Tissue controls for IHC

Background - Selection
Interpretation

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NordiQC
Aalborg Hospital, Denmark
IHC – Biomarker controls

- What is an IHC control for diagnostic IHC?
- What is recommended and best practice?
- What are the pitfalls for the use of controls for IHC?
- How are IHC controls used by laboratories, NordiQC and EQA programmes?
IHC – Biomarker controls

... The biomarker protocol trap – Caution: not for faint-hearted lab personnel !!!!!

**Pre-analytic**
- Fixation
- Time, Type, Volume
- Decalcification Preparation
- Tissue
- Type, Dimension, Laser resection, De-differentiation
- Section Thickness
- Storage Drying

**Post-analytic**
- Interpretation
- Localization
- Positive/Negative - cut-off level

**Analytic**
- Primary antibody
- Clone, Dilution
- Buffer, Time, Temp
- Manual Stainer
- Visualization
- Sensitivity, Specificity

**Pre-treatment**
- Controlment
- Quantification Reporting

With 3 choices for 5 variables in each phase => 4 million protocols....

The right control material will expose right or wrong choices... The biomarker protocol trap – Caution: not for faint-hearted lab personnel !!!!!
Documentation of Immunocytochemistry Controls in the Cytopathologic Literature: A Meta-Analysis of 100 Journal Articles


Absent: Controls were not mentioned.
Vague: Statement such as “appropriate positive and negative controls were included.”
Identical: Controls identical to study samples were described.
Other: Controls were dissimilar or partially similar (i.e., tissue control with smears or tissue control with cell block and ThinPrep samples run), or samples were too scant to include controls.

> 70% of publications based on IHC do not describe controls used to verify data and conclusions....
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results. Without their use, interpretation of staining would be haphazard and the results of doubtful value. More specifically, controls determine if the staining protocols were followed correctly, whether day-to-day and worker-to-worker variations have occurred, and that reagents remain in good working order.
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Reagent controls typically used to validate specificity of the primary and secondary antibodies – to show that the antibody-antigen reaction is due to expression of the target of interest.

- Often referred as negative controls

Tissue controls typically used to show that the IHC staining was successful and capable to demonstrate the target of interest.

- Often referred as positive controls
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results. Reagent control of the primary antibody is crucial for the producer to validate specificity and can include:

- Primary ab tested on knock-out mice
- Primary ab tested on cell lines +/- antigen of interest
- Primary ab tested by western blotting
- Primary ab tested by antigen absorbption
- Primary ab tested on wide range of tissues/neoplasias

To secure specificity of primary ab -
Both by launch and new ab lots.
IHC – Biomarker controls

- **Reagent** and tissue controls are necessary for the validation of immunohistochemical staining results.

- Negative reagent control is for the laboratories of limited use and "impossible" to perform correctly.
  - **Primary ab control** – *negative reagent control*
    - Each primary ab must have its own negative control serum, and thus all the IHC slides performed will be doubled.
Reagent control is of limited use and impossible to perform correctly.

- e.g. mAb clone PS1 CD3, IgG1a, Ig conc 80 ug. Ab is diluted 1:100
- Neg control mouse serum, IgG1a, Ig conc 120 ug. Must be diluted 1:150 to match CD3

By a work-load of 25,000 slides = 50,000 slides.

By a price pr test of 6 euro the total increase will be 150,000 euro...
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

 Negative reagent control is for the laboratories of limited use and "impossible" to perform correctly.

 Primary ab control – *negative reagent control*

 Each primary ab must have its own negative control serum, and thus all the IHC slides performed will be doubled

 WILL NOT EXPOSE IF WRONG OR CONTAMINATED PRIMARY AB HAS BEEN APPLIED!!!!!
IHC – Biomarker controls

FP staining reactions
Not identified by negative reagent controls
The antibody giving the FP would be substituted by control serum and no staining seen giving a "false security"

CD7 mAb clone LP15
– ductal breast carcinoma
Lot. 920 – CD7 + ER
Lot. 11177

CD7 mAb clone LP15
CD7 neg T-cell lymphoma
Lot. 13566 (Actually CD2...)
Lot. 11177
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Negative reagent control is for the laboratories of limited use and "impossible" to perform correctly.

- Secondary ab control – negative reagent control
  - The primary ab is substituted by e.g. diluent in order to monitor binding of the detection system to the tissue. In principle each of all retrieval methods applied in a diagnostic case must have its own negative diluent control.

Question – what is the value?
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Negative reagent control is for the laboratories of limited use and "impossible" to perform correctly.

Secondary ab control – negative reagent control

In principle only relevant and essential for biotin based detection systems.

"The CAP committee has concluded the value of neg. Reagent controls does not outweigh the costs...."
IHC – Biomarker controls

- Labelled Steptavidin-Biotin system
- Labelled Streptavidin-Biotin system – neg control
- Multimer/Polymer based system
- Synaptophysin mAb clone 27G12
IHC – Biomarker controls

- Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

- Tissue controls are the most valuable tool to monitor the specificity and sensitivity for IHC
  - Internal positive and negative tissue control
    - Cells/structures within the patient slide
  - External positive and negative tissue control
    - Slide next to patient slide
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Tissue controls are the most valuable tool to monitor the specificity and sensitivity for IHC.

- **Internal positive tissue control**
  - MMR protein expression in stromal cells
  - CD markers in lymphatic tissue
  - Hormone receptor in breast glands

Only “indicator of IHC analysis was performed”

No general information of sensitivity/specificity
IHC – Biomarker controls

MSH6 in colon adenocarcinoma

Cyclin D1 in B-CLL

Estrogen receptor in breast carcinoma

Internal positive tissue controls indicating IHC assay was performed – Negative staining can lead to restaining (MMR / ISH HER2). In general no information of level of sensitivity.
IHC – Biomarker controls

Excellent studies – but validated on internal controls

Immunohistochemical Prognostic Markers in Diffuse Large B-Cell Lymphoma: Validation of Tissue Microarray As a Prerequisite for Broad Clinical Applications—A Study From the Lunenburg Lymphoma Biomarker Consortium

Daphne de Jong, Andreas Rosenwald, Mukesh Chananabhai, Philippe Gaulard, Wolfram Klapper, Abigail Lee, Birgitta Sander, Christoph Thors, Elias Campo, Thierry Molina, Andrew Norton, Anton Hagenbeek, Sandra Horning, Andrew Lister, John Raemaekers, Randy D. Gascoyne, Gilles Salles, and Edie Weller

blood

2011 117: 7070-7078
Prepublished online May 2, 2011;
doi:10.1182/blood-2011-04-345256

Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg Lymphoma Biomarker Consortium

Gilles Salles, Daphne de Jong, Wolfram Gaulard, Maria Molina, Abigail Lee, Michael Pfue, John Raemaekers, Anton Hagenbeek,

Purpose
The results of immunohistochemical class prediction and prognostic stratification of diffuse large B-cell lymphoma (DLBCL) have been remarkably various thus far. Apart from biologic variations, this may be caused by differences in laboratory techniques, scoring definitions, and inter- and intraobserver variations. In this study, an international collaboration of clinical lymphoma research groups from Europe, United States, and Canada concentrated on validation and standardization of immunohistochemistry of the currently potentially interesting prognostic markers in DLBCL.
### Table 2. Scoring Criteria for Immunohistochemistry in the Second Rotation Round

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Scoring Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Score as positive/negative in tumor cells.</td>
</tr>
<tr>
<td>CD5</td>
<td>No staining, 1%-25%, 26%-50%, 51%-75%, &gt;75%; for the score designated no staining, an internal staining control must be present; T cells serve as internal controls.</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Score as positive/negative in tumor cells; for the score designated as negative, an internal staining control must be present; reactive small B cells and T cells and accessory cells serve as internal controls.</td>
</tr>
<tr>
<td>CD10</td>
<td>Score as positive/negative in tumor cells; for the score designated as negative, an internal control must be present; granulocytes and stromal fibroblasts serve as internal controls.</td>
</tr>
<tr>
<td>bcl-2</td>
<td>No staining (0%-5%), 5%-25%, 26%-50%, 51%-75%, &gt;75%; for the score designated as no staining, an internal staining control must be present; staining intensity is scored as weak (weaker than internal T cells) and strong (equal or stronger than internal T cells).</td>
</tr>
<tr>
<td>Ki67</td>
<td>No staining, 1%-25%, 26%-50%, 51%-75%, 76%-95%, &gt;95%; for the score designated as no staining, an internal staining control must be present.</td>
</tr>
<tr>
<td>MUM1</td>
<td>No staining (0%-5%), 5%-25%, 26%-50%, 51%-75%, &gt;75%; for the score designated as no staining, an internal staining control must be present; activated T cells serve as internal controls.</td>
</tr>
<tr>
<td>bcl-6, laboratory 3</td>
<td>No staining (0%-5%), 5%-25%, 26%-50%, 51%-75%, &gt;75%; for the score designated as no staining, an internal staining control must be present; internal controls may be sparse and consist of T cells.</td>
</tr>
<tr>
<td>bcl-6, laboratory 7</td>
<td>Strong (saturated), strong variable (variable with strong to moderate staining variation), variable weak (variation between weak and moderate staining intensities), and weak (negative with sparsely stained cells); for the score designated as no staining, an internal staining control must be present; internal controls may be very sparse and consist of T cells.</td>
</tr>
</tbody>
</table>

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**NQC CD5, run 34, 2012**

### Table 1. Primary Antibodies and Protocols

<table>
<thead>
<tr>
<th>Antibody and Designation</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
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<td>Novoceastra 4C7</td>
<td>Novoceastra 4C7</td>
<td>Novoceastra 4C7</td>
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<tr>
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</table>

**Best protocol identified**
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Tissue controls are the most valuable tool to monitor the specificity and sensitivity for IHC.

- Internal negative tissue control
  - Cells / structures to be negative
    - E.g. T-cells for CD19, CD20, CD79a...
    - Mantle zone B-cells for Ki67, Bcl-6...
    - Epithelial cells for CD3, CD5, MUM1,...

Information of primary ab specificity
mAb clone LE-CD19

Dako: B-cells positive, T-cells negative
Serotec: B-cells positive, T-cells false positive
IHC – Biomarker controls

Neg. reagent control

MLH1 mAb ES05
Tonsil: pos. control
Carc. with loss: neg. control

MLH1 rmAb EPR3894
Reduced titre......
Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.

Conclusions – Internal tissue controls

- **Internal positive tissue control**
  - Indicative of "successful" IHC result
  - Cannot be recommended as generally reliable for evaluation of appropriate sensitivity

- **Internal negative tissue control**
  - Can provide valuable information of specificity of the primary antibody/protocol
IHC – Biomarker controls

- Reagent and **tissue** controls are necessary for the validation of immunohistochemical staining results.

- Tissue controls are the most valuable tool to monitor the specificity and sensitivity for IHC
  - **External positive** and **negative** tissue control
    - Appropriate sensitivity of the IHC assay
    - Appropriate specificity of the IHC assay

The central tool to monitor the IHC quality and consistency
IHC – Biomarker controls

- External **tissue** controls requirements:
  - Normal tissues
  - Processed as patient material (fixation, decalc.,...)
  - Described expected staining pattern for each ab
  - Negative staining reaction
    - Specificity of protocol / right ab was used...
  - Positive staining reaction
    - Strong (which cells and how – right ab was used)
    - Weak (which cells and how - appropriate sensitivity)
IHC – Biomarker controls

- External **tissue** controls requirements:
  - Normal tissues
  - Processed as patient material (fixation, decalc.,...)

“Control” – positive

“Patient-material” – false negative
IHC – Biomarker controls

- External **tissue** controls requirements:
  - Normal tissues
  - Processed as patient material (fixation, decalc.,...)

Ki67 10% NBF 24 h → Form acid.  
"Control" – positive

Ki67 NBF + Form Acid simultan.  
"Patient-material" – false negative
IHC – Biomarker controls

- External **tissue** controls requirements:
  - Normal tissues
  - Processed as patient material (fixation, decalc.,...)
  - Described expected staining pattern for each ab
  - Negative staining reaction
    - Specificity of protocol / right ab was used...
  - Positive staining reaction
    - Strong (which cells and how – right ab was used)
    - Weak (which cells and how - appropriate sensitivity)
IHC – Biomarker controls

- Low antigen expressors
- **Critical Stain Quality Indicators (CSQI)**
  - essential to evaluate consistency
  - essential to evaluate sensitivity
  - normal tissue (easy to compare)
  - 90% of insufficient staining results in EQA are caused by weak/false negative results and often related to the use of inappropriate positive tissue controls......
IHC – Biomarker controls

CD56: Optimal

Insufficient.......

Appendix used as external positive and negative control

Virtually all nerves strongly positive
The epithelial cells negative
IHC – Biomarker controls

CD56: Optimal

Neuroendocrine carcinoma

Insufficient

Tonsil: As pos. control
IHC – Biomarker controls

- The NordiQC focus areas
  - Central protocol elements for an optimal staining
    - Antibody selected
    - Antibody dilution range / Ready-To-Use
    - Epitope retrieval
    - IHC detection system & stainer platforms
  - Recommendable control and identification of critical quality stain indicators
    *(Which tissue? Which cells?, How must they look?)*
**IHC – Biomarker controls**

**CD5**

**CSQI:**
Dispersed B-cells in mantle zone.

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**Fig. 2a.** Optimal staining for CD5 of the tonsil no. 1 using same protocol as in Fig. 1a – high magnification x200. Dispersed mantle zone B-cells show a distinct moderate staining reaction, while the T-cells show a strong staining reaction. No staining is seen in the germinal centre B-cells.

**Fig. 2b.** Insufficient staining for CD5 of the tonsil no. 1 using same protocol as in Fig. 1b - same field as in Fig. 2a - high magnification x200. Only the T-cells show a distinct staining reaction, while the mantle zone B-cells are false negative.

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**Fig. 4a.** Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

**Fig. 4b.** Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a. The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.
IHC – Biomarker controls

CD23

CSQI:
Activated
B-cells in
mantle z.

Fig. 2a. High magnification of the optimal staining in Fig 1a of the secondary follicle in the tonsil. The activated B-cells show a distinct continuous membranous reaction.

Fig. 2b. High magnification of the insufficient staining in Fig 1b of the secondary follicle in the tonsil (same field as in Fig 2a). The activated B-cells only show a weak imprecise reaction.

Fig. 3a. Optimal staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2b. The majority of the neoplastic cells show a strong and distinct membranous staining.

Fig. 3b. Insufficient staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2b. The neoplastic cells are virtually negative.
IHC – Biomarker controls

CDX2

CSQI:
Pancreatic duct ep.
cells

Fig. 1a. Optimal staining for CDX2 using the mAb clone CDX2-88.
Left: colon; A strong nuclear staining is seen in all the enterocytes with a minimal cytoplasmic reaction.
Right: pancreas; A weak to moderate staining is seen in the majority of the ductal epithelial cells.

Fig. 1b. Staining for CDX2 using the mAb clone CDX2-68 with an insufficient protocol.
Left: colon; A moderate to strong nuclear staining is seen in all the enterocytes.
Right: pancreas; No nuclear staining is seen in the ductal epithelial cells. Also compare with Fig 2b – same protocol.

Fig. 2a. Optimal staining for CDX2 using same protocol as in Fig. 1a.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show an intense staining while the cytoplasmic compartment is weakly stained.
Right: Colon adenocarcinoma with low expression of CDX2: The majority of the neoplastic cells show a moderate to strong nuclear reaction.

Fig. 2b. Insufficient staining for CDX2 using same protocol as in Fig. 1b.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show a moderate staining, while the cytoplasmic compartment is almost negative.
Right: Colon adenocarcinoma with low expression of CDX2: Only scattered neoplastic cells show a weak nuclear reaction.
IHC – Biomarker controls

PAX8:

CSQI:
Ciliated epithelial cells of the Fallopian tube

Fig. 1a. Optimal PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate, HIER in TRS pH 6.1 and a 3-step polymer based detection system. Virtually all the ciliated epithelial cells show a distinct, weak to moderate nuclear staining reaction, while the secretory epithelial cells are strongly labelled.

Fig. 1b. Insufficient PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate with a protocol giving a too low sensitivity (a too low concentration of the primary Ab and a 2-step polymer based detection system) – same field as in Fig. 1a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 1a. Also compare with Fig. 2b, same protocol.

Fig. 2a. Optimal PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong nuclear staining reaction.

Fig. 2b. Insufficient PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1b – same field as in Fig. 2a. Only scattered neoplastic cells show an equivocal staining reaction.
Focus on external tissue controls are needed to standardize and optimize IHC:

- External tissue control "catalogue"
- Type of normal tissue
- Staining pattern expected
  - High expression (right antibody....)
  - Low expression (right sensitivity)
  - Non expression (right specificity)
# IHC – Biomarker controls

<table>
<thead>
<tr>
<th>ASMA (C)</th>
<th>Appendix</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Tonsil</th>
</tr>
</thead>
<tbody>
<tr>
<td>High expression</td>
<td>A moderate to strong staining reaction in virtually all smooth muscle cells in muscularis mucosae</td>
<td>A moderate to strong staining reaction in the smooth muscle cells in vessels</td>
<td>A moderate to strong staining reaction in the smooth muscle cells in vessels</td>
<td>A moderate to strong staining reaction in the smooth muscle cells in vessels</td>
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<tr>
<td>(right ab)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low expression</td>
<td></td>
<td>An at least weak to moderate, staining reaction of the majority of the perisinusoidal cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(right sens.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non expression</td>
<td>No staining reaction in the epithelial cells</td>
<td>No staining in the hepatocytes (except lipofuscin)</td>
<td>No staining reaction in the epithelial cells</td>
<td>No staining reaction in lymphocytes</td>
</tr>
<tr>
<td>(right spec.)</td>
<td></td>
<td></td>
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<tr>
<td>CD4 (M)</td>
<td>Appendix</td>
<td>Liver</td>
<td>Pancreas</td>
<td>Tonsil</td>
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<tr>
<td>High expression (right ab)</td>
<td>The majority of T-cells in lamina propria must show a moderate to strong, distinct predominantly membranous staining reaction.</td>
<td>Dispersed T-cells and Kupffer cells must show a moderate to strong, distinct predominantly membranous staining reaction.</td>
<td>Dispersed T-cells must show a moderate to strong, distinct predominantly membranous staining reaction.</td>
<td>The majority of T-cells, both in the interfollicular T-zones and in the germinal centers must show a moderate to strong, distinct, predominantly membranous staining reaction.</td>
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<tr>
<td>Low expression (right sens.)</td>
<td>Dispersed intra-epithelial T-cells must show an at least weak to moderate, distinct predominantly membranous staining reaction.</td>
<td>The vast majority of the endothelial cells of the liver sinusoids must show an at least weak to moderate, distinct predominantly membranous staining reaction.</td>
<td>The majority of T-cells in lamina propria must show a moderate to strong, distinct predominantly membranous staining reaction.</td>
<td>The germinal centre macrophages must show an at least weak to moderate predominantly membranous staining reaction.</td>
</tr>
<tr>
<td>Non expression (right spec.)</td>
<td>No staining reaction must be seen in the columnar epithelial cells.</td>
<td>No staining reaction must be seen in the hepatocytes.</td>
<td>No staining reaction must be seen in the epithelial cells of the exocrine pancreas or the endocrine cells of the islets of Langerhans.</td>
<td>No staining reaction must be seen in the B-cells</td>
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</tbody>
</table>

CD4 with PR contamination
Mini TMA external tissue control: (Aalborg experience)

4-6 multi-tissue control-blocks for app. 190/220 markers

Control B1: Appendix, hepar, tonsil, pancreas
Control B2: Brain, str. muscle, skin, adrenel gland
Control B3: Lung, prostate, placenta, thyroidea
Control B4: Thymus, kidney, bone marrow, tonsil
Control B5: ..................

Spec. ALK1, Hel., HER2, Hormones, etc
# IHC – Biomarker controls

## B1: Appendix, Hepar, Tonsil, Pancreas

<table>
<thead>
<tr>
<th>B1:</th>
<th>Appendix</th>
<th>Hepar</th>
<th>Tonsil</th>
<th>Pancreas</th>
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<td>ASMA</td>
<td>BCL2</td>
<td>MMR</td>
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<td>S100</td>
<td>CGA</td>
</tr>
<tr>
<td>CD19</td>
<td>CD31</td>
<td>CD2</td>
<td>SYP</td>
<td>SYP</td>
</tr>
<tr>
<td>CD34</td>
<td>CD34</td>
<td>CD3</td>
<td>CD4</td>
<td>PP</td>
</tr>
<tr>
<td>CD117</td>
<td>CD45</td>
<td>CD4</td>
<td>CD5</td>
<td>SMAD4</td>
</tr>
<tr>
<td>CEA</td>
<td>CD68</td>
<td>CD8</td>
<td>CD8</td>
<td>SYP</td>
</tr>
<tr>
<td>CGA</td>
<td>CK Pan</td>
<td>CD10</td>
<td>CD10</td>
<td></td>
</tr>
<tr>
<td>CK20</td>
<td>CK LMW</td>
<td>CD20</td>
<td>CD20</td>
<td></td>
</tr>
<tr>
<td>DOG1</td>
<td>CK8</td>
<td>CD21</td>
<td>CD21</td>
<td></td>
</tr>
<tr>
<td>MMR</td>
<td>CK18</td>
<td>CD23</td>
<td>CD23</td>
<td></td>
</tr>
<tr>
<td>S100</td>
<td>HEPA</td>
<td>CD38</td>
<td>CD38</td>
<td></td>
</tr>
<tr>
<td>SYP</td>
<td>Arginase</td>
<td>CD56</td>
<td>CD56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD79a</td>
<td>CK Pan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD138</td>
<td>CyD1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EMA</td>
<td></td>
</tr>
</tbody>
</table>

**Used together inclusive:**
- HE
- LE
- NE
IHC – Biomarker controls

“Ideal” daily control for the majority of routine markers:

Appendix
Hepar
Pancreas
Tonsil

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

In contrast only using 1 external tissue control, no information is available for the single slide evaluated.
## IHC – Biomarker controls

<table>
<thead>
<tr>
<th></th>
<th>TMA control on all slides</th>
<th>One batch control</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missing reagent FN in patient test</td>
<td>Yes</td>
<td>No – only control slide</td>
<td>Potential internal pos. control only indicator of protocol performed</td>
</tr>
<tr>
<td>Wrong antibody FP in patient test</td>
<td>Yes</td>
<td>No – only control slide</td>
<td></td>
</tr>
<tr>
<td>Inappropriate protocol performance - Drying out etc FN / FP in patient test</td>
<td>Yes</td>
<td>No – only control slide</td>
<td></td>
</tr>
<tr>
<td>Errors seen for all IHC automated and semi-automated IHC platforms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# IHC – Biomarker controls

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>ASMA</td>
<td>B-catenin</td>
<td>CD3</td>
<td>Desmin</td>
<td>Estrogen rec.</td>
</tr>
</tbody>
</table>

**CD79a – membrane / cytoplasmic antigen**

<table>
<thead>
<tr>
<th>MB1</th>
<th>Appendix</th>
<th>Tonsil</th>
<th>Pancreas</th>
<th>Lever</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>The vast majority of plasma cells in lamina propria must show a moderate to strong cytoplasmic staining reaction.</td>
<td>The vast majority of plasma cells must show a moderate to strong cytoplasmic staining reaction. All mantle zone B-cells must show a moderate to strong membranous staining reaction.</td>
<td>B-cells must show a moderate to strong membranous staining reaction.</td>
<td>B-cells must show a moderate to strong membranous staining reaction.</td>
</tr>
<tr>
<td>LE</td>
<td>-</td>
<td>Virtually all the germinal centre B-cells must show a weak to moderate membranous staining reaction.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NE</td>
<td>No staining reaction must be seen in the columnar epithelial cells.</td>
<td>No staining reaction must be seen in the T-cells and squamous epithelial cells.</td>
<td>The epithelial cells of the exocrine pancreas and endocrine cells of the islets of Langerhans must be negative.</td>
<td>No staining reaction must be seen in the hepatocytes.</td>
</tr>
</tbody>
</table>

HE: High expression, LE: Low expression, NE: No expression
The vast majority of plasma cells in lamina propria must show a moderate to strong cytoplasmic staining reaction. No staining reaction must be seen in the columnar epithelial cells.
The vast majority of plasma cells must show a moderate to strong cytoplasmic staining reaction. All mantle zone B-cells must show a moderate to strong membranous staining reaction.

Virtually all the germinal centre B-cells must show a weak to moderate membranous staining reaction.
IHC – Biomarker controls

EQA

Industry

Controls

Laboratory
IHC – Biomarker controls

- Low antigen expressors
- Critical Staining Quality Indicators (CSQI)
  - CGA = peripheral nerves
  - Run 31, NordiQC CGA – 42/170 laboratories failed (25%)
- 12/36 used pancreas as control (High expressor)
- 12/36 used carcinoid as control (High expressor)
- 5/36 used tumour as control (?? expressor)
- 7/36 used appendix as control (High and low expressor)
- 24/36 = 66% of the laboratories failing the CGA assessment used control with only high antigen expression
IHC – Biomarker controls

- Low antigen expressors
- Critical Staining Quality Indicators (CSQI)
  - CGA = peripheral nerves
  - Run 31, NordiQC CGA – 128/170 laboratories passed (75%)

- 7/108 used pancreas as control (High expressor)
- 14/108 used carcinoid as control (High expressor)
- 3/108 used tumour as control (?? expressor)
- 85/108 used appendix as control (High and low expressor)
- 85/108 = 79% of the laboratories passing the CGA assessment used control with low antigen expression
**Chromogranin A Ab-1**

*Click here to view datasheet for research use*

*Click here to view IVD datasheet*

*Compare different antibodies against Chromogranin A*

**Mouse Monoclonal Antibody**

**Description:** Chromogranin A (a protein of 439-amino acid which is encoded on chromosome 14) is present in neuroendocrine cells throughout the body, including the neuroendocrine cells of the large and small intestine, adrenal medulla and pancreatic islets. It is an excellent marker for carcinoid tumors, pheochromocytomas, paragangliomas, and other neuro-endocrine tumors. Coexpression of chromogranin A and neuron specific enolase (NSE) is common in neuroendocrine neoplasms.

**IgIsotype:** IgG1 / kappa

**Clone Designation:** LK2H10

**Immunogen:** Human pheochromocytoma.

**Positive Control:** PC-12 cells. Pancreas.

**Cellular Localization:** Cytoplasmic.

**Molecular Weight:** 68-75kDa

**Species Reactivity:** Human, Monkey, Pig, Rat, and Rabbit. Others.

Formalin-fixed, paraffin-embedded rat pancreas stained with NeoMarkers' Chromogranin A Ab-1 (Cat. #MS-324-P) using peroxidase-conjugate and AEC chromagen. Note cytoplasmic staining of islet cells.

Formalin-fixed, paraffin-embedded human pancreatic tumor stained with NeoMarkers' Chromogranin A Ab-1 (Cat. #MS-324-P) using peroxidase-conjugate and AEC chromagen. Note cytoplasmic staining of tumor cells.
Conclusions:

Focus on external tissue controls are central to standardize and optimize IHC:

- On-slide TMA controls are preferable to 1 batch control
- Internal tissue controls are of limited value
- Negative reagent controls are only essential for biotin-based detection systems
- Negative reagent controls can be valueable for non-biotin based systems e.g. If pigment, frozen sections..
Conclusions:

Focus on external tissue controls are central to standardize and optimize IHC:

- External tissue control "catalogue" (normal preferable) with descriptions of HE, LE and NE
- Accepted and developed by KOL, EQA, Industry, Labs
- Used to validate/verify IHC studies and publications
- Used for both internal and external IHC QC
Conclusions:

Focus on external tissue controls are central to standardize and optimize IHC:

- Usefull to monitor IHC consistency and thus quality indicator of technical precision of individual IHC tests
  - Each day all controls are evaluated and categorized
    - All controls and slides that do not pass are classified with respect to source of error
    - Do we see any errors related to retrieval (morphology), background (drying out at stainer), weak signal (antibody) etc....
IHC is a challenge, technical complex but not mission impossible and rests on 5 legs

- **Use proper controls**
- **Use a robust and specific detection system**
- **Use efficient HIER**
- **Use Ab clones, optimal for the IHC platform**
- **Harmonize and standardize tissue processing**

Alice in Wonderland

Begin at the beginning,' the King said gravely, and go on till you come to the end: then stop.'

For IHC: begin at the end, tune in your protocol: then stop.