

Immunocytochemistry and immunohistochemistry on frozen sections

Overview, considerations and applications

NQC workshop 2018

Ole Nielsen,
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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, δ -Sarc/12C1

Sarcoglycan beta, β -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

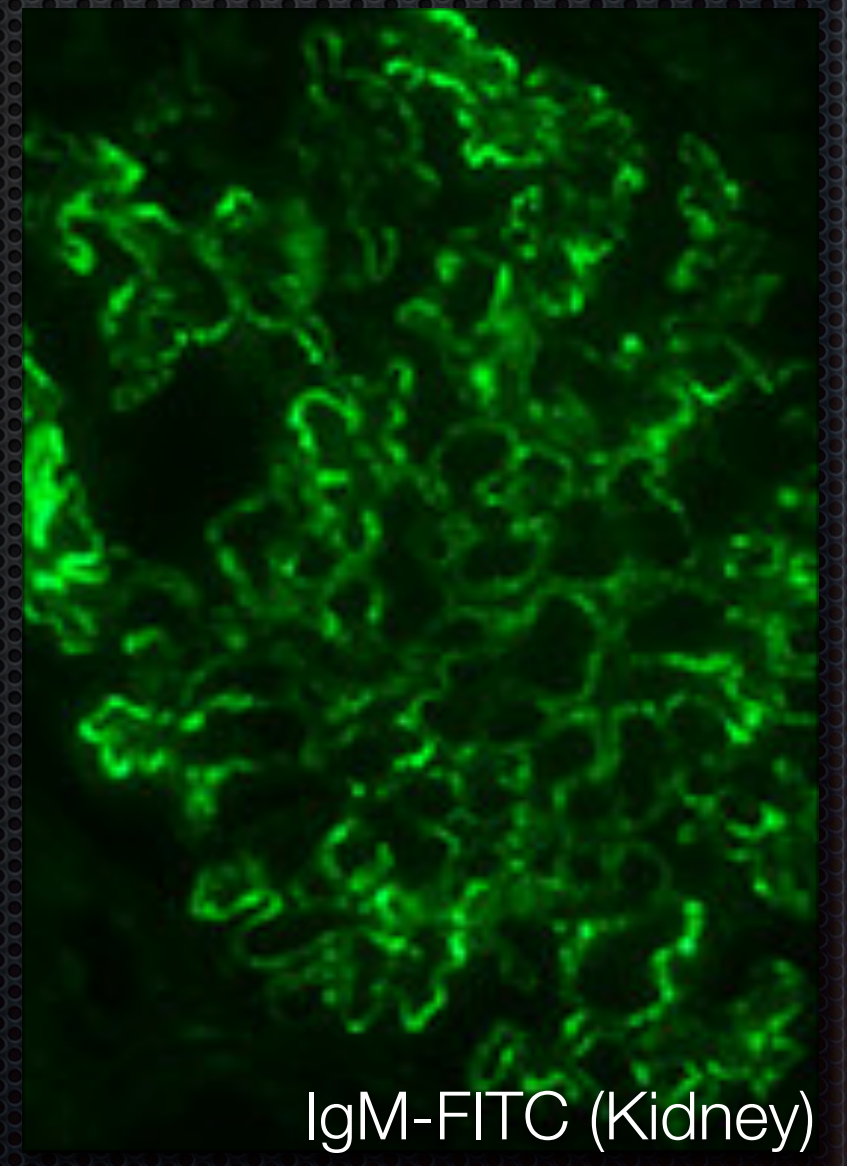
Collagen IV, MAB5

ICC protocols (69) (Odense)

Antibody	Antibody	Antibody
ALK, D5F3 (CD246)	CD71, 10F11	Myosin -Fast, MY32
CA-125, OC125	CD79a, SP18	Napsin A, IP64
Calcitonin, SP17	CD117, YR145	Oct-3/4, N1NK
Calretinin, SP65	CD138, B-A38	p40, BC28
CD1a, EP3622	CDX2, EPR2764Y	P501S, 10E3
CD2, MRQ-11 -T-Cell	CEA, COL-1	p63, 4A4
CD3, 2GV6	Chromogranin A, LK2H10	PAX-8, EP298
CD4, SP35	CK, AE1/AE3	PR, 1E2
CD5, SP19	CK, CAM 5.2	PSA, p
CD8, C8/144B	CK5, XM26	S-100, p
CD10, 56C6	CK7, SP52	Synaptophysin, 27G12
CD14, EPR3653	CK17, SP95	TdT, SEN28
CD15, MMA	CK19, A53-B/A2.26	TG, 2H11/6E1
CD19, SP110	CK20, SP33	TPO, MoAb47
CD20cy, L26	Podoplanin, D2-40	TTF-1, SPT24
CD23, SP23	EMA, E29	Villin, CWWB1
CD30, Ber-H2	Ep-CAM, BS14	Vimentin, V9
CD33, SP266	ER, SP1	Wilms Tumor 1, EP122
CD34, EP88	GATA3, L50-823	
CD42b, MM2/174	Mesotelial Cell, HBME-1	
CD45, 2B11 & PD7/26	Hepa. Spec. Ag, OCH1E5	
CD56, 56C04	Ki67, 30-9	
CD56, MRQ-42	Melan-A, A103	
CD61, 2f2	MITF, 24CA5	
CD68, EBM11	Myogenin, F5D	

IHC on frozen sections

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



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

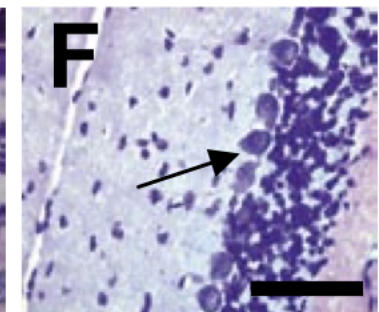
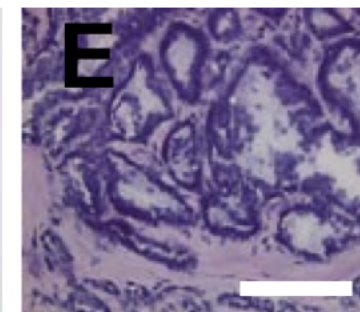
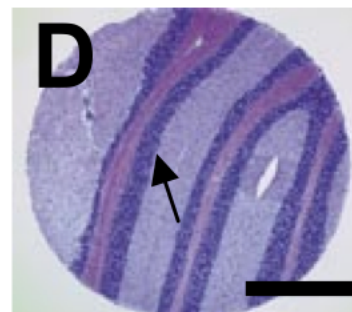
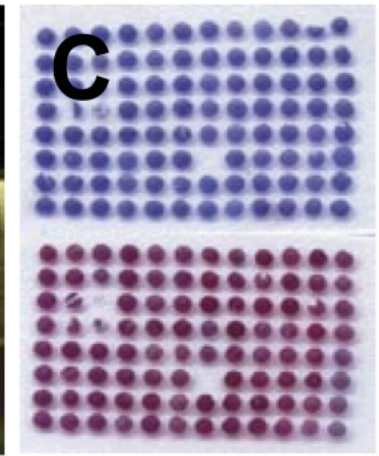
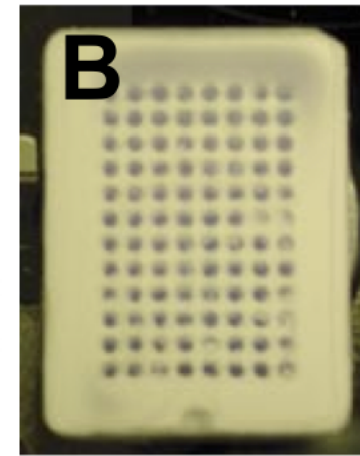
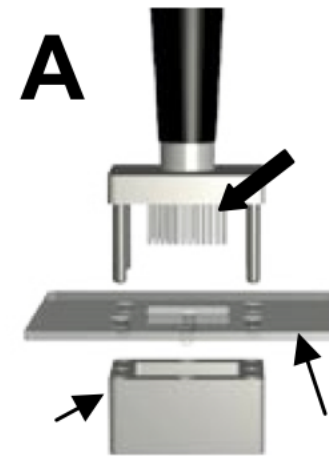
New tissue microarray technology for analyses of gene expression in frozen pathological samples

Lei Zhou¹, Melissa Hodeib², Joseph D. Abad², Leopoldo Mendoza¹, Anilkumar R. Kore¹, and Zhongting Hu²

¹Ambion, Inc., Austin, TX and ²Western University of Health Sciences, CA, USA

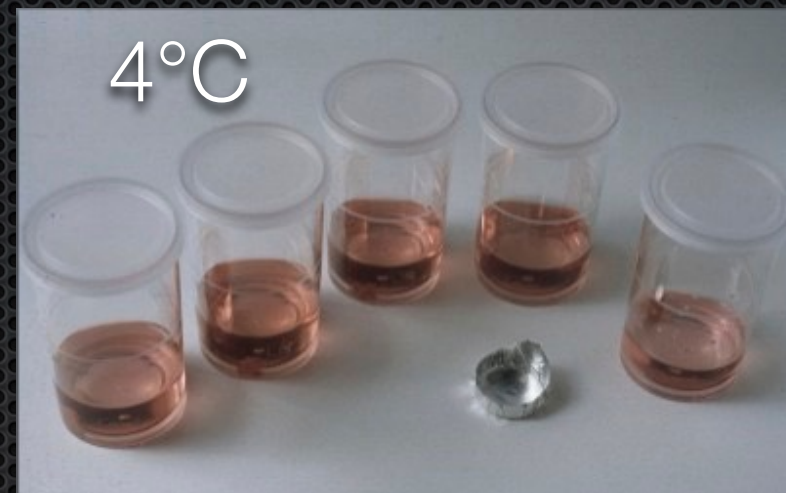
BioTechniques 43:101-105 (July 2007)
doi 10.2144/000112498

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



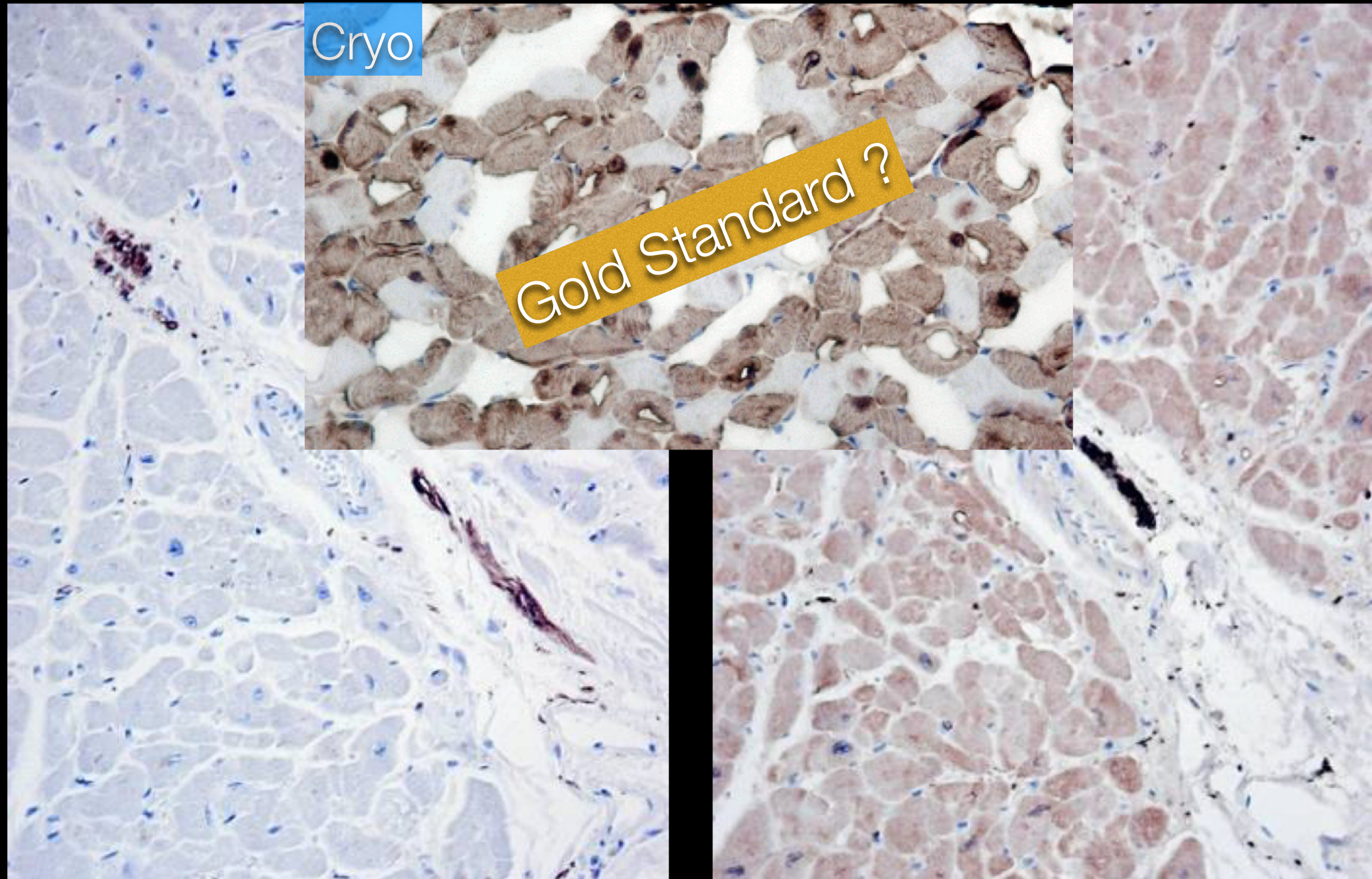
Optimizing biomarker-protocols (IHC-protocols on frozen sections)

- ✦ "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	Frozen Section Fixed in							
	Acetone or Ethanol (10 min)		NBF (30 min)		NBF (Overnight)		FFPE Section	
	Acetone	Ethanol	w/o AR	w/AR	w/o AR	w/AR	w/o AR	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+

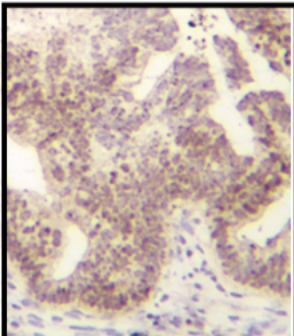
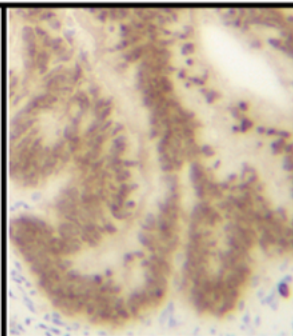
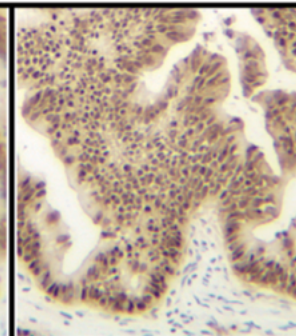
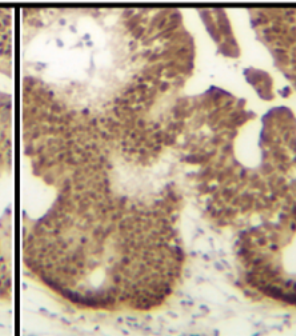
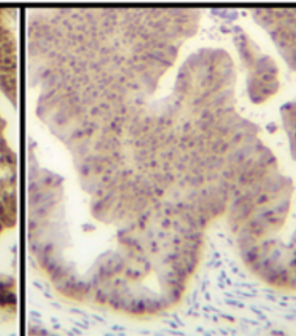
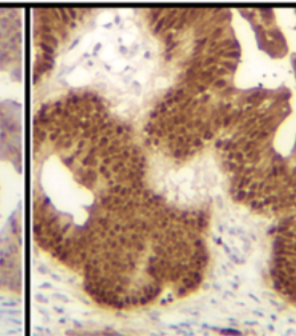
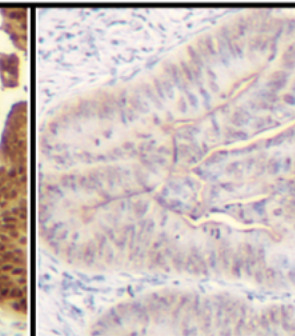
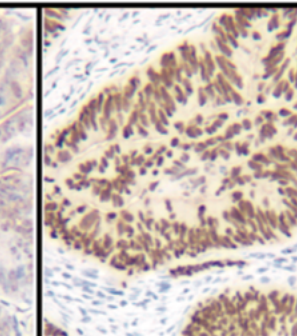
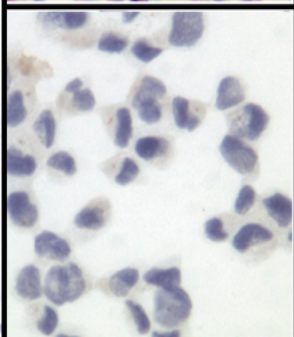
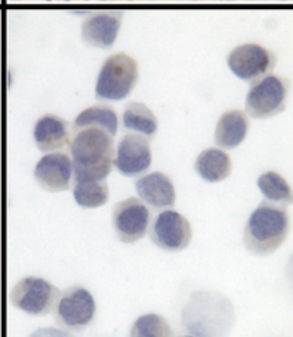
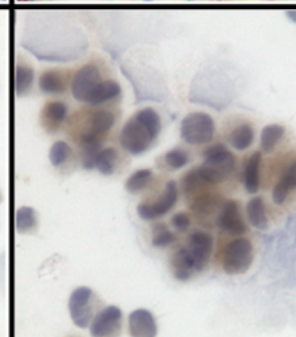
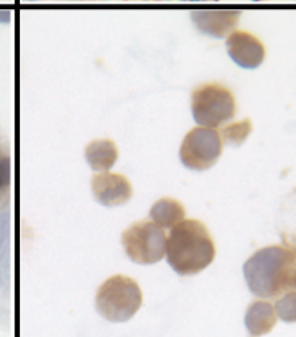
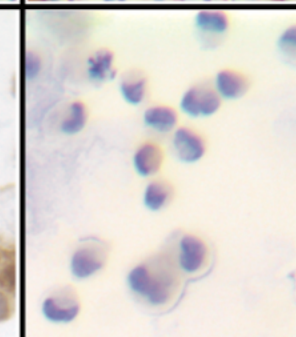
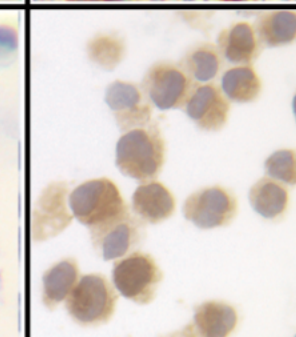
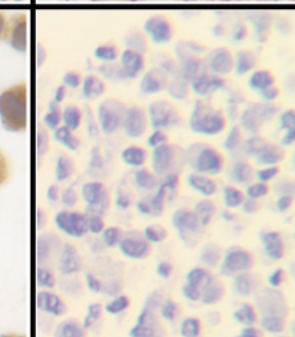
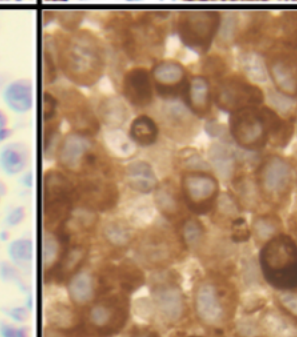
AR, antigen retrieval; CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; FFPE, formalin-fixed, paraffin-embedded; GRP, glucose-regulated protein; NBF, neutral buffered formalin; Rb, retinoblastoma; w/AR, use of the AR pretreatment before the immunohistochemical staining procedure; 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

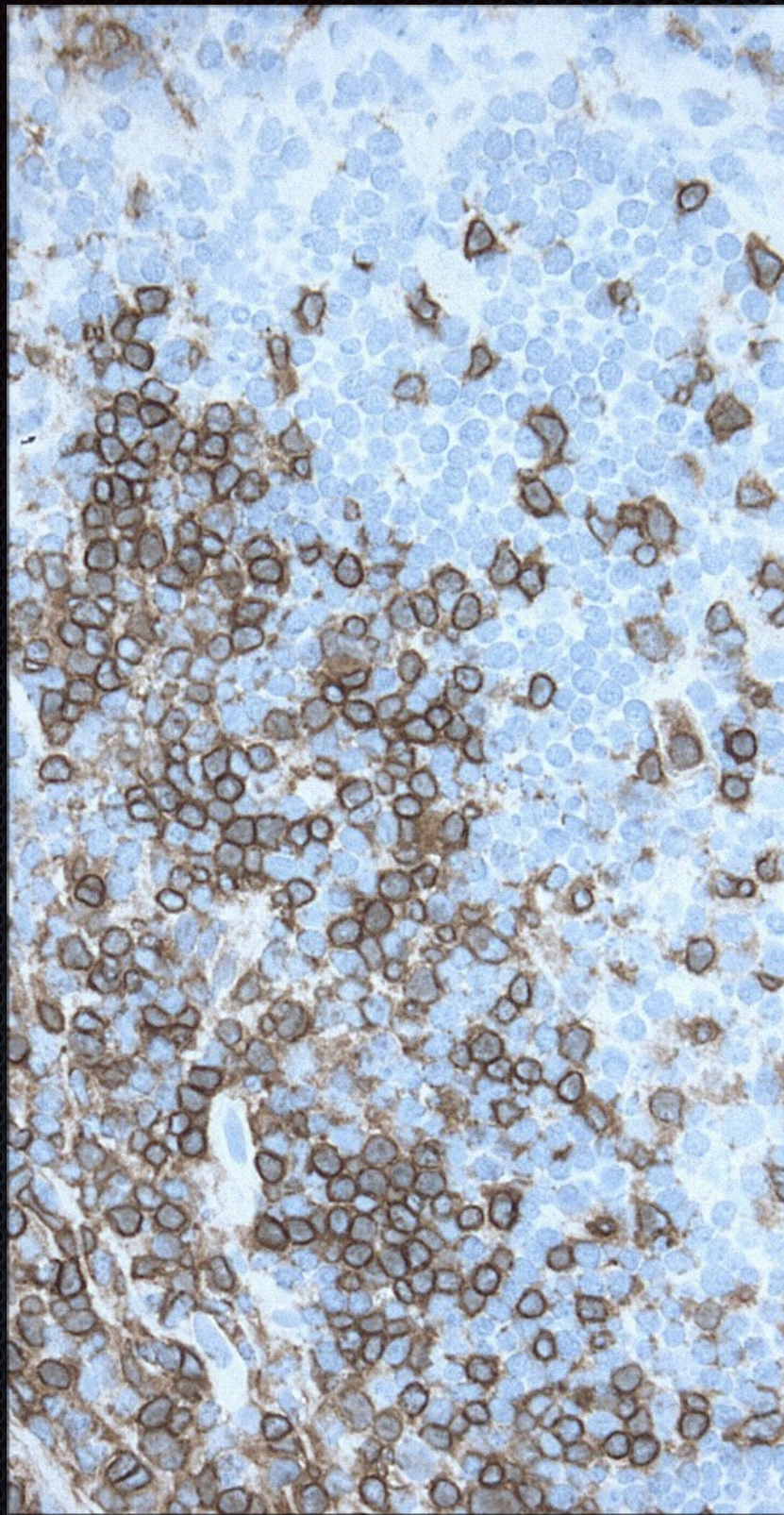
Am J Clin Pathol 2008;129:358-366

	Acetone	Ethanol	NBF (30 min)	NBF (30 min)	NBF ON	NBF ON	FFPE	FFPE
	Acetone	Ethanol	NBF 30 w/o AR	NBF 30 w/AR	NBF ON w/o AR	NBF ON w/AR	FFPE w/o AR	FFPE w/AR
p53								
GRP-78								

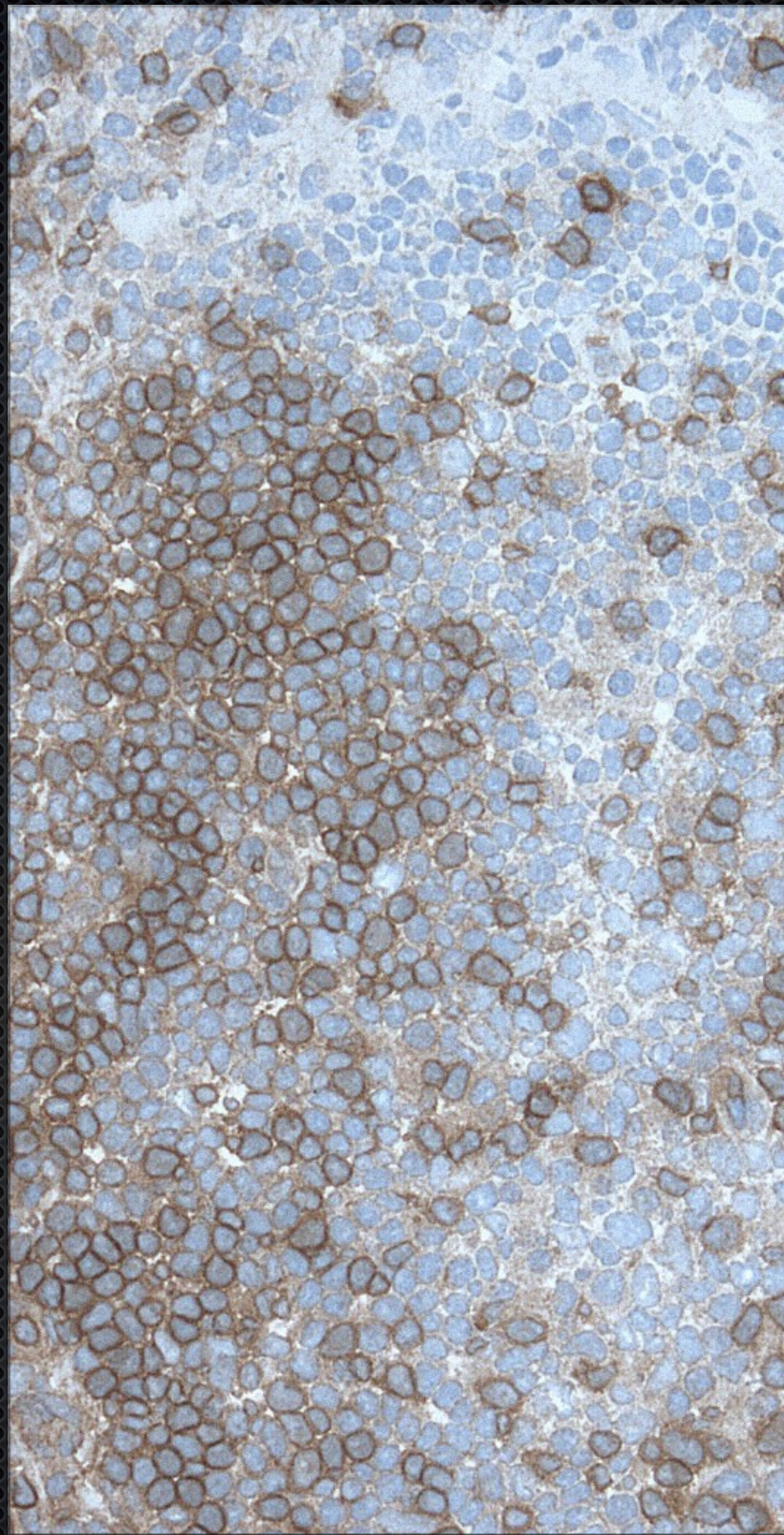
Results:

- ★ 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.

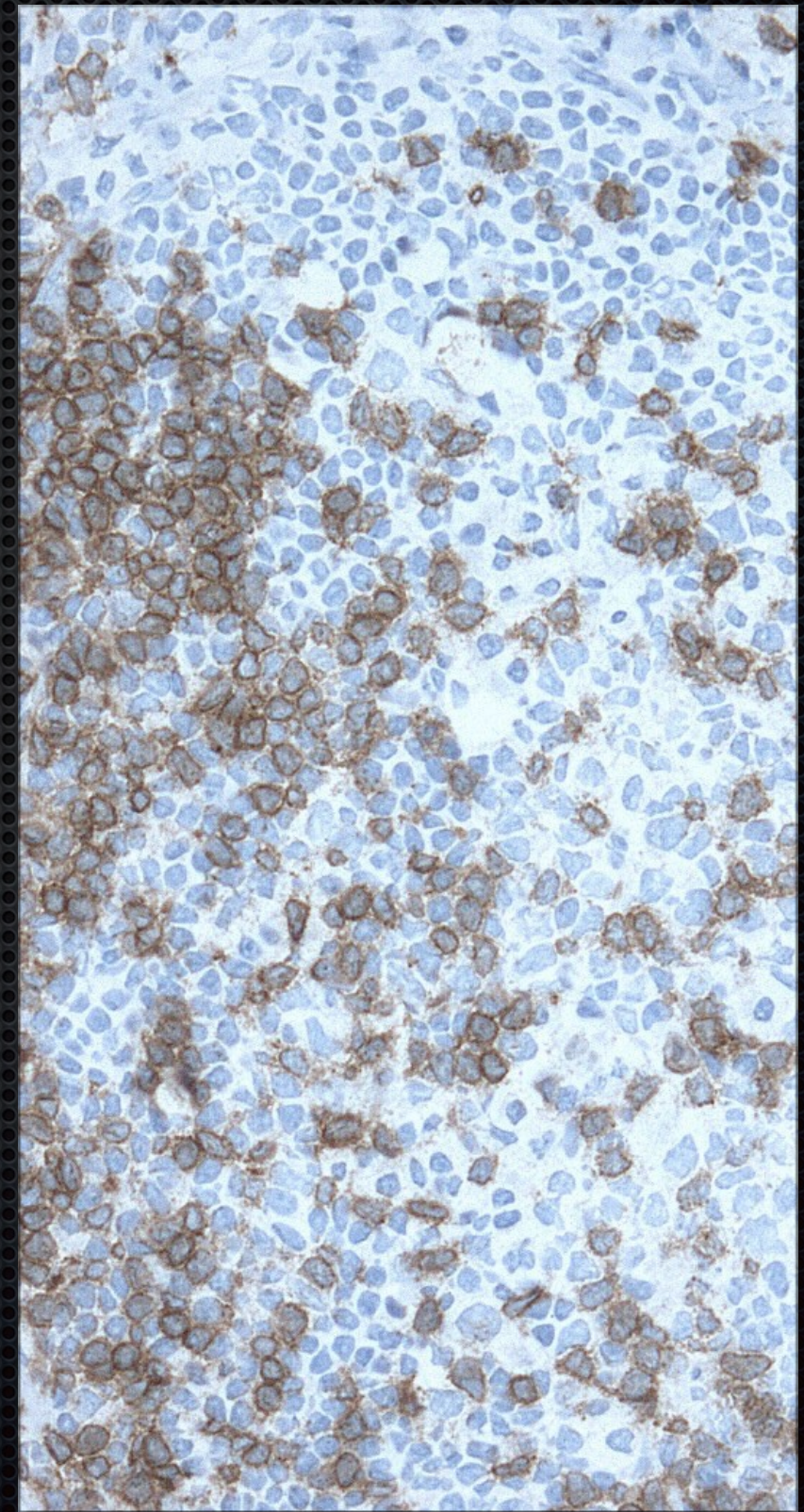
CD3, F7.2.38 (Tonsil) various fixatives



Acetone 10'

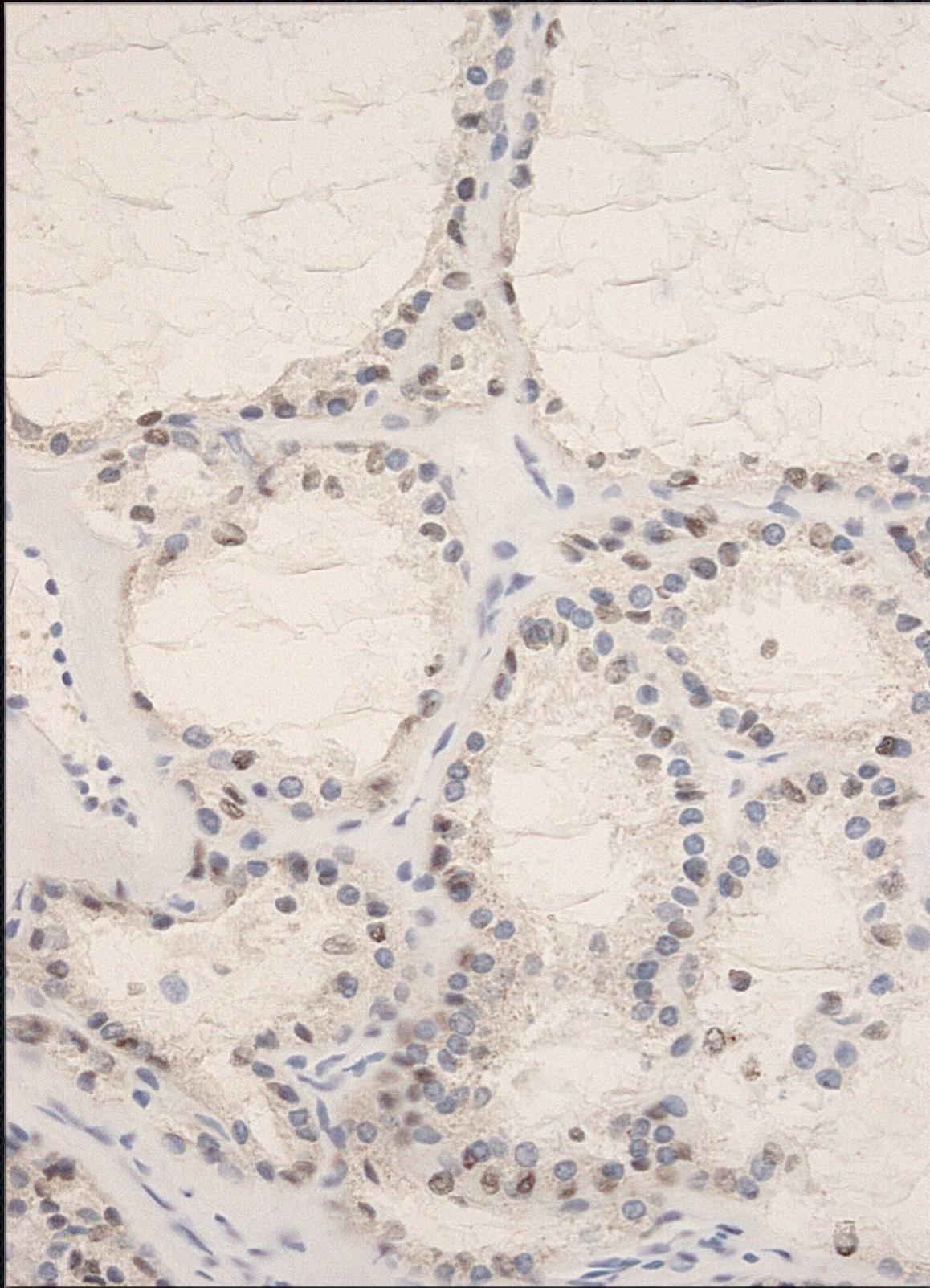


4% NBF 5'

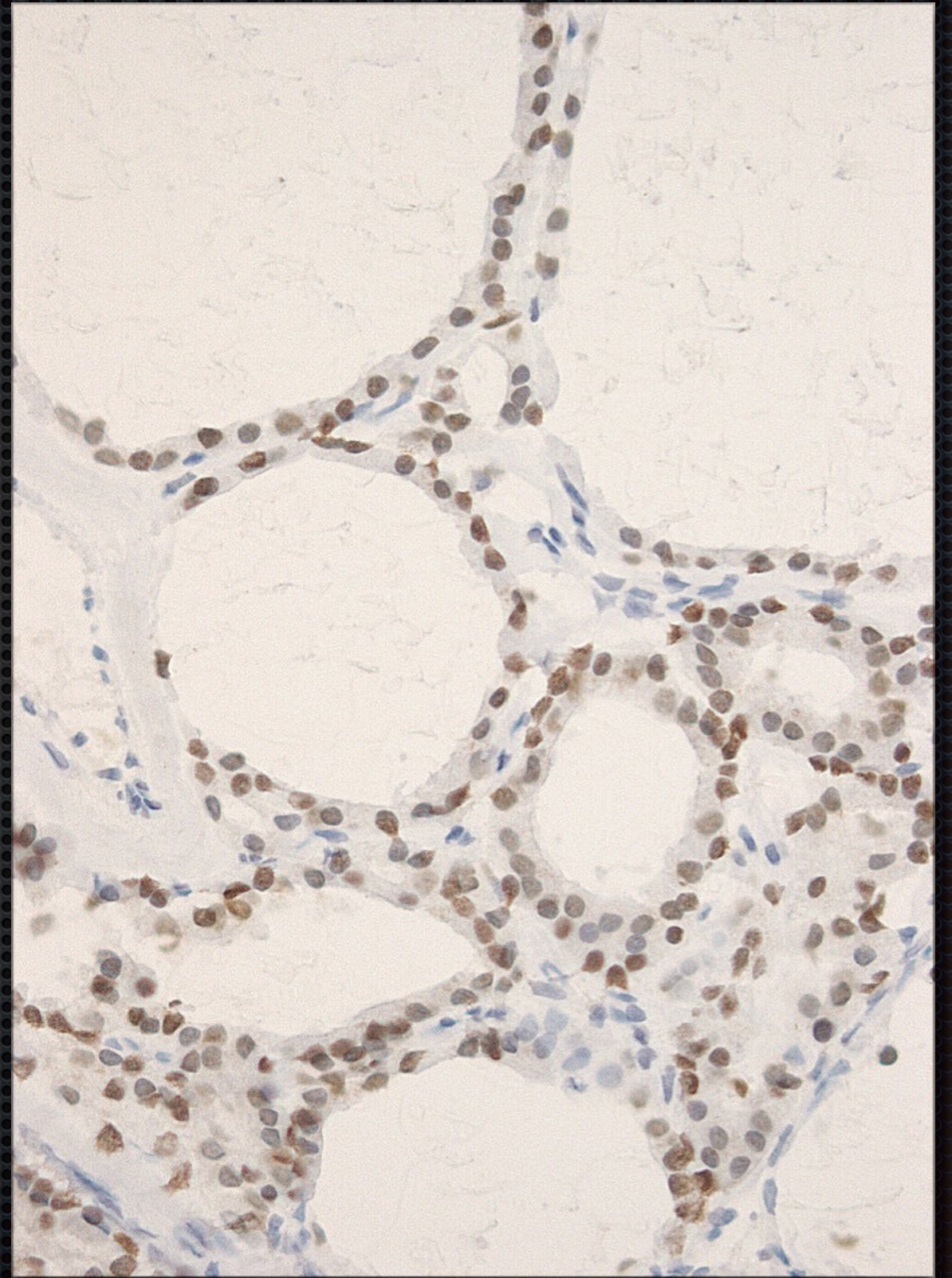


NBF 2' - TEG95° 30s

TTF-1, SPT24 (Thyroid) various fixatives



Acetone 10'

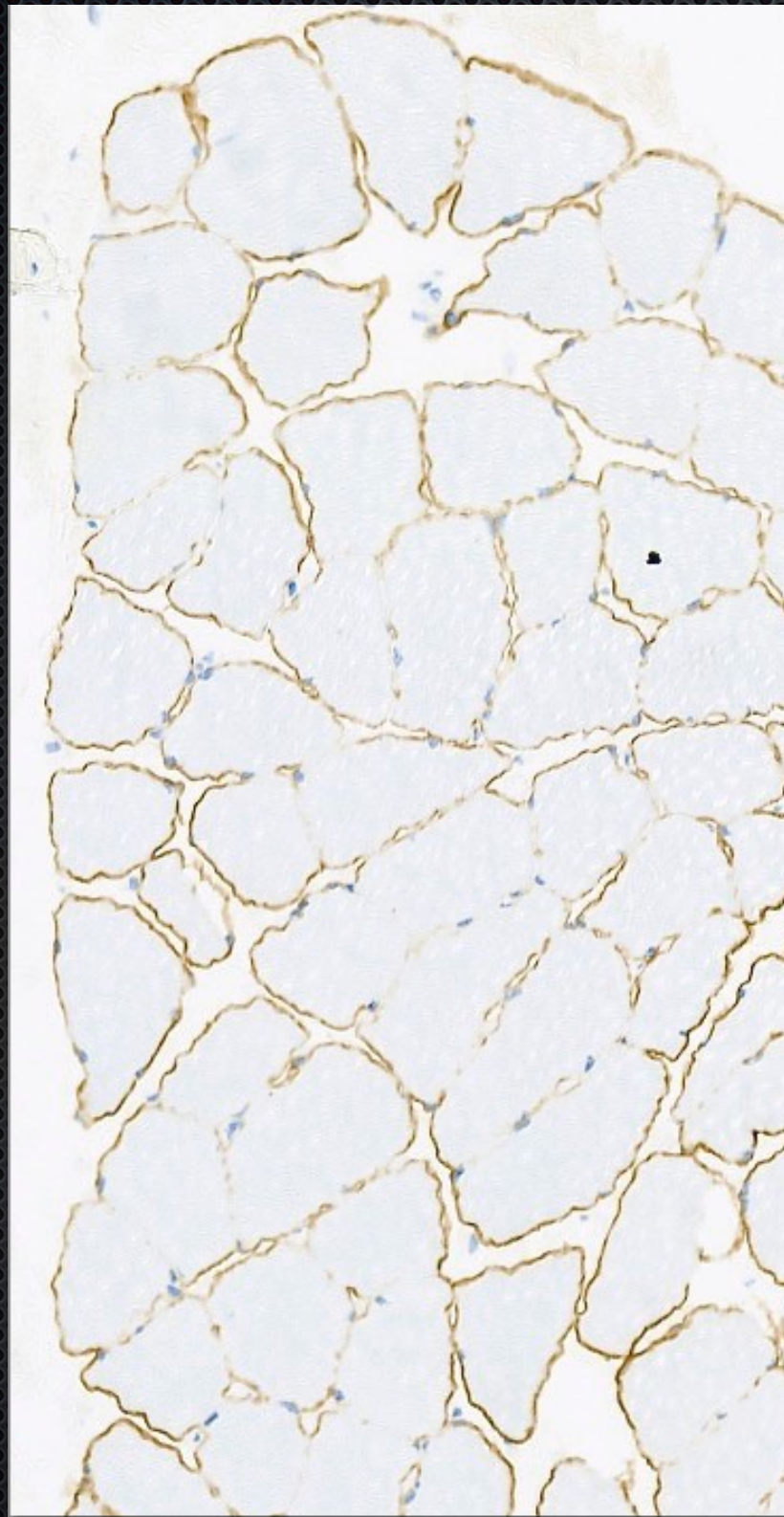


NBF 2' - TEG95° 30s

LAM-A2, Mer3/22B2 - various fixatives



No fixation (muscle)

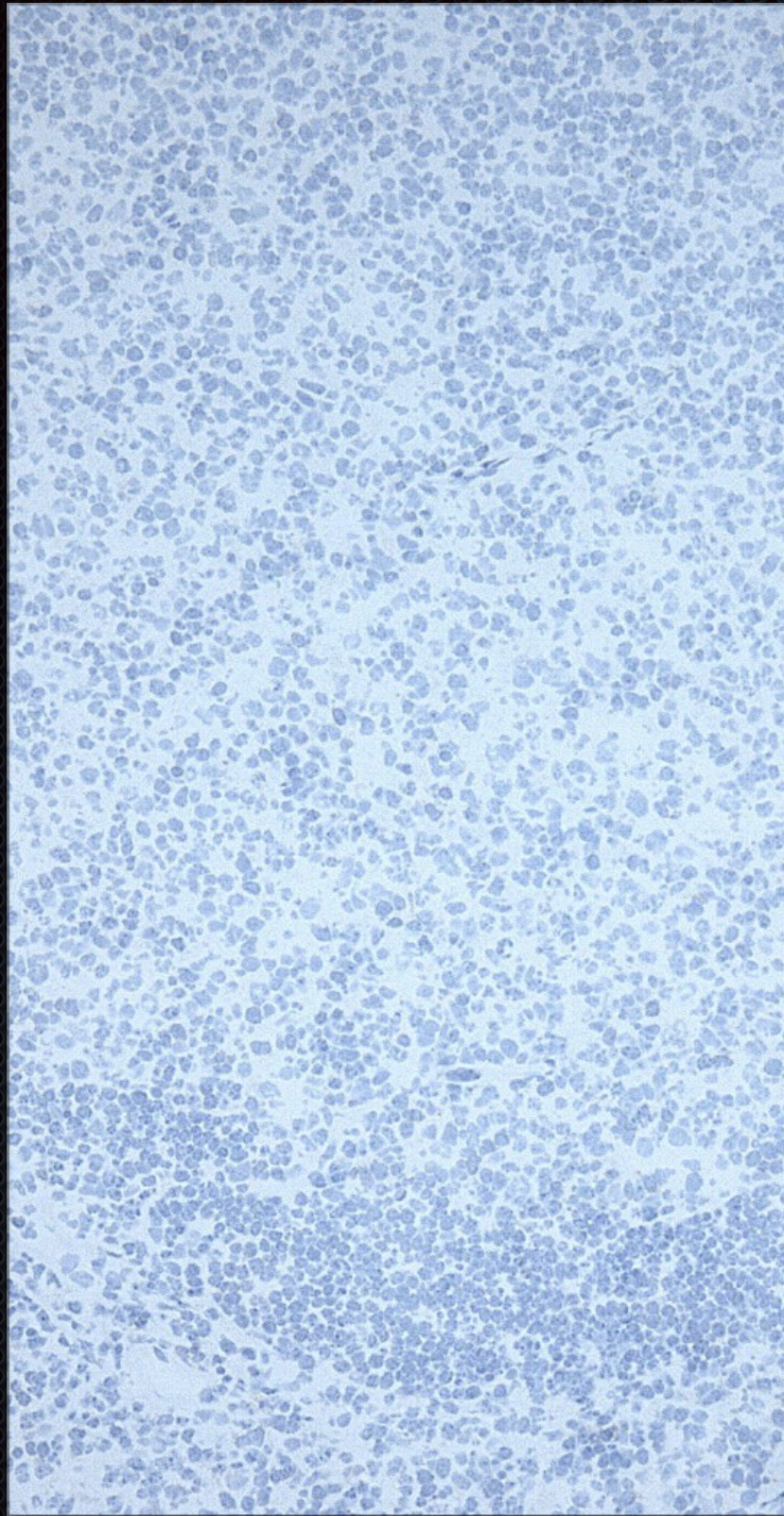


4% NBF 5' (muscle)

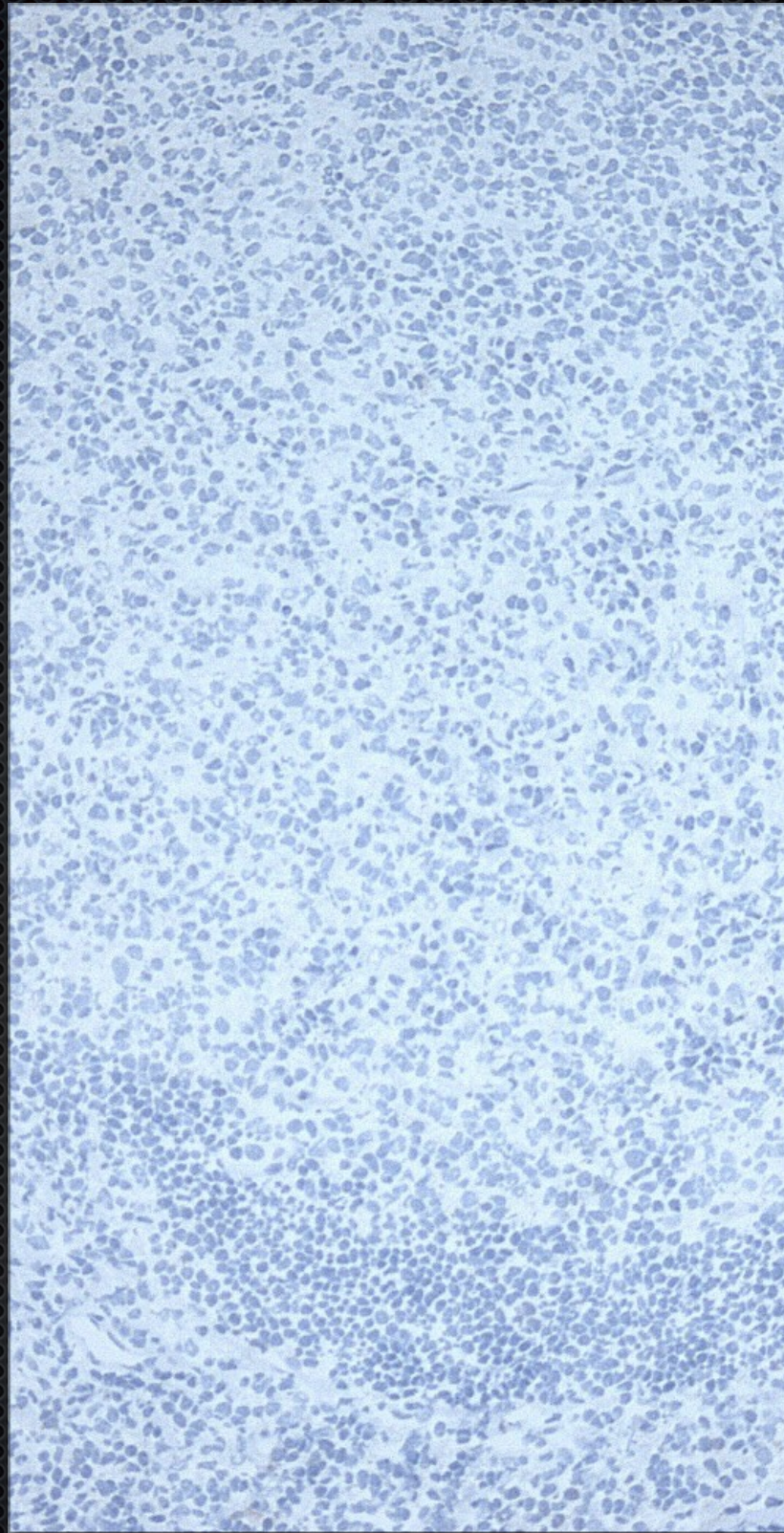


Acetone 10' (muscle)

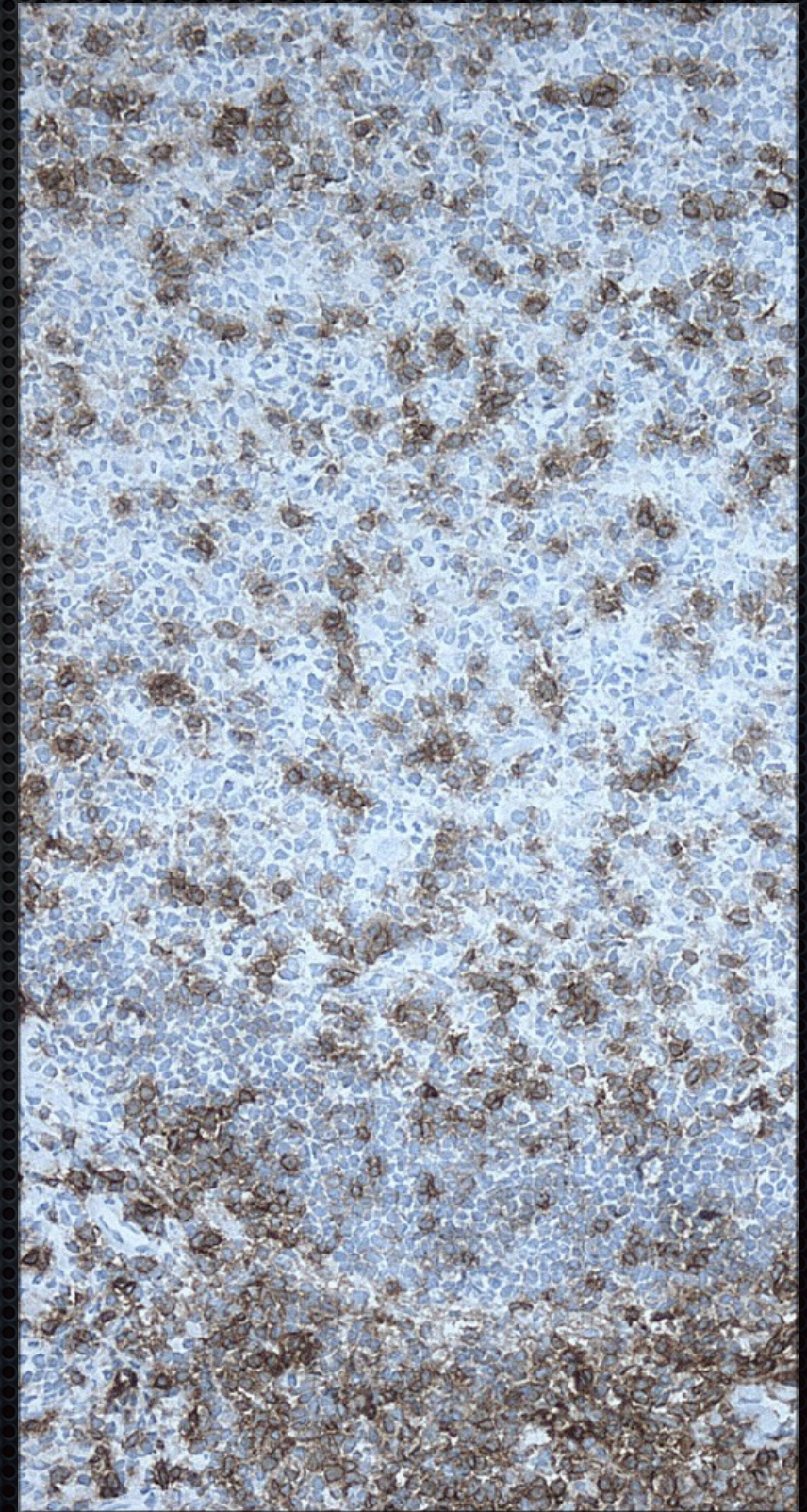
CD5, 4C7 (Tonsil) various fixatives



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				

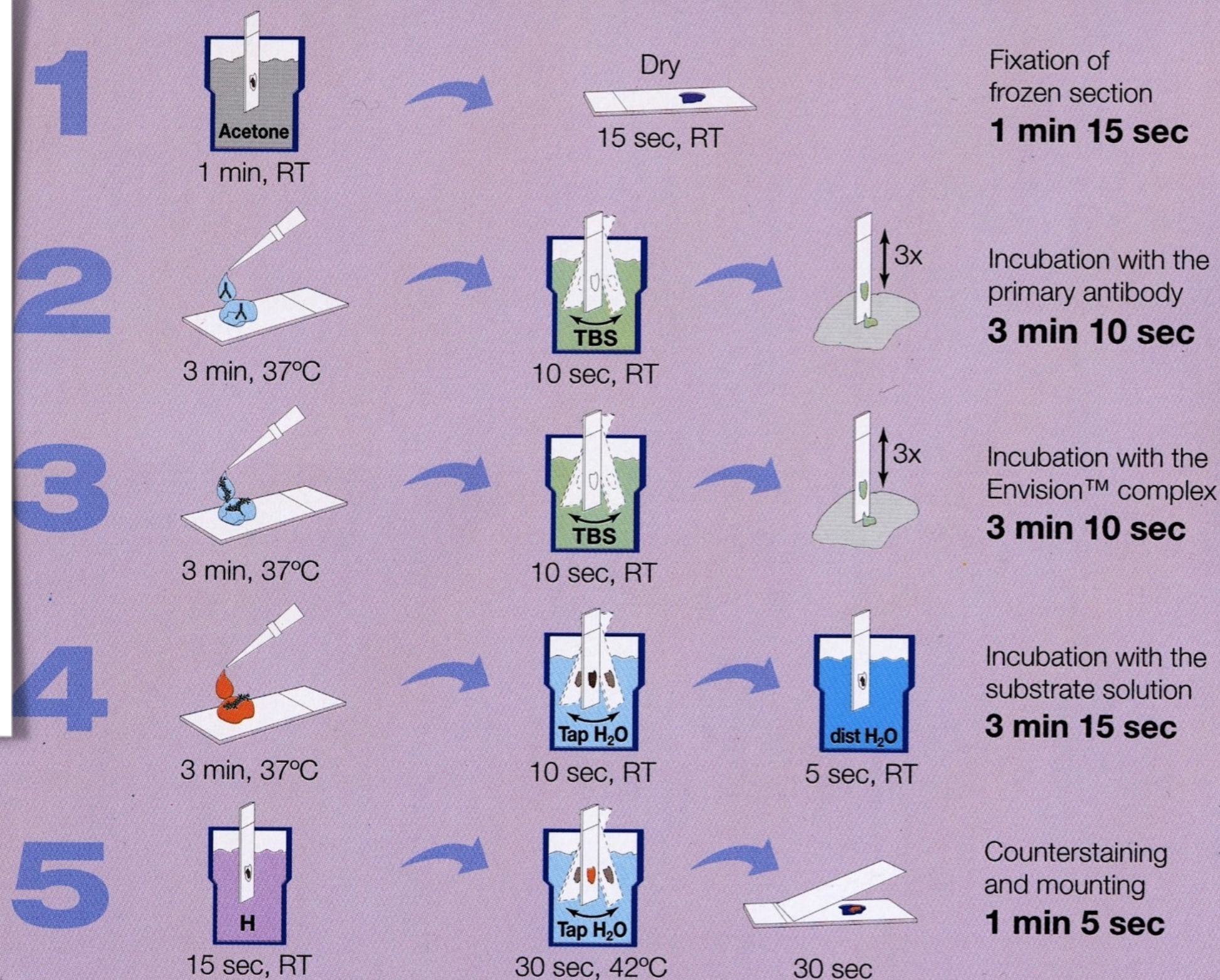
TEG: Tris-EGTA based buffer pH 9.0

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

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

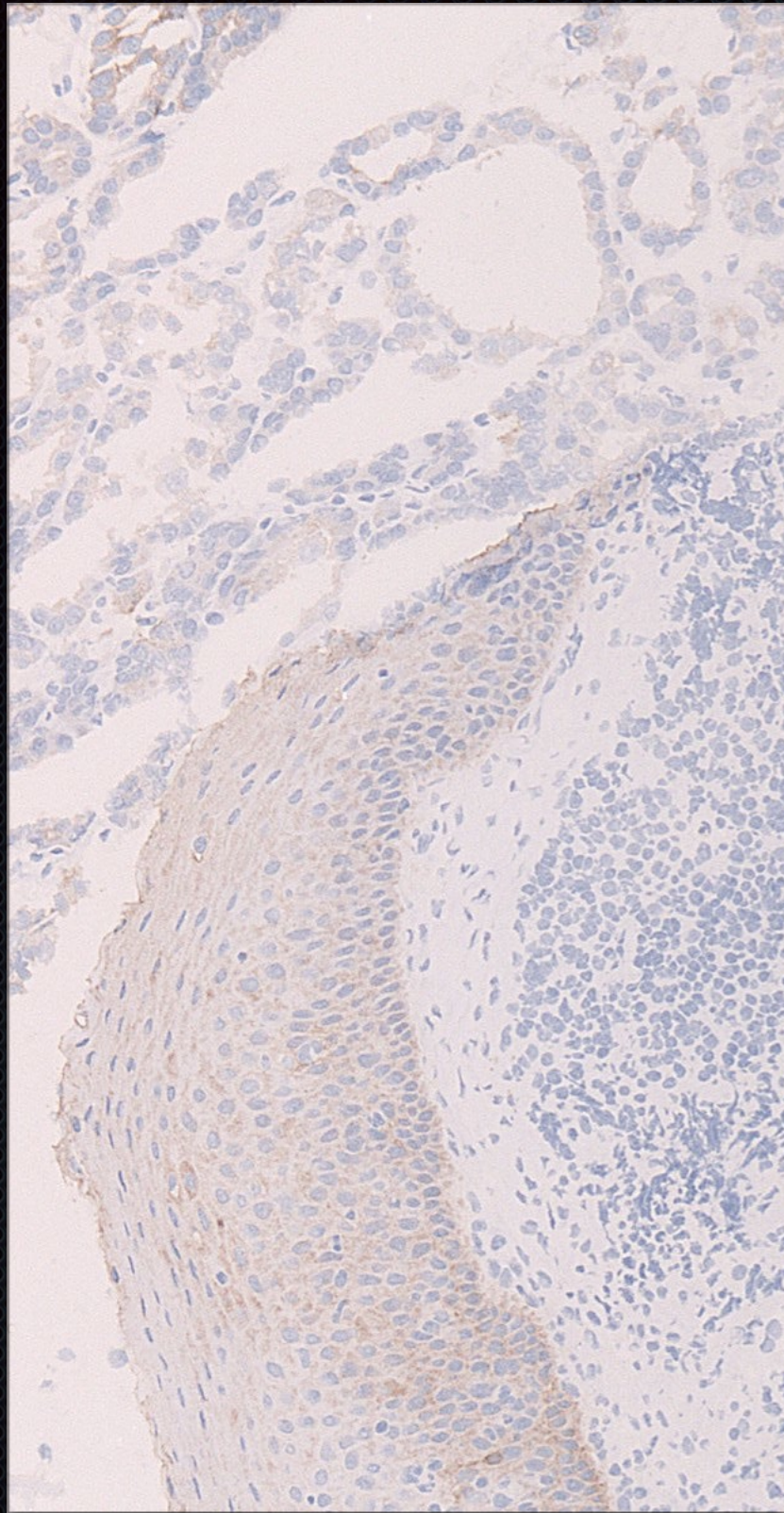
Kämmerer et al



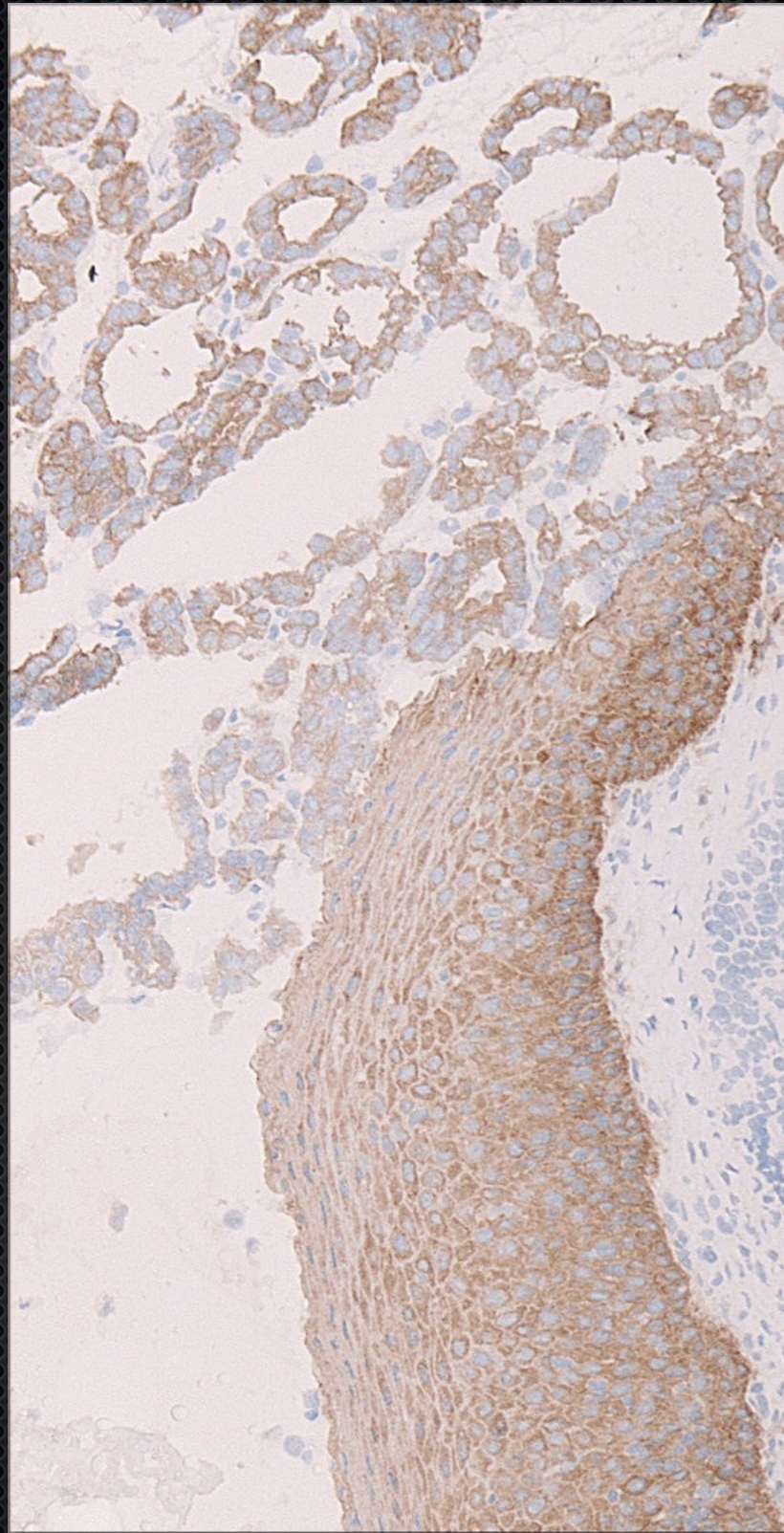
Step-by-step schematic of the rapid EnVision procedure.

RT, Room temperature; TBS, Tris-buffered saline, pH 7.4; H Mayer's hematoxylin

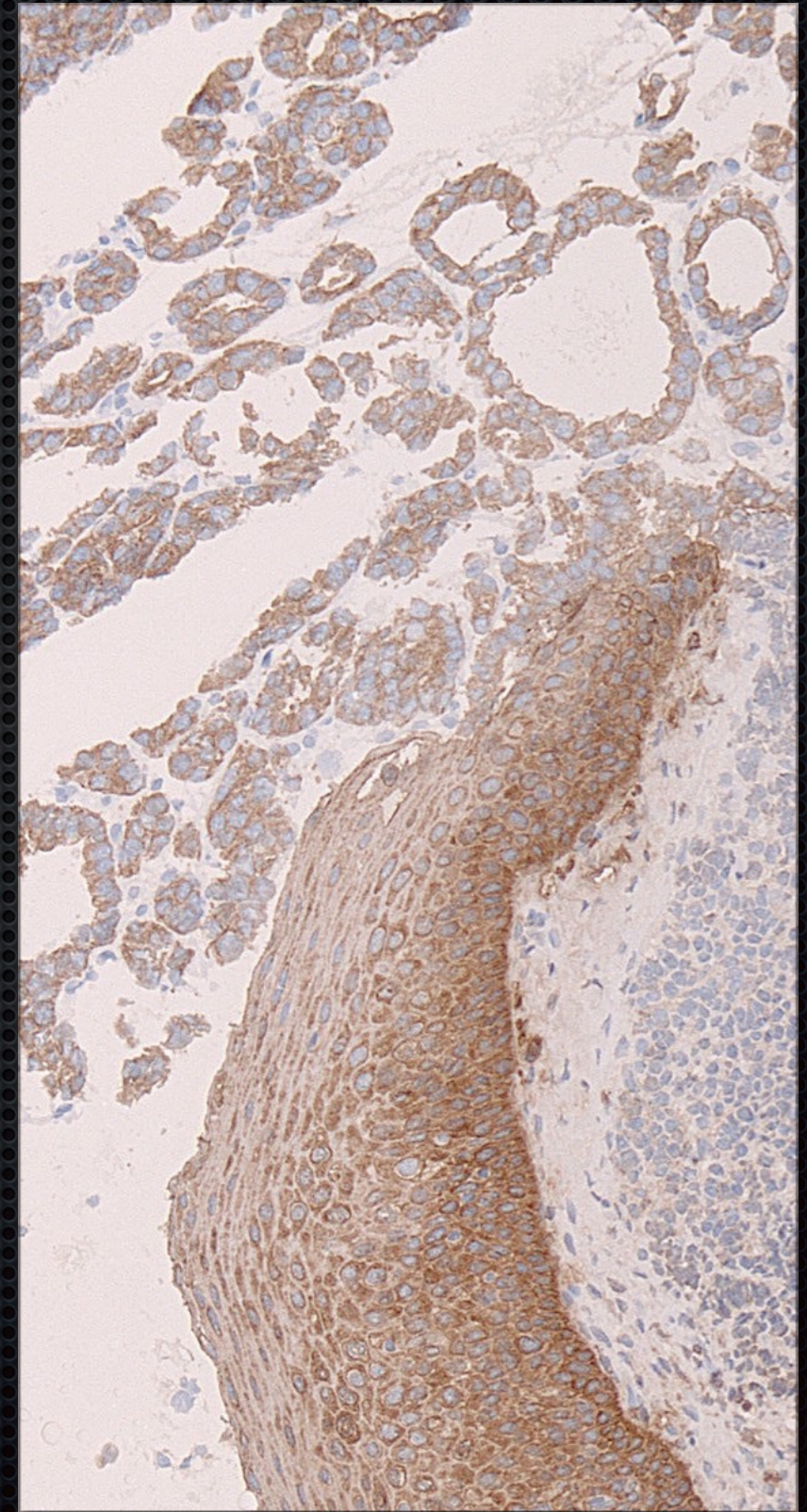
Rapid-IHC - Pan-CK, AE1/AE3



NBF - TEG 60°C



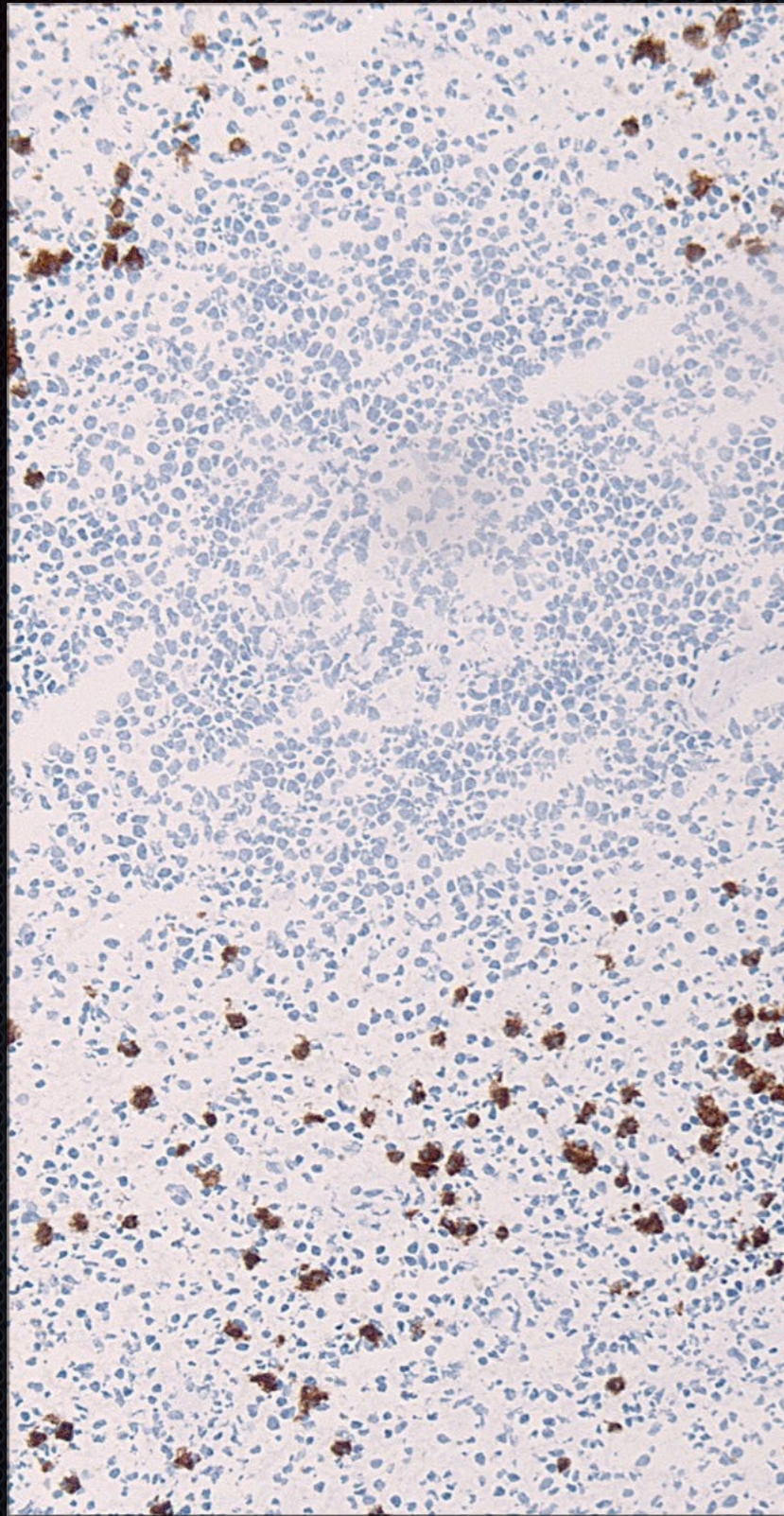
NBF - TEG 80°C



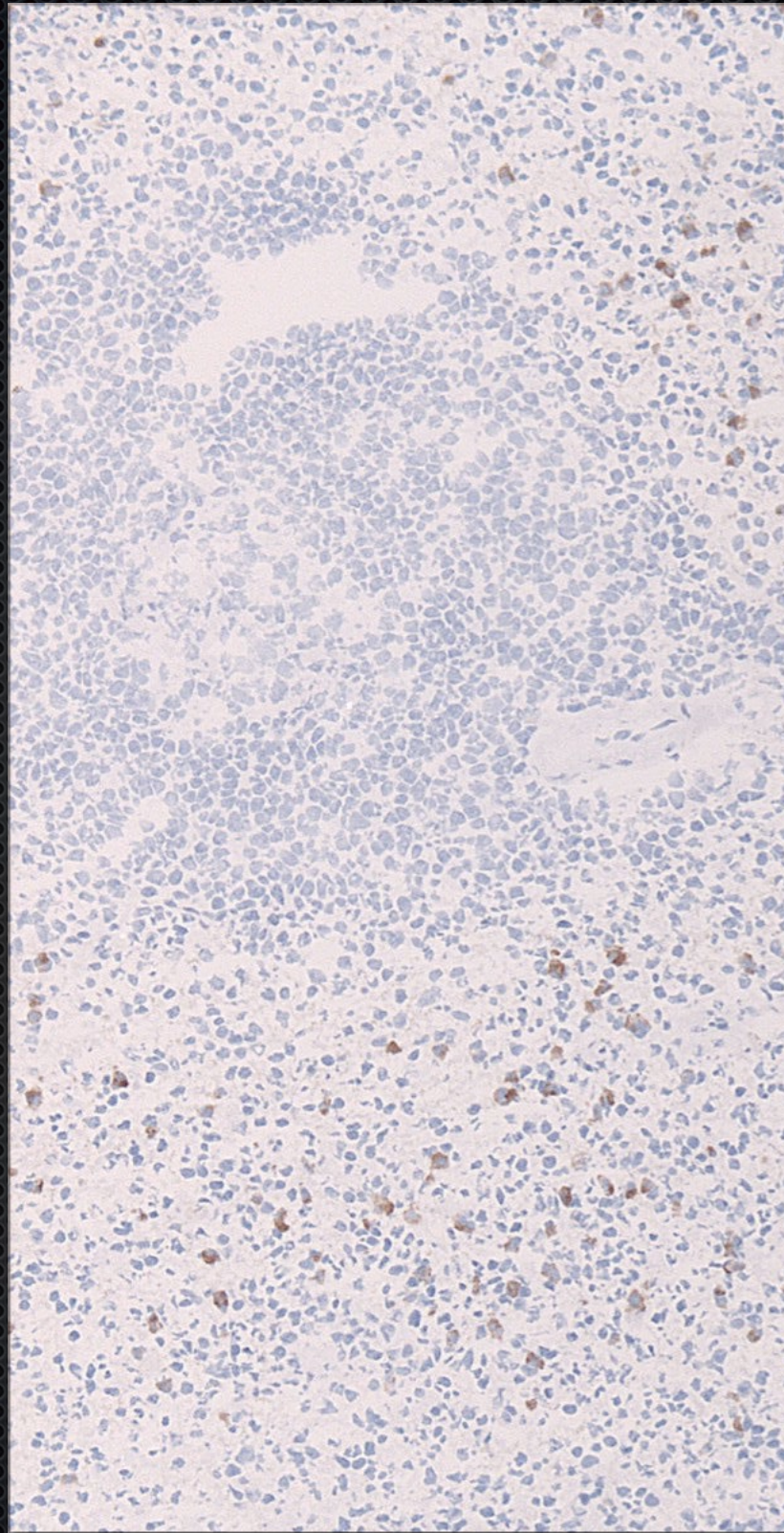
NBF - TEG 95°C

Endogenous peroxidase

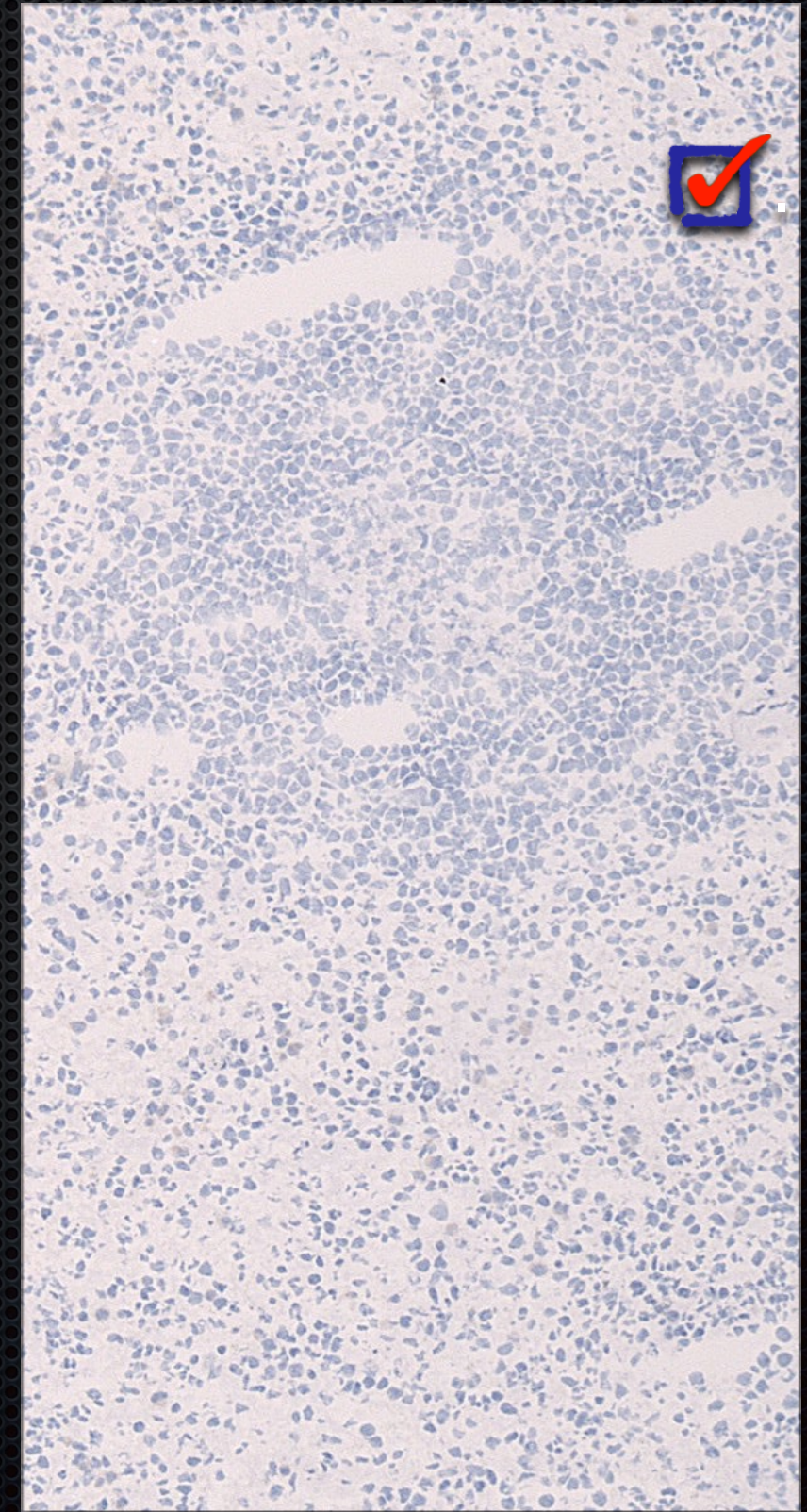
Spleen



NBF - TEG 60°C

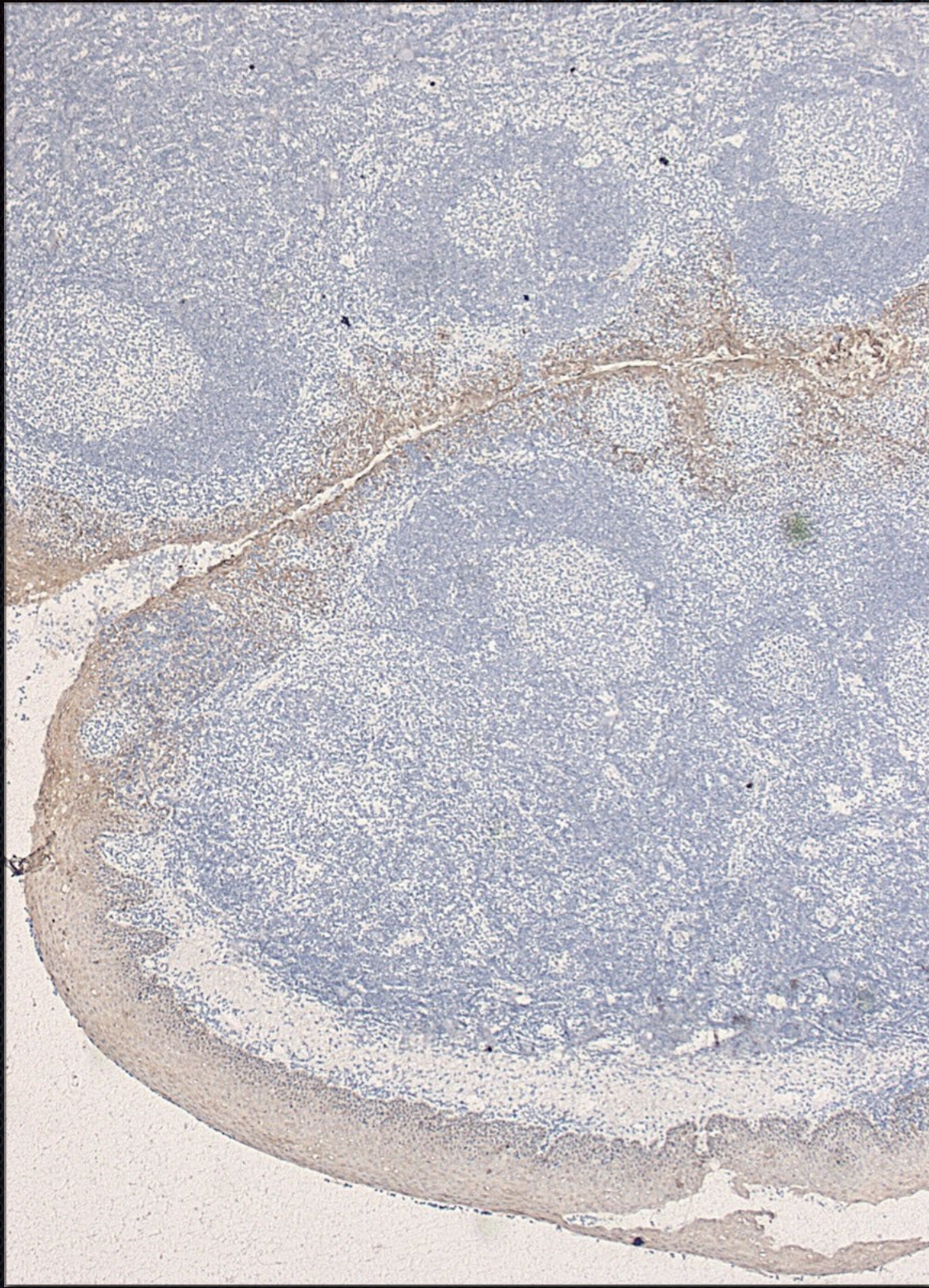


NBF - TEG 90°C

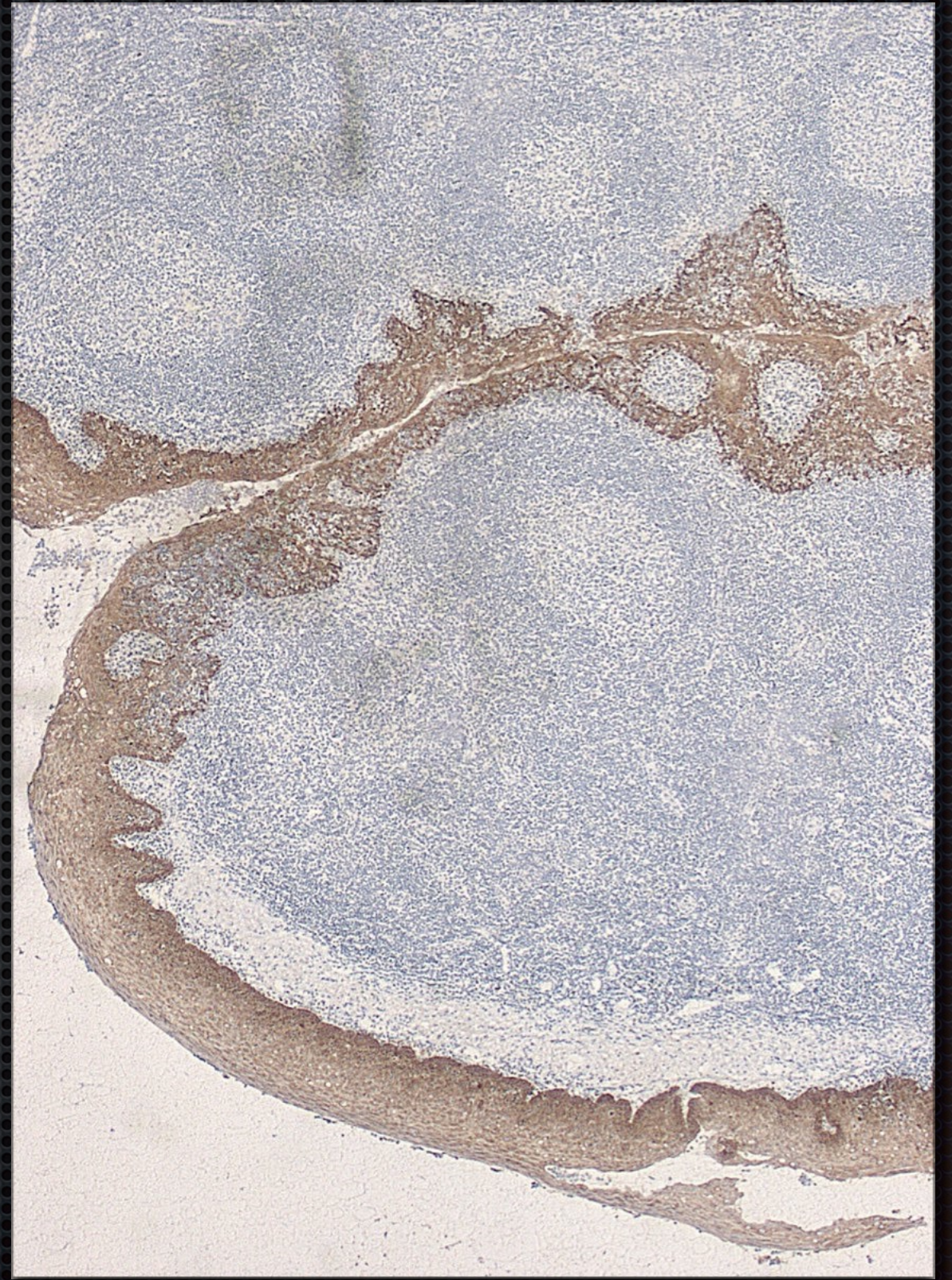


NBF - TEG 95°C

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



Acetone



NBF-TEG95

Rapid-IHC fixation:

Acetone vs NBF-MBO/TEG

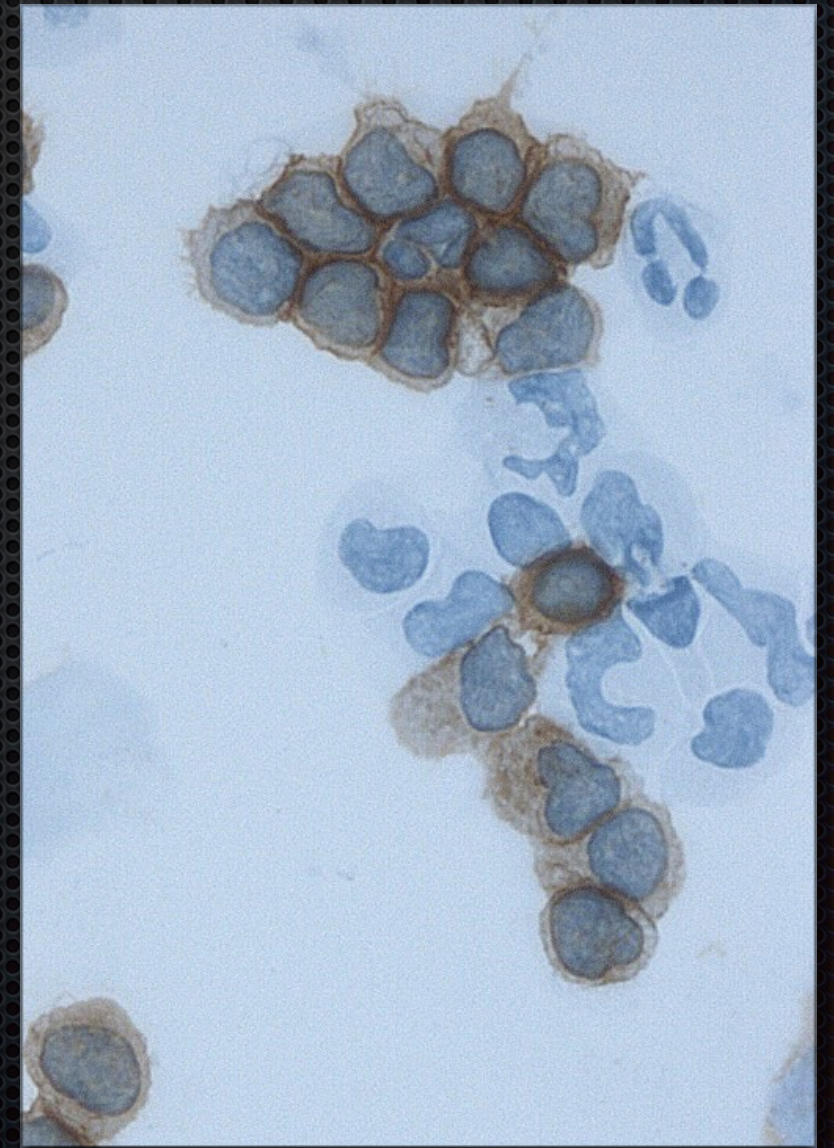
Antibody	Acetone	NBF-MBO/TEG
CK20, Ks20.8	+++ 	++
Melanoma, HMB45	++ 	+
Insulin, 2D11-H5	0	++ 
Melan-A, A103	+++	+++
Ki67, MIB1	+	++ 
Synaptophysin, 27G12	++	+++ 
TTF-1, SPT24	+	++ 
Vimentin, V9	+++	+++

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

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	+++	+++

ICC on cytological material

- * Smears, imprints, cytopins, etc
- * Liquid based cytology (LBC)



Optimizing biomarker-protocols (ICC-protocols on cells)

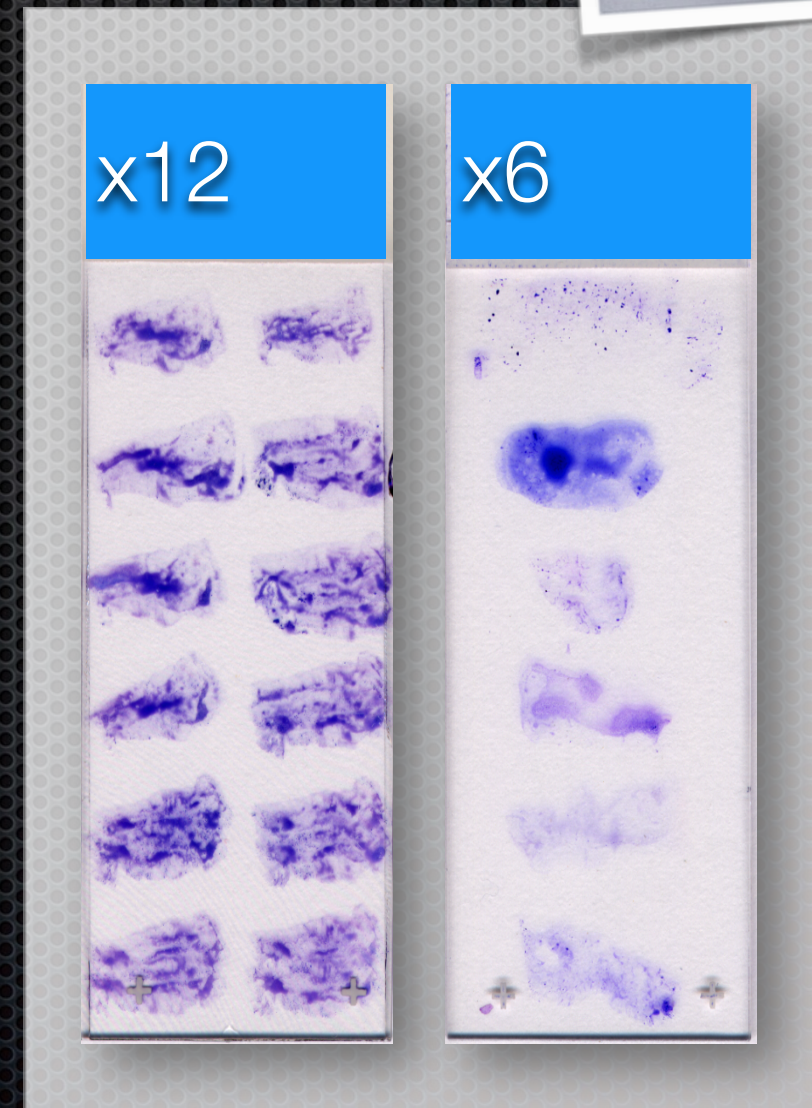
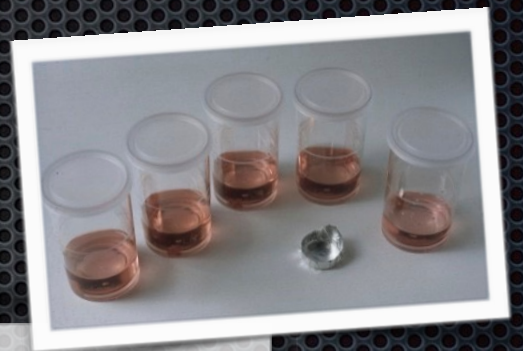
Requirements:

- 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

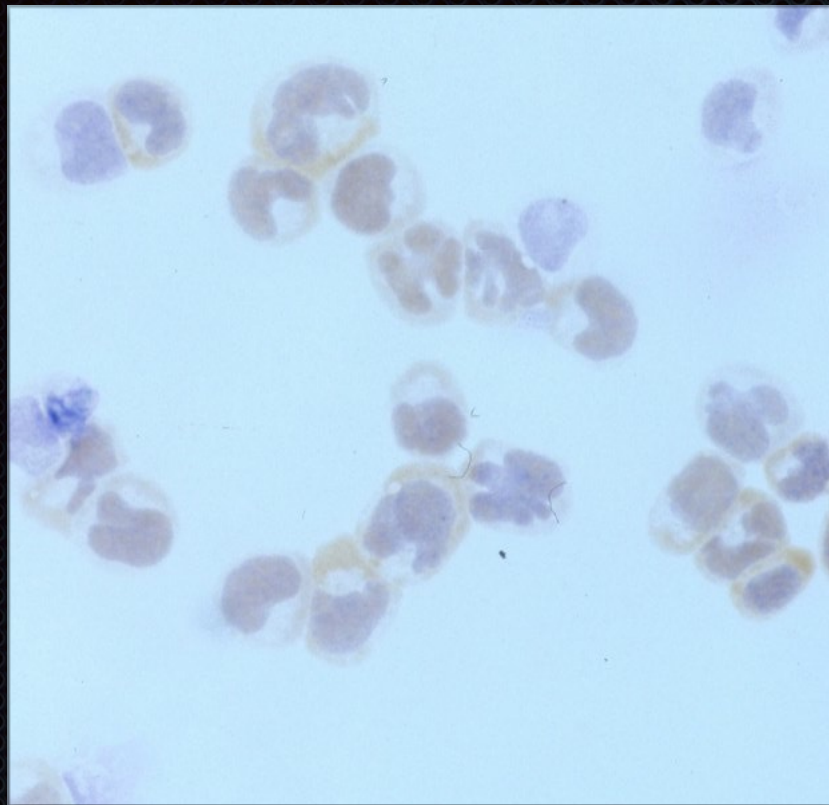
Optimizing biomarker-protocols (ICC-protocols on cells)

✦ Designed “Multi-Imprints” from:

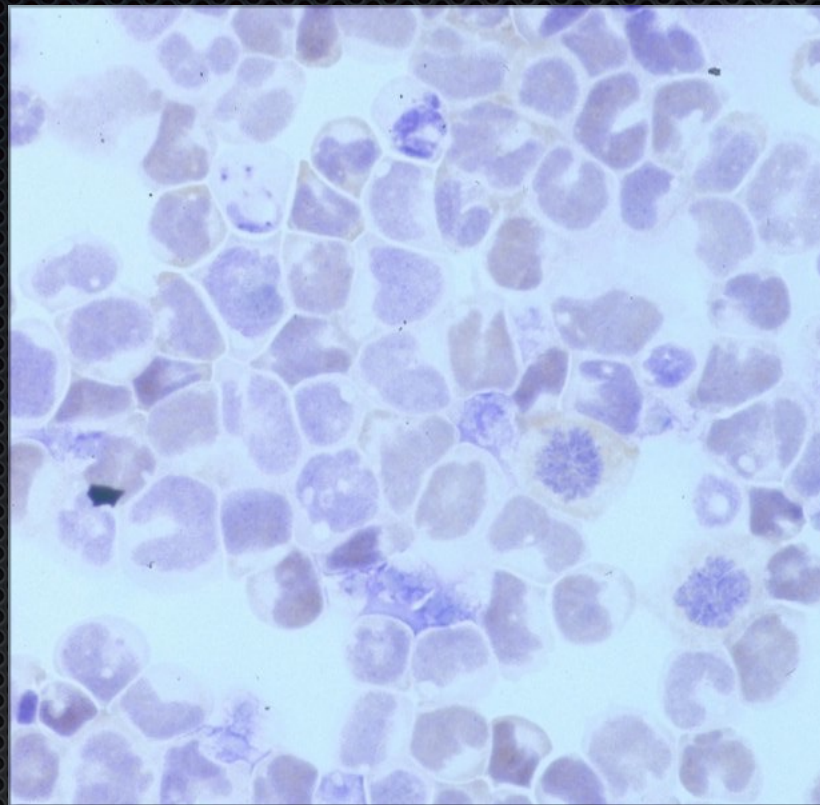
- ✦ Tissue with high expressors
- ✦ Tissue with low expressors
- ✦ Tissue with non-expressors



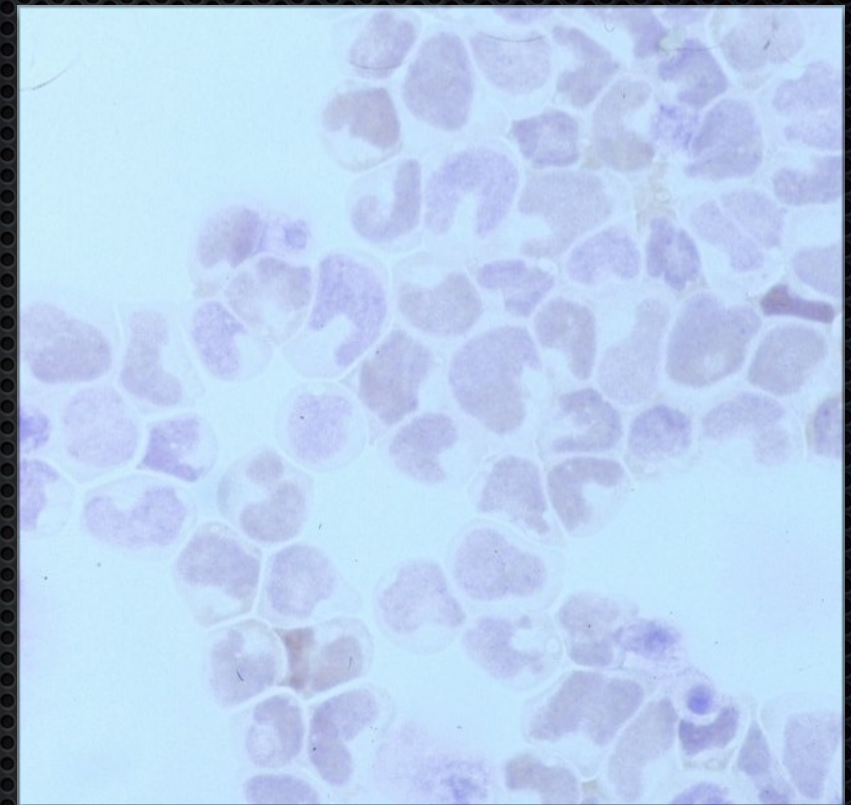
TdT, poly (MOLT4 cells) and various fixatives



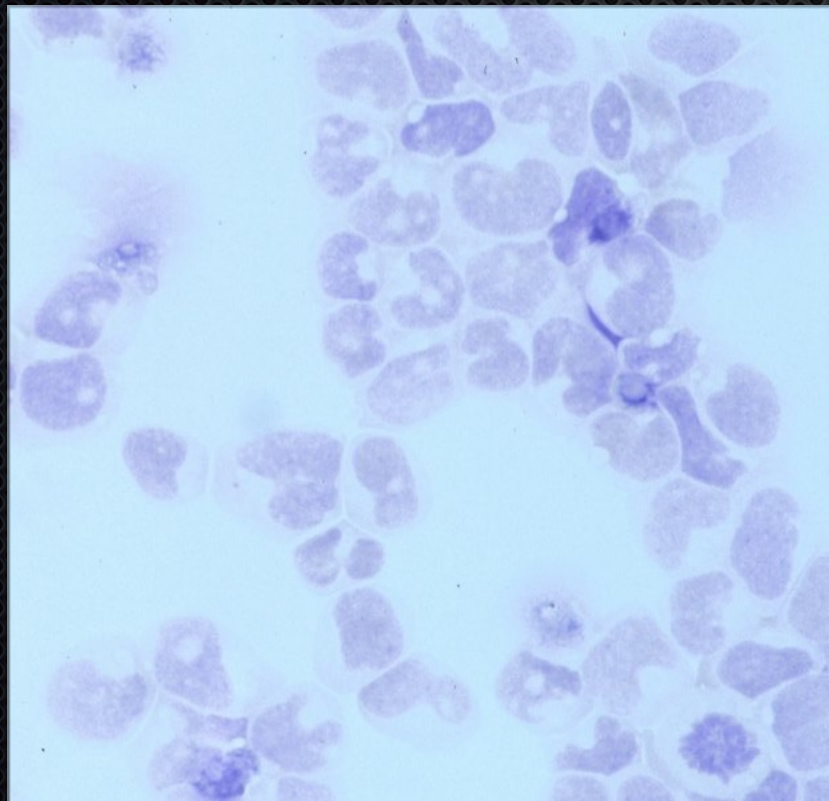
Acetone 5'



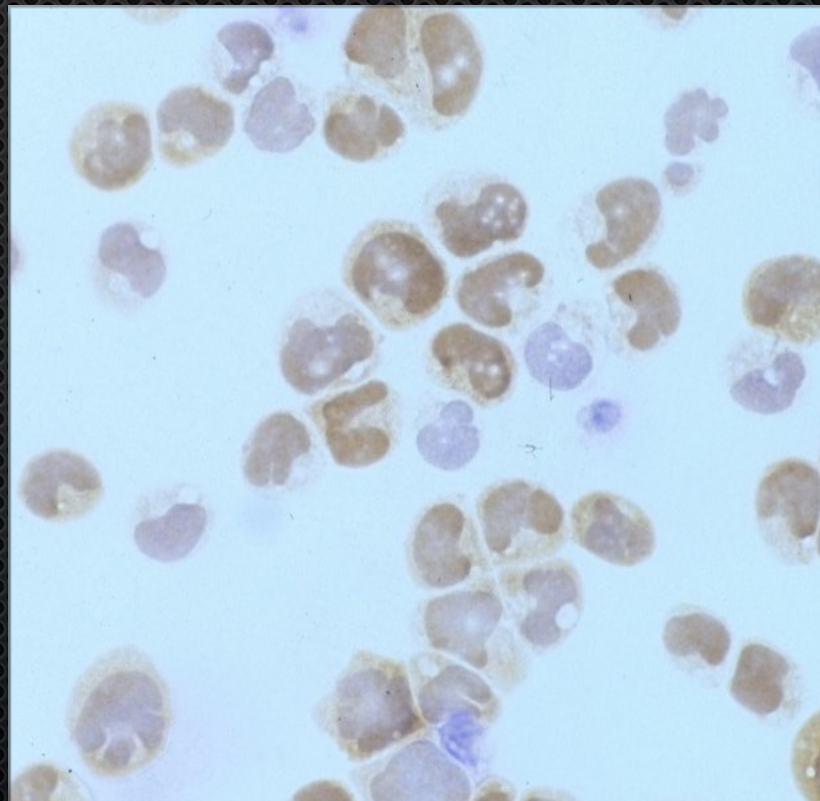
Acetone/Methanol 40s



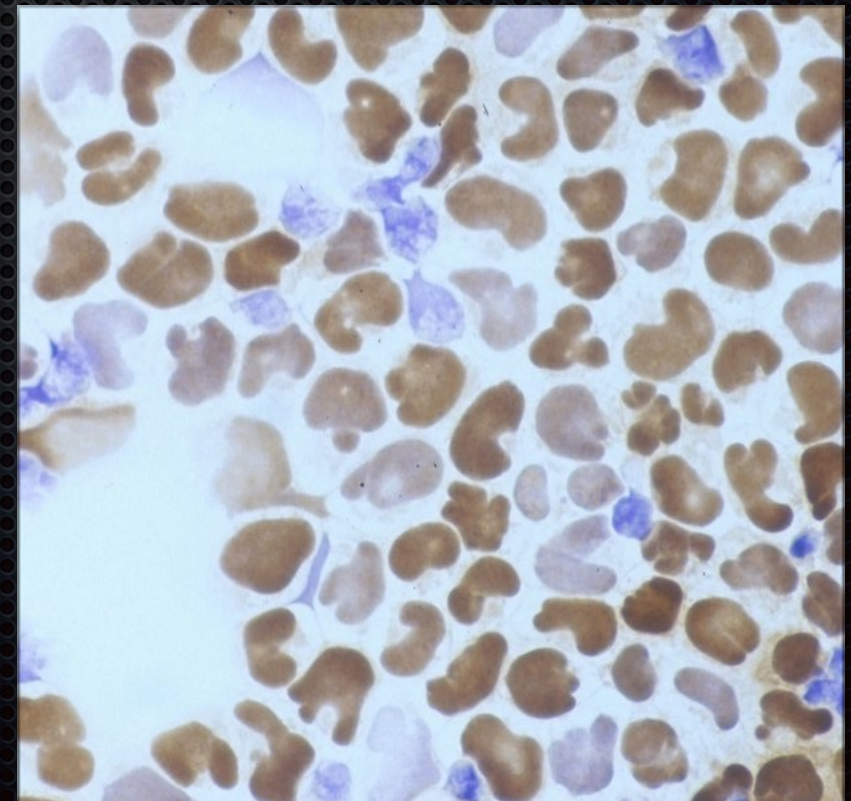
Methanol 5'



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”

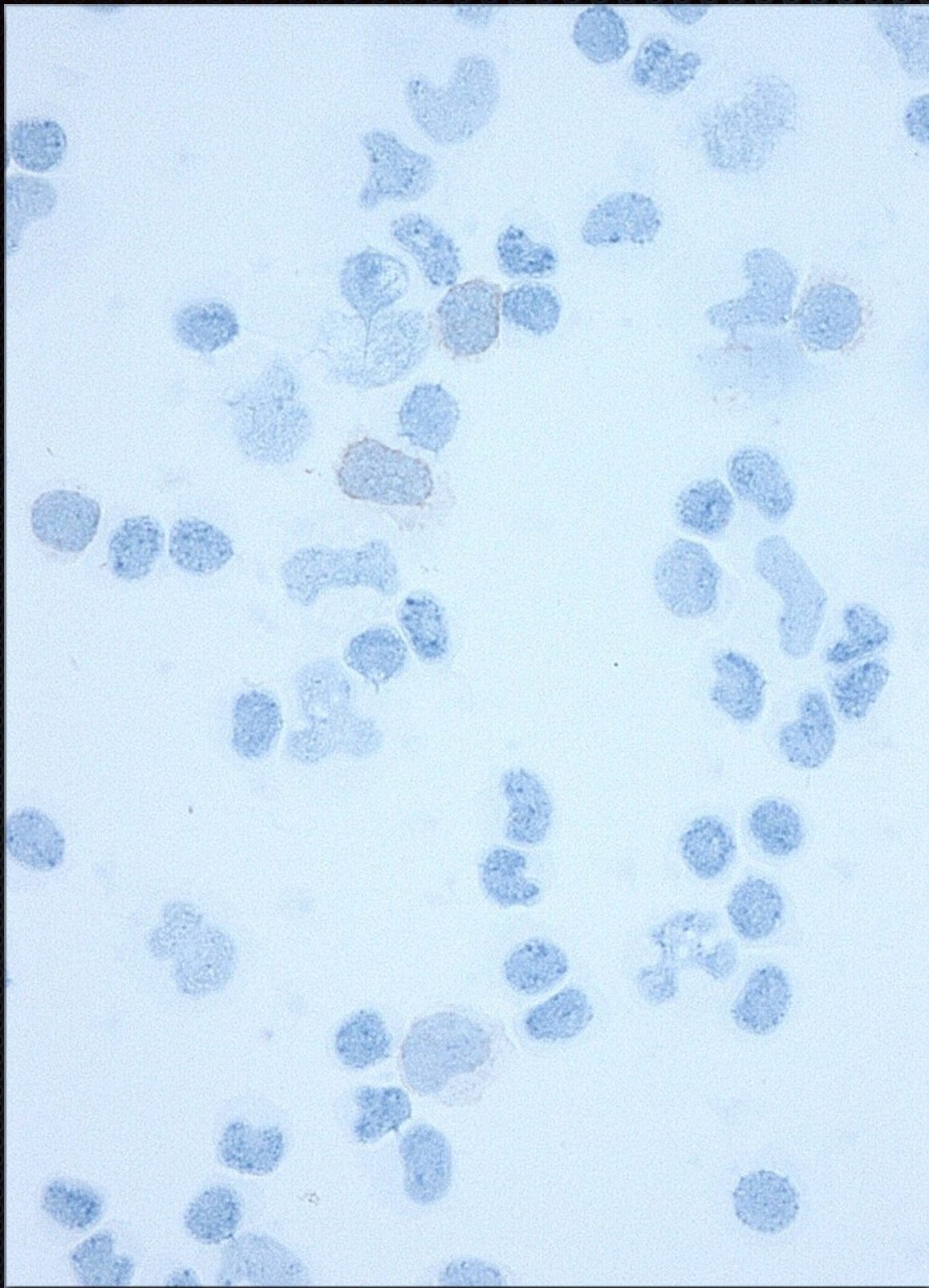
Omnis

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				

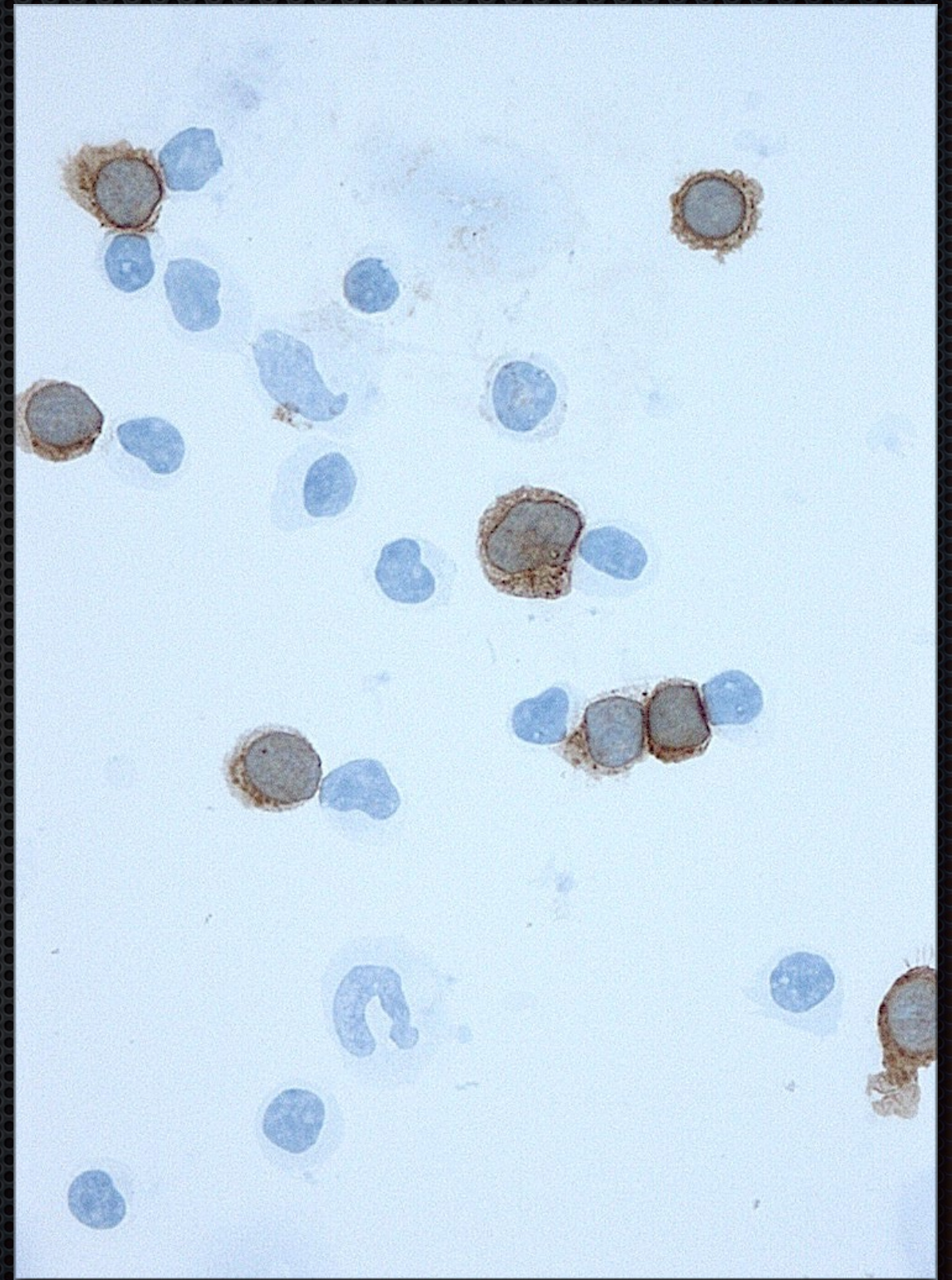
TRS-H: Tris-EDTA based buffer pH9.0

TRS-L: Citrate based buffer pH6.1

CD79a, JCB117 (Buffy coat cytospin) Fixation

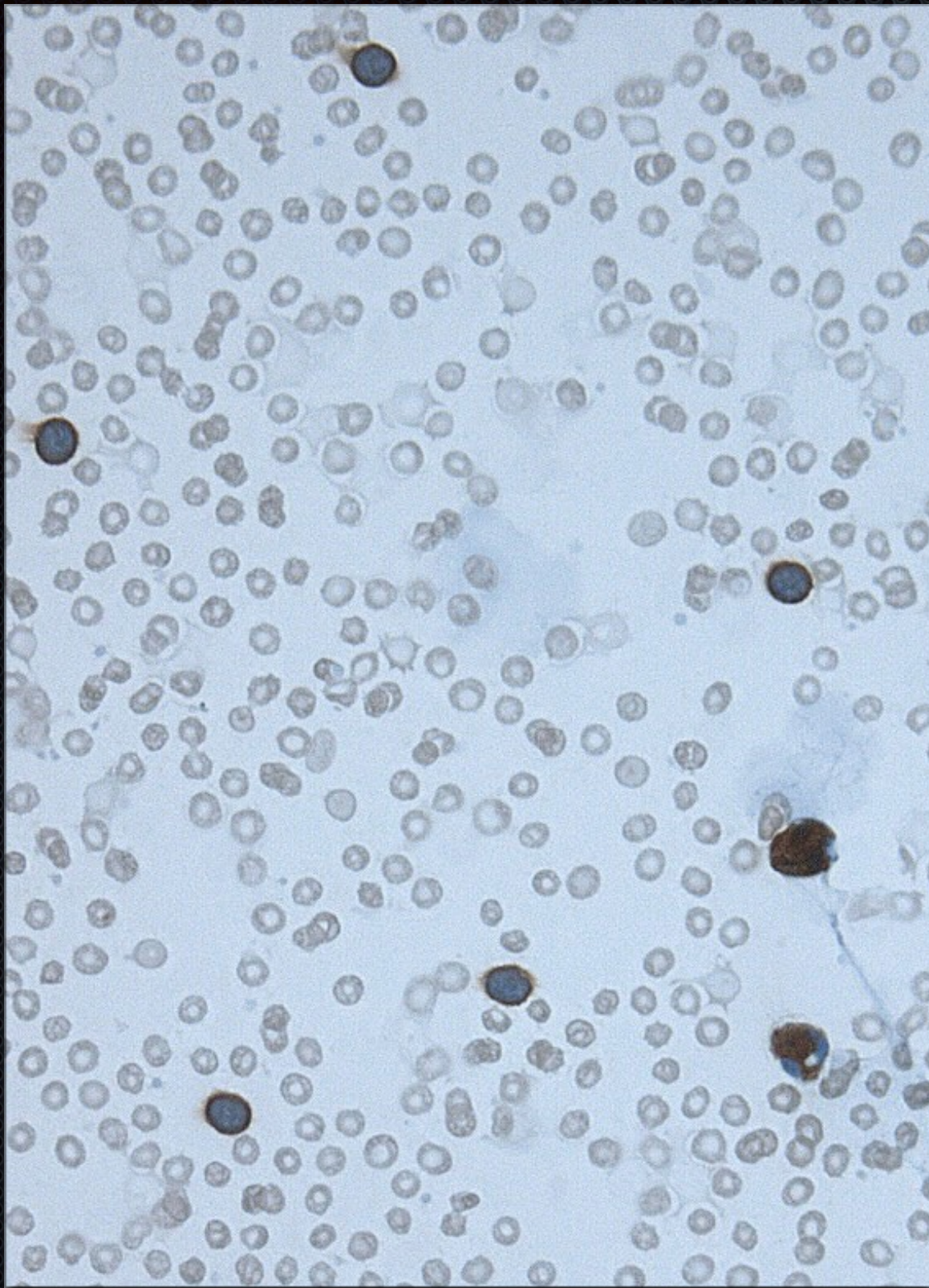


Acetone 10'



NBF 15' - TEG95° 15'

CD79a, JCB117 (PB) Various fixatives

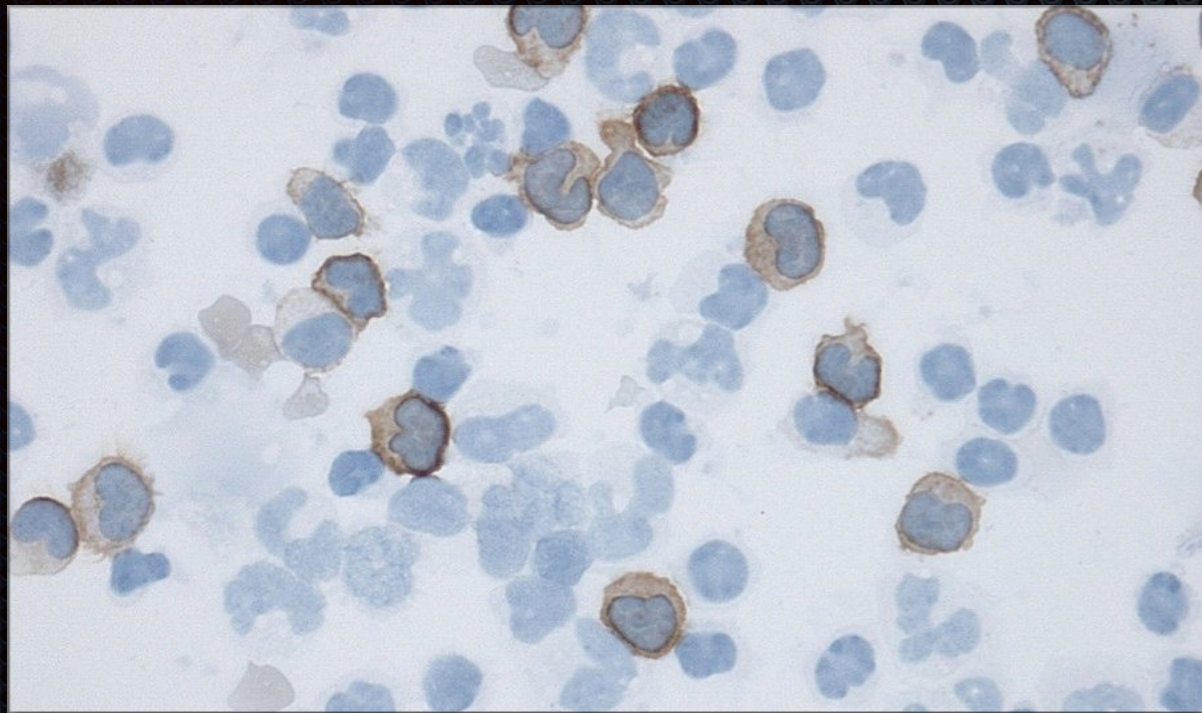


Acetone 10'

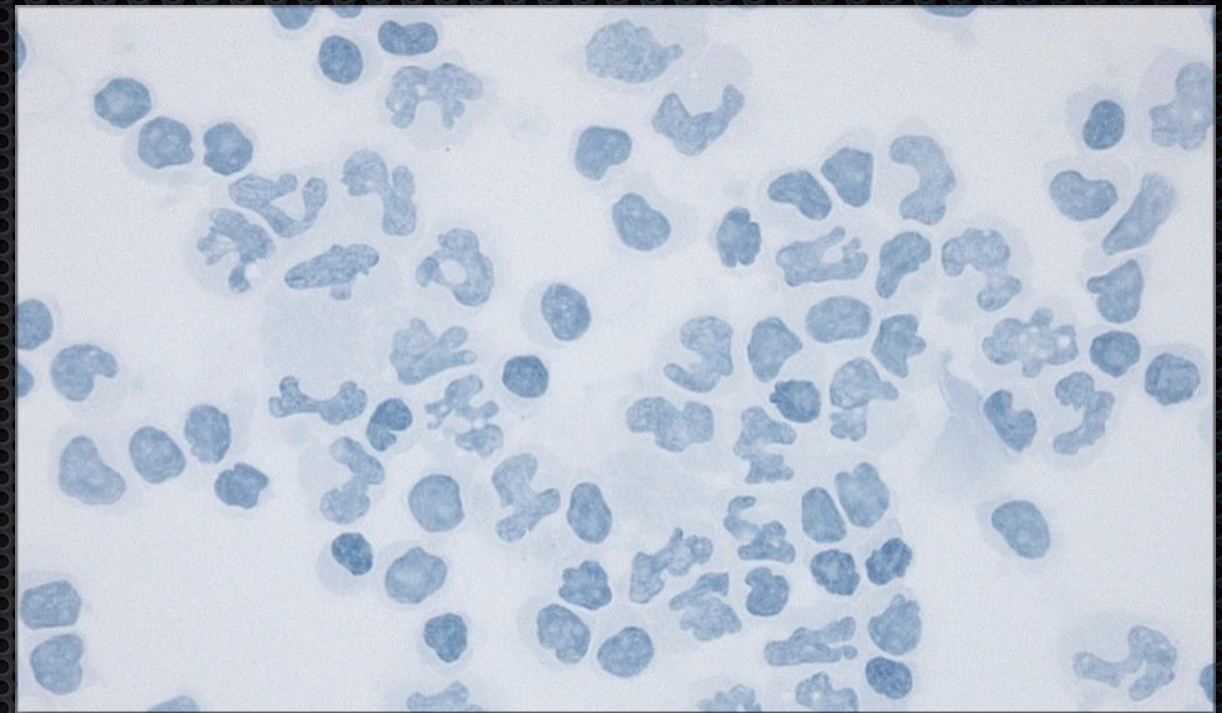


NBF 15' - TEG95° 15'

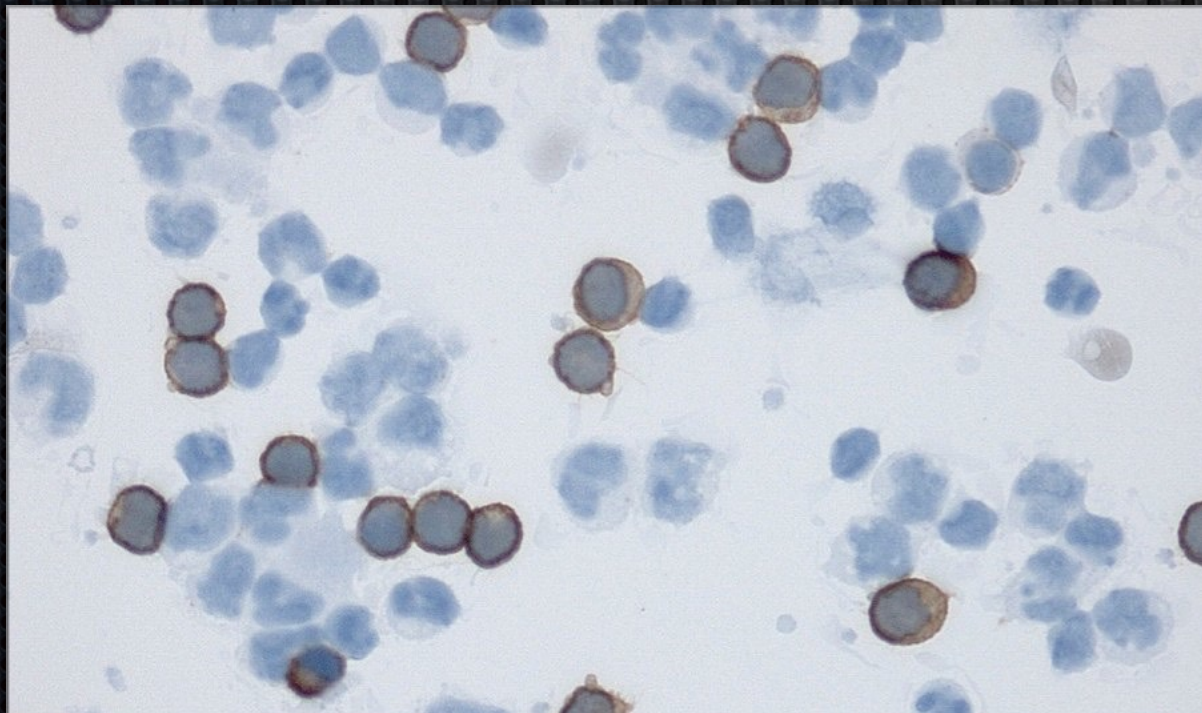
CD8 Abs (Buffy coat PB) various fixatives



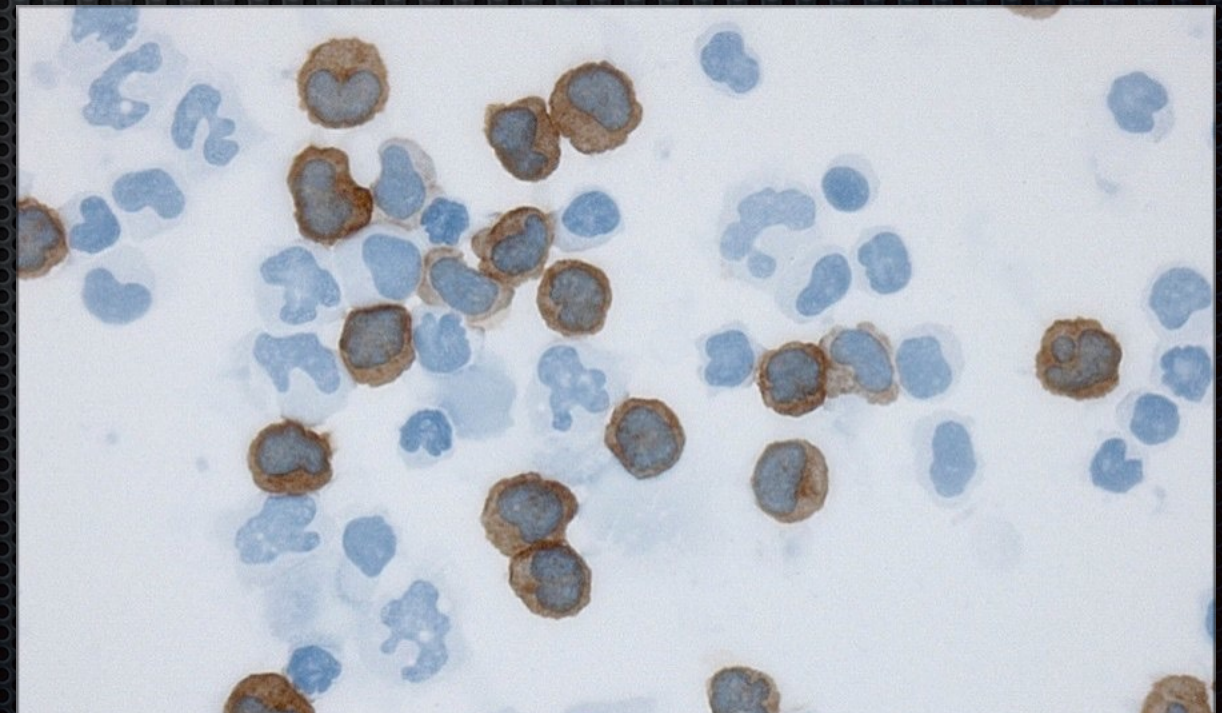
CD8, DK25 - Acetone 10'



CD8, DK25 - NBF 2' - TEG95° 30s

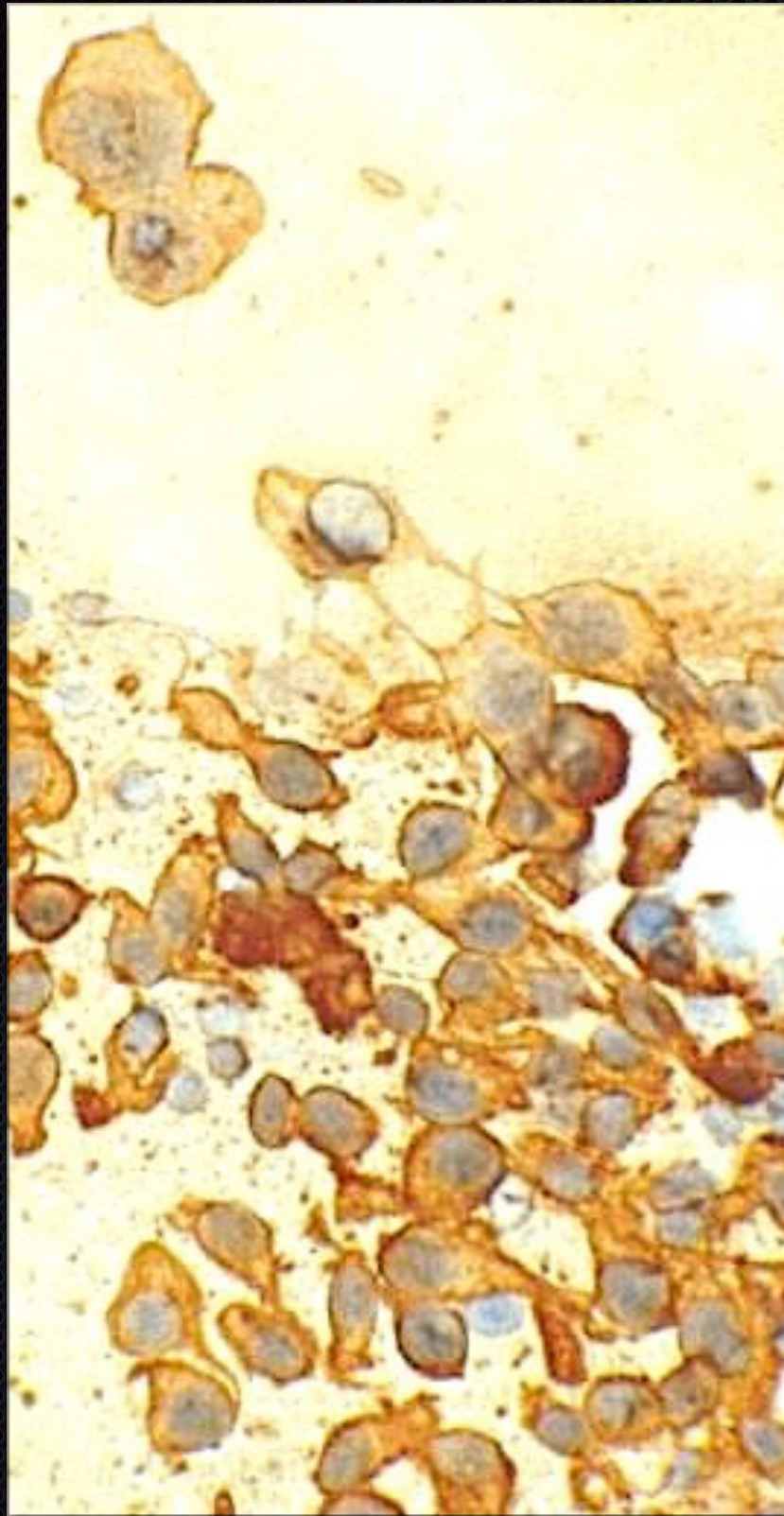


CD8, C8/144B - Acetone 10'

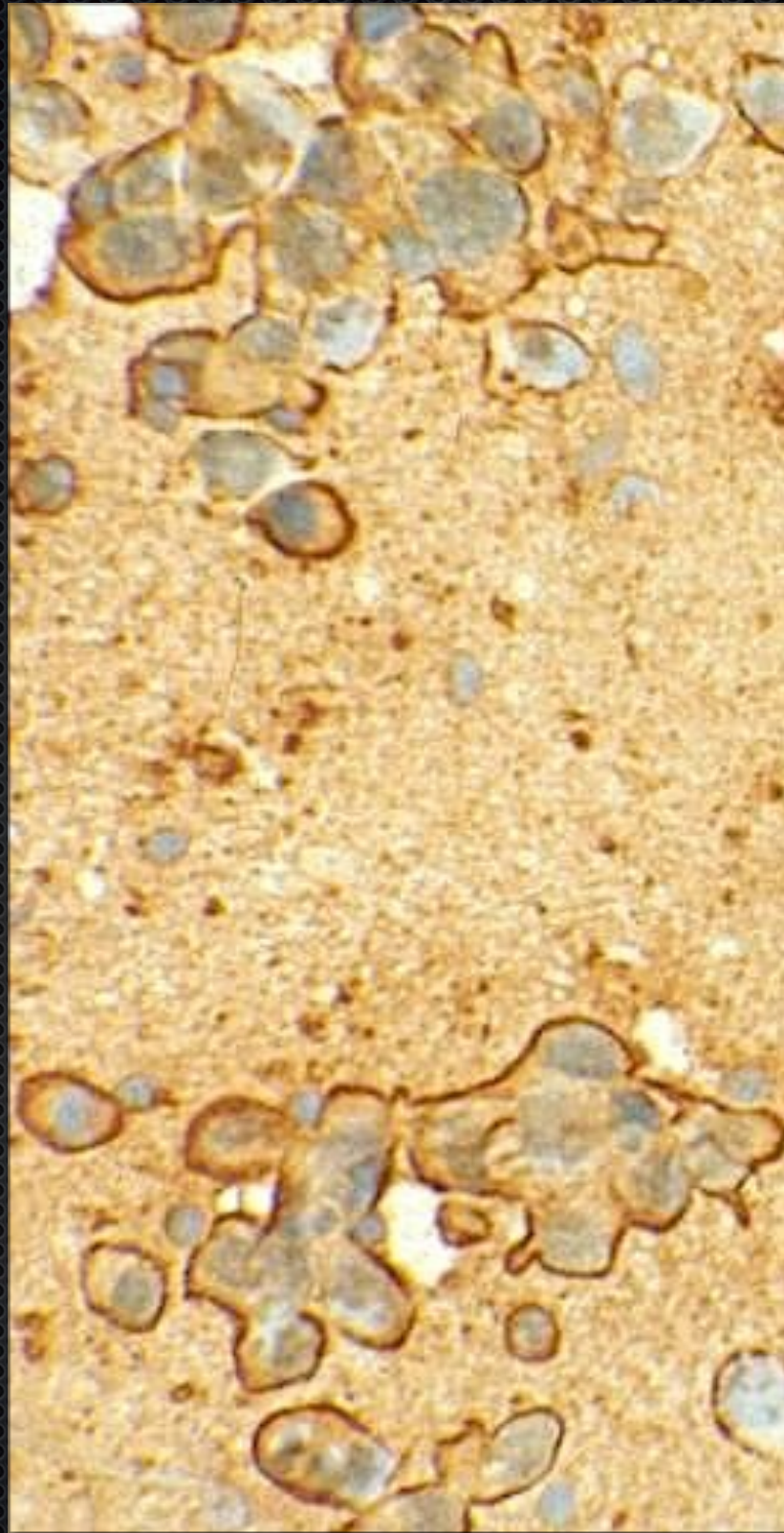


CD8, C8/144B NBF 2' - TEG95° 30s

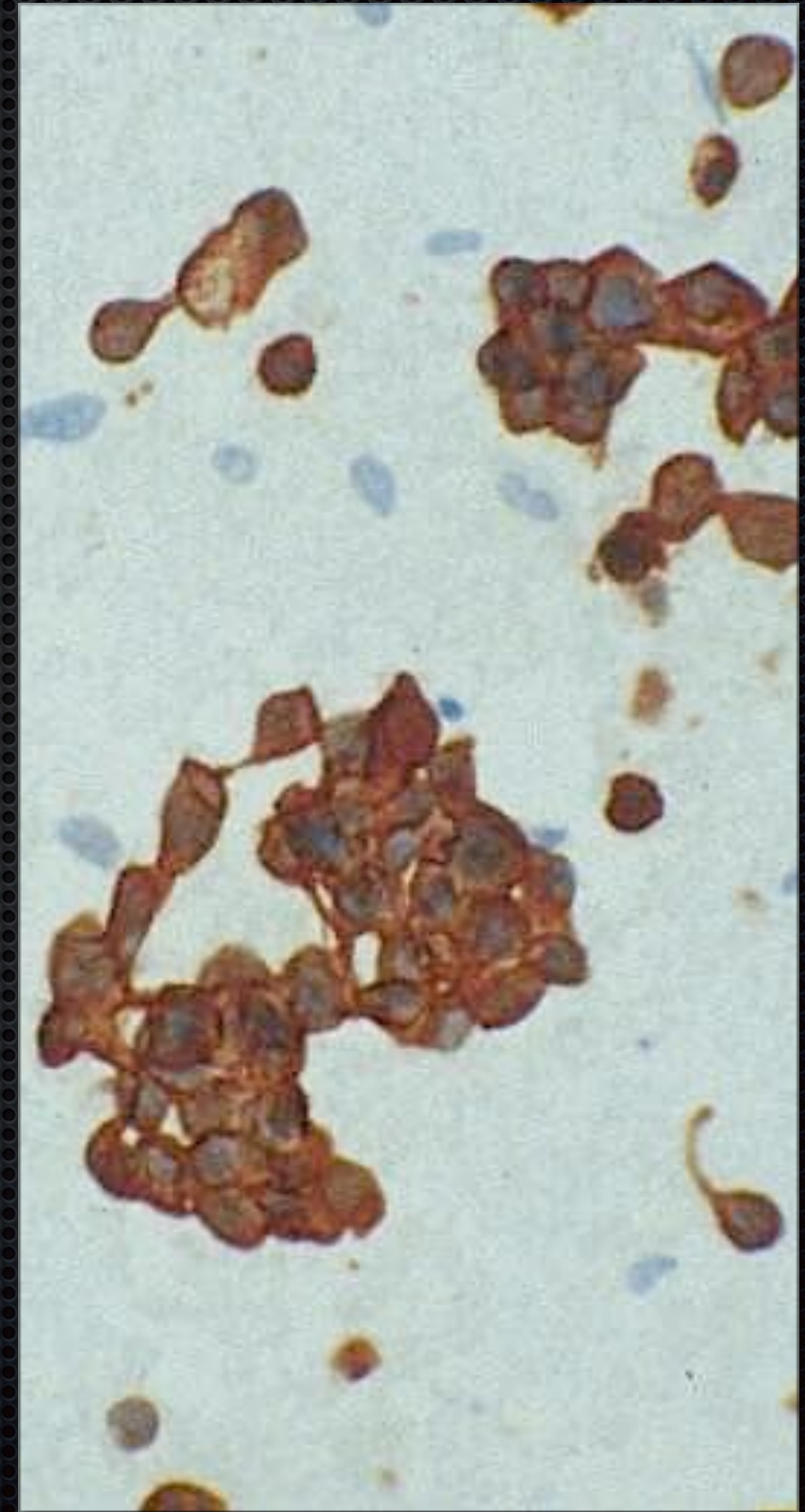
CK7, OV-TL12/30 (Imprint) various fixatives



Acetone 10'

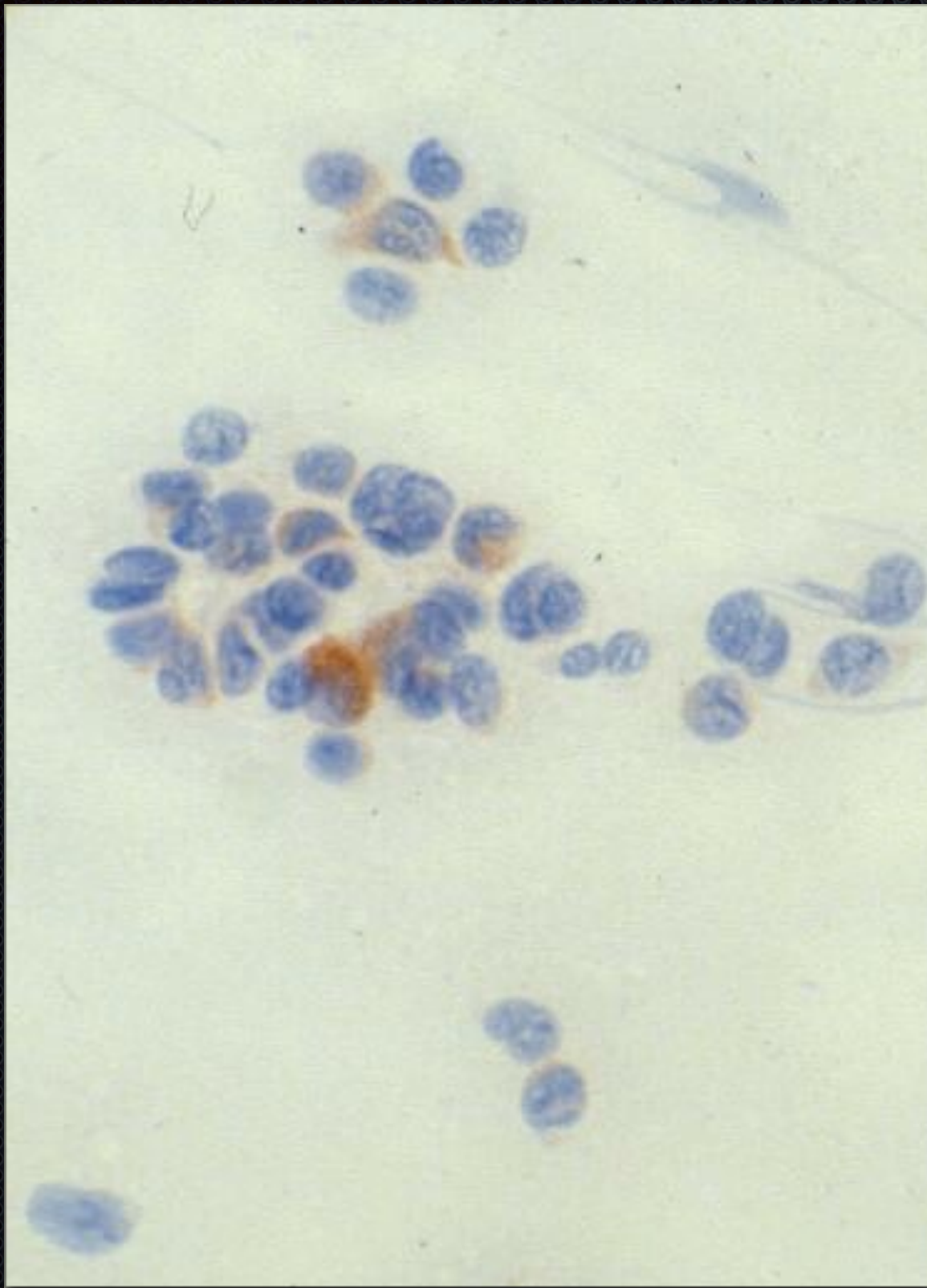


4% NBF 5'

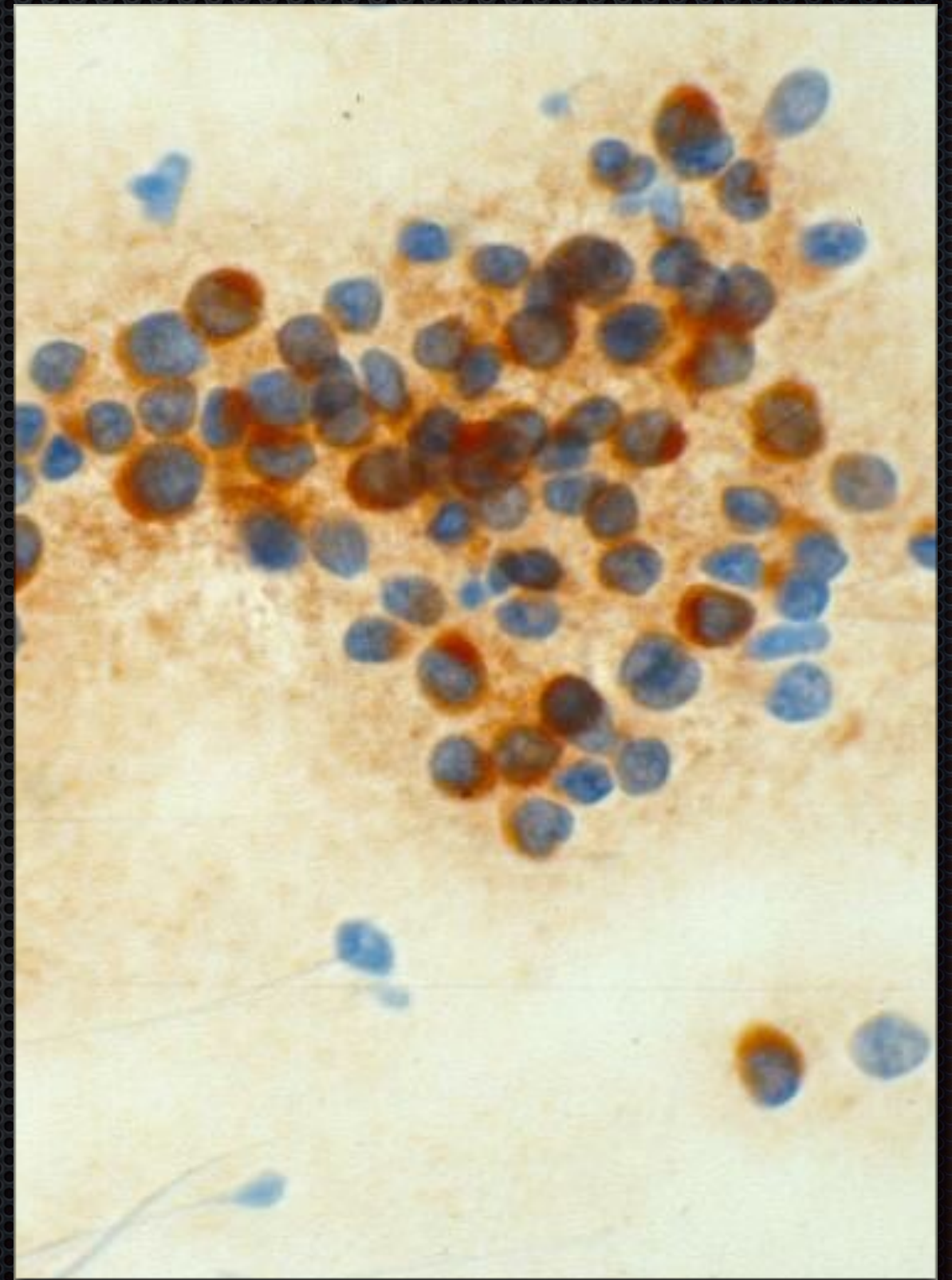


NBF 15' - TEG95° 15

Tyrosinase, T31 1 (Imprint) Various fixatives

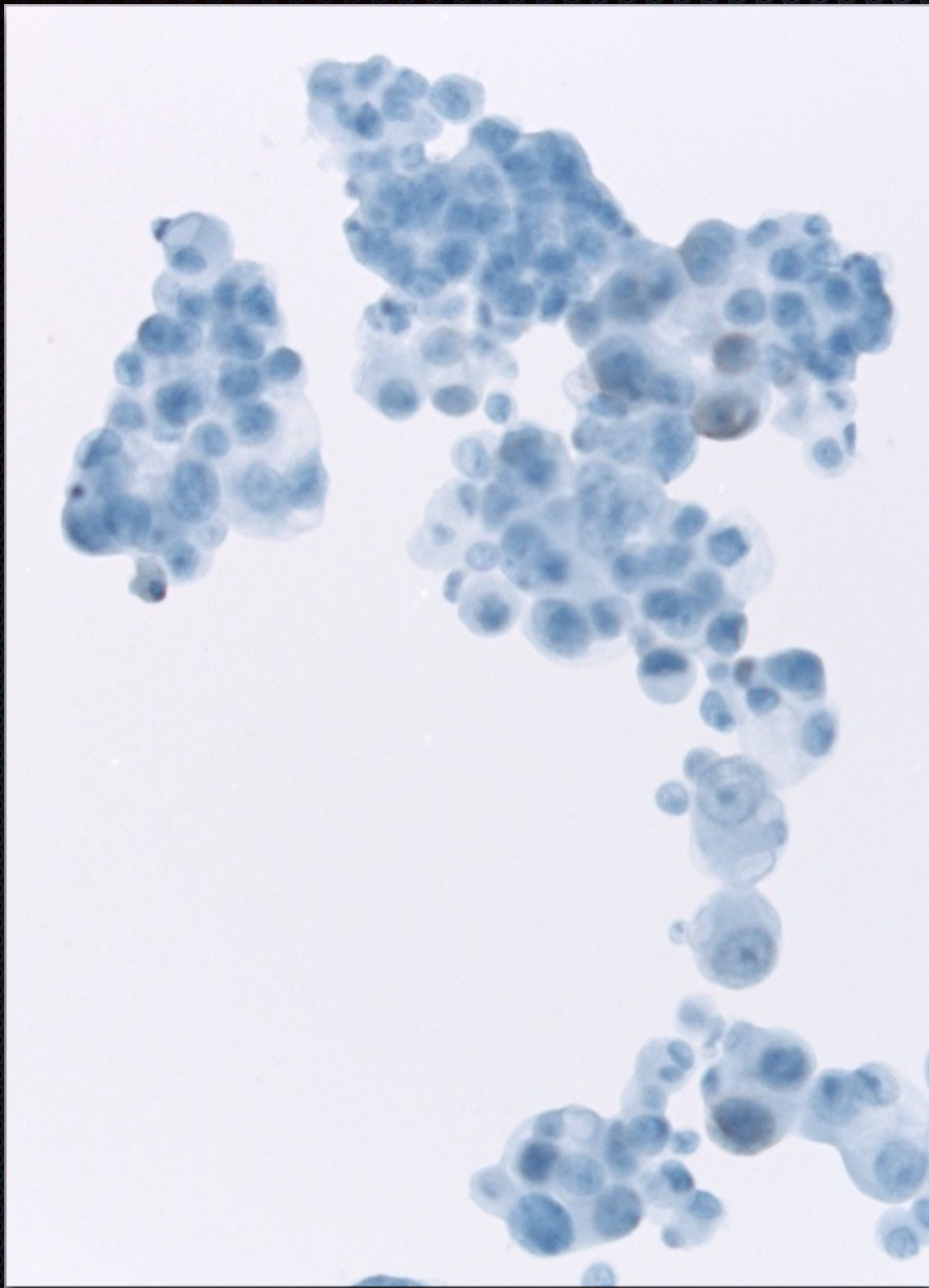


Acetone 10'

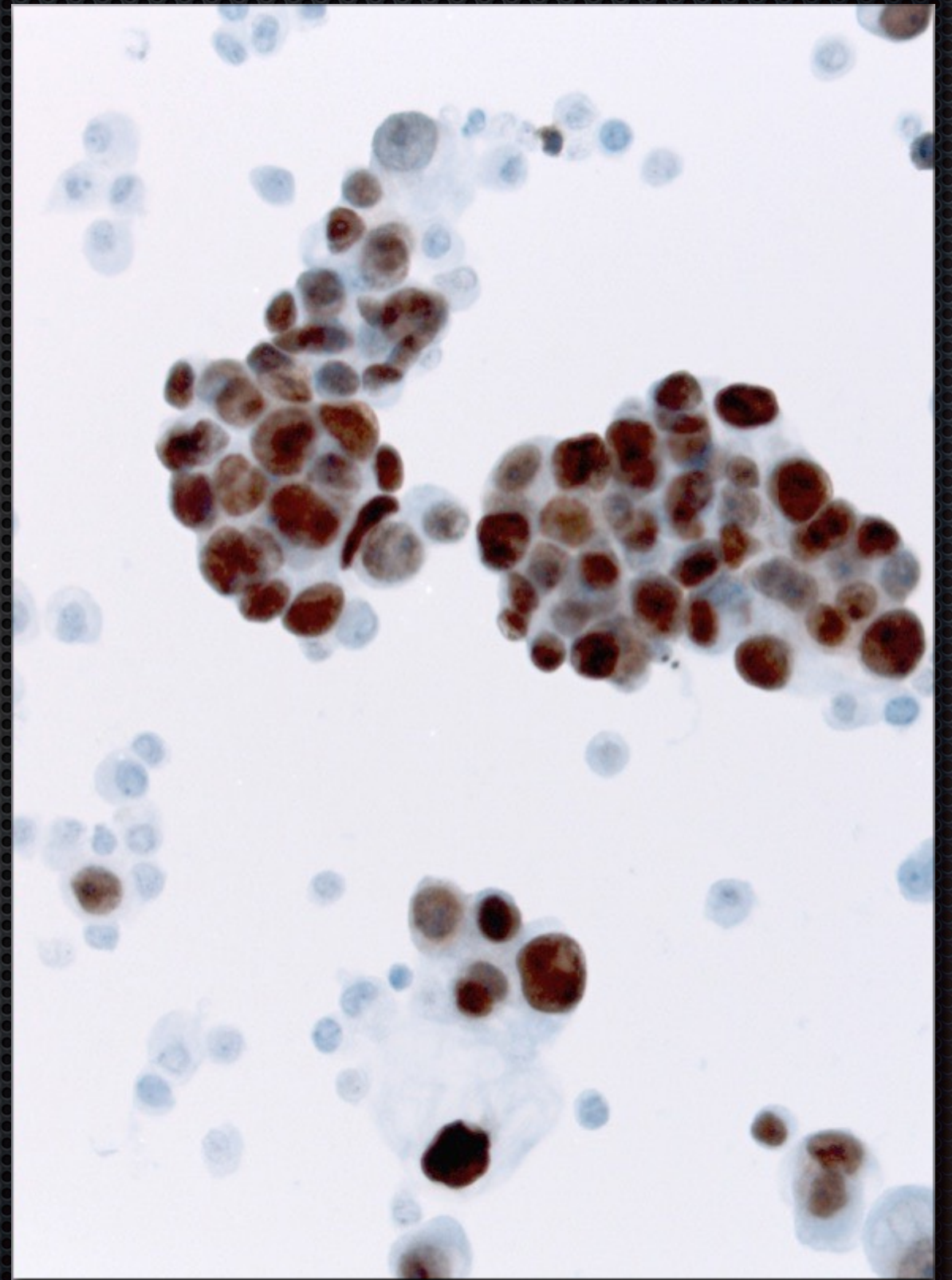


NBF 15' - TEG95° 15'

TTF1, SPT24 (ThinPrep) Various fixatives

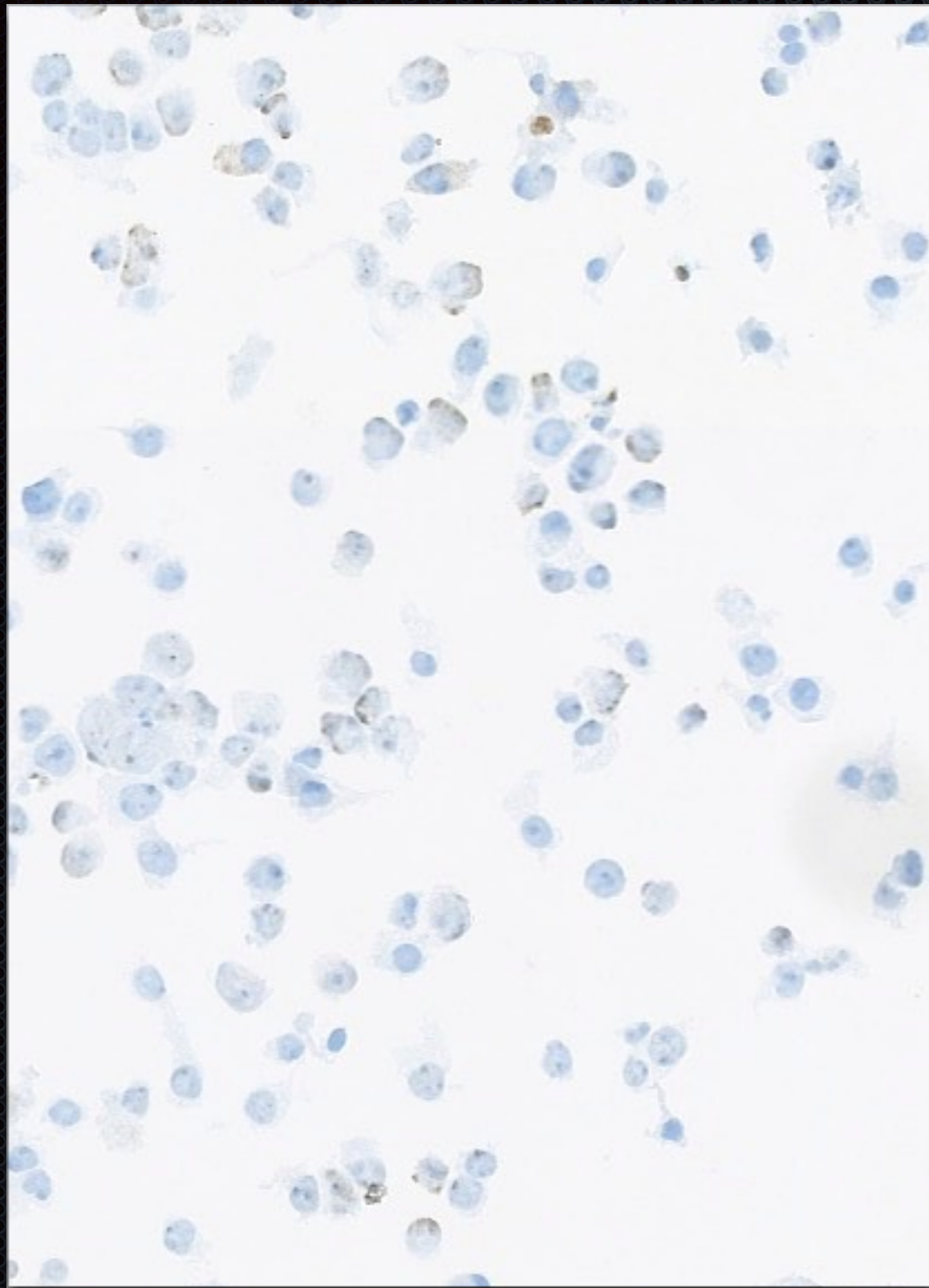


NBF 15' - TEG95° 15'



NBF 15' - MW/TEG 15'

p40, BC28 (LBC - A431 cells) Various fixatives



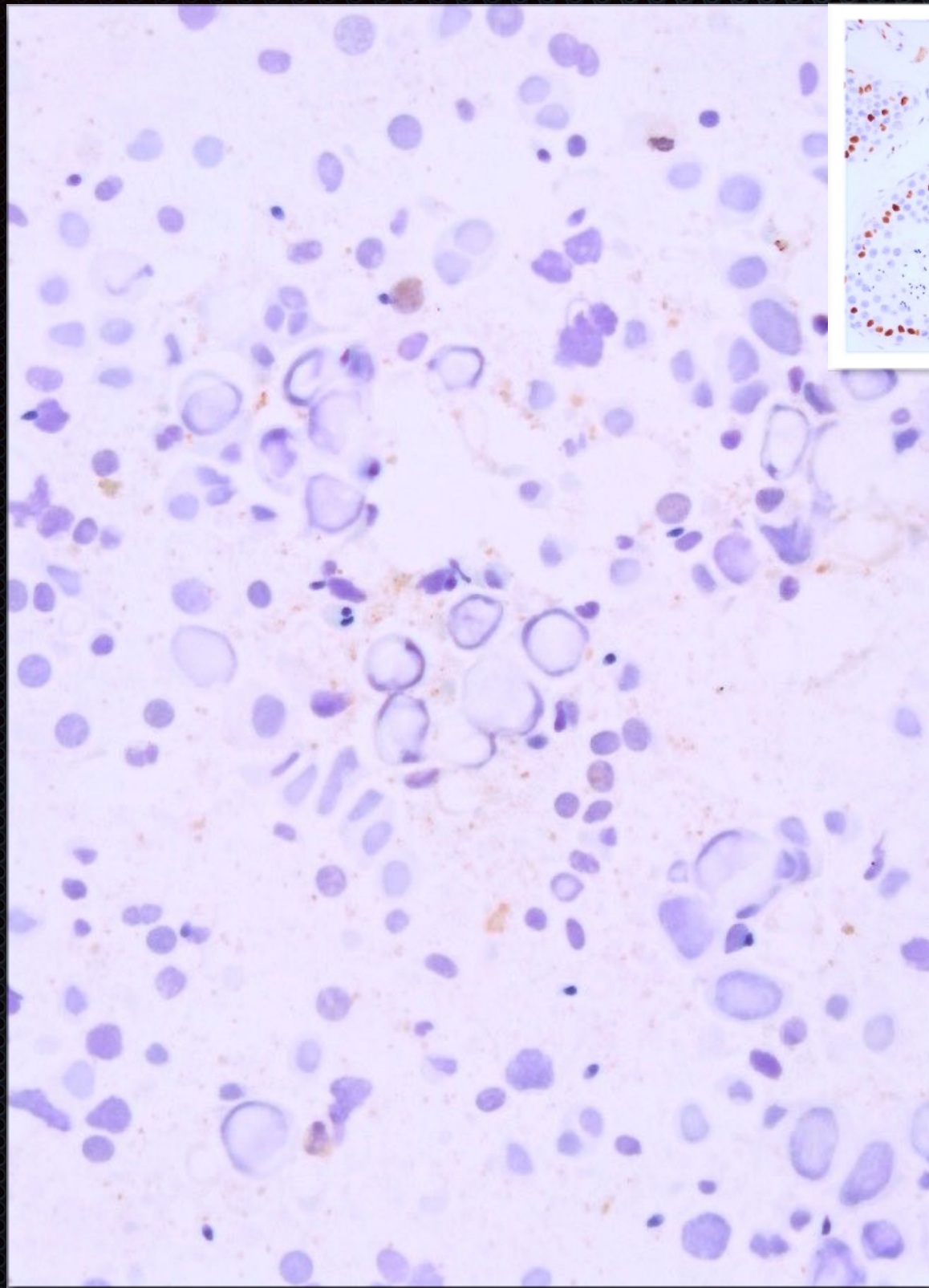
NBF 5'

Ventana BenchMark Ultra

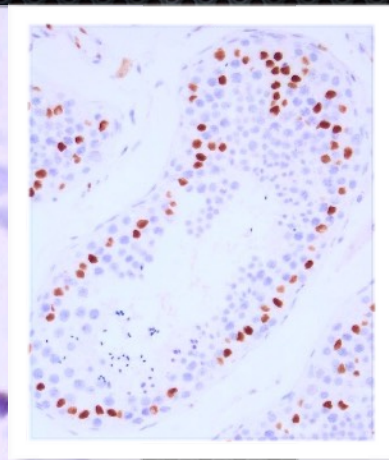


NBF 5' - CC1_32'_95°

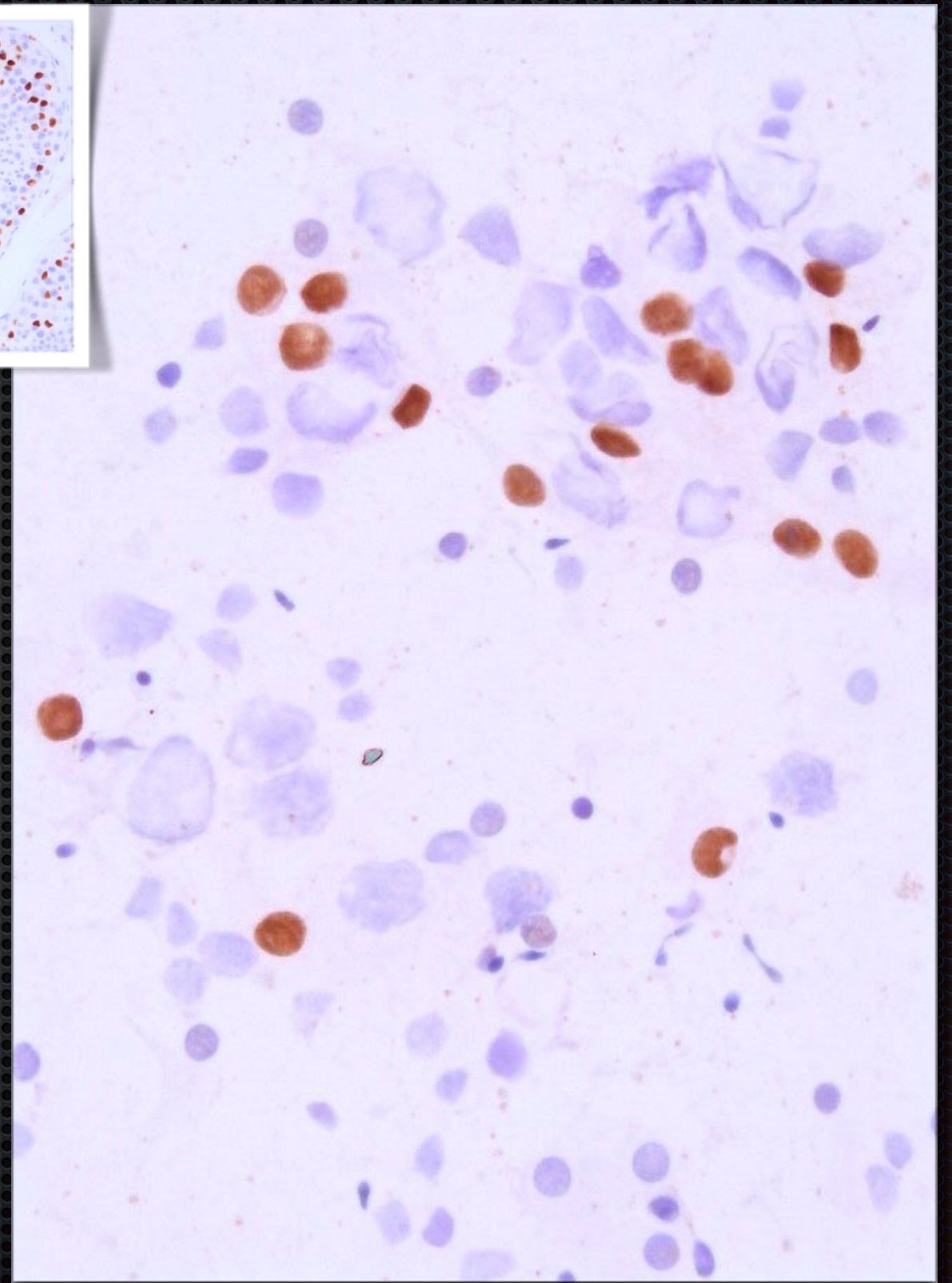
WT-1, EP122 (Imprint, testis) Various fixatives



NBF 5'



Dako OMNIS



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

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

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

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'

4 Abs: Omnis

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

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, δ -Sarc/12C1	None	None
Sarcoglycan beta, β -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

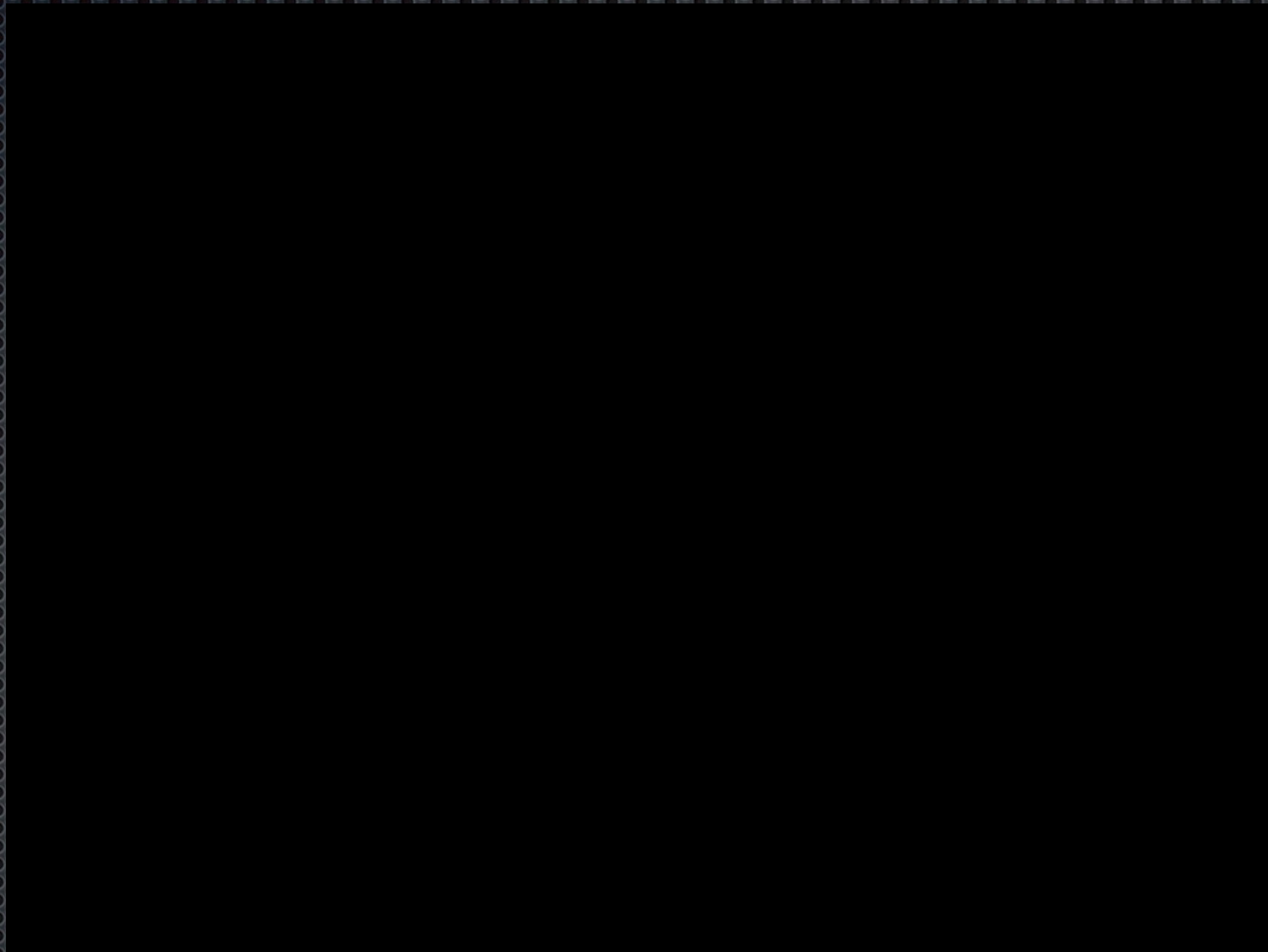
Antibody	Fixation	Retrieval
Cranin, VIA4-1	4% NBF 15 min	CC1_8_90
CD4, SP35	4% NBF 5 min	None
CD45, 2B11 & PD7/26	4% NBF 5 min	None
CD56, 56C04	4% NBF 5 min	None
C5b-9, aE11	4% NBF 5 min	None
CD57, HNK-1	4% NBF 5 min	None
CD8, C8/144B	4% NBF 5 min	None
CD20cy, L26	Acetone 10 min	None
Collagen IV, MAB3	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 safe trip home!