Nordic Immunohistochemical Quality Control

- International organization for quality assurance 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

www.nordiqc.org

Nordic Immunohistochemical Quality Control

General module: Breast cancer IHC module + HER-2 ISH module

www.nordiqc.org

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
1. Tonsil, 24 h.
2. Tonsil, 48 h.
3. Follicular lymphoma, grade I
4. Follicular lymphoma, grade II
5. Diffuse large B-cell lymphoma

- Tissue selection
  - High
  - Low
  - None
  - Expressor

Nordic immunohistochemical Quality Control

Participants

NordiQC assessment results 2003 – 2014

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

Insufficient 32%

Optimal 35%

Borderline 11%

Good 33%
Publications

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

Ettiene E. Torkzadeh, MD, PhD*†, Soon Nielson, HT, CT, CG, Francis McEvoy, MBBS, FRCPA, MBA, FFrS, FRCPath, FRCPath, RCU Pathology, and Mugen Yeh, MBBS

AIMM 2015, 23:1

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

Ettiene E. Torkzadeh, MD, PhD*†, Francis McEvoy, MBBS, FRCPA, MBA, FFrS, FRCPath, RCU Pathology, and Mugen Yeh, MBBS

AIMM 2014, 22:241

Serial sections stained for Estrogen receptor

Lab. A

ER in ductal breast carcinoma

Lab. B

False neg.

Serial sections stained for Estrogen receptor

Lab. A

False neg.

Lab. B

Control: uterine cervix

Serial sections stained for Estrogen receptor

Lab. A

False neg.

Lab. B

Control: uterine cervix

NordiQC runs for HER2 IHC

Optimal

Ampl. 3+

Unampl. 3+

Unampl. 0

Poor

Ampl. 1+

Unampl. 1+

Unampl. 0
NordiQC runs for HER2 IHC

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

Ampl. 3+ Ampl. 2+ Poor

NordiQC general results 2003 – 2013

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 (EBR)**
**Section drying out after HIER**
**Technical platform error**

Unexplained

NordiQC general results 2003 – 2013

Less successful antibodies

- Poor antibodies
- Poor ready-to-use formats
- Less robust antibodies
- Platform dependent antibodies
- Other error-prone antibodies
- Lot-to-lot variation
- Mouse-anti-Golgi (MAG) reaction
- Poor cocktail composition

NordiQC regrets any offence caused to laboratories and companies.

Poor antibodies (few examples)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>High expression</th>
<th>Low expression</th>
<th>Non expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5</td>
<td>CD5/S4/F6</td>
<td>√</td>
<td>FN</td>
<td>-</td>
</tr>
<tr>
<td>CD23</td>
<td>MRMN</td>
<td>√</td>
<td>FN</td>
<td>-</td>
</tr>
<tr>
<td>CD31</td>
<td>1A10</td>
<td>(1)</td>
<td>FN</td>
<td>-</td>
</tr>
<tr>
<td>CD34</td>
<td>SP38</td>
<td>(1)</td>
<td>FN</td>
<td>-</td>
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<tr>
<td>CD138</td>
<td>SF7</td>
<td>(1)</td>
<td>FN</td>
<td>-</td>
</tr>
<tr>
<td>CDX2</td>
<td>SP54</td>
<td>(1)</td>
<td>FN</td>
<td>FP</td>
</tr>
<tr>
<td>PR</td>
<td>CDX2-88</td>
<td>√</td>
<td>FN</td>
<td>FP</td>
</tr>
<tr>
<td>CEA</td>
<td>TF-3HB-1</td>
<td>√</td>
<td>√</td>
<td>FP</td>
</tr>
<tr>
<td>CGA</td>
<td>DAK A3</td>
<td>√</td>
<td>FN</td>
<td>-</td>
</tr>
<tr>
<td>PR</td>
<td>SP2</td>
<td>√</td>
<td>√</td>
<td>FP</td>
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<tr>
<td>SYP</td>
<td>SY38</td>
<td>√</td>
<td>FN</td>
<td>-</td>
</tr>
</tbody>
</table>

Poor antibodies: CD5

<table>
<thead>
<tr>
<th>CD5</th>
<th>N</th>
<th>Sufficient*</th>
<th>Optimal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4C7 conc</td>
<td>145</td>
<td>74%</td>
<td>49%</td>
</tr>
<tr>
<td>SP19 conc</td>
<td>11</td>
<td>91%</td>
<td>46%</td>
</tr>
<tr>
<td>CD5/S4/F6 conc</td>
<td>28</td>
<td>4%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*With optimal protocol settings
Poor antibodies: CD5

TP

FN

Tonsil

B-CLL

SP19

CD5/54/F6

Poor antibodies: CD31

TP

FN

Haemangiosarcoma

FC31

JC70A

1A10

Poor antibodies – MLH1

MLH1 clone ES05

MLH1 clone EPR3894

Poor RTU formats: CD5

CD5 Run 24 | N | Sufficient* | Optimal* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SP19 conc</td>
<td>11</td>
<td>91%</td>
<td>46%</td>
</tr>
<tr>
<td>SP19 RTU Dako</td>
<td>3</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>SP19 RTU VMS</td>
<td>14</td>
<td>79%</td>
<td>(14%)</td>
</tr>
</tbody>
</table>

CD5 Run 34 | N | Sufficient* | Optimal* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>SP19 RTU VMS</td>
<td>33</td>
<td>97%</td>
<td>(97%)</td>
</tr>
</tbody>
</table>

* With optimal protocol settings

Poor RTU formats
Platform dependant antibodies

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>XT / Ultra automated</th>
<th>Bond-max automated</th>
<th>Autostainer semi-automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>1F6</td>
<td>FN</td>
<td>Weak</td>
<td>√</td>
</tr>
<tr>
<td>CD56</td>
<td>MP-42</td>
<td>FN</td>
<td>Weak</td>
<td>√</td>
</tr>
<tr>
<td>CD79a</td>
<td>JCB117</td>
<td>Weak</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>BSAP/Pax5</td>
<td>24</td>
<td>FN</td>
<td>Weak</td>
<td>√</td>
</tr>
<tr>
<td>BCL6</td>
<td>PG-B6p</td>
<td>FN</td>
<td>Weak</td>
<td>√</td>
</tr>
<tr>
<td>SYP</td>
<td>27G12</td>
<td>Weak</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Inappropriate antibody dilution
IgK: Dako pAb A0191

~1:300 ~1:3.000 ~1:30.000

239 IgK tests, 12 Abs:
- 12% optimal
- Dako pAb A0191:
  - 17% optimal
  - TRS/CI 3.000-16.000:
    - 29% optimal
- All other Abs:
  - 0% optimal

Inappropriate visualization system

NordiQC run 41/42 2014 - MMR

MMR MLH1 mAb clone ES55, 1:20 Leica
UltraView + Amplification
OptiView + Amplification (Tyc)

NordiQC run 41 2014 – PMS2 131 labs

Optimal: 47%
Insufficient: 15%

Optimal: 47%
Insufficient: 15%
NordiQC run 41 2014 – PMS2 131 labs

Optimal: 47%
Insufficient: 15%

- Too dilute Ab
- Insufficient HIER
- Insensitive viz system

Inappropriate epitope retrieval
&
Misleading data sheets

Inappropriate retrieval (31%)

Liver

TP
FN

AE1/AE3 + HIER

AE1/AE3 + proteolysis

RCC

TP
FN

Misleading datasheets

Table 1: Recommended Staining Protocols

<table>
<thead>
<tr>
<th>Procedure Type</th>
<th>ES or NeedHIER</th>
<th>BenchMark or BenchMark XT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Cell-Destroying</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Antigen (retrieval)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Enzyme (Protease)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Amplification</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Counterstain</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Leica: RTU – HIER

Proteolysis or UltraVision

Thermo: Conc: HIER

With UltraVision

Dako: RTU – HIER

Conc: Proteolysis or HIER

Leica: RTU – Proteolysis

Conc: HIER

Thermo: Conc: HIER with Quanto

Proteolysis with UltraVision

IHC - NordiQC 2014

Table 2: Proportion of sufficient results for CK-PAN in the seven NordiQC runs performed

<table>
<thead>
<tr>
<th>Participants, n</th>
<th>72</th>
<th>85</th>
<th>113</th>
<th>133</th>
<th>166</th>
<th>202</th>
<th>233</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficient results</td>
<td>53%</td>
<td>50%</td>
<td>62%</td>
<td>60%</td>
<td>65%</td>
<td>60%</td>
<td>67%</td>
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AE1/AE3: Optimal results only obtained by HIER in NordiQC runs

Misleading datasheets – improved information

IHC - NordiQC 2014

Performance history

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Leica: RTU – HIER

Proteolysis or UltraVision

Thermo: Conc: HIER

With UltraVision

Dako: RTU – HIER

Conc: Proteolysis or HIER

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<td>None</td>
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<td>Counterstain</td>
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Proteolysis or UltraVision

Thermo: Conc: HIER

With UltraVision

Dako: RTU – HIER

Conc: Proteolysis or HIER

Leica: RTU – Proteolysis

Conc: HIER

Thermo: Conc: HIER with Quanto

Proteolysis with UltraVision

IHC - NordiQC 2014
By 17th October 2014

Improved datasheets

<table>
<thead>
<tr>
<th>Procedure Type</th>
<th>Method</th>
<th>100% Conformity Antigen Unmasking</th>
<th>Antibody (Primary)</th>
<th>Antibody (Secondary)</th>
<th>Cell Counting</th>
<th>Cell Counting YMB</th>
<th>Corestain</th>
<th>Post Counterstain</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-paraffinisation</td>
<td>Selected</td>
<td>Derived antibody</td>
<td>Proteolysis</td>
<td>VENTANA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen Unmasking</td>
<td>Derived</td>
<td>Proteolysis</td>
<td>VENTANA</td>
<td>Antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody (Primary)</td>
<td>Derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody (Secondary)</td>
<td>Derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Cell Counting</td>
<td>Derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corestain</td>
<td>Derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Counterstain</td>
<td>Derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Use of VENTANA Antibody Diluent with Casein (09-0210) at the ultraBlock step is recommended to reduce staining on non-cells.

Misleading datasheets

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Company</th>
<th>Datasheet</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>CGA</td>
<td>LKH10</td>
<td>VMS</td>
<td>No retrieval</td>
<td>FN</td>
</tr>
<tr>
<td>CK8</td>
<td>5D3</td>
<td>Leica</td>
<td>RTU: HIER</td>
<td>Contound</td>
</tr>
<tr>
<td>CK19</td>
<td>RCK108</td>
<td>BioGenex</td>
<td>Proteolysis</td>
<td>FN</td>
</tr>
<tr>
<td>CK19</td>
<td>B170</td>
<td>Leica</td>
<td>Proteolysis</td>
<td>FN</td>
</tr>
<tr>
<td>CKPan</td>
<td>AE1/AE3</td>
<td>VMS/Dako</td>
<td>Proteolysis</td>
<td>FN</td>
</tr>
<tr>
<td>CD34</td>
<td>QBEnd 10</td>
<td>Leica</td>
<td>RTU: HIER</td>
<td>Contound</td>
</tr>
<tr>
<td>CD34</td>
<td>QBEnd 10</td>
<td>VMS</td>
<td>Changed from no retrieval to HIER</td>
<td>FN</td>
</tr>
<tr>
<td>CD68</td>
<td>KP1</td>
<td>Thermo</td>
<td>Proteolysis</td>
<td>FN</td>
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<td>DES</td>
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<td>FN</td>
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<tr>
<td>PLAP</td>
<td>PL8-F6</td>
<td>BioGenex</td>
<td>No retrieval</td>
<td>FN</td>
</tr>
<tr>
<td>PLAP</td>
<td>PL8-F6</td>
<td>BioGenex</td>
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<td>FN</td>
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<td>3B4</td>
<td>VMS</td>
<td>Proteolysis</td>
<td>FN</td>
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<td>WT1</td>
<td>6F-H2</td>
<td>Dako</td>
<td>RTU: HIER</td>
<td>Contound</td>
</tr>
</tbody>
</table>

Tailored NordiQC recommendations

- Replace less successful antibodies (conc./RTU)
- Calibrate the antibody concentration
- Use HIER (instead of proteolysis or no retrieval)
- Increase HIER time / temperature / buffer pH
  - For 95% of epitopes pH 8-9 is preferable to pH 6
- Use a non-biotin based viz. system
- Use FDA approved kits instead of home-brews
- . . . . .
- Improve the internal QC; Identify the right controls:
  Select well defined normal low expressor cells/tissues

Results of NordiQC recommendations

<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>
</tr>
<tr>
<td>Negative</td>
<td>151</td>
<td>21</td>
<td>14</td>
</tr>
</tbody>
</table>
HER-2 staining results in 17 runs

Roche – NordiQC joint venture

Roche – NordiQC joint venture

Roche – NordiQC joint venture

For each $ saved by the pathology lab by usage of cheaper reagents, the healthcare system is ultimately burdened with ~$6

Immunohistochemical expression of HER2 in breast cancer:
Socioeconomic impact of inaccurate tests


NordiQC, Aalborg, DK, Ventana Medical Systems Inc, Tucson, AZ, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Submitted for publication
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?

External Quality Assurance (EQA)
- Provides objective evidence of lab performance
- Identifies methodological errors
- Provides directions for improvements & controls

The results of the NordiQC work indicate that
- Improvement of IHC is strongly needed
- EQA schemes, industry and KOL must align - describing the requirements for optimal IHC performance.

Collaboration between Companies and EQA schemes
- Define expression patterns for markers
- Identify best controls and stain quality indicators
- Implement these in guidelines and package inserts

- Discontinue poor antibodies
- Guide laboratories
  - platform dependent clones
- Amend inappropriate package inserts.

Welcome to:
2nd NordiQC Conference on Applied Immunohistochemistry
June 9th - 12th 2015, Aalborg, Denmark

www.nordiqc.org
www.nordiqc2015.dk

Thank you for your attention!