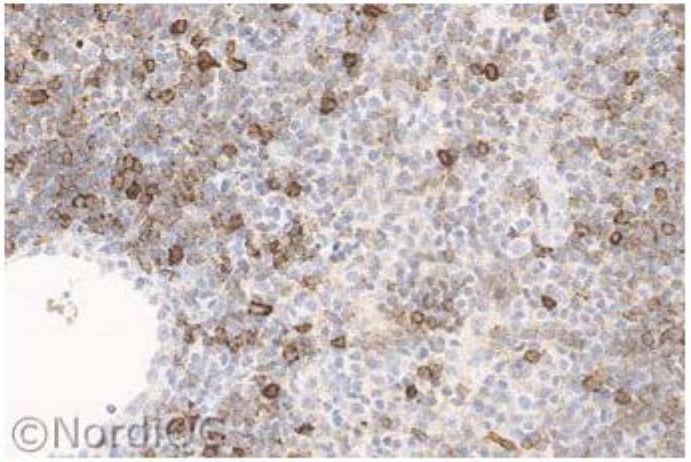



Fig. 4b. Insufficient CD45 LCA staining of the B-CLL using same protocol as in Figs. 1b - 3b. – same field as in Fig. 4a.

The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

# IHC – Protocols and controls for UPT I

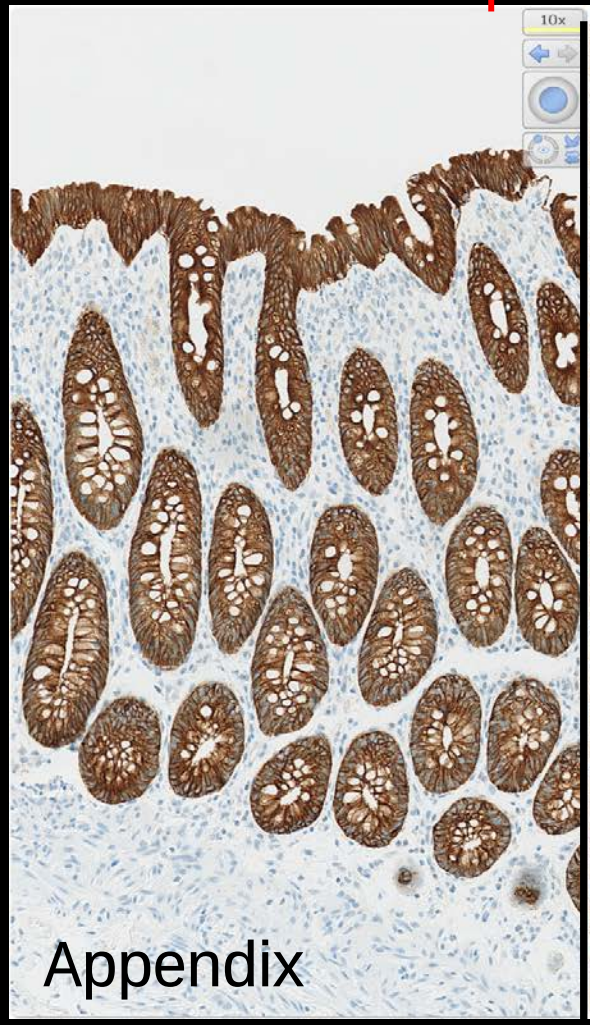
| CKs | Recommendable clones (conc.) | Less successful clones (conc.) | RTU ”plug and play” giving optimal result |
|---|---|---|---|
| CK-Low | mAb 5D3 (8,18)<br>mAb B22.1+B23.1(8,18)<br>mAb C51 (18)<br>mAb DC10 (18)<br>mAb TS1 (8)<br>rmAb EP17 (8)<br>rmAb EP17/30 (8,18,19) | mAb 35BH11<br>mAb CAM5.2 | Dako: mAb DC10<br>Dako: rmAb EP17/EP30<br>Leica: mAb 5D3<br>VMS: mAb B22.1+B23.1 |
| CK-High | mAb XM26 (5)<br>mAb LL002 (14)<br>rmAb EP1601Y (5)<br>rmAb SP27 (5)<br>rmAb SP54 (14)<br><br>*mAb D5/16B4 (5/6)* | mAb 34BE12 | VMS: rmAb SP27 |

# IHC – Protocols and controls for UPT I

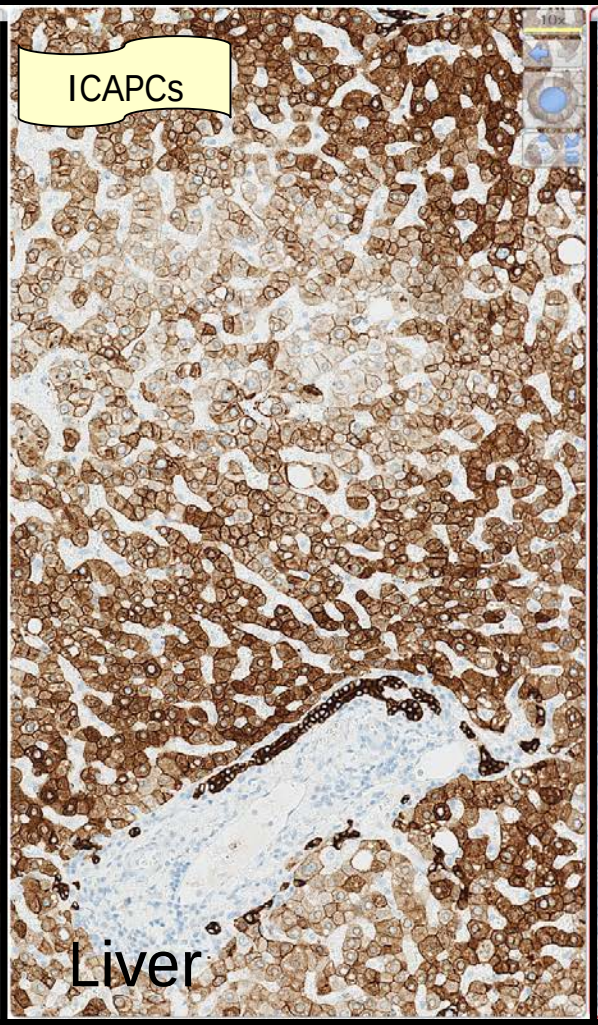
| CKs | Positive tissue control HE | Positive tissue control LE | Negative tissue control NE |
|---|---|---|---|
| CK-Low | Liver: Epithelial cells of bile ducts<br><br>Appendix: Epithelial cells | Liver: Hepatocytes<br><br>Tonsil: Fibroblastic reticulum cells | Tonsil: Lymphocytes<br><br>Appendix: Smooth muscle cells |
| CK-High | Tonsil: Basal squamous epithelial cells | Tonsil: Intermediate squamous epithelial cells | Appendix: Epithelial cells |

# IHC – Protocols and controls for UPT I

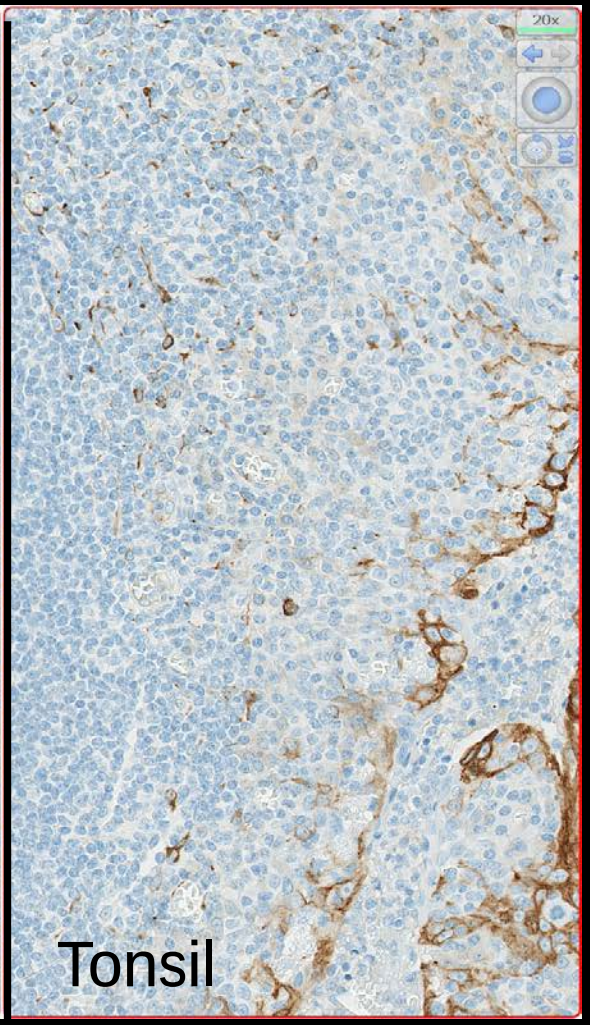
## CK-LMW reaction pattern




A moderate to strong distinct cytoplasmic staining reaction in virtually all columnar epithelial cells.




An at least weak to moderate distinct cytoplasmic staining reaction of the vast majority of the hepatocytes (membrane accentuation).



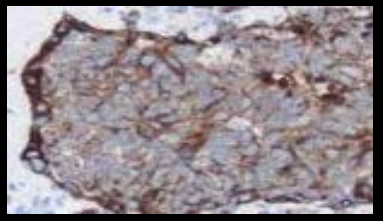

Scattered epithelial cells and fibroblastic reticulum cells can show a weak to moderate staining. No reaction in the vast majority of lymphocytes.

# IHC – Protocols and controls for UPT I

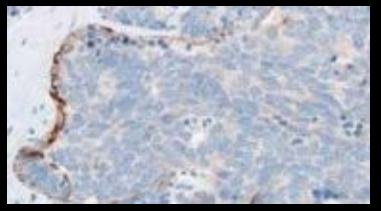
Table 1. **Antibodies and assessment marks for CK-LMW, run 49**

| Concentrated antibodies | Reactivity | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **34βH11** | *CK8* | 1 | In-house | 0 | 0 | 1 | 0 | - | - |
| mAb clone **5D3** | *CK8/18* | 18<br>3<br>3<br>1<br>1 | Leica/Novocastra<br>Thermo/Neomarkers<br>Biocare<br>Biogenex<br>Vector | 3 | 10 | 8 | 5 | 50% | 100% |
| mAb clones **B22.1/B23.1** | *CK8/18* | 6<br>1<br>1 | Cell Marque<br>Bio SB<br>Immunologic | 4 | 4 | 0 | 0 | 100% | 100% |
| mAb clone **BS83** | *CK18* | 1 | Nordic Biosite | 0 | 1 | 0 | 0 | - | - |
| mAb clone **CAM5.2** | *CK8 (7)* | 1 | Cell Marque<br>Zytomed | 0 | 2 | 0 | 0 | - | - |
| mAb clone **C51** | *CK18* | 2<br>1 | Invitrogen/Zymed<br>Biogenex | 0 | 2 | 1 | 0 | - | - |
| mAb clone **DC10** | *CK18* | 20<br>3<br>1<br>1<br>1<br>1<br>1 | Agilent/Dako<br>Thermo/Neomarkers<br>Biogenex<br>Empire Genomics<br>Histolab<br>Immunologic<br>Spring Bioscience | 6 | 15 | 6 | 1 | 75% | 90% |
| mAb clone **K8.8/DC10** | *CK8/18* | 1 | Thermo/Neomarkers | 0 | 0 | 1 | 0 | - | - |
| mAb clone **TS1** | *CK8* | 1 | Leica/Novocastra | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **EP17** | *CK8* | 6 | Epitomics | 6 | 0 | 0 | 0 | 100% | 100% |
| rmAb clones **EP17/EP30** | *CK8/18* | 3 | Agilent/Dako | 2 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | | |
| mAb clone **35βH11 MAB-0051** | *CK8* | 1 | Maixin | 0 | 1 | 0 | 0 | - | - |
| mAb clone **35βH11 760-2637** | *CK8* | 2 | Roche/Ventana | 0 | 0 | 0 | 2 | | |
| mAb clone **35βH11** no product number | *CK8* | 1 | ZS | 0 | 0 | 0 | 1 | - | - |
| mAb clones **5D3 PA0067** | *CK8/18* | 8 | Leica/Novocastra | 2 | 3 | 3 | 0 | 63% | 83% |
| mAb clone **5D3 PDM070-10MM** | *CK8/18* | 2 | Diagnostics BioSystems | 0 | 1 | 1 | 0 | - | - |
| mAb clones **B22.1/B23.1 760-4344** | *CK8/18* | 27 | Roche/Ventana | 16 | 7 | 4 | 0 | 85% | 96% |
| mAb clones **B22.1/B23.1 818M-97/98** | *CK8/18* | 8 | Cell Marque | 4 | 2 | 0 | 2 | 75% | - |
| mAb clones **B22.1/B23.1 MAD-000589QD** | *CK8/18* | 2 | Master Diagnostica | 1 | 1 | 0 | 0 | - | - |
| mAb clone **CAM5.2 345779** | *CK8 (7)* | 29 | Becton Dickinson | 1 | 9 | 8 | 11 | 34% | - |
| mAb clone **CAM5.2 349205** | *CK8 (7)* | 6 | Becton Dickinson | 0 | 0 | 4 | 2 | - | - |
| mAb clone **CAM5.2 790-4555** | *CK8 (7)* | 10 | Roche/Ventana | 3 | 3 | 3 | 1 | 60% | 80% |
| mAb clone **DC10 IR618/IS618** | *CK18* | 12 | Agilent/Dako | 0 | 8 | 4 | 0 | 67% | - |
| mAb clone **DC10 GA618** | *CK18* | 9 | Agilent/Dako | 2 | 5 | 2 | 0 | 78% | 100% |
| rmAb clones **EP17/EP30 IR094** | *CK8/18* | 16 | Agilent/Dako | 16 | 0 | 0 | 0 | 100% | 100% |
| Total | | 213 | | 66 | 75 | 46 | 26 | - | |
| Proportion | | | | 31% | 35% | 22% | 12% | 66% | |

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



Optimal



Poor

Clone !

Retrieval !

Concentration !

# IHC – Protocols and controls for UPT I

**Table 4: Proportion of sufficient results for CK-LMW in the six NordiQC runs performed**

| | Run 9 2003 | Run 16 2006 | Run 20 2007 | Run 25 2009 | Run 33 2011 | Run 38 2013 |
|---|---|---|---|---|---|---|
| Participants, n= | 54 | 66 | 74 | 99 | 141 | 161 |
| Sufficient results | 57 % | 45 % | 67 % | 66 % | 64 % | 77 % |

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

**Table 3. Pass rates for four CK-LMW clones using different epitope retrieval methods**

| | Pass rate for run 16, 20, 25, 33 & 38 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | | HIER | | Prot. pre-treatm. | | HIER + proteolysis | |
| | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient |
| mAb clone CAM 5.2 | 126 | 56 (44 %) | 41 | 14 (34 %) | 66 | 39 (59 %) | 9 | 3 (33 %) |
| mAb clone DC10 | 159 | 151 (95 %) | 158 | 149 (95 %) | 0 | 0 | 2 | 2 (100 %) |
| mAb clone 5D3 | 107 | 66 (62 %) | 80 | 65 (81 %) | 27 | 3 (11 %) | 0 | 0 |
| mAb clone 35BH11 | 54 | 6 (11 %) | 32 | 4 (13 %) | 22 | 2 (11 %) | 0 | 0 |

# IHC – Protocols and controls for UPT I

**Table 4: Proportion of sufficient results for CK-LMW in the six NordiQC runs performed**

| | Run 9 2003 | Run 16 2006 | Run 20 2007 | Run 25 2009 | Run 33 2011 | Run 38 2013 |
|---|---|---|---|---|---|---|
| Participants, n= | 54 | 66 | 74 | 99 | 141 | 161 |
| Sufficient results | 57 % | 45 % | 67 % | 66 % | 64 % | 77 % |

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

CLONE: 5D3

| Code | Name | Configuration | Use |
|---|---|---|---|
| PA0067 | 7ml CK8/18 Bond RTU Primary | Bond ready to use reagent | P(HIER) |
| 5D3-L-CE | 1ml NCL-L-5D3 | Liquid Concentrated Monoclonal Antibody | P (ENZYME) |

**Table 3. Pass rates for four CK-LMW clones using different epitope retrieval methods**

| | Pass rate for run 16, 20, 25, 33 & 38 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | | HIER | | Prot. pre-treatm. | | HIER + proteolysis | |
| | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient |
| mAb clone CAM 5.2 | 126 | 56 (44 %) | 41 | 14 (34 %) | 66 | 39 (59 %) | 9 | 3 (33 %) |
| mAb clone DC10 | 159 | 151 (95 %) | 158 | 149 (95 %) | 0 | 0 | 2 | 2 (100 %) |
| mAb clone 5D3 | 107 | 66 (62 %) | 80 | 65 (81 %) | 27 | 3 (11 %) | 0 | 0 |
| mAb clone 35BH11 | 54 | 6 (11 %) | 32 | 4 (13 %) | 22 | 2 (11 %) | 0 | 0 |

# IHC – Protocols and controls for UPT I

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

| Concentrated antibodies | Dako Autostainer Link / Classic | | Ventana BenchMark XT / Ultra | | Leica Bond III / Max | |
|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | ER2 pH 9.0 | ER1 pH 6.0 |
| mAb clone **5D3** | 36 %<br>4/11** | - | 0 %<br>0/5 | - | 67 %<br>2/3 | - |
| mAb clone **DC10** | 67 %<br>2/3 | - | 64 %<br>7/11 | - | 50 %<br>3/6 | - |

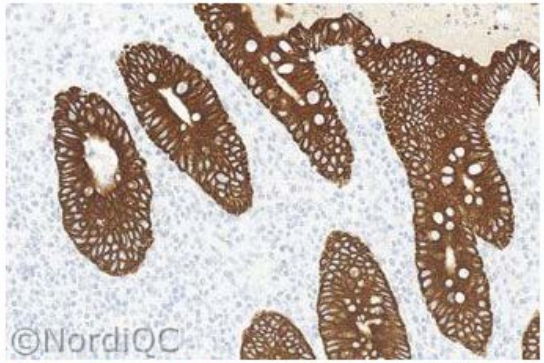



Fig. 1a. Optimal staining for CK-LMW of the appendix using the mAb clone 5D3 for CK 8/18 optimally calibrated, HIER in an alkaline buffer and performed on the Autostainer Link stainer, Dako.
Virtually all the columnar epithelial cells show a strong cytoplasmic staining reaction, while no background staining is seen.
Also compare with Figs. 2a - 3a, same protocol.

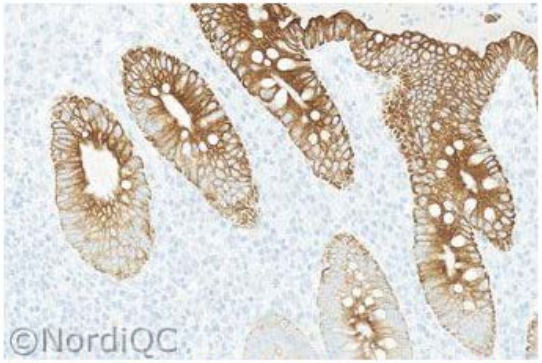



Fig. 1b. Insufficient staining for CK-LMW of the appendix using the mAb clone 5D3 for CK 8/18, HIER in an alkaline buffer and performed on the BenchMark ULTRA stainer, Ventana – same field as in Fig. 1a. The mAb clone 5D3 gave same insufficient staining result by all protocol settings used on the BenchMark stainers.
Only the luminal columnar epithelial cells show a moderate to strong cytoplasmic staining, while virtually no staining is seen in the basal part of the crypts.
Also compare with Figs. 2b - 3b, same protocol.

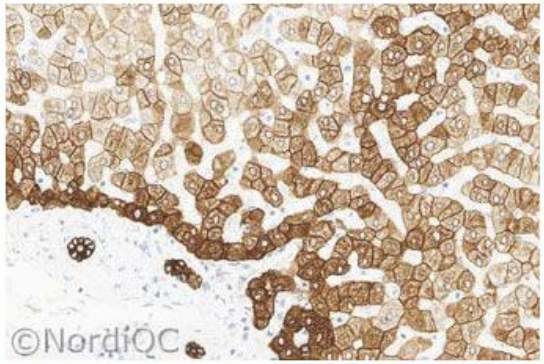



Fig. 2a. Optimal staining for CK-LMW of the liver using the same protocol as in Fig. 1a.
The majority of the hepatocytes show a distinct, moderate staining reaction with a membrane enhancement, while the columnar epithelial cells of the bile ducts show a strong cytoplasmic staining reaction.
Same protocol used in Figs. 1a - 3a.

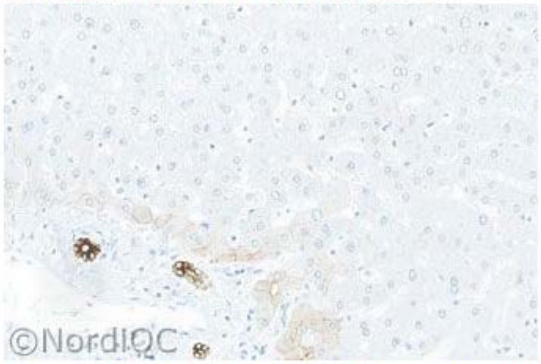


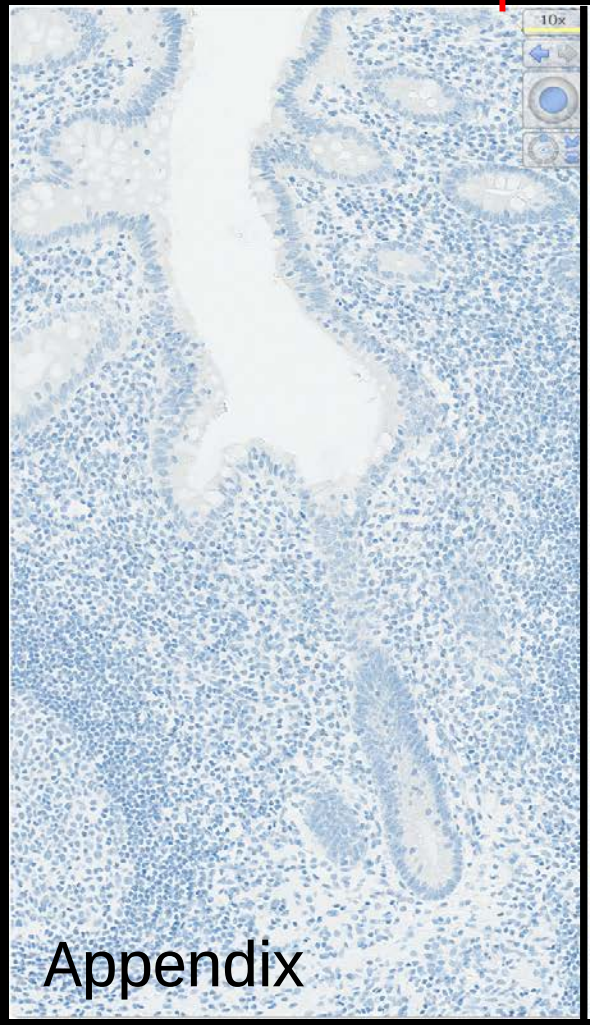

Fig. 2b. Insufficient staining for CK-LMW of the liver using the same protocol as in Fig. 1b - same field as in Fig. 2a.
Only the bile duct epithelial cells are demonstrated, while the hepatocytes are almost negative.
Same protocol used in Figs. 1b - 3b.

mAb clone 5D3
Less successful on VMS

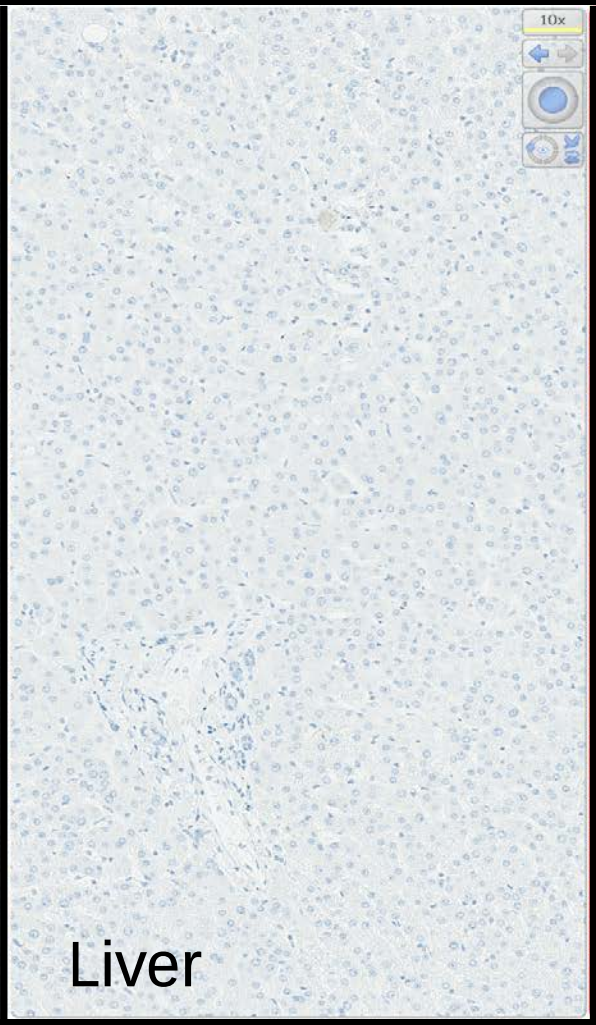
VMS (and all..):
rmAb EP17
rmAb EP17/EP30
mAb DC10
mAb B22.1 + B23.1

# IHC – Protocols and controls for UPT I

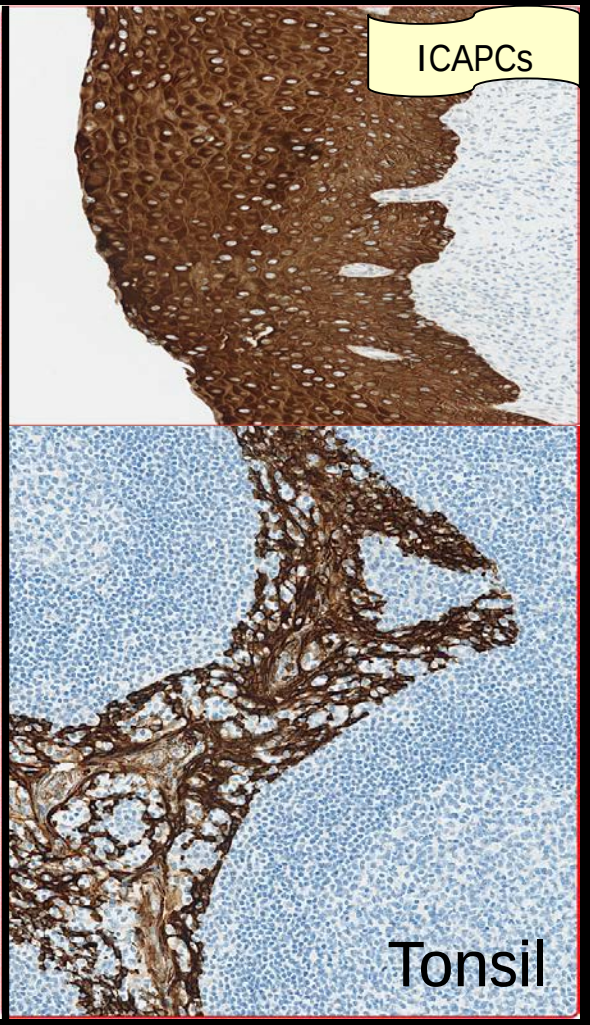
## CK-HMW reaction pattern




No staining should be seen.




No staining should be seen.




Virtually all squamous epithelial cells must show a moderate to strong cytoplasmic staining reaction.

# IHC – Protocols and controls for UPT I

## Assessment Run 38 2013

## Cytokeratin, High Molecular Weight (CK-HMW)

**Material**
The slide to be stained for CK-HMW comprised:

1. Prostate hyperplasia, 2. Esophagus, 3. Liver,
4. Prostate intraepithelial neoplasia (PIN) / prostate adenocarcinoma,
5. Breast ductal carcinoma, 6. Lung squamous cell carcinoma.


All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK-HMW staining as optimal included:

- A strong and distinct cytoplasmic staining reaction of all squamous epithelial cells of the esophagus throughout all the cell layers*.
- A strong and distinct cytoplasmic staining reaction of the majority of the basal cells of the prostate hyperplastic glands and the PIN lesions.
- A moderate to strong cytoplasmic staining reaction of the majority of the neoplastic cells of the lung squamous cell carcinoma.
- No staining of the neoplastic cells of the breast ductal carcinoma and of the epithelial cells of the bile ducts of the liver.

* Antibodies against CK14 only demonstrate the basal squamous epithelial cells.

213 laboratories participated in this assessment. 6 participants used an inappropriate antibody like CK-PAN, CK13 and CK19. Of the remaining 207 laboratories 45 % achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

**Table 3: Proportion of sufficient results for CK-HMW in the five NordiQC runs performed**

| | Run 12 2004 | Run 16 2006 | Run B6 2008 | Run 32 2011 | Run 38 2013 |
|---|---|---|---|---|---|
| Participants, n= | 73 | 87 | 97 | 163 | 207 |
| Sufficient results | 77 % | 88 % | 24 % | 23 % | 45 % |

**Table 1. Abs and assessment marks for CK-HMW, run 38**

| Concentrated Abs | Reactivity | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **34BE12** | CK 1, 5, 10, 14, (19) * | 51<br>2<br>2<br>1<br>1<br>1<br>1<br>1<br>1 | Dako<br>Leica/Novocastra<br>Thermo/Neomarkers<br>Abcam<br>Biocare<br>Bio SB<br>Cell Marque<br>Enzo<br>Gene Tech | 0 | 6 | 54 | 1 | 10 % | 0 % |
| mAb clone **BS42** | Unknown | 1 | Nordic Biosite | 0 | 0 | 1 | 0 | - | - |
| mAb clone **D5/16B4** | CK 5, 6 | 28<br>2<br>1<br>1 | Dako<br>Cell Marque<br>Genemed<br>Zymed | 15 | 13 | 4 | 0 | 88 % | 100 % |
| mAb clone **DE-SQ** | CK 13, 14, 15, 16 | 1 | Thermo/Neomarkers | 0 | 0 | 1 | 0 | - | - |
| mAb clone **LL002** | CK 14 | 6<br>1<br>1 | Leica/Novocastra<br>AbD Serotec<br>Thermo/Neomarkers | 5 | 1 | 2 | 0 | 75 % | 83 % |
| mAb clone **XM26** | CK 5 | 23<br>1 | Leica/Novocastra<br>Diagnostic BioSystems | 19 | 5 | 0 | 0 | 100 % | 100 % |
| mAb clone cocktail **XM26+LL002** | CK 5, 14 | 2<br>2 | Diagnostic BioSystems<br>Zytomed | 1 | 2 | 1 | 0 | - | - |
| mAb clone cocktail **Y4A3+XM26+ LL002** | p63, CK 5, 14 | 1 | Zytomed | 0 | 1 | 0 | 0 | - | - |
| mAb clone **34BE12** + rmAb clone **EP1601Y** | CK 1, 5, 10, 14, (19) * + CK 5 | 1 | Homemade cocktail: Dako/Cell Marque | 0 | 0 | 1 | 0 | - | - |
| mAb clone **XM26** + mAb clone **LL002** | CK 5, 14 | 1 | Homemade cocktail: Leica/Novocastra/ Cell Marque | 0 | 1 | 0 | 0 | - | - |
| **Ready-To-Use Abs** | | | | | | | | | |
| mAb clone **34BE12 IR051** | CK 1, 5, 10, 14, (19) * | 24 | Dako | 0 | 0 | 24 | 0 | 0 % | 0 % |
| mAb clone **D5/16B4 IS/IR780** | CK 5, 6 | 9 | Dako | 3 | 4 | 2 | 0 | 78 % | 78 % |

mAb clone 34BE12 gives an aberrant staining with an unidentified CK-LMW subtype complicating the use as a reliable marker for CK-HMW

**mAb clone XM26 or D5/16B4**

Conc & RTU

Alternatively:
CK5: rmAb EP1601Y & SP27

CK14: rmAb SP53 & mAb LL002

# IHC – Protocols and controls for UPT I

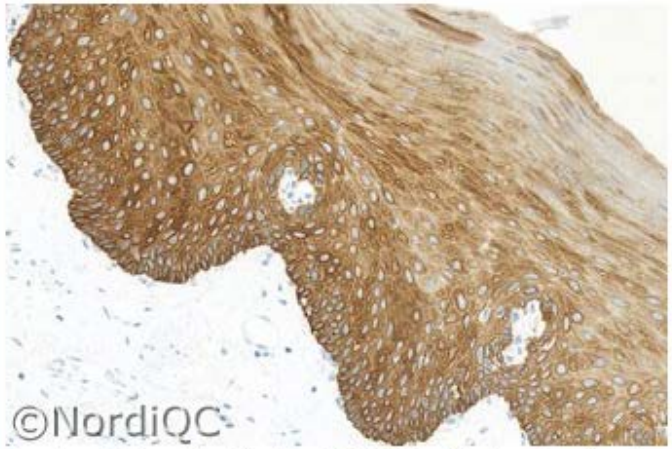



Fig. 1a. Optimal staining for CK-HMW of the tonsil using the mAb clone D5/16B4 against CK5/6 optimally calibrated and with HIER in an alkaline buffer.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen.

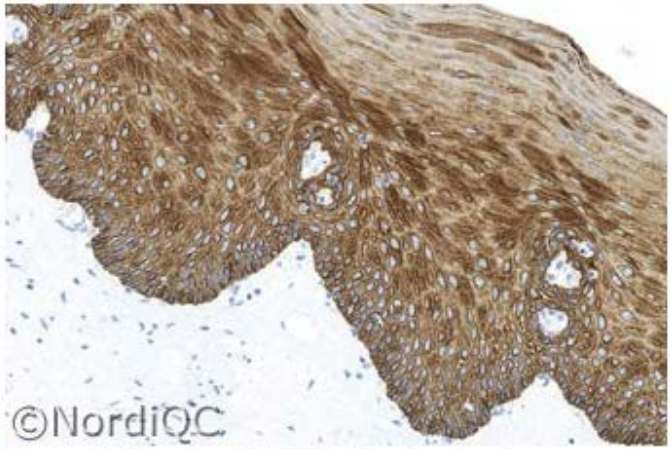



Fig. 1b. Staining for CK-HMW of the tonsil using an insufficient protocol based on the mAb clone 34BE12 against CK-HMW with HIER in an alkaline buffer, same field as in Fig. 1a.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen. However, compare with Fig. 3b, same protocol.

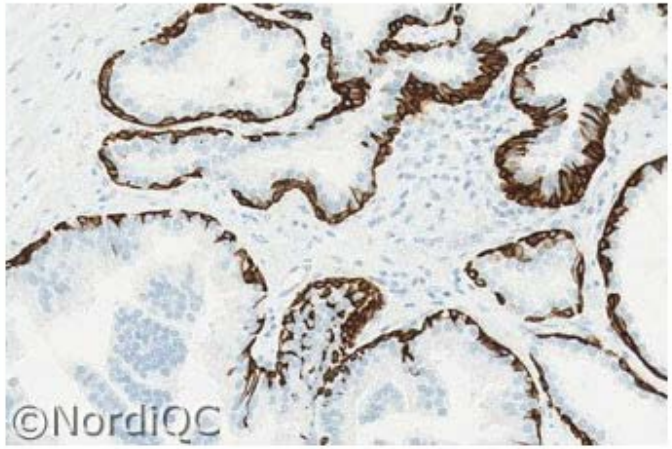



Fig. 2a. Optimal staining for CK-HMW of the prostate hyperplasia/PIN lesion using same protocol as in Fig. 1a.
Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen.

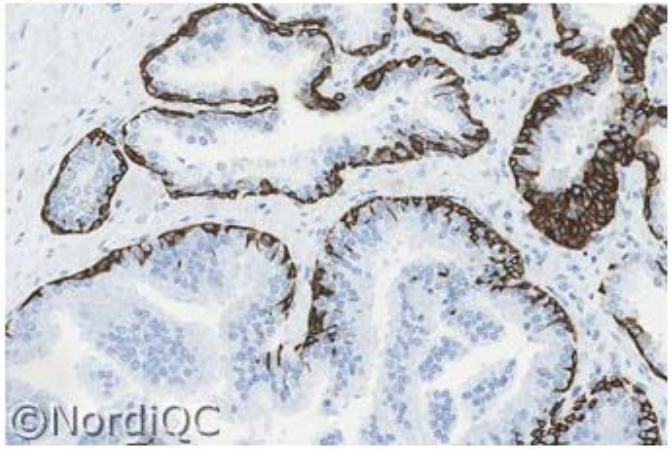



Fig. 2b. Staining for CK-HMW of the prostate hyperplasia/PIN lesion using same insufficient protocol as in Fig. 1b, same field as in Fig. 2a. Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen, same field as in Fig. 2a. However, compare with Fig. 3b., same protocol.

# IHC – Protocols and controls for UPT I

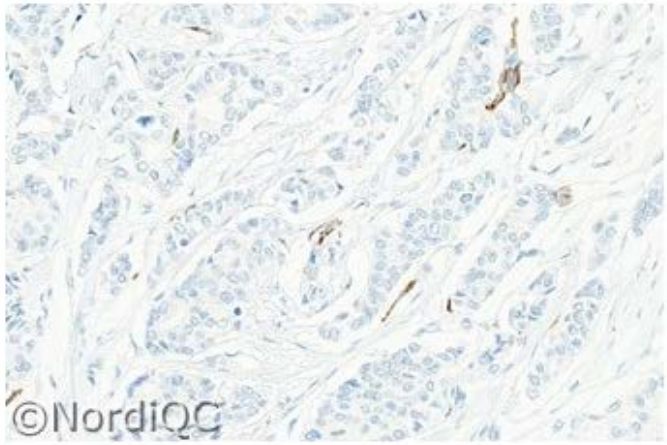



Fig. 3a. Optimal staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1a. & 2a.
The neoplastic cells expressing CK-LMW are negative, while the remnants of entrapped myoepithelial cells expressing the CK-HMW subtypes CK5 & CK14 show a moderate cytoplasmic staining.

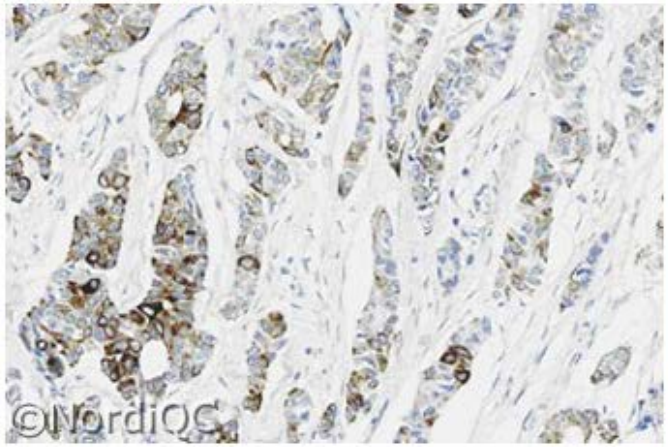



Fig. 3b. Insufficient staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1b. & 2b, same field as in Fig. 3a. A moderate to strong aberrant cytoplasmic staining is seen in the majority of the neoplastic cells. This false positive cross reaction with an unidentified subtype of CK-LMW was typically seen, when the mAb clone 34BE12 was used with HIER.

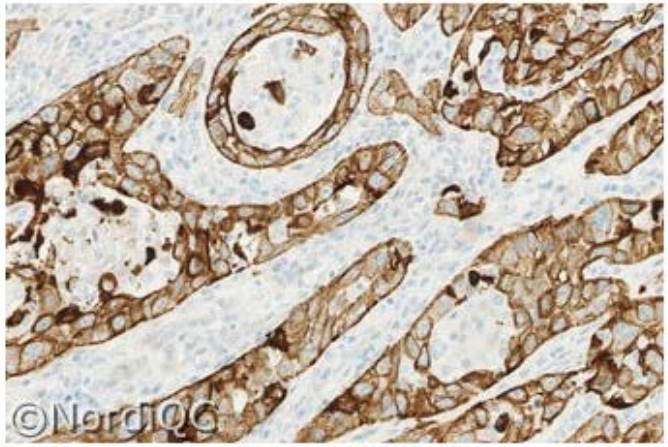



Fig. 4a. Optimal staining for CK-HMW of the lung squamous cell carcinoma using same protocol as in Figs. 1a. - 3a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining.

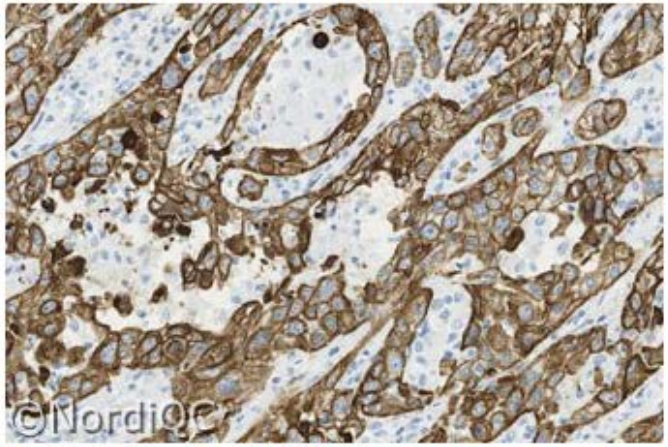
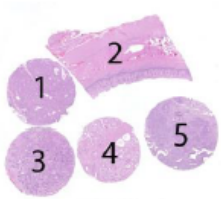



Fig. 4b. Staining for CK-HMW of the lung squamous cell carcinoma using same insufficient protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining. However, as the epithelial cells of the breast ductal carcinoma in Fig. 3b showed same staining characteristics, the staining for CK-HMW is not reliable.

# IHC – Protocols and controls for UPT I

## Assessment Run 46 2016
## CK5

**Material**
The slide to be stained for CK5 comprised:




1: Lung squamous cell carcinoma 2: Esophagus 3: Lung adenocarcinoma
4: Prostate hyperplasia 5: Lung squamous cell carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK5 staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining reaction of all squamous epithelial cells in esophagus throughout all the cell layers.
- A strong and distinct cytoplasmic staining reaction of the majority of basal cells in the hyperplastic prostate glands.
- A moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung squamous cell carcinoma, tissue core no. 1.
- An at least weak to moderate cytoplasmic staining reaction of the majority of neoplastic cells in the lung squamous cell carcinoma, tissue core no. 5.
- No staining of neoplastic cells in the lung adenocarcinoma.

**Participation**

| | |
|---|---|
| Number of laboratories registered for CK5, run 46 | 281 |
| Number of laboratories returning slides | 266 (95%) |

**Results**
266 laboratories participated in this assessment. 181 (68%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful CK5 antibodies
- Too low concentration of the primary Ab
- Insufficient HIER - too short efficient HIER time

**Performance history**
This was the second NordiQC assessment of CK5. The pass rate in this run was improved compared to the previous run from 2004 as shown in table 2.

Table 2. **Proportion of sufficient results for CK5 in the two NordiQC runs performed**

| | Run 12 2004 | Run 46 2016 |
|---|---|---|
| Participants, n= | 74 | 266 |
| Sufficient results | 47% | 68% |

# IHC – Protocols and controls for UPT I

Table 1. **Antibodies and assessment marks for CK5, run 46**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **10C11E6** | 1 | Immunologic | 0 | 1 | 0 | 0 | - | - |
| mAb clone **D5/16 B4** | 74<br>1<br>6<br>3<br>1<br>2<br>1 | Dako/Agilent<br>Invitrogen<br>Cell Marque<br>Zytomed<br>Thermo Scientific<br>Biocare<br>Immunologic | 21 | 34 | 28 | 5 | 63% | 66% |
| mAb **BS42** | 1 | Nordic Biosite | 1 | 0 | 0 | 0 | - | - |
| mAb clone **XM26** | 49<br>2<br>1 | Leica/Novocastra<br>Zytomed<br>Sanbio | 25 | 15 | 11 | 1 | 77% | 79% |
| mAb clone **XM26/LL002** | 1 | Zytomed | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP1601Y** | 8<br>1 | Cell Marque<br>Biocare | 6 | 3 | 0 | 0 | 100% | 100% |
| rmAb clone **SP27** | 3 | Immunologic | 3 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **D5/16 B4 1072** | 1 | Monosan | 0 | 1 | 0 | 0 | - | - |
| mAb clone **D5/16 B4 BMS017** | 1 | Zytomed | 0 | 1 | 0 | 0 | - | - |
| mAb clone **D5/16 B4 IR/IS780** | 36 | Dako/Agilent | 1 | 8 | 22 | 5 | 25% | 67% |
| mAb **D5/16 B4 GA780** | 11 | Dako/Agilent | 1 | 8 | 2 | 0 | 82% | 82% |
| mAb clone **D5/16 B4 790-4554** | 38 | Ventana/Roche/Cell Marque | 15 | 15 | 5 | 3 | 79% | 85% |
| mAb clone **XM26 PA0468** | 2 | Leica/Novocastra | 0 | 2 | 0 | 0 | - | - |
| mAb clone **XM26 PM234** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP1601Y/LL002 760-4939** | 1 | Ventana/Cell Marque | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP1601Y/LL002 905H-8** | 3 | Cell Marque | 1 | 1 | 1 | 0 | - | - |
| rmAb clone **EP1601Y 305R-18** | 2 | Cell Marque | 0 | 1 | 1 | 0 | - | - |
| rmAb clone **EP24/EP67 MAD-000651QD** | 2 | Master Diagnostica | 2 | 0 | 0 | 0 | - | - |
| rmAb clone **SP27 760-4935** | 12 | Ventana /Cell Marque | 11 | 0 | 1 | 0 | 92% | 92% |
| rmAb clone **SP27 RMA-0612** | 1 | Maixin | 0 | 0 | 1 | 0 | - | - |
| Total | 266 | | 88 | 92 | 72 | 14 | - | |
| Proportion | | | 33% | 35% | 27% | 5% | 68% | |

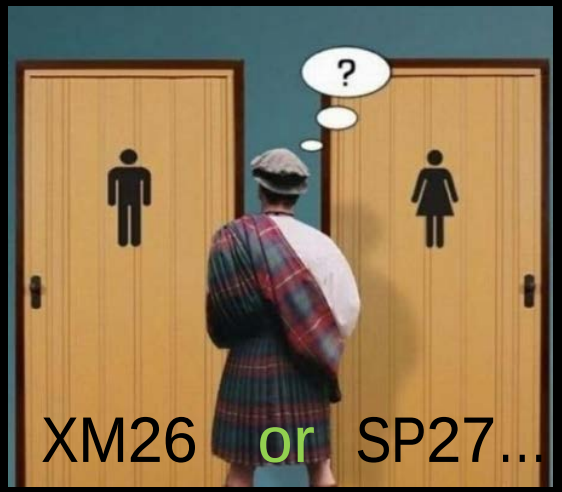
1)Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Clone

HIER buffer

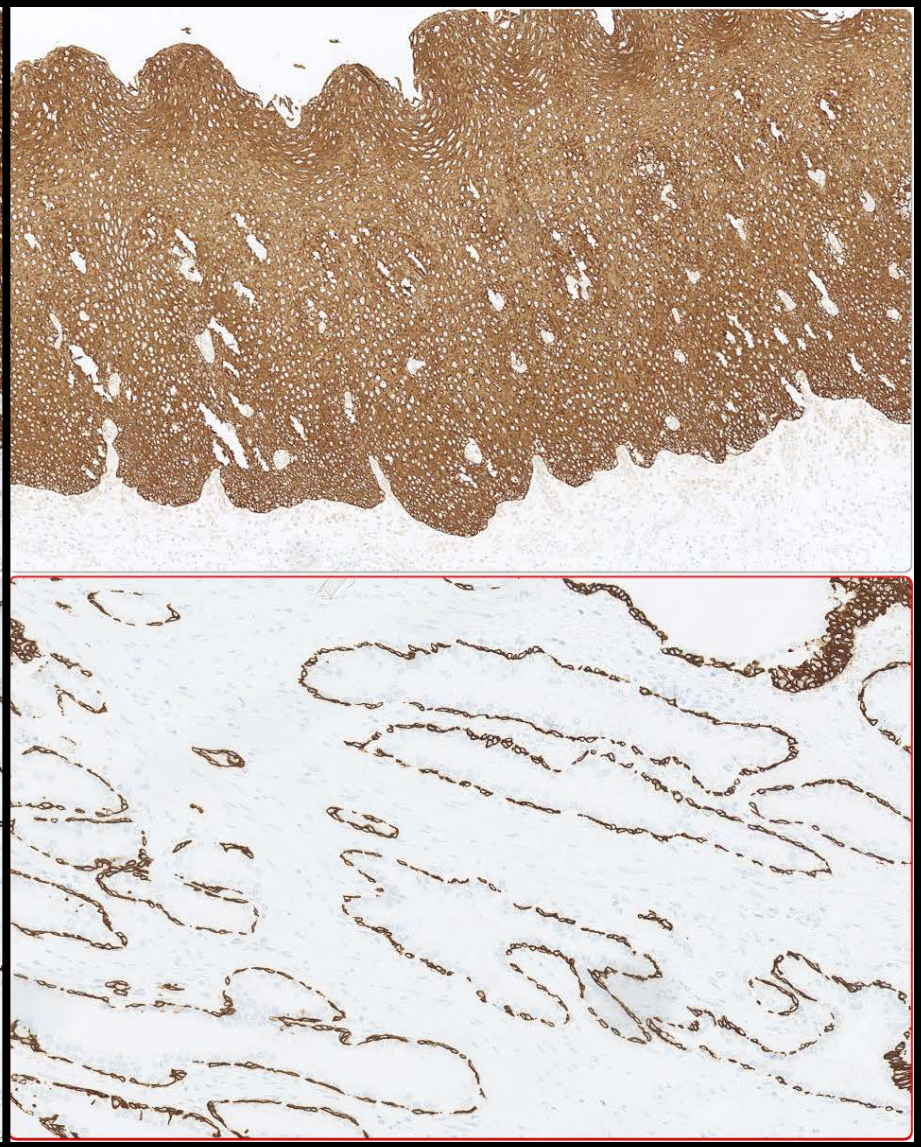
Detection kit

High pH + 3-step



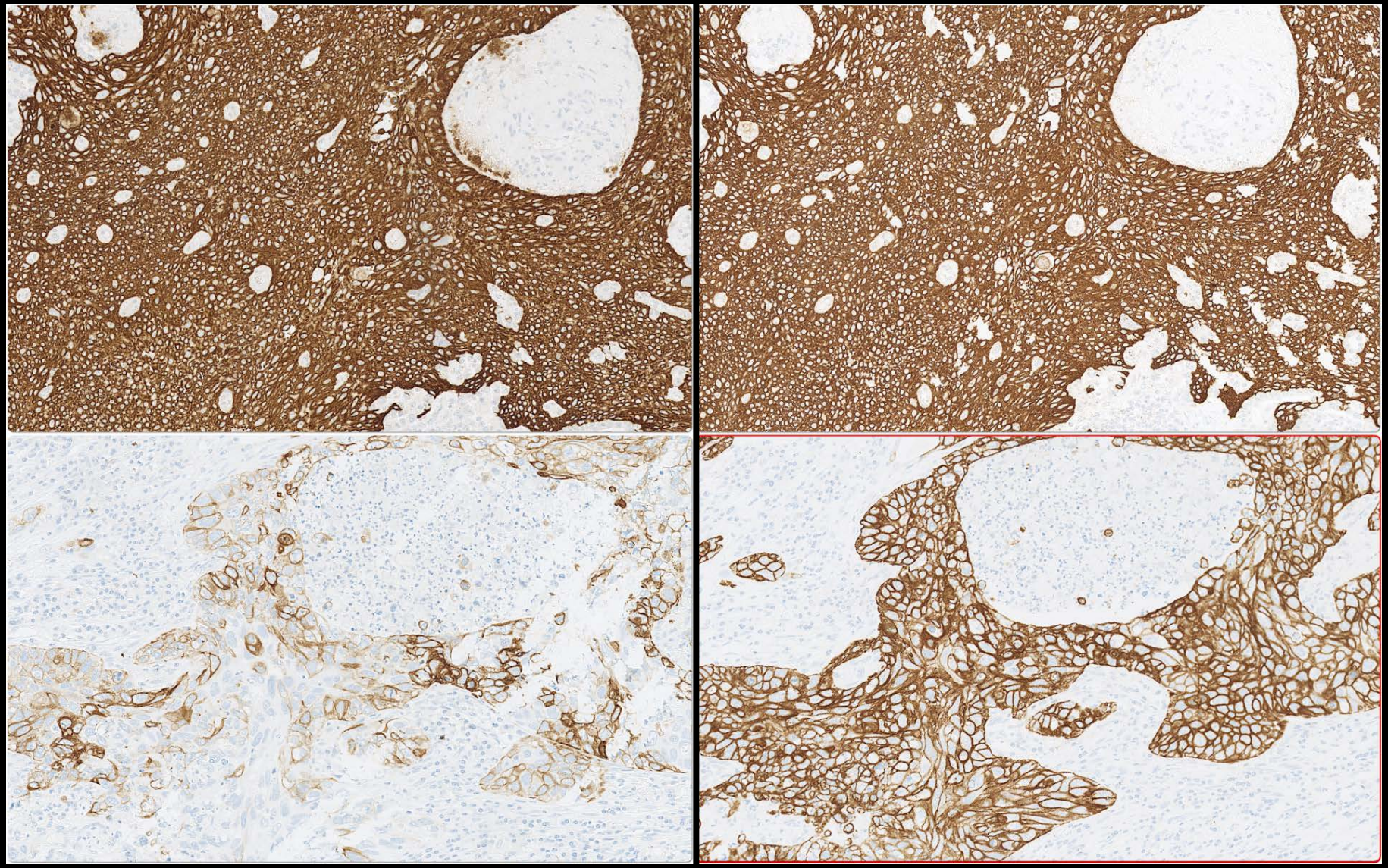
# IHC – Protocols and controls for UPT I

mAb XM26



rmAb SP27

# IHC – Protocols and controls for UPT I

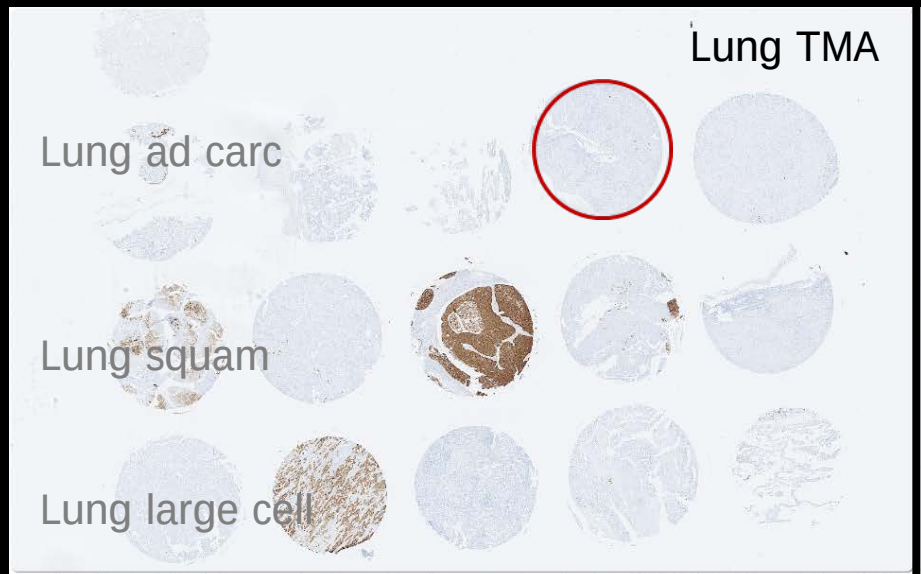


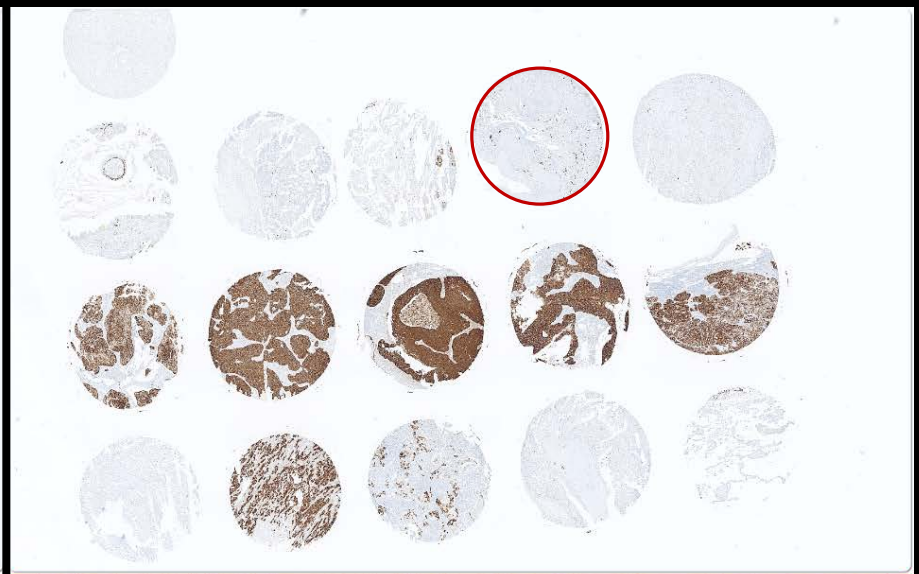
mAb XM26

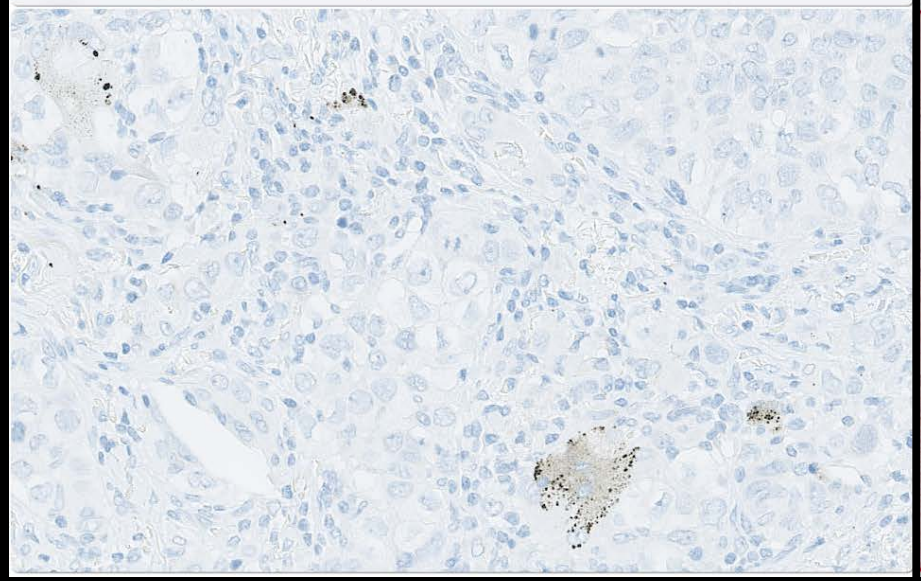
Lung squam cell carcs.

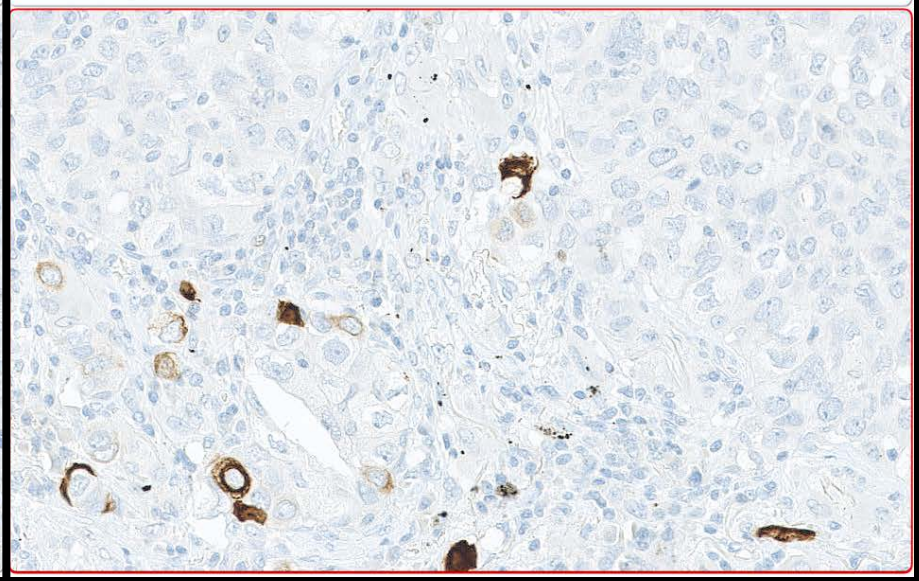
rmAb SP27

# IHC – Protocols and controls for UPT I





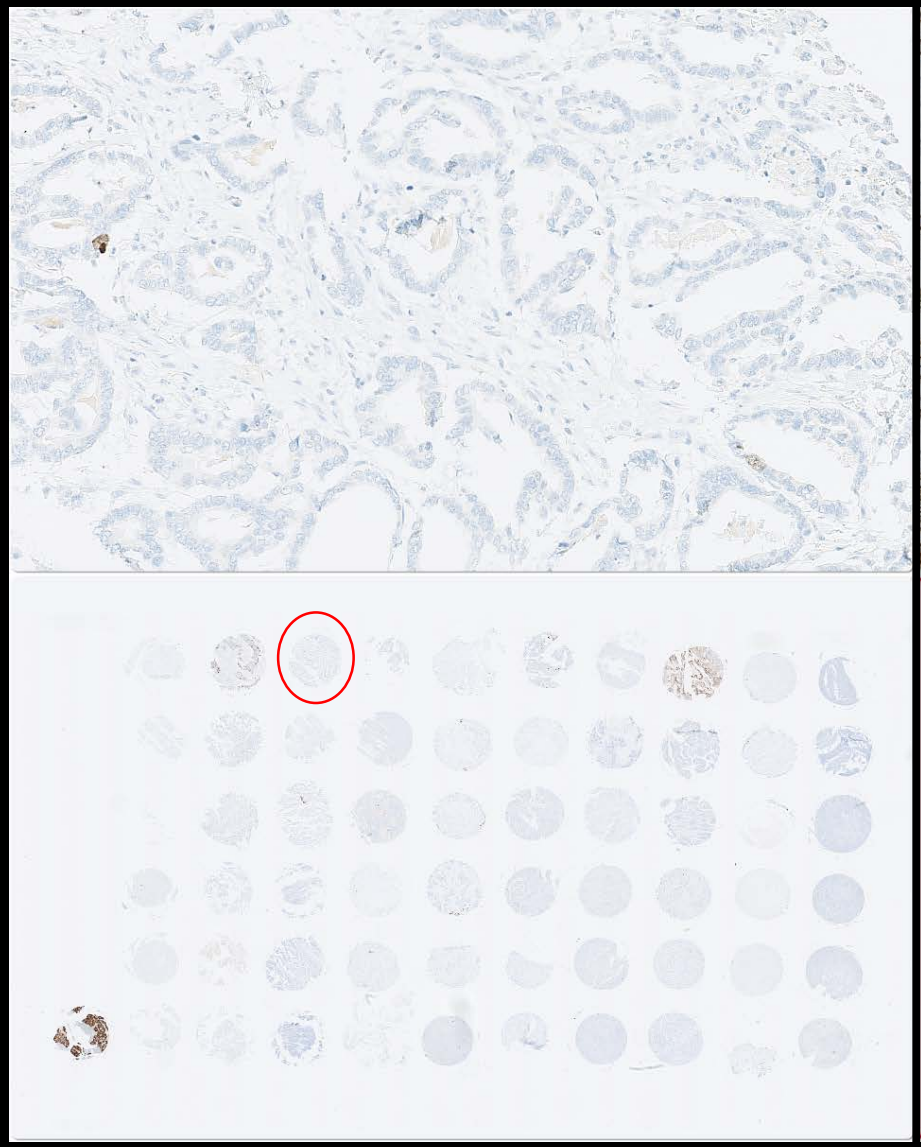


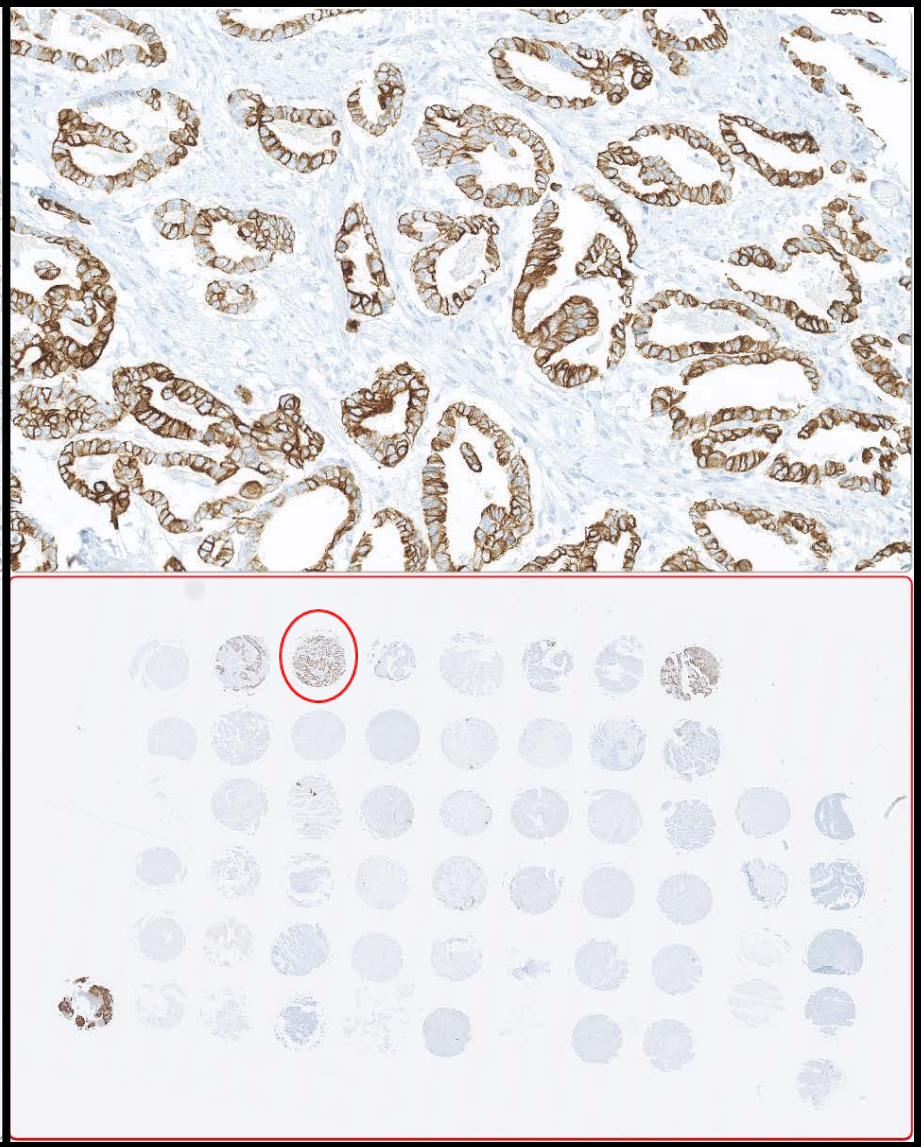
mAb XM26

Lung adenocarc.

rmAb SP27

# IHC – Protocols and controls for UPT I





mAb XM26

TMA Neoplasia
Gastic ad. carc.

rmAb SP27

# IHC – Protocols and controls for UPT I

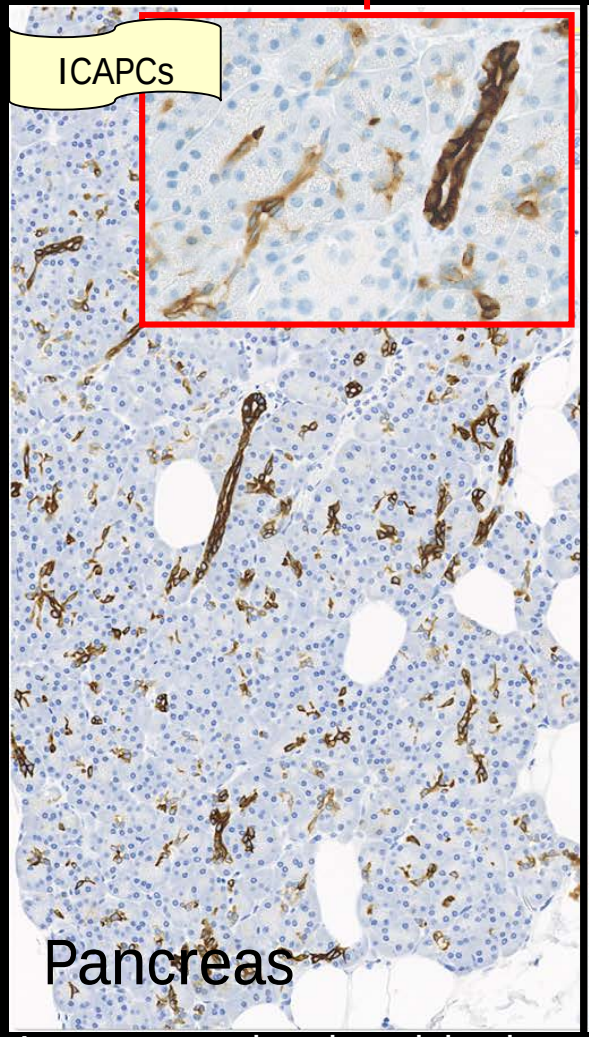
| CKs | Positive tissue control HE | Positive tissue control LE | Negative tissue control NE |
|---|---|---|---|
| CK 7 | Pancreas: Epithelial cells of large ducts<br><br>Tonsil: Squamous epithelial cells | Pancreas: Epithelial cells of intercalating ducts<br><br>Appendix: Endothelial cells | Appendix: Vast majority of epithelial cells<br><br>Tonsil: Lymphocytes |
| CK 19 | Appendix: Virtually all epithelial cells. | Tonsil / Esophagus: Basal squamous epithelial cells | Tonsil: Lymphocytes<br>Appendix: Endothelial cells |
| CK 20 | Appendix: Luminal epithelial cells | Appendix: Epithelial cells, basal crypts | Tonsil: Squamous epithelial cells |

# IHC – Protocols and controls for UPT I

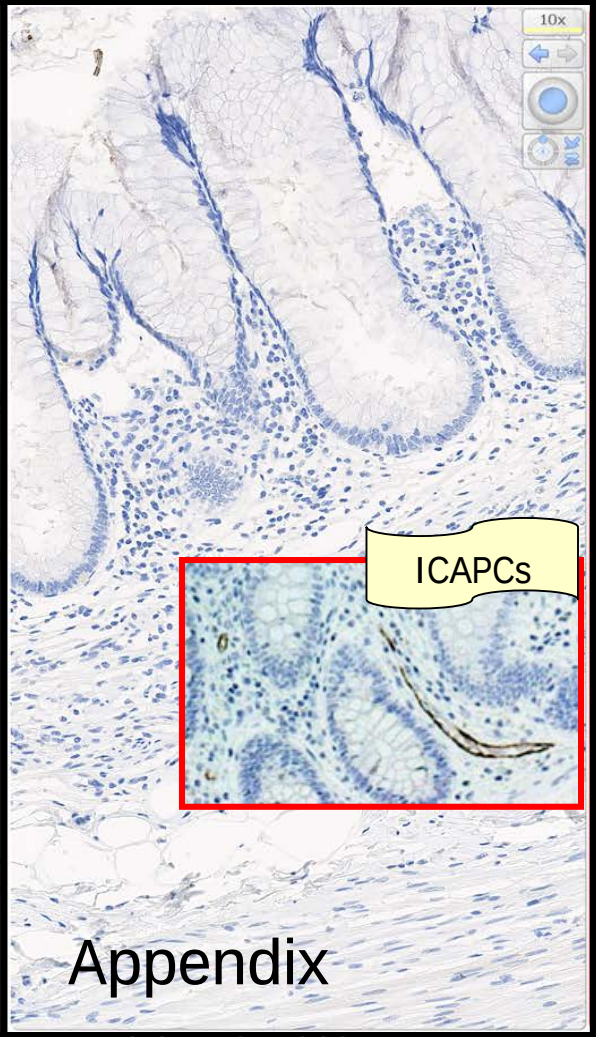
| CKs | Recommendable clones (conc.) | Less successful clones (conc.) | RTU ”plug and play” giving optimal result |
|---|---|---|---|
| CK 7 | mAb OV-TL 12/30<br>mAb RN7<br>rmAb SP52 | | Dako: mAb OV-TL 12/30<br>Leica: mAb RN7<br>VMS: mAb SP52 |
| CK 19 | mAb A53-B/A2.26<br>mAb B170<br>mAb BA17 | mAb Rck108 | VMS: mAb A53-B/A2.26 |
| CK 20 | mAb BS101<br>mAb Ks20.8<br>rmAb E19-1<br>rmAb SP33 | mAb PW31 | Dako: mAb Ks20.8<br>Leica: mAb Ks20.8<br>VMS: rmAb SP33 |

# IHC – Protocols and controls for UPT I

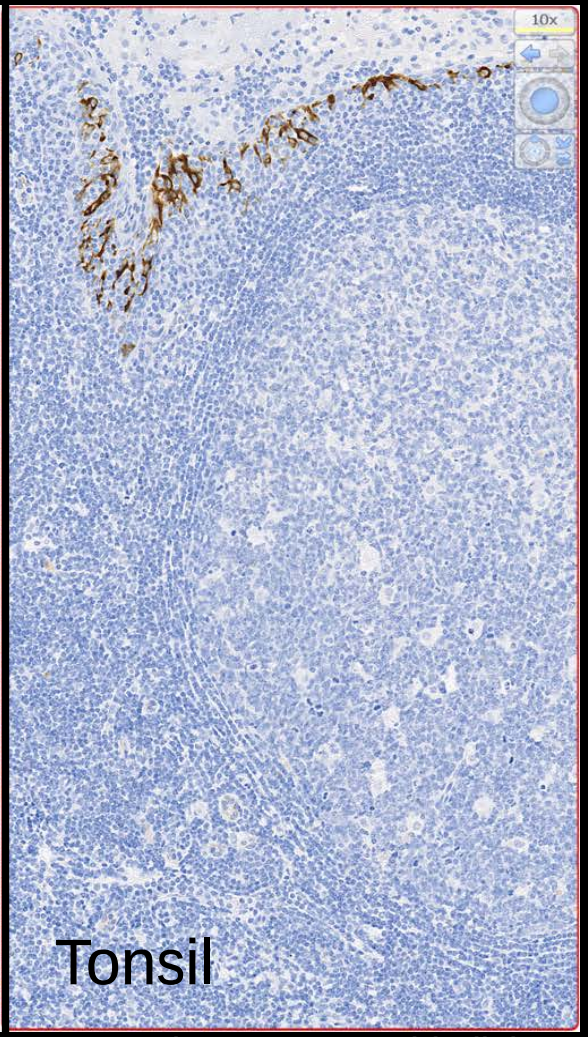
## CK7 reaction pattern




A strong cytoplasmic staining in virtually all epithelial cells of the large pancreatic ducts & weak to moderate cytoplasmic staining in cells of intercalating ducts.



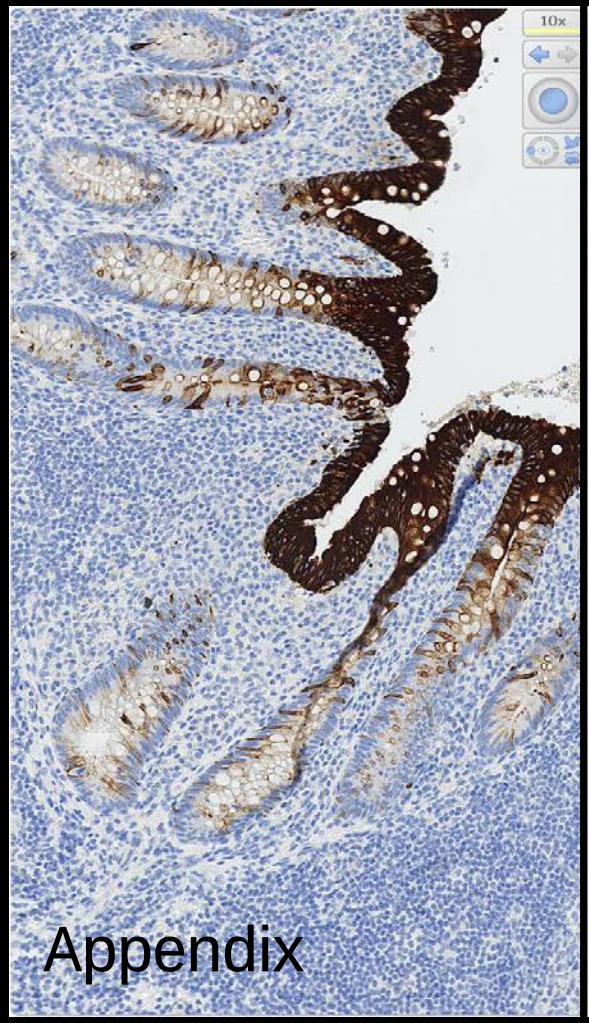

No staining should be seen. Endothelial cells can be demonstrated.



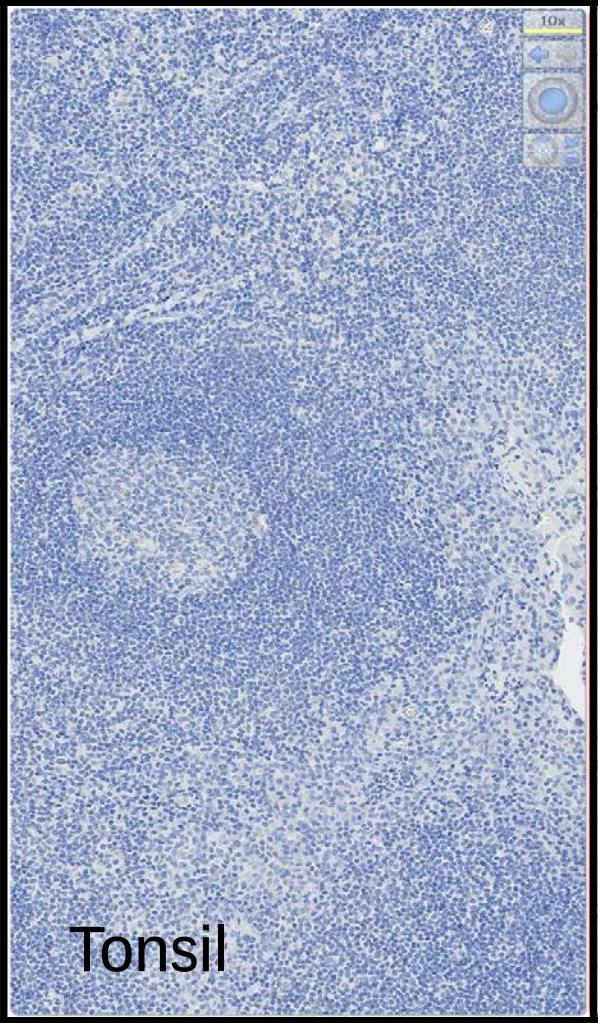

Scattered squamous epithelial cells can show a weak to strong cytoplasmic staining reaction.

# IHC – Protocols and controls for UPT I

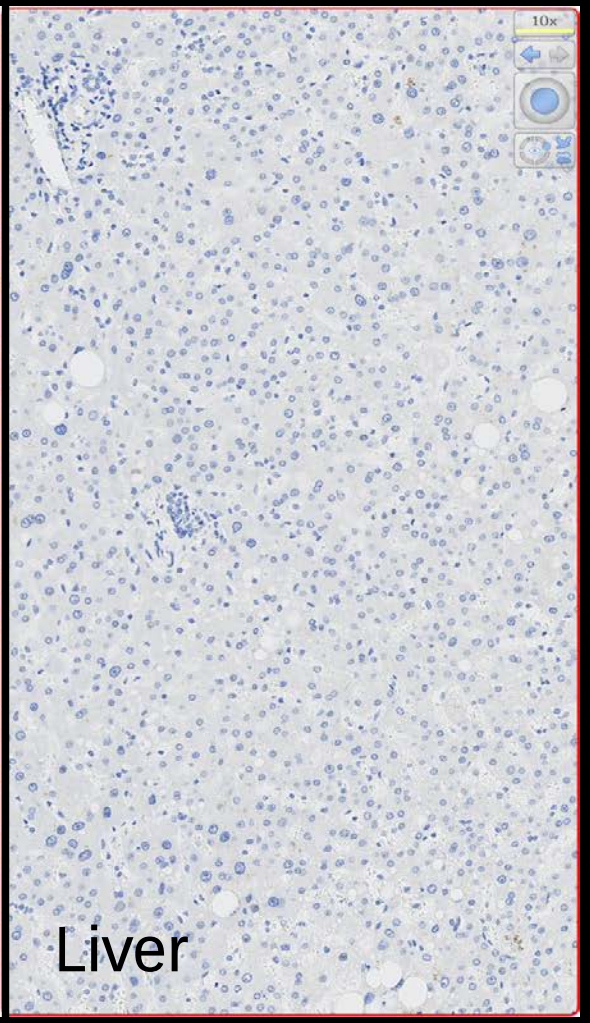
## CK20 reaction pattern




A strong, distinct cytoplasmic staining reaction of all the surface epithelial cells and at least a weak to moderate staining reaction in most crypt cells.




No staining should be seen.




No staining should be seen.

# QA Undifferented tumours and lymphomas

## 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

15 markers

**Relative frequency of lymphoid malignancies**

| 10 | 3 | 1 |
|---|---|---|
| B-Cell | Hodgkin | T-Cell |

## 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 |
| Ki67 | √ | Run B22 | 93 | 69 |
| CD5 | √ | Run 49 | 92 | 68 |
| CD3 | √ | Run 37 | 92 | 66 |
| CD23 | √ | Run 50 | 89 | 55 |
| Pax5 | √ | Run 41 | 84 | 54 |
| CD45 | √ | Run 37 | 82 | 56 |
| BCL2 | √ | Run 28 | 82 | 44 |
| CD79a | √ | Run 45 | 79 | 51 |
| BCL6 | √ | Run 42 | 74 | 30 |
| CD30 | √ | Run 43 | 71 | 34 |
| Lambda | √ | Run 15 | 34 | 15 |
| Kappa | √ | Run 18 | 41 | 14 |
| CD43 | - | - | - | - |

# IHC – Protocols and controls for Lymphomas

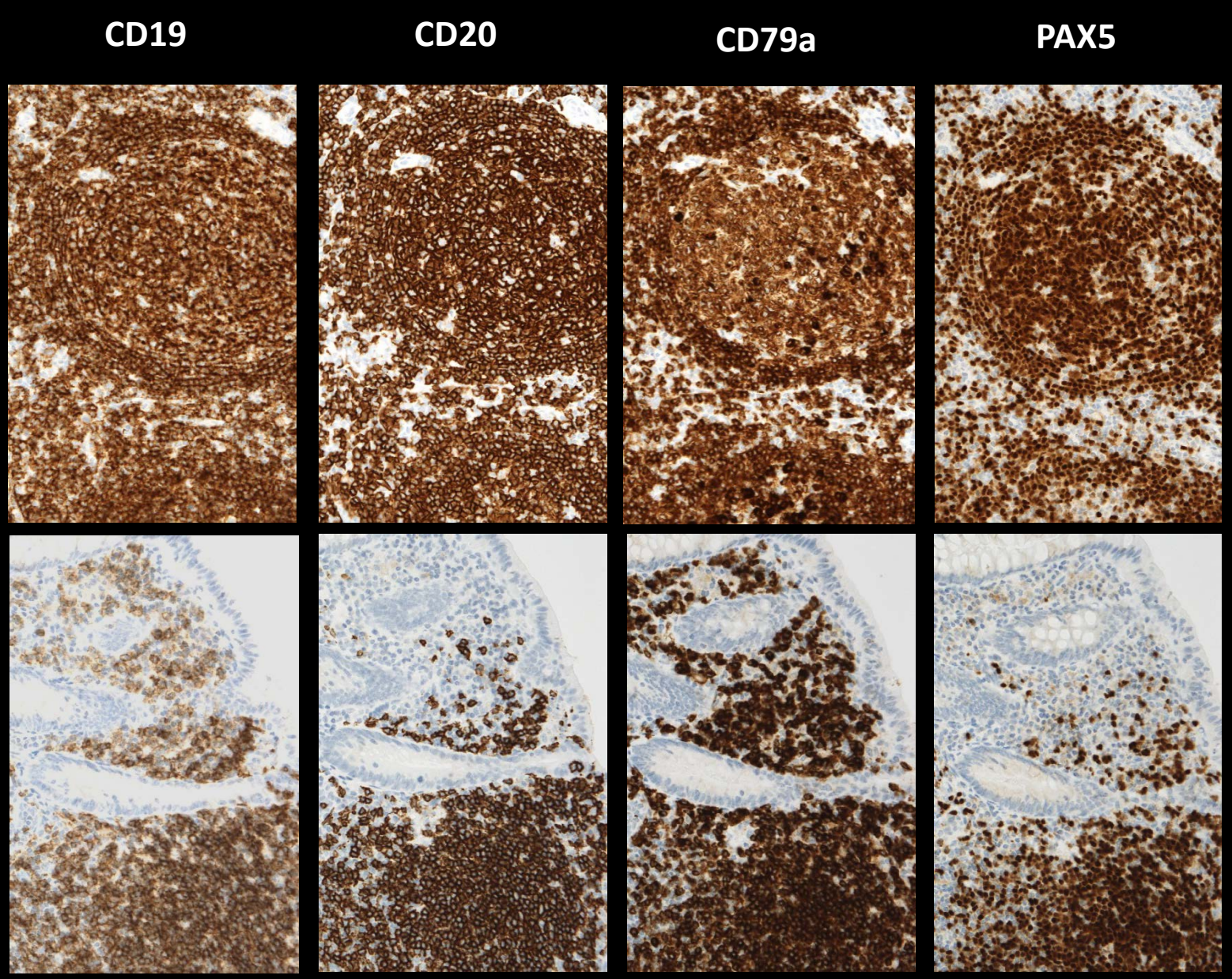
| B-cells | Recommendable clones (conc.) | Less successful clones (conc.) | RTU ”plug and play” giving optimal result |
|---|---|---|---|
| CD19 | mAb LE-CD19<br>mAb BT51E | *mAb LE-CD19.....* | Dako: mAb LE-CD19<br>Leica: mAb BT51E |
| CD20 | mAb L26<br>mAb 7D1<br>rmAb EP7 | pAbs | Dako: mAb L26<br>Leica: mAb L26<br>Ventana: mAb L26 |
| CD79a | mAb JCB117*<br>rmAb SP18 | mAb HM57<br>mAb 11D10<br>mAb 11E3 | Dako: mAb JCB117<br>VMS: rmAb SP18 |
| BSAP | mAb 1EW<br>mAb 24*<br>mAb DAK-Pax5<br>rmAb SP34 | | Dako: mAb DAK-Pax5<br>Leica: mAb 1EW<br>VMS: mAb SP34 |

* Inferior on VMS BenchMark

# IHC – Protocols and controls for Lymphomas

| B-cells | Positive tissue control HE | Positive tissue control LE | Negative tissue control NE |
|---|---|---|---|
| CD19 | Tonsil: Mantle zone, germinal centre & interfollicular B-cells | Appendix: Plasma cells | Appendix: Epithelial cells, muscle cells etc |
| CD20 | Tonsil: Mantle zone, germinal centre & interfollicular B-cells | ------- None--------- | Appendix: Epithelial cells, muscle cells etc |
| CD79a | Tonsil: Mantle zone, & interfollicular B-cells | Tonsil: Germinal centre B-cells | Appendix: Epithelial cells, muscle cells etc |
| BSAP | Tonsil: Mantle zone, germinal centre & interfollicular B-cells | Hodgkin classical: Reed-Sternberg cells | Appendix: Epithelial cells, muscle cells etc |

# IHC – Protocols and controls for Lymphomas



# IHC – Protocols and controls for Lymphomas

J Clin Pathol 1998;51:364–369

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

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

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

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

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

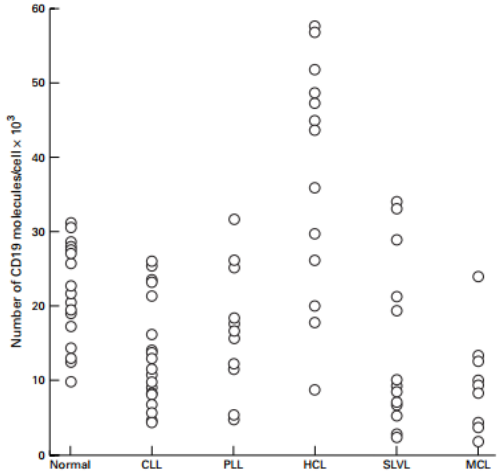



*Figure 2 Distribution of individual ABC values for CD19 in normal peripheral blood B lymphocytes and B cell leukaemias.*

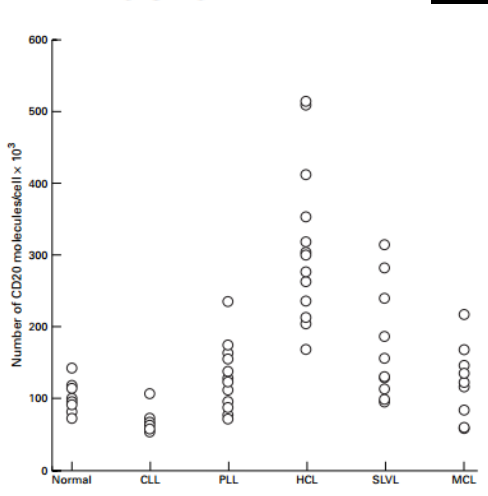



*Figure 4 Individual ABC values for CD20 in normal peripheral blood B cells and B lineage leukaemias.*

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

DIAGNOSTIC PATHOLOGY

RESEARCH Open Access

## The predictive significance of CD20 expression in B-cell lymphomas

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

**Abstract**

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

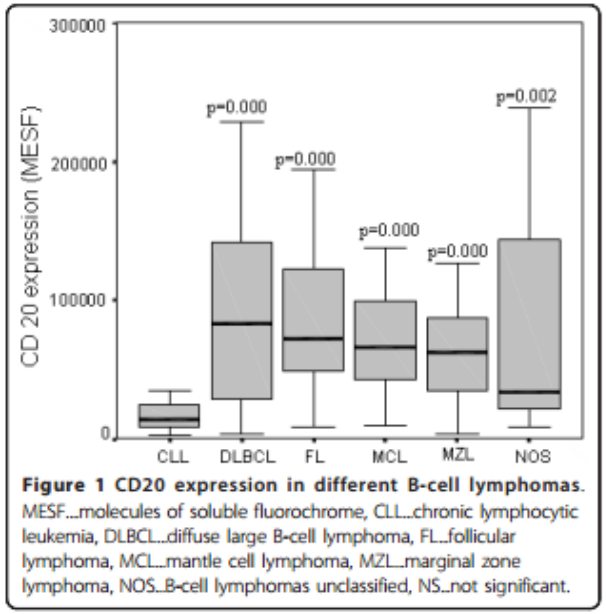
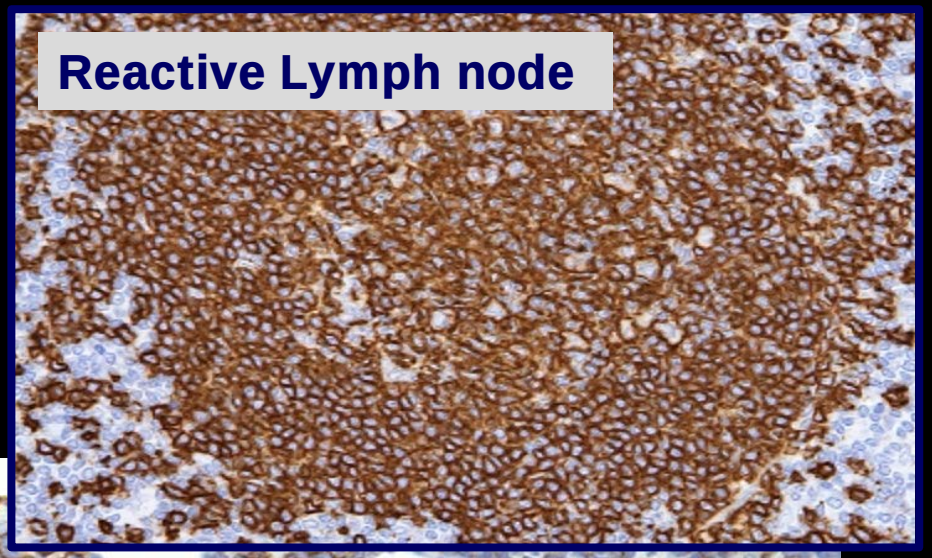


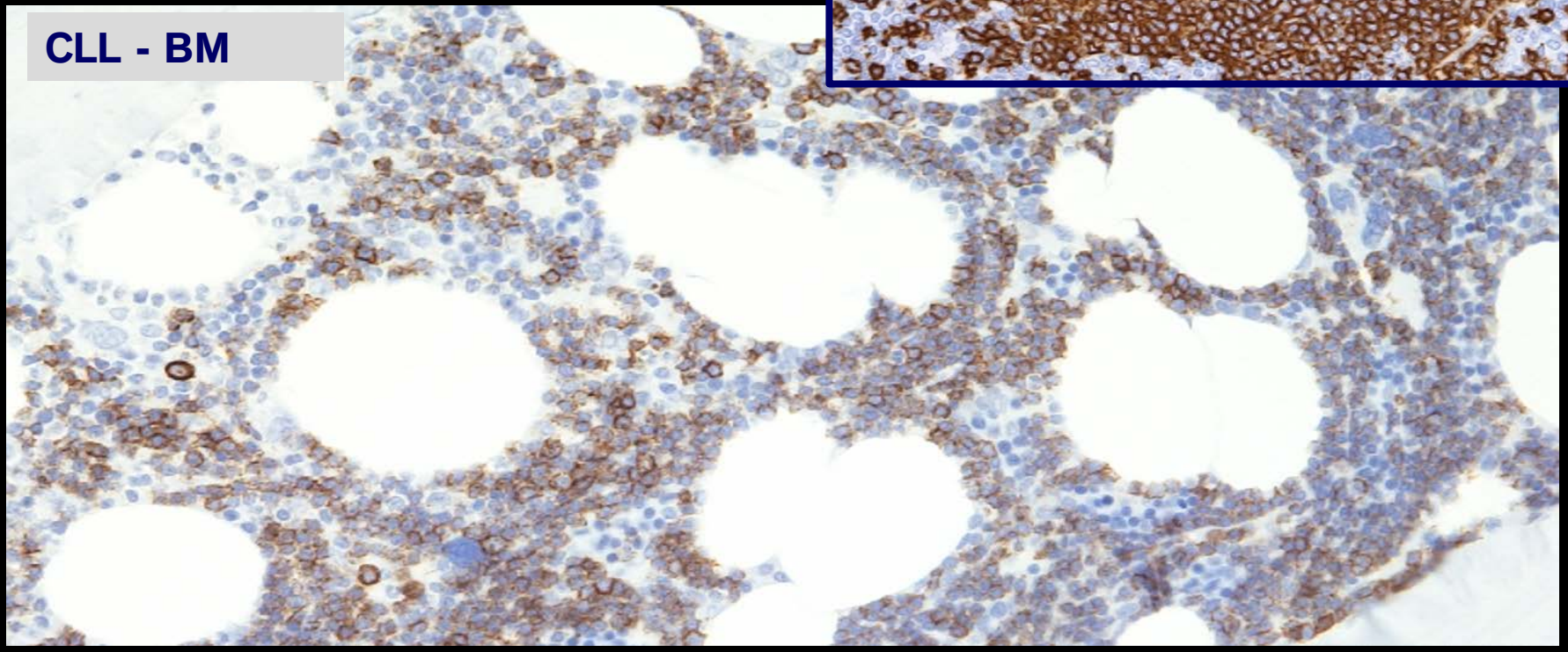

**Figure 1 CD20 expression in different B-cell lymphomas.** MESF...molecules of soluble fluorochrome, CLL...chronic lymphocytic leukemia, DLBCL...diffuse large B-cell lymphoma, FL...follicular lymphoma, MCL...mantle cell lymphoma, MZL...marginal zone lymphoma, NOS...B-cell lymphomas unclassified, NS...not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

CD19 and CD20 can both be reduced or increased in lymphomas compared to normal stage

# IHC – Protocols and controls for Lymphomas

CD20 mAb L26 same protocol



# IHC – Protocols and controls for Lymphomas

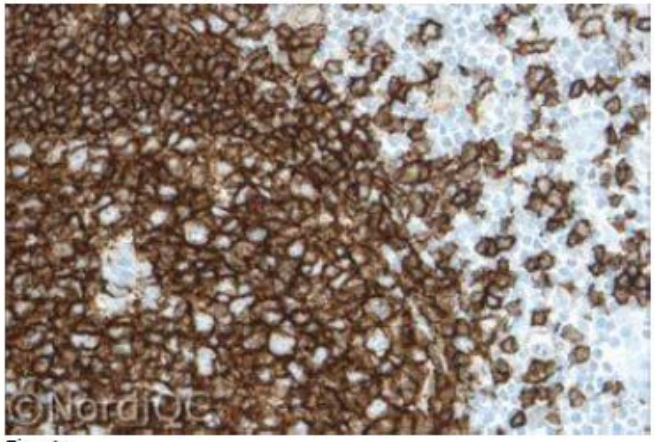


Fig. 1a
Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. HIER was performed using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the B-cells.

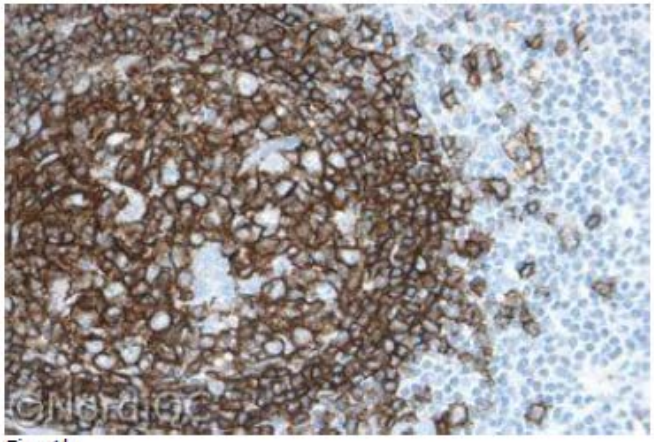


Fig. 1b
Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. No HIER was performed. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors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

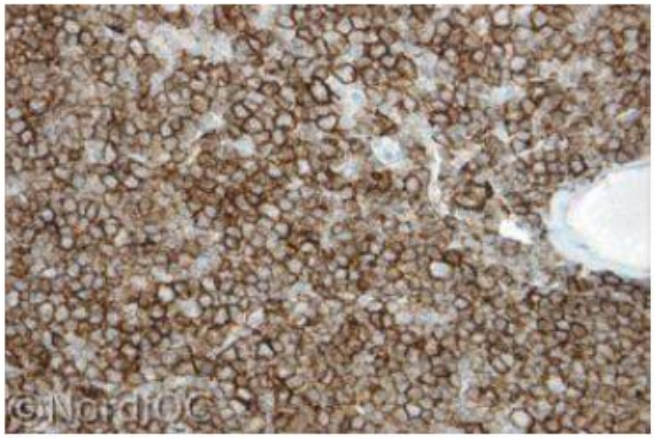


Fig. 2a
B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

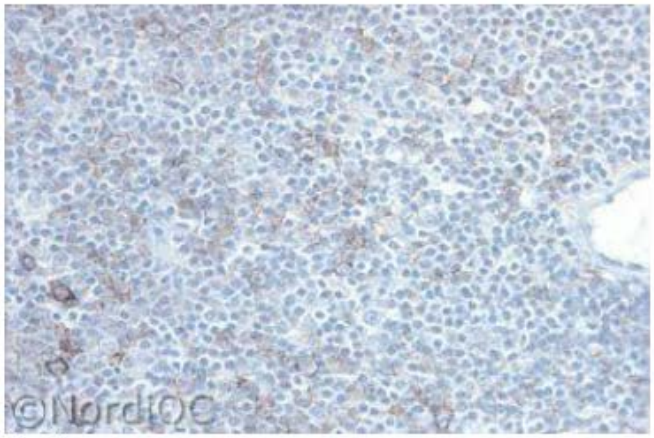


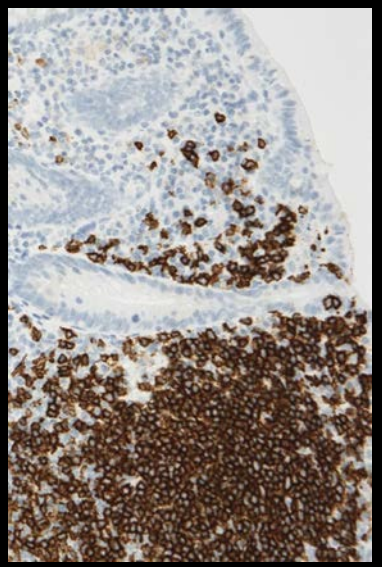
Fig. 2b
B-CLL. Insufficient staining for CD20 using the same protocol as in Fig. 1b. Omitting HIER, only scattered cells are positive. The majority of the neoplastic cells are negative. Compare with the optimal result in Fig. 2a, same field.

CD20 IHC assay

CLL needed to Validate accuracy

Tonsil ASAP

APP neg control



# IHC – Protocols and controls for Lymphomas

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

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

**Suff. (clone L26)**

HIER (preferable in alkaline buffers)

1:75-1:2.000

All detection systems

**Insuff. (clone L26)**

Omission of HIER

Too low conc. of primary Ab

RTU's superior to LDT's

# IHC – Protocols and controls for Lymphomas

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

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

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

**mAb clone LE-CD19 Dako most consistent**

**HIER in alk. pH**

**3-step polymer**

**mAb clone LE-CD19 (Serotec, Biocare …)**

**False positive**

# IHC – Protocols and controls for Lymphomas

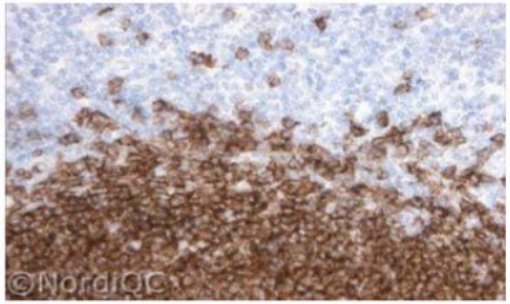


Fig. 1a
Normal tonsil showing an optimal staining for CD19 using the mAb clone LE-CD19 from Dako, diluted 1:50, on the Autostainer platform. HIER was performed using TRS pH 9 (3-in-1) (Dako). A strong and distinct membranous staining reaction is seen in virtually all B-cells. T-cells are negative.

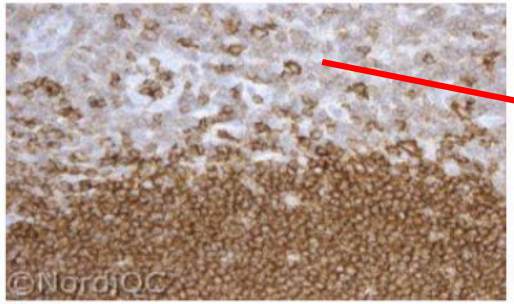


Fig. 1b
Normal tonsil showing an insufficient staining for CD19 using the mAb clone LE-CD19 from Serotec, diluted 1:500, on the Autostainer platform. HIER was performed using Citrate pH 6. In addition to a moderate to strong staining reaction in the normal B-cells (albeit weaker than that seen in Fig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

False Positive (T-cells)
Serotec / Biocare

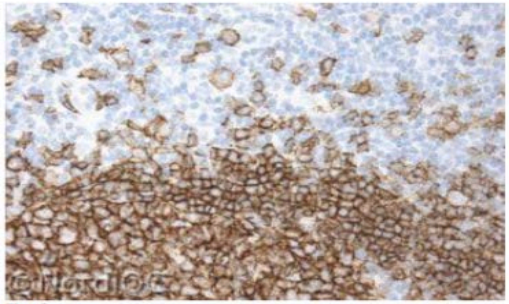


Fig. 2a
Lymphatic tissue in the appendix showing an optimal staining for CD19 using the mAb clone BT51E (RTU) on the BOND-III platform. HIER was performed using Bond Epitope Retrieval Solution 1. A strong and very distinct membranous staining is seen in virtually all B-cells, while the T-cells are negative.

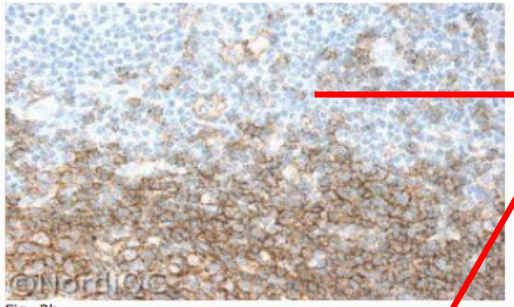


Fig. 2b
Lymphatic tissue in the appendix showing an insufficient staining for CD19 using the mAb clone BT51E, diluted 1:30, on the BenchMark platform. HIER was performed using Cell Conditioning 1. Only a weak to moderate staining is seen in the majority of B-cells. T-cells are negative. Also compare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

Too weak

mAb clone BT51E applied by protocol settings with too low sensitivity

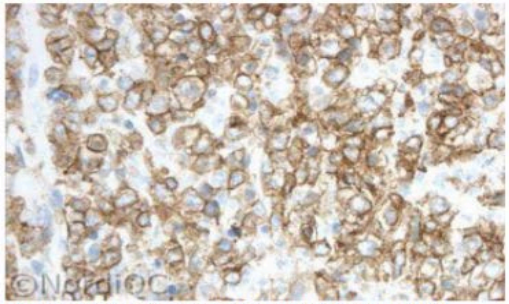


Fig. 3a. Optimal staining reaction for CD19 of the DLBCL. Same protocol used as in Fig. 2a based on the mAb clone BT51E. A moderate to strong membranous staining reaction is seen in virtually all the neoplastic cells.

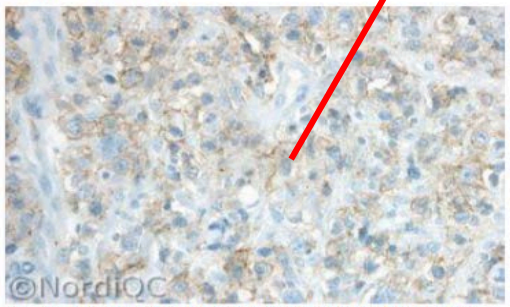


Fig. 3b. Insufficient staining reaction for CD19 of the DLBCL using same protocol as in Fig. 2b. Only a weak staining is seen in scattered neoplastic cells. The majority of the tumour cells are negative. Compare with the optimal protocol in Fig. 3a, same field.

# IHC – Protocols and controls for Lymphomas

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<br>3 | Dako<br>Thermo/NeoMarkers | 37 | 35 | 19 | 6 | 74% | 74% |
| rmAb clone **SP18** | 12<br>3<br>2<br>1<br>1 | Thermo/NeoMarkers<br>Spring Bioscience<br>Cell Marque<br>Nordic Biosite<br>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.

21 %

86 %

## Optimal (clone JCB117)

HIER (preferable alkaline buffer)

1:25-1:600

3 step detection systems

## Optimal (clone SP18)

HIER (CC1)

1:300-1:500

3-step systems

## Insufficient results

Too short inefficient HIER

Too low conc. of primary Ab

Less successful primary Abs

RTU's superior to LDT's

# IHC – Protocols and controls for Lymphomas

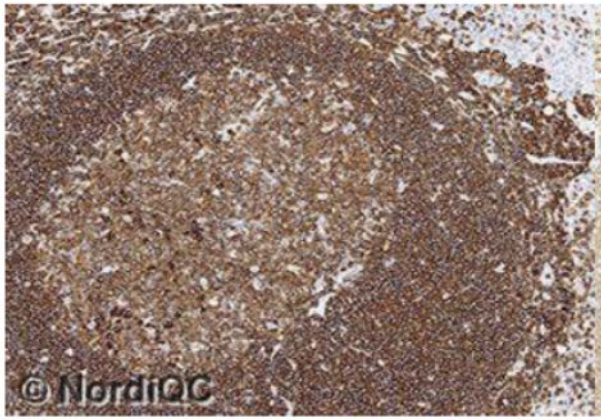



Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone JCB117 as Ready-To-Use format (GA621, Dako), with HIER in TRS High pH 9 for 30 min., a 3-step polymer based detection kit and performed on Omnis, Dako.
Mantle zone B-cells show an intense membranous staining reaction, while the germinal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

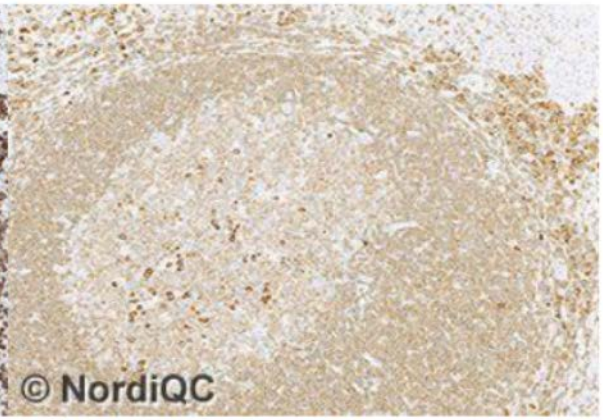



Fig. 1b
CD79a staining of the tonsil using the mAb clone JCB117 with an insufficient protocol – same field as in Fig. 1a.
The primary Ab was used at a titre of 1:500 and a 2-step multimer based detection system providing a too low sensitivity.
The mantle zone B-cells and the late stage germinal centre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

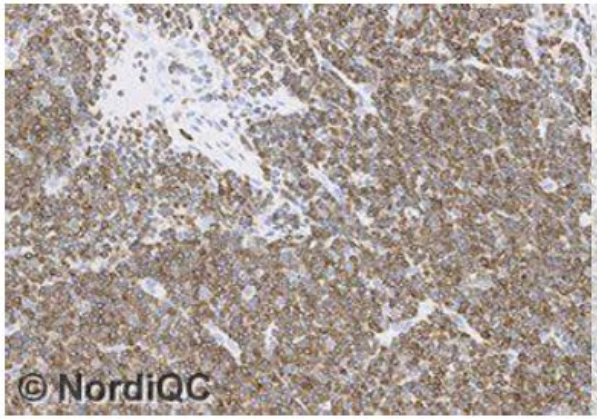



Fig. 2a
Optimal CD79a staining of the B-CLL using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate and distinct membranous staining reaction.
No background reaction is seen.

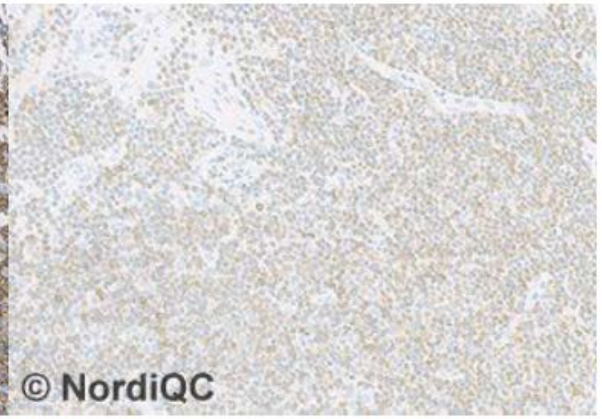



Fig. 2b
Insufficient CD79a staining of the B-CLL using same protocol as in Fig. 1b - same field as in Fig. 2a.
The neoplastic cells only show a weak and equivocal staining reaction.
Also compare with Fig. 3b – same protocol.

**Problem:**

**Too low sensitivity;**

**1. Low concentration of primary Ab**

**2. Detection system**

# IHC – Protocols and controls for Lymphomas

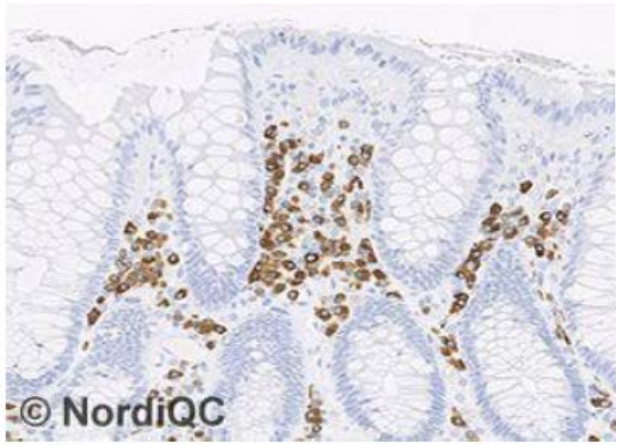



Fig. 4a
Optimal CD79a staining of colon using same protocol as in Figs. 1a - 3a.
Plasma cells show a moderate to strong cytoplasmic staining reaction.
No background reaction is seen.

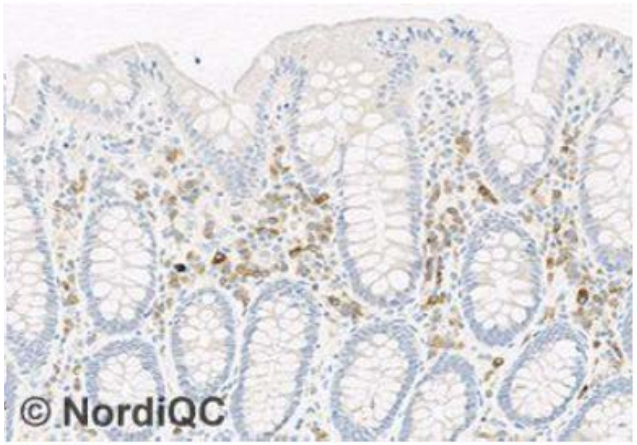



Fig. 4b
CD79a staining of the colon using an insufficient protocol based on the mAb clone 11E3.
The intensity and proportion of plasma cells demonstrated is reduced compared to the level expected.
However also compare with Fig. 5b – same protocol

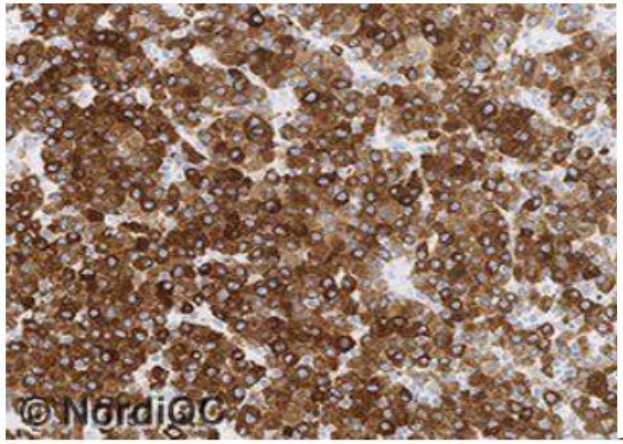



Fig. 5a
Optimal CD79a staining of the plasmacytoma using same protocol as in Figs. 1a - 4a.
Virtually all neoplastic cells show a moderate cytoplasmic staining reaction.

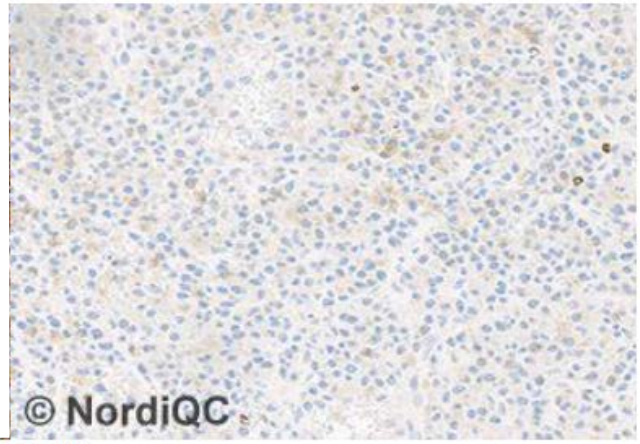



Fig. 5b
Insufficient CD79a staining of the plasmacytoma using same protocol as in Fig. 4b.
Only scattered normal B-cells are demonstrated, while the neoplastic cells are negative.
9 of 9 protocols based on mAb clone 11E3 provided an insufficient result due to a too weak or completely false negative staining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

**Problem:**

**Less successful Ab**

**mAb 11E3**
**or**
**mAb HM57**

# IHC – Protocols and controls for Lymphomas

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% | 88% |
| 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<br>4<br>2 | Cell Marque<br>Spring Biosciences<br>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% | |

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

HIER in high pH

3-step detection

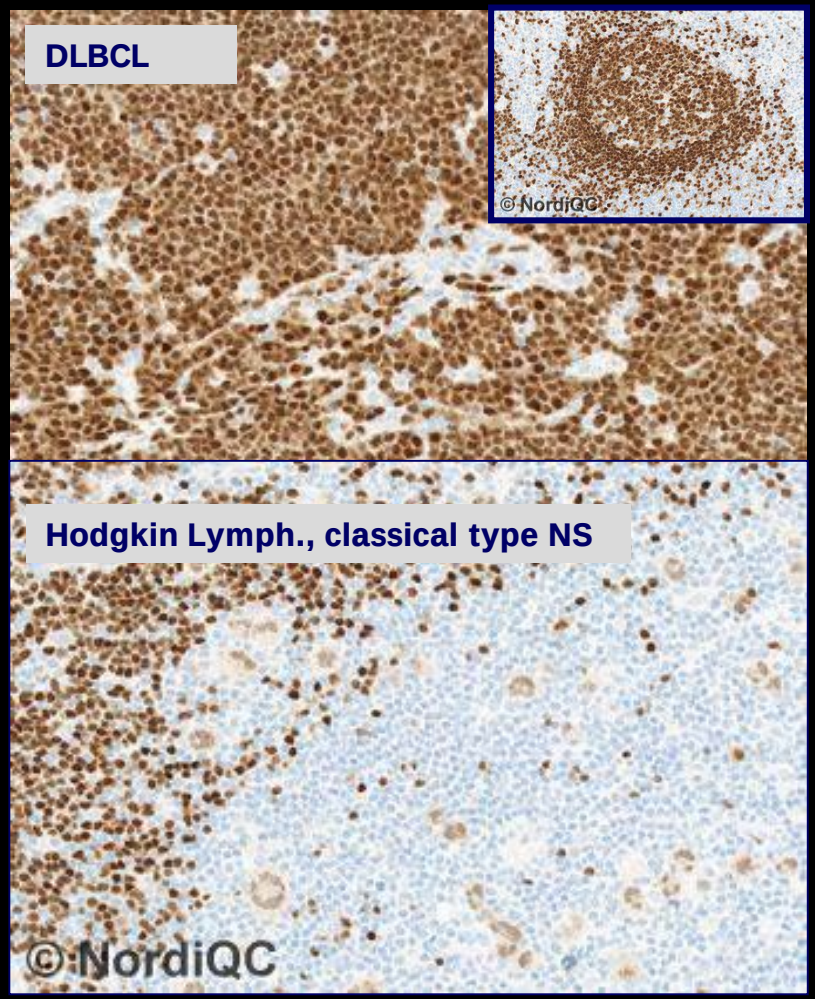
24 + 1EW inferior on VMS

RTU's superior to LDT's

# IHC – Protocols and controls for Lymphomas

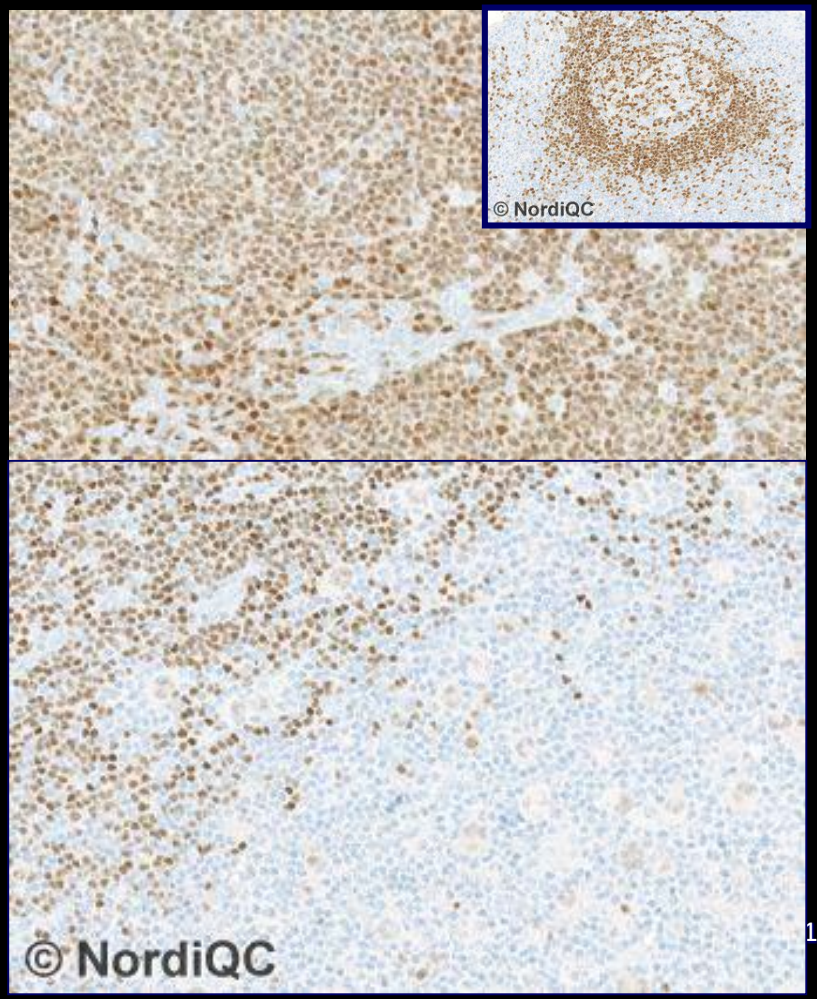
## Optimal

rmAb SP34 , OV (3-step multimer )




## Insufficient

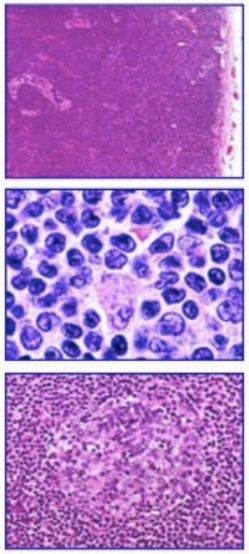
DAK-PAX5: (too low titre), UV (2-step multimer )



## Immunophenotype: Small B-Cell Lymphomas

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

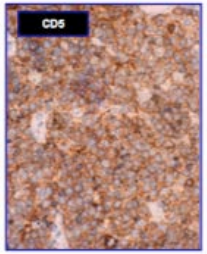
## Mantle Cell Lymphoma

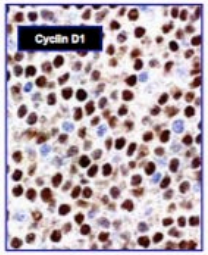


**Morphology**
- small-medium lymphocytes
- cleaved / irregular
- blastoid variant
- nodular / mantle / diffuse

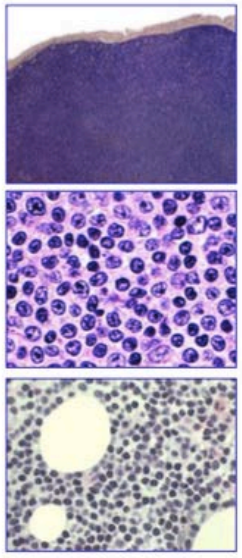
**Immunology**

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



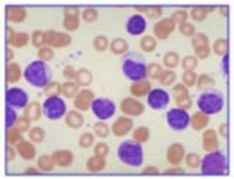

## B-cell Small Lymphocytic Lymphoma (CLL)

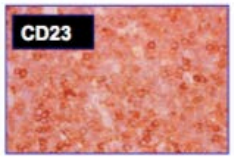


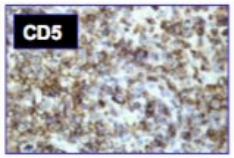
**Morphology**
- small lymphocytes
- proliferation centres

**Immunology**

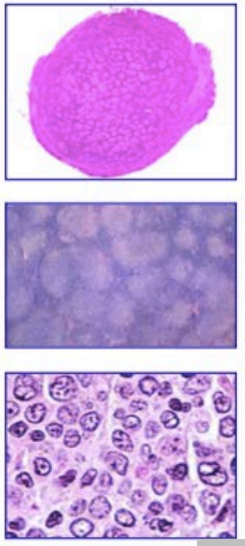
| | |
|---|---|
| surface IgMD weak | |
| CD19, 20, 79a | + |
| CD5 | + |
| CD23 | + |
| CD10, CycD1 | - |





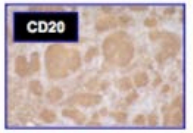

## Follicular Lymphoma

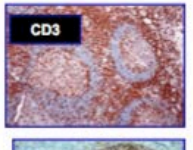


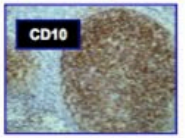
**Morphology**
- germinal centre cells
- CBs & CCs
- follicular

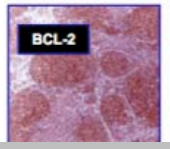
**Immunology**

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



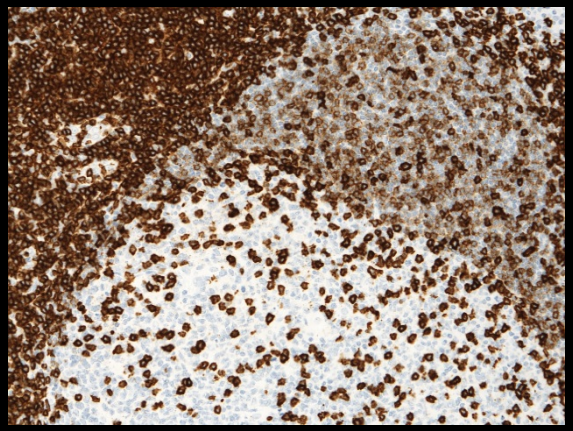

Courtesy: Steve Hamilton-Dutoit

# IHC – Protocols and controls for Lymphomas

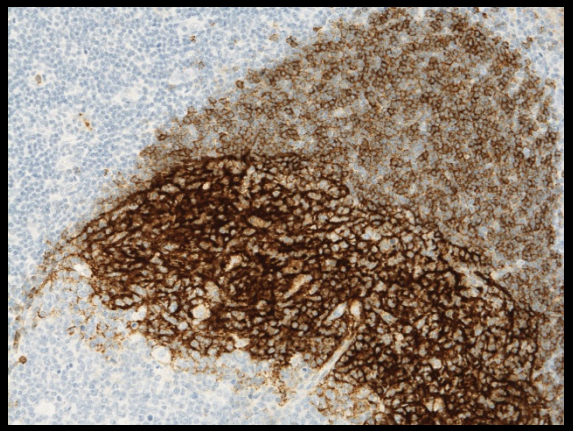
| B-cells | Recommendable clones (conc.) | Less successful clones (conc.) | RTU ”plug and play” giving optimal result |
|---|---|---|---|
| CD5 | mAb 4C7<br>rmAb SP19 | mAb CD5/54/F6<br>rmAb EP77 | Dako: mAb 4C7<br>Leica: mAb 4C7<br>VMS: mAb SP19 |
| CD23 | mAb 1B12<br>mAb DAK-CD23<br>rmAb SP23 | mAb MHM26 | Dako: mAb DAK-CD23<br>Leica: mAb 1B12<br>Ventana: mAb SP23 |
| CyD1 | rmAb SP4<br>rmAb EP12 | mAb DCS6<br>mAb P2D11F11 | Dako: rmAb EP12<br>VMS: rmAb SP4 |

# IHC – Protocols and controls for Lymphomas

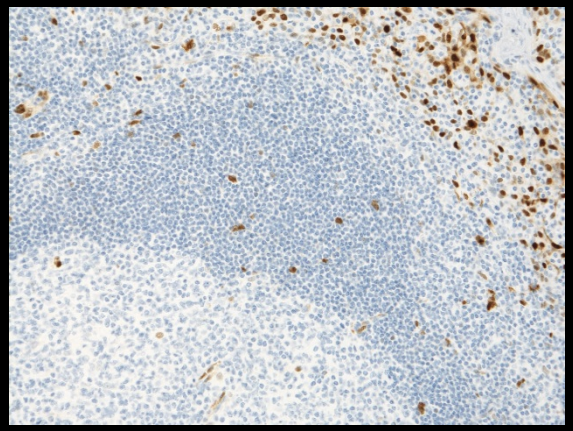
| B-cells | Positive tissue control HE | Positive tissue control LE | Negative tissue control NE |
|---|---|---|---|
| CD5 | Tonsil: T-cells | Tonsil: Mantle zone B-ells | Appendix: Epithelial cells, muscle cells etc |
| CD23 | Tonsil: Follicular dendritic network | Tonsil: Mantle zone B-ells | Appendix: Epithelial cells, muscle cells etc |
| CyD1 | Tonsil: Squamous epithelial cells | Tonsil: Germinal centre macrophages / endothelial cells | Tonsil: Mantle zone B-cells |



CD5



CD23



CyD1

# IHC – Protocols and controls for Lymphomas

**Performance history**

This was the fifth NordiQC assessment of CD5. The overall pass rate was high and has increased significantly compared to the result obtained in run 34, 2012 (see table 2).

Table 2. **Proportion of sufficient results for CD5 in the five NordiQC runs performed**

| | Run 8 2003 | Run 17 2006 | Run 24 2008 | Run 34 2012 | Run 49 2017 |
|---|---|---|---|---|---|
| Participants, n= | 65 | 88 | 119 | 187 | 278 |
| Sufficient results | 65% | 66% | 68% | 79% | 92% |

**Performance history**

This was the fifth NordiQC assessment of CD23. The overall pass rate was significantly higher compared with all previous runs for CD23 (see Table 2).

Table 2. **Proportion of sufficient results for CD23 in the five NordiQC runs performed**

| | Run 8 2003 | Run 19 2007 | Run 24 2008 | Run 34 2012 | Run 50 2017 |
|---|---|---|---|---|---|
| Participants, n= | 59 | 88 | 114 | 181 | 270 |
| Sufficient results | 76% | 54% | 56% | 73% | 89% |

**Performance history**

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

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

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

# IHC – CD5

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

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

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

HIER in high pH

3-step detection

Titre of primary Ab

RTU's superior to LDT's

# IHC – CD5

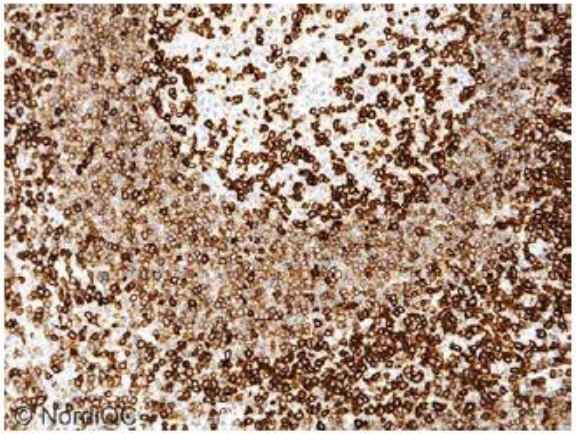



Fig. 2a (x200)
Optimal staining for CD5 in the tonsil, core 2, using same protocol as in Fig. 1a. T-cells are strongly stained and the majority of mantle zone B-cells show a weak to moderate staining reaction.

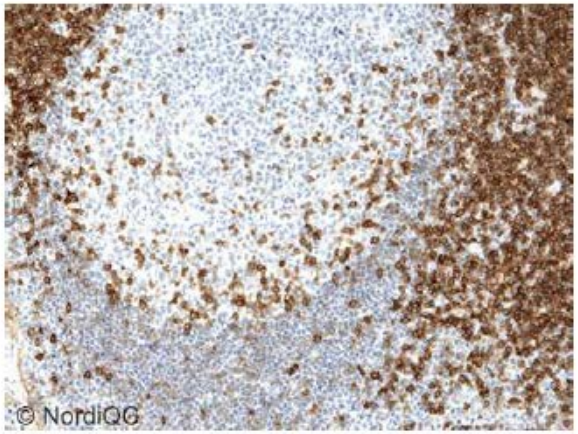



Fig. 2b (x200)
Insufficient staining for CD5 in the tonsil, core 2, using same protocol as in Fig. 1b. The intensity of the staining reaction in T-cells is reduced compared to the result obtained in Fig. 2a. However most important and critical; no staining reaction in the mantle zone B-cells is observed – compare with Fig. 2a (same field).

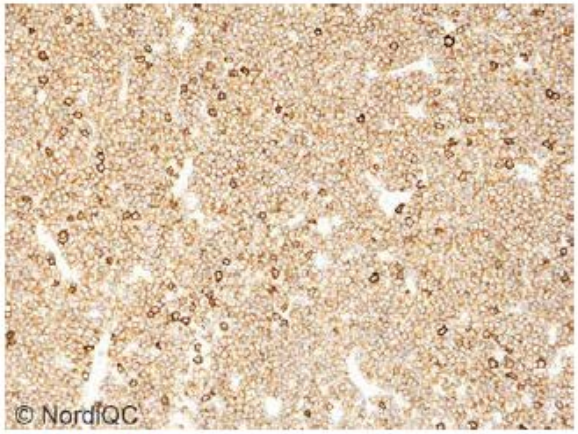



Fig. 4a (x200)
Optimal staining for CD5 of the MCL, core 4, using same protocol as in Figs. 1a - 3a. Virtual all the neoplastic cells show a weak to moderate, distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

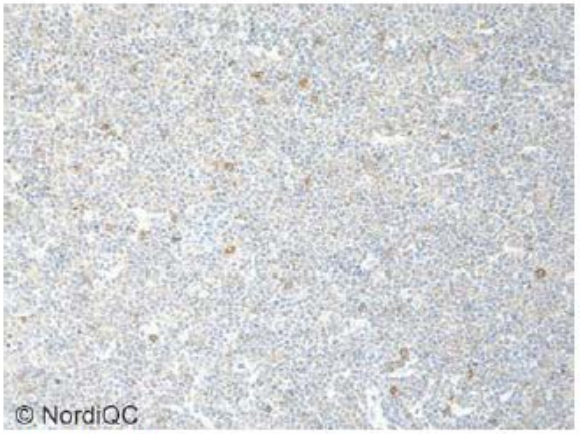



Fig. 4b (x200)
Insufficient staining for CD5 of the MCL, core 4, using same protocol as in Figs. 1b -3b. The neoplastic cells are false negative and only T-cells with reduced intensity are demonstrated - compare with Fig. 4a (same field).

# IHC – CD23

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

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **1B12** | 51<br>3<br>2<br>2<br>2 | Leica/Novocastra<br>Cell Marque<br>Biocare<br>Thermo F. Scientific<br>Monosan | 22 | 27 | 8 | 3 | 82% | 87% |
| mAb clone **DAK-CD23** | 12 | Agilent/Dako | 5 | 4 | 2 | 1 | 75% | 100% |
| mAb clone **BS20** | 1 | Nordic Biosite | 1 | 0 | 0 | 0 | - | - |
| mAb clone **MRQ-57** | 1 | | 0 | 0 | 1 | 0 | - | - |
| mAb clone **MHM6*** | 1 | Agilent/Dako | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP23** | 25<br>3<br>3<br>1<br>1 | Thermo S./ Neomarkers<br>Spring Bioscience<br>Cell Marque<br>Immunologic<br>Diagnostic Biosystems | 20 | 9 | 4 | 0 | 88% | 90% |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **1B12 PA0169** | 9 | Leica/Novocastra | 8 | 0 | 1 | 0 | 89% | 100% |
| mAb clone **1B12**[3] **PA0169** | 3 | Leica/Novocastra | 0 | 2 | 1 | 0 | - | - |
| mAb clone **1B12 123M-18** | 1 | Cell Marque | 0 | 0 | 1 | 0 | - | - |
| mAb clone **1B12 PM100** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| mAb clone **1B12 PDM143** | 1 | Diagnostic Biosystems | 0 | 0 | 1 | 0 | - | - |
| mAb clone **DAK-CD23 IR781** | 31 | Agilent/Dako | 24 | 5 | 1 | 1 | 94% | 92% |
| mAb clone **DAK-CD23 IR781**[4] | 7 | Agilent/Dako | 3 | 4 | 0 | 0 | 100% | - |
| mAb clone **DAK-CD23 GA781** | 15 | Agilent/Dako | 14 | 1 | 0 | 0 | 100% | 100% |
| mAb clone **DAK-CD23 GA781**[5] | 1 | Agilent/Dako | 0 | 1 | 0 | 0 | - | - |
| mAb clone **GR013 8262-C010** | 1 | Sakura | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP23 790-4408** | 78 | Roche/Ventana | 43 | 34 | 1 | 0 | 99% | 99% |
| rmAb clone **SP23 123R-17/18** | 5 | Cell Marque | 3 | 1 | 1 | 0 | 80% | 100% |
| rmAb clone **SP23 MAD-00333QD** | 3 | Master Diagnostica | 2 | 0 | 0 | 1 | - | - |
| rmAb clone **SP23 M3231** | 2 | Spring Bioscience | 0 | 2 | 0 | 0 | - | - |
| rmAb clone **SP23 RMA-0504** | 1 | Maixin | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **SP23 IR800*** | 1 | Agilent/Dako | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP75 123R-27/28** | 1 | Cell Marque | 1 | 0 | 0 | 0 | - | - |
| pAb **AR460-5/10R** | 1 | Biogenex | 0 | 0 | 0 | 1 | - | - |
| Total | 270 | | 149 | 92 | 22 | 7 | - | |
| Proportion | | | 55% | 34% | 8% | 3% | 89% | |

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

HIER in high pH – 1B12 & SP23

HIER in low pH – DAK-CD23

3-step detection

Titre of primary Ab

RTU's superior to LDT's

# IHC – CD23


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

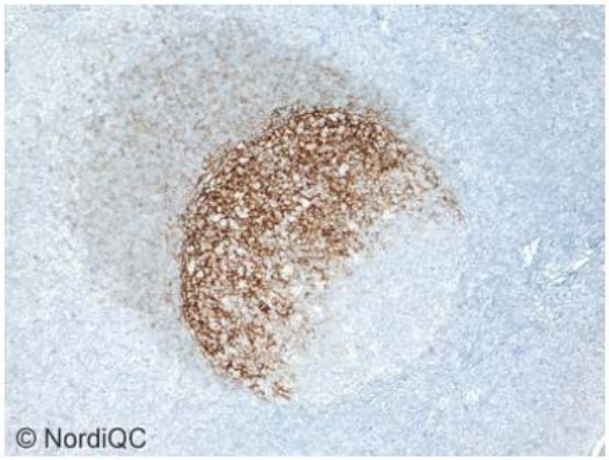



Fig. 1b (x100)
Insufficient staining reaction for CD23 of the tonsil using the mAb clone 1B12 as concentrate (too diluted, 1:50), HIER in CC1 and with a detection system giving a too low sensitivity (UltraView, Ventana) - same protocol used in Figs. 2b - 3b. The intensity of the staining reaction is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

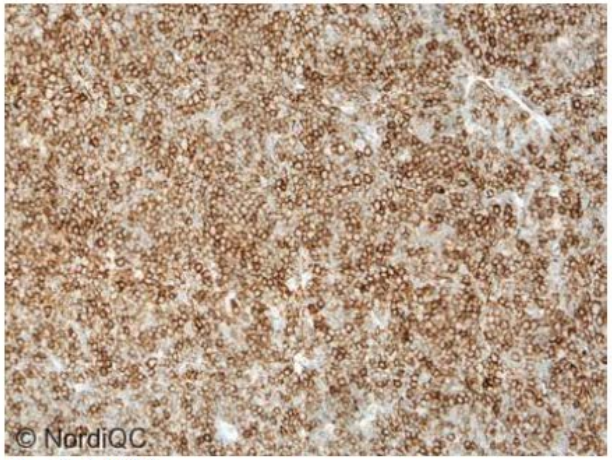



Fig. 3a (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1a and 2a. The vast majority of the neoplastic cells show a strong

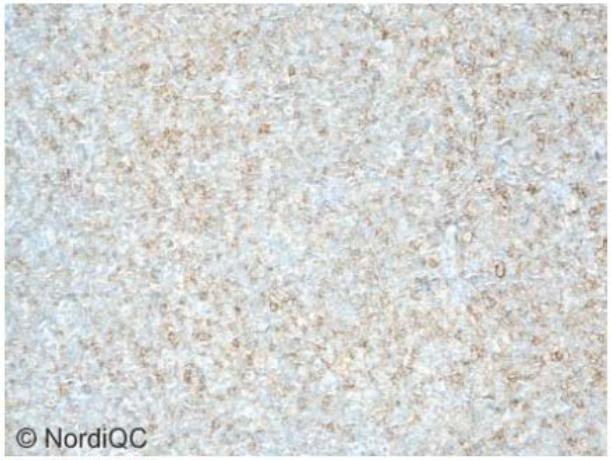



Fig. 3b (x200)
Insufficient staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.

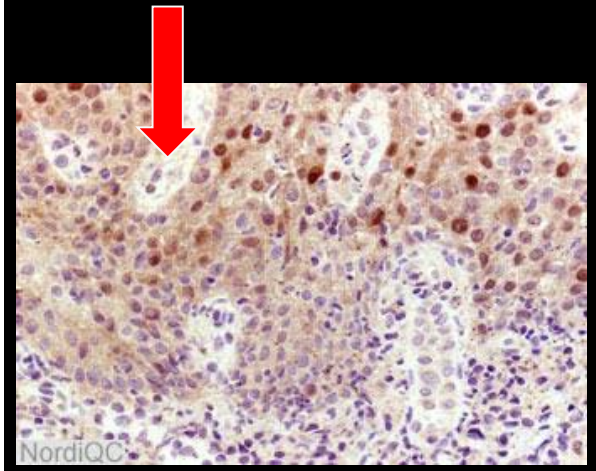
# IHC – Cyclin D1

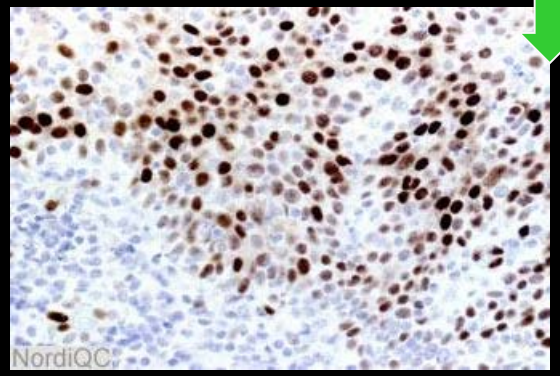
Table 1. **Antibodies and assessment marks for CyD1, run 47**

| 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<br>1<br>1 | Dako/Agilent<br>Cell Marque<br>Epitomics | 8 | 6 | 1 | 0 | 93% | 98% |
| rmAb clone **SP4** | 69<br>6<br>5<br>4<br>2<br>1<br>1<br>1<br>1 | Thermo/Neomarkers<br>Cell Marque<br>Biocare<br>Spring Bioscience<br>Zytomed<br>Immunologic<br>Maixin<br>Nordic Biosite<br>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

| Ab use | Run 9 2003 | Run 47 2015 |
|---|---|---|
| SP4 / EP12 | 5% (3/57) | 98% (251/257) |
| DCS6 / P2D11F11 | 86% (49/57) | 2% (5/257 |



# IHC – Cyclin D1

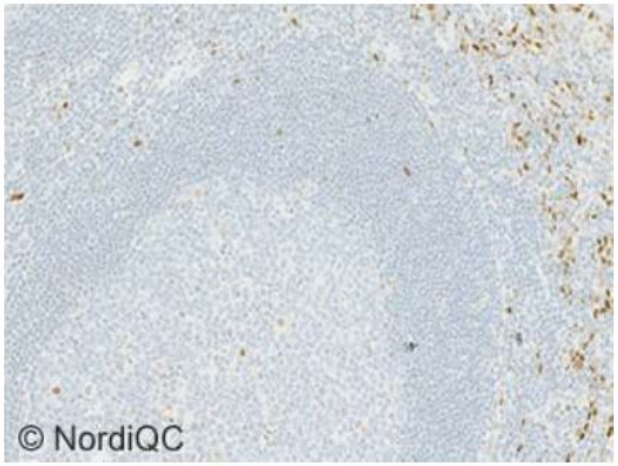



Fig. 2a
Optimal staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1a. High power field x200.
Virtually all squamous epithelial cells, dispersed endothelial cells and germinal centre macrophages show a moderate to strong nuclear staining reaction. The vast majority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

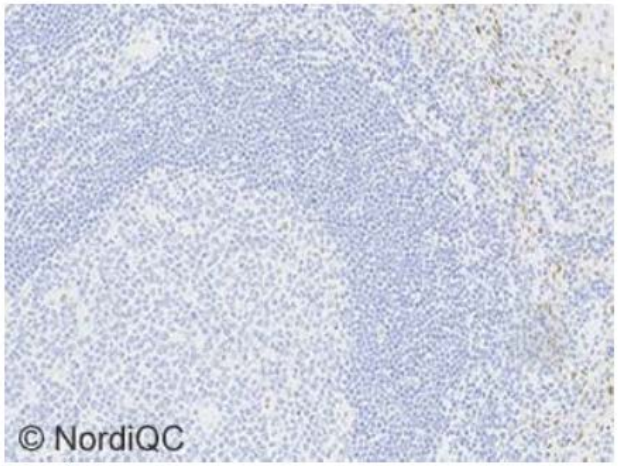



Fig. 2b
Insufficient staining for Cyclin D1 of the tonsil, tissue core no. 1, using same protocol as in Fig. 1b - same field as in Fig. 2a.
Only scattered squamous epithelial cells show a weak and equivocal staining reaction, while endothelial cells and germinal centre macrophages are negative.
Also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

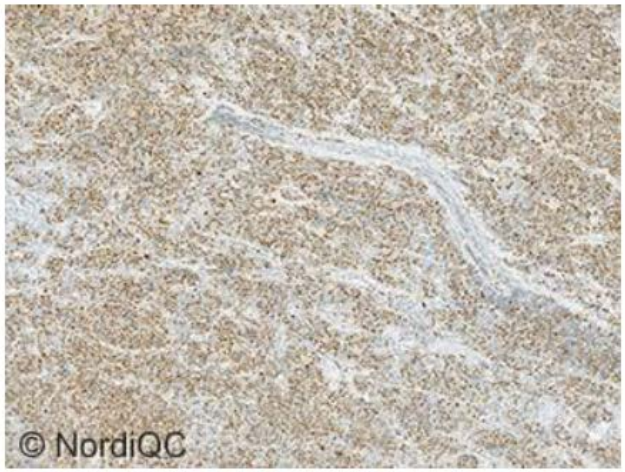



Fig. 3a
Optimal staining for Cyclin D1 of the mantle cell lymphoma, tissue core no. 4, using same protocol as in Figs. 1a & 2a.
Virtually all the neoplastic cells show a distinct, moderate

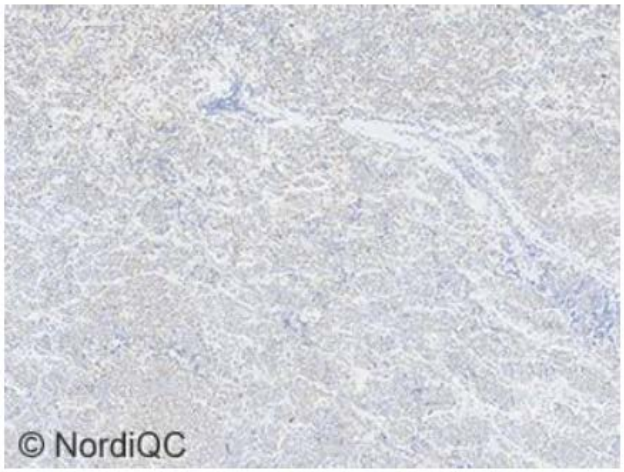



Fig. 3b
Insufficient staining for Cyclin D1 of the mantle cell lymphoma, tissue core no. 4, using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion of positive cells and the intensity of the

# IHC – Protocols and controls for Lymphomas

## Overall for haematololymphoid markers

**Use primary Abs with the highest optimal score rates**

NordiQC assessments

**Perform a careful calibration / validation of LDTs and verification of RTUs**

Use appropriate iCAPCs – right tissues / right staining pattern

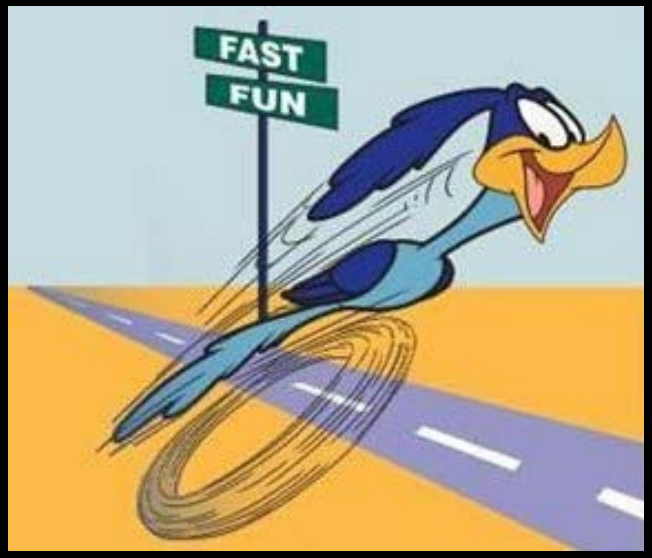
**Use efficient HIER in alkaline buffers (a few exceptions require low pH)**

**Proteolysis virtually a no-go**

**Use a sensitive and specific polymer/multimer based detection system**

3-step systems are preferable

# IHC – Protocols and controls for Lymphomas




The fast lane.....

Hands-out will be supportive....