Immunohistochemistry in the classification of neoplasias of the alimentary tract

&

External Quality Assurance of Immunohistochemistry for GI cancer markers

Mogens Vyberg, director, NordiQC
GI tumours of uncertain origin

Carcinoma or GIST/sarcoma?

Primary or secondary?
Important IHC markers of the alimentary tract

- **Epithelial markers**
  - CK-pan, CK20, CK7, CK5, EpCAM, Claudin4
- **Lower GI tract markers**
  - CEA, CDX2, SATB2, Cadherin 17
- **MMR-proteins**
  - pMLH1, pMSH2, pMSH6, pPMS2
- **Neuroendocrine markers**
  - SYP, CGA
- **Hepatocellular differentiation markers**
  - Glypican 3, can. CD66a/CD10, Arginase, GS6
- **Mesenchymal markers**
  - CD117, DOG1, SOX10, β-catenin
<table>
<thead>
<tr>
<th>Epitope</th>
<th>Epitope description</th>
<th>Latest assessment</th>
<th>Year</th>
<th>Recommended protocols</th>
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<td>Alpha-Smooth Muscle Actin</td>
<td>Run 44</td>
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<td>Bcl-2</td>
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Assessment Run 47 2016
Pan Cytokeratin (CK-PAN)

Material
The slide to be stained for CK-PAN comprised:

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

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<td>85</td>
<td>103</td>
<td>105</td>
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<td>168</td>
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<td>53%</td>
<td>58%</td>
<td>62%</td>
<td>60%</td>
<td>65%</td>
<td>60%</td>
<td>67%</td>
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</table>

Conclusion
The mAb clone cocktails AE1/AE3, AE1/AE3/SD3, AE1/AE3/PCK26 and mAb clone BS5 can all be recommended for demonstration of CK-PAN. The epitope retrieval method must be specifically tailored to each of the clones/cocktails applied. The Ready-To-Use systems from Dako based on mAb clone cocktail AE1/AE3 were in this assessment most successful and provided the highest proportion of sufficient and optimal results.
PAN-CK Inappropriate retrieval

AE1/AE3 + HIER

Liver

AE1/AE3 + proteolysis

RCC

TP

FN

TP

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

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<td>Participants, n=</td>
<td>72</td>
<td>85</td>
<td>103</td>
<td>123</td>
<td>168</td>
<td>202</td>
<td>233</td>
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<td>Sufficient results</td>
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<td>58%</td>
<td>62%</td>
<td>60%</td>
<td>65%</td>
<td>65%</td>
<td>67%</td>
</tr>
</tbody>
</table>

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

Dako: RTU - HIER
Leica: RTU - Proteolysis
Thermo: Conc: HIER Quanto - Proteolysis UltraVision
VMS: RTU - Proteolysis ⇔ Proteolysis + HIER

Misleading data sheets + Wrong control material used
EpCAM (Epithelial cell adhesion molecule, EP4, MOC31, …)

+(-)
- Adenocarcinomas (of most types)
- Neuroendocrine neoplasms
EpCAM (Epithelial cell adhesion molecule, EP4, MOC31, …)

-/+ 
- Lobular breast carcinoma
- Hepatocellular carcinoma
- Squamous cell carcinoma
- Renal cell carcinoma
- Embryonal carcinoma
- Epithelioid mal. mesothelioma
- Adrenal cortical carcinoma
- Choroid plexus carcinoma
EpCAM protocol

Colon adenocarcinoma

Lab A optimal with EP4
Retrieval: TRS pH 6.1 DAKO
    Diva2 pH 6.2 Thermo
    CC1+Proteolysis VMS
Clone BS14: TRIS-EDTA a.o.

Lab B insufficient with EP4
Retrieval: Any other buffer without concomitant proteolysis
EpCAM antibodies – renal cell carcinoma

SPM491  Ber-EP4  Ab71916

with optimal protocols
Claudin 4

Integral membrane protein, which belongs to the claudin family. The protein is a component of **tight junction**.

“Never” found in mesothelioma
Claudin 4 vs. EpCAM

Mal. mesothelioma

Claudin 4

Mal. mesothelioma

EpCAM
Drosophila caudal related homeobox gene 2 product: Nuclear transcription factor for intestinal differentiation

- Intestine
  - all cell types incl. endocrine
- Intestinal metaplasia
  - chronic gastritis
  - Barrett’s esophagus
- Pancreas/bil.tract
CDX-2 protein in adenocarcinoma

- Colorectum  +(-)
- Mucinous ovar.  +(-)
- Esoph./Stom.  +/-
- Mucinous lung  +/-
- Pancr./biliary  -/+ 
- Prostate  -(+)
- Urothelial  -(+)
- Endometrioid  -(+)
- Yolk sac tumour  +
Endometrioid carcinoma: ER & CDX-2
Quality assurance of immunohistochemical Cdx2 detection in carcinomas

Results

Mean H-score and proportion of positives

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>EPR* CONC</th>
<th>EPR* RTU</th>
<th>DAK-CDX2</th>
<th>AMT-28</th>
<th>CDX2-88</th>
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<td>High Ex</td>
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<td>240</td>
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<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>98%</td>
<td>96%</td>
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<td>Low Ex</td>
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<td>27</td>
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<td>95%</td>
<td>48%</td>
<td>58%</td>
<td>19%</td>
<td>13%</td>
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rmAb EPR2764Y (Ventana, CellMarque)

Demonstration of CDX2 is Highly Antibody Dependant

Martine Borrisholt, MS, Soren Nielsen, HT, and Mogens Vyberg, MD

(Appl Immunohistochem Mol Morphol 2013;21:64–72)
CDX2
Colon adenocarc.
Optimized protocols
Cadherin 17

- Calcium dependent adhesion molecules
- CAD17 = Liver-Intestine (LI-) Cadherin
- Regulated by CDX2
- Intestine (uniform)
- Pancreas/biliary tract (heterogeneous)
Cadherin 17

+ Adenocarcinoma, colon (incl. medullary MMR prot. defic.)
Endocrine neoplasm of small intestine
+- or -/+ Adenocarcinomas of esophagus, stomach, pancreas / biliary tract
-(+)
Adenocarcinomas of lung, endometrium, ovary, breast
Endocrine neoplasms of lung and pancreas
Squamous cell carcinoma
CAD17 and CDX2 – Colon adenocarcinoma

Cadherin 17: rmAb SP183, CM, 1:50, CC1M/16M/UV
CDX2: rmAb EPR2764Y, CM
CAD17 and CDX2 – Colon adenocarcinoma

Cadherin 17: rmAb SP183, CM, 1:50, CC1M/16M/UV

CDX2: rmAb EPR2764Y, CM
**SATB2** – rMab SP281 CM

_**Special Adenine Thymine-rich sequence-binding protein 2**_

- Nuclear matrix-associated transcription factor of intestine, neurons and osteoblasts
- Colorectal adenocarcinomas positive in 90%
- Renal cell carcinoma positive in 30%
- Other carcinomas usually negative (10-20% focal)
- Neuroendocrine neoplasms usually negative
Colon adenocarcinoma

Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

James R. Conner, MD, PhD and Jason L. Hornick, MD, PhD
Carcinoembryonic antigen (CD66e)

- Adhesion molecule esp. associated with intestine
Carcinoembryonic antigen (CD66e) in adenocarcinomas

- Colorectal  +
- Medull. thyroid  +
- Pancreas/biliary tract  +/–
- Stomach  +/–
- Lung
- Ovary, mucinous  +/–
- Ovary, non-muc.  –/+
- Prostate  –
- Kidney  –
- Liver  (!)
- Mesothelioma  (!)  –
Carcinoembryonic antigen – which antibody?

<table>
<thead>
<tr>
<th>Concentrated Abs</th>
<th>N</th>
<th>Vendor</th>
<th>Optimal</th>
<th>Good</th>
<th>Borderl.</th>
<th>Poor</th>
<th>Suff. 1</th>
<th>Suff. OPS²</th>
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<td>Dako</td>
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<td>34</td>
<td>6</td>
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<td>91 %</td>
<td>96 %</td>
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<td>NeoMarkers</td>
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<td>100 %</td>
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<td>Ready-To-Use Abs</td>
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<td>Ventana</td>
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<td>0</td>
<td>0</td>
<td>13</td>
<td>0 %</td>
<td>-</td>
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</table>
Carcinoembryonic antigen – which antibody?

Normal liver

Clone II-7

Clone TF3H8-1
Carcinoembryonic antigen – which antibody?

Mal. mesothelioma

mAb II-7

pAb or TF3H8-1
MLH1

- Improper calibration of Ab titre
- Less successful Ab clone G168-728 (8/9 insuff.)
- Less sensitive detection systems:
MLH1 loss

False neg. internal control

3-step polymer

2-step polymer
- Improper calibration of Ab titre
- Poor Ab clone 25D12 (17/17 insuff.)
- Insufficient HIER

25D12 – combined false negative and positive
Poor RTU formats: Chromogranin A

Well diff endocrine carcinoma

LK2H10 with optimized protocol

pAb RTU
Company 1

mAb LK2H10 RTU
Company 2

mAb LK2H10 RTU
Company 3
Poor RTU formats: Chromogranin A

Small cell carcinoma

LK2H10 REF

pAb RTU
Company 1

mAb LK2H10 RTU
Company 2

mAb LK2H10 RTU
Company 3
Liver tumour of unknown origin – hepatocellular carcinoma?
Glypican 3

- Hepatocellular carcinoma +/-
- Yolk sac tumour +
- Chorionic carcinoma +
- Merkel cell carcinoma +/-
- Colorectal adenocarcinoma -/+ 
- Gastric adenocarcinoma -/+ 
- Ovarian clear cell carcinoma -/+ 
- Emb. carcinoma –(+) 
- Ovarian serous carcinoma –(+) 
- Liposarcoma +/-
Table 1. Antibodies and assessment marks for GLP3, run 42

<table>
<thead>
<tr>
<th>Concentrated antibodies</th>
<th>n</th>
<th>Vendor</th>
<th>Optimal</th>
<th>Good</th>
<th>Borderline</th>
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<td>94%</td>
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<tr>
<td>Total</td>
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<td>35</td>
<td>34</td>
<td>8</td>
<td>3</td>
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<tr>
<td>Proportion</td>
<td></td>
<td></td>
<td>44%</td>
<td>42%</td>
<td>10%</td>
<td>4%</td>
<td>86%</td>
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</tbody>
</table>

Note: 1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see balance.
Poor calibration of conc.: Glypican 3
CD66a (biliary glycoprotein-1)

CEA-like cell adhesion molecule ("pCEA")
- Bile canaliculi
- Many epithelia
- Trophoblast
canCD66a (biliary glycoprotein-1)

Hepatocellular carcinoma
~70% canalicular staining
Immunohistochemistry in the classification of neoplasias of the alimentary tract & External Quality Assurance of Immunohistochemistry for GI cancer markers

Mogens Vyberg, director, NordiQC