

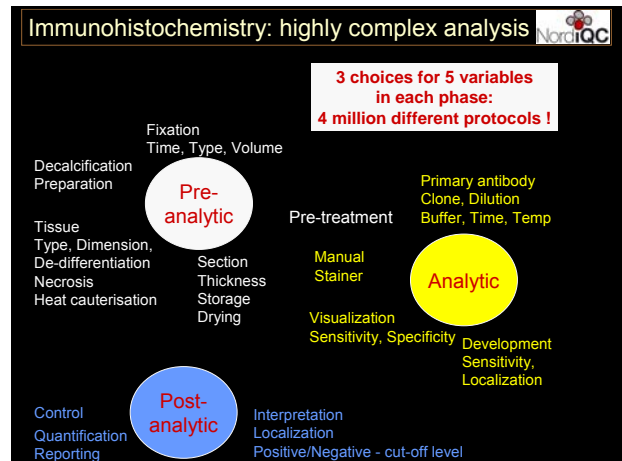
DSCH

Best Laboratory Practise and Standardization of Immunohistochemistry Testing

Pitfalls in immunohistochemistry

Seminar, Copenhagen, May 18th 2011

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Immunohistochemistry: analytical part

Major causes of insufficient stains: "Leading"

- Less successful / problematic antibodies: 18 %
- Inappropriate antibody dilution: 39 %
- Inappropriate epitope retrieval: 31 %
- Other inappropriate lab. performance: 12 %
 - Platform problems
 - Inappropriate calibration / home brews
 - Endogenous biotin reaction (EBR)
 - Section drying-out after HIER
 - Technical stainer errors
 - Unexplained

Less successful / problematic antibodies incl. RTUs: 18 %

Less succesful Abs: Estrogen receptor alpha



Nordic immunohistochemical Quality Control

Home • Participation • Assessments • Epitopes • Protocols • Techniques • Links

Recommended EK protocols • Recommended EK control tissue

Assessment Run B10 2010 Estrogen receptor (ER)

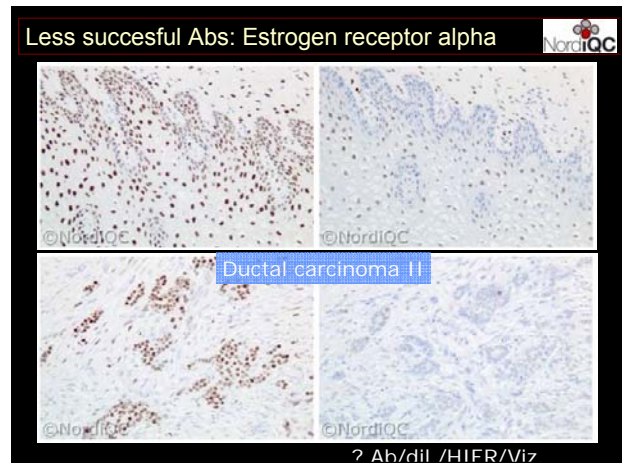
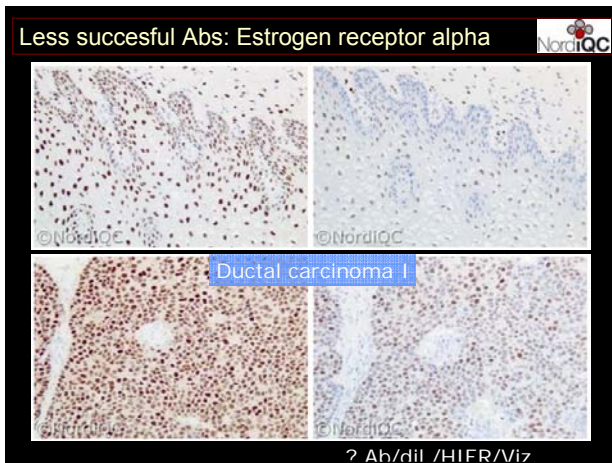
The slide to be stained for ER comprised the following 7 tissues:

No.	Tissue	ER extension*	ER intensity*
1.	Uterine cervix	00 - 90 %	Moderate to strong
2.	Breast lobular carcinoma	60 - 80 %	Weak to moderate
3.	Breast ductal carcinoma	10 - 30 %	Weak
4.	Breast ductal carcinoma	Negative	Negative
5.	Breast ductal carcinoma	10 - 30 %	Weak
6.	Breast ductal carcinoma	60 - 80 %	Weak to moderate
7.	Breast ductal carcinoma	50 - 70 %	Moderate to strong

*ER status and staining pattern as characterized by NordiQC reference laboratories using the mAb clone 6F11 and the mAb clone SP1.

Table 1. Abs and assessment marks for ER, run B10

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. rps ²
rmAb clone SP1	32	NeoMarkers	20	10	6	2	79 %	79 %
	4	Dako						
	1	Immunologic Spring						
mAb clone 6F11	29	Novocastra/Leica	9	7	8	9	40 %	70 %
	2	Vector						
	1	BioCare						
	1	Monoclonal						
mAb clone IDS	24	Immunologic	5	3	5	15	29 %	44 %
	2	Zytomed						
mAb clones IDS+6F11	4	NeoMarkers	0	1	3	0		
Ready-To-Use Abs								
rmAb clone SP1, 790-4324/25	60	Ventana	56	3	0	1	98 %	98 %
rmAb, clone SP1, 15/IR151	22	Dako	0	6	5	3	64 %	100 %
rmAb clone SP1, RM-9101-R7	4	NeoMarkers	0	2	1	1		
mAb/rmAb clones 6F11 + SP1, PH300	1	Biocare	1	0	0	0		
mAb clone 6F11, PA0151	1	Novocastra/Leica	0	0	0	1		
mAb clone IDS, IR654	1	Dako	0	0	0	1		
mAb clone IDS, N1575	1	Dako	0	0	0	1		
mAb clones IDS+ER-2-123, SK10/K4071	4	Dako	0	2	1	1		
Total	197		99	34	29	35		
Proportion			50 %	17 %	15 %	18 %	57 %	



Less successful antibodies – CD31

Recommended CD31 protocols • Recommended CD31 control tissue

Assessment Run 26 2009

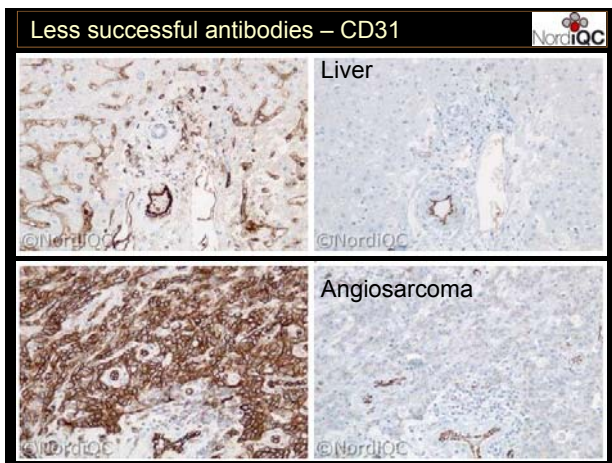
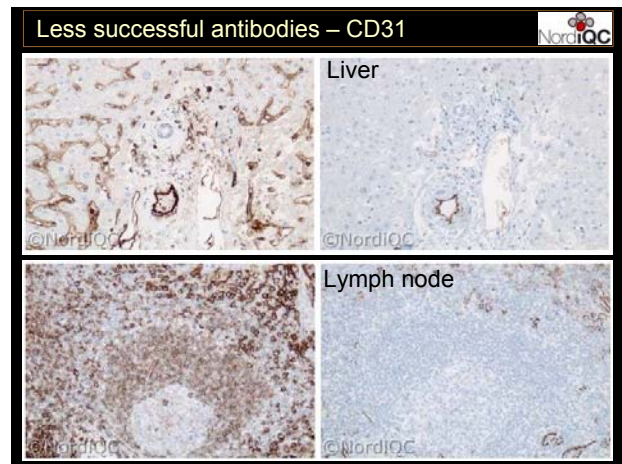
CD31

The slide to be stained for CD31 comprised:
1. Appendix, 2. Tonsil, 3. Liver, 4. Angiosarcoma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD31 staining as optimal included:

Table 1. Abs and scores for CD31, run 26

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
mAb clone JC70A	83	Dako NecMarkers	16	36	20	21	56 %	77 %
mAb clone 1A10	1	ID Labs Master Diagnostica	0	0	1	3	-	-
mAb clone 1A10	1	Sanovia SIGNET	0	0	0	0	-	-
mAb clone 1A10	3	Novocastra NecMarkers	0	0	0	0	-	-
Ready-To-Use Abs								
mAb clone JC70A, IR610	8	Dako	2	5	1	0	88 %	100 %
mAb clone JC70A, N1596	1	Dako	0	1	0	0	-	-
mAb clone 1A10, 760-4246	10	Ventana	0	0	0	10	0 %	0 %



Less successful RTUs – CDX2

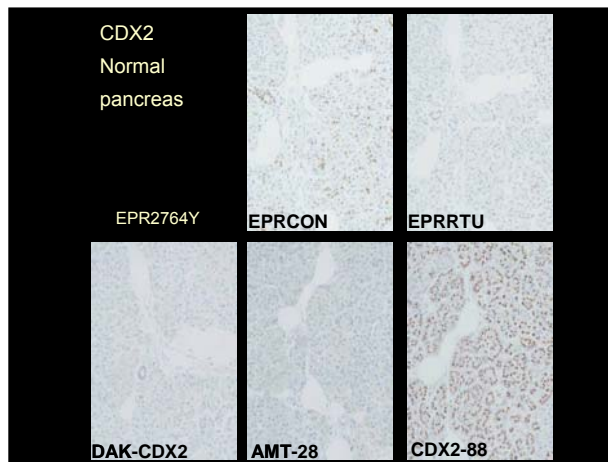
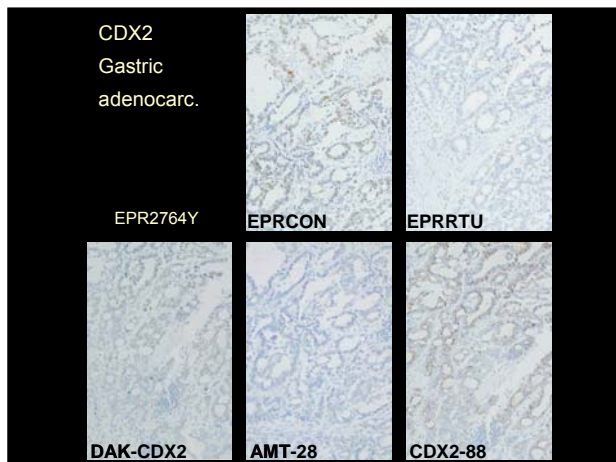
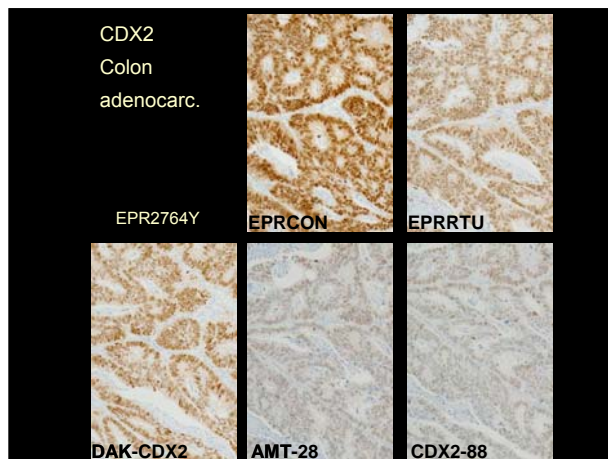
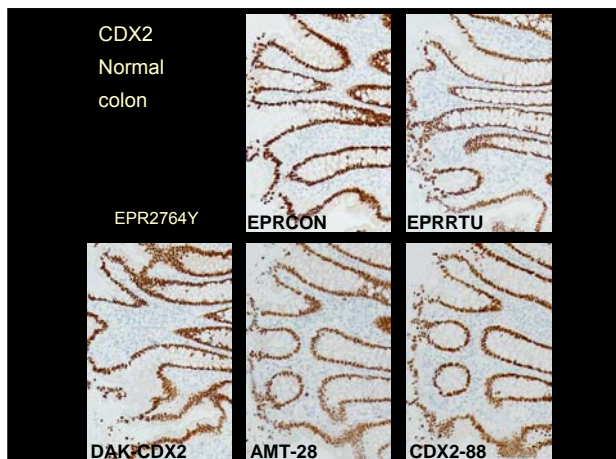
Recommended CDX2 protocols • Recommended CDX2 control tissue

Assessment Run 27 2009

CDX2

Table 1. Abs and assessment marks for CDX2, run 27

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
mAb clone CDX2-08	35	BioGenex Biocare	7	6	9	16	36 %	50 %
mAb clone ANT28	21	Novocastra	3	4	5	9	33 %	60 %
mAb clone DAK-CDX2	4	Dako	2	2	0	0	-	-
mAb clone SFI-2	1	Master Diagnostica	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone DAK-CDX2 IS080/IR080	15	Dako	13	2	0	0	100 %	100 %
mAb clone CDX2-88, AM392	6	BioGenex	0	0	0	6	-	-
mAb clone CDX2-88, IP226	1	Biocare	0	0	0	1	-	-
mAb clone CDX2-08, E087	2	Linaris	0	1	1	0	-	-
mAb clone LPR276-4Y, 760-4380	4	Ventana Cell Marque	1	2	1	1	60 %	100 %
Total	93		26	17	16	34		
Proportion			28 %	18 %	17 %	37 %	46 %	-



Less successful Abs – CDX2

105 CDX2+ carcinomas:
Mean H-score and proportion of positives

	N	EPR* CON	EPR RTU	DAK- CDX2	AMT- 28	CDX2 88
High Expressors	55	264	238	232	169	152
		100%	100%	100%	98%	96%
Low Expressors	50	60	28	29	8	5
		100%	64%	72%	24%	22%

*mAb EPR2764Y 1:25, (Ventana, Cell Marque)
All antibodies used by CC1S & UltraView + amp.
Four compared on Dako Autostainer with same results

Less successful Abs – CEA

Recommended CEA protocols * Recommended CEA control tissue
Assessment Run 27 2009
CEA

Table 1. Abs and assessment marks for CEA, run 27

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. 1	Suff. OPs ²
mAb clone II-7	78	Dako	37	34	6	1	91 %	96 %
mAb clone COL-1	5	NeoMarkers						
	2	Zymed						
	1	Biocare	6	2	2	0	90 %	100 %
mAb clone 12-140-10	1	Master Diagnostica						
	1	Zytomed						
	3	Novocastra Vector	0	0	1	3	-	-
mAb clone PARLAN 4	1	Bio-Science AG	0	0	0	2	-	-
	1	Euro Diagnostica						
mAb clone 001-94-11M-P	1	BioGenex	0	0	0	1	-	-
mAb clone TF3H8-1	1	BioGenex	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone II-7, 15622/IR622	11	Dako	11	0	0	0	100 %	100 %
mAb clone TF3H8-1, 760-2507	13	Ventana	0	0	0	13	0 %	0 %

Less successful Abs – CEA (CD66e)

Normal liver

©NordiQC

II-7 TF3H8-1

Cross reacting with CD66a

Less successful Abs – CEA

Malignant mesothelioma

No cross reaction Cross reaction

Normal liver

No cross reaction Cross reaction

Less successful antibodies – Synaptophysin

Recommended SYP protocols • Recommended SYP control tissue

Assessment Run 29 2010

Synaptophysin (SYP)

Table 1. Abs and assessment marks for SYP, run 29

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. nps ²
mAb ^{3D} clone 27G12	60	Novocastra / Leica Mnosan	19	28	8	6	77 %	78 %
mAb clone Snp88	10	Biogenex	4	0	3	3	67 %	83 %
mAb clone Sy38	0	Dako	0	0	2	6	0 %	0 %
rmAb clone SP11	2	NesMarkers	2	6	1	3	67 %	100 %
1	Spring Bioscience							
1	Master Diagnostica							
1	Unknown							
rmAb clone Z66	1	Zymed	0	1	0	0	-	-
pAb A0010	12	Dako	0	2	4	6	17 %	-
pAb CMC111	2	Cell Marque	0	0	2	0	-	-
pAb NCL-Synapp	2	Novocastra / Leica	0	0	1	1	-	-
pAb RB-1461	1	NesMarkers	0	1	0	0	-	-
pAb SIGNET-3261-1000	1	Signet Lab	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone Sy38, IR776	10	Dako	0	1	0	9	10 %	-
mAb clone Sy38, N1566	1	Dako	0	0	0	1	-	-

Less successful antibodies – Synaptophysin

27G12 Sy38

SCLC

Problematic antibodies – Synaptophysin

Blood group A Blood group A

Snp88 27G12

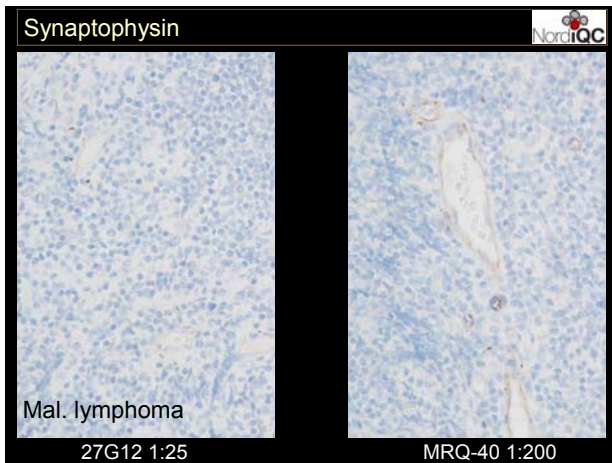
Blood group 0

MAG

Synaptophysin

Prostate carcinoma

27G12 1:25 MRQ-40 1:200



Less successful antibodies: CD117

Recommended CD117 protocols • Recommended CD117 control tissue

Assessment Run 26 2009

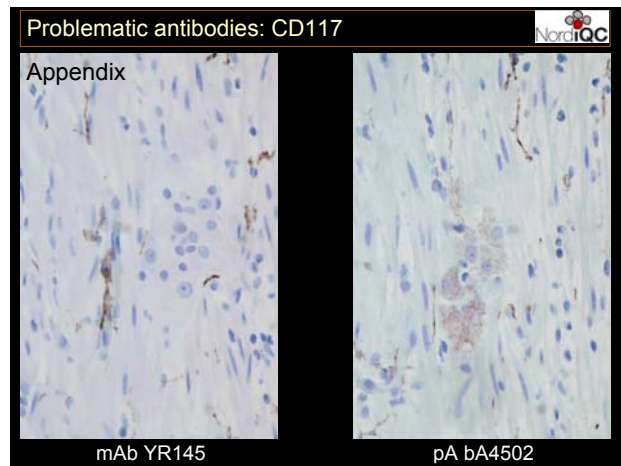
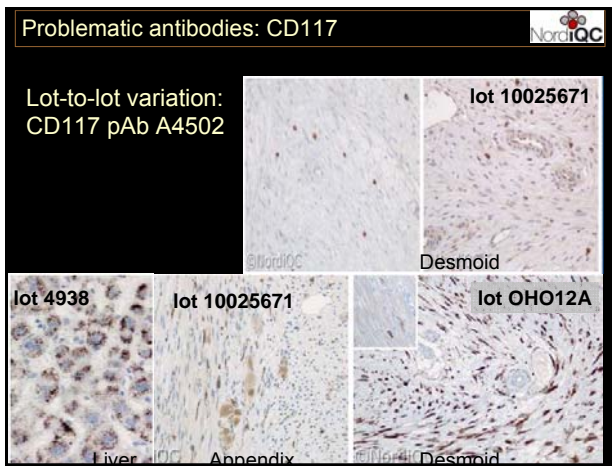
CD117

The slide to be stained for **CD117** comprised:
1. Appendix, 2. Desmoid tumour, 3-5. Gastrointestinal stromal tumour (GIST).
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD117 staining as optimal included:

Table 1. Abs and scores for CD117, run 26

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
pAb A4502	113	Dako	29	70	12	2	90%	92%
pAb RB-9030-P	2	NeoMarkers	0	0	2	0	-	-
rmAb clone YR145	2	Epitomics	3	0	1	0	-	-
	1	Cell Marque						
	1	Master Diagnostica						
Ready-To-Use Abs								
rmAb clone 9.7	8	Veritana	0	1	7	0	12%	-
mAb clone TS95	1	Menarini	0	0	0	1	-	-
Total	128		32	71	22	3	81%	92%
Proportion			25%	56%	17%	2%	81%	92%



Chromogranin A

Recommended CCA protocols

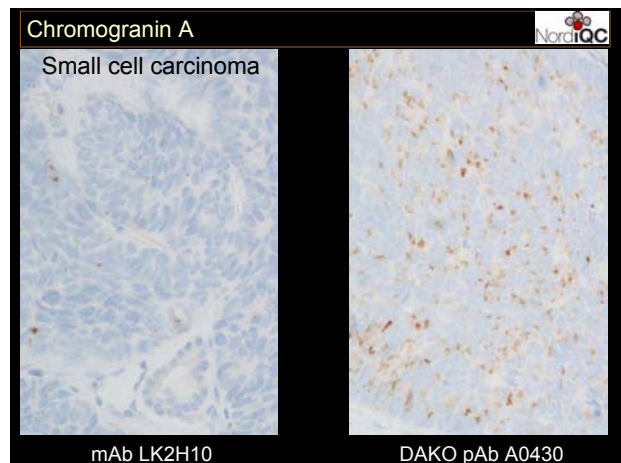
Assessment Run 31 2011

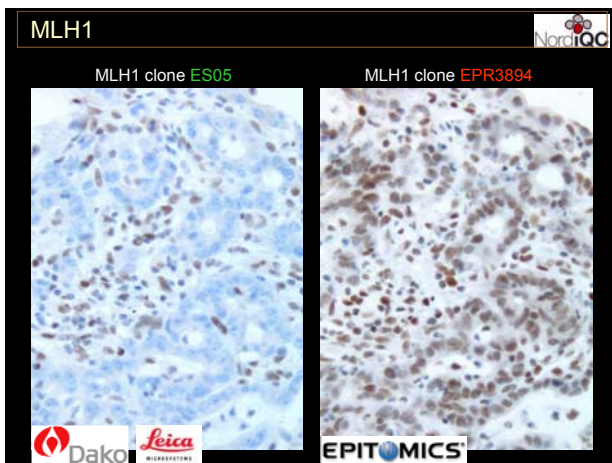
Chromogranin A (CGA)

Table 1. Abs and assessment marks for CGA, run 31

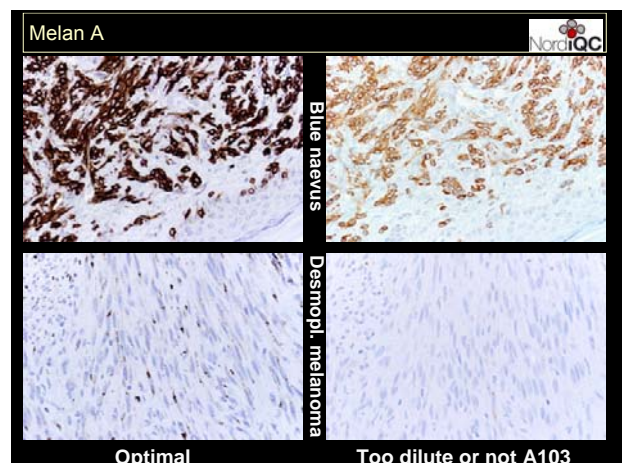
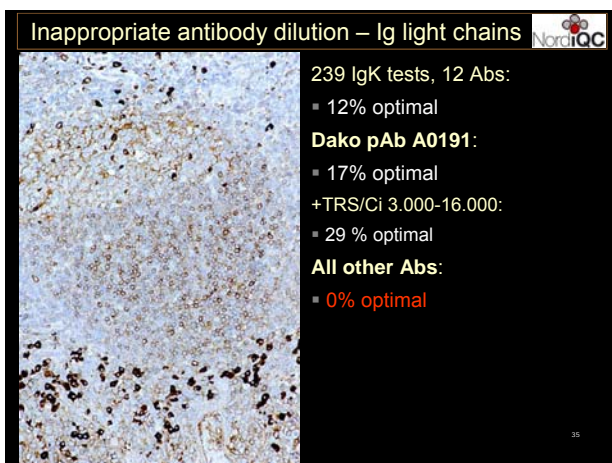
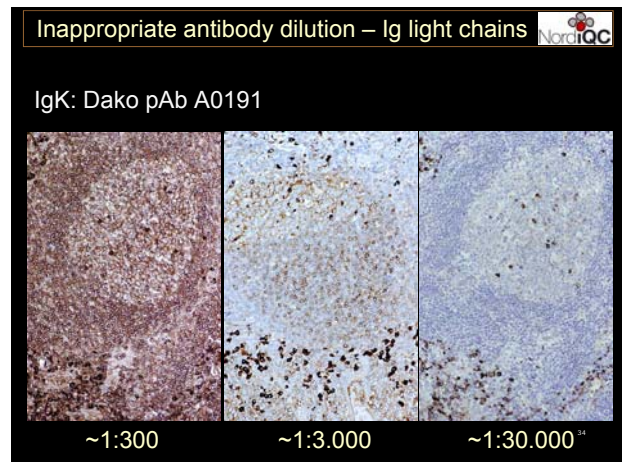
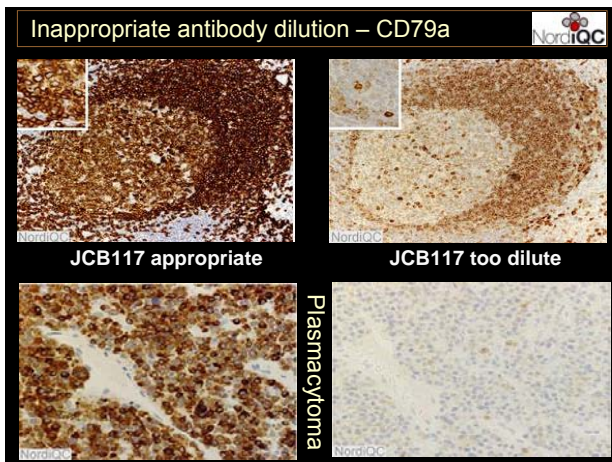
Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
mAb clone LK2H10	13	NeoMarkers	5	13	6	0	75%	91%
	5	BioGenex						
	2	Chemicon/Millipore						
	2	Leica/Novocastra						
	1	EuroProxima						
	1	Zytomed						
mAb clones LK2H10 + PHE5	8	NeoMarkers	3	5	3	0	93%	90%
	3	Biocare						
mAb clone DAK-A3	16	Dako	0	2	12	2	13%	-
mAb clone SH7	4	Leica/Novocastra	0	2	0	2	-	-
rmAb clone SP12	3	Spring Bioscience	0	0	5	1	0%	-
	1	DSG						
	1	Master Diagnostica						
	1	NeoMarkers						
pAb A0430	53	Dako	36	15	2	0	96%	100%

(Abs) used and marks are summarized.

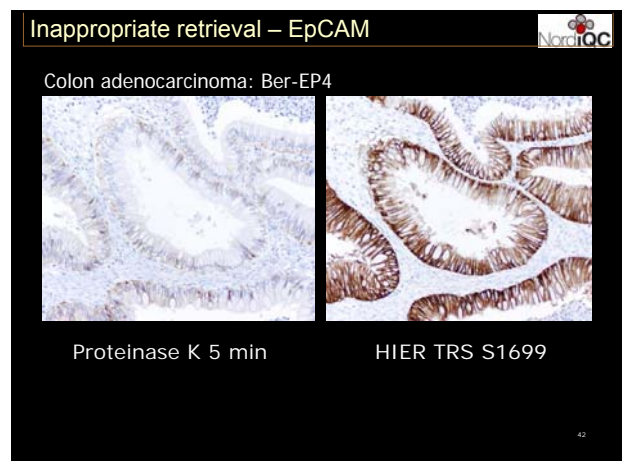
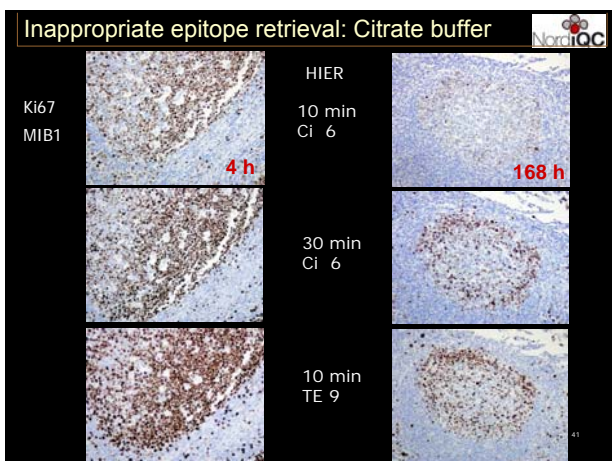
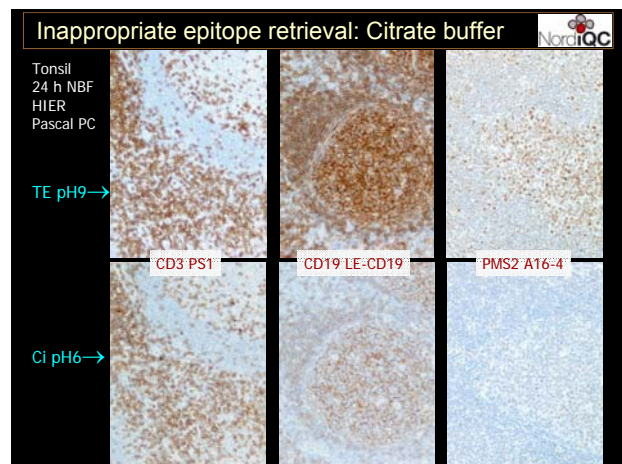
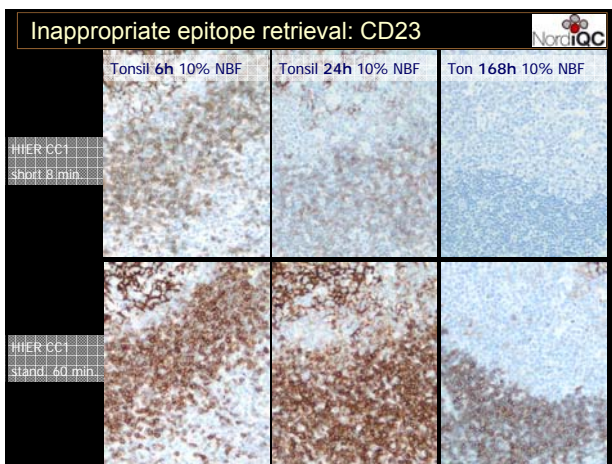
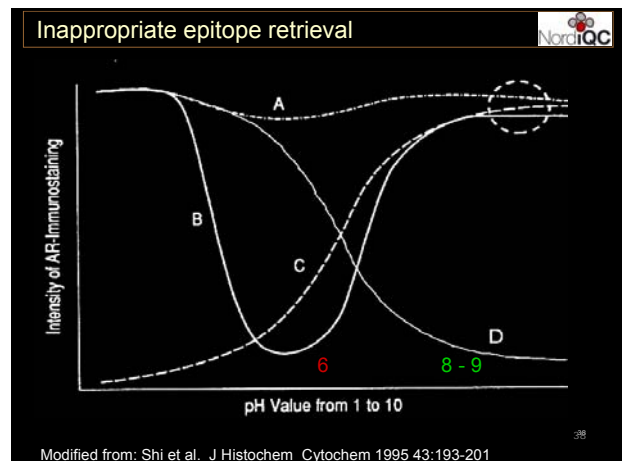


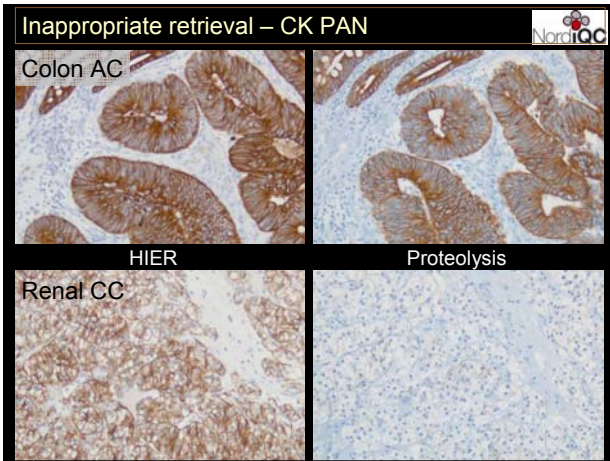
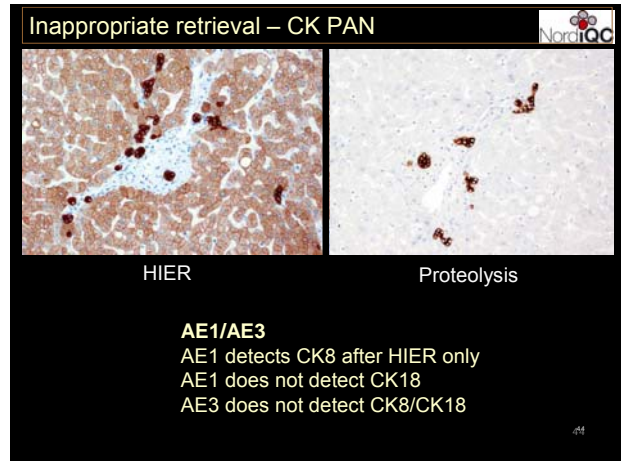
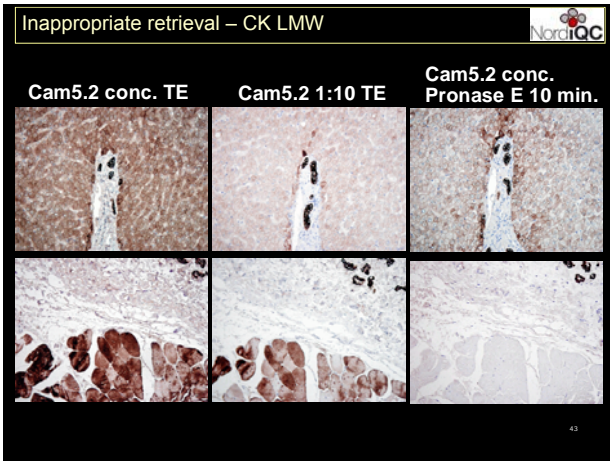


Inappropriate antibody dilution
39 %



Inappropriate epitope retrieval
31 %





Inappropriate vendor information

Dako

Monoclonal Mouse Anti-Human Cytokeratin Clones AE1/AE3

ENGLISH Code N1590

Specimen preparation Paraffin Sections

Dako AE1/AE3 can be used on formalin-fixed, paraffin-embedded tissue sections. Tissue pretreatment for epitope enhancement is required.

The following enzymes can be used for pretreatment of formalin-fixed, paraffin-embedded tissues: Proteolytic Enzyme, RTU (code S3007), Proteinase K, RTU (code S3002), Pepsin (code S3002), Pronase (code S2013) or Trypsin (code S2012) for 5 minutes. Rinse thoroughly with distilled water and continue with the staining procedure of the detection system instructions.

As an alternative to enzyme pretreatment, heat-induced epitope retrieval can be used. Heat-induced epitope retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95–99 °C), a steamer (95–99 °C) or an autoclave (121 °C). For greater adherence of tissue sections to glass slides, the use of Silanized Slides (code S3003) is recommended. Target Retrieval Solution (code S1700) or 10x Concentrate (code S1699) is recommended using a 20-minute heating protocol. After thermal treatment, allow the jar with buffer and slides to cool for 20 minutes at room temperature. Rinse well with buffer.

Misleading datasheet: AE1/AE3

2011

Inappropriate vendor information

Dako

FLEX Monoclonal Mouse Anti-Human Cytokeratin Clone AE1/AE3 Ready-to-Use (Dako Autostainer/Autostainer Plus)

Code IS063

Correct datasheet: AE1/AE3

Pre-treatment with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues using EnVision™ FLEX Target Retrieval Solution, High pH (10x) (Dako Autostainer/Autostainer Plus) (Code K8010/K8014).

2011

47

Inappropriate vendor information

VENTANA

Anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody

Catalog numbers 760-2595 (50 Tests) 760-2135 (250 Tests)

Misleading datasheet: AE1/AE3/PCK26

2011

Table 1. Recommended Staining Protocols for Anti-Pan Keratin (AE1/AE3/PCK26)

Procedure Type	Platform/Method	
	ES or NexES IHC	BenchMark or BenchMark XT
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None Required	None Required
Enzyme (Protease)	Protease 1, 4 minutes	Protease 1, 4 minutes
Antibody (Primary)	Pan Keratin, approximately 16 minutes	Pan Keratin, approximately 16 minutes
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin, 2 to 4 minutes	Hematoxylin, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

Inappropriate vendor information

December 2007
ISSUE N° 11

Correct guide line:
AE1/AE3/PCK26

Fig 2:
Enzymatic and heat pre-treatment:
mild CC1 and Protease 3 for 4 min, CK-Pan incubated for 8 min ultraView™ DAB

IHC – Quality control

Placental Alkaline Phosphatase (PLAP)

Clone: PLAP6
Isotype: IgG
Source: Mouse
Immunogen: Purified human placental alkaline phosphatase
Specificity: Placental alkaline phosphatase
Localization: Cytoplasm
Pre-treatment: None

Placentas stained with anti-PLAP using AEC chromogen

Ready-to-use (Manual): AM228-5M
Ready-to-use (Automated): AM228-10M, AX228-YCD, Xmatrix®
Concentrated: MU228 UC
Recommended Positive Control: FG-228M
Recommended Barrier Control: FB-228M

Vendor recommendations

clone PL8-F6

1:50 Recommendation 1:50 Own optim. + HIER

Placenta

Seminoma

Other inappropriate lab. performance

12 %

Platform dependent antibodies

Antigen	Clone	XT / Ultra	Autostainer	Bond-max
CD4	1F6, 4B12	FN (3% ^{H2O2})	√	√
CD4	SP35	√	√	√
CD5	4C7	FP	√	√
CD5	SP19	√	√	√
CD79a	JCB117	(√) Weak	√	√
CD79a	SP18	√	√	√
BSAP	24	FN	√	(√) Weak
BSAP	SP34	√	√	√
BCL6	PG-B6p	FN (3% ^{H2O2})	√	√
BCL6	G1191E/A8	√	√	√

Platform dependent antibodies – PAX5

Recommended BSAP protocols • Recommended BSAP control tissue

Assessment Run 28 2010

B-cell specific activator protein (BSAP, Pax5)

Table 1. Abs and assessment marks for BSAP, run 28

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. OPs ²
mAb clone 24	22	BD Bioscience	6	13	5	5	86 %	74 %
	5	Immunologic						
	1	Abcam						
	1	Zeta						
mAb clone 1Ew	11	Novocestra	3	5	1	2	73 %	80 %
mAb clone BC/24	5	Biocare	0	4	1	0	80 %	-
mAb clone DAK-Pax5	5	Dako	4	1	0	0	100 %	100 %
	1	Cell Marque						
mAb clone SP34	1	NeoMarkers	1	1	0	1	-	-
	1	Spring Bioscience						
pAb RB-9406	14	NeoMarkers	4	4	2	4	57 %	100 %
Ready-To-Use Abs								
mAb clone DAK-Pax5, IR650	3	Dako	2	5	0	0	-	-
mAb clone SP34, 790-4420	4	Ventana/Cell Marque	2	2	0	0	-	-
mAb clone 24, 760-4270	5	Ventana/Cell Marque	0	0	4	1	-	-
mAb clone 24, 312H-17, CMA462	3	Cell Marque	0	1	0	2	-	-

Platform dependent antibodies – PAX5

Hodgkin lymphoma NS

clone SP34
RTU VMS/CM
x200

clone 24
RTU VMS/CM
x200

Platform dependent antibodies SF1

Steroidogenic factor SF1 – mAb clone N1665 R&D systems

1:100, PC TE pH 9, EnV+, AS+

1:100, CC1 pH 8.5, UI.W., Ultra

Platform dependent antibodies – CD56

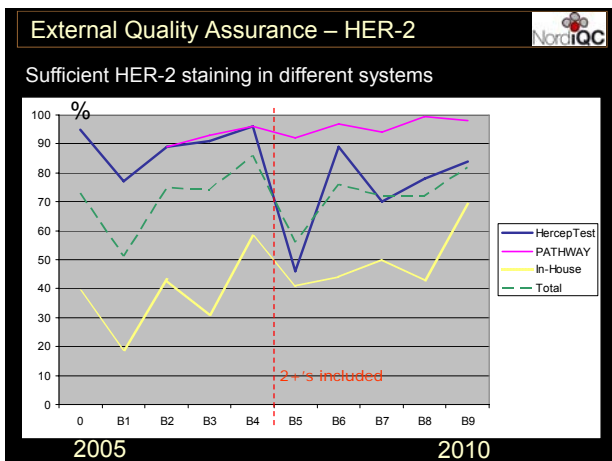
Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
mAb clone 1B6	33	Leica/Novocstra	3	11	12	9	40 %	75 %
mAb clone 123C3	1	Novoson	0	0	0	0	0 %	0 %
mAb clone 123C3	1	Dynabeads/Zymed	0	0	0	0	0 %	0 %
mAb clone 123C3	1	BioSisa	0	0	0	0	0 %	0 %
mAb clone 123C3	1	Moran	0	0	0	0	0 %	0 %
mAb clone 123C3	1	Nova Kern	0	0	0	0	0 %	0 %
mAb clone 123C3	1	Spring Bioscience	0	0	0	0	0 %	0 %
mAb clone 123C3.05	23	NeoMarkers/Thermo	2	10	8	9	41 %*	100 %
mAb clone CD564	9	Leica/Novocstra	1	2	2	4	33 %	-
mAb clone MRQ-42	4	Cell Marque	3	1	0	0	100 %	-
mAb clone 56C04	2	NeoMarkers/Thermo	1	2	1	0	100 %	-
mAb clone BC56C04	1	Biocare	0	0	0	1	0 %	-
Ready-To-Use Abs								
mAb clone 123C3	17	Dako	9	8	0	0	100 %	100 %
mAb clone 123C3	11	Ventana/Cell Marque	0	0	1	10	0 %*	-
mAb clone 123C3.05	5	Ventana	0	1	2	2	20 %*	-
mAb clone CD564	3	Leica/Novocstra	1	2	0	0	100 %	-
mAb clone CD564	1	NeoMarkers/Thermo	0	0	2	0	0 %	-
mAb clone 123C3.05	1	Novoson	0	0	1	0	0 %	-
mAb clone MR-RTU1949	1	Biocare	0	1	0	0	100 %	-
mAb clone BC56C04	1	Cell Marque	1	0	0	0	100 %	-
mAb clone MRQ-42	1	Cell Marque	1	0	0	0	100 %	-
Total	153		27	46	38	42	48 %	-

mAb clone 123C3: Non-VMS 81% passed VMS 13% passed (no optimal)

rmAb MRQ-42: VMS 100% passed

Inappropriate system calibration / home brews

Table 1. The IHC systems/Abs used and the assessment marks given:	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. ops ²
FDA approved HER-2 systems								
PATHWAY mAb clone 4B5, 790-2991, CONFIRM™	71	Ventana	67	4	0	0	100 %	100 %
rmAb clone 4B5, 800-2996								
HerceptTest™ K5204, K5206, K5207, SK001	50	Dako	30	3	0	9	82 %	85 %
CE IVD approved HER-2 systems								
Oradex™ mAb clone CB11, TA9145	6	Leica	5	0	0	1	83 %	100 %
Abs for in-house HER-2 systems, conc. Ab.								
pAb A0485	36	Dako	16	10	1	9	72 %	80 %
mAb clone CB11	5	Novocstra/Leica	3	4	0	1	88 %	88 %
mAb clone 10A7	1	BioGenex	0	1	0	0	-	-
rmAb clone SP3	19	NeoMarkers	3	1	0	0	100 %	100 %
	3	Zytomed	1	0	0	0	100 %	100 %
	1	DCS	1	0	0	0	100 %	100 %
	1	Spring	1	0	0	0	100 %	100 %
	1	Ventana	1	0	0	0	100 %	100 %



Inappropriate system calibration / home brews

Ampl.: >6 2,5 – 2,9 1,2 – 1,5 1,0 – 1,2

HER-2 IHC

Optimal

Poor

Poor

