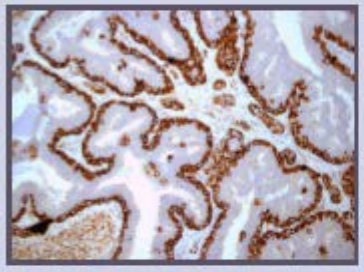

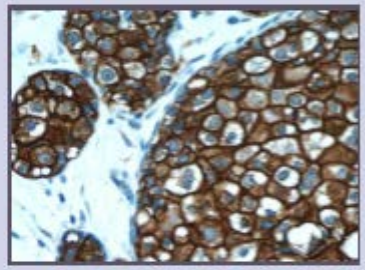

NordiQC TATA MEDICAL CENTER ISIMm International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

QA of IHC

Lung pathology

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

NordiQC

IHC - Protocols and controls for Lung tumours

Lung panel:

- TTF-1
- Napsin A
- p63 / p40
- CK5
- ALK
- PD-L1
- Calretinin
- Podoplanin
- Vimentin
- WT1
- CA125

- Is it primary lung ?
- Is it adeno or squamous ?
- Which teraphy ?
- Is it mesothelioma ?

IHC - Protocols and controls for Lung tumours

	Recommendable clones (conc.)*	Less successful clones (conc.)	RTU ”plug and play”** giving optimal result
TTF-1	mAb SPT24 (mAb 8G7G3/1) rmAb SP141 rmAb EP229	(mAb 8G7G3/1)	VMS: mAb SP141 Leica: mAb SPT24
Napsin A	mAb IP64 mAb MRQ-60	pAb 352-7 pAb 760-4446	
p63	mAb 4A4 mAb DAK-p63	mAb 7JUL	Dako: mAb DAK-p63 VMS: mAb 4A4
p40	mAb BC28 rmAb ZR8	pAbs……	VMS: mAb BC28

* Potential to provide optimal result by a laboratory developed test (LDT)

** Using the protocol settings as recommended by the vendor – incubation, retrieval, detection kit.

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	Positive tissue control HE*	Positive tissue control LE**	Negative tissue control NE***
TTF-1	Thyroid: Fol. colum. epith. cells Lung: Pneumocytes type II, basal epithelial cells	Lung: Columnar epithelial cells of terminal bronchi	Appendix: Columnar epithelial cells
Napsin A	Lung: Pneumocytes type II, basal epithelial cells	Kidney: Epithelial cells of proximale tubules	Appendix: Columnar epithelial cells
p63	Tonsil / esophagus: Squam. epith. cells	Tonsil: Scattered lymphocytes and endothelial cells	Appendix.: Col. epith. cells
p40	Tonsil / esophagus: Squam. epith. cells	Placenta: Cytothrophoblasts	Appendix: Col. epith. cells

* HE = High expression

** LE = Low expression

*** NE = No expression

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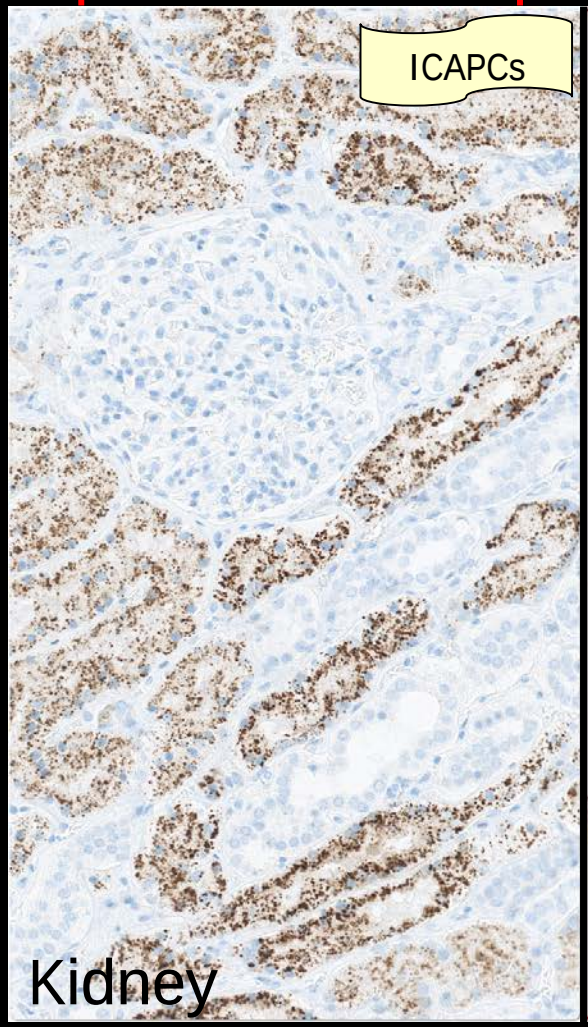
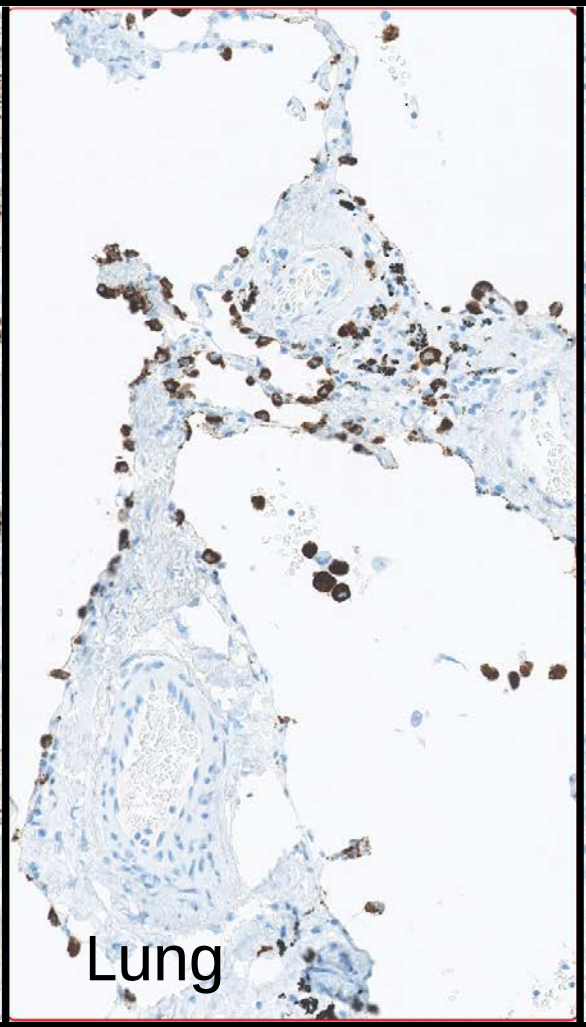
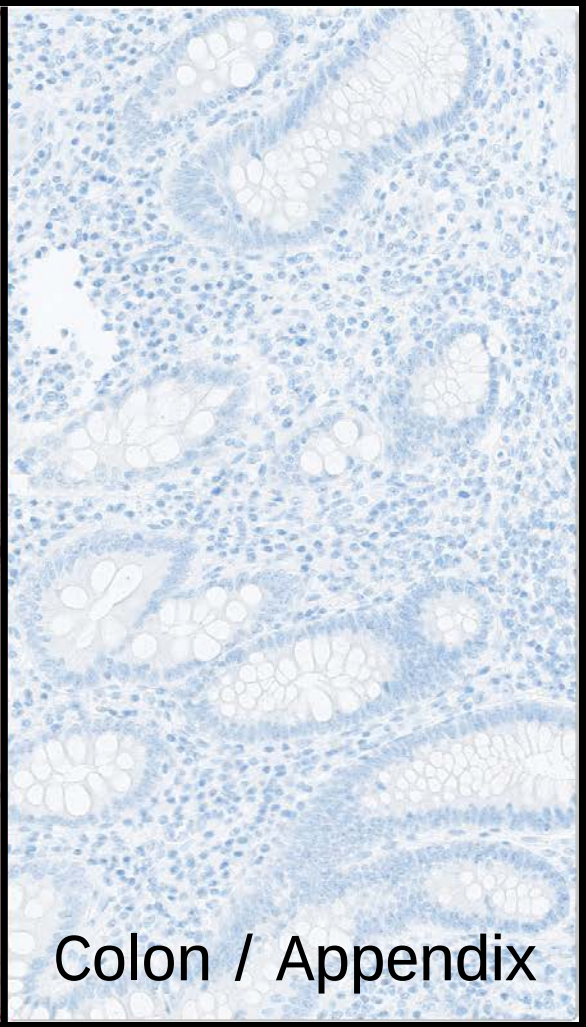
Napsin A reaction pattern



A moderate to strong distinct cytoplasmic granular staining reaction in virtually all epithelial cells in proximale tubules.



A strong distinct cytoplasmic staining reaction of all alveolar macrophages and pneumocytes type II.



No staining reaction

IHC - Protocols and controls for Lung tumours

Assessment Run 44 2015

Napsin A

Material
The slide to be stained for Napsin A comprised:

1. Colon, 2. Kidney, 3-4. Lung adenocarcinomas, 5. Lung, 6. Renal clear cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing Napsin A staining as optimal included:

- An at least moderate, granular cytoplasmic staining reaction of virtually all type II pneumocytes and alveolar macrophages in the lung
- An at least moderate, granular cytoplasmic staining reaction of the majority of the epithelial cells of the proximal tubules in the kidney
- A moderate to strong, granular cytoplasmic staining reaction of the majority of the neoplastic cells in the renal clear cell carcinoma and lung adenocarcinomas
- Negative staining reaction of normal columnar epithelial cells and macrophages in lamina propria in the colon

Participation

Number of laboratories registered for Napsin A, run 44	174
Number of laboratories returning slides	162 (93%)

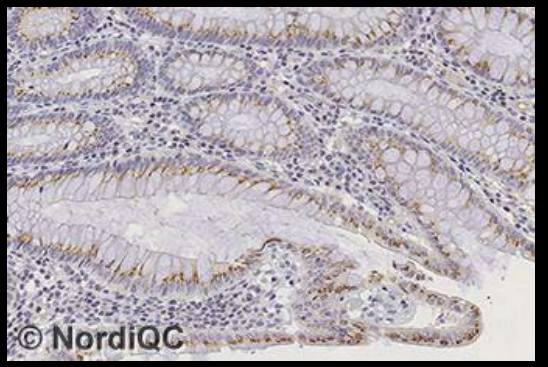
Results
162 laboratories participated in this assessment. 126 laboratories (78%) achieved a sufficient mark (optimal or good). Abs used and assessment marks are summarized in table 1 (see page 2)

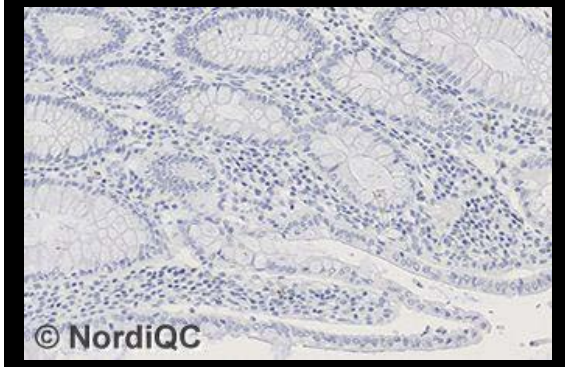
The most frequent causes of insufficient staining were:
- Less successful performance of polyclonal Napsin A antibodies
- Too low concentration of the primary Ab

Performance history
This was the second NordiQC assessment of Napsin A. A significant increase of the pass rate was seen compared to run 39 in 2013 (see table 2).

Table 2. **Proportion of sufficient results for Napsin A in the two NordiQC runs performed**

	Run 39 2013	Run 44 2015
Participants, n=	104	162
Sufficient results	58%	78%





False positive..
False negative..

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Table 1. **Antibodies and assessment marks for Napsin A, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **IP64**	86	Leica/Novocastra	39	39	6	2	91%	92%
mAb clone **MRQ-60**	8	Cell Marque	3	4	1	0	88%	100%
mAb, clone **TMU-Ad02**	4 3	Biocare IBL	1	2	4	0	43%	-
rmAb clone **KCG1.1**	2 2 1 1	Zytomed Diagnostic Biosystems Abcam Acris	1	5	0	0	100%	-
rmAb clone **BC15**	1	Zytomed	1	0	0	0	-	-
mAb, clone **BS10**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EPR6252**	1	Abcam	1	0	0	0	-	-
pAb **352A-7x**	8	Cell Marque	0	1	1	6	13%	
Ready-To-Use antibodies								
mAb clone **MRQ-60 760-4867**	18	Ventana/Cell Marque	1	16	1	0	84%	-
mAb clone **MRQ-60 352M-98**	3	Cell Marque	0	3	0	0	-	-
mAb clone **MRQ-60 MAD-000633QD**	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone **BC15 API 3043**	1	Biocare	0	0	1	0	-	-
mAb clone **IP64 AM701-5M**	1	BioGenex	0	0	1	0	-	-
mAb clone **IP64 ZM-0473**	1	ZSGB-BIO	0	1	0	0	-	-
rmAb clone **EP205 352R-18**	1	Cell Marque	1	0	0	0	-	-
mAb clone **MX015 MAB-0704**	1	Maixin	0	1	0	0	-	-
pAb **760-4446**	12	Ventana/Cell Marque	0	1	0	11	8%	
pAb **PPM428DS**	1	Biocare	0	0	0	1	-	-
pAb **MP-394-DS6**	1	Menapath	0	0	0	1	-	-
pAb **RAB-0639**	1	Maxim	0	1	0	0	-	-
Total	162		49	77	15	21	-	
Proportion			30%	48%	9%	13%	78%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

IP64 the secure Choice

pAbs – no-go

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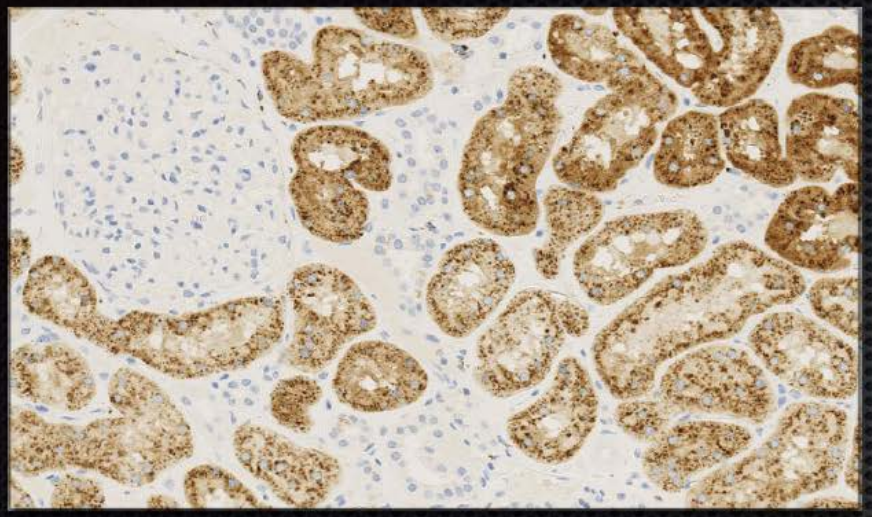
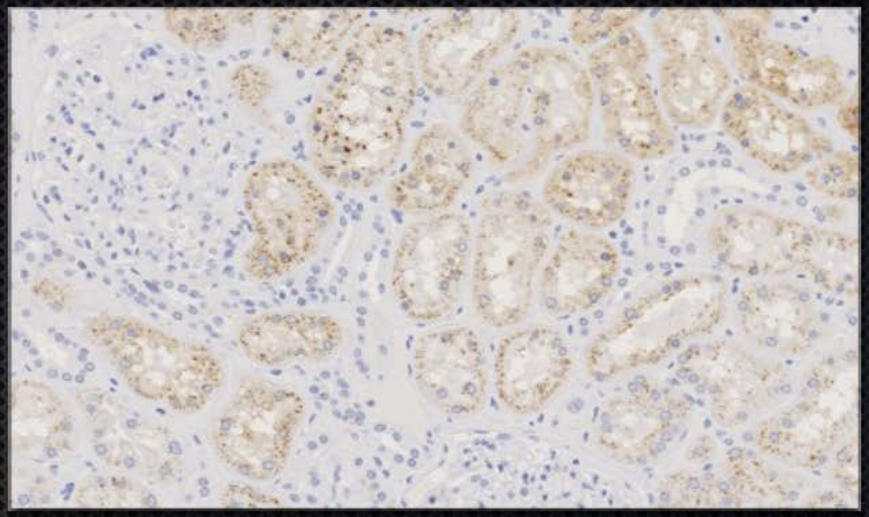


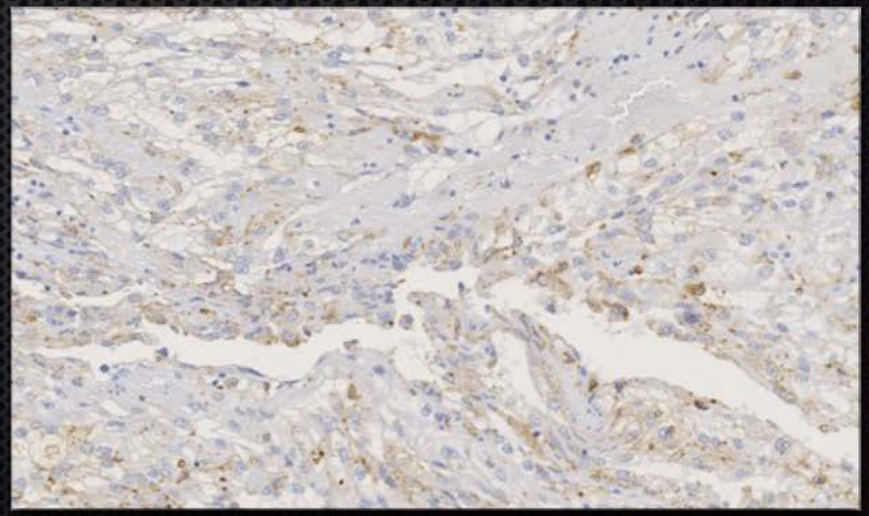
Kidney



Optimal-IP64-1/100-HIER/pH9-BOND

Insuff.-IP64-1/400-HIER/pH6-BOND

RCC



Optimal-IP64-1/100-HIER/pH9-BOND

Insuff.-IP64-1/400-HIER/pH6-BOND

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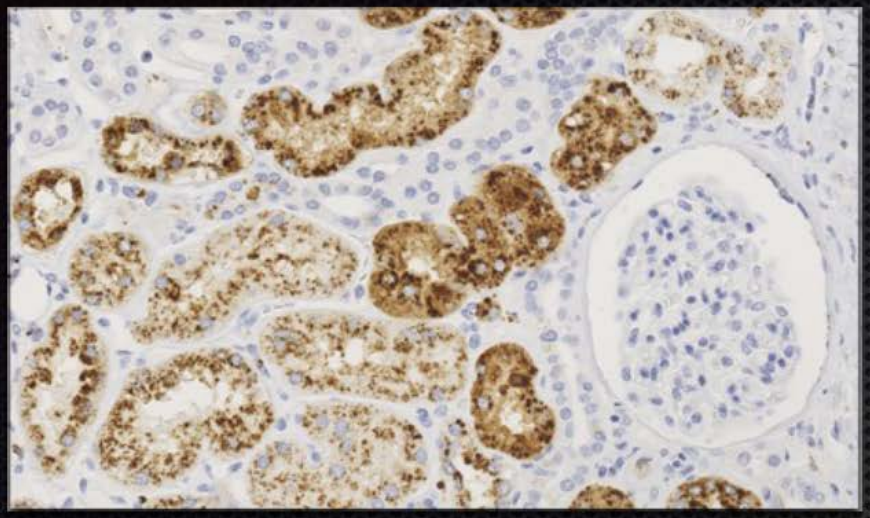
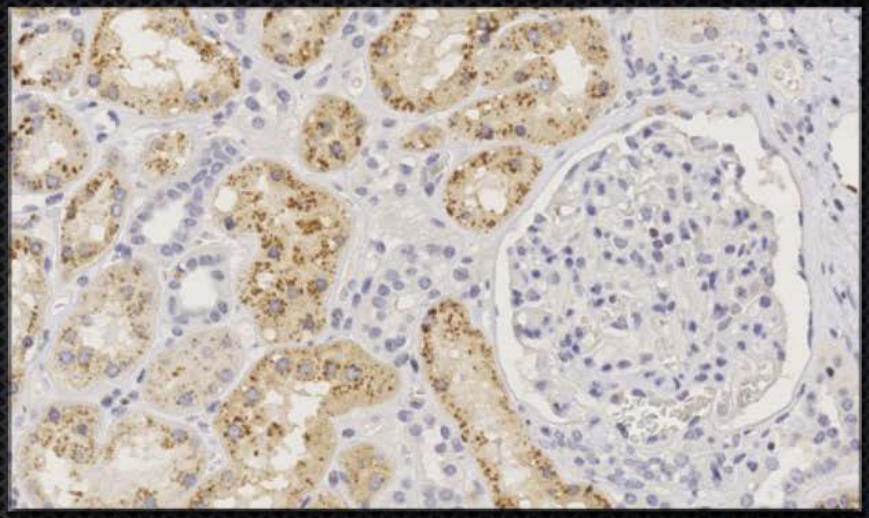
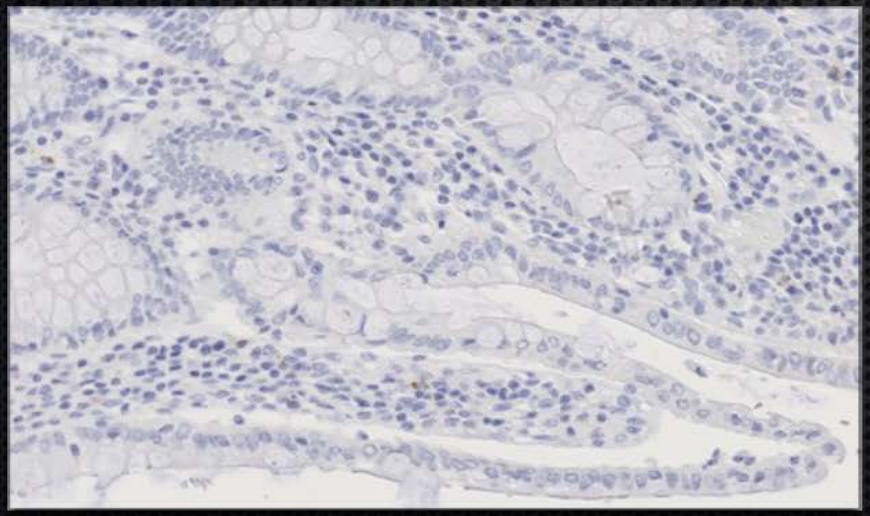


Kidney



Optimal-IP64-1/100-HIER/pH8.5-VBM

Insuff-pAb760-4446-HIER/pH8.5-VBM



Colon

Optimal-IP64-1/100-HIER/pH8.5-VBM

Insuff-pAb760-4446-HIER/pH8.5-VBM

IHC - Protocols and controls for Lung tumours

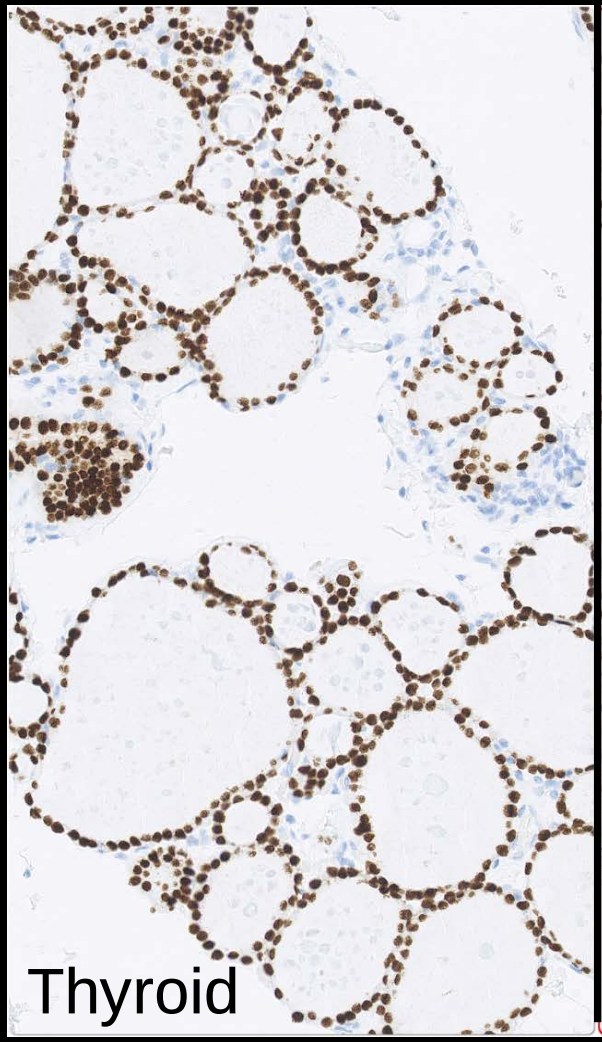
Napsin A

Basic protocol settings for an optimal staining result (NQC)

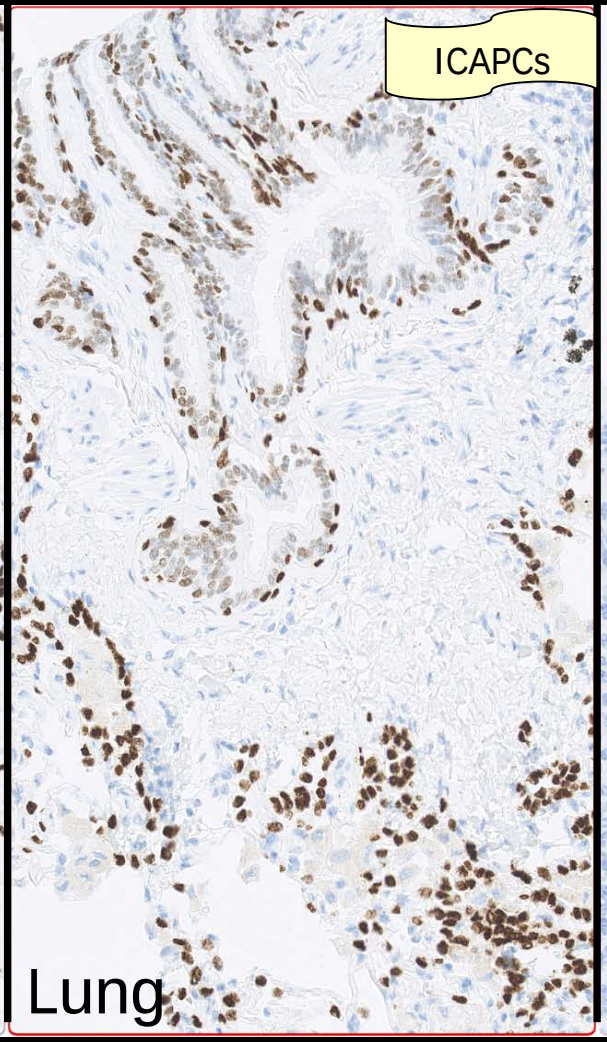
	Retrieval	Titre	Detection	RTU	Detection
mAb IP64	HIER	1:20-400	2- & 3-step	-	-
mAb MRQ-60	HIER high	1:200-500	3-step	*Ventana*	*3-step+AMP*

IHC - Protocols and controls for Lung tumours

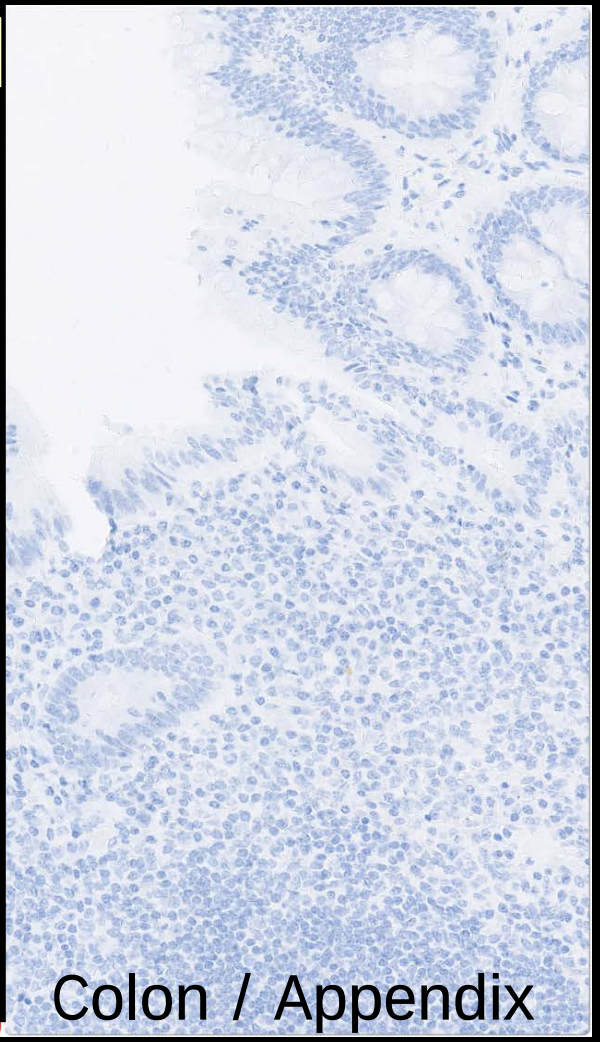
TTF-1 reaction pattern



A moderate to strong distinct nuclear staining reaction in virtually all follicular epithelial cells.



An at least weak but distinct nuclear staining reaction of the vast majority of epithelial cells of terminal bronchi.



No staining reaction.

IHC - Protocols and controls for Lung tumours

Assessment Run 46 2016

Thyroid transcription factor-1 (TTF1)

Material
The slide to be stained for TTF1 comprised:

1. Thyroid gland, 2. Liver, 3. Normal lung, 4-6. Lung adenocarcinoma 7. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a TTF1 staining as optimal included:

- A strong, distinct nuclear staining reaction of all type II pneumocytes, Clara cells and basal cells of the terminal bronchioles in the lung.
- A moderate to strong, distinct nuclear staining reaction of the vast majority of columnar epithelial cells of the terminal bronchioles in the lung.
- A strong, distinct nuclear staining reaction of all the follicular epithelial cells in the thyroid gland.
- A strong nuclear staining reaction of the majority of neoplastic cells in the two lung adenocarcinomas no. 5 & 6 and at least weak to moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma no. 4.
- No nuclear staining reaction of the colon adenocarcinoma.
- No nuclear staining reaction of the liver. Cytoplasmic staining in the hepatocytes was accepted when using mAb 8G7G3/1.

Participation

Number of laboratories registered for TTF1, run 46	287
Number of laboratories returning slides	272 (95%)

Results
272 laboratories participated in this assessment. 67% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Less successful primary Ab (the mAb clone 8G7G3/1)
- Too low concentration of the primary antibody
- Use of detection systems with low sensitivity

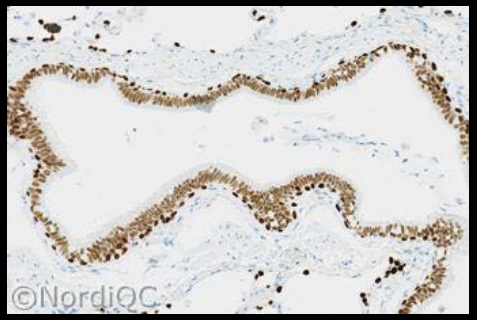
Performance history
This was the sixth NordiQC assessment of TTF1. A minor decrease of the pass rate was seen compared to run 39 in 2013 (see table 2).

Table 2. **Proportion of sufficient results for TTF1 in the last 5 NordiQC runs performed**

	Run 19 2007	Run 23 2008	Run 33 2011	Run 39 2013	Run 46 2015
Participants, n=	99	125	183	227	272
Sufficient results	24%	45%	60%	71%	67%

Clone !!!

(Titre)
(HIER settings)
(Detection kit)



IHC - Protocols and controls for Lung tumours

Table 1. **Antibodies and assessment marks for TTF1, run 46**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8G7G3/1**	15 3 6 1	Dako/Agilent Thermo/NeoMarkers Cell Marque Zeta Corp.	0	0	14	11	0%	-
mAb clone **SPT24**	120 9 5 2	Leica/Novocastra Monosan Immunologic BioCare	76	43	14	3	88%	89%
rmAb clone **EP229**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP141**	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **8G7G3/1 790-4398**	16	Ventana/Roche	0	1	7	8	7%	-
mAb clone **8G7G3/1 IR056**	31	Dako/Agilent	0	1	24	6	3%	-
rmAb clone **SP141 790-4756**	50	Ventana/Roche	30	17	3	0	94%	94%
mAb clone **SPT24 PA0364**	8	Leica/Novocastra	7	1	0	0	100%	100%
mAb clone **SPT24 MAD-000486QD**	2	Master Diagnostica SL	2	0	0	0	-	-
mAb clone **SPT24 API 3126**	1	BioCare	1	0	0	0	-	-
mAb clone **MX011 MAB-0677**	1	Maixin	1	0	0	0	-	-
Total	272		118	64	62	28	-	
Proportion			43%	24%	23%	10%	67%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Table 4. **The overall pass rate in the last 4 runs for the mmAb clones SPT24, 8G7G3/1 and the rmAb clone SP141**

	SPT24 All protocol settings		**SP141* All protocol settings**		**8G7G3/1 All protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal	Sufficient	Optimal
Participants	90% (429/479)	64% (308/479)	94% 59/63	65% 41/63	7% (17/259)	0% (0/259)

* Because rmAb clone SP141 is only recently introduced, data represents Run 39 and 46 only

Clone !!!

(Titre)
(HIER settings)
(Detection kit)

RTUs superior

IHC - Protocols and controls for Lung tumours

TTF1 / RUN 39 2013

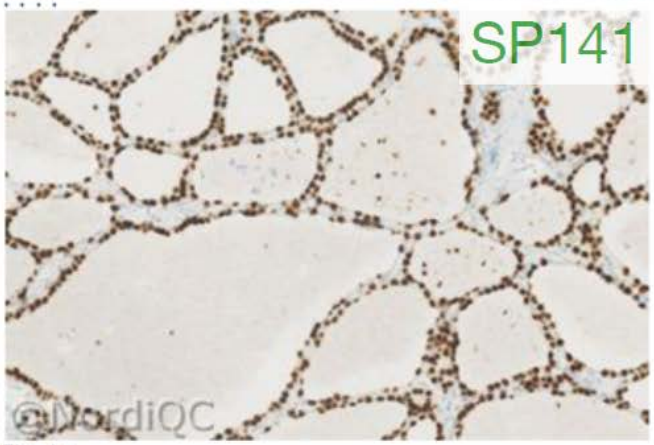


Fig. 1a
Optimal TTF1 staining of the thyroid gland using the rmAb clone SP141 (Ventana, RTU) optimally calibrated with HIER in an alkaline buffer (CC1) and performed on the BenchMark Ultra, Ventana. A strong nuclear staining reaction is seen in virtually all follicular epithelial cells. No background staining is seen.

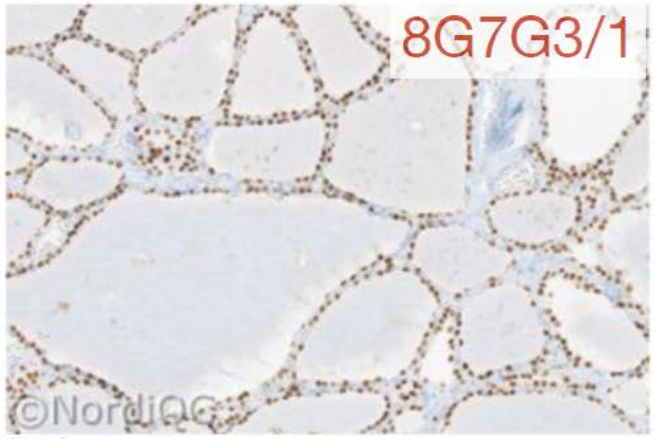


Fig. 1b
Insufficient TTF1 staining of the thyroid gland using the mAb clone 8G7G3/1 (Ventana, RTU) with HIER in an alkaline buffer (CC1) and performed on the BenchMark Ultra, Ventana. A moderate nuclear staining reaction is seen in the majority of follicular epithelial cells – same field as in Fig. 1a. Also compare with Figs. 2b, 3b and 4b – same protocol.

Primary antibody with a too low sensitivity.

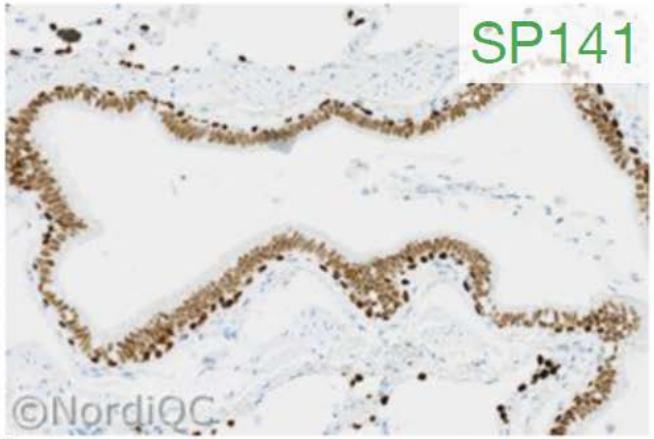


Fig. 2a
Optimal TTF1 staining of the lung using same protocol as in Fig. 1a. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a moderate nuclear staining reaction. No background staining is seen.

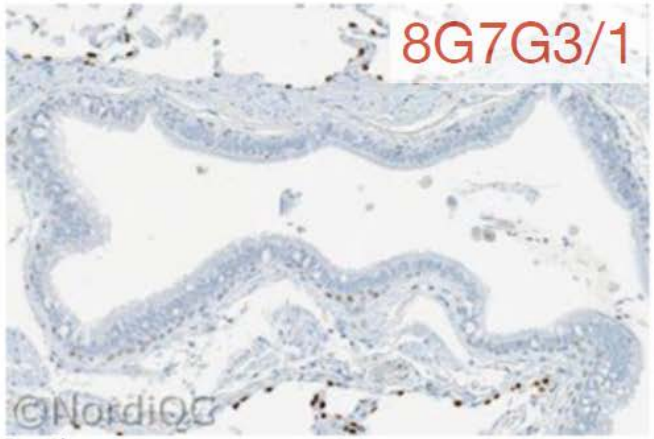


Fig. 2b.
Insufficient TTF1 staining of the lung using same protocol as in Fig. 1b. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show only a weak to moderate positive nuclear staining reaction and no reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

IHC - Protocols and controls for Lung tumours

TTF1 / RUN 39 2013

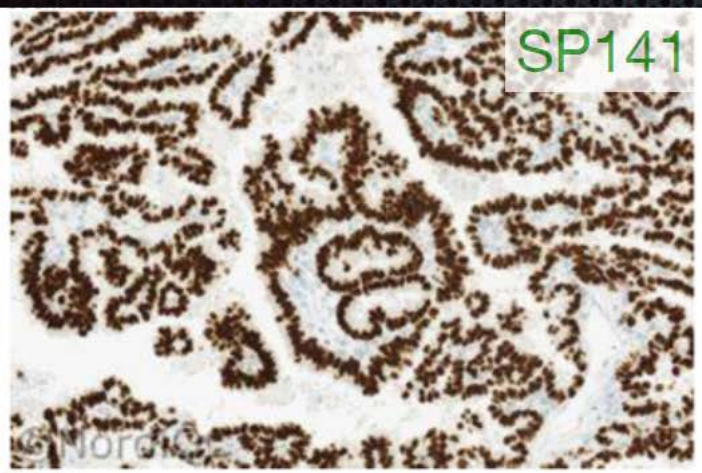


Fig. 3a
Optimal TTF1 staining of the lung adenocarcinoma no. 7 (high level expression of TTF1) using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background staining is seen.

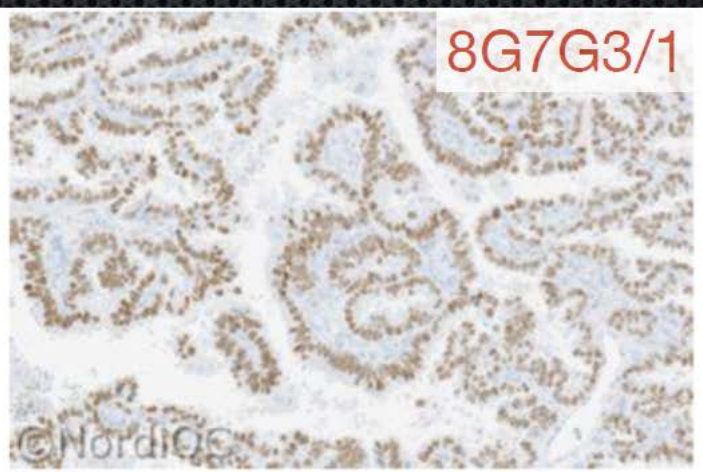


Fig. 3b
Insufficient TTF1 staining of the lung adenocarcinoma no. 7 using same protocol as in Figs. 1b & 2b. Despite a high level of TTF1 expression of the neoplastic cells only a moderate nuclear staining reaction is seen – same field as in Fig. 3a.

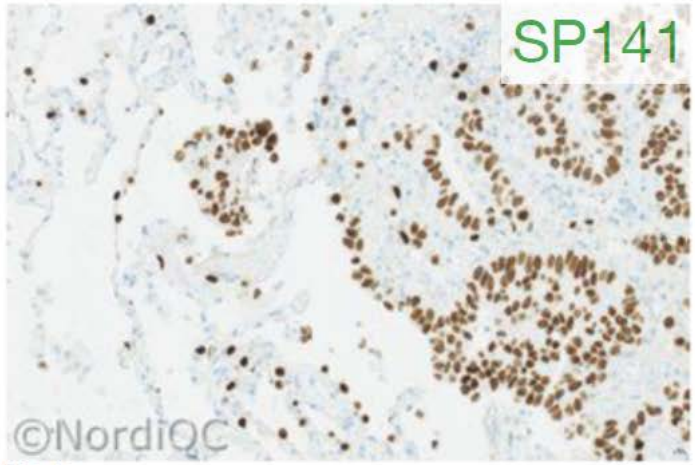


Fig. 4a
Optimal TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1a, 2a & 3a. Tumour (right side) with adjacent normal lung tissue. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. Strong reaction is also seen in all type II pneumocytes. No background staining is seen.

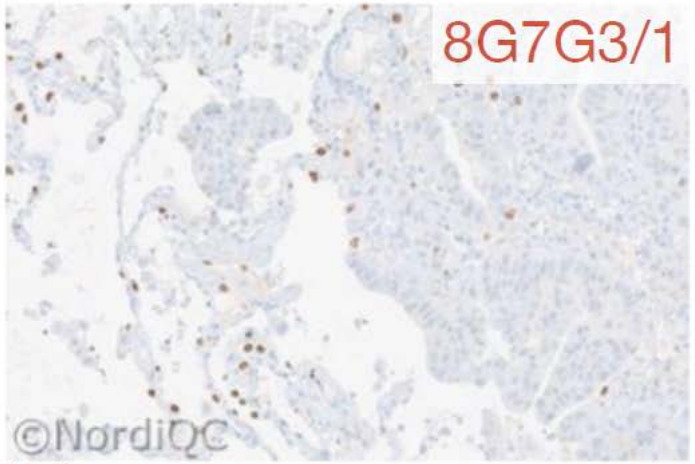


Fig. 4b
Insufficient TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1b, 2b & 3b. Despite a moderate positive staining reaction in the majority of type II pneumocytes - both in the normal tissue and within the tumour tissue - virtually all neoplastic cells are negative – same field as in Fig. 4a.

IHC - Protocols and controls for Lung tumours

TTF-1

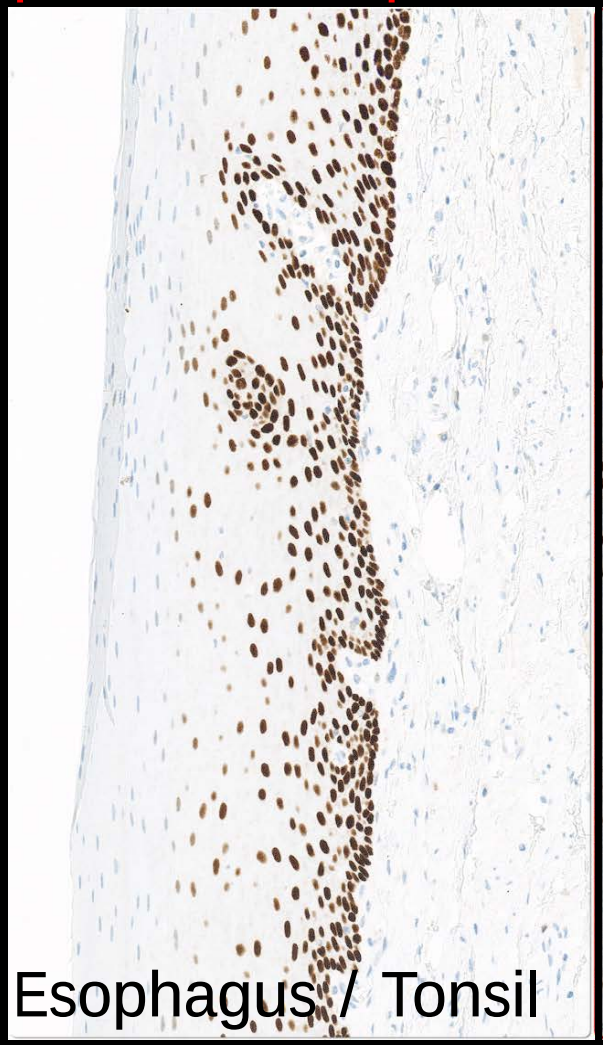
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb SPT24	HIER	1:30-500	2- & 3-step	Leica	3-step
rmAb SP141	HIER high	-	-	Ventana	2- & 3-step
rmAb EP229*	HIER high	1:50-200	2- & 3-step	-	-

* In-house data

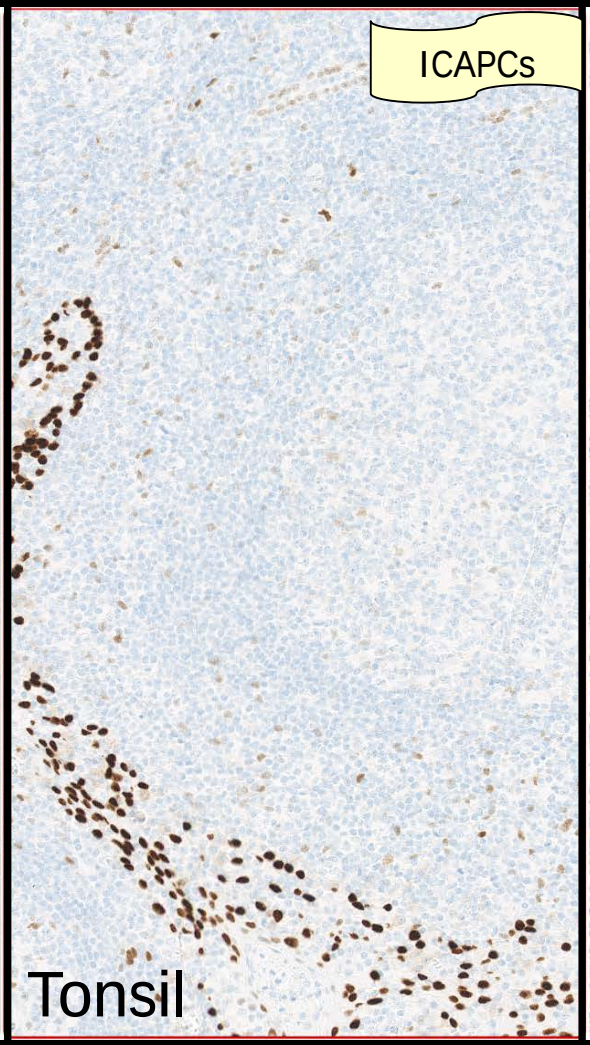
IHC – Protocols and controls – Part V

p63 reaction pattern



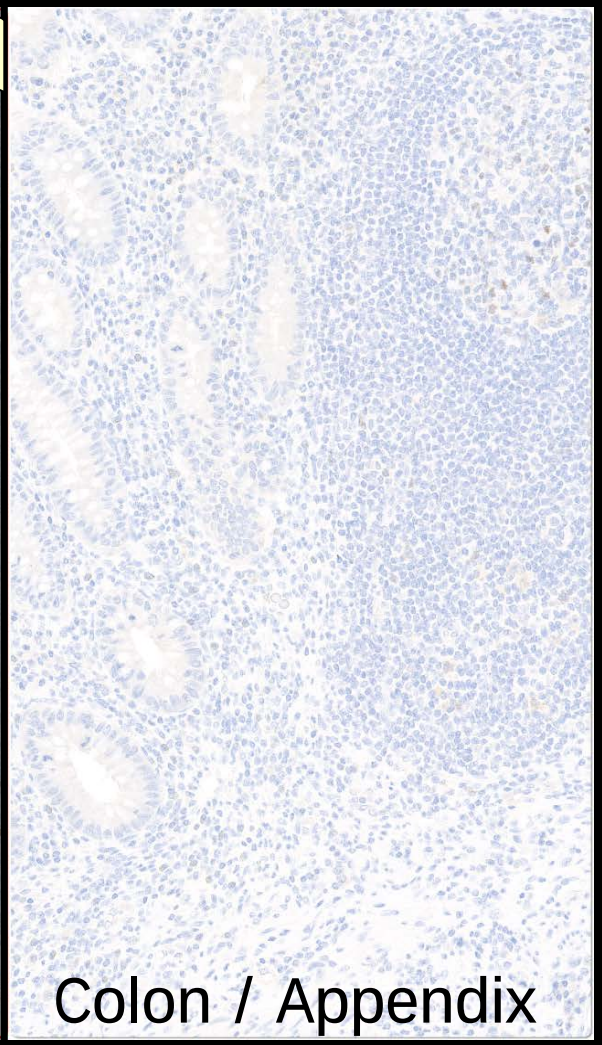
Esophagus / Tonsil

A moderate to strong distinct nuclear staining reaction in the vast majority of squamous epithelial cells.



Tonsil

An at least weak but distinct nuclear staining reaction of scattered lymphocytes and endothelial cells.



Colon / Appendix

No staining reaction in columnar epithelial cells – scattered lymphocytes can be expected to be demonstrated.

IHC - Protocols and controls for Lung tumours

Assessment Run 48 2016

p63

Material
The slide to be stained for p63 comprised:

1. Placenta, 2. Tonsil, 3. Lung adenocarcinoma, 4-5. Lung squamous cell carcinoma, 6. Prostate hyperplasia

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a p63 staining as optimal included:

- An at least weak to moderate, distinct nuclear staining reaction of dispersed cytotrophoblastic cells in the placenta.
- A moderate to strong, distinct nuclear staining reaction in almost all squamous epithelial cells in the tonsil and an at least weak nuclear reaction in scattered lymphocytes in the tonsil.
- A moderate to strong, distinct nuclear staining reaction in basal cells in the hyperplastic prostate glands.
- A moderate to strong, distinct nuclear staining reaction in virtually all tumour cells of the lung squamous cell carcinomas.
- No staining reaction in the vast majority of tumour cells of the lung adenocarcinoma.
- No staining reaction in secretory cells of the hyperplastic prostate glands.
- No or only a week cytoplasmic reaction in cells with strong p63 expression.

Participation

Number of laboratories registered for p63, run 48	299
Number of laboratories returning slides	274 (92%)

Results
274 laboratories participated in this assessment. 224 (82%) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (see page 2)

The most frequent causes of insufficient staining reactions were:
- Less successful primary antibodies
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval (HIER)
- Detection systems with low sensitivity

Performance history
This was the fourth NordiQC assessment of p63. Compared to the previous run in 2014 (Run 41), an increase in pass rate was seen (table 2).

Table 2. **Proportion of sufficient results for p63 in four NordiQC runs**

	Run 16 2006	Run B8 2009	Run 41 2014	Run 48 2016
Participants, n=	68	113	236	274
Sufficient results	83%	95%	70%	82%

Clone

4A4
DAK-p63

HIER settings
High pH

Detection kit
3-step

IHC - Protocols and controls for Lung tumours

Table 1. **Antibodies and assessment marks for p63, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4A4**	26 4 3 3 2 2 1 1 1 1 1 1	BioCare Medical ImmunoLogic Dako Zeta Corporation Thermo Scientific Zytomed Systems BioGenex Diagnostic BioSystems Klinipath Minarini Nordic Biosite Santa Cruz	13	20	11	2	72%	76%
mAb clone **DAK-p63**	47	Dako	20	21	6	0	87%	91%
mAb clone **7JUL**	12	Leica/Novocastra	0	1	3	8	8%	-
mAb clone **SFI-6**	2	DCS Immunoline	0	0	2	0	-	-
rmAb clone **BSR6**	1	Nordic Biosite	0	0	1	0	-	-
rmAb clone **DBR16.1**	1	Diagnostic Biosystems	1	0	0	0		
rmAb clone **EPR5701**	1	Epitomics	0	0	1	0	-	-
Unknown Ab	1	Unknown	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **4A4 790-4509**	102	Ventana	59	36	5	2	93%	95%
mAb clone **DAK-p63 IR662**	46	Dako	21	23	2	0	96%	94%
mAb clone **4A4 PM163**	3	BioCare	1	1	1	0	-	-
mAb clone **7JUL PA0103**	5	Leica/Novocastra	0	0	3	2	-	-
mAb clone **4A4 AM418**	2	BioGenex	0	1	0	1	-	-
mAb clone **4A4 ARB-56695**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **MX013 MAB-0694**	1	Maixin	0	1	0	0	-	-
mAb clone **4A4 MAD-000479QD**	3	Master Diagnostica SL	3	0	0	0	-	-
Total	274		120	104	35	15	-	
Proportion			44 %	38 %	13 %	5 %	82 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

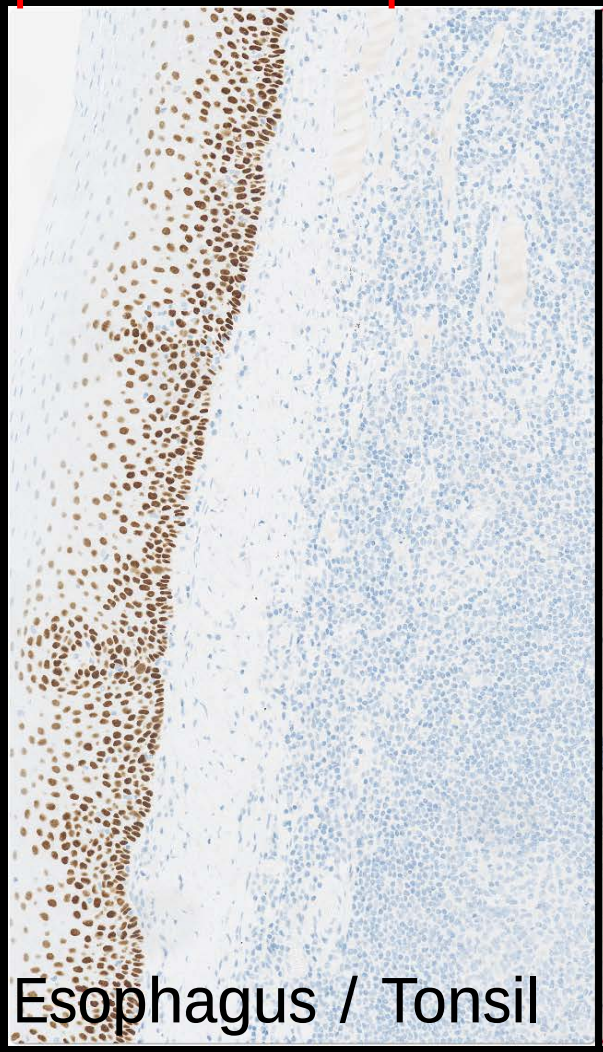
Clone

HIER settings
Detection kit

RTU superior

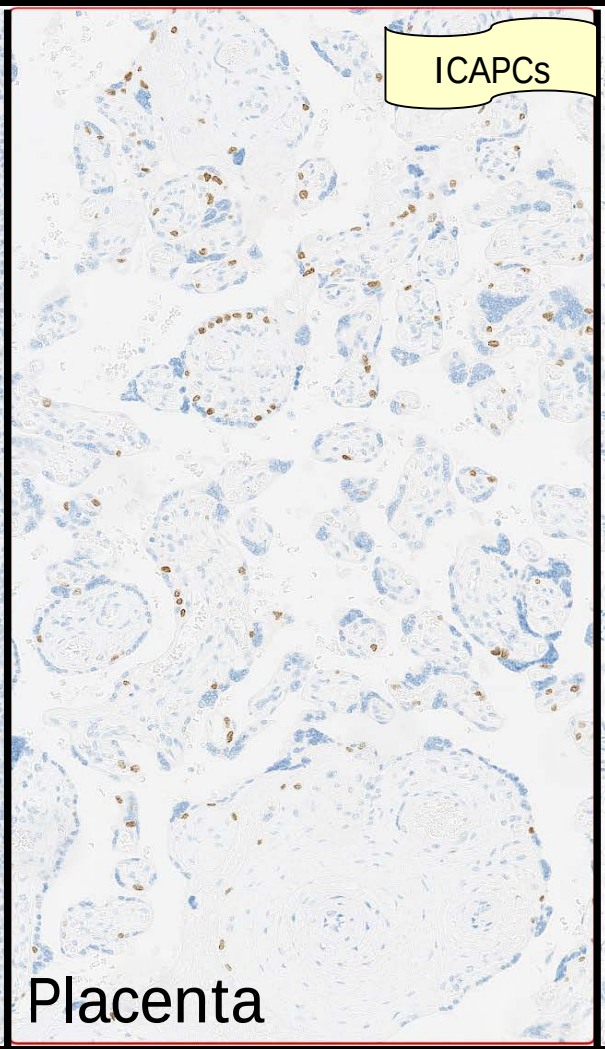
IHC - Protocols and controls for Lung tumours

p40 reaction pattern



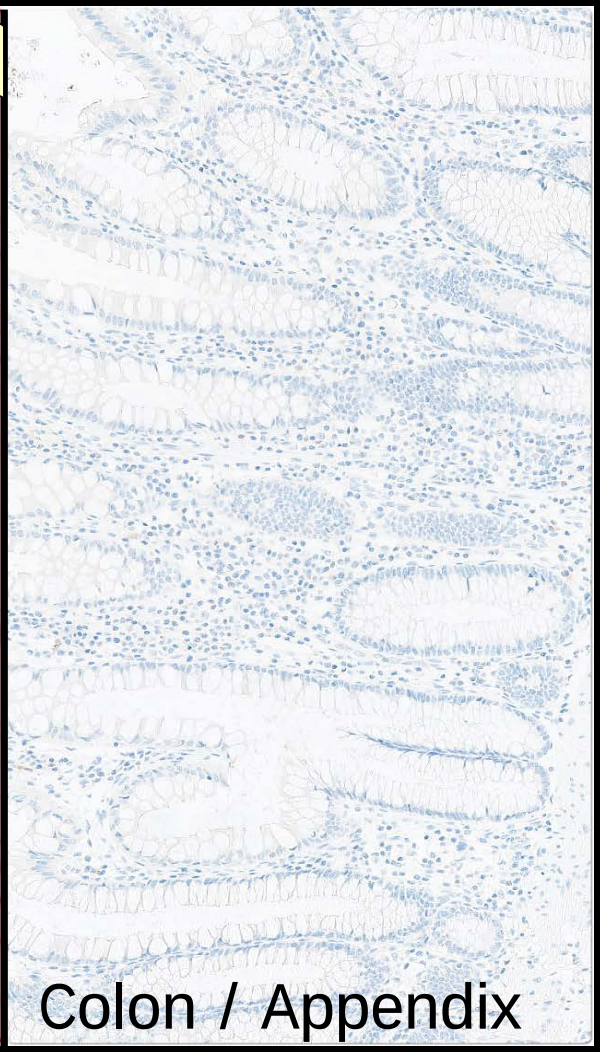
Esophagus / Tonsil

A moderate to strong distinct nuclear staining reaction in the vast majority of squamous epithelial cells.



Placenta

An at least weak to moderate and distinct nuclear staining reaction of dispersed cytothrophoblasts.



Colon / Appendix

No staining reaction in columnar epithelial cells and lymphocytes.

IHC - Protocols and controls for Lung tumours

Assessment Run 48 2016

p40 (ΔNp63)

Material
The slide to be stained for p40 comprised:

1. Placenta, 2. Tonsil, 3. Lung adenocarcinoma, 4-5. Lung squamous cell carcinoma, 6. Prostate hyperplasia

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing p40 staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of virtually all squamous epithelial cells in the tonsil and basal cells lining the hyperplastic glands in the prostate
- An at least weak to moderate, distinct nuclear staining reaction of dispersed cytotrophoblastic cells in the placenta
- A moderate to strong, distinct nuclear staining reaction of the vast majority of neoplastic cells in the lung squamous cell carcinoma
- No staining reaction of neoplastic cells in the lung adenocarcinoma
- No staining reaction of other cells including lymphocytes in the tonsil

Participation

Number of laboratories registered for p40, run 48	209
Number of laboratories returning slides	188 (90%)

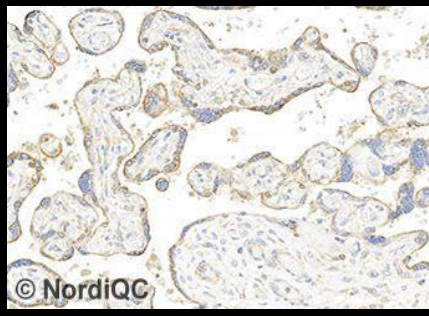
Results
188 laboratories participated in this assessment, 74% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

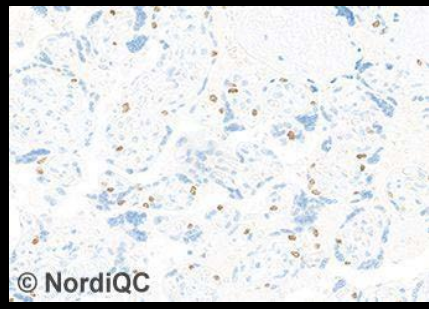
The most frequent causes of insufficient staining were:
- Use of less successful primary antibodies
- Too low concentration of the primary antibody
- Use of less sensitive detection systems

Performance history
This was the second NordiQC assessment of p40. A significantly increased pass rate was observed (table 2).

Table 2. **Proportion of sufficient results for p40 in two NordiQC runs**

	Run 44 2015	Run 48 2016
Participants, n=	129	188
Sufficient results	56%	74%





Clone / Ab
Titre
HIER settings
Detection kit

Sensitivity / Specificity

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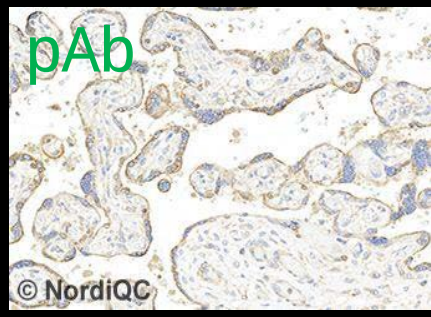
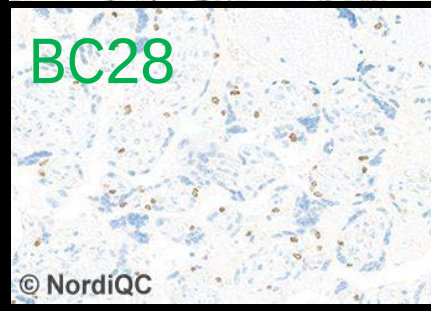
Table 1. **Antibodies and assessment marks for p40, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC28**	77 6 2 2 1	Biocare Zytomed Menarini abcam Nordic Biosite	52	24	10	2	86%	89%
rmAb clone **ZR8**	12 1 1	Immunologic Zeta Corporation BioSB	1	6	2	5	50%	67%
pAb **AC13030**	8	Biocare	0	2	6	0	-	-
pAb **RP163**	5	Diagnostic Biosystems	0	1	1	3	-	-
pAb **PC373**	4	Calbiochem, Merck	0	1	0	3	-	-
pAb **RBK054**	3	Zytomed	0	0	1	2	-	-
pAb **PI049**	1	DCS	0	1	0	0	-	-
pAb **PP123**	1	Pathnsitu	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **BC28 API/IPI/AVI 3066**	13	Biocare	5	8	0	0	100%	100%
mAb clone **BC28 790-4950**	39	Ventana	19	15	5	0	87%	94%
mAb clone **BC28 MSG097**	1	Zytomed	1	0	0	0	-	-
mAb clone **ZR8 MAD-000686QD**	3	Master Diagnostica	0	2	1	0	-	-
pAb **API 3030**	6	Biocare	0	0	4	2	-	-
pAb **RAB-066**	1	Maixin	0	1	0	0	-	-
pAb **A00112**	1	Loxo GmbH	0	0	1	0	-	-
Total	188		78	61	32	17	-	
Proportion			42%	32%	17%	9%	74%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.





Clone / Ab
Titre
HIER settings
Detection kit

RTU superior

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p40 / RUN 44 2015

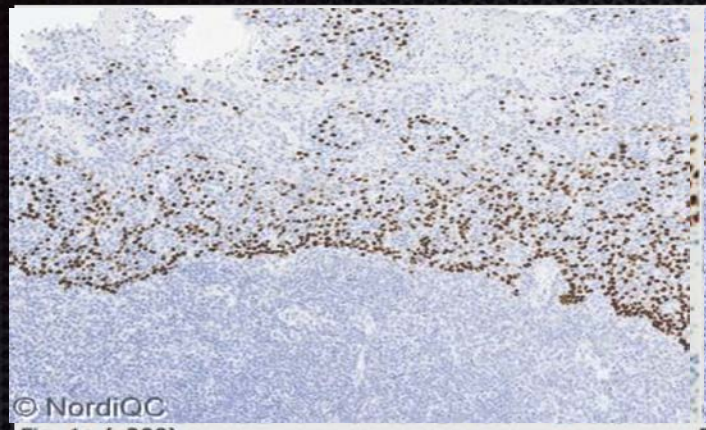

Fig. 1a (x200)
Optimal p40 staining of the tonsil using the mAb clone BC28 as a concentrate, optimally calibrated, HIER in an alkaline buffer (TRS pH 9.0, Dako), and a 3-step polymer based detection system (FLEX+, Dako). A moderate to strong nuclear staining reaction is seen in the majority of the squamous epithelial cells. No background staining is seen. Same protocol used in Figs. 1a - 4a.

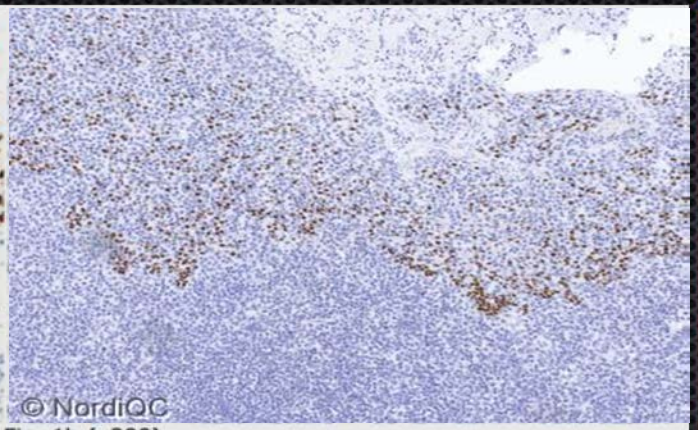

Fig. 1b (x200)
Insufficient p40 staining of the tonsil using the mAb clone BC28. The protocol provided an overall too low sensitivity most likely due to a combination of a too low concentration of the primary Ab and use of a 2-step polymer based detection system with a moderate sensitivity (FLEX, Dako)- compare with Fig. 1a (same field). The intensity and proportion of cells demonstrated is reduced. Also compare with Figs. 2b - 4b, same protocol.

Combination of a too low concentration of the primary Ab and use of a less sensitive 2-step polymer based detection system!

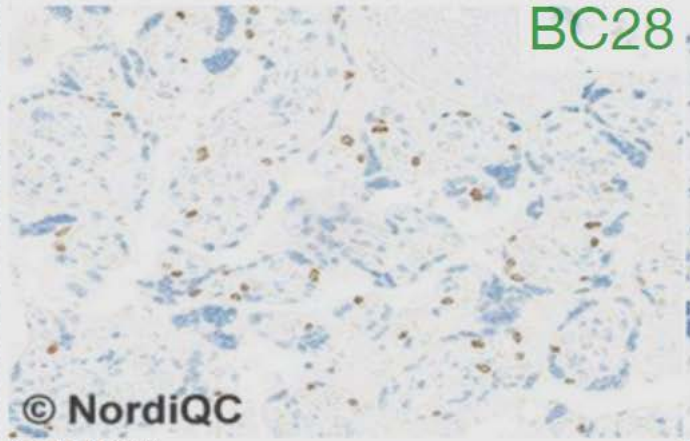

Fig. 2a (x200)
Optimal p40 staining of the placenta using same protocol as in Fig. 1a. Scattered cytothrophoblastic cells show a weak to moderate, distinct nuclear staining reaction.

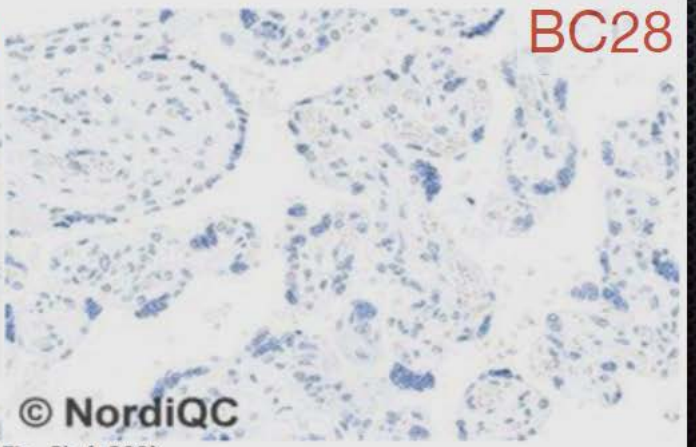

Fig. 2b (x200)
Insufficient p40 staining of the placenta using same protocol as in Fig. 1b. Virtually no staining reaction of cytothrophoblastsic cells is seen. Also compare with Figs. 3b and 4b, same protocol.

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p40 / RUN 44 2015

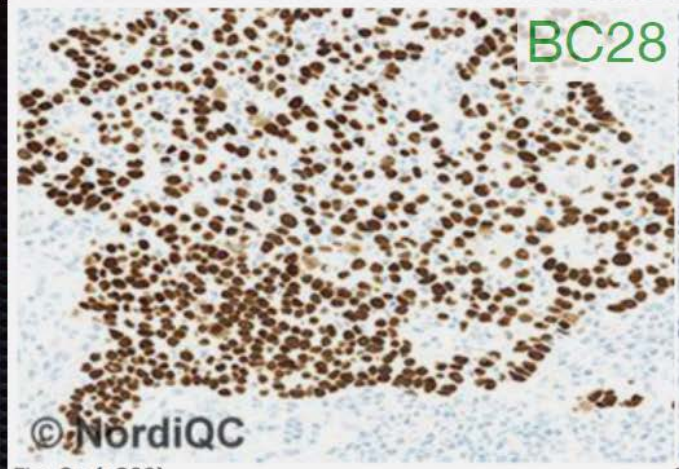

Fig. 3a (x200)
Optimal p40 staining of the lung squamous cell carcinoma using same protocol as in Figs. 1a & 2a.Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen.

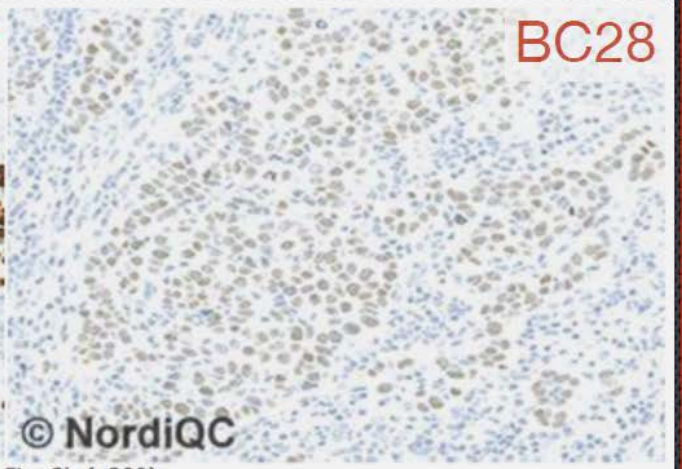

Fig. 3b (x200)
Insufficient p40 staining of the lung squamous cell carcinoma using same protocol as in Figs. 1b & 2b. The intensity and proportion of cells demonstrated is significantly reduced.

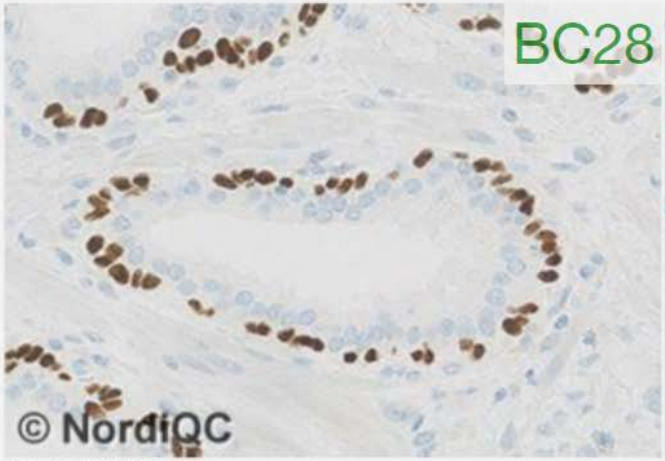

Fig. 4a (x400)
Optimal p40 staining of the prostate hyperplasia using same protocol as in Figs. 1a - 3a. The basal cells are distinctively demonstrated as a moderate to strong nuclear staining reaction is observed.

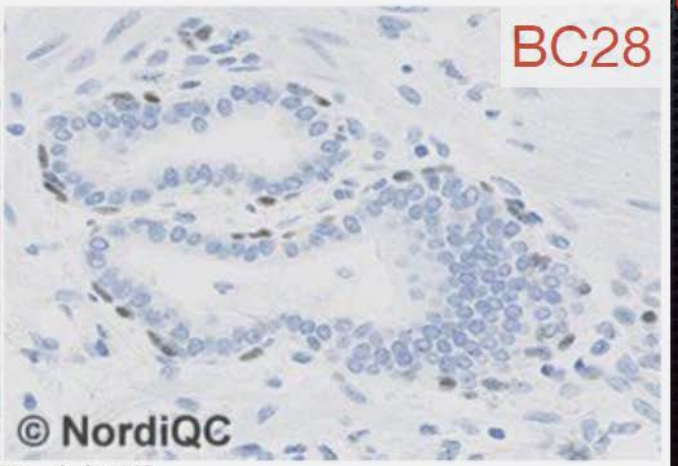

Fig. 4b (x400)
Insufficient p40 staining of the prostate hyperplasia using same protocol as in Figs. 1b – 3b. Only a weak and equivocal nuclear staining reaction in the basal cells is observed.

Combination of a too low concentration of the primary Ab and use of a less sensitive 2-step polymer based detection system!

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p40

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb BC28	HIER	1:25-100	3-step	Ventana	3-step

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	Recommendable clones (conc.)*	Less successful clones (conc.)	RTU ”plug and play”** giving optimal result
ALK Lung	mAb 5A4 mAb OTI1A4 rmAb D5F3	mAb ALK1 rmAb SP8	VMS: rmAb D5F3
PD-L1 Lung	mAb 22C3 mAb E1L3N rmAb 28-8 rmAb ZR3		Dako: mAb 22C3 Dako: 28-8 VMS: SP263
Calretinin	mAb 5A5 mAb CAL6 mAb DAK-Calret 1 rmAb SP65 pAb 18-0211	pAb 232	Dako: mAb DAK-Calret 1 Leica: mAb CAL6 VMS: rmAb SP65
Podop.	mAb D2-40		Dako: mAb D2-40

* Potential to provide optimal result by a laboratory developed test (LDT)

** Using the protocol settings as recommended by the vendor – incubation, retrieval, detection kit.

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	Positive tissue control HE*	Positive tissue control LE**	Negative tissue control NE***
ALK Lung	ALCL: Neoplastic cells *Lung adenocarc.: Neoplastic cells*	Appendix: Ganglion cells + axons	Appendix: Columnar epithelial cells
PD-L1 Lung	*Lung carcinoma TPS≥50%*	*Lung carcinoma TPS≥1% <50% Tonsil: Macrophages*	*Lung carcinoma TPS<1%*
Calretinin	Appendix: Macrophages and periheral nerves	Adrenal gland: Cortical epithelial cells	Appendix: Columnar epithelial cells Tonsil: Lymphocytes
Podop.	Tonsil: Lymphatic endothelial cells	*Tonsil: Follicular dendritic network*	Appendix.: Col. epith. cells

* HE = High expression

** LE = Low expression

*** NE = No expression

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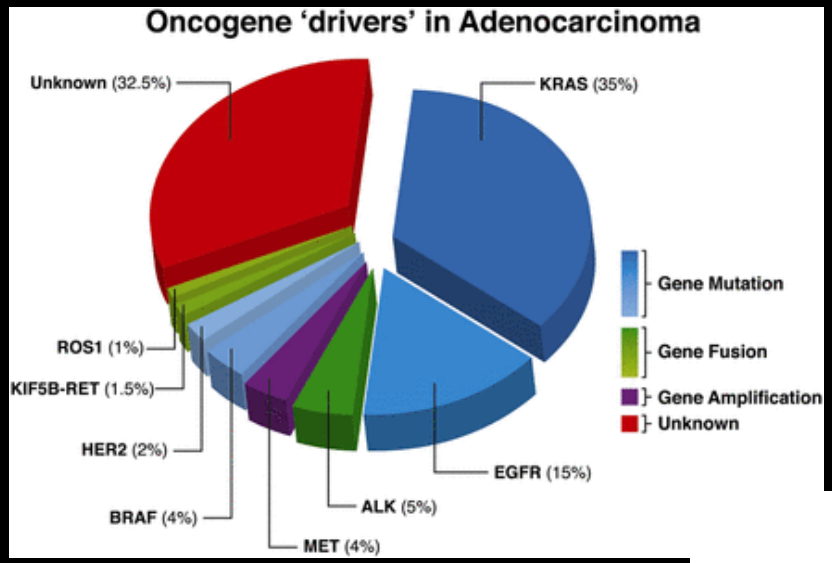

IHC; Detects ALK independent of fusion partner

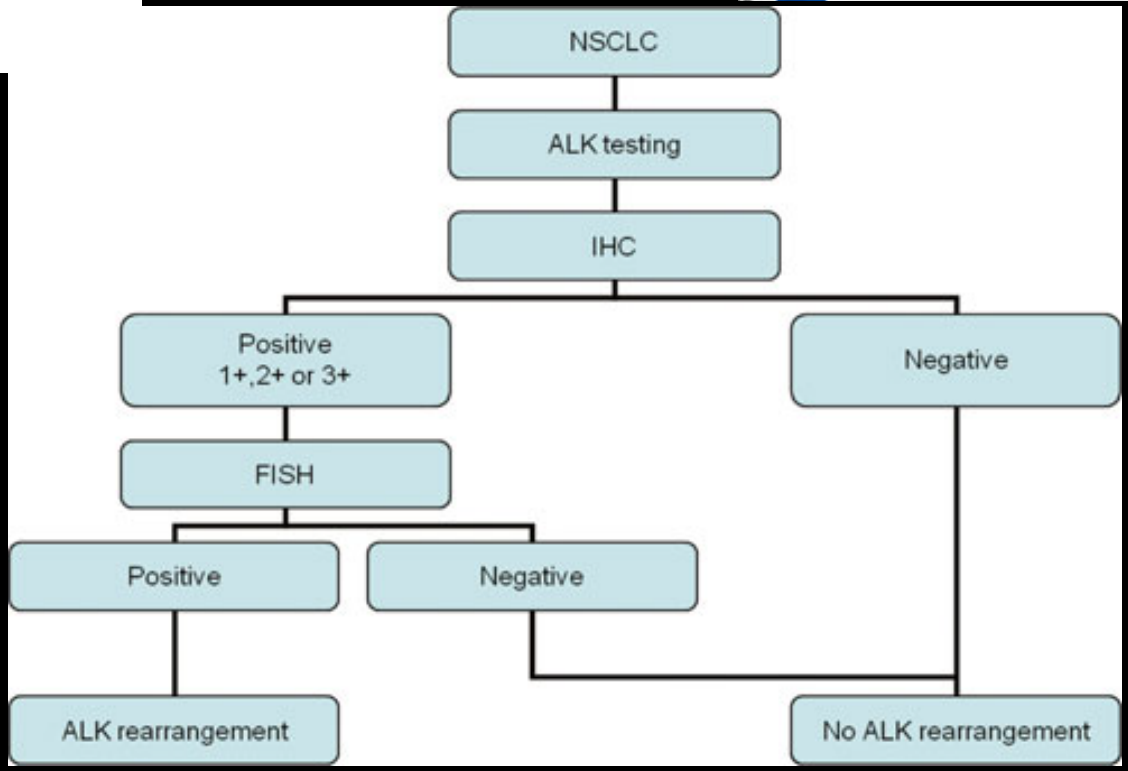

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Table 2 IHC scoring systems

	Score			
	0	1+	2	3
Yi et al. [46]	No staining	Faint cytoplasmic staining	Moderate smooth cytoplasmic staining	Intense granular cytoplasmic staining in ≥10 % of tumour cells
Kim et al. [27]	No staining	Faint or weak staining intensity with >5 % tumour cells or any staining intensity with ≤5 % tumour cells [a]	Moderate staining >intensity with >5 % tumour cells [b]	Strong and granular staining intensity with >5 % tumour cells [c]

[a] Average of 14.7 % positively stained cells

[b] Average of 58.2 % positively stained cells

[c] Average of 97.3 % positively stained cells

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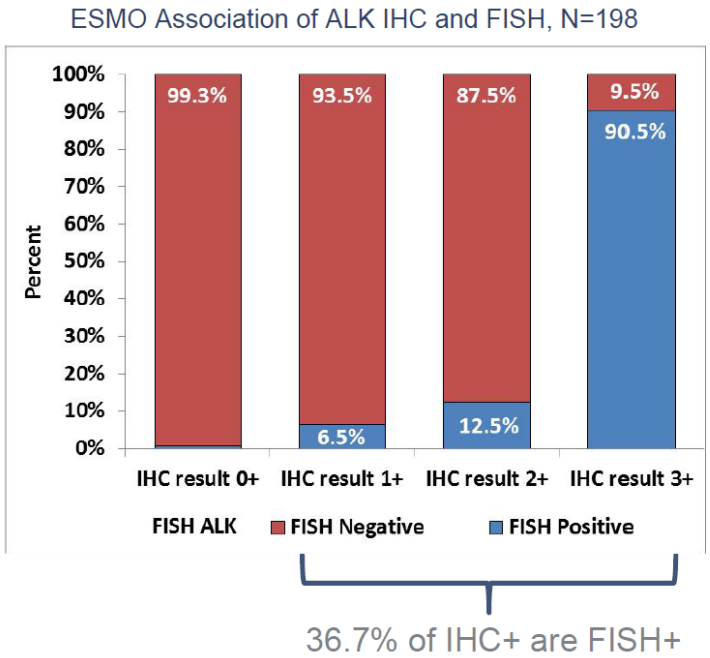
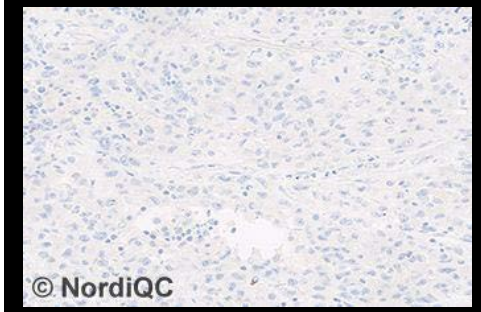
DOI 10.1007/s00428-012-1281-4

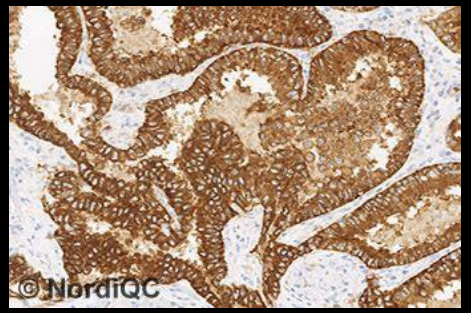
REVIEW AND PERSPECTIVES

EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations

Erik Thunnissen · Lukas Bubendorf · Manfred Dietel · Göran Elmberger · Keith Kerr · Fernando Lopez-Rios · Holger Moch · Wlodzimierz Olszewski · Patrick Pauwels · Frédérique Penault-Llorca · Giulio Rossi







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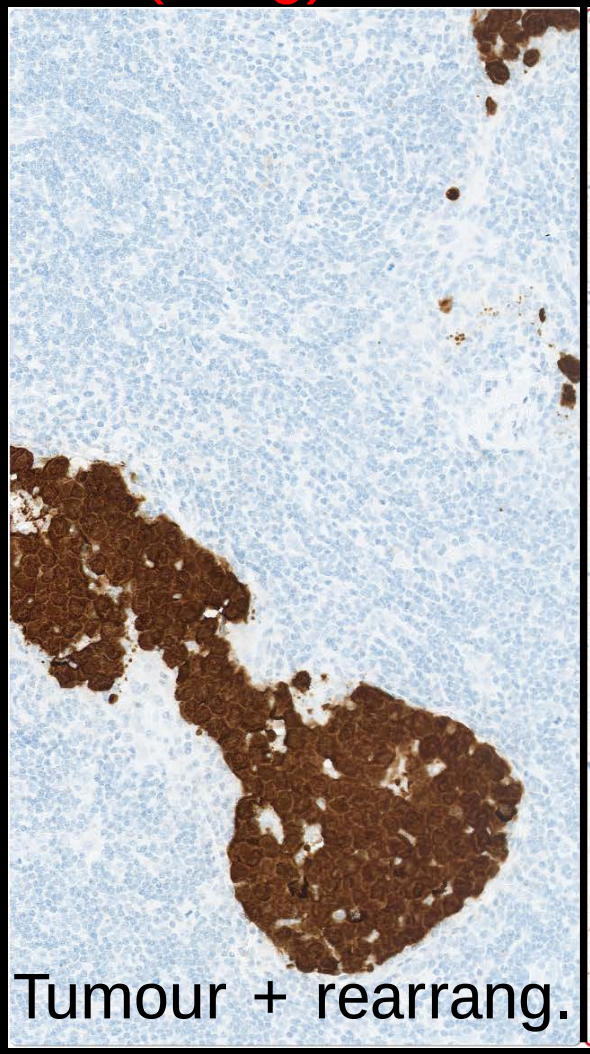
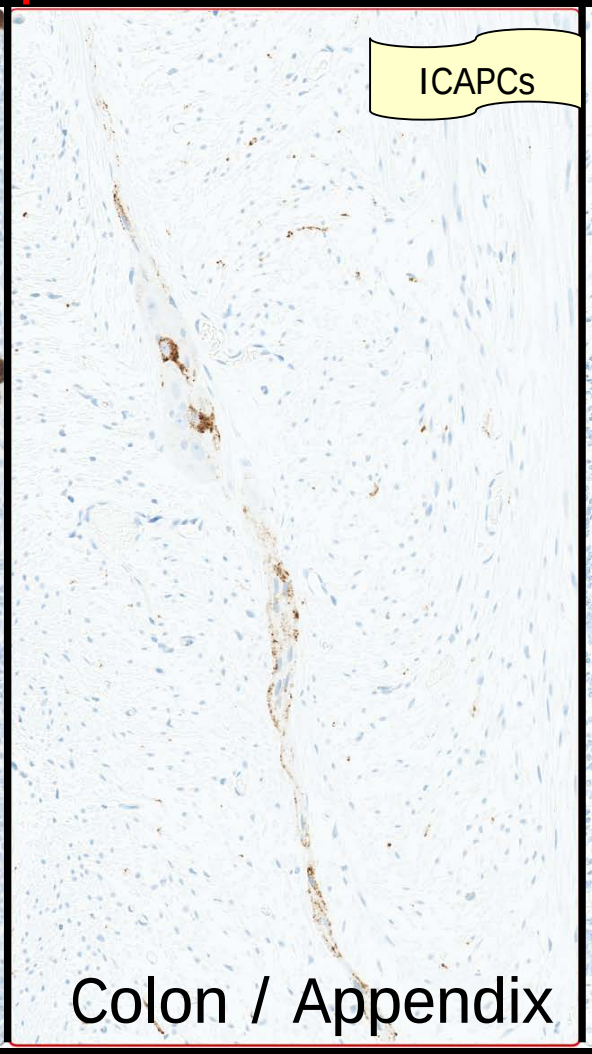
ALK (Lung) reaction pattern



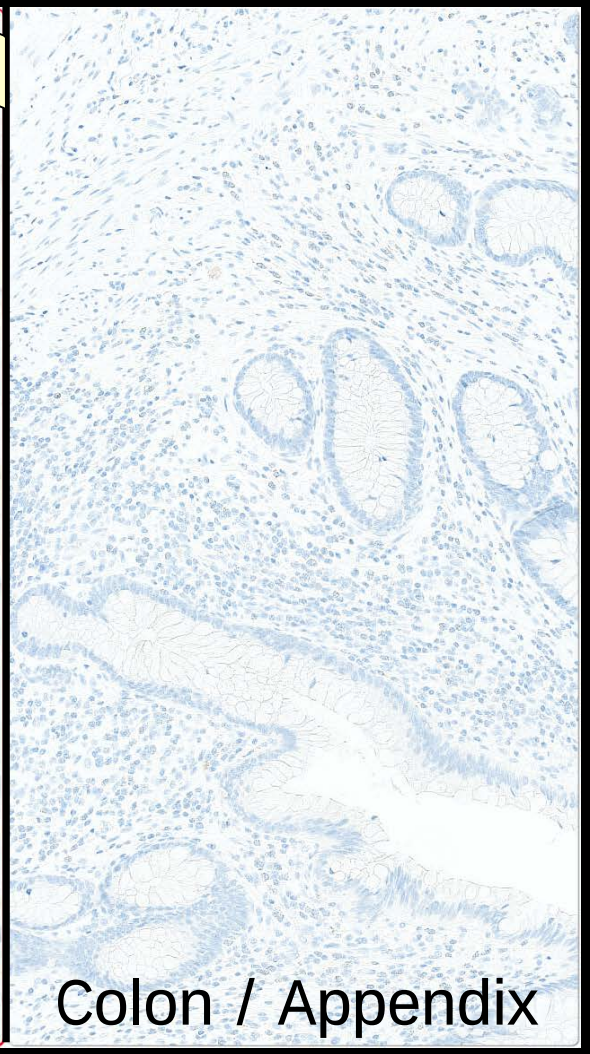
Tumour + rearrang.

A weak to strong distinct staining reaction in the vast majority of neoplastic cells



Colon / Appendix

An at least weak to moderate and distinct cytoplasmic staining reaction of ganglion cells.



Colon / Appendix

No staining reaction in columnar epithelial cells and lymphocytes.

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Table 1. **Antibodies and assessment marks for lu-ALK, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5A4**	46 3 2 1 1 1	Leica/Novocastra Thermo/NeoMarkers Monosan Abcam Biocare Zytomed	24	16	13	1	74%	81%
mAb clone **ALK1**	8	Dako	0	0	3	5	0%	-
mAb clone **OTI1A4**	5	ORIGENE	4	1	0	0	100%	100%
rmAb clone **D5F3**	21 1	Cell Signaling PrimeBioMed	18	2	1	1	91%	95%
rmAb clone **SP8**	2	Thermo/NeoMarkers	0	0	1	1	-	-
Ready-To-Use antibodies								
mAb clone **5A4 PA0306**	3	Leica/Novocatra	0	1	2	0	-	-
mAb clone **5A4 API3041**	1	Biocare	1	0	0	0	-	-
mAb clone **5A4 MAB-0281**	1	Maixin	1	0	0	0	-	-
mAb **5A4 MAD-001720QD**	1	Master Diagnostica	0	0	0	1	-	-
mAb **ALK1 IR641**	15	Dako	0	0	4	11	0%	-
mAb clone **ALK1 790/800-2918**	10	Ventana	0	1	6	3	10%	-
mAb clone **ALK1 204M-18**	1	Cell Marque	0	0	0	1	-	-
mAb clone **ALK1 GA641**	1	Dako	0	0	0	1	-	-
rmAb clone **D5F3 790-4794**	47	Ventana	41	4	2	0	96%	96%
rmAb clone **D5F3 790-4843** (CDx assay)	4	Ventana	3	0	1	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Total	176		93	25	33	25	-	
Proportion			53%	14%	19%	14%	67%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Clone

Titre

Detection kit

Calibration level...

One protocol for ALCL

One protocol for lung

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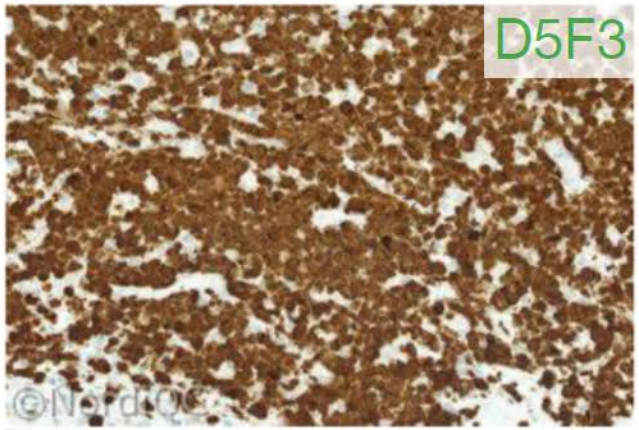

Fig. 1a.
Optimal ALK staining of the ALCL with ALK rearrangement using the rmAb clone D5F3 as RTU format (Ventana), showing an intense nuclear and cytoplasmic staining reaction. Despite the intense staining reaction, a high signal-to-noise ratio is provided and no background staining is seen. Also compare with Figs. 2a - 4a, same protocol.

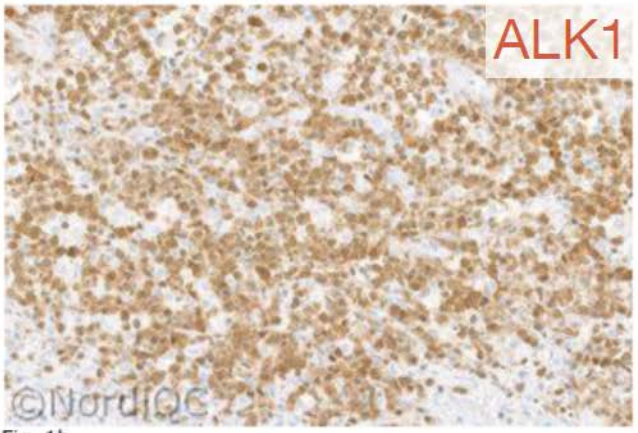

Fig. 1b
Insufficient ALK staining of the ALCL with ALK rearrangement using a protocol based on the mAb clone ALK1 - same field as in Fig. 1a. The vast majority of the neoplastic cells are demonstrated, however also compare with Fig. 2b, same protocol.

Primary antibody with a too low sensitivity.

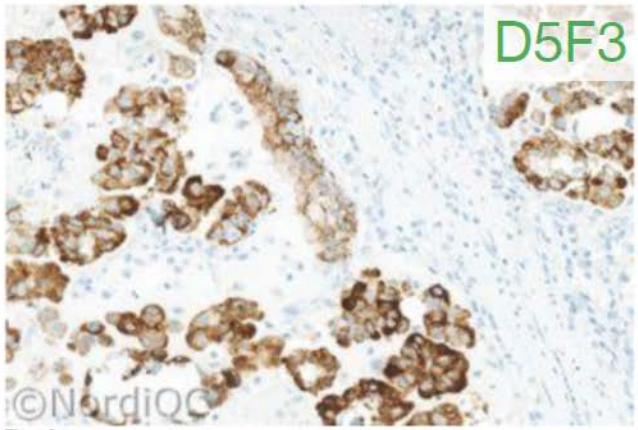

Fig. 2a
Optimal ALK staining of the lung adenocarcinoma with ALK rearrangement using same protocol as in Fig. 1a.
The majority of the neoplastic cells show a moderate to strong granular cytoplasmic staining reaction. No background staining is seen.

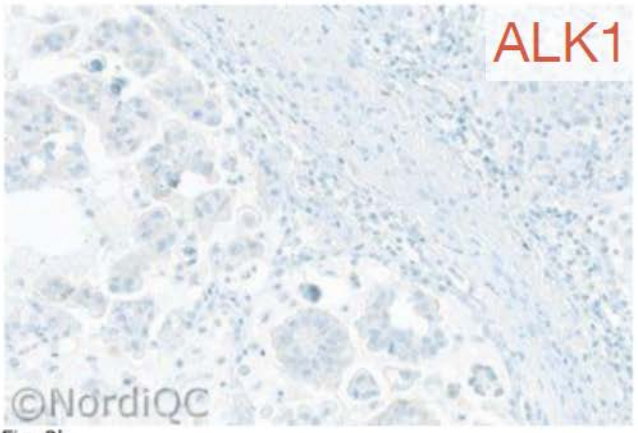

Fig. 2b
Insufficient ALK staining of the lung adenocarcinoma with ALK rearrangement using same protocol as in Fig. 1b - same field as in Fig. 2a. Only scattered neoplastic cells show a weak cytoplasmic staining reaction, while the vast majority are negative.

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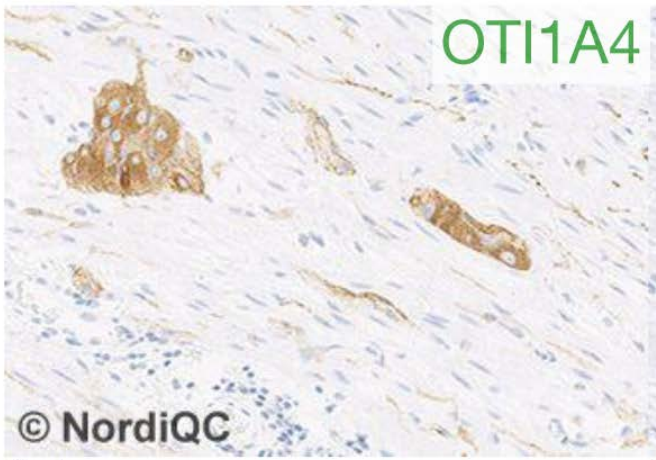

Fig. 4a
Optimal ALK staining of the appendix using same protocol as in Figs. 1a - 3a. The ganglion cells of the myenteric plexus show a moderate, distinct cytoplasmic staining reaction, while the axons show a weak to moderate staining reaction.

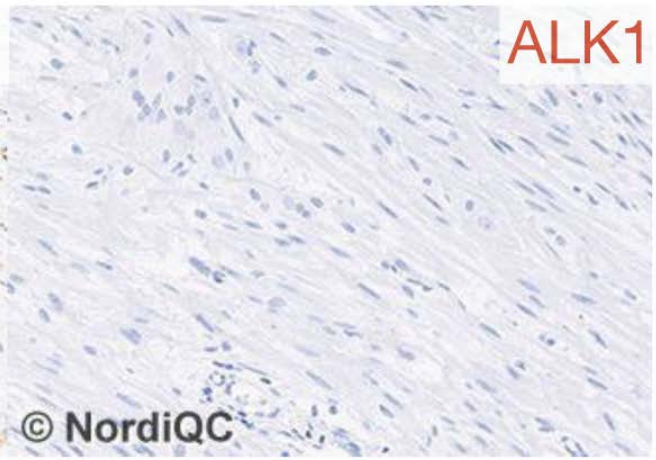

Fig. 4b
Insufficient ALK staining of the appendix using same protocol as in Figs. 1b - 3b. - same field as in Fig. 4a. The ganglion cells and axons are unstained.

Primary antibody with a too low sensitivity.

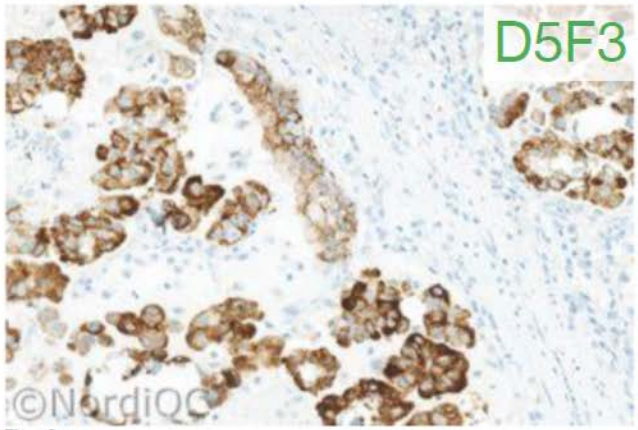

Fig. 2a
Optimal ALK staining of the lung adenocarcinoma with ALK rearrangement using same protocol as in Fig. 1a.
The majority of the neoplastic cells show a moderate to strong granular cytoplasmic staining reaction. No background staining is seen.

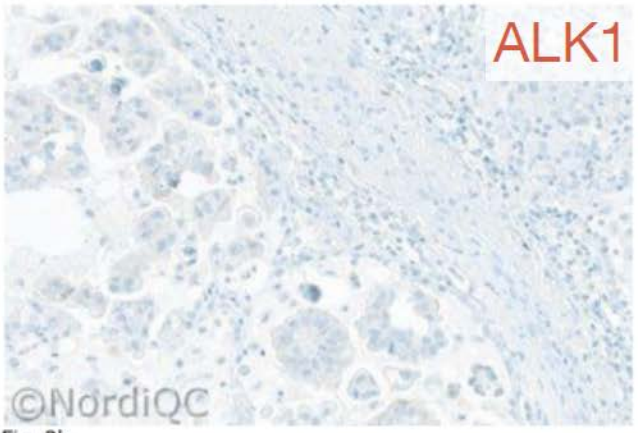

Fig. 2b
Insufficient ALK staining of the lung adenocarcinoma with ALK rearrangement using same protocol as in Fig. 1b - same field as in Fig. 2a. Only scattered neoplastic cells show a weak cytoplasmic staining reaction, while the vast majority are negative.

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ALK lung

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 5A4	HIER high	1:10-50	3-step	-	-
mAb OTI1A4	HIER high	1:50-1.000	3-step	-	-
rmAb D5F3	HIER high	1:50-250	3-step	Ventana	3-step+AMP

IHC - Protocols and controls for Lung tumours

Assessment Run C1 2017

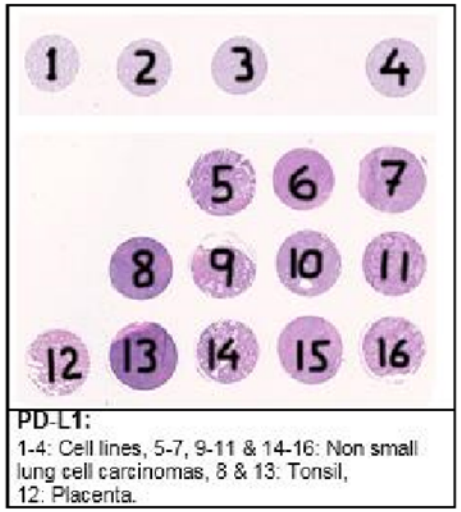
PD-L1

The first assessment in this new NordiQC Companion module C1 focused on the accuracy of the PD-L1 IHC assays performed by the participating laboratories to identify patients with NSCLC to be treated with immune therapy as either first line treatment (Keytruda®) or second line treatment (Keytruda® and Opdivo®). The PD-L1 expression levels in the circulated material used for the assessment were characterized by two CE IVD / FDA approved companion and complementary IHC assays, 28-8 pharmDX, SK005 Dako/Agilent and 22C3 pharmDX, SK006 Dako/Agilent. The associated cut-off values and interpretation guidelines were used accordingly to these two PD-L1 IHC assays.

Material

The slides to be stained for PD-L1 comprised the following 16 materials

	PD-L1 IHC TPS score*	Eglible for treatment**
Cell line controls* **		
1. Cell line 1	NA	NA
2. Cell line 2	NA	NA
3. Cell line 3	NA	NA
4. Cell line 4	NA	NA
Tissue controls		
8. Tonsil	NA	NA
13. Tonsil	NA	NA
12. Placenta	NA	NA
NSCLC		
5. NSCLC	No <1%	No
9. NSCLC	No <1%	No
14. NSCLC	No <1%	No
10. NSCLC	Low 1-49%	Yes
15. NSCLC	Low 1-49%	Yes
6. NSCLC	High ≥50%	Yes
7. NSCLC	High ≥50%	Yes
11. NSCLC	High ≥50%	Yes
16. NSCLC	High ≥50%	Yes



PD-L1:
1-4: Cell lines, 5-7, 9-11 & 14-16: Non small lung cell carcinomas, 8 & 13: Tonsil, 12: Placenta.

* Tumour proportion score (TPS) determined by PD-L1 IHC 28-8, SK005 & 22C3, SK006 Dako performed in NordiQC reference lab.
** Using present recommendations for cut-off value of TPS of 1-49% and ≥ 50% for second line (Keytruda® and Opdivo®) and first line treatment (Keytruda®), respectively.
*** Cell lines, Horizon Discovery, prod. Id HD788.

PD-L1 status
In NSCLC

For treatment

1’ line – Keytruda

2’ line – Keytruda
2’ line – Opdivo

IHC - Protocols and controls for Lung tumours

Table 1. **Assessment marks for IHC assays and antibodies run C1, PD-L1 IHC**

			Sufficient		Insufficient			
CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
22C3 pharmDX, **SK006**	12	Dako/Agilent	10	1	0	1	92%	92%
22C3 pharmDX, **SK006**[4]	2	Dako/Agilent	0	0	1	1	-	-
28-8 pharmDX, **SK005**	7	Dako/Agilent	3	3	1	0	86%	86%
SP263, 790-4905	16	Ventana/Roche	9	2	2	3	69%	77%
SP142, 740-4859	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	13	Dako/Agilent	1	1	4	7	15%	-
mAb clone **E1L3N**	8	Cell Signaling	1	1	1	5	25%	-
mAb **CAL10**	1	Biocare	0	0	1	0	-	-
rmAb clone **28-8**	6	Abcam	0	1	1	4	17%	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-
Antibodies for laboratory developed PD-L1 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP142**	1	Spring Biosystems	0	0	0	1	-	-
Total	68		25	9	11	23	-	-
Proportion			37%	13%	16%	34%	50%	-

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
4) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana Benchmark and Dako Omnis).

Optimal
TPS status as pr ref.

Good
TPS status as pr ref.
TPS % might be imprecise

Borderline
TPS status NOT as pr ref.
Impact on treatment option (one tumour)

Poor
TPS status NOT as pr ref.
Impact on treatment option (more tumours)

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Table 1. **Assessment marks for IHC assays and antibodies run C1, PD-L1 IHC**

			Sufficient		Insufficient			
CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
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28-8 pharmDX, **SK005**	7	Dako/Agilent	3	3	1	0	86%	86%
SP263, 790-4905	16	Ventana/Roche	9	2	2	3	69%	77%
SP142, 740-4859	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	13	Dako/Agilent	1	1	4	7	15%	-
mAb clone **E1L3N**	8	Cell Signaling	1	1	1	5	25%	-
mAb **CAL10**	1	Biocare	0	0	1	0	-	-
rmAb clone **28-8**	6	Abcam	0	1	1	4	17%	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-
Antibodies for laboratory developed PD-L1 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP142**	1	Spring Biosystems	0	0	0	1	-	
Total	68		25	9	11	23	-	
Proportion			37%	13%	16%	34%	50%	

1) Proportion of sufficient stains (optimal or good).
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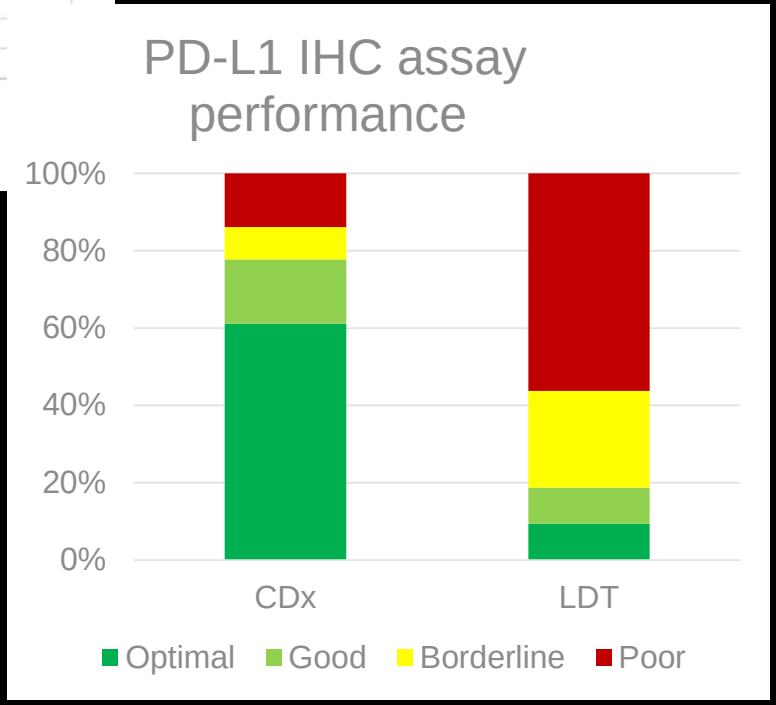
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
4) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana Benchmark and Dako Omnis).



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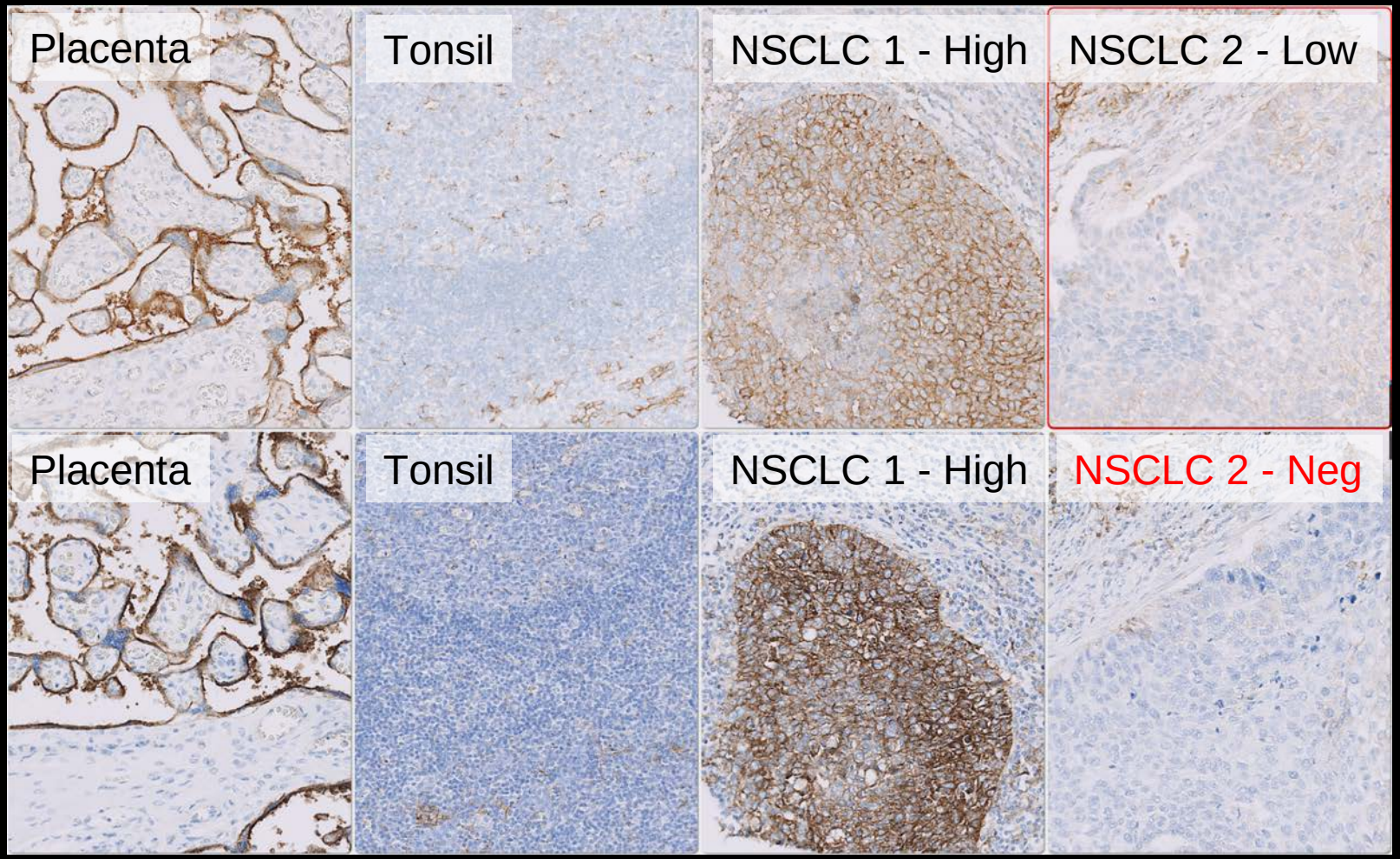

pharmDx
SK006

LDT M3653
22C3

IHC - Protocols and controls for Lung tumours

Lung Cancer 113 (2017) 102–105

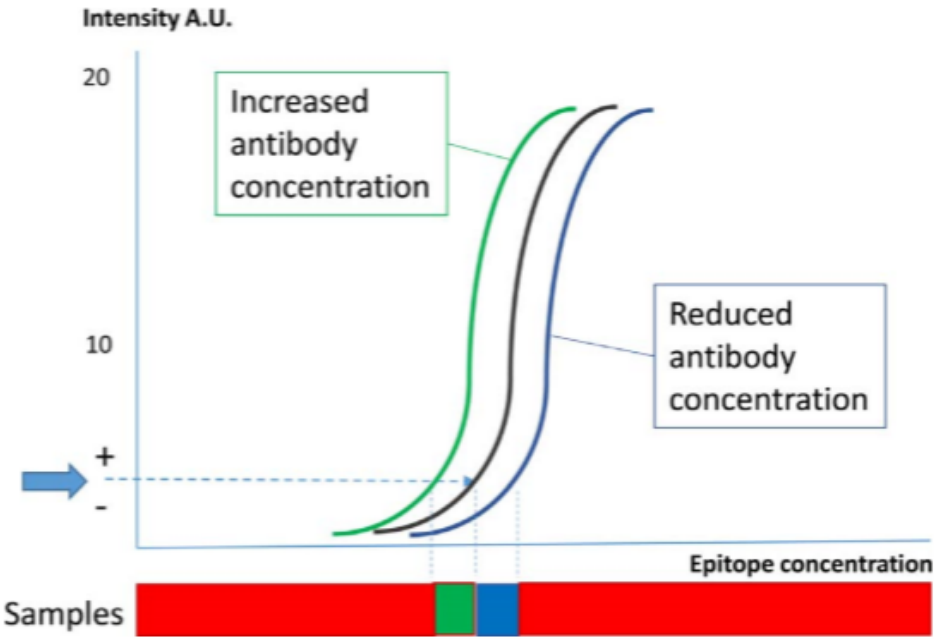

Fig. 1. The critical sample set is determined by examining i) positive samples in the clinical validated assay [black curve] with a 2 fold reduced concentration of the primary antibody [blue curve] and ii) negative samples with a 2 fold increase in concentration [green curve]. Cases reversing in the test outcome are critical samples [green box and blue box] with an epitope concentration close the positivity threshold, while samples outside this range are not informative [red boxes] for clinical validation of different IHC tests. In practice < 10% of the samples are critical samples. Note that the width of the green and blue box is arbitrary, as a standard for (any) epitope concentration is lacking. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

E Thunissen et al.
Lung cancer 113 (2017) 102-105
PD-L1 IHC in NSCLC with a global and methodological perspective

E Thunissen et al.
Arch Pathol Lab Med. 2017-0106-SA
doi: 10.5858/arpa.

10 samples PD-L1 neg (clear neg – non-critical)
10 samples PD-L1 pos (clear pos – non-critical)
+
20 samples critical PD-L1 level
(% and expression level close at threshold levels)

NordiQC

Validation and development of laboratory developed IHC assays for PD-L1 are challenged by the two following issues:

1. Access to well characterized and verified PD-L1 IHC expression levels in app. 40-100 samples.

   For Keytruda thus 40- 100 NSCLCs evaluated by SK006

   90-95% concordance between LDT and CDx must be reached.

2. No alternative validated method as support – e.g. FISH as for HER2 IHC.

Table 1. **Assessment marks for IHC assays and antibodies run C1, PD-L1 IHC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
22C3 pharmDX, **SK006**	12	Dako/Agilent	10	1	0	1	92%	92%
22C3 pharmDX, **SK006**[4]	2	Dako/Agilent	0	0	1	1	-	-
28-8 pharmDX, **SK005**	7	Dako/Agilent	3	3	1	0	86%	86%
SP263, 790-4905	16	Ventana/Roche	9	2	2	3	69%	77%
SP142, 740-4859	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	13	Dako/Agilent	1	1	4	7	15%	-
mAb clone **E1L3N**	8	Cell Signaling	1	1	1	5	25%	-
mAb **CAL10**	1	Biocare	0	0	1	0	-	-
rmAb clone **28-8**	6	Abcam	0	1	1	4	17%	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-
Antibodies for laboratory developed PD-L1 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP142**	1	Spring Biosystems	0	0	0	1	-	-
Total	68		25	9	11	23	-	-
Proportion			37%	13%	16%	34%	50%	-

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
4) RTU system developed for the Agilent/Dako´s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana Benchmark and Dako Omnis).

(*Appl Immunohistochem Mol Morphol* 2017;25:151–159)

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine. Part 3: Technical Validation of Immunohistochemistry (IHC) Assays in Clinical IHC Laboratories

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From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path)

IHC - Protocols and controls for Lung tumours

Conclusions run NordiQC run C1;

PD-L1 IHC is a technical challenge.

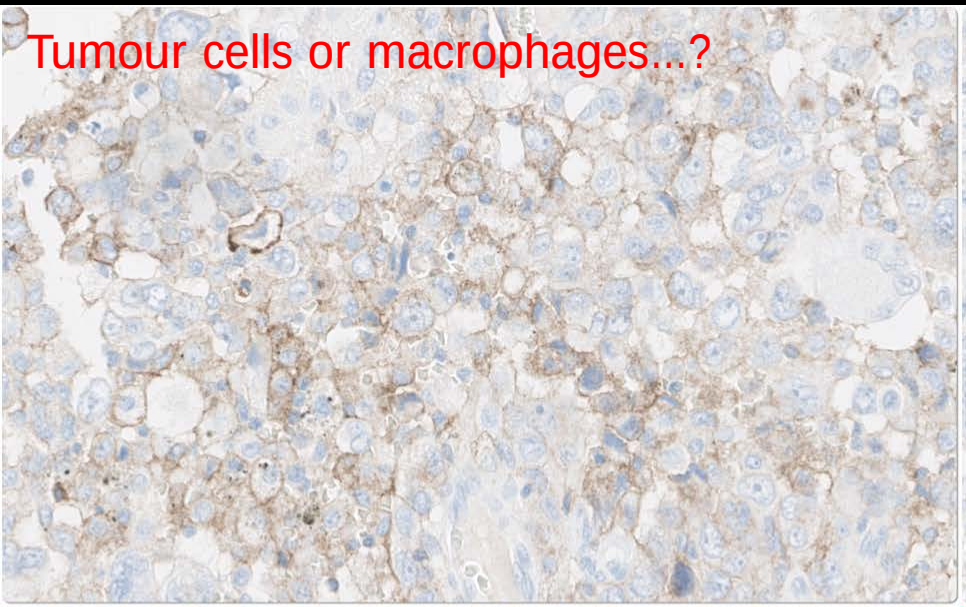
1. NordiQC data indicates that specific CDxs are prefered for the specific use of drug – e.g. SK006 for Keytruda (most likely also SK005 and SP263 giving comparable analytical results).

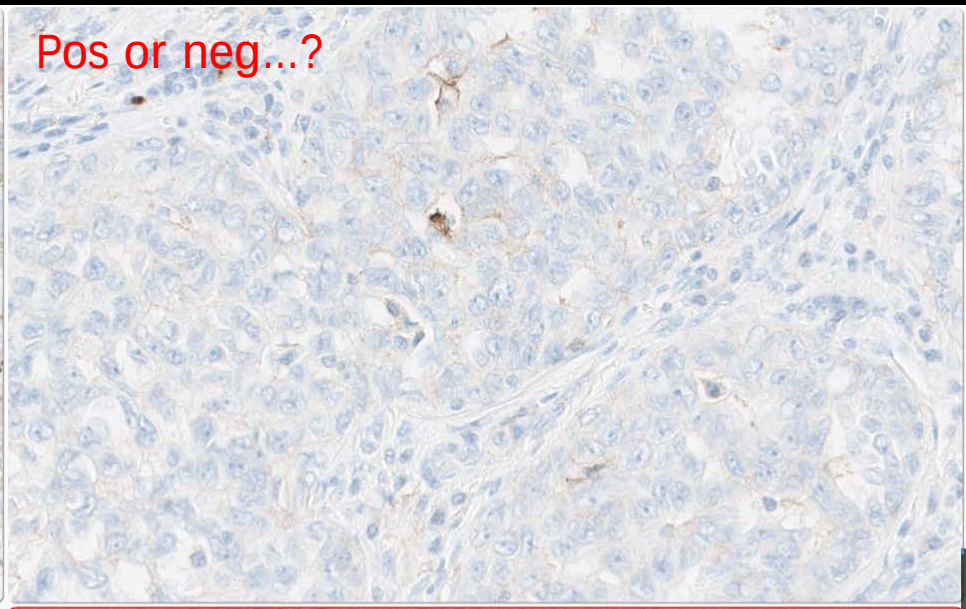
2. LDTs can be applied if no access to CDx IHC device – however inferior choice – **require access to reference**.

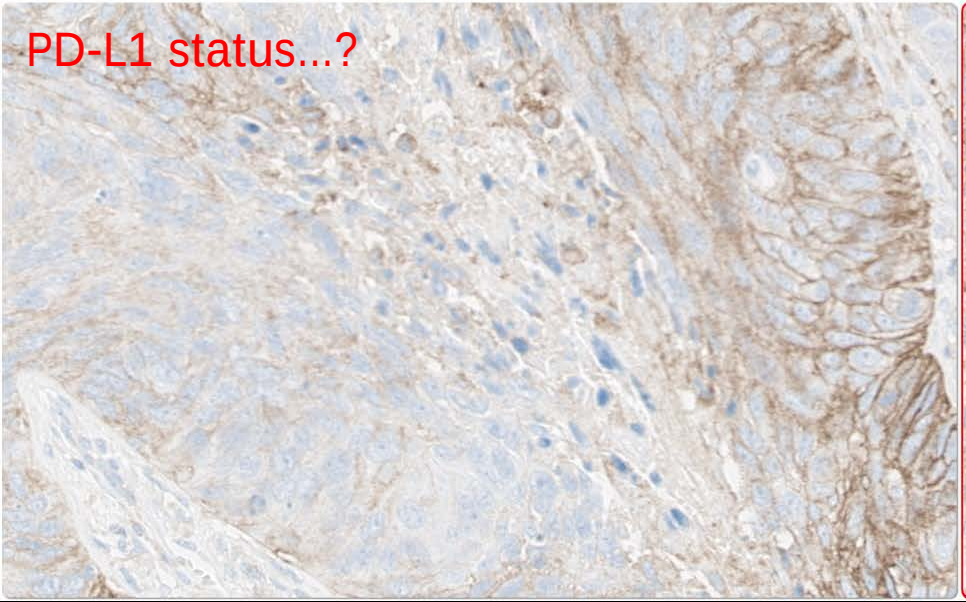
3. On-slide controls including tonsil and NSCLCs with dynamic and clinical range of PD-L1 expression is best practice.

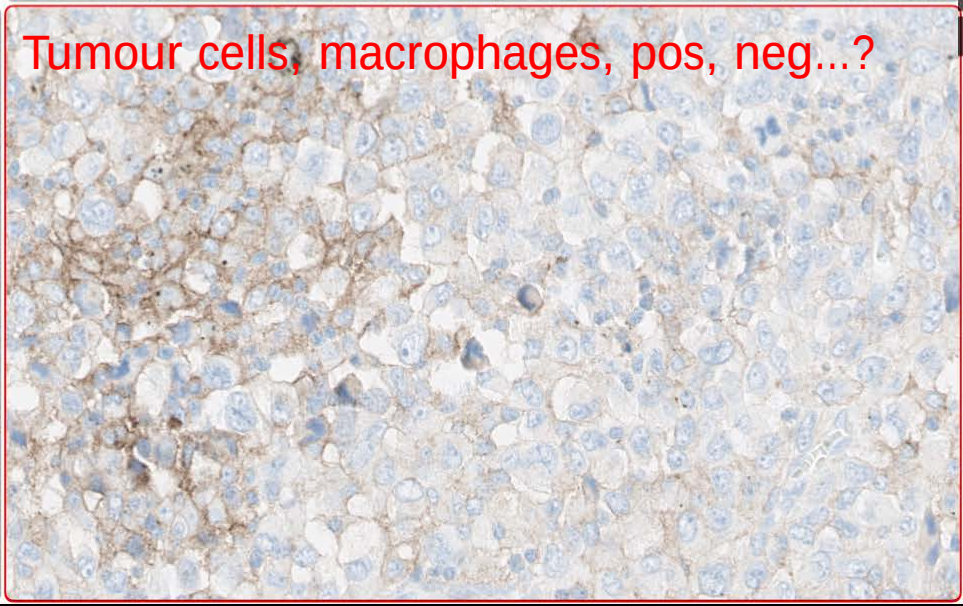
4. Interpretation for PD-L1 additional challenge.....

IHC - Protocols and controls for Lung tumours



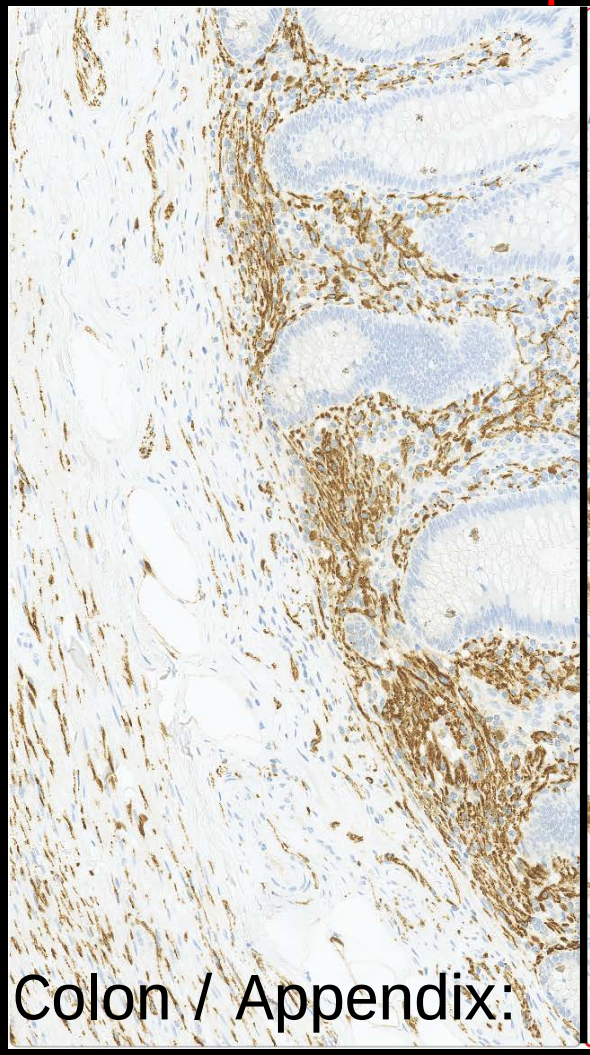






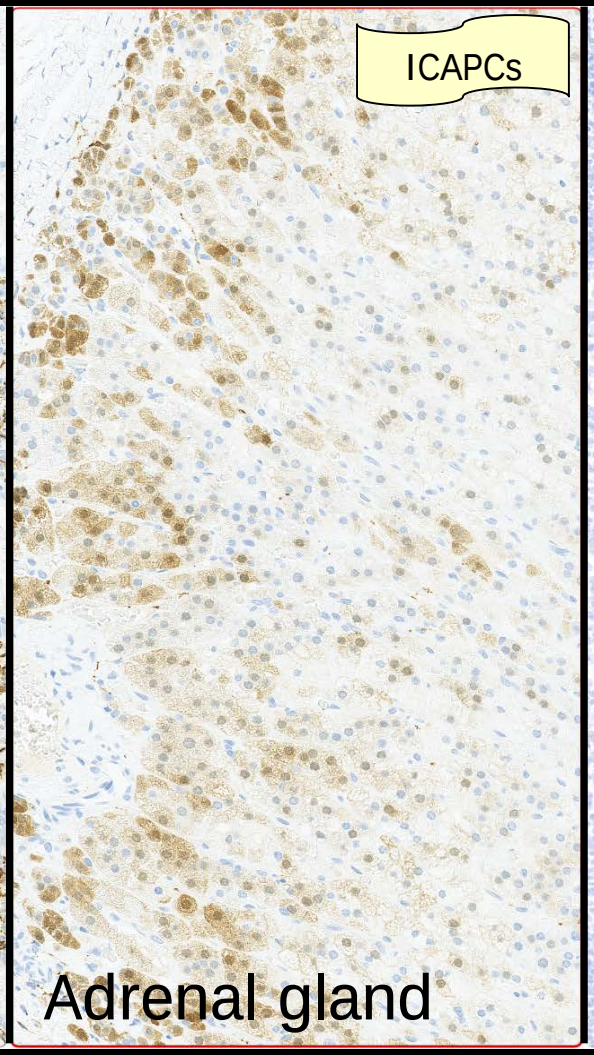
IHC - Protocols and controls for Lung tumours

Calretinin reaction pattern



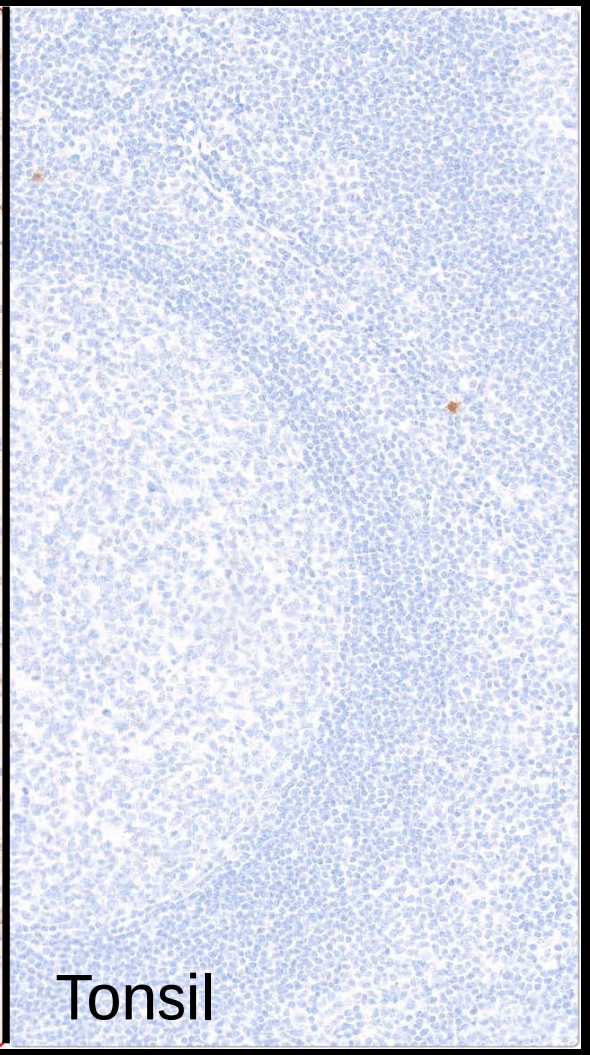
Colon / Appendix:

A moderate to strong distinct nuclear and cytoplasmic staining reaction in ganglion cells and macrophages.



Adrenal gland

An at least weak to moderate and distinct nuclear and cytoplasmic staining reaction of most cortical epithelial cells.



Tonsil

No staining reaction epithelial cells and lymphocytes – scattered macrophages can be seen.

IHC - Protocols and controls for Lung tumours

Table 1: **Antibodies and assessment marks for CR, run 45**

	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mmAb clone **2E7**	1	Immunologic	1	0	0	0	-	-
mmAb clone **5A5**	21 1	Leica/Novocastra Immunologic	3	10	8	1	59%	56%
mmAb clone **CAL6**	6 1	Leica/Novocastra Monosan	4	1	0	2	71%	-
mmAb clone **DAK-Calret 1**	35	Dako	10	13	9	3	66%	87%
rmAb clone **SP13**	3 1 2	Thermo/Neomarkers Spring Bioscience Cell Marque	1	2	2	1	50%	-
pAb **18-0211**	16	Invitrogen/Zymed	2	8	6	0	63%	-
pAb **232A**	5	Cell Marque	0	1	2	2	20%	-
pAb **61-0006**	1	Genemed	0	1	0	0	-	-
pAb **7699/3H**	1	Swant	0	0	0	1	-	-
pAb **RBK003**	1	Zytomed	0	0	1	0	-	-
Ready-To-Use antibodies								
mmAb clone **CAL6 PA0346**	8	Leica/Novocastra	2	3	2	1	63%	-
mmAb clone **DAK-Calret 1 IS/IR627**	38	Dako	9	17	10	2	68%	79%
rmAb **SP13 RMA-0524**	1	Maixin	1	0	0	0	-	-
rmAb **SP13 232R-18**	1	Cell Marque	0	1	0	0	-	-
rmAb **SP13 MAD-000315QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **SP65 790-4467**	64	Ventana	52	8	2	2	94%	94%
pAb **232A-78**	1	Cell Marque	0	1	0	0	-	-
pAb **PP092**	1	BioCare	0	1	0	0	-	-
Total	210		85	68	42	15	-	
Proportion			41%	32%	20%	7%	73%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Clone dependent on IHC platform

VMS: No-go for DAK-Calret 1

Efficient HIER
3-step > 2-step

3-step systems gave sufficient rate of 94-100% versus 50-60% for 2-step..... (DAK-Calret 1 and 5A4 as LDT)

IHC - Protocols and controls for Lung tumours

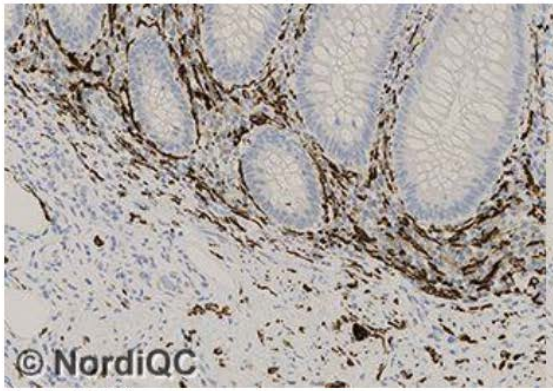


Fig. 1a (x200)
Optimal CR staining of the appendix using the mmAb clone DAK-Calret 1 (Dako) in a RTU format (Dako IR627) and with an incubation time of 20 min. after HIER in an alkaline buffer (TRS pH 9, Dako). Staining was performed on the Dako Autostainer using a 3-step polymer system (EnVision Flex+). A strong, distinct cytoplasmic and nuclear staining of the peripheral nerves is seen. No reaction is seen in the columnar epithelial cells (same protocol used in Figs. 2a - 4a). Compare with Fig. 1b.

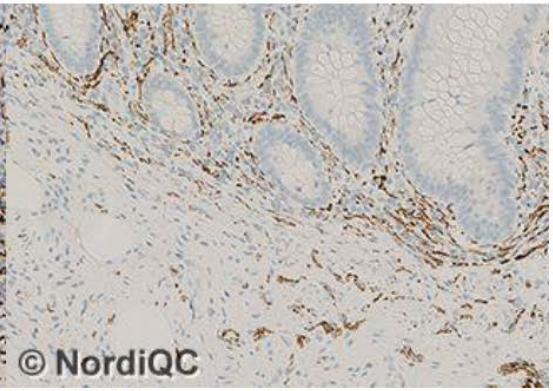


Fig. 1b (x200)
Insufficient CR staining of the appendix using the mmAb clone DAK-Calret 1 (Dako) in a RTU format (Dako IR627) and with an incubation time of 32 min. after HIER in an alkaline buffer (CC1, pH 8,5, Ventana). Staining was performed on the Ventana BenchMark using a 3-step multimer system (UltraView with amplification). Although the same RTU product and similar protocol settings were used, the CR staining on the Ventana BenchMark was significantly weaker. A moderate, distinct cytoplasmic and nuclear staining of the peripheral nerves is seen. Compare with Fig. 1a. – same field. Also compare with Figs. 2b, 3b and 4b – same protocol.

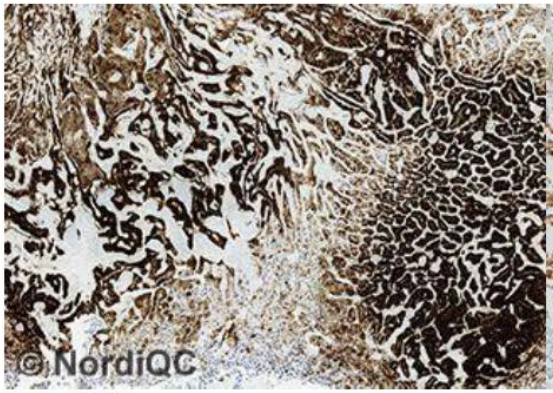


Fig. 2a (x100)
Optimal CR staining of the mesothelioma in core no. 5 ("high expressor") using the same protocol as in Fig. 1a. A strong, distinct cytoplasmic and nuclear staining of virtually all the tumour cells is seen. Compare with Fig. 2b.

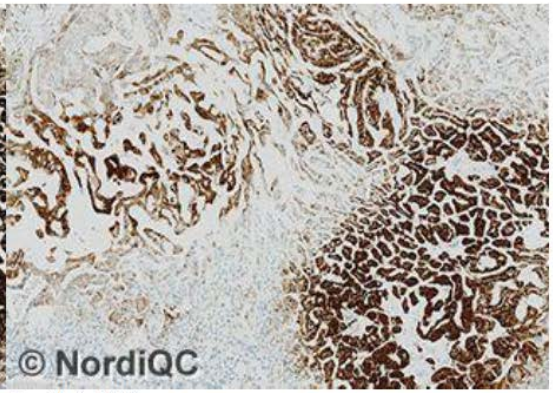


Fig. 2b (x100)
Insufficient CR staining of the mesothelioma in core no. 5 ("high expressor") using the same protocol as in Fig. 1b. Only a moderate, distinct cytoplasmic and nuclear staining of the majority of the tumour cells is seen. Compare with Fig. 2a - same field.

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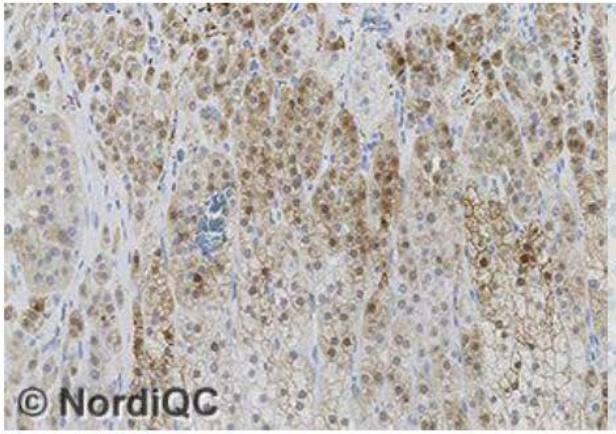

Fig. 3a (x200)
Optimal CR staining of the adrenal gland ("low expressor") using the same protocol as in Fig. 1a and 2a. A moderate, distinct cytoplasmic and nuclear staining of the majority of the cortical epithelial cells is seen. Compare with Fig. 3b.

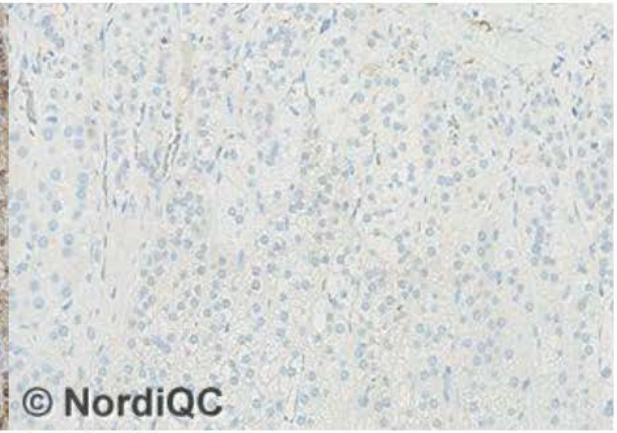

Fig. 3b (x200)
Insufficient CR staining of the adrenal gland ("low expressor") using the same protocol as in Fig. 1b and 2b. No staining of the cortical epithelial cells is seen. Compare with Fig. 3a – same field.

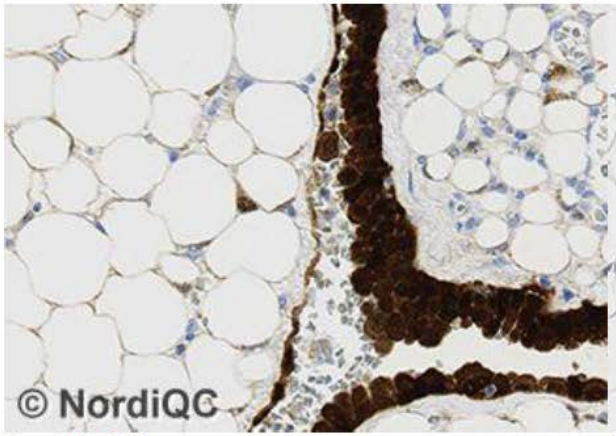

Fig. 4a (x400)
Optimal CR staining of the mesothelioma in core no. 6 ("medium expressor") using the same protocol as in Fig. 1a, 2a and 3a. A strong, distinct cytoplasmic and nuclear staining of virtually all the tumour cells is seen. In addition to the positive tumour cells a weak to moderate, distinct cytoplasmic and nuclear staining is seen in virtually all the fat cells. Compare with Fig. 4b.

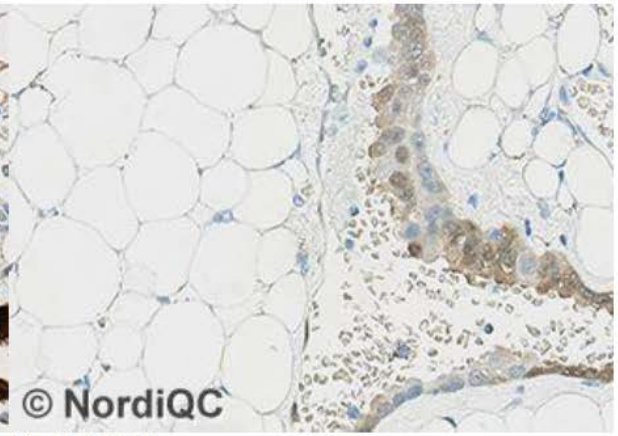

Fig. 4b (x400)
Insufficient CR staining of the mesothelioma in core no. 6 ("medium expressor") using the same protocol as in Fig. 1b, 2b and 3b. The majority of the tumour cells are virtually negative and no staining is seen in the fat cells. Compare with Fig. 4a – same field.

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Calretinin

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 5A5	HIER high	1:25-100	3-step	-	-
mAb CAL6	HIER high	1:15-50	3-step	Leica	3-step
mAb DAK-Calret1	HIER high	1:20-200	3-step	Dako	3-step
rmAb SP65	HIER high	-	-	Ventana	2- & 3-step

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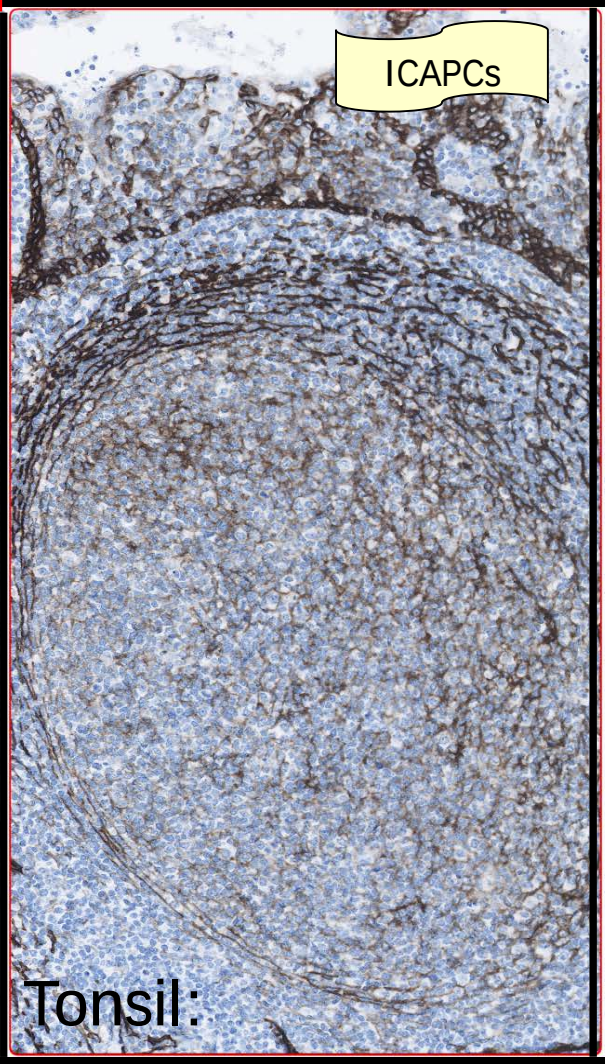
Podoplanin reaction pattern

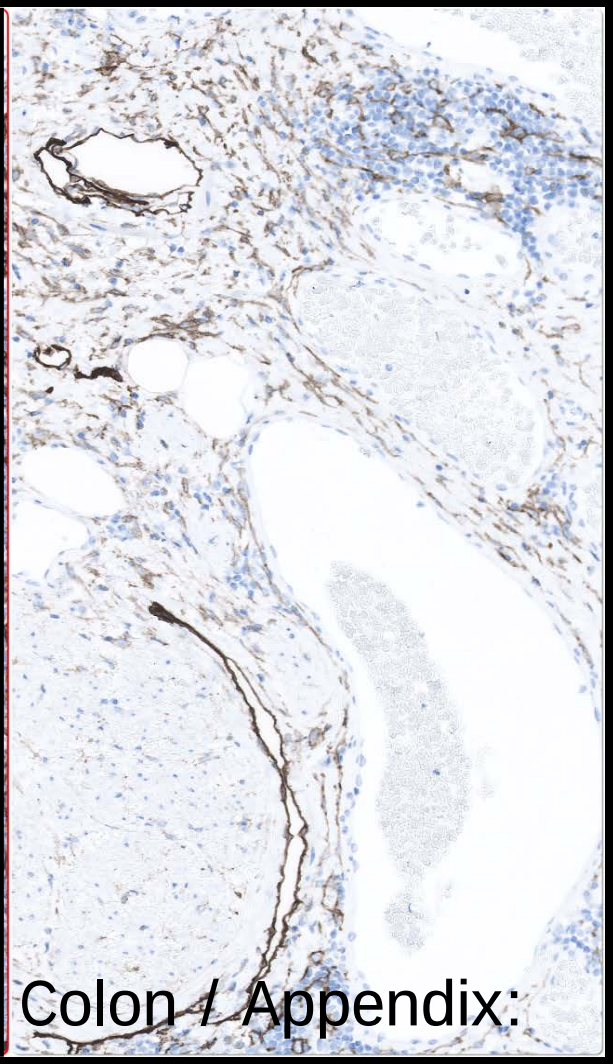
Tonsil:

A moderate to strong distinct cytoplasmic staining reaction basal squamous epithelial cells and lymphatic endothelial cells.



Tonsil:

An at least weak to moderate and distinct staining reaction of the follicular dendritic network.



Colon / Appendix:

No staining reaction of endothelial cells of blood vessels

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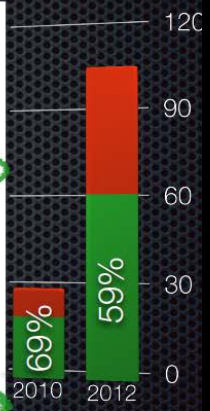
Podoplanin / RUN 36 2012

Pass: 59 %

Table 1. Abs and assessment marks for Podop, run 36

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **D2-40**	48 3 2 1 1 1	Dako Signet Biocare Cell Marque Immunologic Zytomed	10	20	22	4	54 %	56 %
mAb clone **AB3**	1	AngioBio	0	1	0	0	-	-
mAb clone **18H51**	1	Acris	0	0	0	1	-	-
rmAb clone **EP215**	1	Epitomics	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **D2-40 IS/IR072**	15	Dako	11	4	0	0	100 %	100 %
mAb clone **D2-40 N1607**	3	Dako	0	3	0	0	-	-
mAb clone **D2-40 760-4395**	21	Ventana/Cell Marque	0	8	13	0	38 %	-
mAb clone **D2-40 322M-17/18**	2	Cell Marque	0	1	1	0	-	-
mAb clone **D2-40 MON-RTU1092**	1	Monosan	0	1	0	0	-	-
mAb clone **D2-40 MAD-000402QD**	1	Master Diagnostica	0	1	0	0	-	-
Total	102		21	39	37	5	-	
Proportion			21 %	38 %	36 %	5 %	59 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.



* Poor performance of the mAb clone D2-40 on the Ventana BenchMark platform.

Platform	No. of labs	Passrate	Optimal
Ventana	50	38 %	4 %
Non-Ventana	46	85 %	39 %

2012 – OptiView not widely implemented.....

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Podoplanin / RUN 36 2012

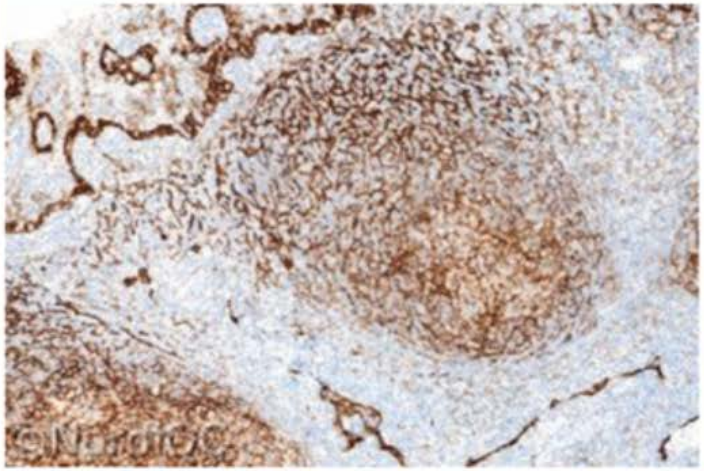

Fig. 1a. Optimal staining for Podop of the tonsil using the mAb clone D2-40 optimally calibrated as a concentrate, HIER in BERS2, Leica and a 3-step polymer based detection system. The basal squamous epithelial cells and the lymphatic endothelial cells show a strong cytoplasmic staining reaction, while a moderate staining reaction is seen in the follicular dendritic cells in the germinal centres.

Fig. 1b. Insufficient staining for Podop of the tonsil using the mAb clone D2-40 as a concentrate with a too low sensitivity (too low conc. of the primary Ab and HIER in citrate pH 6). Only a weak cytoplasmic staining reaction is demonstrated in the cells with a high antigen expression as the basal squamous epithelial cells, whereas no staining is seen in the follicular dendritic cells – compare with Fig. 1a.

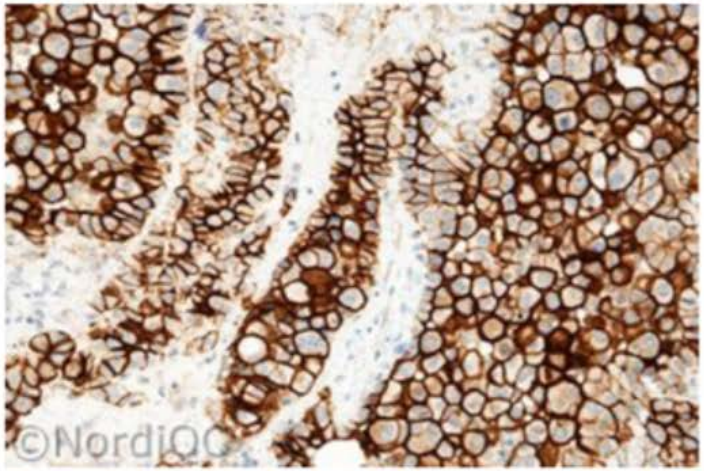

Fig. 2a. Optimal staining for Podop of the mesothelioma using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction.

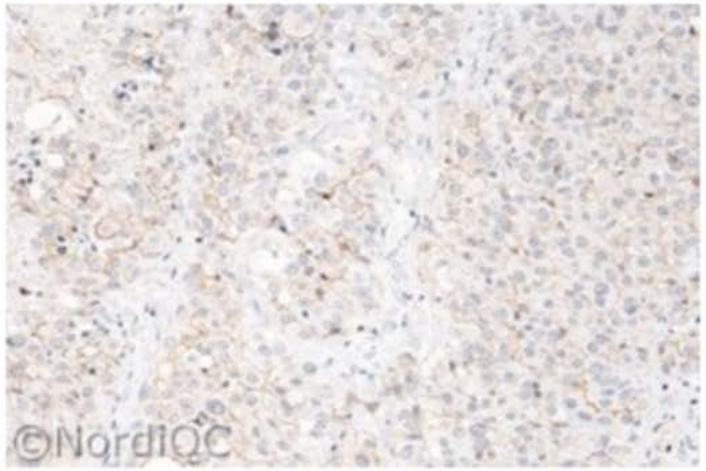

Fig. 2b. Insufficient staining for Podop of the mesothelioma using the same protocol as in Fig. 1b. The proportion and the intensity of the cells demonstrated is significantly reduced – compare with Fig. 2a.

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Podoplanin

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb D2-40	HIER high	1:10-50	2- & 3-step	Dako	2- & 3-step

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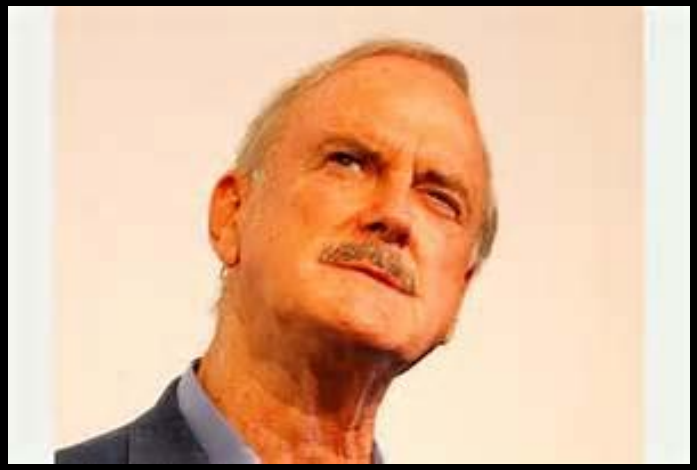

Hmmmm just wonder ???