Immunocytochemistry and immunohistochemistry on frozen sections

Overview, considerations and applications

NQC workshop 2018

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IHC on cryostat sections (55) (Odense)

Antibody
MyoD1, EP212
CD56, MRQ-42
Serca1, VE121G9
C1q-FITC
Albumin-FITC
Fibrinogen-FITC
C3-FITC
Lambda-FITC
Kappa-FITC
IgA-FITC
IgM-FITC
IgG-FITC
Dystrophin, 34C5
Desmin, DE-R-11
Collagen IV, CIV22
Collagen VI, VI-26
Pax-7, P3U1
Laminin alfa2, 4H8-2
Laminin alfa2, Mer3/22B2
Calpain, 12A2

Antibody
Dysferlin, Ham1/7B6
Sarcoglycan g, 35DAG/21B5
Sarcoglycan delta, ∂-Sarc/12C1
Sarcoglycan beta, ß-SARC/5B1
Laminin alfa5, 4C7
Caveolin 3, 26
Emerin, 4G5
Utrophin, DRP2/20C5
Serca2, IID8
Myosin neonatal, WB-MHCn
Myosin slow, WB-MHCs
Myosin -Fast, MY32
Laminin beta2, C4
Laminin beta1, 4E10
Dystrophin, DY8/6C5
Dystrophin, DY4/6D3
Dystroglycan beta, NCL-43DAG
Actinin alfa, RBC2/1B6
Sarcoglycan alfa, Ad1/20A6

Antibody
Cranin, VIA4-1
CD4, SP35
CD45, 2B11 & PD7/26
CD56, 56C04
C5b-9, aE11
CD57, HNK-1
CD8, C8/144B
CD20cy, L26
Collagen IV, MAB3
Collagen IV, MAB1
GFAP, p
HLA-DR, CR3/43
HLA-ABC, W6/32
CD68, EBM11
Collagen IV, MAB5

ICC protocols (64) (Odense)

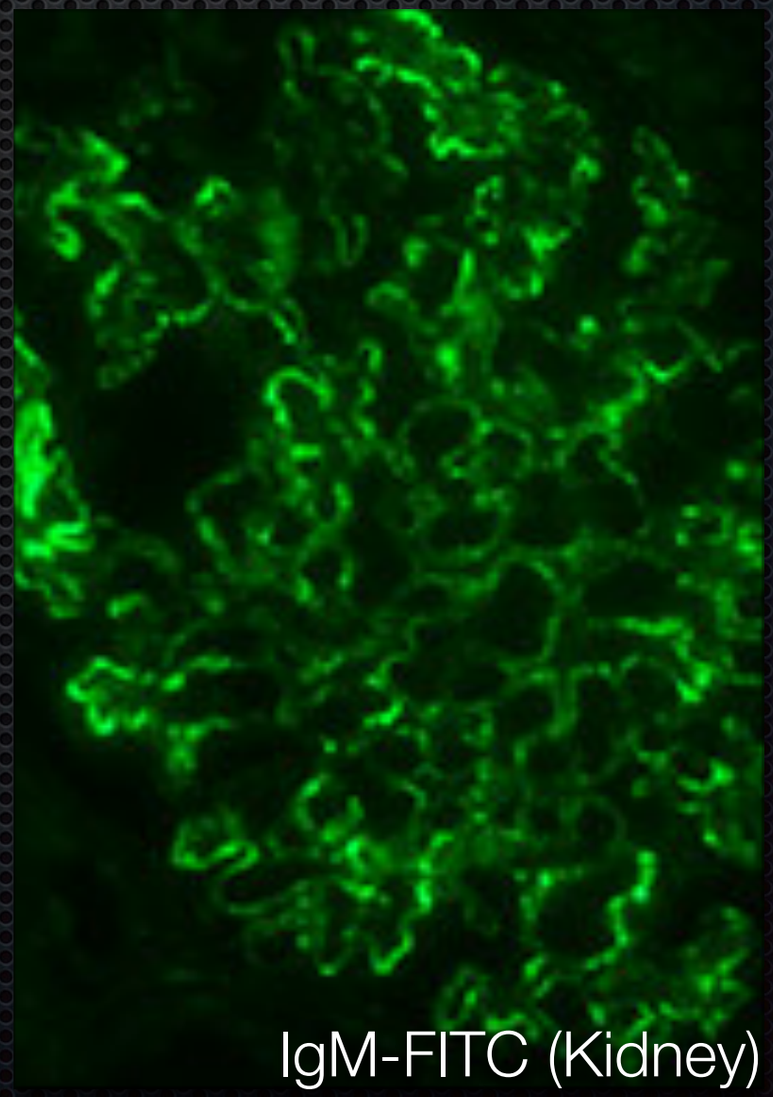
Antibody
PAX-8, ZR-1 (Zeta)
CD117, YR145
CD14, 7 -
CD19, SP110
CD1a, EP3622
CD2, MRQ-11
CD20cy, L26
CD23, SP23
CD3, 2GV6
CD33, PWS44
CD34, EP88
CD4, SP35
CD45, 2B11 & PD7/26
CD5, SP19
CD56, MRQ-42
CD71, 10F11
CD8, C8/144B
CK20, SP33
CA-125, OC125
CDX2, EPR2764Y
EMA, E29
Ep-CAM, Ber-EP4

Antibody
CD15, MMA
CD30, Ber-H2
CD61, 2f2
TdT, SEN28
CK17, SP95
CK19, A53-B/A2.26
CK5, XM26
CK7, SP52
CK, AE1/AE3
CK, CAM 5.2
CD68, EBM11
Calretinin, SP65
CEA, Col-1
GATA3, L50-823
Mesotelial Cell, HBME-1
Napsin A, IP64
P501S, 10E3
TPO, MoAb47
Vimentin, V9
Melan-A, A103
S-100, p
Synaptophysin, 27G12

Antibody
CD10, 56C6
CD42b, MM2/174
CD79a, SP18
HEPA, OCH1E5
PSA, p
TTF-1, SPT24
Villin, CWWB1
WT1, 6F-H2
Calcitonin, poly
ER, SP1
Oct-3/4
PR, 1E2
TG, 2H11/6E
CD56, 56C04
Podoplanin. D2-40
MITF, 24CA5
Ki67, 30-9
p63, 4A4
CGA, LK2H10
p40, BC28

IHC on frozen sections

1. Skin and kidney biopsies (IF)
2. Skeletal muscle biopsies (HRP)
3. Peroperative diagnostics (HRP)
4. Others...



IgM-FITC (Kidney)

Optimizing biomarker-protocols (IHC-protocols on parafin sections)

Requirements:

- Use a robust, specific and sensitive detection system
- Use designed TMA-blocks (Multiblocks)
- Optimize epitope retrieval using a Test Battery
- Optimize conc./incubation-time/temp. for primary Ab
- Test more than one antibody

Optimizing biomarker-protocols (IHC-protocols on frozen sections)

Requirements:

- Use a robust, specific and sensitive detection system
- Use designed TMA-blocks (Multiblocks)
- Optimize fixation and epitope retrieval using a Test Battery
- Optimize conc./incubation-time/temp. for primary Ab
- Test more than one antibody

Optimizing biomarker-protocols (IHC-protocols on frozen sections)

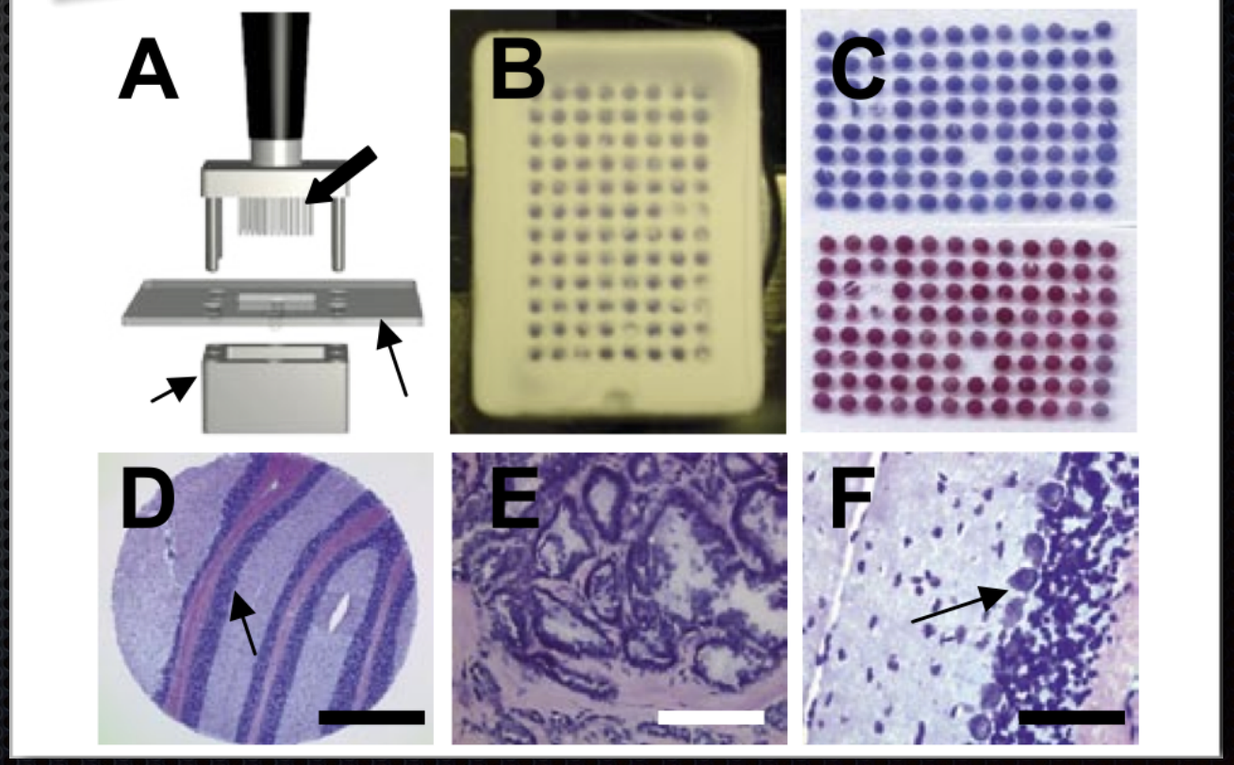
- Designed TMAs:
  - Tissue with high expressors
  - Tissue with low expressors
  - Tissue with non-expressors

New tissue microarray technology for analyses of gene expression in frozen pathological samples

Lei Zhou[1], Melissa Hodeib[2], Joseph D. Abad[2], Leopoldo Mendoza[1], Anilkumar R. Kore[1], and Zhongting Hu[2]
[1]Ambion, Inc., Austin, TX and [2]Western University of Health Sciences, CA, USA

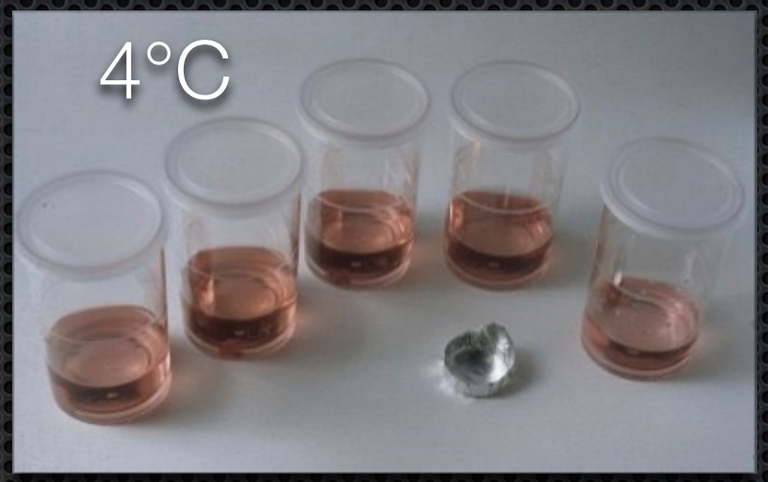
BioTechniques 43:101-105 (July 2007)
doi 10.2144/000112498

0.2% formalin,
20% gelatin,
10% sucrose,
1% agarose,
and PBS



Optimizing biomarker-protocols (IHC-protocols on frozen sections)

- ”Designed” TMAs:
- Tissue with high expressors
- Tissue with low expressors
- Tissue with non-expressors

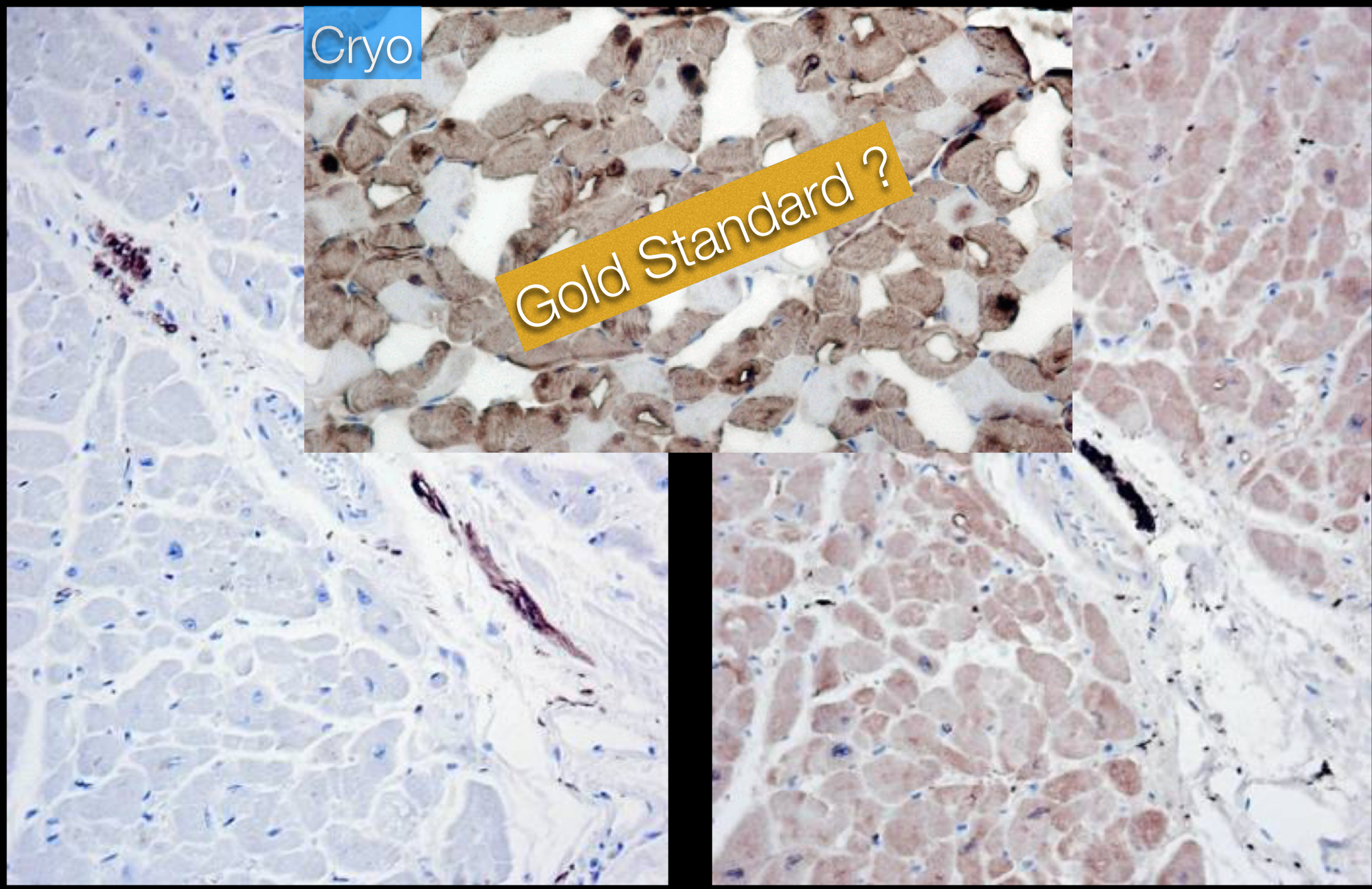






S-100 protein

To HIER or not..



Proteolytic

HIER

Mogens Vyberg

Evaluation of the Value of Frozen Tissue Section Used as "Gold Standard" for Immunohistochemistry

Shan-Rong Shi, MD, Cheng Liu, Llana Pootrakul, PhD, Laurie Tang, MS, Andrew Young, Ryan Chen, Richard J. Cote, MD, and Clive R. Taylor, MD, PhD

Am J Clin Pathol 2008;129:358-366

Table 2
Comparison of Immunohistochemical Staining Results*

Antibodies Tested 26	Frozen Section Fixed in: Acetone or Ethanol (10 min) — Acetone	Acetone or Ethanol (10 min) — Ethanol	NBF (30 min) — w/o AR	NBF (30 min) — w/AR	NBF (Overnight) — w/o AR	NBF (Overnight) — w/AR	FFPE Section — w/o AR	FFPE Section — w/AR
ER	2+	2+	2+	3+	–	3+	–	3+
MIB-1	3+	3+	2+	3+	±	3+	–	3+
p53	3+	3+	3+	3+	2+	3+	1+~2+	3+
p27	–	±	–	3+	–	3+	2+	3+
Rb protein	2+	±	2+	3+	±	3+	–	3+
p21	±	±	2+	3+	1+	3+	–	3+
Pan-keratin	3+	2+	3+	3+	1+	3+	–	3+
S-100	–	–	1+	3+	–	3+	±	3+
Vimentin	3+	2+	2+	3+	1+	3+	1+	3+
CK7	3+	2+	3+	3+	2+	3+	1+	3+
CK20	3+	3+	3+	3+	2+	3+	–	3+
Desmin	3+	3+	2+	3+	2+	3+	2+	3+
Actin	3+	3+	3+	3+	2+	3+	2+	3+
Factor VIII antigen	3+	3+	3+	3+	3+	3+	–	3+
CEA	3+	3+	2+	3+	2+	3+	1+	3+
GRP 78	±	±	2+	3+	±	3+	±	3+
Melanosome Melan A	3+	1+	2+	3+	2+	3+	±	3+
Survivin	2+	2+	2+	3+	±	3+	–	3+
bcl-2 Oncoprotein	3+	1+	2+	3+	1+	3+	–	3+
CD45	3+	3+	3+	2+	2+	2+	2+	3+
HER2/neu	3+	2+	2+	3+	2+	3+	1+	3+
CD15	–	2+	3+	2+	2+	3+	±	3+
CD20	3+	3+	3+	3+	2+	3+	1+	3+
CD3	3+	3+	2+	3+	–	3+	–	3+
CD68	3+	3+	2+	1+	±	2+	–	3+
E-cadherin	3+	1+	2+	3+	1+	3+	–	3+

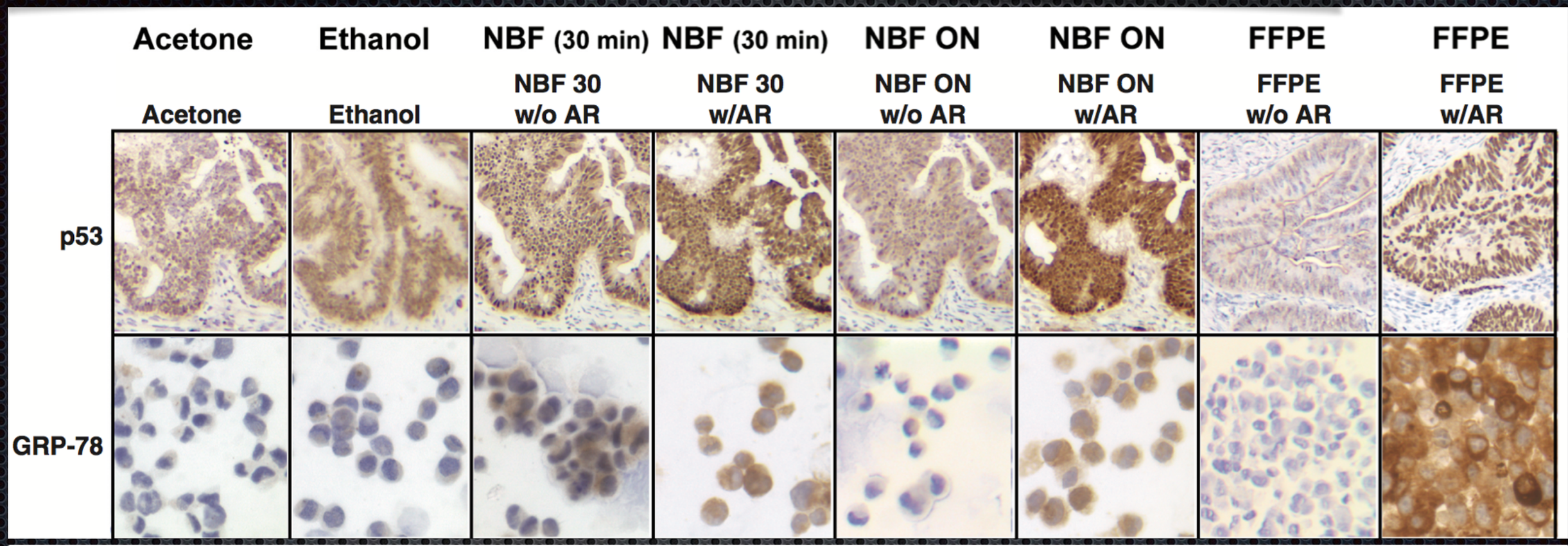
AR, antigen retrieval; CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; FFPE, formalin-fixed, paraffin-embedded; GRP, glucose-regulated protein; NBF, neutral buffered formalin; Rb, retinoblastoma; w/AR, use of the AR pretreatment before the immunohistochemical staining procedure; w/o AR, without use of the AR pretreatment.

* Scoring was as follows: 1+, weak; 2+, moderate; 3+, strong; –, negative; ±, focal or questionable weakly positive.

Evaluation of the Value of Frozen Tissue Section Used as "Gold Standard" for Immunohistochemistry

Shan-Rong Shi, MD, Cheng Liu, Llana Pootrakul, PhD, Laurie Tang, MS, Andrew Young, Ryan Chen, Richard J. Cote, MD, and Clive R. Taylor, MD, PhD

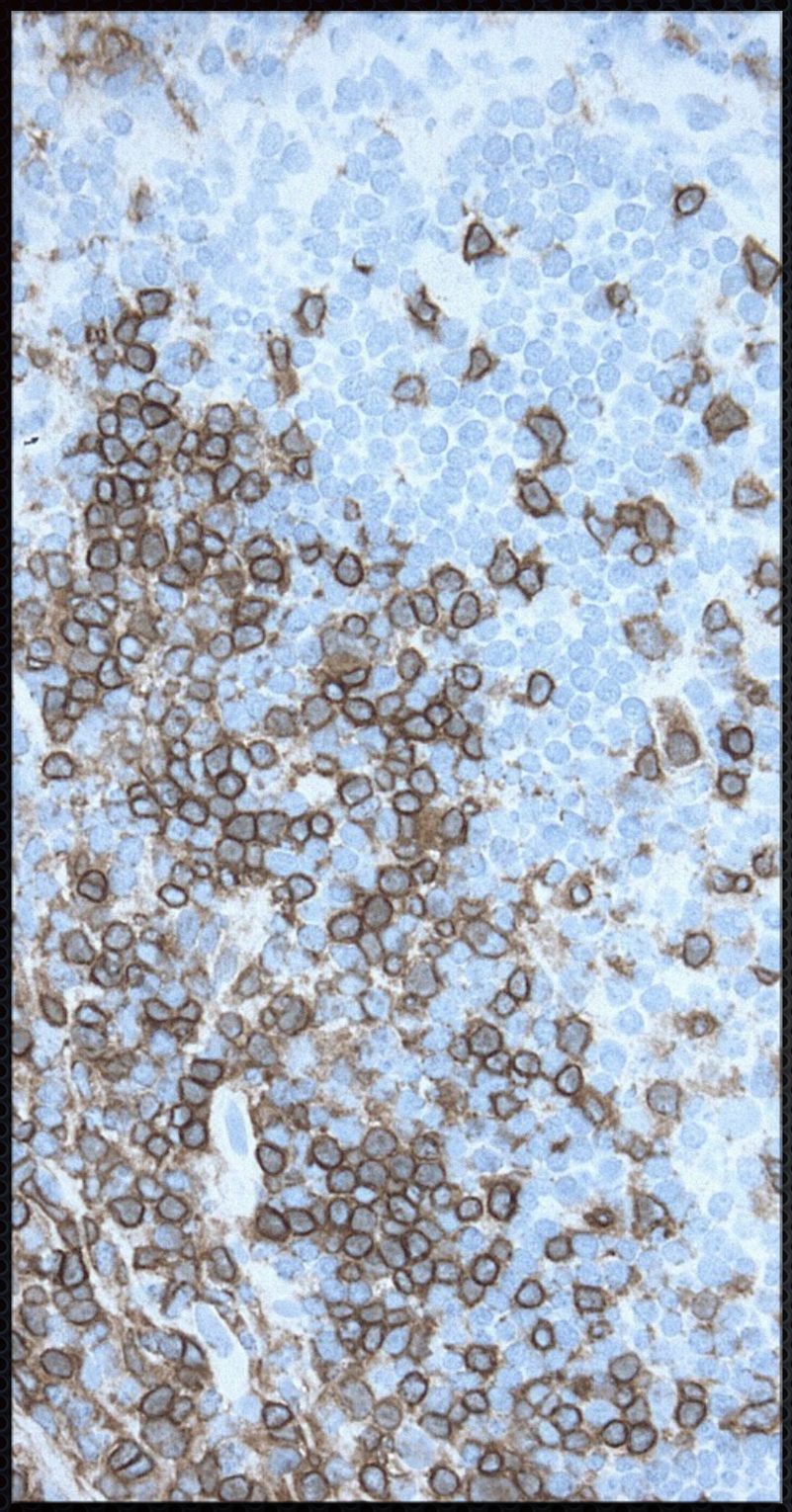
Am J Clin Pathol 2008;129:358-366



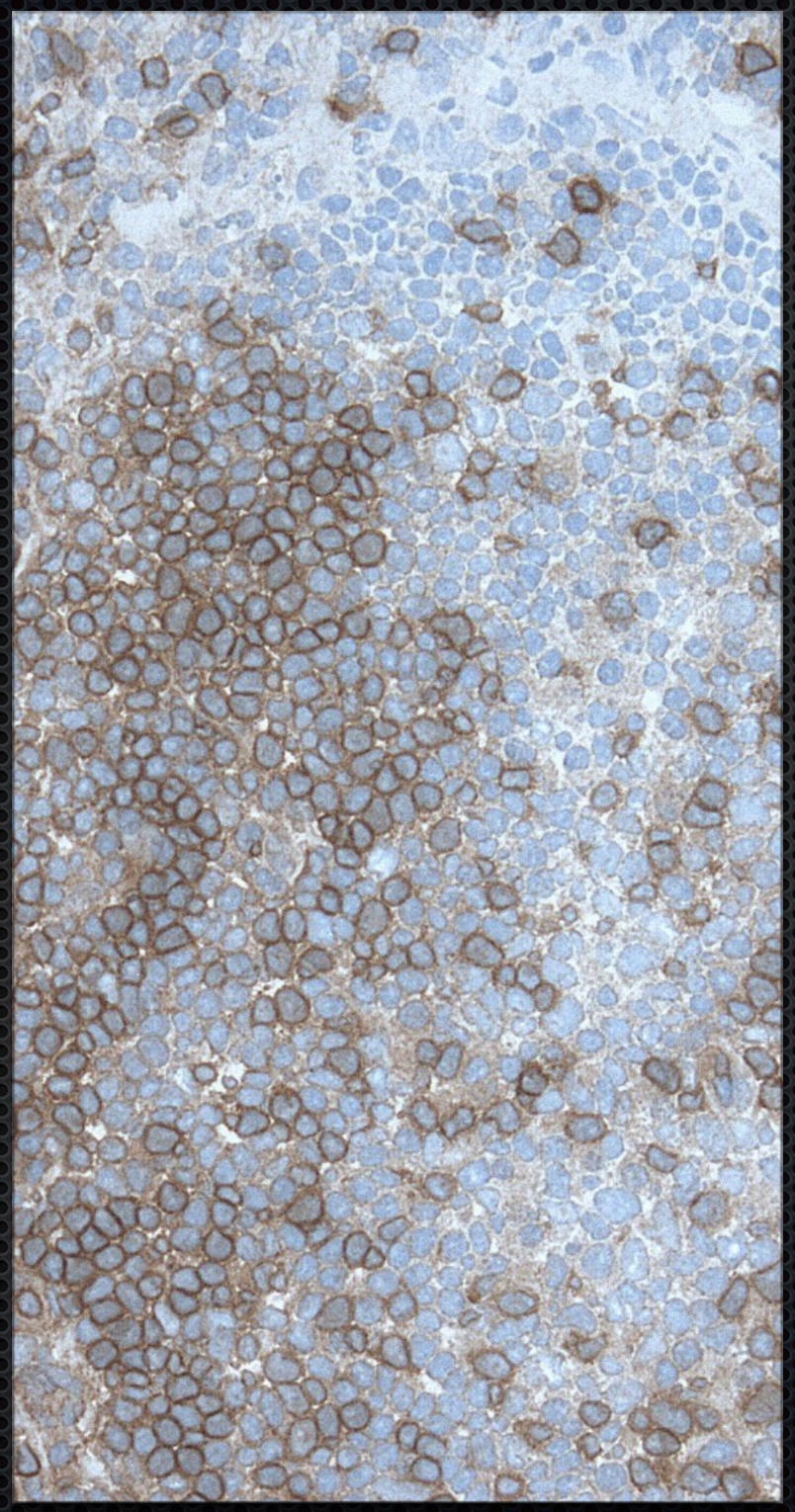
Results:

- ★ *16/26 (62%) showed immunohistochemical results indistinguishable between acetone- and NBF-fixed sections.*
- ★ *8/26 (31%) showed better immunohistochemical signals following NBF and AR.*
- ★ *2 gave better immunohistochemical results for acetone-fixed sections.*
- ★ *In most cases, NBF yielded stronger signals with less background and better morphology.*
- ★ *In evaluating new antibodies, a combination of acetone- and NBF-fixed frozen sections should be used.*

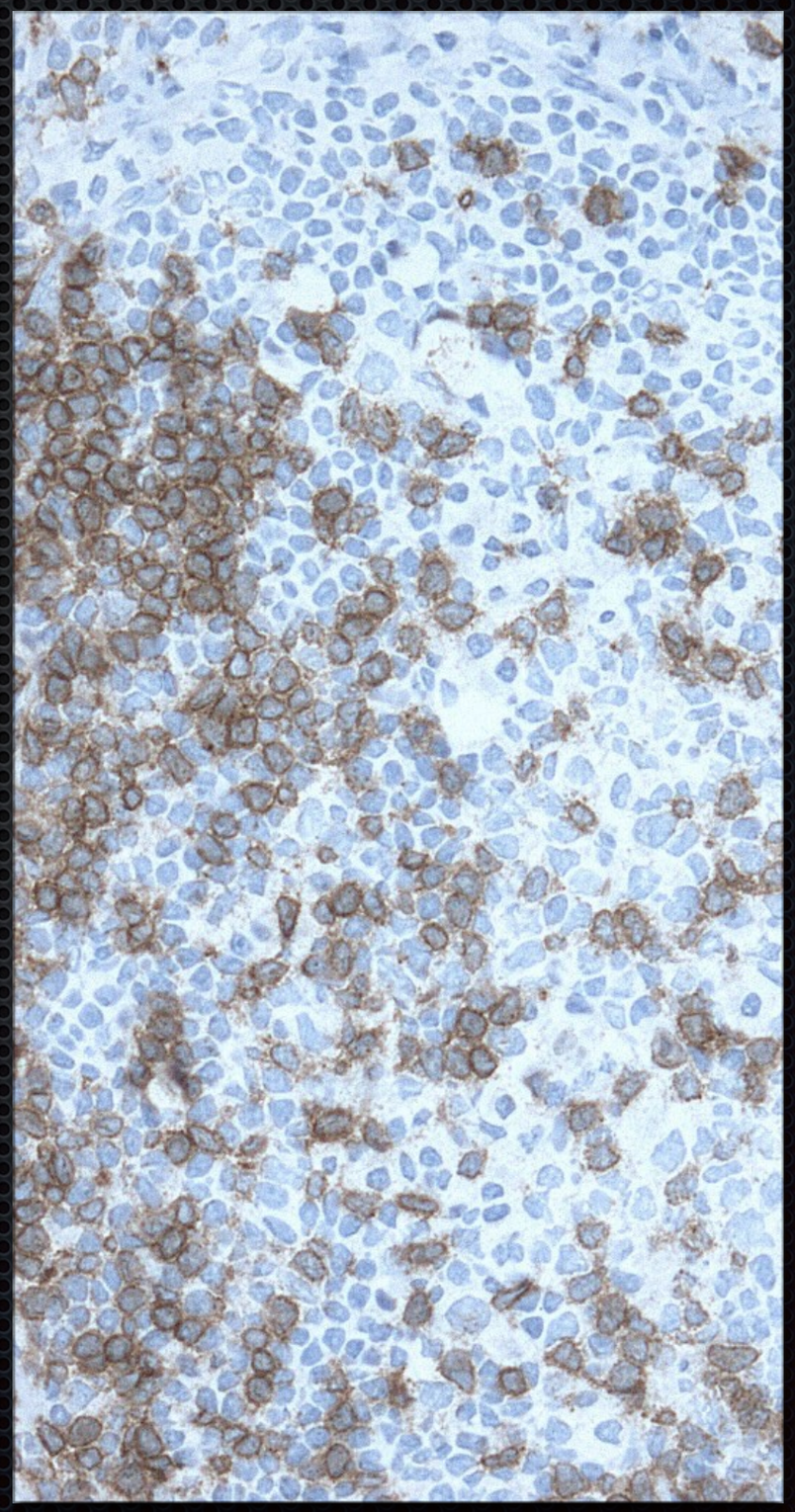
CD3, F7.2.38 (Tonsil) various fixatives



Acetone 10’

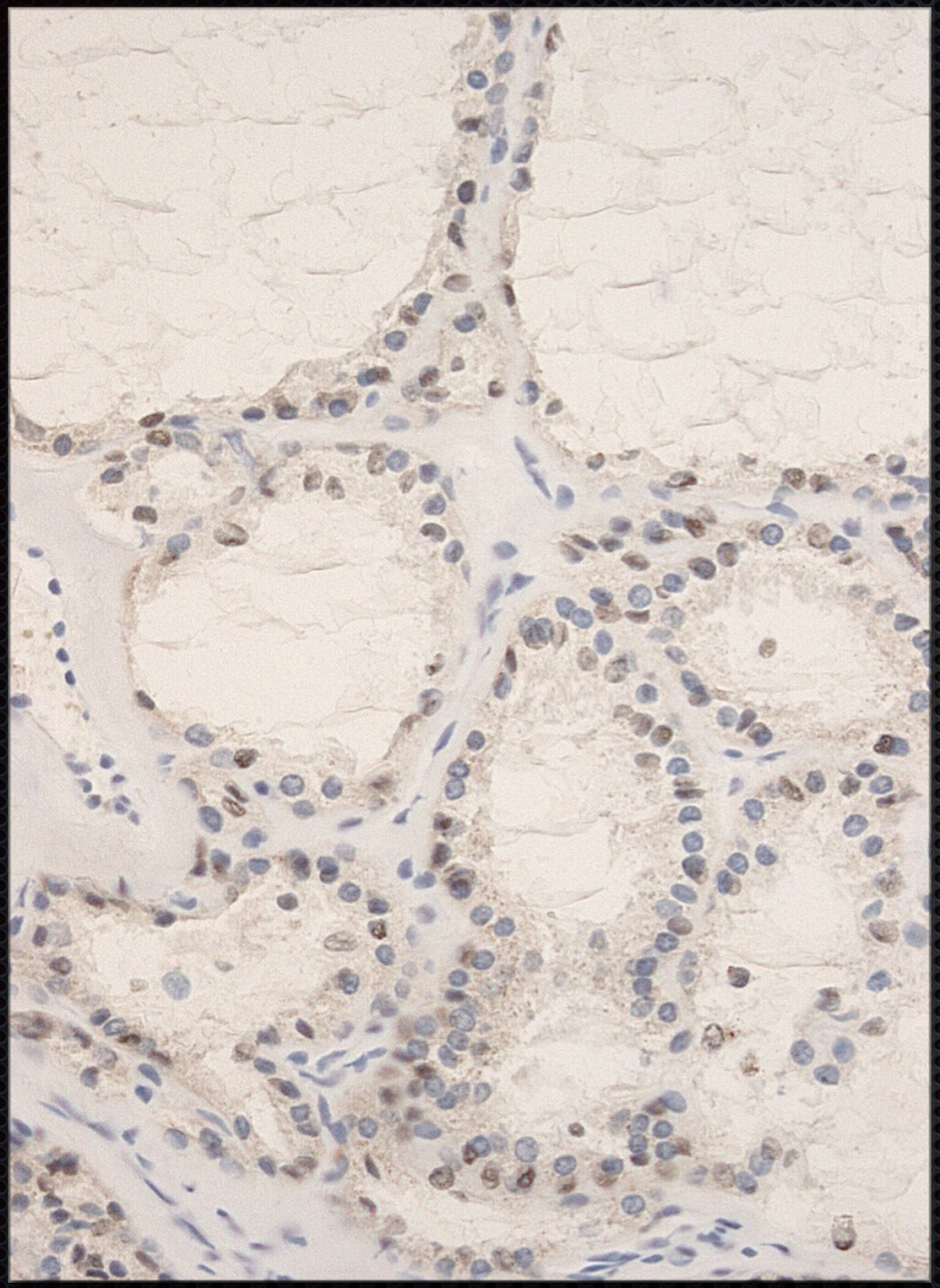


4% NBF 5’

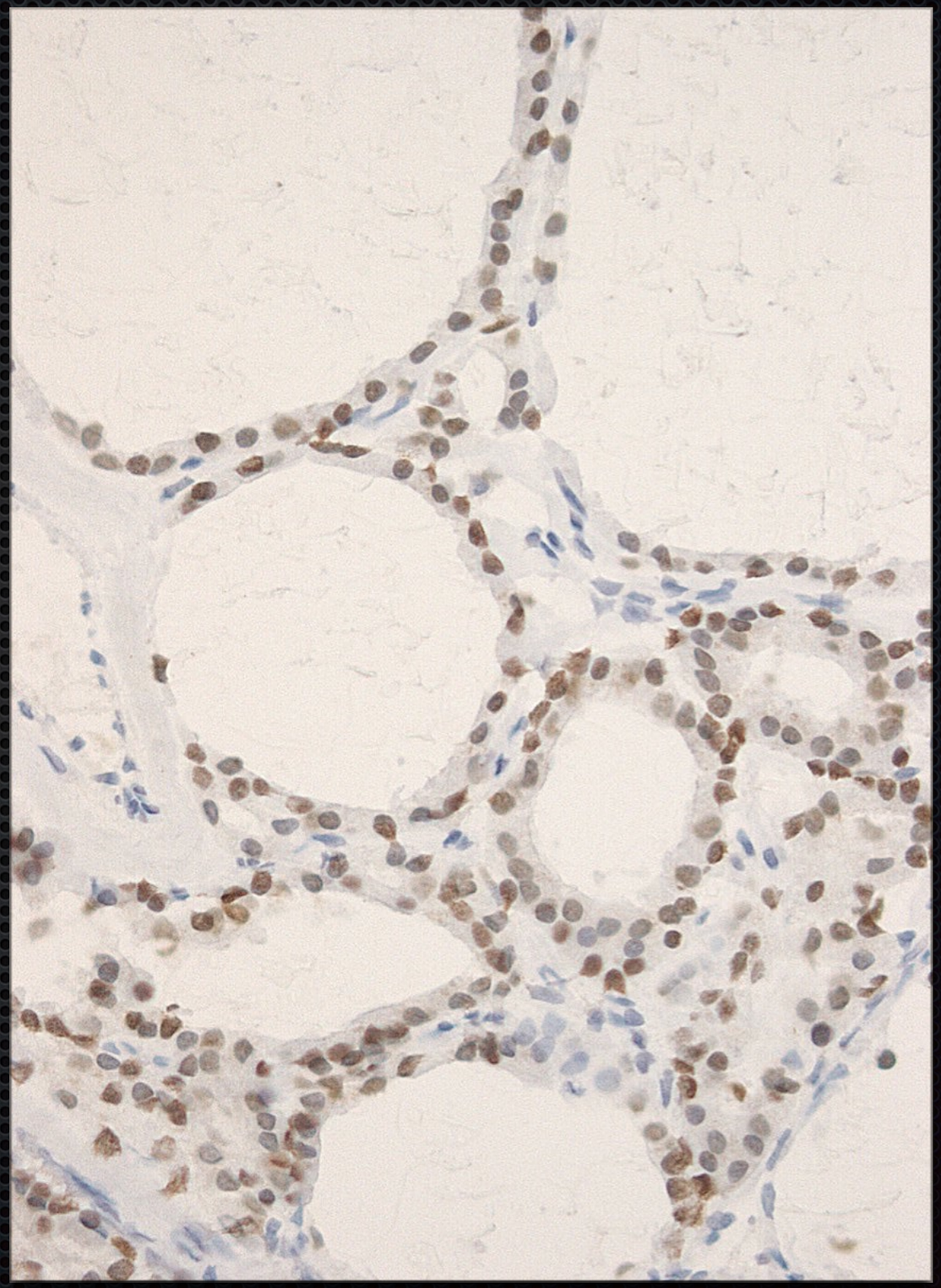


NBF 2’ - TEG95° 30s

TTF-1, SPT24 (Thyroid) various fixatives

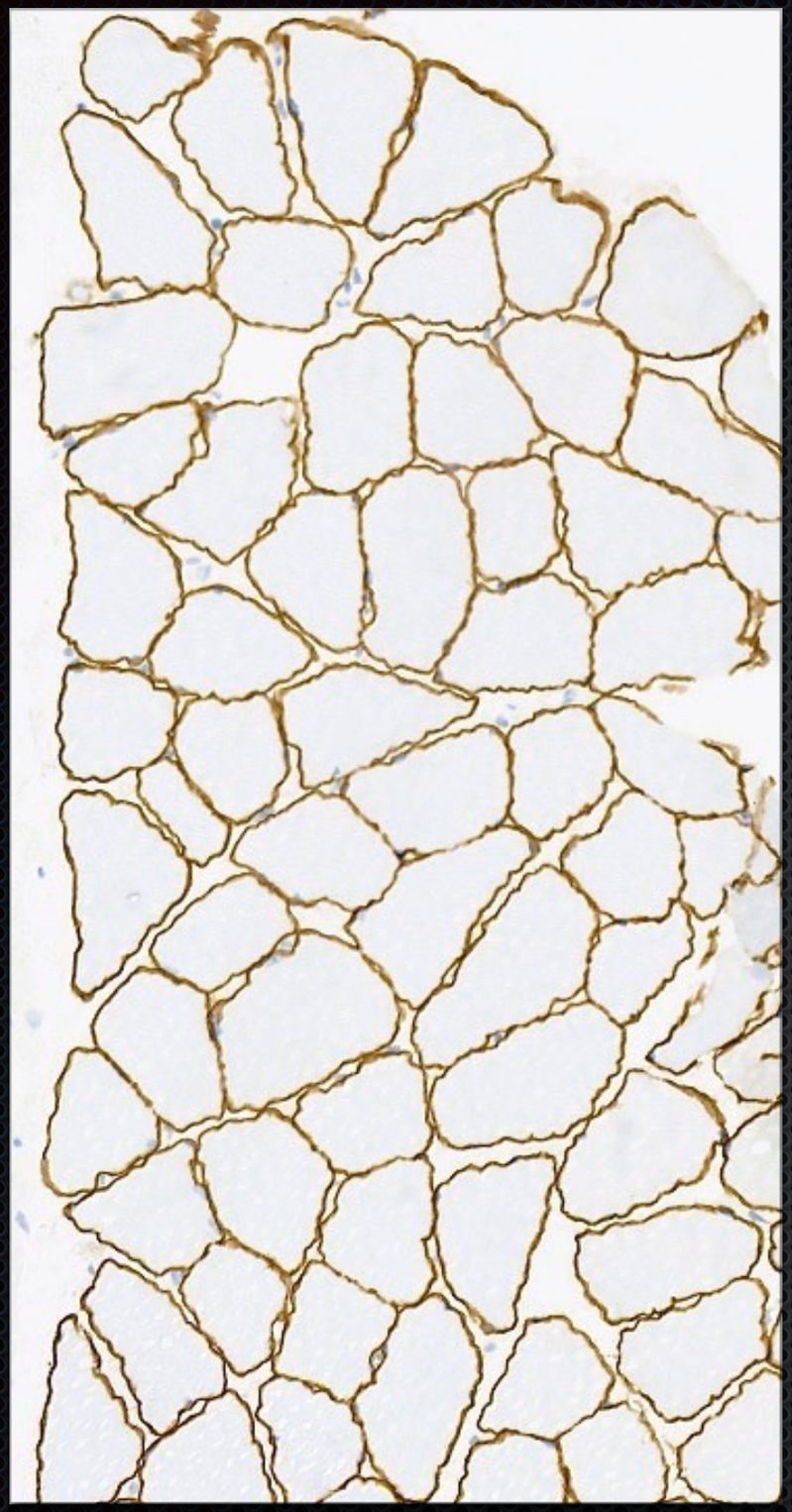


Acetone 10’



NBF 2’ - TEG95° 30s

LAM-A2, Mer3/22B2 - various fixatives



No fixation (muscle)

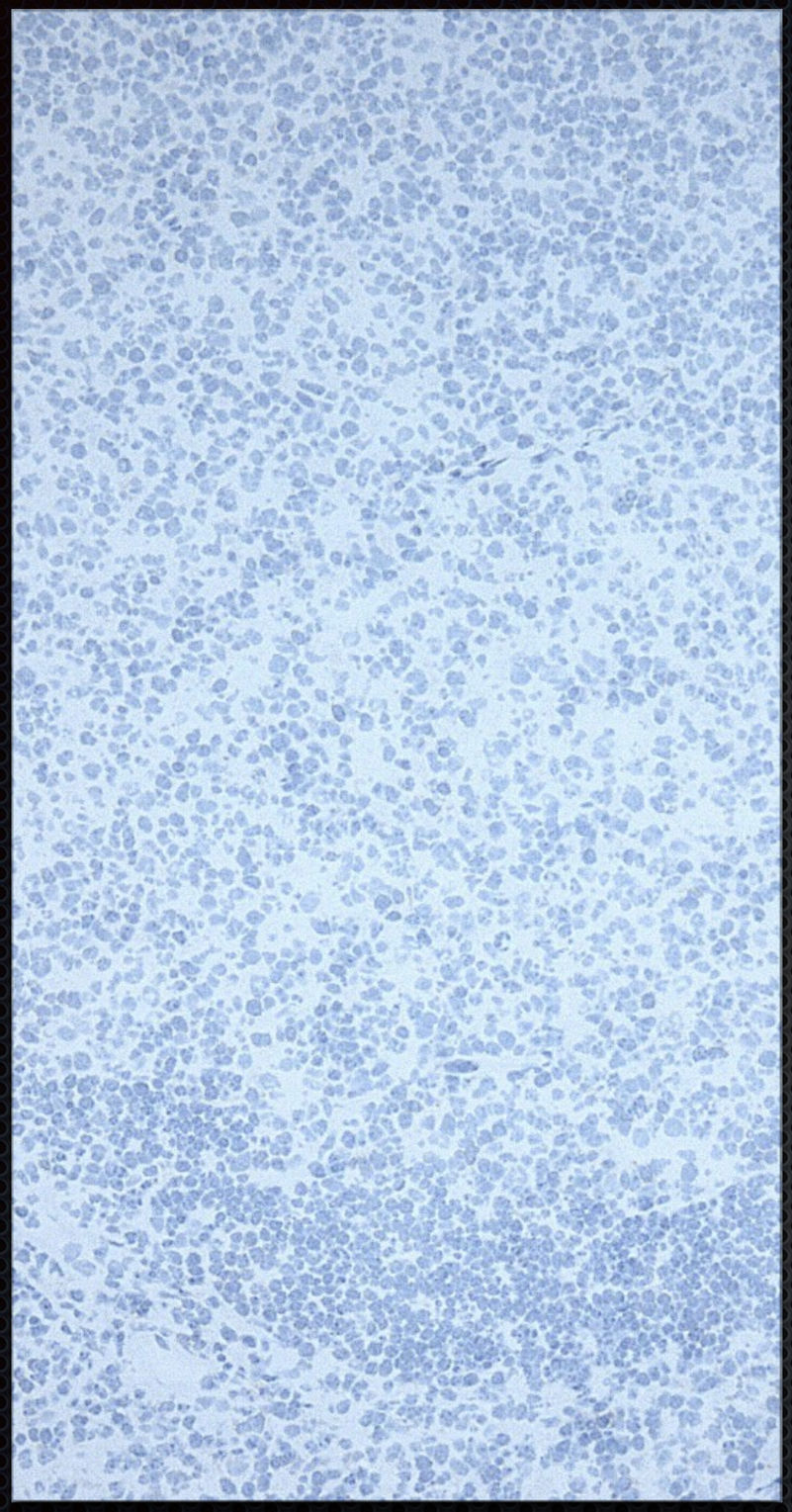


4% NBF 5’ (muscle)

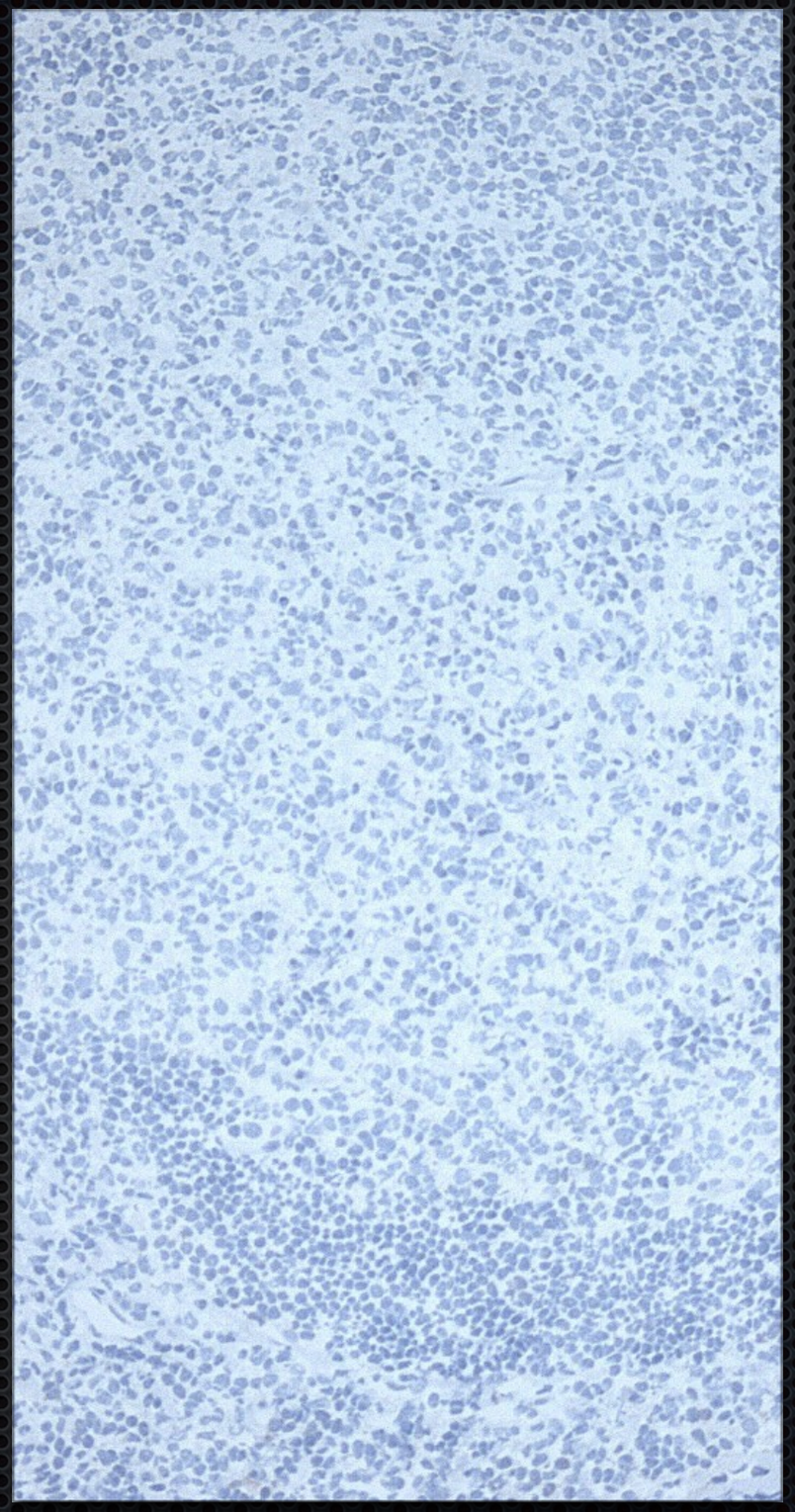


Acetone 10’ (muscle)

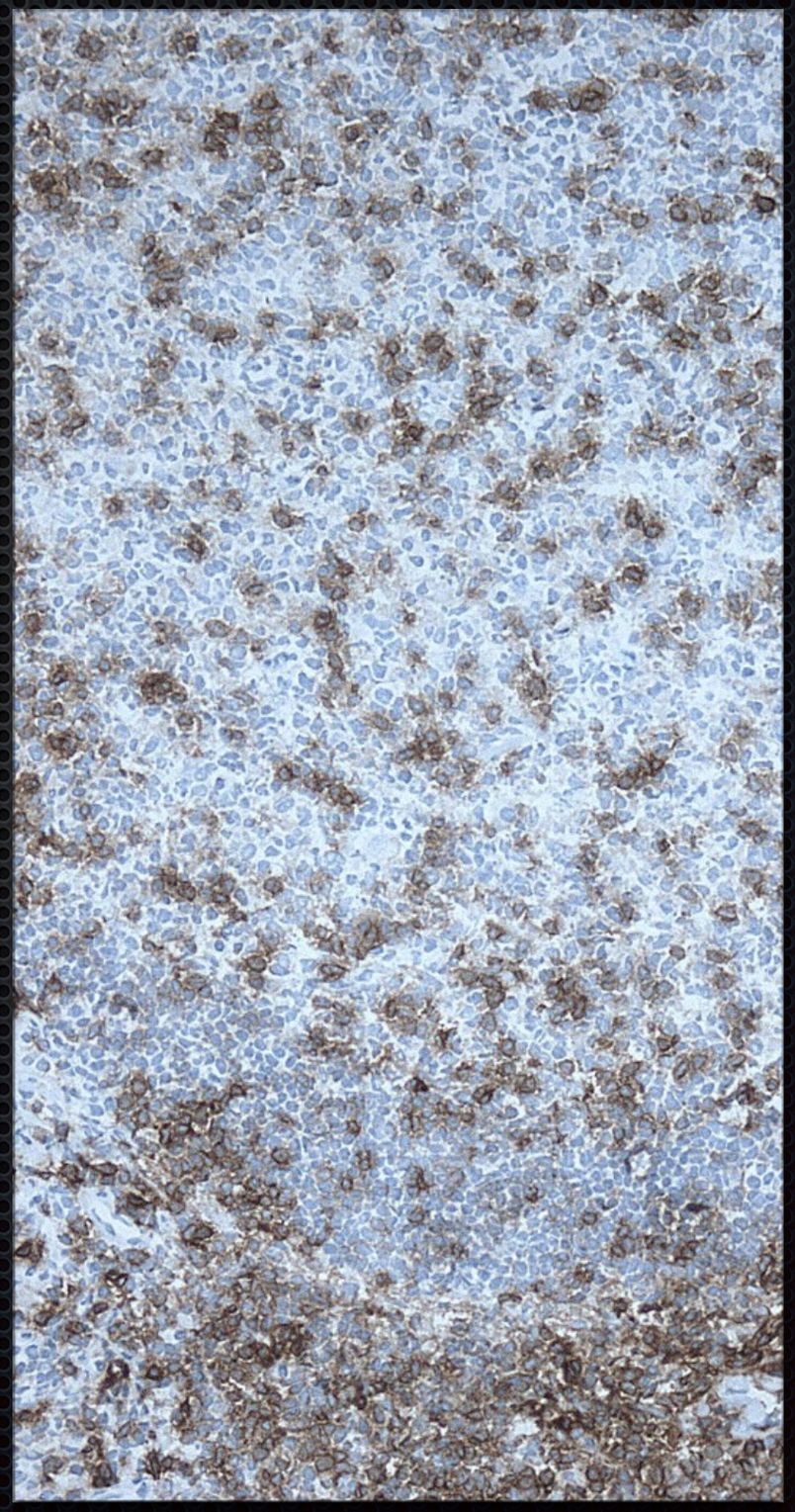
CD5, 4C7 (Tonsil) various fixatives



Acetone 10’



4% NBF 5’



NBF 2’ - TEG95° 30s

Optimizing biomarker-protocols (IHC-protocols on frozen sections)

Fixation/Epitope retrieval “Test Battery”

No	Method			
1	No fixation (“drying”)	60 min	No retrieval	
2	Acetone	10 min	No retrieval	
3	4% NBF	5 min	No retrieval	
4	4% NBF	**2**-30 min >>>>	TEG, pH9 (95°C)	30 sec
5	The vendors recommendations			

TEG: Tris-EGTA based buffer pH 9.0

Volume 49(5): 623–630, 2001
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

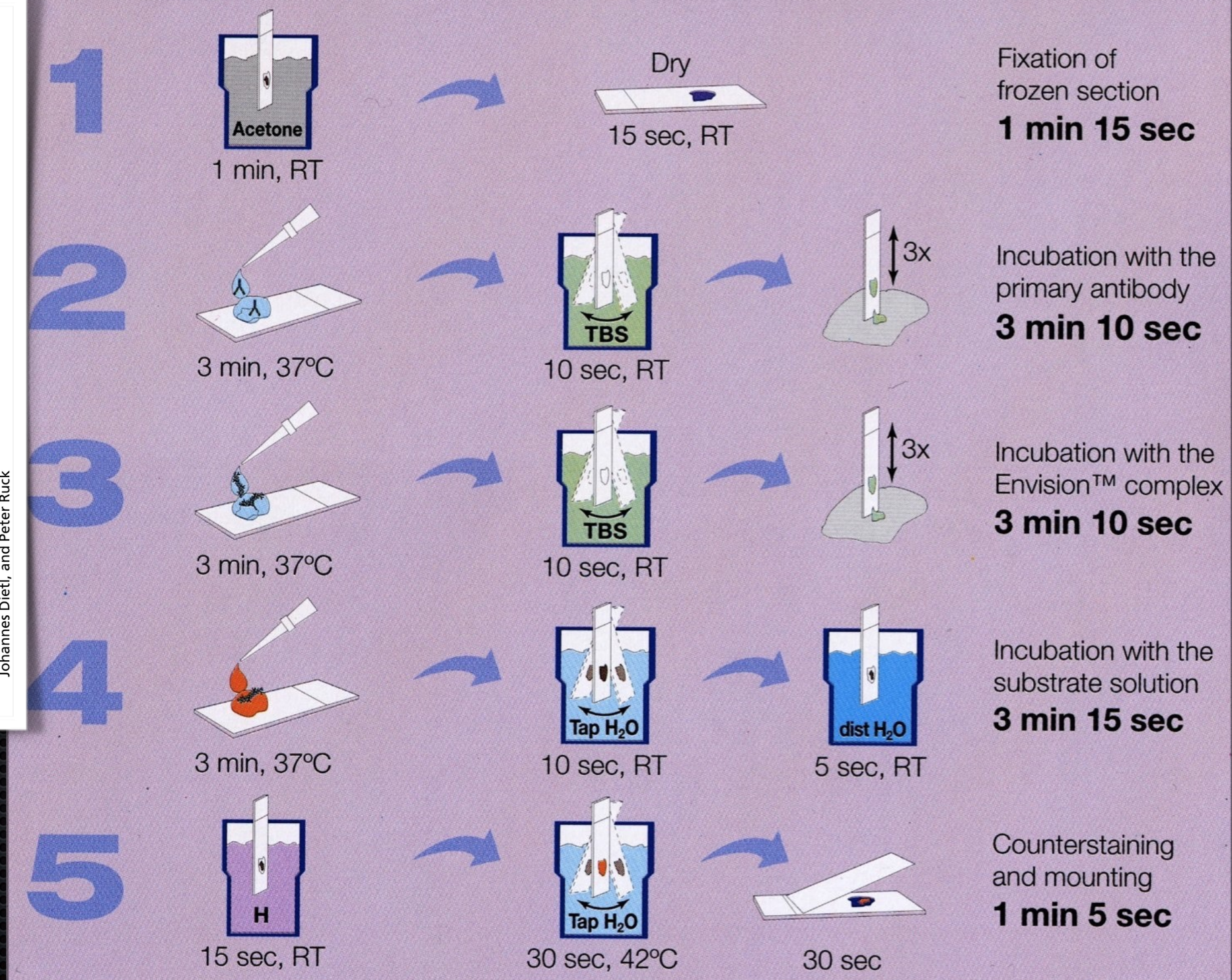
ARTICLE

A New Rapid Immunohistochemical Staining Technique Using the EnVision Antibody Complex

Ulrike Kämmerer, Michaela Kapp, Andrea Maria Gassel, Thomas Richter, Christian Tank, Johannes Dietl, and Peter Ruck

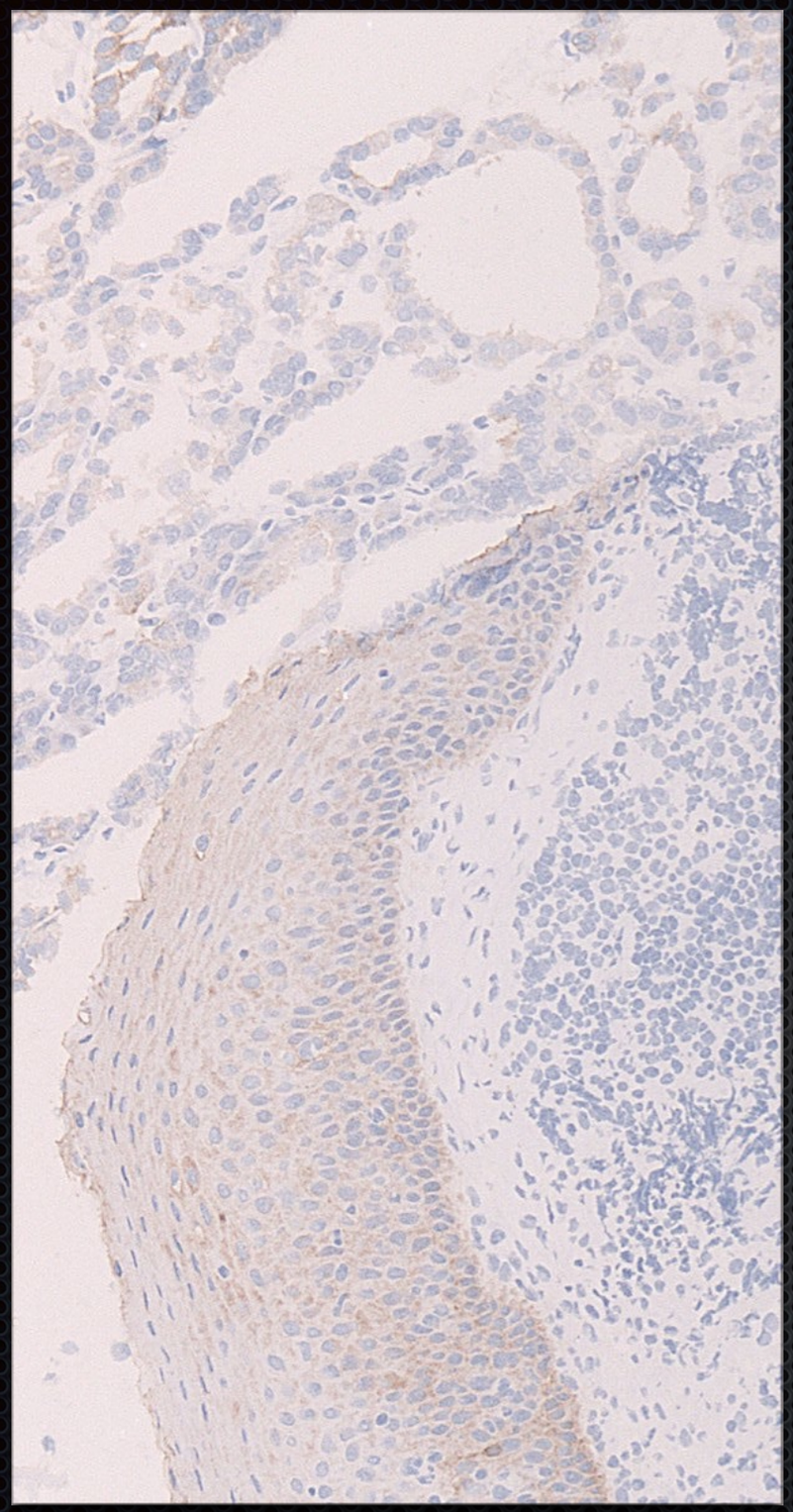
Kämmerer et al

Figure 1: Rapid staining protocol for DAKO Envision™ on frozen sections

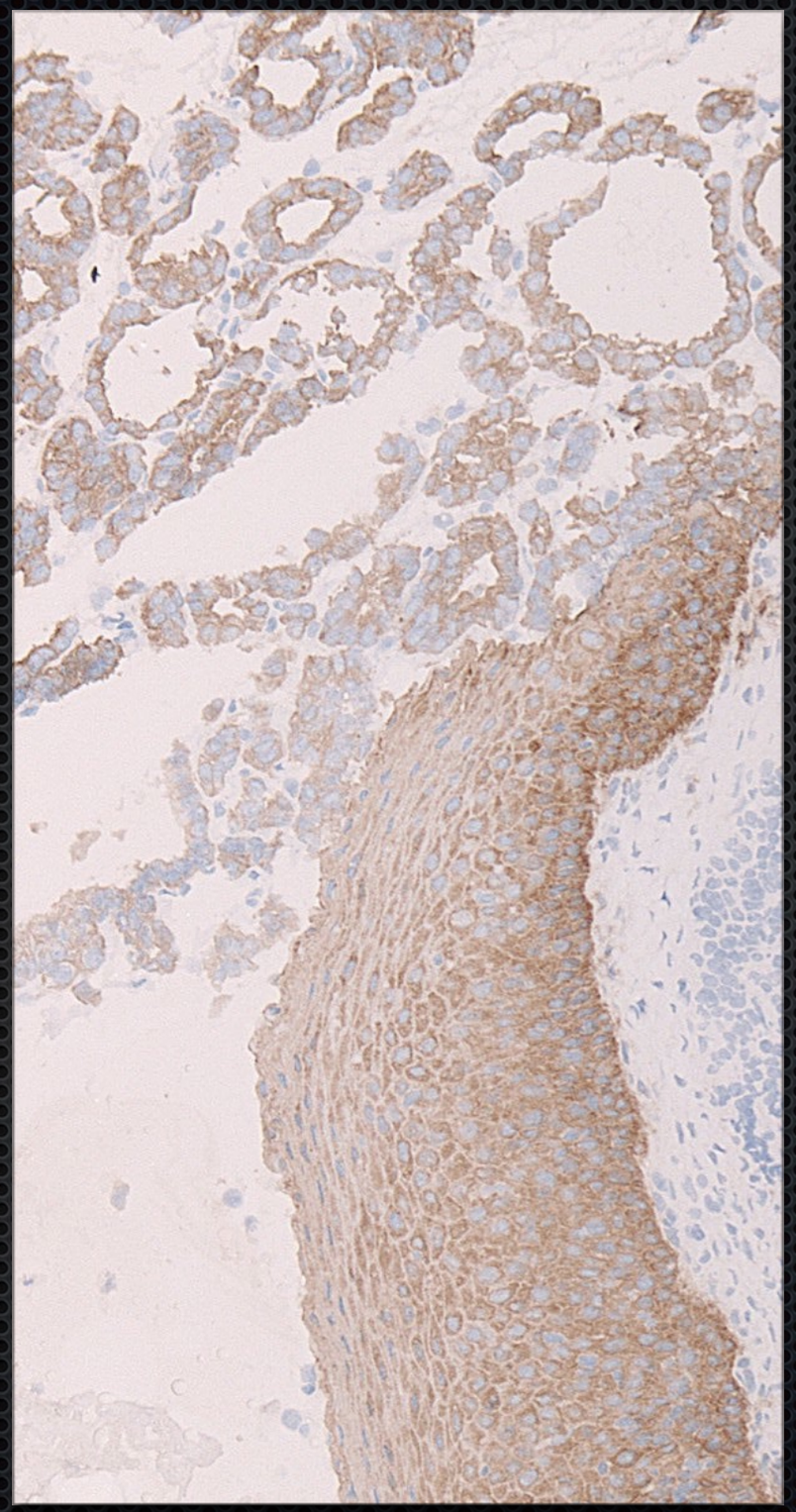


Step-by-step schematic of the rapid EnVision procedure.
RT, Room temperature; TBS, Tris-buffered saline, pH 7.4; H Mayer's hematoxylin

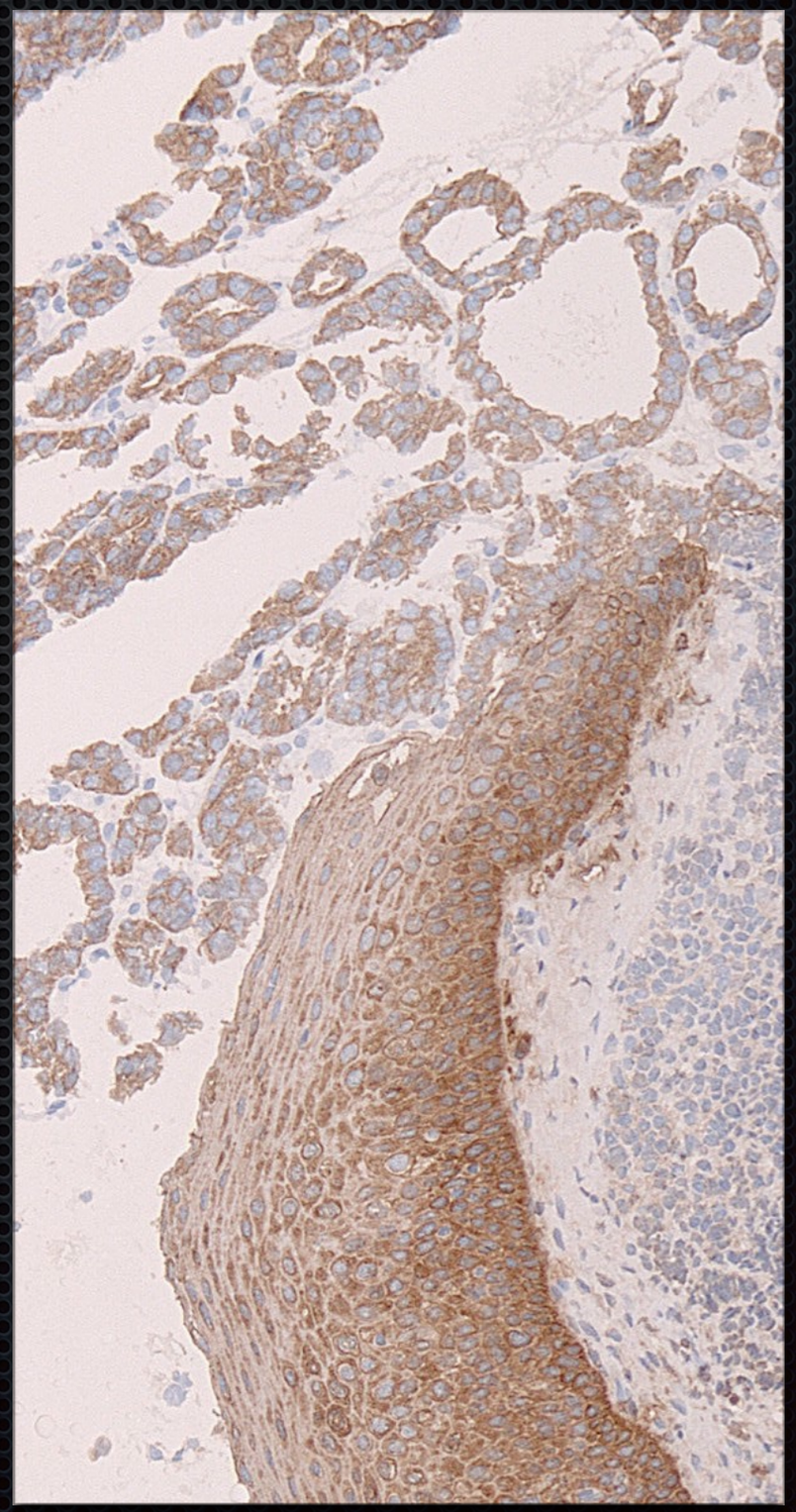
Rapid-IHC - Pan-CK, AE1/AE3



NBF - TEG 60°C



NBF - TEG 80°C

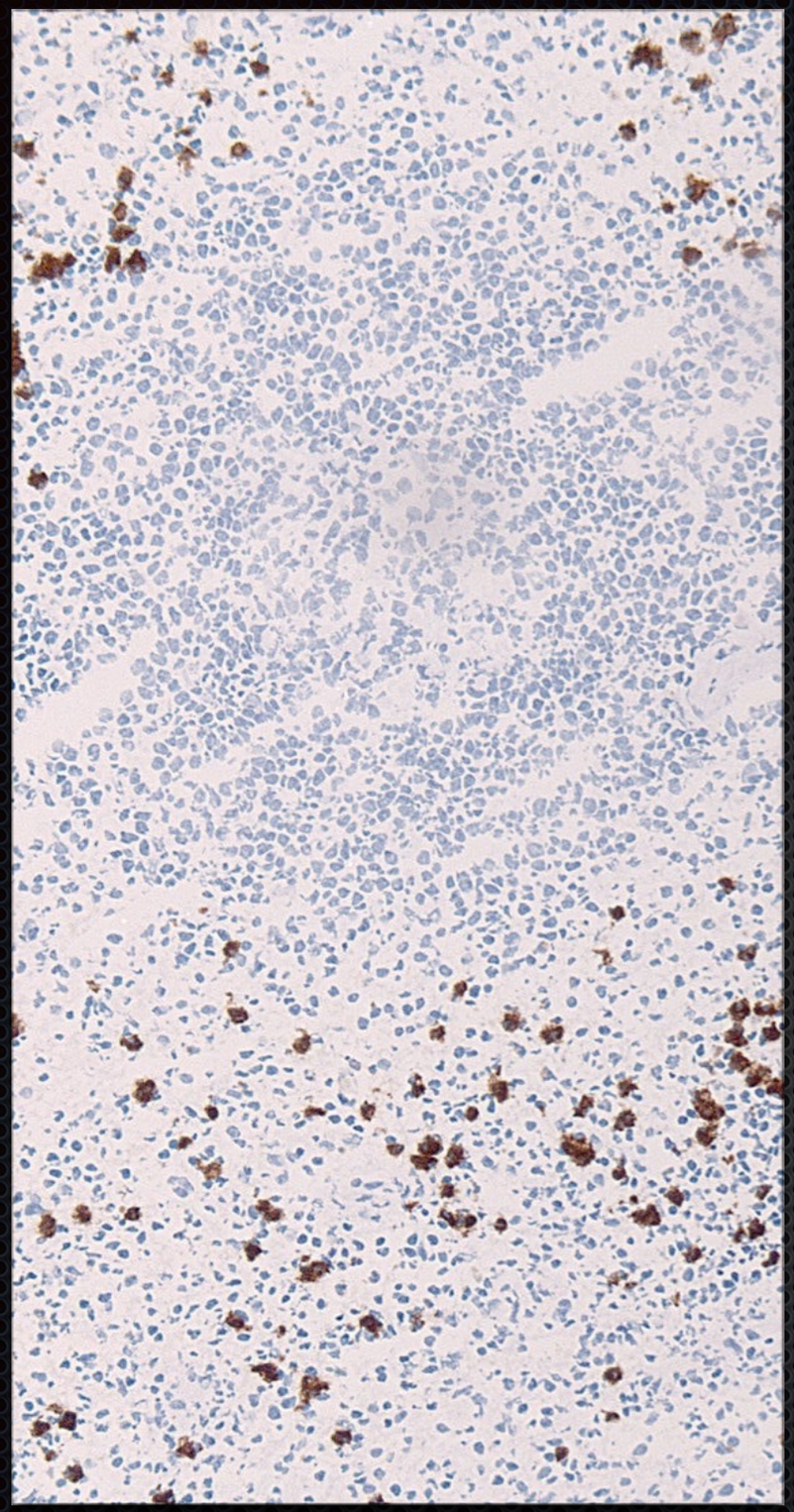


NBF - TEG 95°C

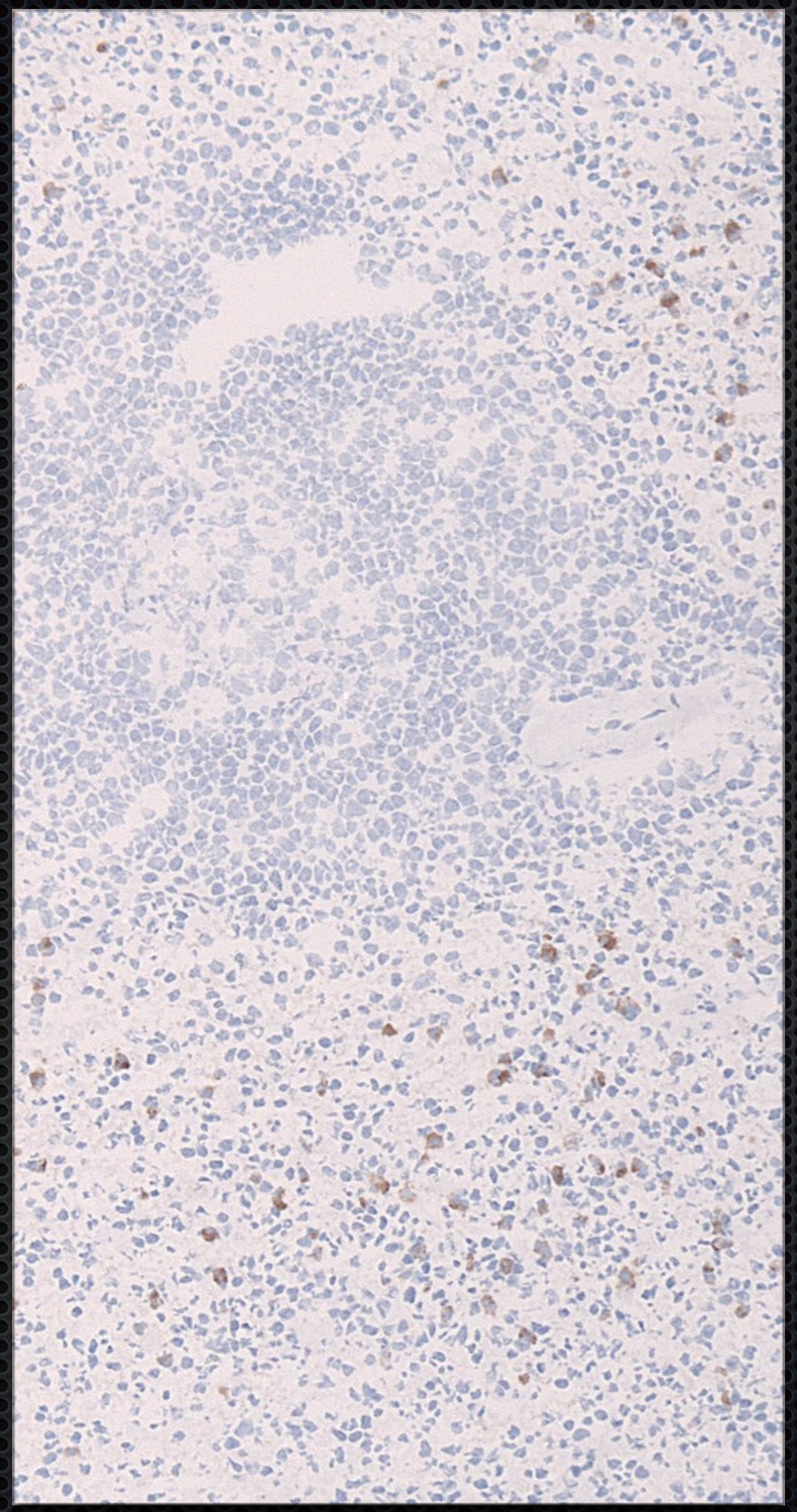
Endogenous peroxidase

Spleen

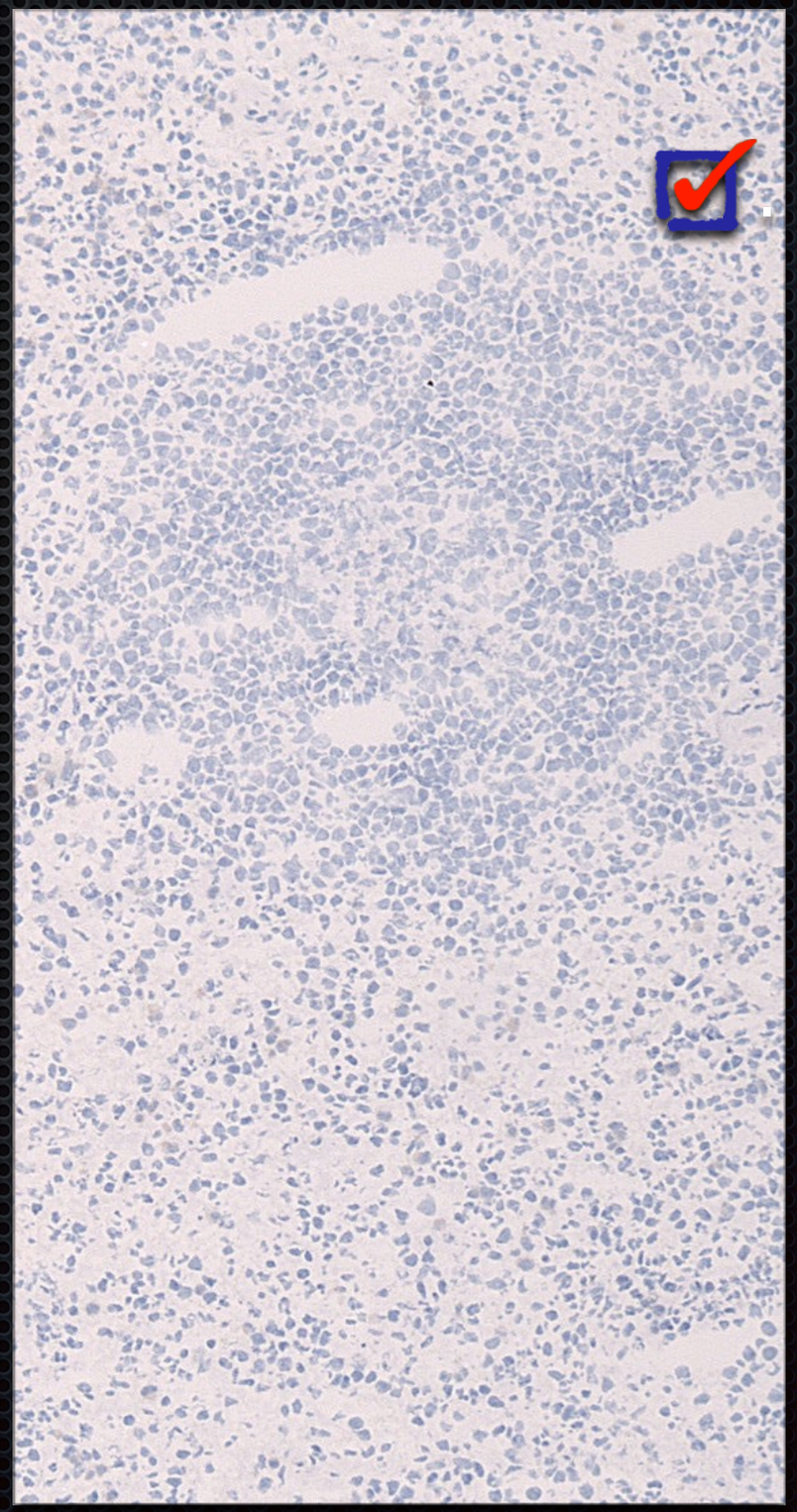
NordiQC



NBF - TEG 60°C

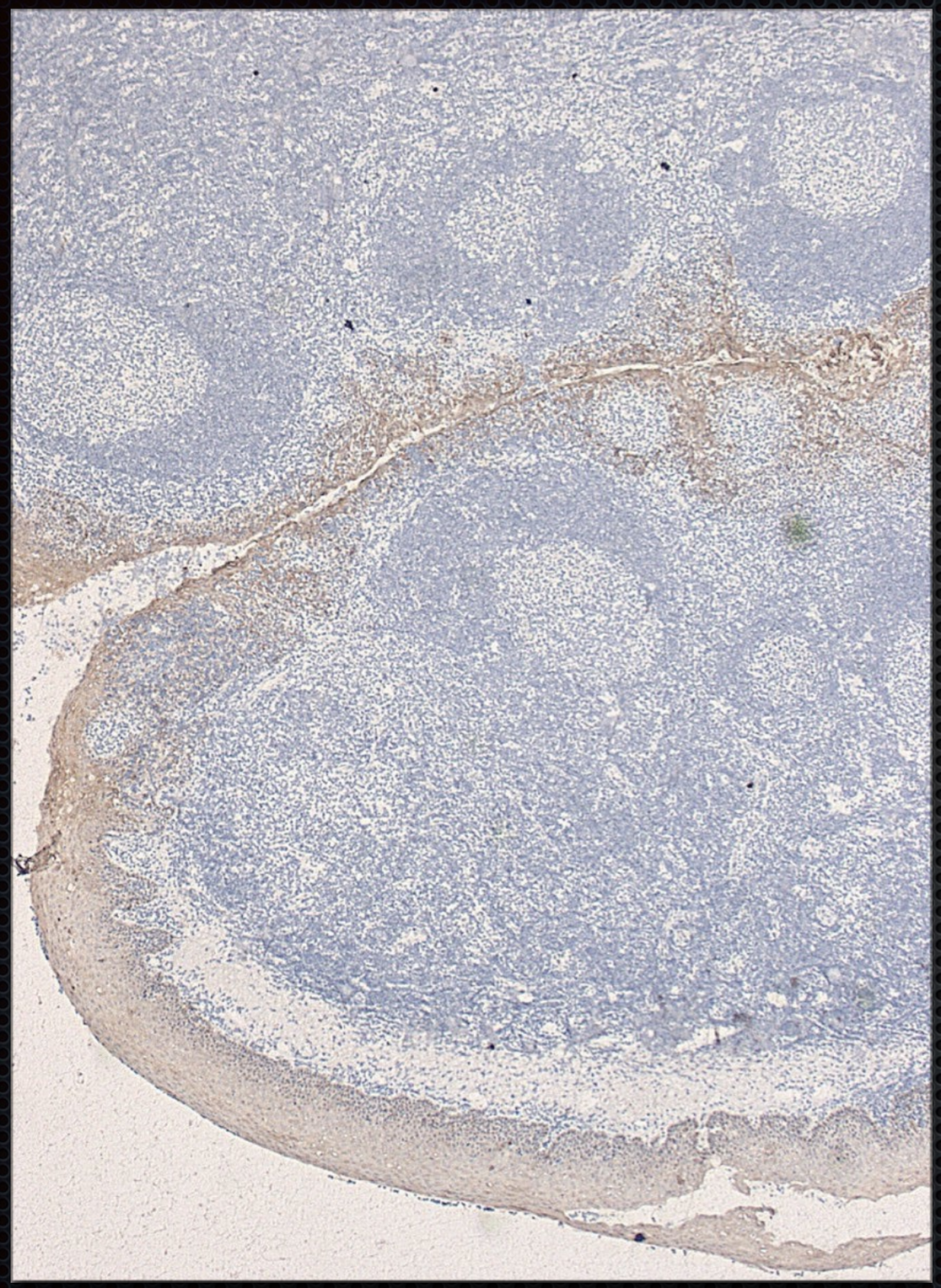


NBF - TEG 90°C

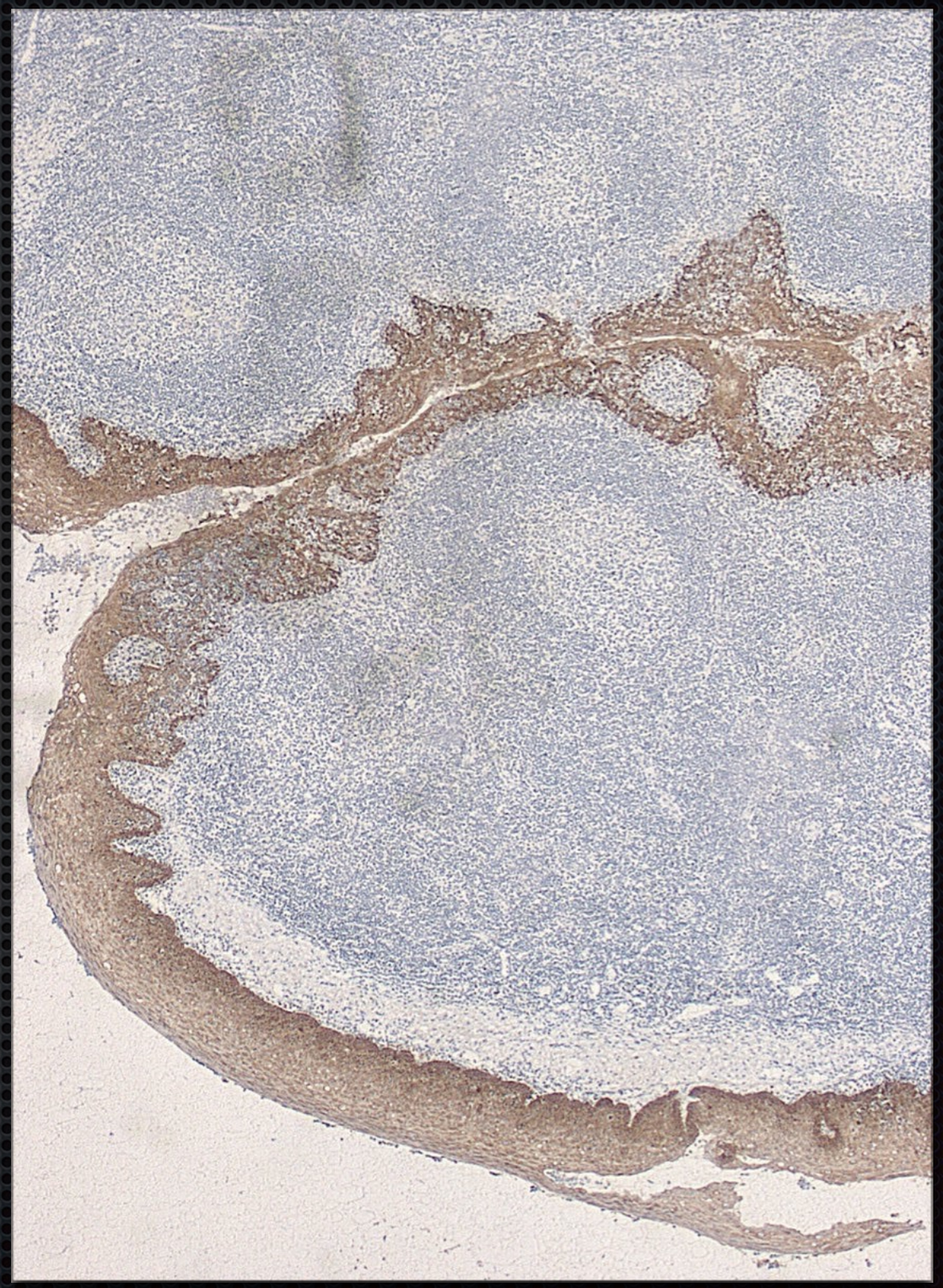


NBF - TEG 95°C

Pan-CK, AE1/AE3 (Tonsil) various fixatives



Acetone



NBF-TEG95

Rapid-IHC fixation:

Acetone vs NBF-MBO/TEG

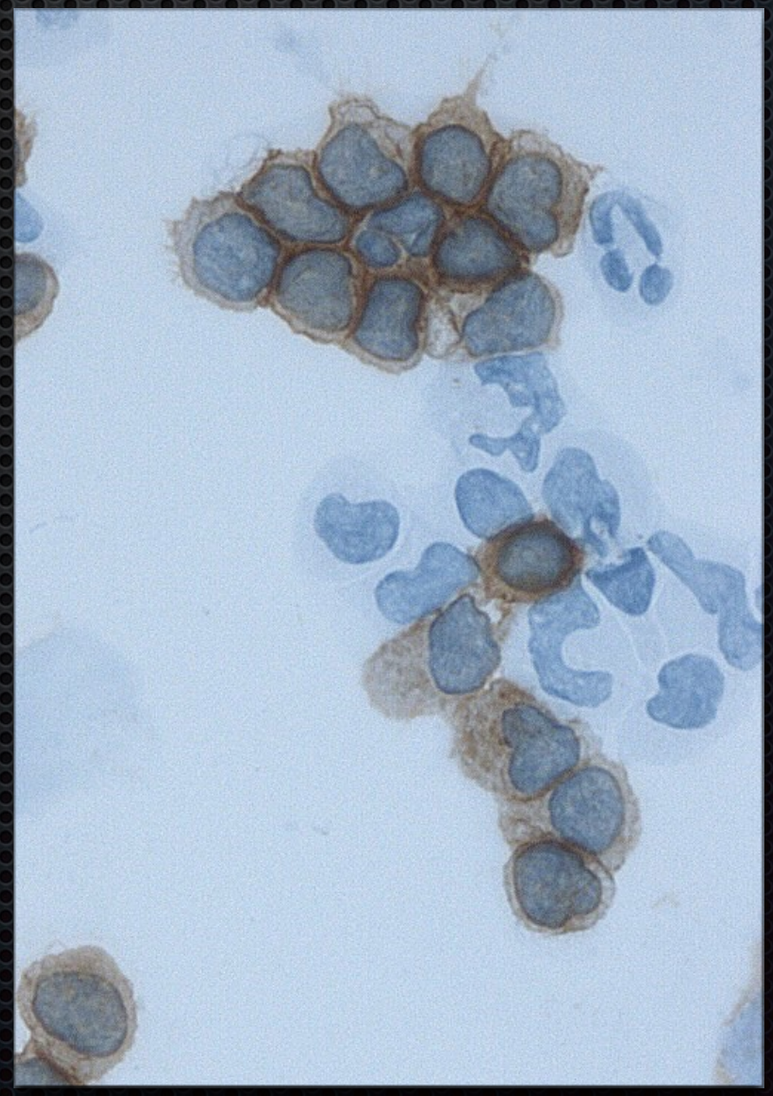
Antibody	Acetone	NBF-MBO/ TEG
CK20, Ks20.8	+++	++
Melanoma, HMB45	++	+
Insulin, 2D11-H5	0	++
Melan-A, A103	+++	+++
Ki67, MIB1	+	++
Synaptophysin, 27G12	++	+++
TTF-1, SPT24	+	++
Vimentin, V9	+++	+++

Antibody	Acetone	NBF-MBO/ TEG
CD3, F7.2.38	+	++
CD5, 4C7	0	++
CD20cy, L26	++	+++
CD30, Ber-H2	+	++
CD45, 2B11+PD7/26	+++	+++
CDX2, ATM28	0	+
CEA, Col-1 (CD66e)	+++	+++
CK, AE1/AE3	++	+++
CK7, OV-TL12/30	+++	+++

+++ Strong. / ++ Moderat. / + Veak. / 0 Negative

ICC on cytological material

- Smears, imprints, cytospins, etc
- Liquid based cytology (LBC)



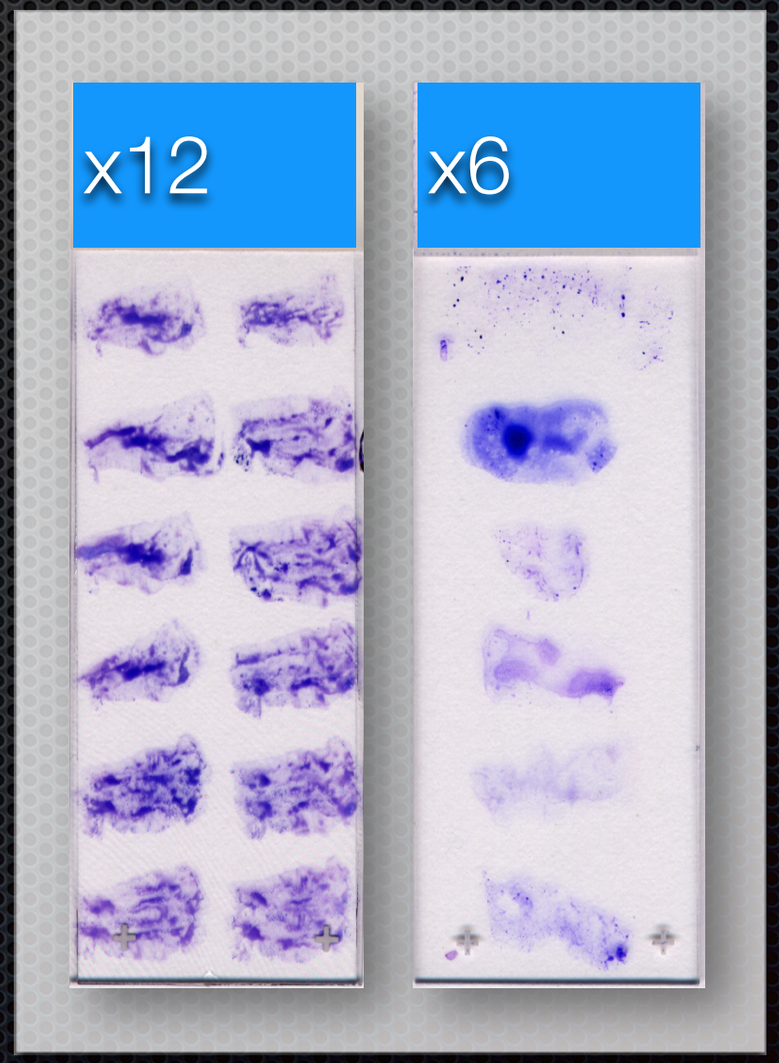
Optimizing biomarker-protocols (ICC-protocols on cells)

Requirements:

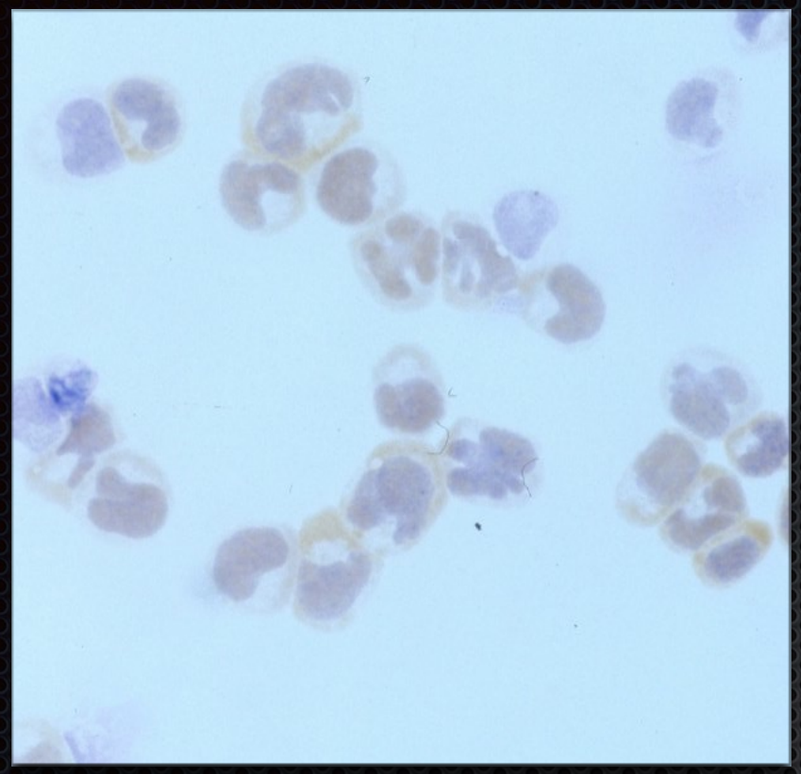
- Use a robust, specific and sensitive detection system
- Use designed “Multi-imprints” and cell lines
  - Classic imprint/smears
  - LBC
- Optimize fixation and epitope retrieval using a Test Battery
- Optimize conc./incubation-time/temp. for primary Ab
- Test more than one antibody

Optimizing biomarker-protocols (ICC-protocols on cells)

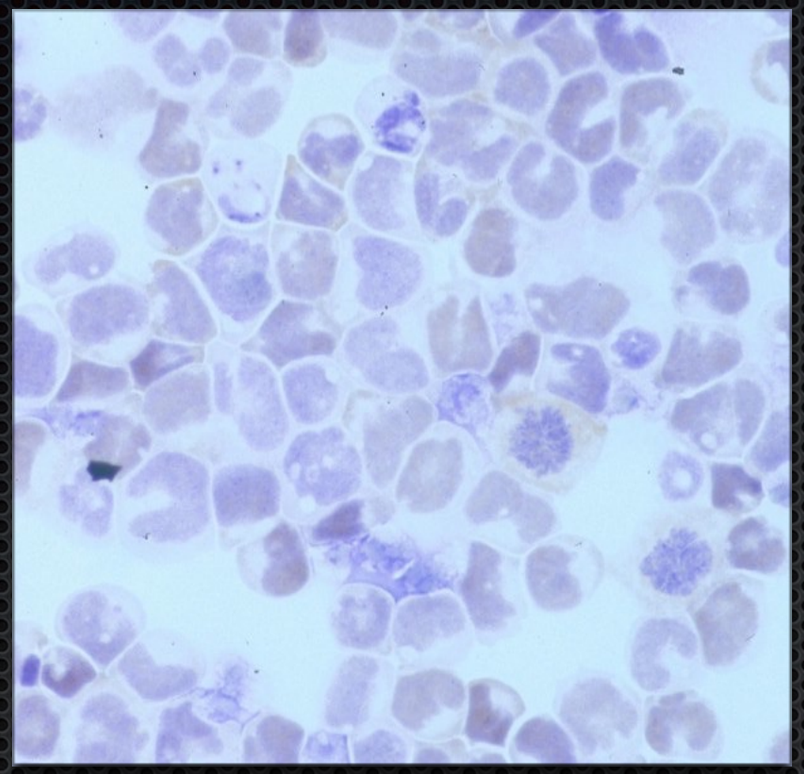
- Designed “Multi-Imprints” from:
  - Tissue with high expressors
  - Tissue with low expressors
  - Tissue with non-expressors



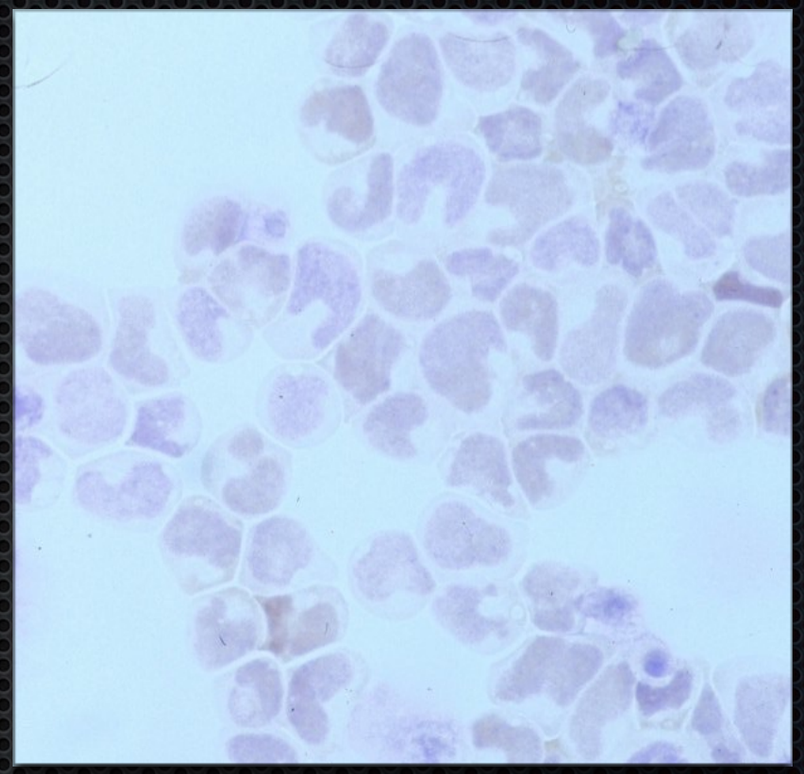
TdT, poly (MOLT4 cells) and various fixatives



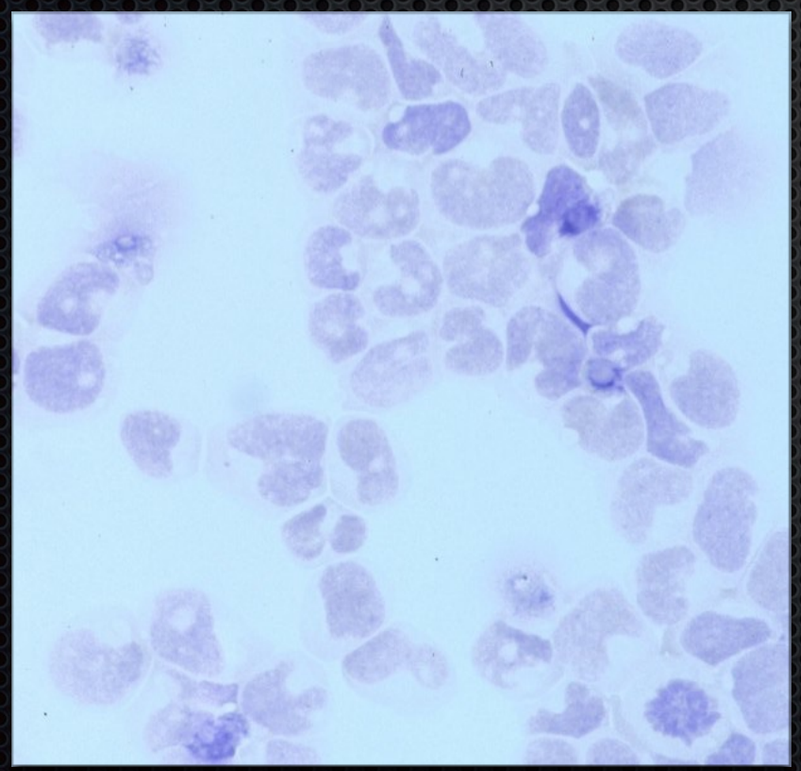
Acetone 5’



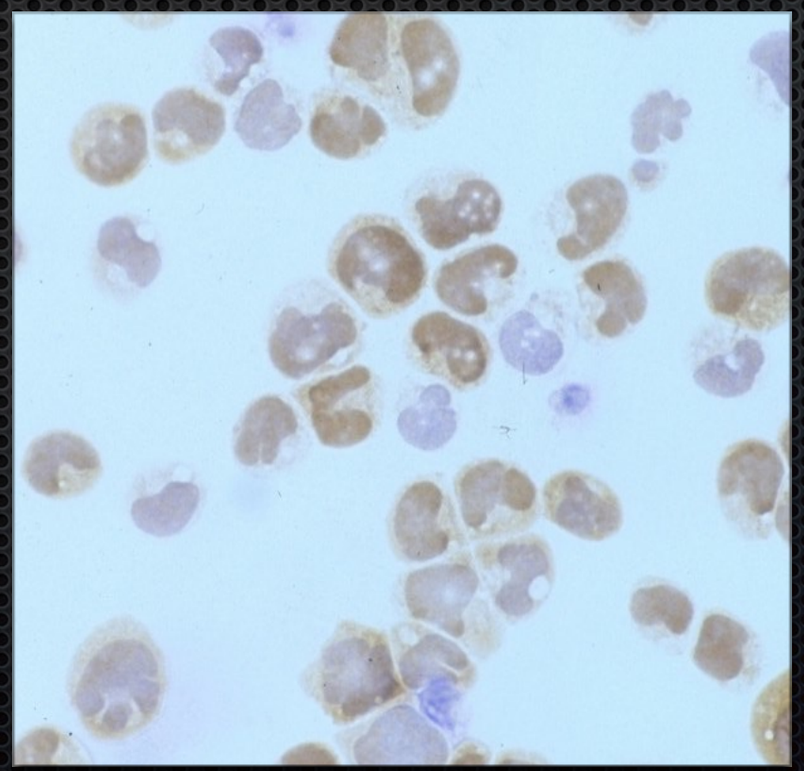
Acetone/Methanol 40s



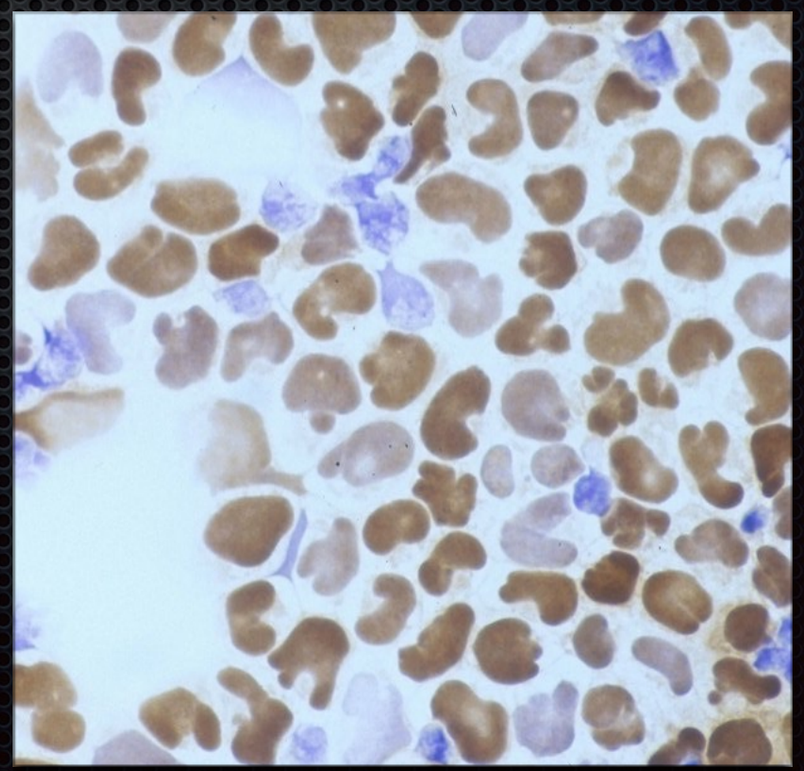
Methanol 5’



Ethanol 5’



NBF 5’



NBF 15’ - TritonX100 5’

Optimizing biomarker-protocols (ICC-protocols on cells)

Fixation/Epitope retrieval “Test Battery”

Manuel

No	Method				
1	No fixation (“drying”)	60 min		No retrieval	
2 ★	Acetone	10 min		No retrieval	
3 ★	4% NBF	5 min		No retrieval	
4	4% NBF	10 min	>>>>	0,5%TritonX-100	5 min
5 ★	4% NBF	15 min	>>>>	TEG, pH9 (95°C)	15 min
6	4% NBF	15 min	>>>>	MW/TRS pH6.1 (100°C)	15 min
7	4% NBF	15 min	>>>>	MW/CIT pH6 (100°C)	15 min
8 ★	The vendors recommendations				

TEG: Tris-EGTA based buffer pH 9,0. TRS: Target Retrival Solution pH 6,1 (Dako S1700). CIT: Citrate buffer pH 6,0

Optimizing biomarker-protocols (ICC-protocols on classical cyt. material)

Fixation/Epitope retrieval “Test Battery”

BenchMark

No	Method				
1	No fixation (“drying”)	60 min		No retrieval	
2 ★	Acetone	10 min		No retrieval	
3 ★	4% NBF	5 min		No retrieval	
4 ★	4% NBF	30 min	>>>>	CC1, pH8.5 (95°C)	8 min
5	4% NBF	30 min	>>>>	CC1, pH8.5 (95°C)	32 min
6 ★	The vendors recommendations				

CC1: Tris-EDTA based buffer pH8.5

Optimizing biomarker-protocols (ICC-protocols on LBC material)

Fixation/Epitope retrieval “Test Battery”

BenchMark

No	Method				
1	No fixation			No retrieval	
2 ★	4% NBF	5 min		No retrieval	
3 ★	No fixation		>>>>	CC1, pH8.5 (95°C)	8 min
4	No fixation		>>>>	CC1, pH8.5 (95°C)	32 min
5	4% NBF	30 min	>>>>	CC1, pH8.5 (95°C)	8 min
6 ★	4% NBF	30 min	>>>>	CC1, pH8.5 (95°C)	32 min
7 ★	The vendors recommendations				

CC1: Tris-EDTA based buffer pH8.5

Optimizing biomarker-protocols (ICC-protocols on classical cyt. material)

Fixation/Epitope retrieval “Test Battery”

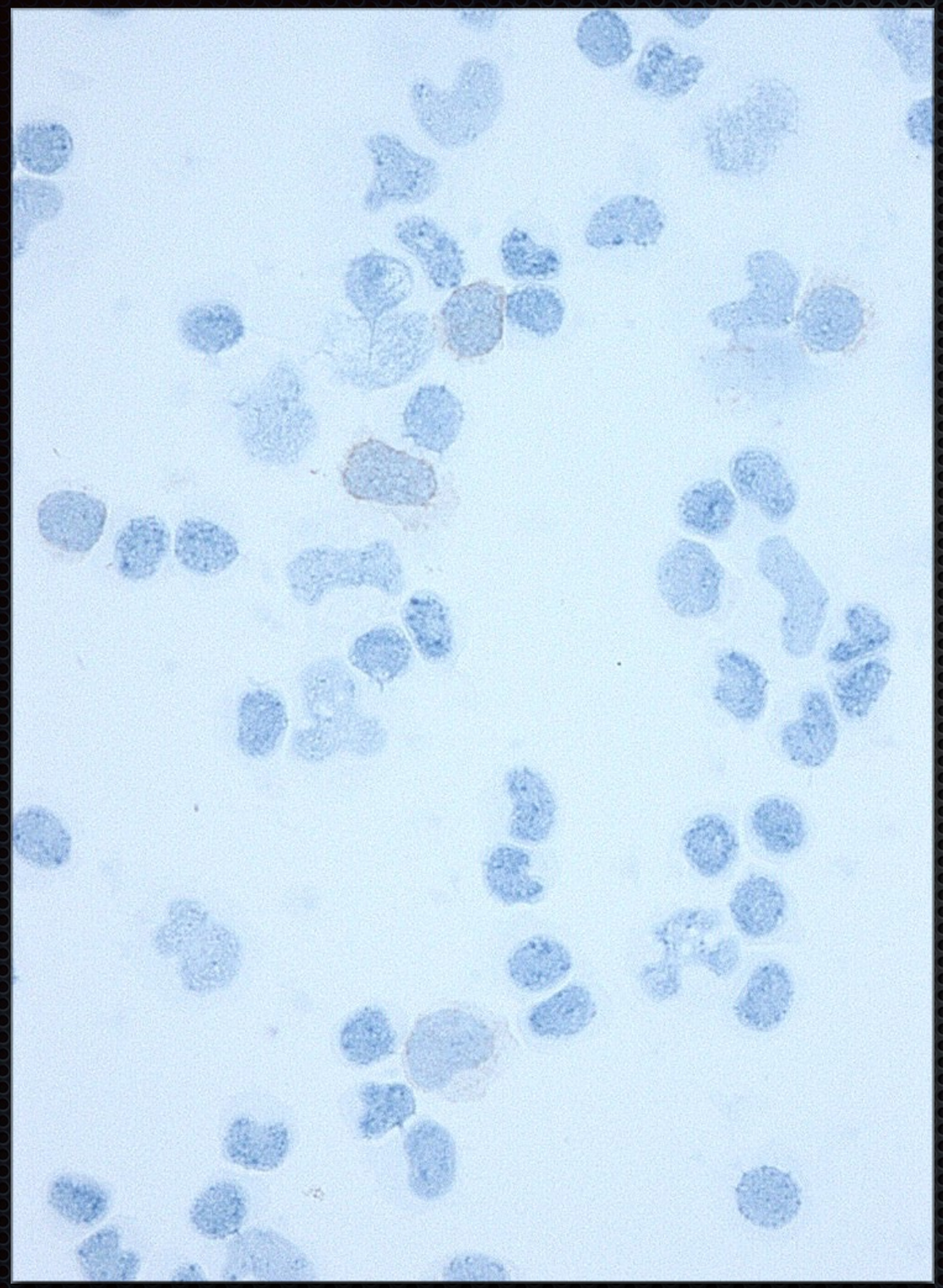
Omnis

No	Method				
1	No fixation (“drying”)	60 min		No retrieval	
2 ★	Acetone	10 min		No retrieval	
3 ★	4% NBF	5 min		No retrieval	
4 ★	4% NBF	30 min	>>>>	TRS-H, pH9.0 (97°C)	5 min
5	4% NBF	30 min	>>>>	TRS-H, pH9.0 (97°C)	10 min
6 ★	4% NBF	30 min	>>>>	TRS-L, pH6.1 (97°C)	5 min
7 ★	The vendors recommendations				

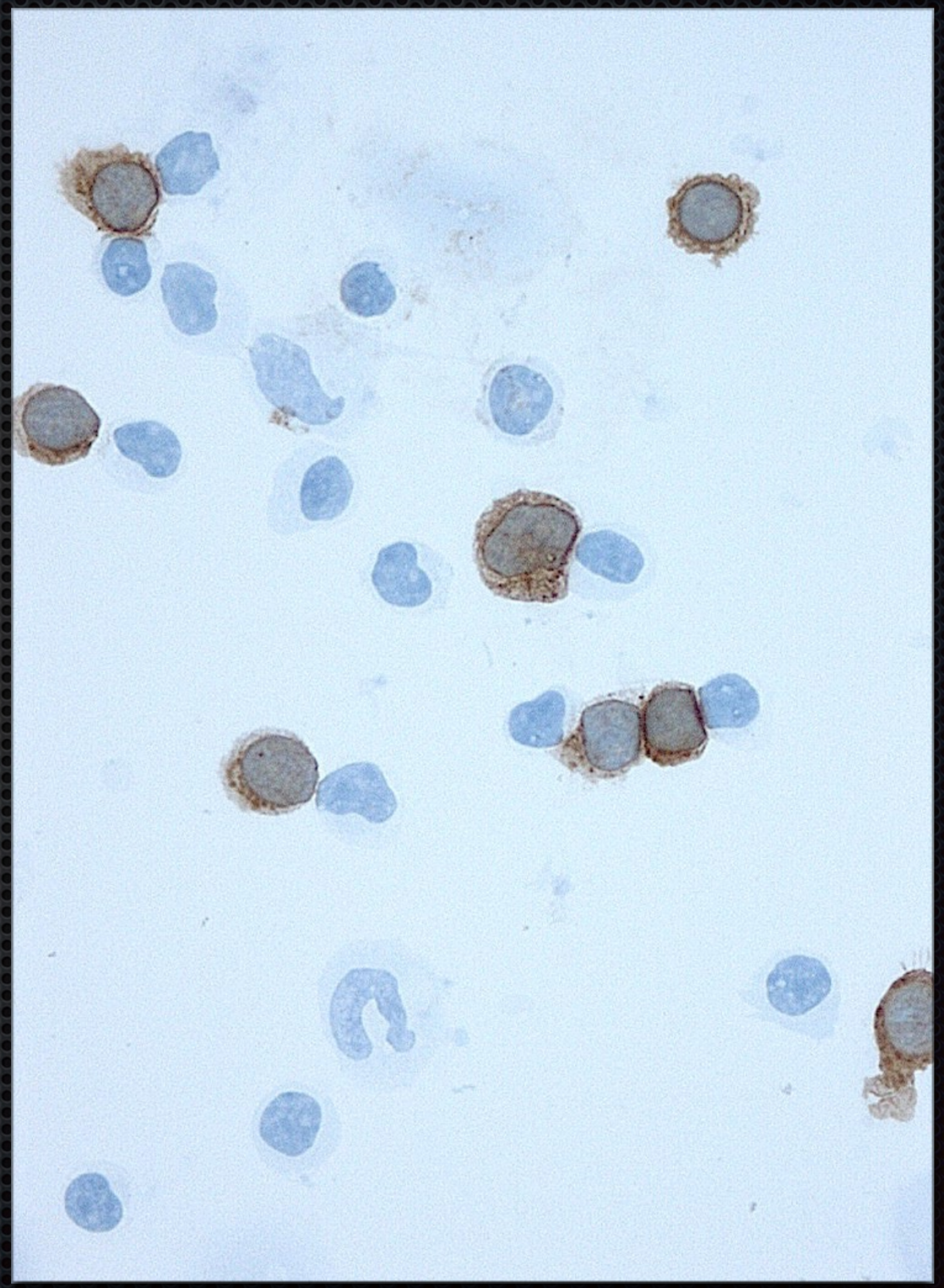
TRS-H: Tris-EDTA based buffer pH9.0

TRS-L: Citrate based buffer pH6.1

CD79a, JCB117 (Buffy coat cytospin) Fixation

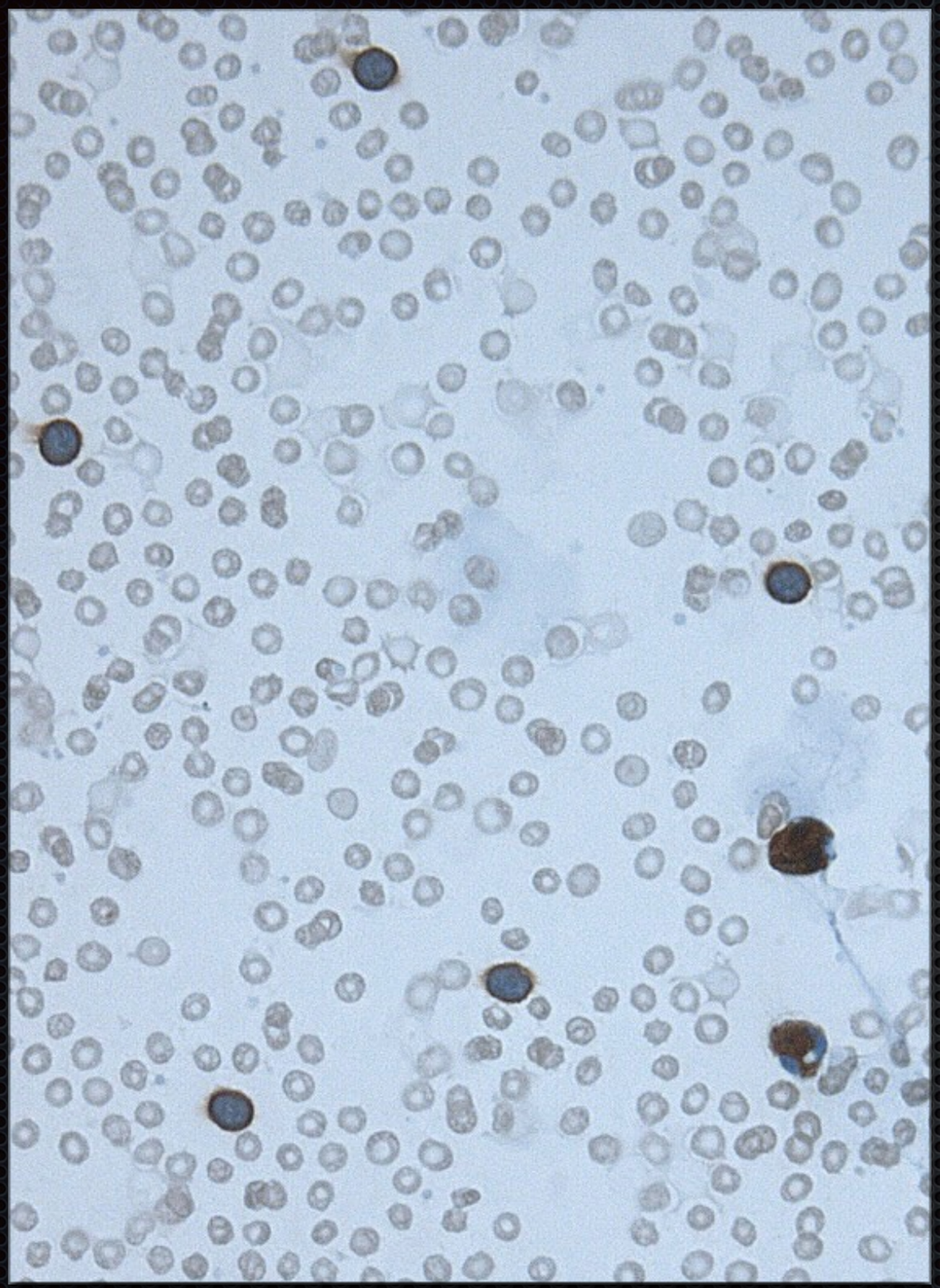


Acetone 10’



NBF 15’ - TEG95° 15’

CD79a, JCB117 (PB) Various fixatives

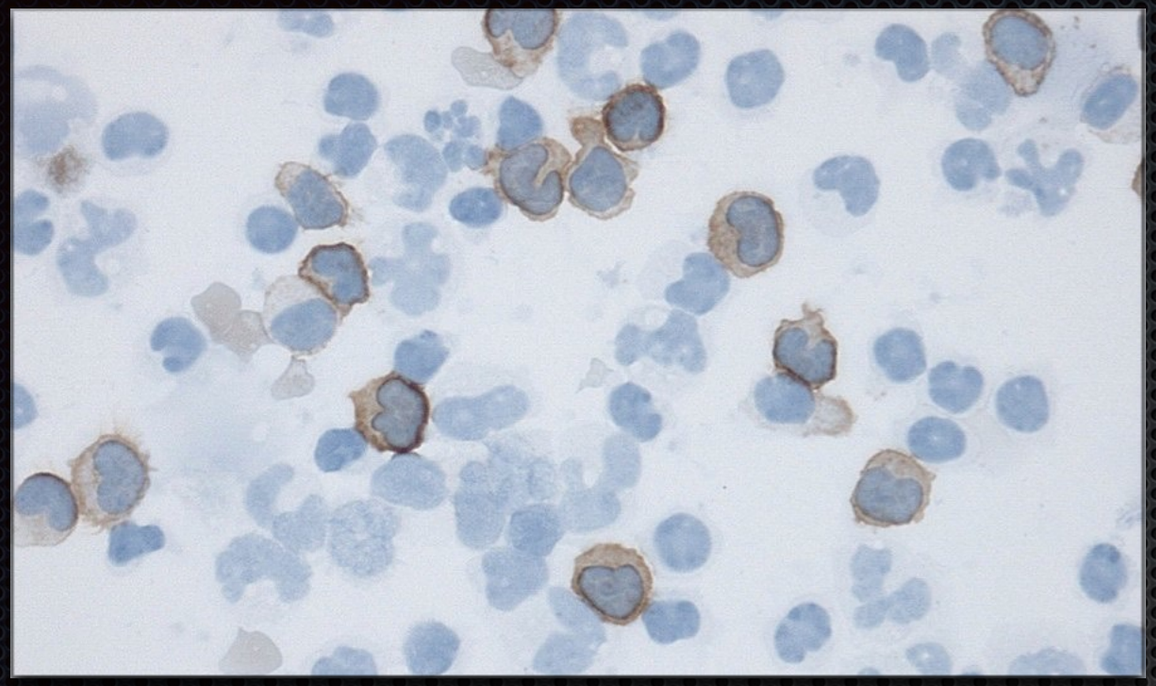


Acetone 10’

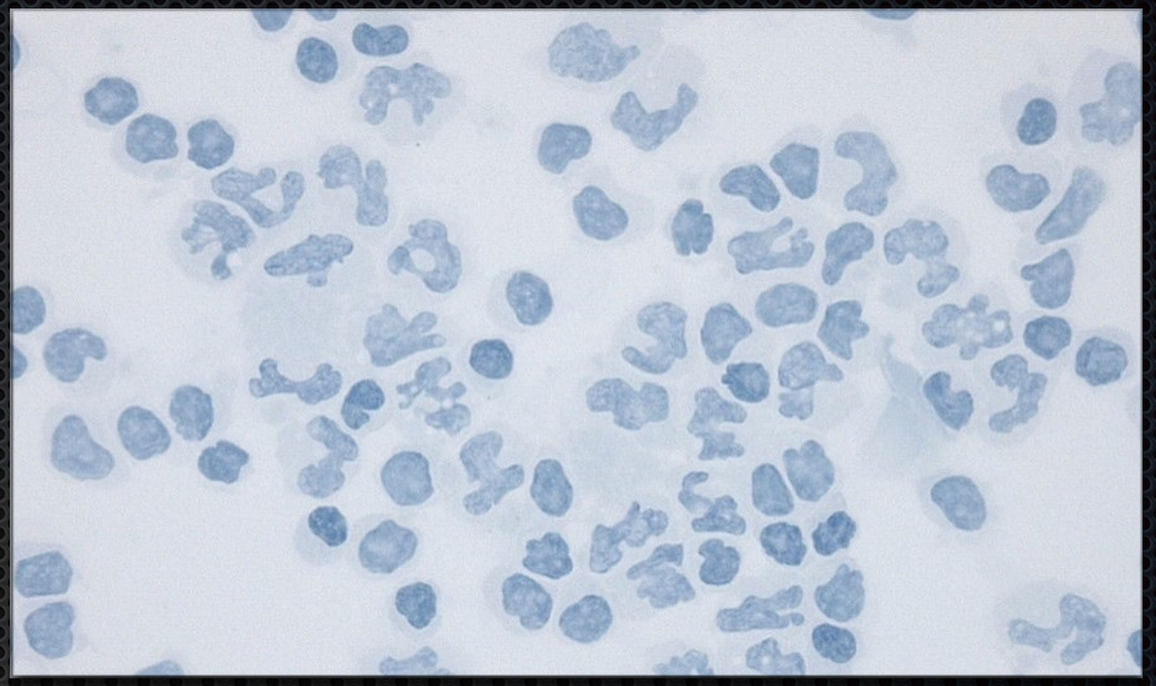


NBF 15’ - TEG95° 15’

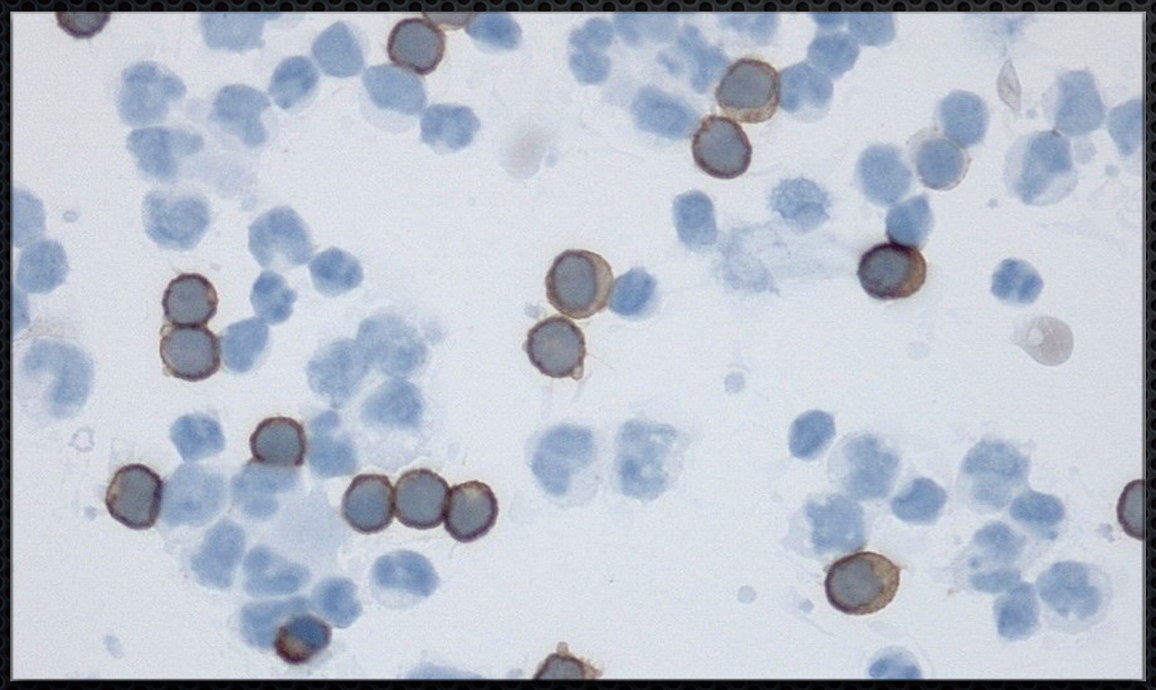
CD8 Abs (Buffy coat PB) various fixatives



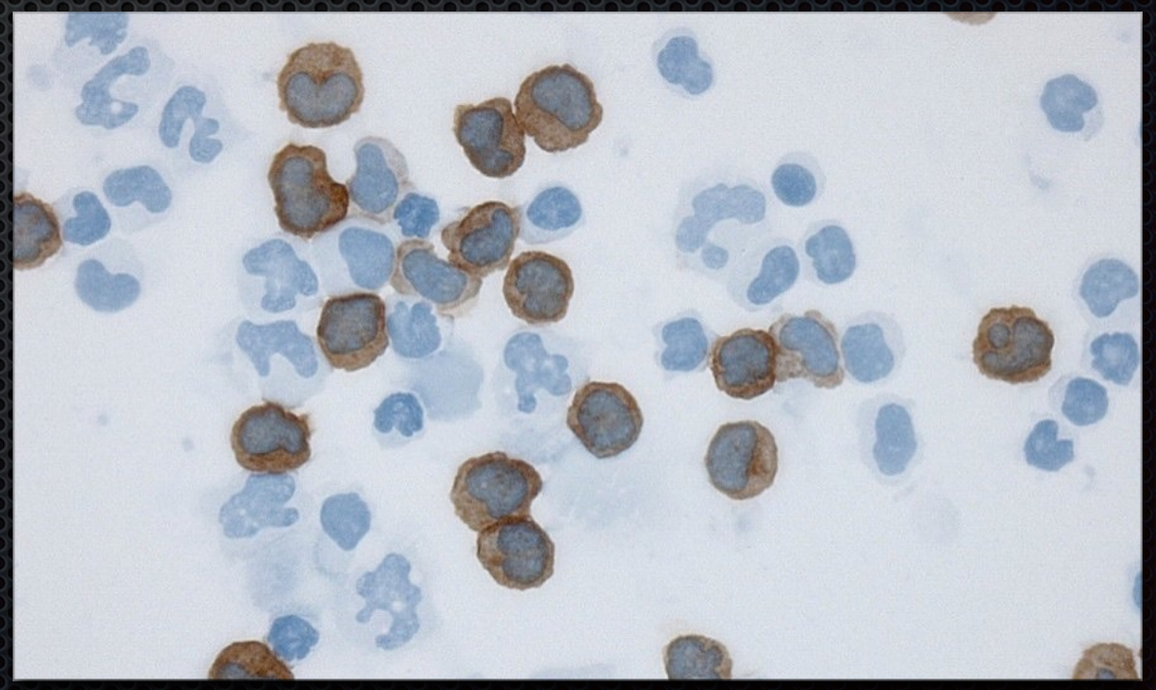
CD8, DK25 - Acetone 10’



CD8, DK25 - NBF 2’ - TEG95° 30s

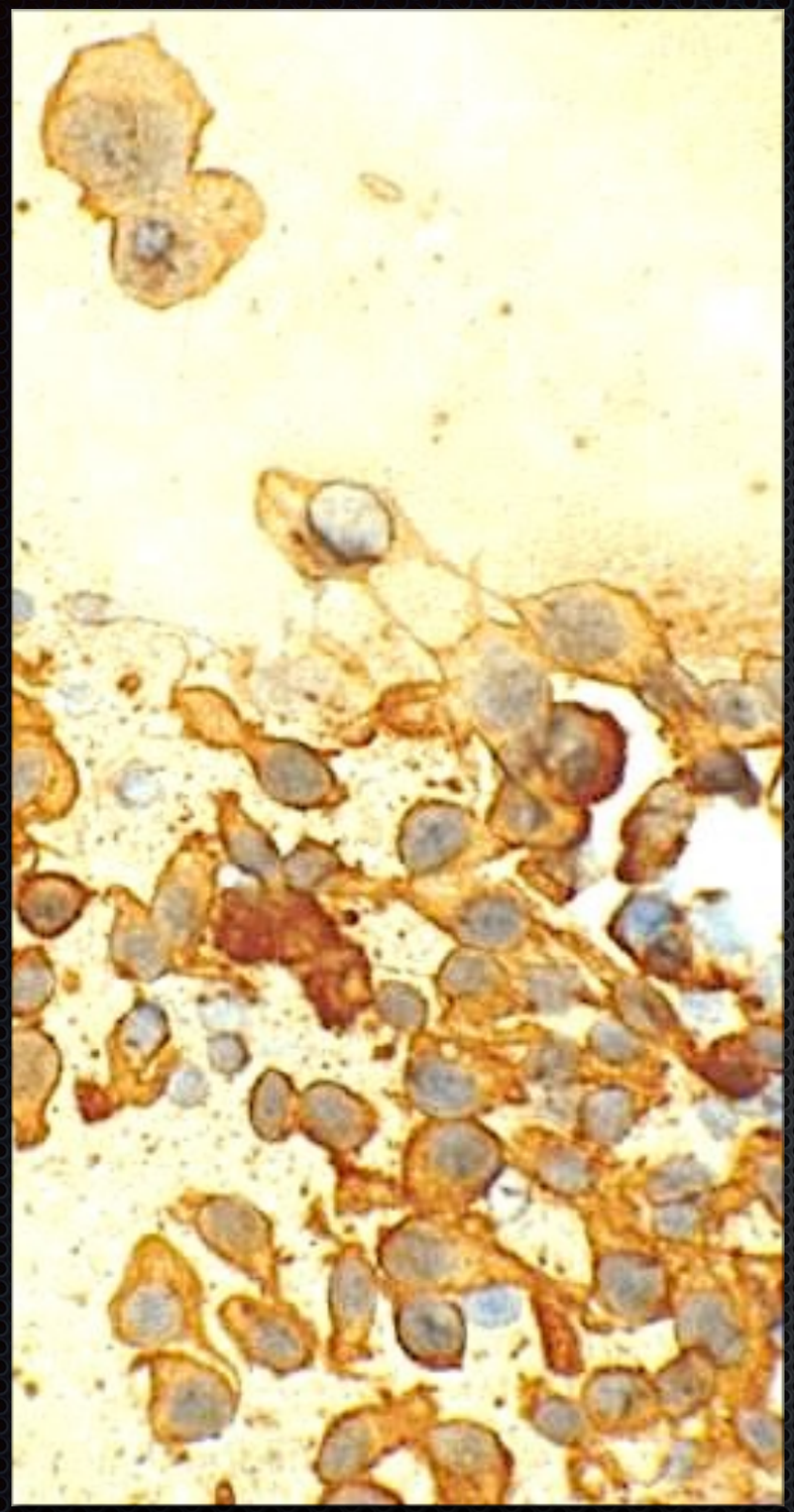


CD8, C8/144B - Acetone 10’

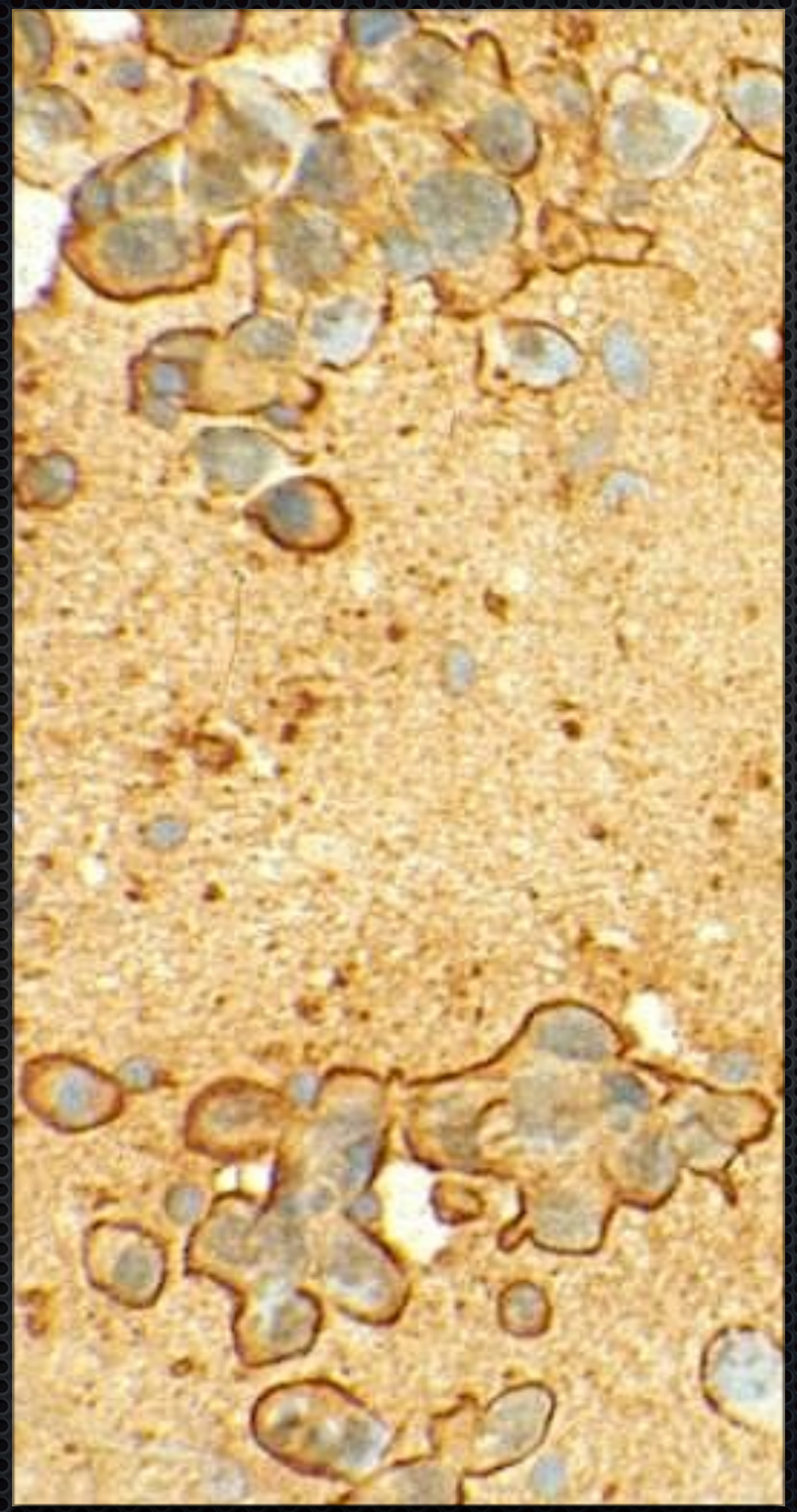


CD8, C8/144B NBF 2’ - TEG95° 30s

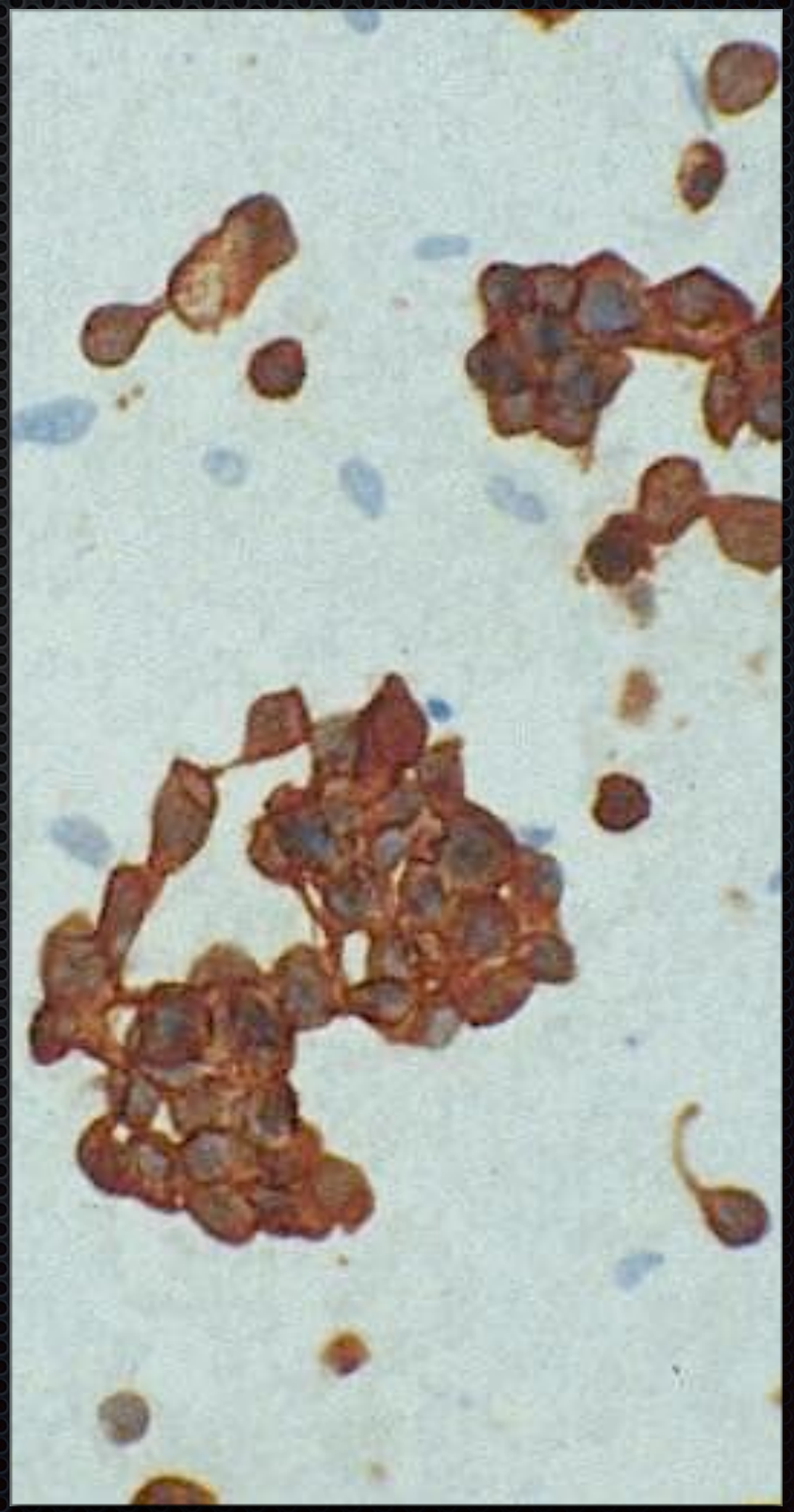
CK7, OV-TL12/30 (Imprint) various fixatives



Acetone 10’

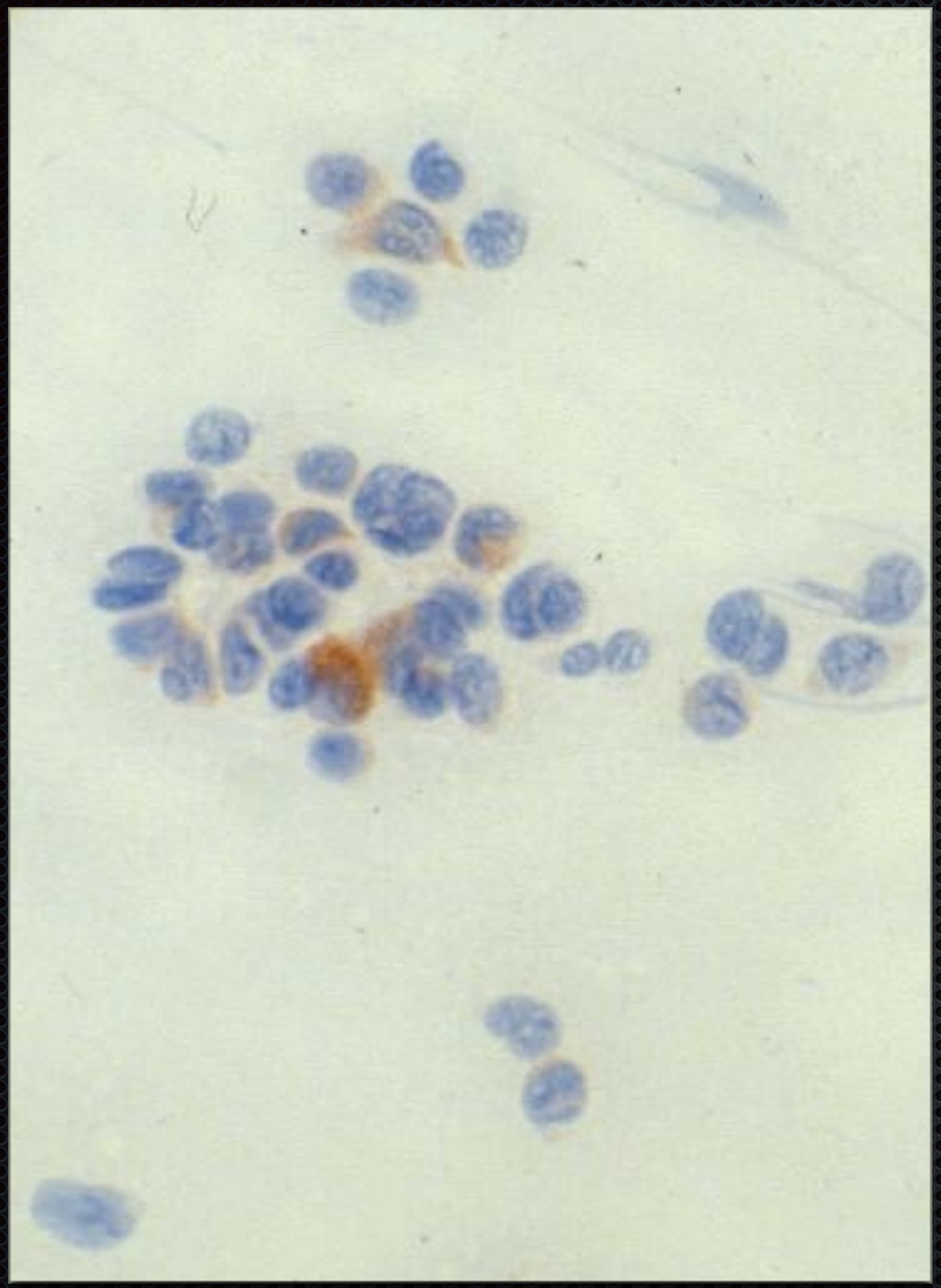


4% NBF 5’

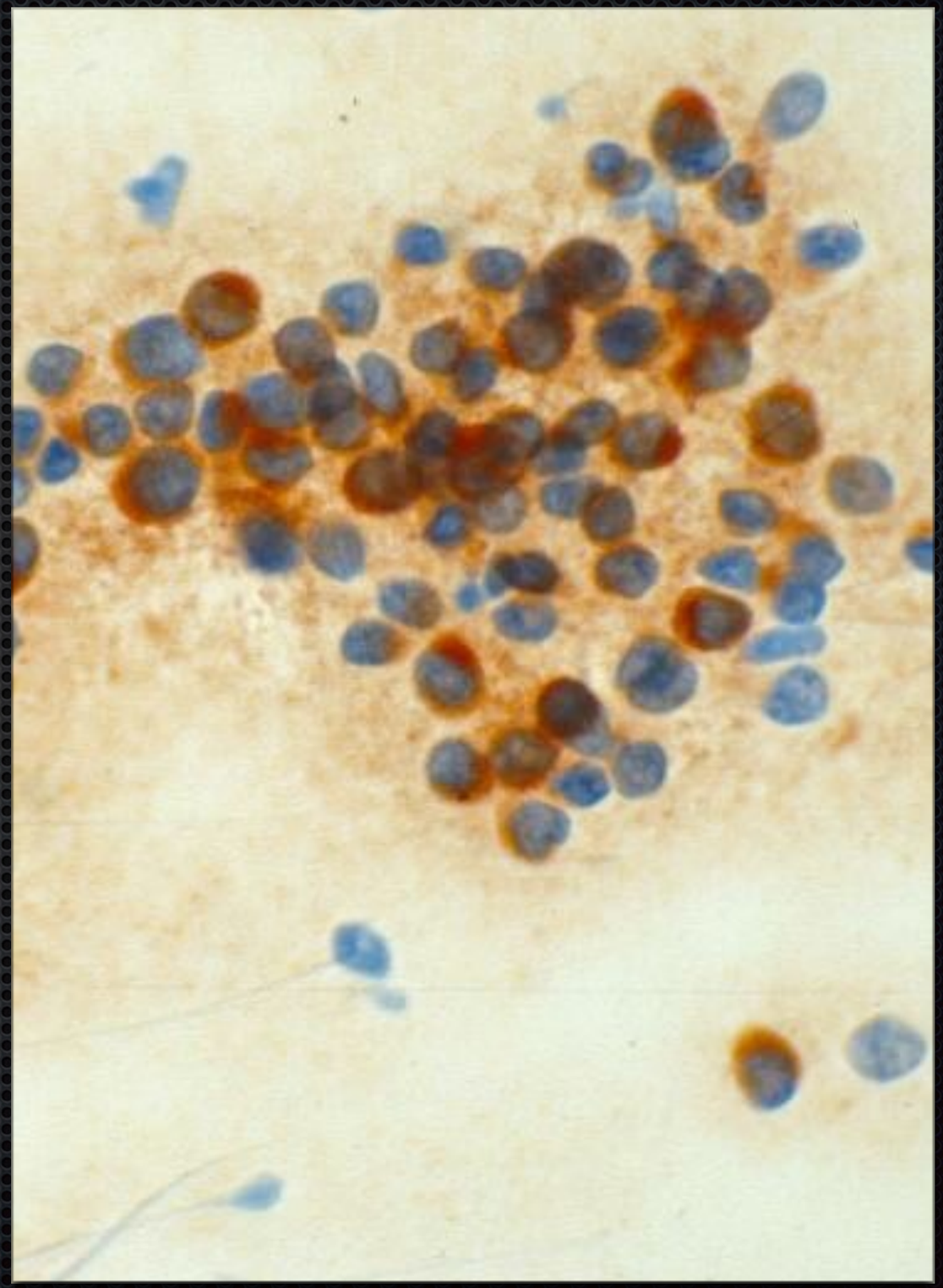


NBF 15’ - TEG95° 15

Tyrosinase, T311 (Imprint) Various fixatives

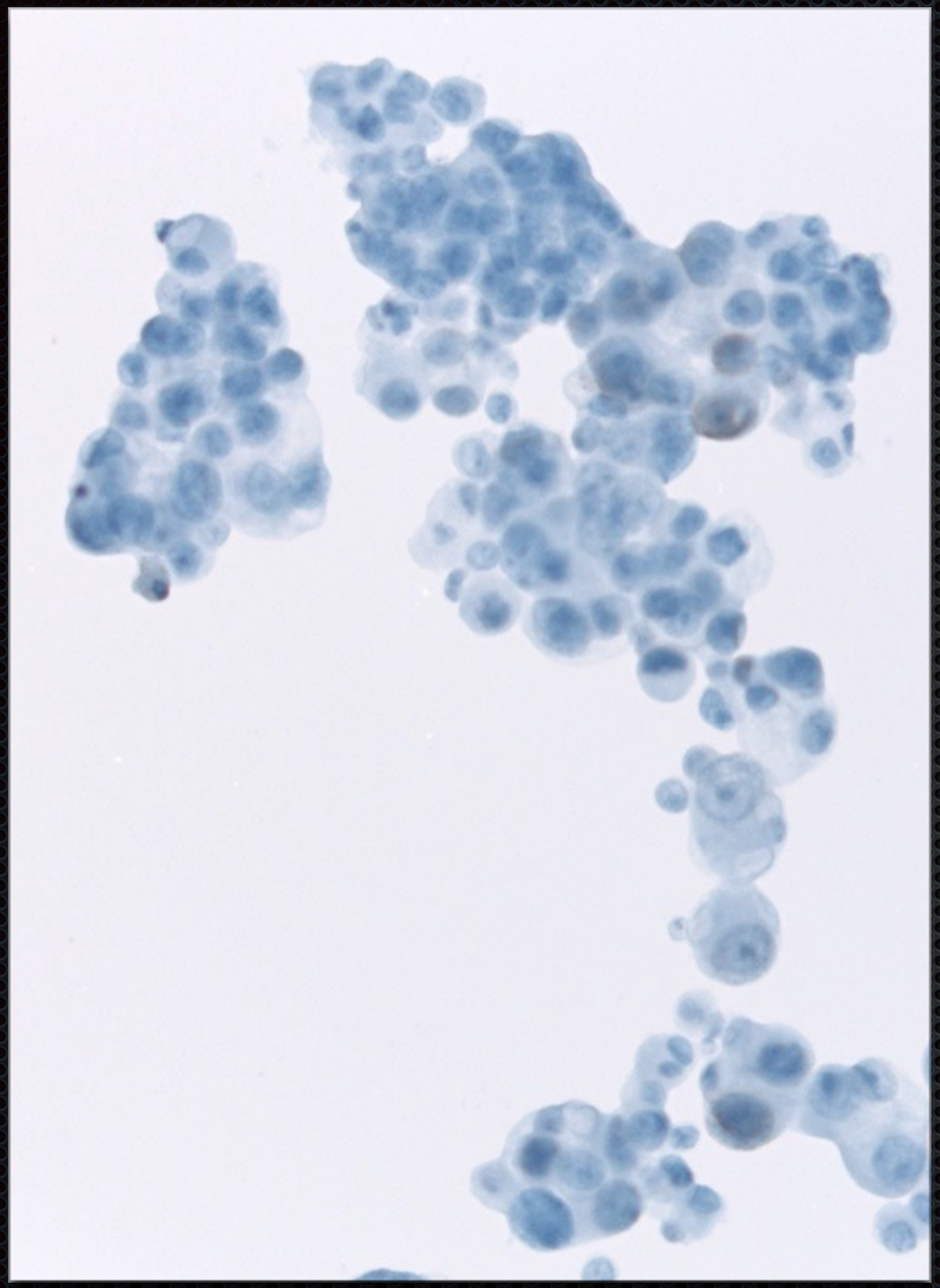


Acetone 10’

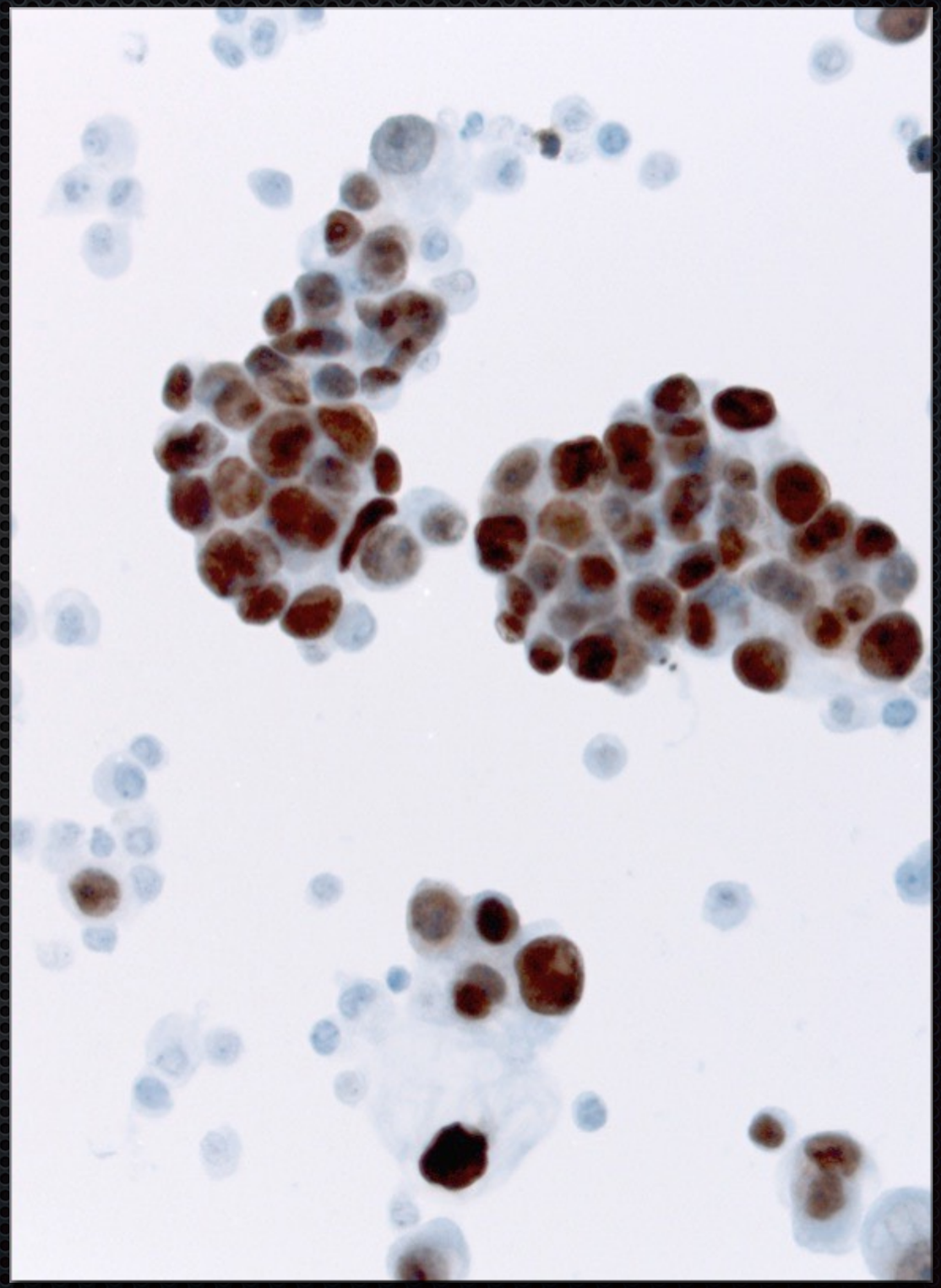


NBF 15’ - TEG95° 15’

TTF1, SPT24 (ThinPrep) Various fixatives



NBF 15’ - TEG95° 15’



NBF 15’ - MW/TEG 15’

p40, BC28 (LBC - A431 cells) Various fixatives

Ventana BenchMark Ultra

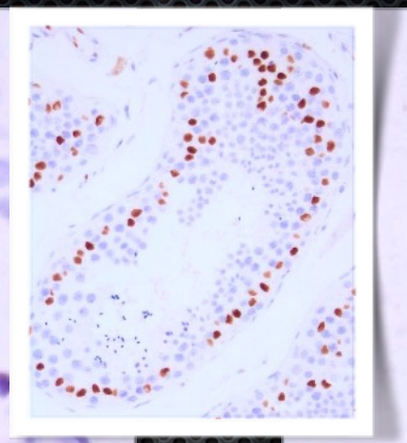


NBF 5’



NBF 5’ - CC1_32’_95°

WT-1, EP122 (Imprint, testis) Various fixatives



Dako OMNIS

NBF 5’

NBF 30’ - TRS-H_5’_95°

Cytology: Fixation and retrieval (Odense)

Antibody	Fixation	Retrieval
PAX-8, ZR-1 (Zeta)	4% NBF 15 min	CC1_32_95
CD117, YR145	4% NBF 30 min	CC1_8_95
CD14, 7 -	4% NBF 30 min	CC1_8_95
CD19, SP110	4% NBF 30 min	CC1_8_95
CD1a, EP3622	4% NBF 30 min	CC1_8_95
CD2, MRQ-11	4% NBF 30 min	CC1_8_95
CD20cy, L26	4% NBF 30 min	CC1_8_95
CD23, SP23	4% NBF 30 min	CC1_8_95
CD3, 2GV6	4% NBF 30 min	CC1_8_95
CD33, PWS44	4% NBF 30 min	CC1_8_95
CD34, EP88	4% NBF 30 min	CC1_8_95
CD4, SP35	4% NBF 30 min	CC1_8_95
CD45, 2B11 &	4% NBF 30 min	CC1_8_95
CD5, SP19	4% NBF 30 min	CC1_8_95
CD56, MRQ-42	4% NBF 30 min	CC1_8_95
CD71, 10F11	4% NBF 30 min	CC1_8_95
CD8, C8/144B	4% NBF 30 min	CC1_8_95
CK20, SP33	4% NBF 30 min	CC1_8_95
CA-125, OC125	4% NBF 30 min	CC1_8_95
CDX2, EPR2764Y	4% NBF 30 min	CC1_8_95
EMA, E29	4% NBF 30 min	CC1_8_95
Ep-CAM, Ber-EP4	4% NBF 30 min	CC1_8_95
HEPA, OCH1E5	4% NBF 30 min	CC1_8_95
PSA, p	4% NBF 30 min	CC1_8_95
TTF-1, SPT24	4% NBF 30 min	CC1_8_95
Villin, CWWB1	4% NBF 30 min	CC1_8_95
WT1, 6F-H2	4% NBF 30 min	CC1_8_95
Calcitonin, poly	4% NBF 30 min	CC1_8_95
ER, SP1	4% NBF 30 min	CC1_8_95
Oct-3/4	4% NBF 30 min	CC1_8_95
[illegible]	[illegible]	[illegible]
TG, 2H11/6E	4% NBF 30 min	CC1_8_95
[illegible]	[illegible]	[illegible]
Podoplanin, D2-40	4% NBF 30 min	CC1_8_95
MITF, 24CA5	4% NBF 30 min	CC1_8_95
Ki67, 30-9	4% NBF 30 min	CC1_8_95
p63, 4A4	4% NBF 30 min	CC1_8_95
CGA, LK2H10	4% NBF 5 min	CC1_8_95
p40, BC28	4% NBF 5 min	CC1_32_95

39 Abs: NBF (5’-30’) HIER pH9 (8’-32’)

Antibody	Fixation	Retrieval
CD15, MMA	4% NBF 5 min	None
CD30, Ber-H2	4% NBF 5 min	None
CD61, 2f2	4% NBF 5 min	None
TdT, SEN28	4% NBF 5 min	None
CK17, SP95	4% NBF 5 min	None
CK19, A53-B/A2.26	4% NBF 5 min	None
CK5, XM26	4% NBF 5 min	None
CK7, SP52	4% NBF 5 min	None
CK, AE1/AE3	4% NBF 5 min	None
CK, CAM 5.2	4% NBF 5 min	None
CD68, EBM11	4% NBF 5 min	None
Calretinin, SP65	4% NBF 5 min	None
CEA, Col-1	4% NBF 5 min	None
GATA3, L50-823	4% NBF 5 min	None
Mesotelial Cell,	4% NBF 5 min	None
Napsin A, IP64	4% NBF 5 min	None
P501S, 10E3	4% NBF 5 min	None
TPO, MoAb47	4% NBF 5 min	None
Vimentin, V9	4% NBF 5 min	None
Melan-A, A103	4% NBF 5 min	None
S-100, p	4% NBF 5 min	None
Synaptophysin,	4% NBF 5 min	None

22 Abs: NBF 5’

Antibody	Fixation	Retrieval
CD10, 56C6	Acetone 10 min	None
CD42b, MM2/174	Acetone 10 min	None
CD79a, SP18	Acetone 10 min	None

3 Abs: Acetone 10’

Antibody	Fixation	Retrieval
CEA, Col-1	4% NBF 30 min	TRS-L_5_95
Ep-CAM, BS14	4% NBF 30 min	TRS-L_5_95
PAX-8, EP298	4% NBF 30 min	TRS-H_10_95
WT1, EP122	4% NBF 30 min	TRS-H_5_95

4 Abs: Omnis

ThinPrep (LBC): Fixation and retrieval (Odense)

Antibody	Fixation	Retrieval
CD1a, EP3622	4% NBF 30 min	CC1_32_100
CD56, MRQ-42	4% NBF 30 min	CC1_32_100
CK19, A53-B/A2.26	4% NBF 30 min	CC1_32_100
CK, CAM 5.2	4% NBF 30 min	CC1_32_100
CK, KL1	4% NBF 30 min	CC1_32_100
E-Cadherin, 36	4% NBF 30 min	CC1_32_100
Napsin A, IP64	4% NBF 30 min	CC1_32_100
TTF-1, SPT24	4% NBF 30 min	CC1_32_100
CD56, 56C04	4% NBF 30 min	CC1_32_100
Ki67, 30-9	4% NBF 30 min	CC1_32_100

Antibody	Fixation	Retrieval
CD3, 2GV6	4% NBF 30 min	CC1_32_95
CD5, SP19	4% NBF 30 min	CC1_32_95
CK7, SP52	4% NBF 30 min	CC1_32_95
Calretinin, SP65	4% NBF 30 min	CC1_8_95
CK18, DC10	4% NBF 5 min	CC1_32_100
CDX2, EPR2764Y	4% NBF 5 min	CC1_32_100
p40, BC28	4% NBF 5 min	CC1_32_100
P501S, 10E3	None	CC1_32_100
PSA, p	None	CC1_32_100
Melan-A, A103	None	CC1_32_100
MITF, 24CA5	None	CC1_32_100
p63, 4A4	None	CC1_32_100
CD7, CBC.37	None	None

Cryo section: Fixation and retrieval (Odense)

Antibody	Fixation	Retrieval
MyoD1, EP212	None	None
CD56, MRQ-42	None	None
Serca1, VE121G9	None	None
C1q-FITC	None	None
Albumin-FITC	None	None
Fibrinogen-FITC	None	None
C3-FITC	None	None
Lambda-FITC	None	None
Kappa-FITC	None	None
IgA-FITC	None	None
IgM-FITC	None	None
IgG-FITC	None	None
Dystrophin, 34C5	None	None
Desmin, DE-R-11	None	None
Collagen IV, CIV22	None	None
Collagen VI, VI-26	None	None
Pax-7, P3U1	None	None
Laminin alfa2, 4H8-2	None	None
Laminin alfa2, Mer3/22B2	None	None
Calpain, 12A2	None	None

Antibody	Fixation	Retrieval
Dysferlin, Ham1/7B6	None	None
Sarcoglycan g, 35DAG/21B5	None	None
Sarcoglycan delta, ∂-Sarc/12C1	None	None
Sarcoglycan beta, ß-SARC/5B1	None	None
Laminin alfa5, 4C7	None	None
Caveolin 3, 26	None	None
Emerin, 4G5	None	None
Utrophin, DRP2/20C5	None	None
Serca2, IID8	None	None
Myosin neonatal, WB-MHCn	None	None
Myosin slow, WB-MHCs	None	None
Myosin -Fast, MY32	None	None
Laminin beta2, C4	None	None
Laminin beta1, 4E10	None	None
Dystrophin, DY8/6C5	None	None
Dystrophin, DY4/6D3	None	None
Dystroglycan beta, NCL-43DAG	None	None
Actinin alfa, RBC2/1B6	None	None
Sarcoglycan alfa, Ad1/20A6	None	None

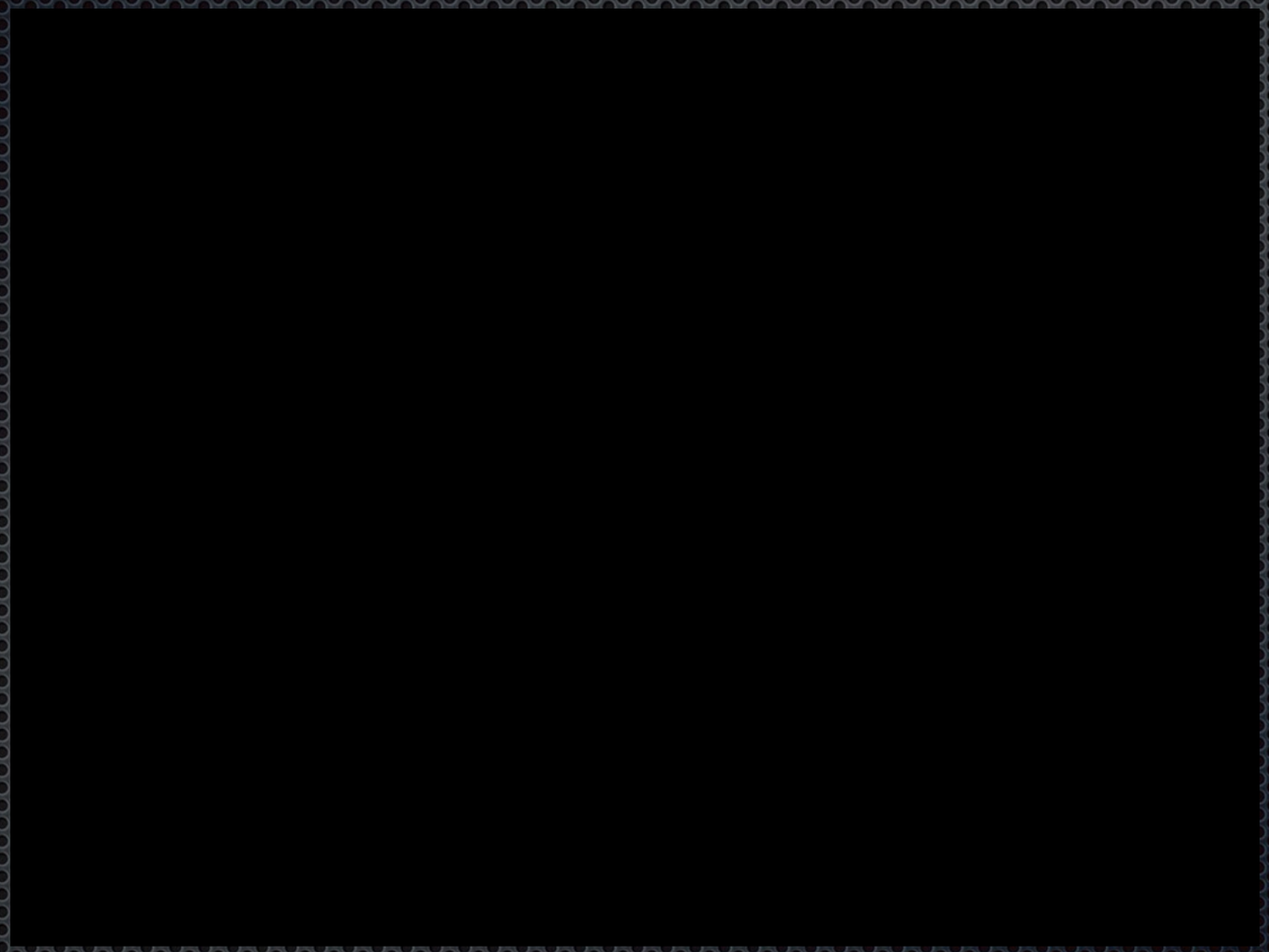
Antibody	Fixation	Retrieval
Cranin, VIA4-1	4% NBF 15 min	CC1_8_90
CD4, SP35	4% NBF 5 min	None
CD45, 2B11 & PD7/26	4% NBF 5 min	None
CD56, 56C04	4% NBF 5 min	None
C5b-9, aE11	4% NBF 5 min	None
CD57, HNK-1	4% NBF 5 min	None
CD8, C8/144B	4% NBF 5 min	None
CD20cy, L26	Acetone 10 min	None
Collagen IV, MAB3	Acetone 10 min	None
Collagen IV, MAB1	Acetone 10 min	None
GFAP, p	Acetone 10 min	None
HLA-DR, CR3/43	Acetone 10 min	None
HLA-ABC, W6/32	Acetone 10 min	None
CD68, EBM11	Acetone 10 min	None
Collagen IV, MAB5	Acetone 10 min	Glycin/Urea

40 Ab: No Fixation and no retrieval (mainly muscle markers and markers for IF)

Summary

- Optimizing the fixation and epitope retrieval procedures are very important elements in optimizing biomarker protocols for both frozen sections and cytological materials.
- The optimization of the fixation and epitope retrieval procedures should be based on a Test Battery approach !

Thank you for your attention!



Have a save trip home!