

NordiQC Nordic immunohistochemical Quality Control
 Institute of Pathology, Aalborg Hospital, P.O. Box 561, DK-9100 Aalborg, Denmark

NordiQC seminar in collaboration with
 Danish Society for Cytochemistry and Histochemistry

DSCH

Best Laboratory Practice and Standardization of Immunohistochemistry Testing

Requirements for internal and external quality controls


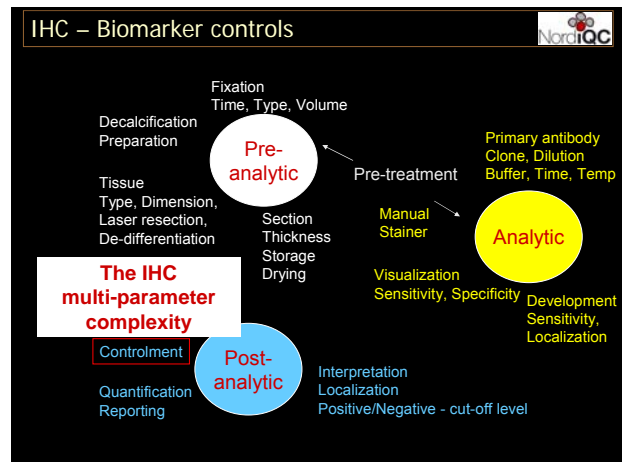
Søren Nielsen
 Scheme Manager
 NordiQC
 Aalborg Hospital, Denmark

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 and EQA programmes ?

IHC – Biomarker controls

- 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.

IHC – Biomarker controls

The basal fundament for a technical optimal IHC performance:

- Appropriate tissue fixation and processing
- Appropriate and efficient epitope retrieval
- Appropriate choice of antibody/clone
- Robust, specific & sensitive detection system
- Appropriate choice of control material

IHC – Biomarker controls

REVIEW ARTICLE

Appl Immunohistochem Mol Morphol • Volume 15, Number 2, June 2007

Recommendations for Improved Standardization of Immunohistochemistry

Neal S. Goldstein, MD, Stephen M. Hewitt, MD, PhD, Clive R. Taylor, MD, DPhil, Hadi Yaziji, MD, David G. Hicks, MD, and Members of Ad-Hoc Committee On Immunohistochemistry Standardization

Preanalytic
Test selection
Specimen type, acquisition, transport time
Fixation type and time
Tissue processing, type, and temperature
Analytic
ARI procedure
Patented, control selection
Reagent validation
Technician training/certification
Laboratory certification
Postanalytic
Control evaluation
Results interpretation
Results reporting
Pathologist, experience and CME

“Whether there is an internal positive control cell or structure present on the test (patient) slide and descriptive documentation regarding the intensity and proportion of internal control cells or structures that were immunoreactive”.

IHC – Biomarker controls

Diagnostic Cytopathology, Vol 39, No 4

Documentation of Immunocytochemistry Controls in the Cytopathologic Literature: A Meta-Analysis of 100 Journal Articles

Carol Colasacco, MLIS, S.C.T.I.A.S.C.P.I., C.T.A.G.I.,¹ Sharon Mount, MD,^{1,2} and Gladwyn Leiman, M.B.B.Ch., F.I.A.C., F.R.C.Path.^{1,2}

ICC Controls in the Literature

- Other 15%
- Identical 13%
- Absent 54%
- Vague 18%

Fig. 1. Description of immunocytochemistry controls in articles reviewed.

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.

IHC – Biomarker controls

Am J Clin Pathol 2010;133:354-365
DOI: 10.1309/AJCPDY21XMF4HJWK

Canadian Association of Pathologists-Association canadienne des pathologistes National Standards Committee/Immunohistochemistry

Best Practice Recommendations for Standardization of Immunohistochemistry Tests¹

Emina Emilia Torlakovic, MD, PhD,¹ Robert Riddell, MD, FRCPath, FRCPC,² Diponkar Banerjee, MChB, FRCPC, PhD,³ Hala El-Zimanyi, MD, MS, FRCPC,⁴ Dnyana Pillaydjie, MD, FRCPC,⁵ Peter Djuve, MS,⁶ Anthony Magliocco, MD, FRCPC,⁷ Penny Barnes, MD, FRCPC,⁸ Richard Berendt, MD, FRCPC,⁹ Donald Cook, MD, FRCPC,¹⁰ Blake Gilks, MD, FRCPC,¹¹ Gaynor Williams, MD, PhD,¹² Bayardo Perez-Ondamez, MD, FRCPC,¹³ Bret Wehli, MD, FRCPC,¹⁴ Paul E. Swanson, MD,¹⁵ Christopher N. Otis, MD,¹⁶ Soren Nielsen, HT, CT,¹⁷ Mogens Vyberg, MD,¹⁷ and Jagdish Butany, MBBS, MS, FRCPC¹³

Key Words: Clinical immunohistochemistry, Standards, Canadian Association of Pathologists

IHC – Biomarker controls

- Appropriate choice of control material

- Control material to evaluate the performance of the biomarker in the range of laboratory tissue processing procedures
- Control material to evaluate the diagnostic use of the biomarker (sensitivity / specificity)
- Control material to evaluate the consistency of the biomarker assay - day to day
- Control material evaluate the performance of the biomarker - laboratory to laboratory

IHC – Biomarker controls

- Appropriate choice of control material

- Control material to evaluate the performance of the biomarker in the range of laboratory tissue processing procedures
 - fixed and processed as patient material
 - similar fixation conditions (type, duration)
 - similar tissue processing (decalcification)
 - similar tissue processing (dehydration, etc)

IHC – Biomarker controls

Arch Pathol Lab Med—Vol 131, January 2007

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M, Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hageny, D. Craig Allred, Richard J. Coit, Mitchell Dossert, Patricia L. Fitzgerald, Weidat M. Harari, Amy Langer, Lisa M. McShane, Soosung Park, Alan D. Pritchard, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catherine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gill M. Vance, Marc van de Vijver, Thomas M. Wheeler, Daniel F. Hayes

Appl Immunohistochem Mol Morphol • Volume 16, Number 6, December 2008

Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry

Hadi Yaziji, MD,* Clive R. Taylor, MA, MD, D.Phil.† Neal S. Goldstein, MD,‡ David J. Dabbs, MD,§ Elizabeth H. Hammond, MD,|| Bryan Hewlett, ART (CSMLS), MLT,† (CMLTQ),* Alison D. Floyd, PhD,† Todd S. Barry, MD,§ Alvin W. Martin, MD,** Stanil Badve, MD,†† Frederick Bacikner, MD,†† Richard W. Curtin, MD,‡‡ Richard N. Eisen, MD,§§ Paul E. Swanson, MD,||| Stephen M. Hewitt, MD, PhD,*** Mogen Vyberg, MD,||| and David G. Hicks, MD**** and Members of the Standardization Ad-Hoc Consensus Committee

6 - 48h

8 - 72h

IHC – Biomarker controls

(Am J Surg Pathol 2011;35:545-552)

The Effect of Prolonged Fixation on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Cancer: A Prospective Study

Leung Clu Tong, BA, MD,* Nahid Nelson, BSc, PhD,† Jim Tsourigianis, BSc, MLT,‡ and Anna Marie Mulligan, MB, MSc, FRCPath*†

13 hours versus 79 hours in 10% NBF (the week-end dilemma.....)
101 breast carcinomas:

- 99 % Concordance between short fixation and long fixation for ER (SP1)
- 95 % Concordance between short fixation and long fixation for PR (1E2)
- 98 % Concordance between short fixation and long fixation for HER2 (A0485)

IHC – Biomarker controls

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	1+	1+	1+	1+
Tumour 2	3+	3+	3+	3+
Tumour 3	0	0	0	0
Tumour 4	1+	1+	1+	1+
Tumour 5	0	0	0	0
Tumour 6	3+	3+	3+	3+
Tumour 7	0	0	0	0
Tumour 8	0	0	0	0
Tumour 9	0	0	0	0

Breast carcinomas, HER-2 PATHWAY, rmAb 4B5
(CC1 Mild, Ab inc. 20 min. 36°C, UltraView DAB)

IHC – Biomarker controls

4 h 24 h
48 h 168 h

Breast carcinoma 3+, HER-2 PATHWAY, rmAb 4B5

IHC – Biomarker controls

4 h 24 h
48 h 168 h

Breast carcinoma 1+, HER-2 PATHWAY, rmAb 4B5

IHC – Biomarker controls

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	1+	1+	1+	1+
Tumour 2 3+	3+	3+	3+	3+
Tumour 3	0	0	0	0
Tumour 4	1+	1+	1+	1+
Tumour 5	0	0	0	0
Tumour 6 3+	3+	3+	3+	3+
Tumour 7	0	0	0	0
Tumour 8	0	0	0	0
Tumour 9	0	0	0	0

HER2 ER

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	+	+	+	+
Tumour 2	-	-	-	-
Tumour 3	-	-	-	-
Tumour 4	+	+	+	+
Tumour 5	+	+	+	+
Tumour 6	+	+	+	+
Tumour 7	-	-	-	-
Tumour 8	+	+	+	+
Tumour 9	+	+	+	+

PR ECAD

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	+	+	+	+
Tumour 2	-	-	-	-
Tumour 3	+	+	+	+
Tumour 4	+	+	+	+
Tumour 5	+	+	+	+
Tumour 6	+	+	+	+
Tumour 7	+	+	+	+
Tumour 8	+	+	+	+
Tumour 9	+	+	+	+

Conclusion: IHC biomarkers not affected by NBF fixation time and patient material and control material can be fixed from 4 - 168h in 10% NBF ... **but**

IHC – Biomarker controls

Internal SISH validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	-	-	-	FN
Tumour 2 Amp	+	+	+	+
Tumour 3	(?)	-	FN	FN
Tumour 4	-	-	FN	FN
Tumour 5	-	-	-	-
Tumour 6 Amp	+	+	+	+
Tumour 7	FN	-	-	FN
Tumour 8 poly.	-	-	-	FN
Tumour 9 poly.	-	-	-	FN

HER-2 ISH: 9/36 cores could not be assessed..!
Breast carcinomas, Dual SISH CCrb ext, P3. 8 m

IHC – Biomarker controls

4 h 24 h
48 h 168 h

Breast carcinoma, 1+ Dual SISH CCrb ext, P3. 8 m

IHC – Biomarker controls

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Decalcification

Bone
Bone is a specialized connective tissue that offers protection to internal organs and works with the musculature to provide physical support and movement. Bone has a hard, rigid nature due to the inclusion of minerals in its osseous matrix. This crystalline component of the bone, known as hydroxyapatite, serves as a reservoir for a multitude of inorganic ions that are subsequently utilized by various physiological systems. It is composed of calcium, phosphate and hydroxyl ions along with small amounts of magnesium, fluoride, carbonate, strontium, potassium and several other ions.

Although some labs process mineralized bone for resin embedding, most routine labs demineralize, or "decalcify", bone specimens to make them soft enough to section in paraffin. Bone specimens can be particularly challenging because they are often submitted with the surrounding cartilage and soft tissue that can be easily damaged when exposed to many of the common decalcification solutions. Decalcification is time consuming, which delays diagnostic reporting, but procedures can be expedited with microwave exposure. Monitoring of individual specimens is required.

Technical Manual

- Histology
- Cryotomy
- Fixation
- Decalcification
- Stain
- Decalcification

ASB 01-00948 (31x4 area)

– fixed and processed as patient material

- similar fixation conditions (type, duration)
- similar tissue processing (decalcification)
- similar tissue processing (dehydration, etc)

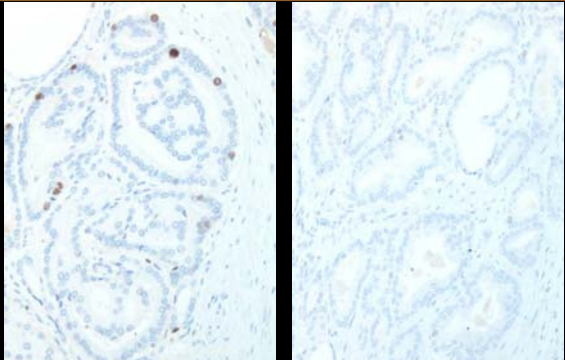
IHC – Biomarker controls

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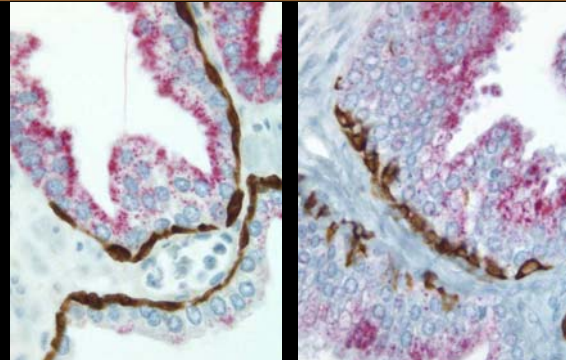
IHC – Biomarker controls



Prostate – Ki67, mAb clone 30.9

10 % NBF 24h → 24h 10 % form. acid 10 % NBF + 10 % form. acid 24h

IHC – Biomarker controls



Prostate – PIN cocktail – p504s/CK5/p63

10 % NBF 24h → 24h 10 % form. acid 10 % NBF + 10 % form. acid 24h

IHC – Biomarker controls

BMC Clinical Pathology
BioMed Central

Research article
Preservation of biomolecules in breast cancer tissue by a formalin-free histology system
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Transforming the future of pathology

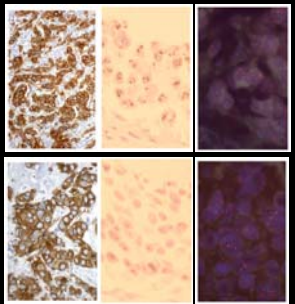
The Tissue-Tek® Xpress™ Rapid Tissue Processor lives up to its name by processing up to 120 specimens per hour... a rate never possible in the laboratory until now.

IHC – Biomarker controls

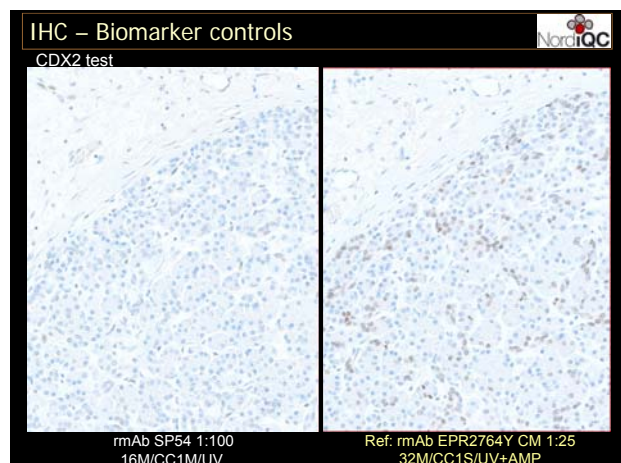
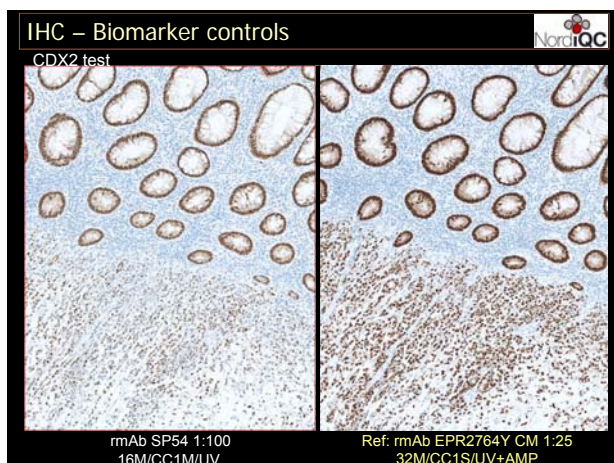
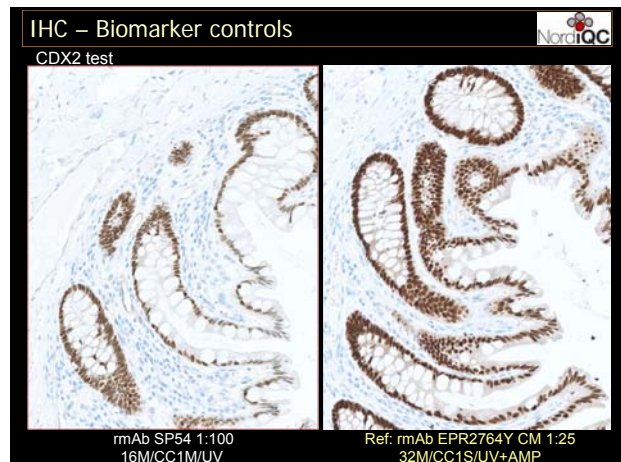
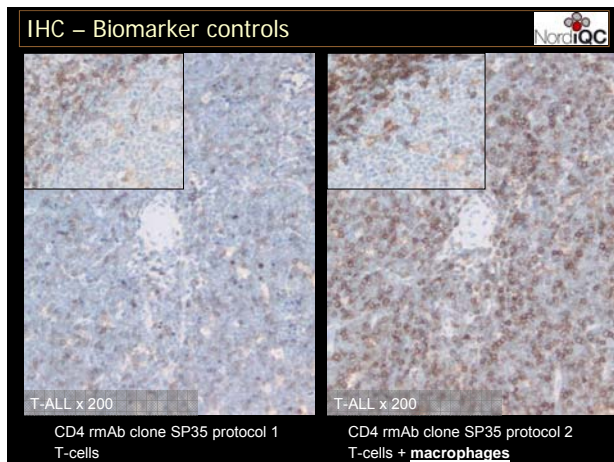
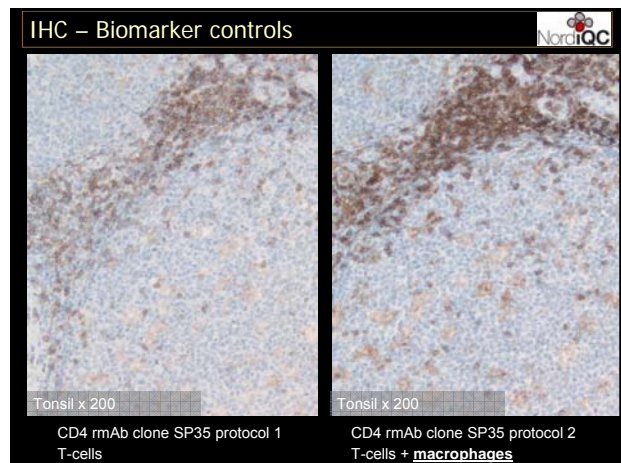
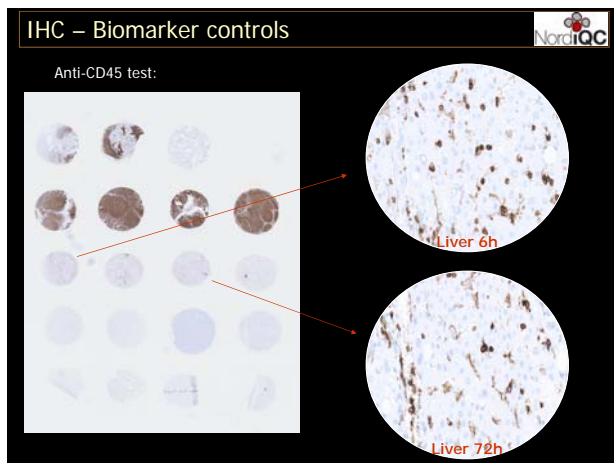
B. HER2

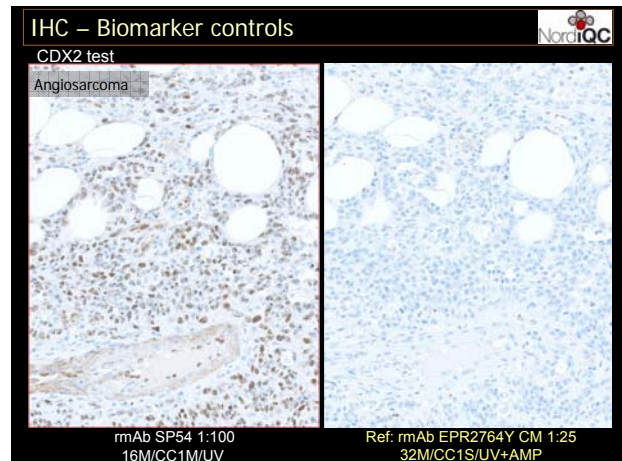
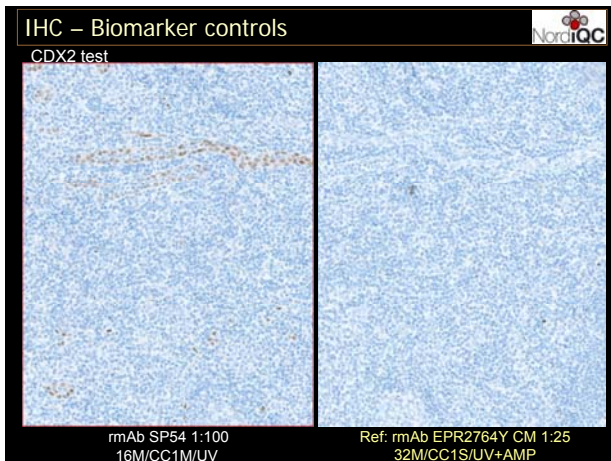
HER2 FISH	HER2 IHC UFPE	HER2 IHC FFPE
0	0	0
Not Amp	1	0
Not Amp	1	0
Not Amp	0	1
Not Amp	0	1
Not Amp	1	1
Not Amp	1	1
Not Amp	0	2
Not Amp	1	2
Not Amp	2	2
Amp	2	2
Amp	3	2
Amp	3	2
Amp	3	3
Amp	3	3
Amp	3	3
Amp	3	3
Amp	3	3
Amp	3	3

Results: The immunohistochemical reaction for HER2 was similar in molecular- and formalin-fixed samples to that of formalin-fixed counterparts after elimination of antigen retrieval step for the HercepTest (Spearman Rank R = 0.84, p < 0.05).



UM
NBF





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 related to a too low sensitivity

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

IHC – Biomarker controls

Identification of relevant tissue & construction of QC material

Multi-tissue blocks with cells/structures with different levels of antigen expression & reflecting the diagnostic application

Includes both normal and clinical relevant tissue – 10% NBE.

IHC – Biomarker controls

CGA

CSQI: Periph. nerves

Fig. 1a. Optimal CGA staining of the appendix mucosa using the mAb clone IC2H10 optimally calibrated and with HIER in an alkaline buffer (x200). The neuroendocrine cells show a strong cytoplasmic staining reaction. A weak diffusion of the staining is seen in the vicinity of the positive cells.

Fig. 1b. CGA staining of the appendix mucosa using an insufficient protocol based on the mAb clone IC2H10 without HIER (x200) – same field as in Fig. 1a. The neuroendocrine cells show a strong cytoplasmic staining reaction. However, compare with Figs. 2b & 3b – same protocol.

Fig. 2a. Optimal CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1a. (x400). Both the axons of the peripheral nerves and the ganglion cells show a weak to moderate distinct granular staining reaction, while the smooth muscle cells are negative.

Fig. 2b. Insufficient CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1b. (x400). No staining reaction is seen in the axons of the peripheral nerves and the ganglion cells – same field as in Fig. 2a.

IHC – Biomarker controls

CGA

CSQI: Periph. nerves

Fig. 2a. Optimal CGA staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a moderate cytoplasmic staining reaction.

Fig. 2b. Insufficient CGA staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak and equivocal staining reaction – same field as in Fig. 2a.

Fig. 2a. Optimal CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1a. (x400). Both the axons of the peripheral nerves and the ganglion cells show a weak to moderate distinct granular staining reaction, while the smooth muscle cells are

Fig. 2b. Insufficient CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1b. (x400). No staining reaction is seen in the axons of the peripheral nerves and the ganglion cells – same field as in Fig. 2a.

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 carcinoïd as control (High expressor)
- 5/36 used tumour as control (?? expressor)
- 5/36 used appendix as control (High and low expressor)
- 2/36 used Multi block 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 carcinoïd as control (High expressor)
- 3/108 used tumour as control (?? expressor)
- 27/108 used appendix as control (High and low expressor)
- 58/108 used Multi block as control (High and low expressor)
- 85/108 = 79% of the laboratories passing the CGA assessment used control with low antigen expression

IHC – Biomarker controls

Antibodies

Chromogranin A Ab-1

[Click here to view database for research use](#)

[Click here to view IVD database](#)

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 carcinosarcoma, pheo-chromocytoma, paragangliomas, and other neuro-endocrine tumors. Coexpression of chromogranin A and neuron specific enolase (NSE) is common in neuroendocrine neoplasms.

IgG isotype: IgG1 / kappa

Clone Designation: LK2H10

Immunogen: Human pheochromocytoma.

Positive Control: PC-12 cells, Pancreas.

Cellular Localization: Cytoplasmic.

Molecular Weight: 69-75kDa

Species reactivity: Human, Monkey, Pig, Rat, and Rabbit. Others.

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

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NeoMarkers
Roche

IHC – Biomarker controls

Dako

FLEX Ready-to-Use Atlas of Stains - 3rd Edition

Which tissue
Which cells
How shall they be stained:

Let's connect.

Figure 10c. Tonsil. The mantle/marginal zone B cells are HE structures showing a moderate to strong membranous and cytoplasmic staining reaction, while the germinal center B cells are LE structures showing a moderate to strong staining reaction. Plasma cells show a strong cytoplasmic staining reaction. The enclosed cellular structures are representative of the HE and LE structures.

Figure 20c. Colon. The peripheral nerves show a distinct nuclear and cytoplasmic staining reaction. The ganglion cells are HE structures with a moderate to strong staining reaction, whereas the axons are LE structures and are weak to moderately stained. The enclosed cellular structures are representative of the HE and LE structures.

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

Fig. 2b. Insufficient staining for CD23 of the B-CLL no. 4 using same protocol as in Fig. 1b and 2. b. 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 CDX2-88 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. 2 - 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 almost negative. 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

MB K1	APPENDIX	TONSIL	PANCREAS	HEPAR
ASMA Alfa-smooth muscle actin (Cytopl)	Smooth muscle cells in vessels and in muscle layers. Myofibroblasts lining the epithelial surface	Smooth muscle cells in vessels	Smooth muscle cells in vessels	Smooth muscle cells in the liver sinusoids
B-CATENIN Beta-catenin (Membrane)	Membranes of columnar epithelial cells. Endothelial and follicular dendritic cells	Membranes of squamous epithelial cells	Membranes of acinar epithelial cells (ducts) and endocrine cells	Hepatocytes - weak membranous
BCL2 Bcl2-oncoprotein (Cytopl)	A weak to moderate staining in the epithelial cells in the basal crypts	All peripheral lymphocytes and T-cells in germinal centres - Germinal center B-cells are negative	Weak reaction of the epithelial cells.	Weak reaction of the epithelial cells in bile ducts.
BCL6 Bcl6-protein (Nuclear)	Germinal center B-cells	Germinal center B-cells and basal squamous cells		
CD2 Membrane	All T-cells - Scattered intraepithelial T-cells	All T-cells - Scattered T-cells in germinal centres	T-cells	T-cells
CD3 Membrane	All T-cells - Scattered intraepithelial T-cells	All T-cells - Scattered T-cells in germinal centres	T-cells	T-cells
CD4 Membrane	50 - 80 % of T-cells	50 - 80 % of T-cells and Germinal center macrophages	T-cells	Kupfer cells and sinusoid endothelial cells
CD5 Membrane	All T-cells - Scattered intraepithelial T-cells	All T-cells - Scattered B cells in the mantle zone must show a weak membranous staining	T-cells	T-cells

= CSQI
Critical Stain Quality Indicator

IHC – Biomarker controls

- Low antigen expressors
- Critical Staining Quality Indicators (CSQI)
 - essential to evaluate consistency
 - essential to evaluate sensitivity
 - qualitative IHC biomarkers (CD markers etc)
 - quantitative IHC biomarkers (ER, HER-2..)

IHC – Biomarker controls

Optimal

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

Poor

Ampl. 3+ Ampl. 1+ Unampl. 1+ Unampl. 0

IHC – Biomarker controls

Optimal

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

Poor

Ampl. 3+ Ampl. 2+ Unampl. 3+ Unampl. 1

IHC – Biomarker controls

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ORIGINAL ARTICLE

Proficiency testing of immunohistochemical biomarker assays in breast cancer

Reinhard von Wasielewski · Svenja Hasselmann · Josef Rüschoff · Annette Fissler-Eckhoff · Hans Kreipe

Average of 140 laboratories in 4 HER2 IHC assessments
On TMA's in 4' run:

100% concordance 0/1
41% concordance 2+
91% concordance 3+

IHC – Biomarker controls 




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



Alice in Wonderland

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

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

IHC – Biomarker controls 



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

Alice in Wonderland

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

IHC is a challenge, technical complex but not mission impossible and rests on 5 legs

- Use proper controls
 - Normal tissue
 - Defined staining criteria
 - Non-, low and high antigen expressor
 - Recommended by both Vendors and EQA programmes