

NORDIQC DATA FOR LUNG MARKERS

Antibody selection, protocols and controls

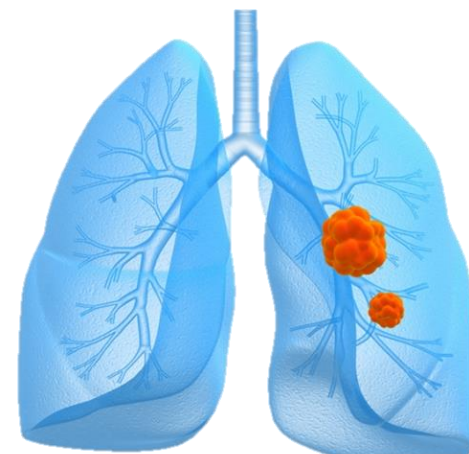
NordiQC Seminar, October 2nd-4th 2024

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NORDIQC EQA DATA FOR IHC LUNG MARKERS

Marker	Purpose	Last run	Pass rate	No of labs
TTF1	<u>Lung</u> vs non-lung <u>Adenocarcinoma</u> vs squam.	Run 68, 2023	81%	401
Napsin A	<u>Lung</u> vs non-lung	Run 66, 2022	83%	321
Calretinin	Lung vs <u>mesothelioma</u>	Run 64, 2022	76%	350
WT1	Lung vs <u>mesothelioma</u>	Run 55, 2019	91%	291
BAP1	Reactive mesothelioma vs <u>malignant mesothelioma</u>	Run 71, 2024	63%	224
EpCAM	<u>Lung</u> vs mesothelioma	Run 69, 2023	48%	372
INSM1	NSCLC vs <u>SCLC</u>	Run 71, 2024	72%	156
CGA	NSCLC vs <u>SCLC</u>	Run 70, 2024	75%	370
SYP	NSCLC vs <u>SCLC</u>	Run 66, 2022	70%	390
CD56	NSCLC vs <u>SCLC</u>	Run 64, 2022	72%	364
p40	Adenocarcinoma vs <u>squam.</u>	Run 67, 2023	85%	344
CK5	Adenocarcinoma vs <u>squam.</u>	Run 65, 2022	71%	311
ALK (lung)	Predictive for Crizotinib	Run 65, 2022	77%	256
PD-L1 TPS/CPS	Predictive for Keytruda, Imfinzi, Opdivo.....	Run C15, 2024	88%	255

Scheduled for
assessment within
the next year



CLONE PERFORMANCE FOR SELECTED LUNG MARKERS

Marker	Successful clones	Less successful clones
TTF1	mAb SPT24, rmAb SP141	mAb 8G7G3/1
Napsin A	mAbs IP64 & MRQ-60	pAbs
Calretinin	mAbs DAK-Calret & CAL6, rmAb SP65	pAbs
WT1	mAbs 6F-H2 & WT49	-
BAP1	mAb C-4 & BSB-109, rmAb EPR22826-65	pAb
EpCAM	mAbs BS14, Ber-EP4, MOC-31 & VU-1D9	mAb Ber-EP4
INSM1	rmAbs MRQ-70 & ZR395	mAb A-8 <i>(might depend on vendor)</i>
CGA	mAb LK2H10	mAbs DAK-A3 & 5H7
SYP	mAbs DAK-SYNAP & 27G12, rmAbs MRQ-40 & SP11	-
CD56	rmAb MRQ-42	mAbs 123C3 & CD564
p40	mAb BC28, rmAbs DAK-p40 & ZR8	pAbs
CK5	mAb XM26, rmAb SP27	mAb D5/16 B4
ALK (lung)	mAbs 5A4 & OTI1A4, rmAb D5F3	mAb ALK1
PD-L1 TPS/CPS	mAb 22C3, rmAb SP263	(rmAb SP142)



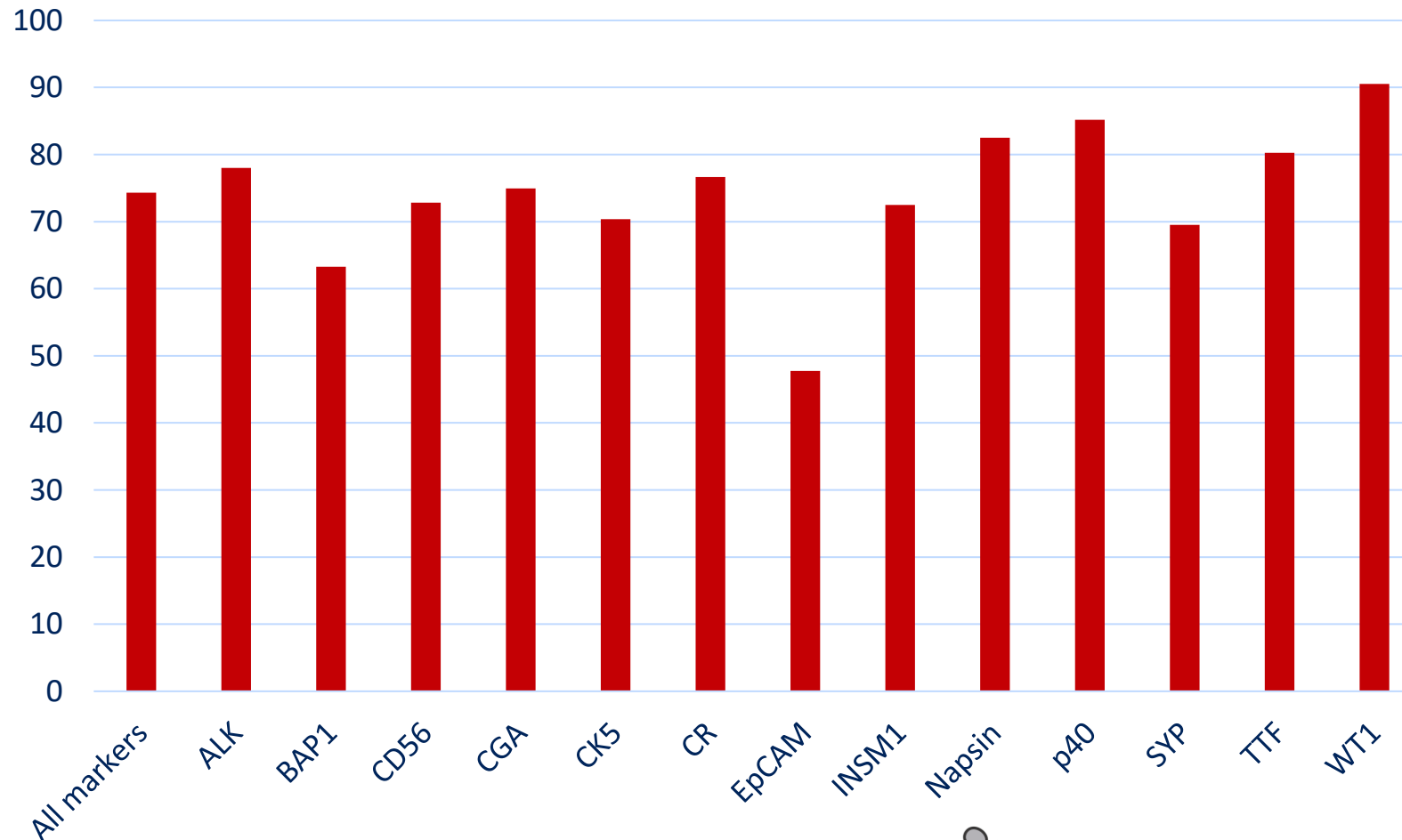
KEY-POINTS FOR BEST PROTOCOLS

- Clone selection
- RTUs – “Plug and Play” or “Play and Plug”?
- Efficient HIER – typically in high pH buffer
- 3 layer detection system
- Use of iCAPS



KEY-POINTS FOR BEST PROTOCOLS

Pass rates for lung markers



Lung markers in the General Module:

Overall pass rate: **74%**
(3.183/4.284), ranging from
48% for EpCam till 91% for WT1.

KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

Overall pass rate: **74%** (3.183/4.284)

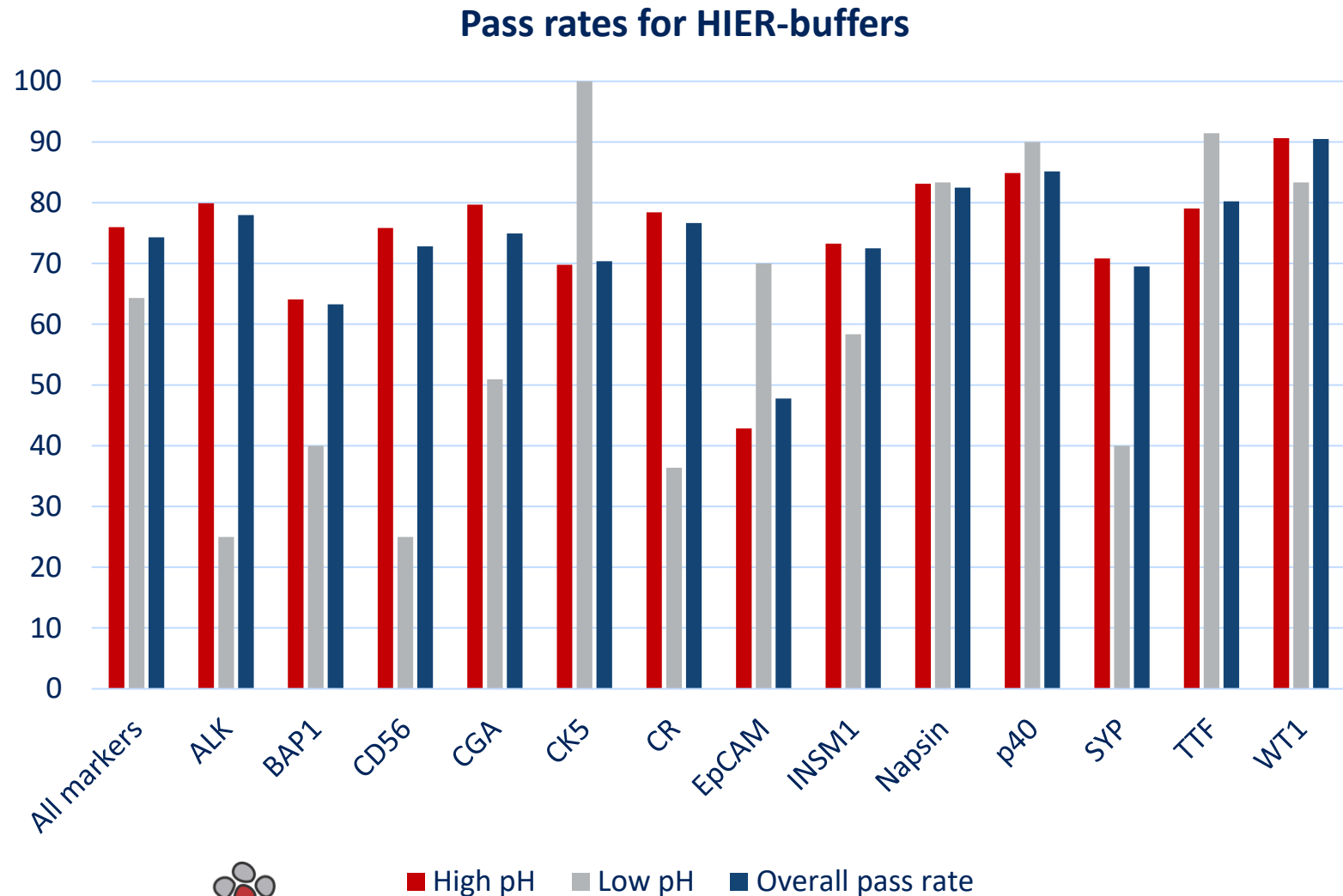
HIER in High pH: **76%**

Ranging from 43% for EpCAM till 91% for WT1

HIER in Low pH: 64%

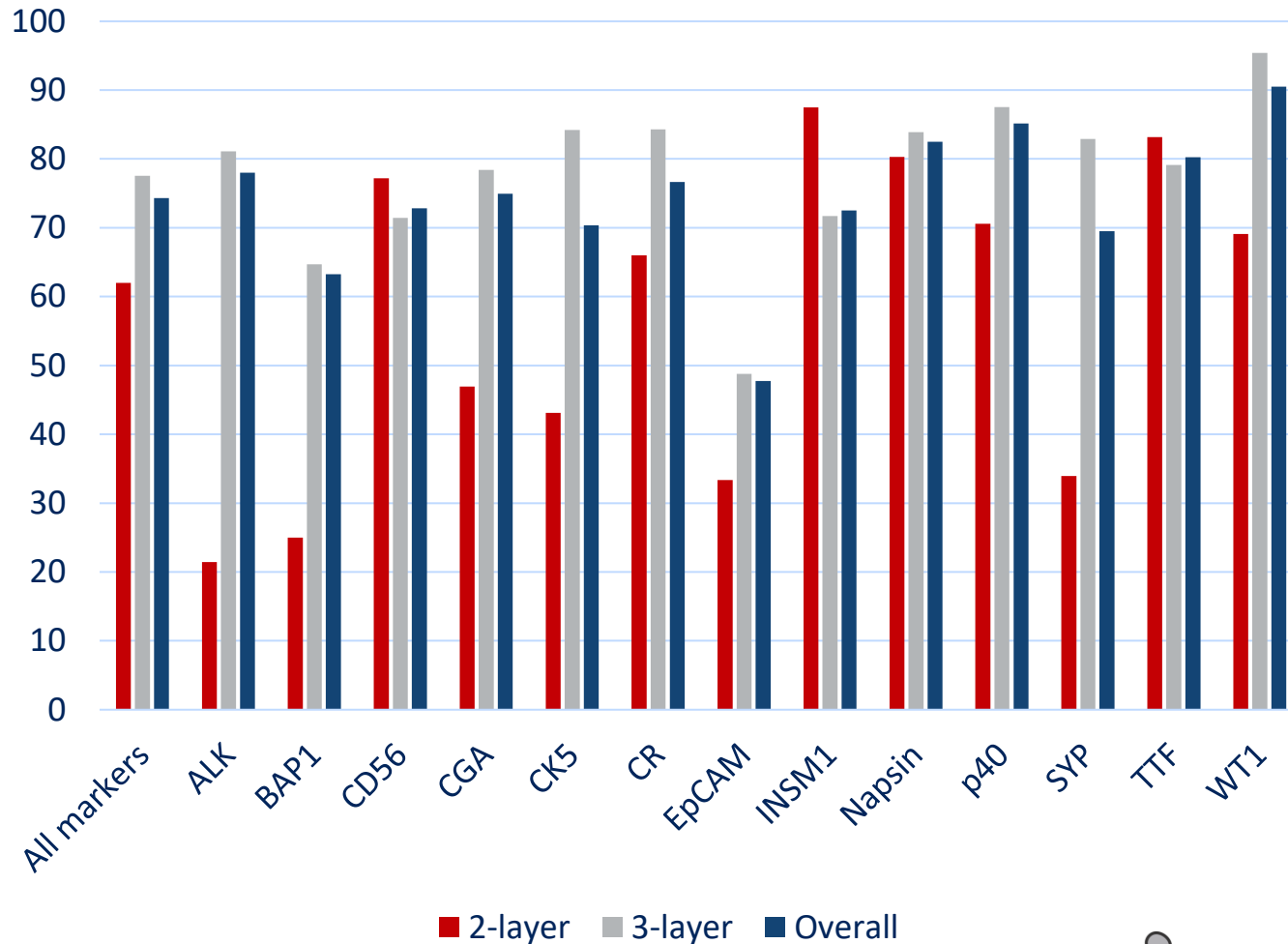
*Ranging from 25% for ALK and CD56 till 100% for CK5**

**9/9 participants used a low pH buffer on a Leica platform.*



KEY-POINTS FOR BEST PROTOCOLS

Detection systems, pass rates



Lung markers in the General Module:

3-layer detection system: 78%

- OptiView, UltraView DAB + amplification
- EnVision Flex +, Flex++ (4-layer)
- Bond Refine

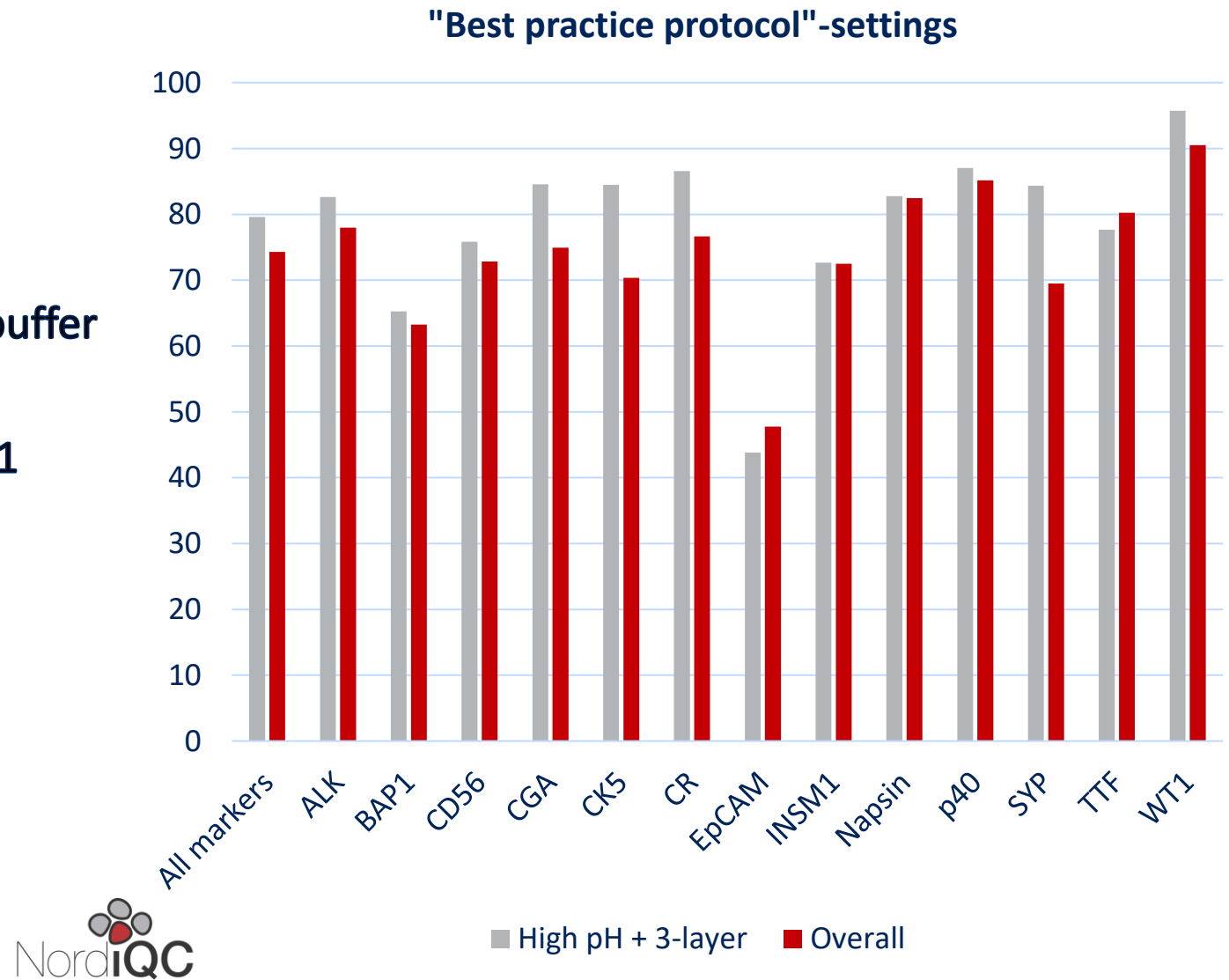
2-layer detection system: 62%

KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

- Overall pass rate: **74%**
- “Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: **80%**
- Ranging from 44% for EpCAM till 96% for WT1

*Clone selection is NOT included.



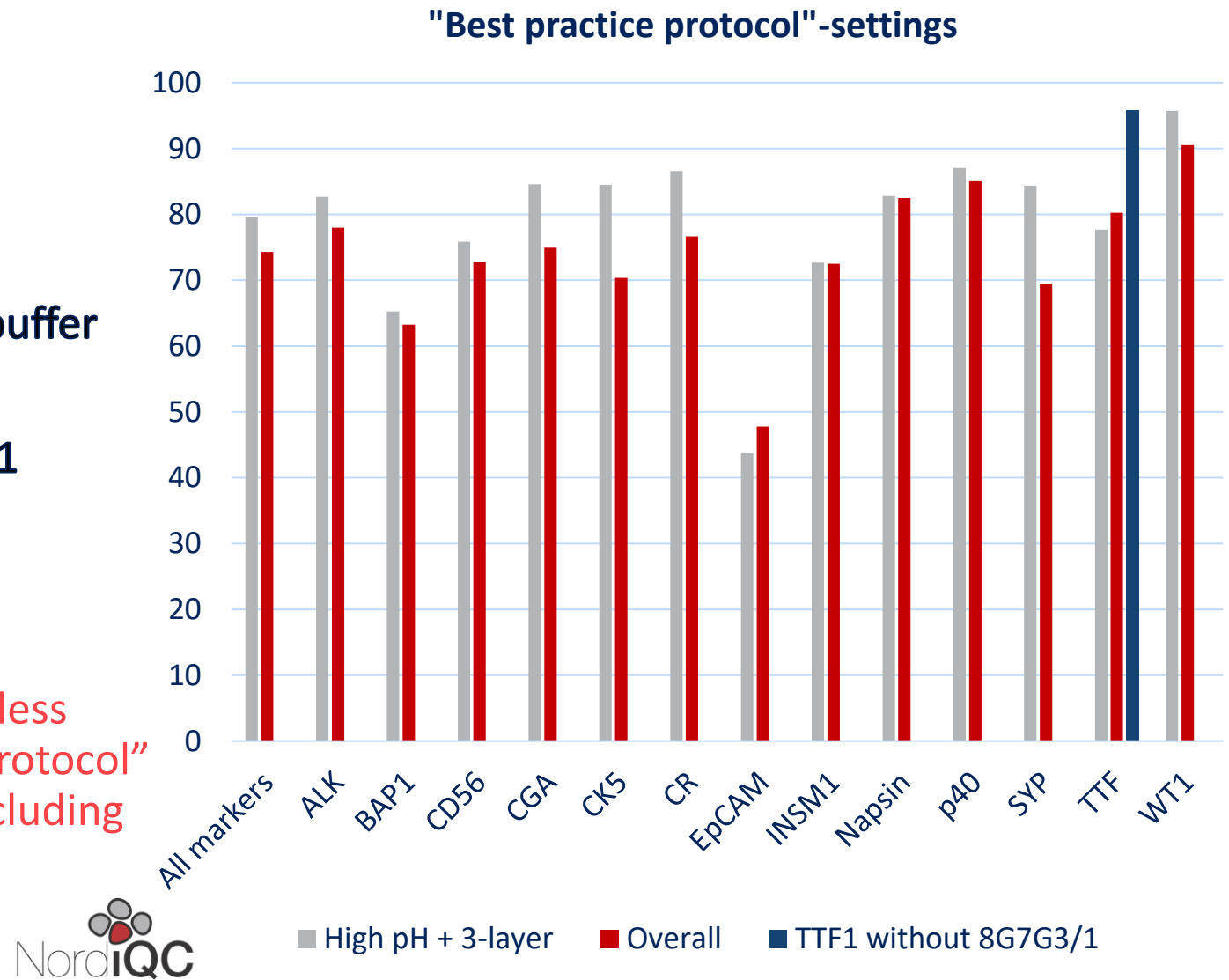
KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

- Overall pass rate: **74%**
- “Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: **80%**
- Ranging from 44% for EpCAM till 96% for WT1

*Clone selection is NOT included.

- E.g. TTF; if not counting participant using the less successful clone 8G7G3/1, the “best practice protocol” pass rate would be **96%** compared to 78% if including the clone.





NOW TIME TO LOOK
AT SOME SPECIFIC
MARKERS

Table 1. Antibodies and assessment marks for CR, run 64

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 2E7	1	BioGenex	0	0	1	0	-	-
mAb clone 5A5	1	Monosan	1	0	0	0	-	-
mAb clone ZM85	1	Zeta Corporation	0	1	0	0	-	-
mAb clone CAL6	19	Leica Biosystems	12	4	1	2	84%	63%
mAb clone DAK-Calret 1	25	Dako/Agilent	6	12	6	2	69%	23%
rmAb clone BSR235	1	Thermo Scientific	1	0	0	0	-	-
rmAb clone SP13	1	Nordic Biosite	1	0	0	0	-	-
	1	Cell Marque						
	1	Zytomed Systems						
	1	Abcam	0	2	2	2	33%	-
	1	Epredia						
pAb 18-0211	1	Diagnostic Biosystems						
	1	Zeta Corporation						
pAb 232A	6	Invitrogen/Thermo S. Zymed	5	1	1	0	86%	71%
	1	Cell Marque	0	0	0	1	-	-
pAb 61-0006	1	Genemed	1	0	0	0	-	-
pAb, CP092C	1	Biocare Medical	0	1	0	0	-	-
pAb RBK003	1	Zytomed Systems	0	1	0	0	-	-
pAb CR7696	1	Swant	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone CAL6 PA0346³	8	Leica Biosystems	4	4	0	0	100%	50%
mAb clone CAL6 PA0346⁴	10	Leica Biosystems	3	3	3	1	60%	30%
mAb clone DAK-Calret 1 IS/IR627³	16	Dako/Agilent	3	5	7	1	50%	19%
mAb clone DAK-Calret 1 IS/IR627⁴	43	Dako/Agilent	5	15	11	12	47%	12%
mAb clone C5G4 CCM-0222	1	Celnovte Biotechnology	1	0	0	0	-	-
mAb clone IHC523 IHC523	1	GenomeMe	1	0	0	0	-	-
rmAb SP13 232R	4	Cell Marque	2	0	1	1	-	-
rmAb SP13 MAD-000315QD	1	Master Diagnostica	0	0	1	0	-	-
rmAb BSR235 MAD-000784QD	2	Master Diagnostica	0	0	1	1	-	-
rmAb RM324 8522-C010	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone SP65 790-4467³	2	Ventana/Roche	2	0	0	0	-	-
rmAb clone SP65 790-4467⁴	177	Ventana/Roche	120	38	18	1	89%	68%
pAb 232A	2	Cell Marque	0	0	1	1	-	-
pAb IP092	1	Biocare Medical	0	0	1	0	-	-
pAb HAP134	1	PathnSitu	0	1	0	0	-	-
pAb 08-1211	1	Invitrogen/Thermo S.	0	0	1	0	-	-
Total	339		169	88	56	26	-	
Proportion			50%	26%	16%	8%	76%	

CALRETININ – PITFALLS

Table 2. Proportion of optimal results for CR for the most commonly used antibodies as concentrates on the 4 main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra	Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5 CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone CAL6	-	-	10/10 ** (100%)	-	-	1/1	-
mAb clone DAK-Calret 1	1/1	-	0/4	-	0/2	2/4	1/2
pAb 18-0211	1/1	-	2/2	-	1/3	1/1	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** (number of optimal results/number of laboratories using this buffer)

Less successful performance on the fully-automated Dako Omnis and Ventana BenchMark platforms for the most widely used conc. Abs

RTU products for Ventana and Leica users

Table 3. Proportion of sufficient and optimal results for CR for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND mAb CAL6 PA0346	100% (8/8)	50% (4/8)	63% (5/8)	25% (2/8)
Dako AS mAb DAK-Calret 1 IR/IS627	50% (8/16)	19% (3/16)	75% (6/8)	38% (3/8)
VMS Ultra/XT rmAb SP65 790-4467	(2/2)	(2/2)	89% (154/173)	67% (116/173)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer are integrated.

Omnis users cannot use the Autostainer RTU: 36% pass rate (12/33)

UltraView:
88% pass rate (65% optimal)

OptiView:
100% pass rate (78% optimal)

WT1 – PITFALLS

Table 1. Antibodies and assessment marks for WT1, Run 55

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 6F-H2	52	Dako/Agilent						
	13	Cell Marque						
	2	BioCare						
	2	DCS						
	2	Diagnostic BioSystems						
	2	Immunologic						
	1	Zeta						
mAb clone WT49	13	Leica						
	1	Immunologic						
rmAb clone D817F	3	Cell Signaling						
rmAb clone EP122	3	Epitomics						
	1	Cell Marque						
pAb RB-9367-P	1	Neomarkers						
Ready-To-Use Antibodies								
mAb clone 6F-H2 760-4397	92	Ventana/Cell Marque	40	37	14	1	84%	94%
mAb clone 6F-H2 IR055/IS055	33	Dako/Agilent	30	3	0	0	100%	100%
mAb clone 6F-H2 IR055/IS055 ³	25	Dako/Agilent	21	3	1	0	96%	-
mAb clone 6F-H2 IR055/IS055 ⁴	9	Dako/Agilent	5	3	1	0	-	-
mAb clone 6F-H2 348M-98 ⁵	14	Cell Marque	5	7	2	0	86%	-
mAb clone 6F-H2 MAD-005671QD	2	Master Diagnostica	2	0	0	0	-	-
mAb clone MX012 MAB-0678	1	Maixin	1	0	0	0	-	-
mAb clone WT49 PA0562	17	Leica	17	0	0	0	100%	100%
mAb clone WT49 PA0562 ⁶	1	Leica	1	0	0	0	-	-
rmAb clone EP122 8340	1	Sakura	1	0	0	0	-	-
Total	291		176	87	25	3	-	
Proportion			60%	30%	9%	1%	90%	

1) Proportion of sufficient stains (optimal or good)

2) Proportion of sufficient stains with optimal protocol settings only, see below.

3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on the Dako/Agilent full-automatic platform (Dako Omnis).

4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on different platforms (e.g. Ventana Benchmark, BioCare IntelliPath and Leica Bond).

5) RTU format not developed for a specific platform, but used by laboratories on the Ventana Benchmark platform.

6) RTU system developed for the Leica Bond system, but used on the Ventana Benchmark platform.

Table 4. Proportion of sufficient and optimal results for WT1 for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone 6F-H2, 760-4397	80% (20/25)	20% (5/25)	85% (57/67)	52% (35/67)
Dako AS mAb clone 6F-H2, IR055/IS055	100% (21/21)	95% (20/21)	100% (12/12)	83% (10/12)
Leica Bond mAb clone WT49, PA0562	100% (8/8)	100% (8/8)	100% (9/9)	100% (9/9)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

The most successful modifications were based on combined retrieval and use of OptiView, giving a pass rate of 96% with 66% optimal.

Concentrated Abs can be used on Omnis, but limited data..

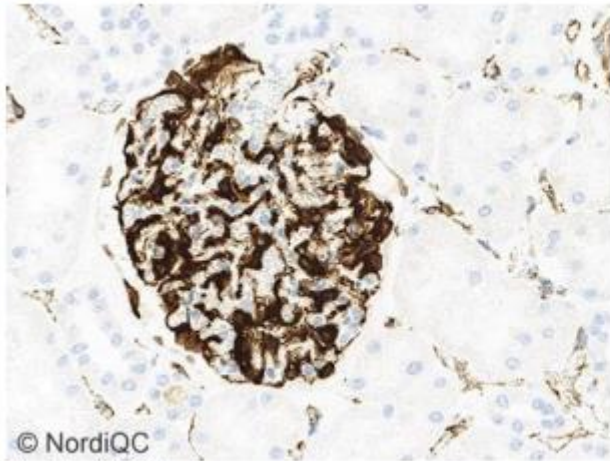
Table 3. Proportion of optimal results for WT1 for the most commonly used antibodies as concentrates on the four main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone 6F-H2	8/9** 89%	1/1	2/6 33%	-	10/24 42%	4/12 33%	-	8/13 62%	1/2
mAb clone WT49	2/3	-	1/1	-	4/5 80%	-	-	3/4	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

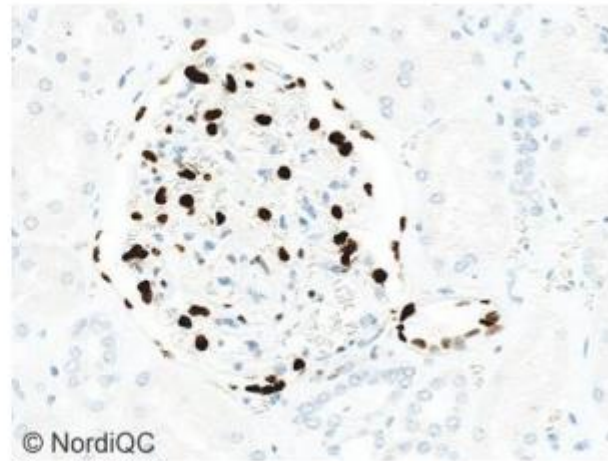
** Number of optimal results/number of laboratories using this buffer

WT1 – PITFALLS



© NordiQC
Fig. 7a (x300)

If using HI ER as single pre-treatment, both a nuclear and cytoplasmic staining reaction is seen.



© NordiQC
Fig. 7b (x300)

If using a combined pre-treatment using HI ER followed by a weak proteolysis, only a nuclear staining reaction is seen.

mAb clone 6F-H2:

Pre-treatment method determines the outcome.

Depending on the purpose of the test, a combined pre-treatment is making the interpretation easier.

A cytoplasmic cross-reaction can be used for vascular lesions, that will be negative if using the combined pre-treatment.

Int J Clin Exp Pathol 2014;7(5):2536-2543
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Original Article

Diagnostic utility of WT-1 cytoplasmic stain in variety of vascular lesions

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Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone BS14	25	Nordic Biosite	19	6	0	0	100%	76%
mAb clone Ber-Ep4	40	Dako/Agilent	5	11	30	3	33%	10%
	8	Cell Marque						
	1	Thermo Scientific						
mAb clone MOC-31	16	Dako/Agilent	1	14	4	1	75%	5%
	1	Biocare Medical						
	2	Cell Marque						
	1	Leica Biosystems						
mAb clone VU-1D9	9	Thermo Scientific	4	8	2	1	80%	27%
	1	Merck Millipore						
	4	Diagnostic Biosystems						
	1	Monosan						
mAb clone C-10	2	Santa Cruz	0	0	2	0	-	-
mAb clone ZM131	1	Zeta Corporation	0	0	0	1	-	-

Ready-To-Use antibodies

mAb clone Ber-Ep4 760-4383³	13	Ventana/Roche	0	0	13	0	0%	0%
mAb clone Ber-Ep4 760-4383⁴	86	Ventana/Roche	1	20	60	5	24%	1%

mAb clone Ber-Ep4 248M-98	15	Cell Marque	0	1	13	1	7%	0%
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mAb clone Ber-Ep4 IR/IS637³	1	Dako/Agilent	0	0	1	0	-	-
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mAb clone Ber-Ep4 IR/IS637⁴	15	Dako/Agilent	3	2	10	0	33%	20%
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mAb clone Ber-Ep4 GA637³	44	Dako/Agilent	12	30	2	0	95%	27%
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mAb clone Ber-Ep4 GA637⁴	16	Dako/Agilent	3	8	5	0	69%	19%
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mAb clone Ber-Ep4 PM107	3	Biocare Medical	0	0	1	2	-	-
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mAb clone Ber-Ep4 MAD-001709QD	3	Master Diagnostica	0	0	2	1	-	-
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mAb clone Ber-Ep4 PDM131	2	Diagnostic Biosystems	0	0	0	2	-	-
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mAb clone Ber-Ep4 P-E002	1	Quartett	0	0	0	1	-	-
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mAb clone Ber-Ep4 BMS048	1	Zytomed Systems	0	1	0	0	-	-
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mAb clone Ber-Ep4 GM080402	2	Gene Tech	0	1	1	0	-	-
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mAb clone MOC-31 790-4561¹	3	Ventana/Roche	0	1	2	0	-	-
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mAb clone MOC-31 790-4561⁴	10	Ventana/Roche	2	6	2	0	80%	20%
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mAb clone MOC-31 248M-18	4	Cell Marque	0	1	3	0	-	-
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mAb clone MOC-31 CEM-0051	1	Celnovte	0	0	1	0	-	-
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mAb clone VU-1D9 PDM077	2	Diagnostic Biosystems	0	1	0	1	-	-
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mAb clone VU-1D9 8230-C010	3	Sakura FineTek	0	1	2	0	-	-
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mAb clone BS14 8377-C010	1	Sakura FineTek	1	0	0	0	-	-
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mAb clone SPM491 ab228023	1	abcam	0	0	0	1	-	-
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mAb clone MX066 MAB-0850	1	Fuzhou Maxin	0	1	0	0	-	-
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mAb clone BP6056 BX50051	1	Biolyntx	0	0	1	0	-	-
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mAb clone IHC567 IHC567-7/25	1	GenomeMe	0	0	1	0	-	-
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rmAb clone EP155 AN820	1	BioGenex	0	0	0	1	-	-
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rmAb clone BY118 BFM-0436	1	Bioin Biotechnology	0	0	1	0	-	-
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rmAb clone DA056 RMA1A068	1	Shenzhen Dartmon Biotechnology	0	0	0	1	-	-
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Total	345		51	113	159	22		
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Proportion			15%	33%	46%	6%	48%	
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EP-CAM – PITFALLS

Table 2. Proportion of optimal results for EpCAM for the most commonly used antibodies as concentrate on the four main IHC systems*

Concentrated antibodies	Dako Autostainer Link/Classic		Dako Omnis		Ventana BenchMark GX /XT/ Ultra / Ultra Plus			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 2/3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone BS14	-	-	1/2**	-	6/7 (86%)	10/13 (77%)	-	1/2	-
mAb clone Ber-EP4	0/1	1/1	-	5/6 (83%)	0/2	0/4	0/1	0/1	0/2
mAb clone MOC-31	0/1	-	-	1/3	0/4	-	-	-	0/1
mAb clone VU-1D9	-	-		0/1	4/5 (80%)		-	0/1	-

Less successful performance of the Ventana RTU. Conc. formats of e.g. mAb BS14 and VU-1D9 can be used on BenchMark platforms.

Table 3. Proportion of sufficient and optimal results for EpCAM for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
BenchMark XT/Ultra mAb Ber-EP4 760-4383	0% (0/13)	0% (0/13)	25% (21/85)	1% (1/85)
Autostainer +/-Link mAb Ber-EP4 IS/IR637	(0/1)	(0/1)	40% (4/10)	30% (3/10)
Omnis mAb Ber-EP4 GA637	95% (42/44)	27% (12/44)	73% (11/15)	27% (3/11)
BenchMark XT/Ultra mAb MOC-31 790-4561	(1/3)	(0/3)	80% (8/10)	20% (2/10)

RTU for Dako Omnis a obtained high pass rate, but few optimal. Use of a 3-layer detection system for IR637 increases sufficient results.

Table 1. Antibodies and assessment marks for CK5, run 65								
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff ¹	OR ²
mAb clone D5/16 B4*	35 2 1 1 2	Dako/Agilent Cell Marque Millipore Epredia Zytomed	6	15	19	1	51%	15%
mAb clone XM26	2 3 64 3	Abcam Diagnostic BioSystems Leica Biosystems Monosan	56	11	4	1	93%	78%
mAb clone IHC556*	1	GenomeMe	0	0	1	0	-	-
mAb clone ZM186	1	Zeta Corporation	0	0	1	0	-	-
rmAb clone BSR55	2	Nordic Biosite	1	0	1	0	-	-
rmAb clone EP1601Y	3	Cell Marque	0	1	2	0	-	-
rmAb clone EP24/EP67*	2	Cell Marque	0	2	0	0	-	-
rmAb clone EP24	1	Epitomics	0	1	0	0	-	-
rmAb clone EP42	1	Epitomics	1	0	0	0	-	-
rmAb clone SP27	1	Immunologic	1	0	0	0	-	-
rmAb clone QR027	1	Quartett	0	1	0	0	-	-
mAb clone XM26/SF13**	1	DCS Innovative Diagnostik-Systeme	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone D5/16 B4* 790-4554³	6	Ventana/Roche	0	3	3	0	50%	0%
mAb clone D5/16 B4* 790-4554⁴	46	Ventana/Roche	9	23	11	3	70%	20%
mAb D5/16 B4* GA780³	13	Dako/Agilent	0	1	12	0	8%	0%
mAb D5/16 B4* GA780⁴	26	Dako/Agilent	0	9	16	1	35%	0%
mAb clone D5/16 B4* IR/IS780³	4	Dako/Agilent	0	1	2	1	-	-
mAb clone D5/16 B4* IR/IS780⁴	9	Dako/Agilent	1	1	4	3	22%	11%
mAb clone D5/16 B4* 3295-C010	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone RM226 8408-C010	1	Sakura Finetek	0	1	0	0	-	-
mAb clone XM26 PA0468³	7	Leica Biosystems	2	4	1	0	86%	29%
mAb clone XM26 PA0468⁴	9	Leica Biosystems	8	1	0	0	100%	89%
mAb clone XM26 PM234	3	Biocare Medical	2	1	0	0	-	-
rmAb clone EP1601Y 305R-17/18	4	Cell Marque	0	2	2	0	-	-
rmAb clone EP42 AN853-10M	1	BioGenex	0	1	0	0	-	-
rmAb clone EP24/EP67* MAD-000651QD	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone EP24/EP67* MRH1159	1	PathnSitu	0	1	0	0	-	-
rmAb clone SP27 760-4935³	21	Ventana/Roche	21	0	0	0	100%	100%
rmAb clone SP27 760-4935⁴	29	Ventana/Roche	26	3	0	0	100%	90%
rmAb clone C9E33 CCR-0973	1	Celnovte	0	0	1	0	-	-
mAb clone 150A8C1 PA018	1	Abcarta	0	0	1	0	-	-
Total	311		136	84	81	10		
Proportion			44%	27%	26%	3%	71%	

CK5 – PITFALLS

Table 2. Proportion of optimal results for CK5 for the most commonly used antibodies as concentrates on the four main IHC systems*

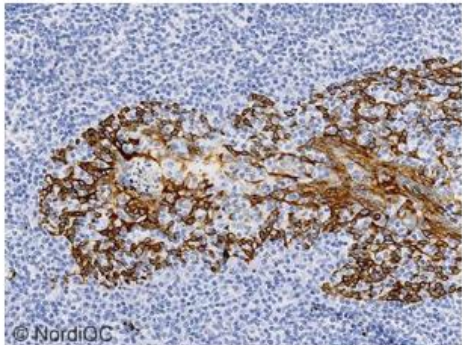
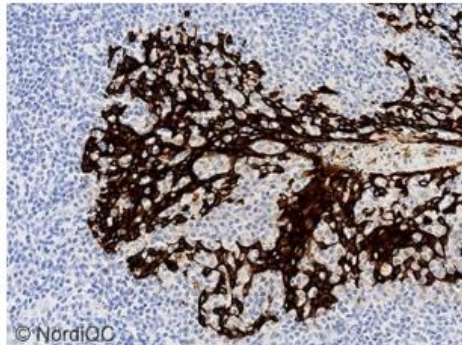
Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone D5/16 B4	0/2	-	0/2	-	5/12 (42%)	1/1	-	0/5 (0%)	0/2
mAb clone XM26	1/4	-	24/26 (92%)	-	17/24 (71%)	1/1	-	12/12 (100%)	1/1

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
 ** (number of optimal results/number of laboratories using this buffer).

Table 3. Proportion of sufficient and optimal results for CK5 for the most commonly used RTU IHC systems

RTU systems	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone D5/16 B4, 790-4554	50% (3/6)	0% (0/6)	70% (32/46)	20% (9/46)
Dako Omnis mAb clone D5/16 B4, GA780	8% (1/13)	0% (0/13)	36% (9/25)	0% (0/25)
Dako Autostainer mAb clone D5/16 B4, IR/IS780	(1/4)	(0/4)	0% (0/6)	0% (0/6)
Leica Bond mAb clone XM26, PA0468	86% (6/7)	29% (2/7)	100% (9/9)	89% (8/9)
Ventana Benchmark rmAb clone SP27, 760-4935	100% (21/21)	100% (21/21)	100% (27/27)	89% (24/27)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
 ** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.



Less successful performance of the mAb D5/16 B4 both as RTU and Conc.

mAb XM26 obtained optimal results on the main systems.

rmAb SP27 with a pass rate of 100%. However, the specificity is reduced compared to e.g. XM26...

OPEN

NordiQC Assessments of Keratin 5 Immunoassays
 Christian Thomsen, MD,* Ole Nielsen, HT,† Søren Nielsen, HT,* Rasmus Roge, MD,*‡ and Mogens Vyberg, MD*‡

Left: XM26 // Right: D5/16 B4

Table 1. Antibodies and assessment marks for ALK (lung), run 65

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 5A4	26	Leica Biosystems	8	9	14	4	49%	23%
	2	Monosan						
	1	Abcam						
	1	DBS						
	2	Biocare Medical						
mAb clone OTI1A4*	2	Zytomed Systems						
	1	Invitrogen						
	19	Origene						
	1	Nordic Biosite						
mAb clone IHC509	1	Cell Signaling	16	6	0	0	100%	73%
rmAb clone D5F3	1	Zeta Corporation	0	0	1	0	-	-
rmAb clone ALK1	19	GenomeMe	7	9	3	0	84%	36%
rmAb clone QR017	3	Cell Signaling	0	0	0	4	-	-
rmAb clone SP8	1	Dako/Agilent	0	0	0	0	-	-
rmAb clone ZR305	1	Cell Marque	0	0	0	0	-	-
Ready-To-Use antibodies								

mAb clone 5A4 PA0306**/PA0831 (VRPS) ³	2	Leica Biosystems	1	1	0	0	-	-
mAb clone 5A4 PA0306*/PA0831 (LMPS) ⁴	10	Leica Biosystems	4	3	2	1	70%	40%
mAb clone 5A4 API3041	1	BioCare	0	0	1	0	-	-
mAb clone 5A4 CAM-0170	1	Celnovte	0	1	0	0	-	-
mAb clone 5A4 MAD-0017200D	1	Master Diagnostica	0	0	1	0	-	-
mAb clone ALK1 GA641	3	Dako/Agilent	0	0	0	3	-	-
mAb clone ALK1 IR641	4	Dako/Agilent	0	0	0	4	-	-
mAb clone ALK1 790/800-2918 (LMPS) ⁴	10	Ventana/Roche	1	0	1	8	10%	10%
mAb clone 137E9E8 PA132	1	Abcarta	0	0	0	1	-	-
mAb clone OTI1A4 / 1A4 8344-C010	1	Sakura Finetek	1	0	0	0	-	-
mAb clone OTI1A4 / 1A4 GA785 (VRPS) ³	12	Dako/Agilent	12	0	0	0	100%	100%
mAb clone OTI1A4 / 1A4 GA785 (LMPS) ⁴	4	Dako/Agilent	4	0	0	0	-	-
rmAb clone D5F3 790-4794 (VRPS) ³	73	Ventana/Roche	62	7	1	3	95%	85%
rmAb clone D5F3 790-4794 (LMPS) ⁴	48	Ventana/Roche	36	9	3	0	94%	75%
rmAb clone SP8 RMPD007	1	Diagnostic BioSystems	0	0	0	1	-	-
Total	256		152	46	28	30		
Proportion			59%	18%	11%	12%	77%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).

2) Proportion of Optimal Results (≥5 assessed protocols).

3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).

4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).

*) OTI1A4 is called 1A4 by some vendors

**) Product no. PA0306 has been terminated and replaced by PA0831.

ALK-LUNG – PITFALLS

rmAb clone ALK1 is not "fit for purpose" for lung diagnostic!

- Be sure to order the right product as both Dako and Ventana have different clones on the market!

Table 4. Proportion of sufficient and optimal results for ALK (lung) for the most commonly used RTU IHC systems

RTU-systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT rmAb D5F3 790-4794	95% (69/73)	85% (62/73)	93% (41/44)	80% (35/44)
Dako Omnis mAb OTI1A4 GA785	100% (12/12)	100% (12/12)	(4/4)	(4/4)
Leica BOND mAb 5A4 PA0306/PA0831	(2/2)	(1/2)	75% (6/8)	50% (4/8)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

RTU products for the automated systems, working as plug-and-play



INSM1 – PITFALLS

Table 1b. Concentrated antibodies and assessment marks for INSM1, run 71

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone A-8	1	Diagnostic Biosystems	1	-	-	-	-	-
	5	Gennova	1	2	1	1	60%	20%
	1	Master Diagnostica	-	-	1	-	-	-
	1	Monosan	1	-	-	-	-	-
	2	Nordic Biosite	-	1	1	-	-	-
	50	Santa Cruz	10	15	13	12	50%	20%
	5	Zeta Corporation	4	1	-	-	100%	80%
mAb clone BSB-123	3	Bio SB	-	3	-	-	-	-
	1	LS Bio	-	1	-	-	-	-
rmAb clone MSVA-456R	1	MS Validated Antibodies	-	1	-	-	-	-
rmAb clone BP6240	1	Biolyx Biotechnology	1	-	-	-	-	-
rmAb clone EPR23199-37-6-1	1	Abcam	-	-	1	-	-	-
rmAb clone MRQ-70	27	Cell Marque	19	6	2	-	93%	70%
rmAb clone QR116	1	Quartett	-	-	-	1	-	-
rmAb clone RBT-INSM1	2	Bio SB	-	1	1	-	-	-
rmAb clone ZR395	2	Zeta Corporation	2	-	-	-	-	-
Total	104		39	31	20	14		
Proportion			38%	30%	19%	13%	68%	

Table 2. Proportion of optimal results for INSM1 for the most commonly used antibody concentrates on the four main IHC systems*

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone A-8	2/5** (40%)	1/1	6/12 (50%)	0/2	2/30 (7%)	1/2	5/10 (50%)	0/1
rmAb clone MRQ-70	1/2	-	5/9 (56%)	-	12/14 (86%)	-	1/2	-

The performance and stability of mAb clone A-8 seems to depend on the vendor. Many different lot numbers from the Santa Cruz Ab were submitted, with varying results:

- Optimal
- Good – poor signal-to-noise ratio, weak
- Borderline/poor – false positive and/or false negative

INSM1 – PITFALLS

Table 1c. Ready-To-Use antibodies and assessment marks for INSM1, run 71

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb A-8 AMB44-5M	2	BioGenex	1	-	-	1	-	-
mAb clone A-8 MAD-000777QD	10	Master Diagnostica/ Vitro SA	2	6	2	-	80%	20%
mAb clone A-8 PDM586	3	Diagnostic Biosystems	1	1	-	1	-	-
mAb clone A-8 MAB-1017	1	Fuzhou Maixin	-	1	-	-	-	-
mAb clone BSB-123 BSB 3553/4/5	11	Bio SB	-	7	2	2		
mAb clone DA267 DMRD0168	1	Dartmon Biotechnology	1	-	-	-	-	-
mAb clone BY059 BFM-0177	1	Bioin Biotechnology	-	1	-	-	-	-
rmAb clone IHC741 IHC741	1	GenomeMe	-	1	-	-	-	-
rmAb clone BLR272L API3299	1	Biocare Medical	-	1	-	-	-	-
rmAb clone 315I4E7 PA598	1	Abcarta	-	1	-	-	-	-
rmAb clone GR013 GT246802	1	Gene Tech	1	-	-	-	-	-
rmAb clone INSM1/6286R ANC07-5M	1	BioGenex	-	-	-	1	-	-
rmAb clone MRQ-70 475-97/98	16	Cell Marque	9	6	-	1	94%	56%
rmAb clone RBT-INSM1 BSB-3780-3/7/15	1	Bio SB	-	1	-	-	-	-
rmAb clone ZR395 Z2751RP	1	Zeta Corporation	1	-	-	-	-	-
Total	52		16	26	4	6		
Proportion			31%	50%	8%	11%	81%	

No RTUs from “main” vendors

Applied on BenchMark Ultra and Dako Omnis

Applied on BenchMark Ultra, Dako Omnis and Leica Bond

Table 2a. Overall results for PD-L1 TPS/CPS, run C15

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
CE-IVD / FDA approved PD-L1 assays*	143	97	33	9	4	91%	68%
Laboratory developed PD-L1 assays based on concentrated antibodies	66	37	14	12	3	77%	56%
PD-L1 assays based on Ready-To-Use antibodies without predictive claims	46	32	11	3	0	93%	69%
Total	255	166	58	24	7		
Proportion		65%	23%	9%	3%	88%	

Table 2b. Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 TPS/CPS, run C15

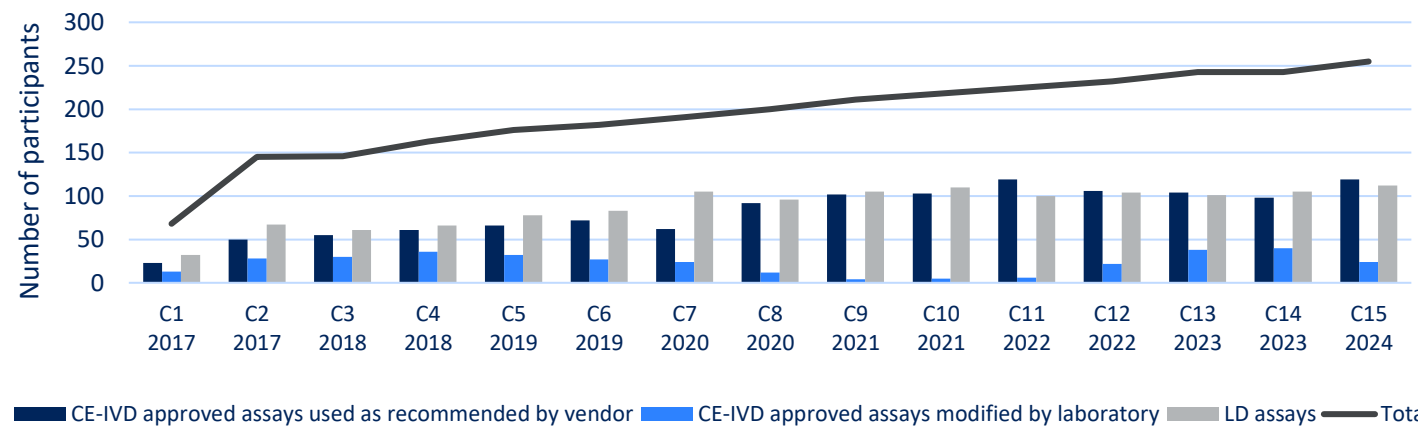
CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone SP263, 741-4905 (VRPS) ³	39	Ventana/Roche	16	19	1	3	90%	41%
rmAb clone SP263, 741-4905 (LPMS) ⁴	5	Ventana/Roche	3	1	1	-	80%	60%
rmAb clone SP263, 740-4907 (VRPS) ³	13	Ventana/Roche	9	4	-	-	100%	69%
mAb clone 22C3 pharmDX, SK006 (VRPS) ³	25	Dako/Agilent	17	4	3	1	84%	68%
mAb clone 22C3 pharmDX, SK006 (LPMS) ⁴	8	Dako/Agilent	6	1	1	-	88%	75%
mAb clone 22C3 pharmDX, GE006 (VRPS) ³	39	Dako/Agilent	38	1	-	-	100%	97%
mAb clone 22C3 pharmDX, GE006 (LPMS) ⁴	11	Dako/Agilent	8	2	1	-	91%	73%
rmAb clone 28-8 pharmDX, SK005 (VRPS) ³	3	Dako/Agilent	-	1	2	-	-	-
Total	143		97	33	9	4		
Proportion			68%	23%	6%	3%	91%	

Table 2d. Assessment marks for Ready-To-Use antibodies⁶ for PD-L1 TPS/CPS, run C15

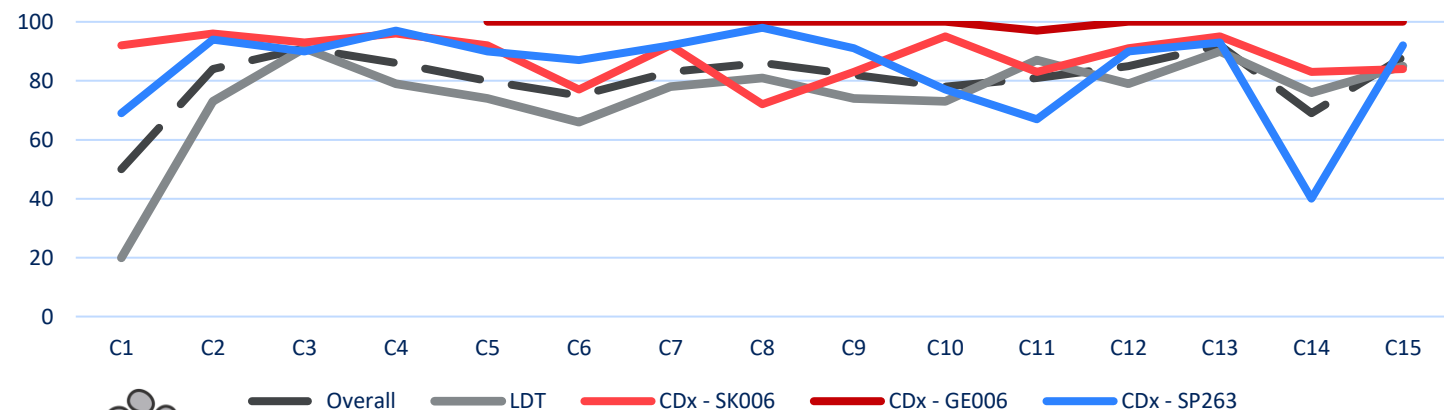
Ready-To-Use antibodies ⁶	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone SP263, 790-4905 ⁶ (VRPS) ³	14	Ventana/Roche	12	2	-	-	100%	86%
rmAb clone SP263, 790-4905 ⁶ (LPMS) ⁴	21	Ventana/Roche	15	5	1	-	95%	71%
rmAb clone SP142, 790-4860 (LPMS) ⁴	1	Ventana/Roche	1	-	-	-	-	-
rmAb clone 73-10 PA0832	4	Leica Biosystems	1	3	-	-	-	-
rmAb MX070C MAB-0854	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone AC37 PA168	1	Abcarta	1	-	-	-	-	-
rmAb clone BP6099 I12052E	1	Biolyntx	-	1	-	-	-	-
rmAb clone E1L3N P05B01	1	MEDx Translational Medicine	-	-	1	-	-	-
rmAb GR110 GT256202	1	Gene Tech	-	-	1	-		
rmAb clone RM320 8263-C010	1	Sakura Finetek	1	-	-	-		
Total	46		32	11	3	0		
Proportion			69%	24%	7%	0%	93%	

PD-L1

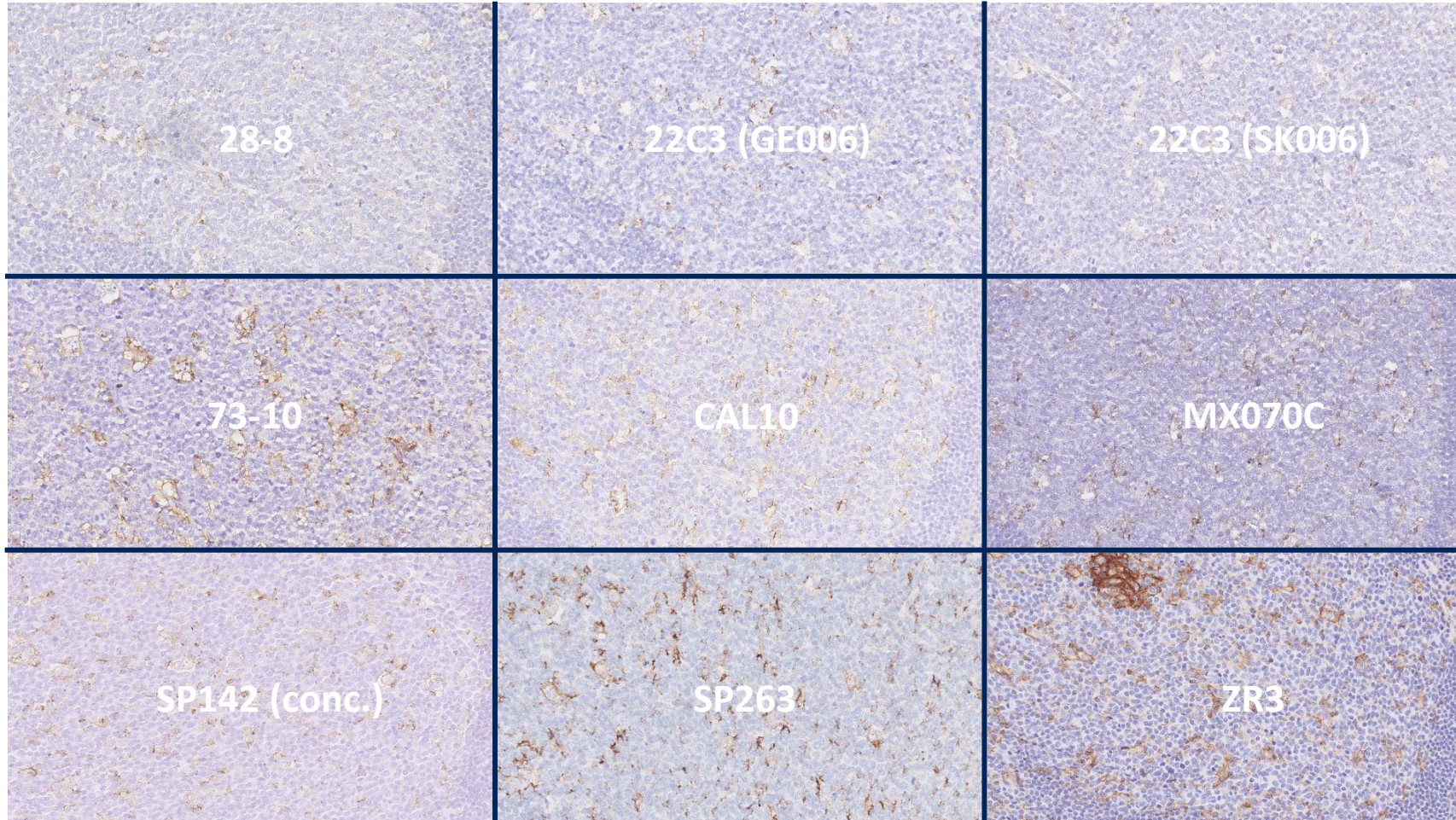
Use of IHC assays in PD-L1 TPS/CPS run C1-C15



Pass rate - PD-L1 assays for TPS/CPS, NordiQC



PD-L1 – ICAPS - TONSIL



In tonsil, a weak to moderate staining reaction in germinal center macrophages should be seen.



Different assays → different staining patterns.

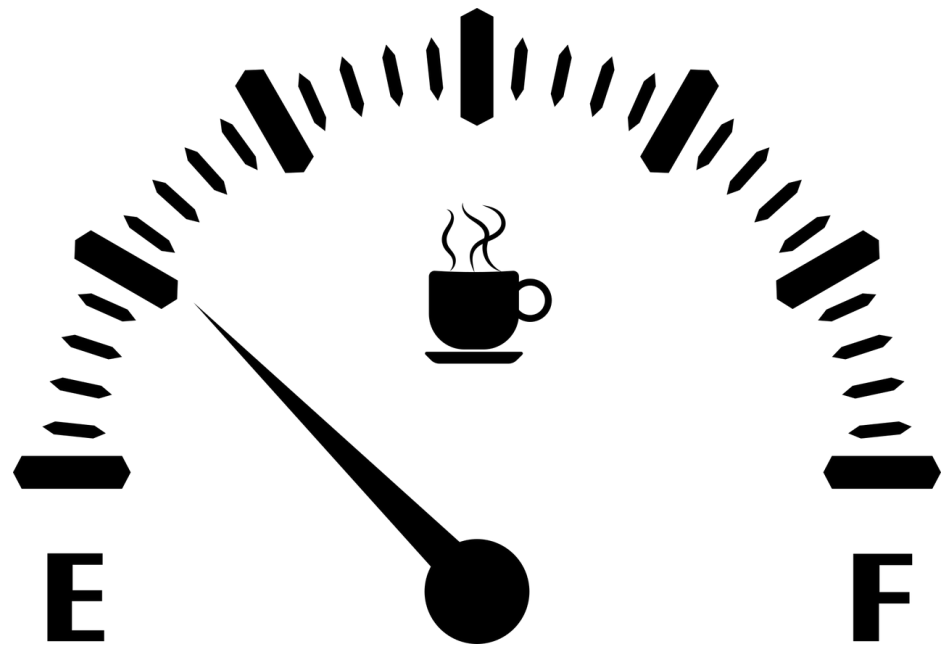
All 9 assays achieved an optimal score for PD-L1 TPS/CPS.

ICAPS FOR SELECTED LUNG MARKERS

Marker	IHC critical assay performance controls	Negative tissue controls
	Low expression	No expression
TTF1	Lung: Columnar epithelial cells of terminal bronchi.	Tonsil: All cell types. Link
Napsin A	Kidney: Epithelial cells of proximal tubules.	Appendix/Colon: Epithelial cells and macrophages. Link
Calretinin	Adrenal gland: Cortical epithelial cells.	Appendix/Colon: Epithelial cells. Link
WT1	Kidney: Podocytes and parietal epithelial cells of Bowman's capsule.	Kidney: Epithelial cells of the tubules. Link
BAP1	Tonsil: Mantle zone lymphocytes and germinal centre lymphocytes.	Malignant Mesothelioma: Neoplastic cells Link
INSM1	Pancreas: Endocrine islets of Langerhans.	Appendix: Columnar epithelial cells and smooth muscle cells. Link
CGA	Appendix/Colon: Axons and ganglion cells in the nerve plexus.	Appendix/Colon: Epithelial cells and smooth muscle cells. Link
SYP	Appendix/Colon: Neuroendocrine and scattered goblet cells in epithelial mucosa.	Appendix/Colon: Smooth muscle cells Link
CD56	Tonsil: NK-cells and scattered T-cells.	Appendix/Colon: Epithelial cells. Link
p40	Placenta: Dispersed cytotrophoblastic cells.	Tonsil: Lymphocytes. Link
CK5	Pancreas: Scattered epithelial cells of intercalated ducts.	Liver. All cell types. Link
ALK (lung)	Appendix/Colon: Dispersed axons of nerve cells.	Tonsil: All cell types. Link
PD-L1 TPS/CPS	Tonsil: Germinal center macrophages and T-cells.	Tonsil: Stratified normal squamous epithelial cells and vast majority of lymphocytes. Link



THANK YOU FOR YOUR ATTENTION!



BONUS – ROS1

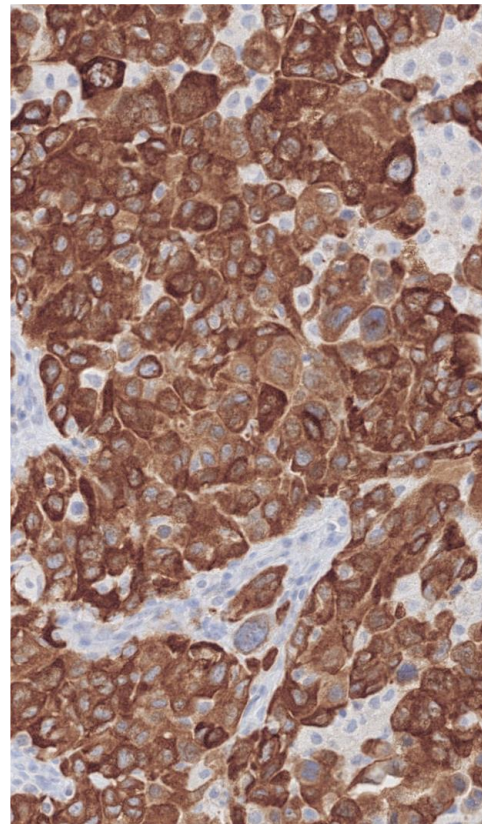
No NordiQC data available for ROS1.

For these stains, the Ventana RTU based on rmAb SP384 is used.

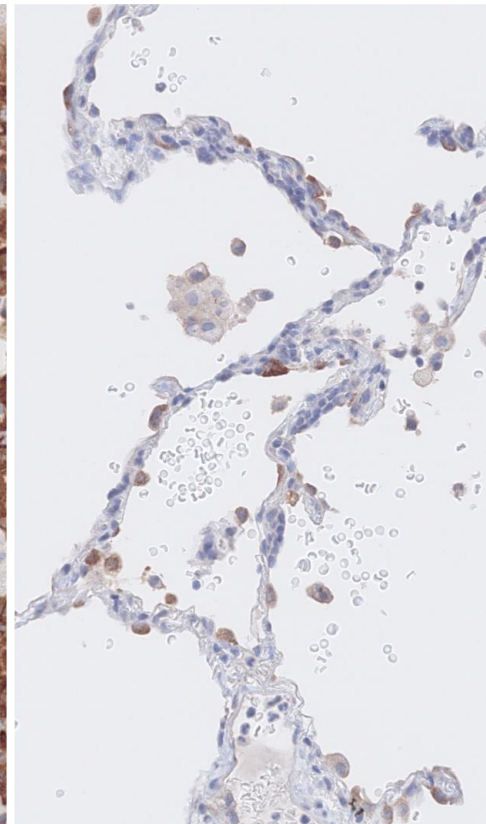
Positive controls:
Tumor with known ROS1-translocation
Type II-pneumocytes in normal lung

Negative control:
Appendix

Tumor with ROS1-
translocation
(lung adenocarc.)



Normal lung



Appendix

