NordiQC External Quality Assurance in Immunohistochemistry
Serial sections stained for Estrogen receptor

Optimally processed ductal breast carcinoma tissue

Lab. A

Lab. B
Serial sections stained for Estrogen receptor

Lab. A
High expressor

Lab. B
Low expressor
False neg.

AALBORG UNIVERSITY HOSPITAL
Serial sections stained for Estrogen receptor

Tonsil

Uterine cervix

Lab. A

Lab. B

Controls

False neg.
Serial sections stained for Estrogen receptor

Clone SP1/EP1/1D5 in 225 labs
Clone 6F11 in 15/37 labs

False pos. (mRNA=0)

External Quality Assurance!
“Through the inquiry, the public learned that between 1997 and 2005 nearly 400 of about 1,000 breast cancer patients received incorrect test results of the ER status of their breast tumors.”

“There are no good data on the quality of ER testing in the United States. The scary thing about the debacle in Canada is that we would never have known about this if results hadn’t been checked in a central lab.”
Suboptimal IHC assays may be due to:

- Preanalytical issues
  - Fixation too short, too late, decalcification too soon…
- Analytical issues:
  - Less successful / too dilute antibody clones/RTUs
  - Insufficient epitope retrieval
  - Insensitive visualization systems
  - Platform problems
- Post-analytical issues
  - Interpretation criteria, interobserver variation …
Nordic immunohistochemical Quality Control

- International organization for **proficiency testing** of IHC
- Founded 2003 by Nordic pathologists
- Independent, scientific, not-for-profit organisation
- Institute of Pathology, Aalborg University Hospital, DK

- General module: 3 runs/year
  - 15-18 different marker challenges
- Breast cancer IHC module: 2 runs/year
  - HER-2, ER/PR, Ki67/E-Cad ...
- HER-2 ISH module: 2 runs/year
  - BRISH, FISH
- Companion module: 2 runs/year
  - PD-L1 ...

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www.nordiqc.org
Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordiQC)

Mogens Vyberg\textsuperscript{1,2} · Søren Nielsen\textsuperscript{1}
Serial sections stained for PD-L1 in two Labs A and B: A1 shows optimal staining of tonsil, while B1 shows a false negative reaction in the histiocytes and a too faint reaction in the epithelial cells. A2 shows optimal staining of a non-small cell lung carcinoma, >50% of the tumour cells are positive. While B2 shows a faint reaction in few cells. Only with the result obtained in Lab A would the patient be offered 1. line treatment.

Results - run 52, B25, H13 & C3

20-Apr-2018
The general results for the runs 52, B25, H13 & C3 are available on the website. Individual results are available after logging in.
# NordiQC assessment scheme 2019

<table>
<thead>
<tr>
<th>Module</th>
<th>Winter</th>
<th>Spring</th>
<th>Autum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td>Run 55</td>
<td>Run 56</td>
<td>Run 57</td>
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<td></td>
<td>ASMA Bcl-6 CK5</td>
<td>C-MYC EpCAM</td>
<td>ALK (lung) Bcl-2</td>
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<tr>
<td></td>
<td>SOX10 WT1</td>
<td>MLA MLH1 p16</td>
<td>CD117 CK8/18</td>
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<tr>
<td></td>
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<td>PAX8</td>
<td>MSH2</td>
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<tr>
<td><strong>Breast</strong></td>
<td>Run B27</td>
<td>Run B28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER HER2 IHC</td>
<td>ER HER2 IHC</td>
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</tr>
<tr>
<td><strong>HER2</strong></td>
<td>Run H15</td>
<td>Run H16</td>
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<td></td>
<td>HER2 ISH</td>
<td>HER2 ISH</td>
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<tr>
<td><strong>Companion</strong></td>
<td></td>
<td>Run C5</td>
<td>Run C6</td>
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<tr>
<td></td>
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<td>PD-L1</td>
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## Dates

<table>
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<tr>
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<th>Winter</th>
<th>Spring</th>
<th>Autum</th>
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<tbody>
<tr>
<td>Protocol submission</td>
<td>3 Dec 2018</td>
<td>1 Feb</td>
<td>1 Aug</td>
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<tr>
<td>Protocol submission</td>
<td>3 Jan</td>
<td>13 Mar</td>
<td>4 Sep</td>
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<td>closes</td>
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<td>Shipping of slides</td>
<td>9 Jan</td>
<td>21 Mar</td>
<td>12 Sep</td>
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<td>Deadline for slide</td>
<td>13 Feb</td>
<td>1 May</td>
<td>11 Oct</td>
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<td>return</td>
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<tr>
<td>Assessment Breast</td>
<td>15 Mar - 16 Mar</td>
<td>7 Nov - 8 Nov</td>
<td></td>
</tr>
<tr>
<td>Assessment HER2</td>
<td>21 Mar</td>
<td></td>
<td>16 Nov</td>
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<tr>
<td>Assessment Companion</td>
<td></td>
<td>28 May - 29 May</td>
<td>20 Nov</td>
</tr>
<tr>
<td>Publication of</td>
<td>20 Apr</td>
<td>9 Jul</td>
<td>7 Dec</td>
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<tr>
<td>results</td>
<td></td>
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</tbody>
</table>
New protocol ID 635, CDX2, run 48

### Staining platform
- **Staining platform**: Ventana Benchmark Ultra

### Primary antibody
- **Primary antibody clone**: Cell Marque (235-Rxx) - EPR2764Y
- **Lot number**: 1523802K
- **Dilution factor**: 1:400
- **Diluent buffer**: Dako - Antibody Diluent (K8006)
- **Incubation time (minutes)**: 32
- **Incubation temperature (Celsius)**: 36

### Epitope Retrieval, HIER
- **Epitope retrieval, HIER**: YES
- **Device**: On Board / On Machine
- **HIER buffer**: Ventana - Ultra CC1 (950-224)
- **Efficient Heating Time (minutes)**: 48
- **Max. heating temperature (Celsius)**: 99

### Epitope Retrieval, proteolysis
- **Epitope retrieval, proteolysis**: NO

### Visualization system
- **Visualization system**: OptiView DAB IHC Detection Kit - 760-700
- **Amplification**: None
- **Incubation time linker (minutes)**: 8
- **Incubation time polymer (minutes)**: 8
- **Incubation temperature (Celsius)**: 36
## Modify protocol ID 635, CDX2, run 48

### Staining platform

| Staining platform | Ventana Benchmark Ultra |

### Primary antibody

<table>
<thead>
<tr>
<th>Primary antibody clone</th>
<th>Cell Marque (235-Rxx) - EPR2764Y</th>
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<td>1523802K</td>
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<tr>
<td>Dilution factor: 1:400</td>
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<tr>
<td>Diluent buffer</td>
<td>Dako - Antibody Diluent (K8006)</td>
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<tr>
<td>Incubation time (minutes)</td>
<td>32</td>
</tr>
<tr>
<td>Incubation temperature (Celsius)</td>
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### Epitope Retrieval, HIER

<table>
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<tr>
<th>Epitope retrieval, HIER</th>
<th>YES</th>
<th>NO</th>
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<tbody>
<tr>
<td>Device</td>
<td>On Board / On Machine</td>
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</tr>
<tr>
<td>HIER buffer</td>
<td>Ventana - Ultra CC1 (950-224)</td>
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</tr>
<tr>
<td>Efficient Heating Time (minutes)</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Max. heating temperature (Celsius)</td>
<td>99</td>
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</tr>
</tbody>
</table>

### Epitope Retrieval, proteolysis

| Epitope retrieval, proteolysis | YES  | NO  |

### Visualization system

<table>
<thead>
<tr>
<th>Visualization system</th>
<th>OptiView DAB IHC Detection Kit - 760-700</th>
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<tbody>
<tr>
<td>Amplification</td>
<td>None</td>
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<tr>
<td>Incubation time linker (minutes)</td>
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<tr>
<td>Incubation time polymer (minutes)</td>
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<td>Incubation temperature (Celsius)</td>
<td>36</td>
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### Protocol submission

<table>
<thead>
<tr>
<th>Run</th>
<th>Module</th>
<th>Epitope</th>
<th>Protocol status</th>
<th>Slide received by NordiQC</th>
<th>Action</th>
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<tr>
<td>49</td>
<td>General Module</td>
<td>CD5</td>
<td>✓</td>
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<td>2017-02-13</td>
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<tr>
<td>49</td>
<td>General Module</td>
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<td>✓</td>
<td>2017-02-13</td>
<td></td>
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<tr>
<td>49</td>
<td>General Module</td>
<td>MLH1</td>
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<td>2017-02-13</td>
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<tr>
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<td>NKKX3.1</td>
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<td>General Module</td>
<td>PSA</td>
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<td>2017-02-13</td>
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<tr>
<td>B23</td>
<td>Breast Cancer Module</td>
<td>ER</td>
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<tr>
<td>B23</td>
<td>Breast Cancer Module</td>
<td>HER2 IHC</td>
<td>✓</td>
<td>2017-02-13</td>
<td></td>
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<tr>
<td>C1</td>
<td>Companion Diagnostic Module</td>
<td>PD-L1</td>
<td>✓</td>
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</table>

### Module status

- **49** General Module: Slides sent
- **B23** Breast Cancer Module: Slides sent
- **H11** HER2-ISH Module: Slides sent
- **C1** Companion Diagnostic Module: Slides sent

### Status

- **Open**: Homepage open for protocol submission. New protocols can be created, edited and deleted.
- **Closed**: Homepage closed for new protocol submission. Protocols already submitted can be edited. NordiQC are preparing to send slides.
- **Slides sent**: Slides for the submitted protocols have been sent to participants. Only protocol corrections are allowed.
Test material

Multi-tissue FFPE blocks
10% NBF 24-48 h (ASCO/CAP guidelines ...)

- Normal and clinically relevant tumour tissues
- Different levels of antigen expression
  - high, moderate, low, none

2 unstained slides for each marker send to the participants
1 stained slide returned for central assessment
The slide to be stained for Bcl-6 comprised:
1-2. Tonsils, 24 h/48 h
3. Follicular lymphoma, grade I
4. Follicular lymphoma, grade II
5. Diffuse large B-cell lymphoma

Tissue selection:
• **High Expressor**
  • to confirm antibody
• **Low Expressor**
  • to ensure sensitivity
• **No-Expressor**
  • to ensure specificity
Assessment Run 42 2014

Bcl-6 protein (Bcl-6)

Material

The slide to be stained for Bcl-6 comprised:

- Tonsil, 24h fixation
- Tonsil, 48h fixation
- Follicular lymphoma grade 1
- Follicular lymphoma grade II
- Diffuse large B-cell lymphoma, non-Germinatal Centre B-cell type (DLBCL non-GCB), 6. DLBCL GCB.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Bcl-6 staining as optimal included:

- Adequate to strong distinct nuclear staining reaction of virtually all normal germinal centre B-cells in the tonsil fixed 24h.
- Adequate to moderate distinct nuclear staining reaction of the majority of the squamous epithelial cells in the tonsil fixed 48h.
- Adequate to strong distinct nuclear staining reaction of the neoplastic cells in the follicular lymphomas.
- Adequate to moderate distinct nuclear staining reaction of the majority of the neoplastic cells in the DLBCL, GCB subtype, tissue core no. 6.
- No or only a nuclear staining reaction in dispersed neoplastic cells of the DLBCL, non-GCB subtype, tissue core no. 5.

* The tonsil fixed for 48h (tissue core no. 2) was excluded from the assessment due to an aberrant inconsistent staining reaction in the circled material.

Participation

Number of laboratories registered for Bcl-6, run 42: 244
Number of laboratories returning slides: 228 (93%)

Results

228 laboratories participated in this assessment. Of these, 168 (74%) 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:

- Too low concentration of the primary antibody
- Less successful performance of the mAb clone PG-B6p
- Use of low sensitivity detection systems

Performance history

This was the third NordiQC assessment of Bcl-6. An increased pass rate was seen compared to the two previous runs 17, 2006 and 28, 2010 (see table 2).

Table 2: Proportion of sufficient results for Bcl-6 in the three NordiQC runs performed

<table>
<thead>
<tr>
<th>Run 17 2006</th>
<th>Run 28 2010</th>
<th>Run 42 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n=</td>
<td>69</td>
<td>132</td>
</tr>
<tr>
<td>Sufficient results</td>
<td>42%</td>
<td>46%</td>
</tr>
</tbody>
</table>

Conclusion

The mAbs clones GI191E/A8, LN22 and PG-B6p could all be used to produce optimal staining results for Bcl-6. Irrespective of the clone applied, efficient HIER in alkaline buffer, use of a high sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an.

Aalborg University Hospital
PDF file e-mailed to participants with assessment marks and – when needed – explanations and recommendations.
NordiQC assessment results 2006 – 2015

General module ~ 20,000 slides (~100,000 core sections)

- Optimal: 35%
- Good: 33%
- Borderline: 21%
- Poor: 11%

Insufficient 32%

- too weak / false neg.: ~ 90%
- over-stained / false pos.: ~ 10%

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NordiQC assessment results 2006 – 2015

Breast cancer module ~ 9,000 slides (~35,000 core sections)

- Insufficient 21%
  - 9% too weak / false neg.
  - 12% over-stained / false pos.
- Optimal 58%
- Good 21%

Insuff. { too weak / false neg.: ~ 90%
          over-stained / false pos.: ~ 10%

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NORTH DENMARK REGION
Major causes of insufficient stains in ~9,000 slides

Less successful antibodies/RTUs 17 %
Inappropriate antibody dilution 20 %
Inappropriate epitope retrieval 27 %
Inappropriate detection kit 19 %
Other inappropriate lab. performance 17 %

Endogenous biotin reaction
Section drying-out after HIER
Technical platform error

. . . .
Unexplained
Standardization of Negative Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Panel

Emina E. Torlakovic, MD, PhD,*†‡ Emina E. Torlakovic, MD, PhD,*†‡ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),§‖¶
John Garratt, RT,†‡¶ Blake Gilks, MD, FRCPG,†‡** Elizabeth Hyjek, MD, PhD,*
Merdol Ibrahim, PhD,†‡ Rodney Miller, MD,‡‡ Soren Nielsen, HT, CT,§§‖
Eugen B. Petcu, MD, PhD,§ Paul E. Swanson, MD,¶¶ Clive R. Taylor, MD, PhD,¶¶
and Mogens Vyberg, MD§§"" AIMM 2014, 22:241

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,*† Soren Nielsen, HT, CT,‡§ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),‖¶‖ John Garratt, RT,†*** Blake Gilks, MD, FRCPG,† ††
Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*§§ Elizabeth Hyjek, MD, PhD,*
Merdol Ibrahim, PhD,‖‖ Keith Miller, FIBMS,‖‖ Eugen Petcu, MD, PhD,‖
Paul E. Swanson, MD,¶¶¶¶ Xiaoge Zhou, MD,***††† Clive R. Taylor, MD, PhD,¶¶¶¶
and Mogens Vyberg, MD‡§

AIMM 2015, 23:1
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 1: Fit-for-Purpose Approach to Classification of Clinical Immunohistochemistry Biomarkers

Carol C. Cheung, MD, PhD, JD,*↑ Corrado D’Arrigo, MB, ChB, PhD, FRCPath,‡§∥ Manfred Dietel, MD, PhD,∥ Glenn D. Francis, MBBS, FRCPA, MBA, FFSc (RCPA),#**↑↑ C. Blake Gilks, MD,↑↑ Jacqueline A. Hall, PhD,§∥∥ Jason L. Hornick, MD, PhD,*↑↑ Merdol Ibrahim, PhD,∥∥ Antonio Marchetti, MD, PhD,** Keith Miller, FIBMS,∥∥ J. Han van Krieken, MD, PhD,↑↑↑ Soren Nielsen, BMS,↑↑↑↑↑↑ Paul E. Swanson, MD,∥∥∥∥ Clive R. Taylor, MD,*↑↑↑ Mogens Vyberg, MD,↑↑↑↑↑↑ Xiaoge Zhou, MD,↑↑↑↑↑↑ and Emina E. Torlakovic, MD, PhD,*↑↑↑↑↑↑

From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path)
IHC – Biomarker controls

Go for Low antigen expressors ~

Critical Assay Performance Controls (CAPCs)

- essential to evaluate sensitivity
- essential to assure consistency

Normal tissues - easier to ensure the quality

- 90 % of insufficient staining results in EQA are caused by weak/false negative results
- often related to the use of inappropriate positive tissue controls......
NordiQC EQA: Estrogen Receptor in 13 runs

Participants

PASS RATE (%)
IHC – Optimal performance

ER 1D5 1:100
HIER Ci pH 6
Results of NordiQC recommendations

Pass rate (optimal + good) by participant status

<table>
<thead>
<tr>
<th>Estrogen receptor</th>
<th>New participants</th>
<th>’Old’ participants</th>
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</thead>
<tbody>
<tr>
<td>Run 10, 2004</td>
<td>57%</td>
<td>71%</td>
</tr>
<tr>
<td>Run B15, 2010</td>
<td>70%</td>
<td>86%</td>
</tr>
<tr>
<td>Run B19, 2015</td>
<td>51%</td>
<td>73%</td>
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<tr>
<td>Average</td>
<td>59%</td>
<td>77%</td>
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</table>
HER-2 staining results in 17 runs

- **HercepTest™**: Red line
- **PATHWAY®**: Blue line
- **Oracle™**: Green line
- **Laboratory Dev.**: Yellow line
- **Total**: Black line
NordiQC runs for HER2 IHC

Optimal

Ampl. 3+
Ampl. 2+

Unampl. 2+
Unampl. 0

Poor

Ampl. 3+
Ampl. 1+

Unampl. 1+
Unampl. 0
NordiQC runs for HER2 IHC

Ampl. 3+  Ampl. 2+  Optimal  Unampl. 2+  Unampl. 0

Ampl. 3+  Ampl. 2+  Poor  Unampl. 3+  Unampl. 1
Every $1 saved by laboratories by using cheaper reagents could potentially result in approximately $6 additional costs to the healthcare system.
Lung ALK

The immunoassay must fit for the purpose:
- Identify the antibody useful for the specific task

The right external controls must be used:
- Tissue with high epitope expression to identify the right antibody
  - Anaplastic large cell lymphoma
- Normal and neoplastic tissue with moderate/low epitope expression to assure the sensitivity:
  - Appendix
  - ALK-positive lung adenocarc.
- Tissue with no epitope expression to assure the specificity
  - e.g., liver
Lung ALK – run 45, 176 labs

5A4

© NordiQC
Optimal

© NordiQC
Lung adenocarcinoma

© NordiQC
Poor

© NordiQC

Appendix
Results of NordiQC recommendations

419 advices for 11 markers

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Improved</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>268</td>
<td>195</td>
<td>73</td>
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<tr>
<td>Negative</td>
<td>151</td>
<td>21</td>
<td>14</td>
</tr>
</tbody>
</table>
Almost 1/3 of all IHC stains produced by NordiQC participants are still insufficient!

- New labs
- New antibodies, techniques, platforms
- Increasing demands

How many IHC stains produced by labs not participating in an EQA scheme are insufficient?

How many scientific publications are based on insufficient IHC stains?

What are the consequences for the patients?
A HISTORY OF MURPHY’S LAW

by Nick T. Spark

"Whatever can go wrong, will go wrong."

When you believe in automation and stop thinking
NordiQC External Quality Assurance in Immunohistochemistry

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital, Aalborg, Denmark