

# Immunocytochemistry and immunohistochemistry on frozen sections

Overview, considerations and applications

NQC workshop 2019

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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

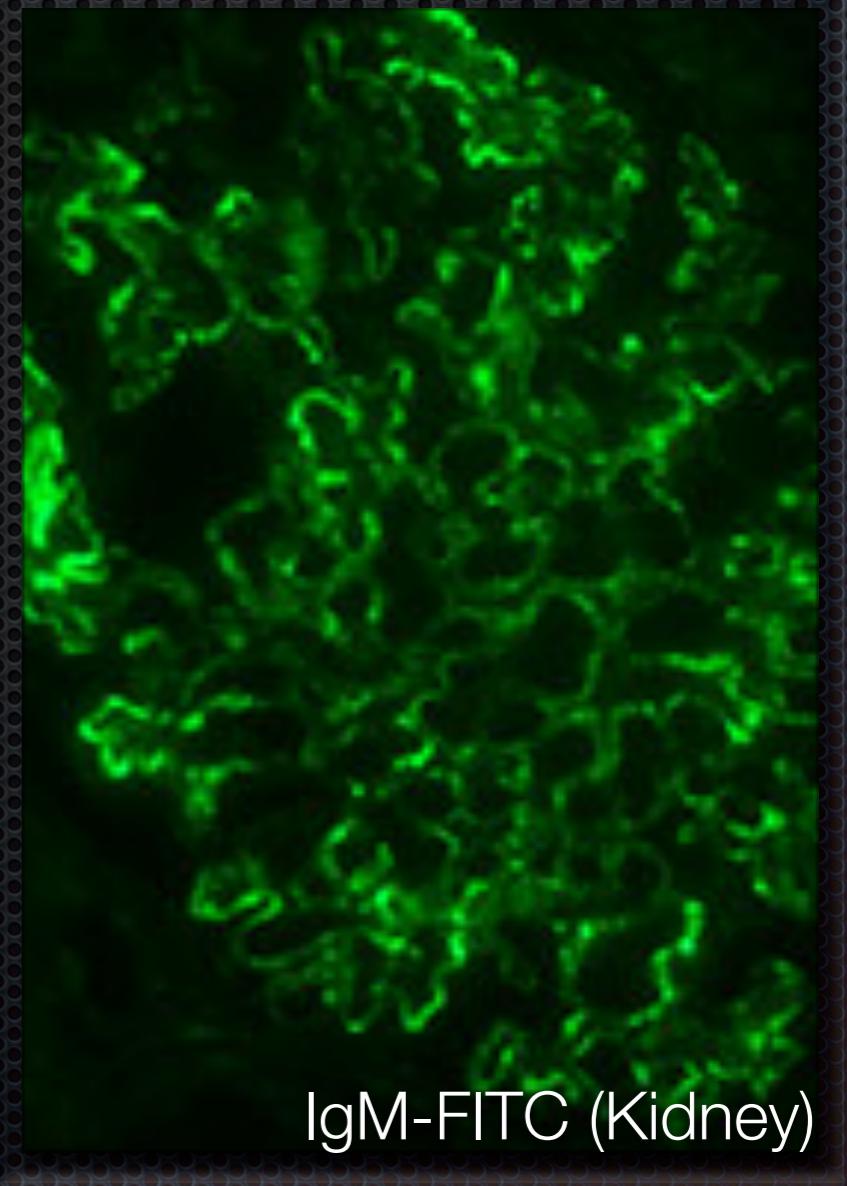
Collagen IV, MAB5

# 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 paraffin 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)

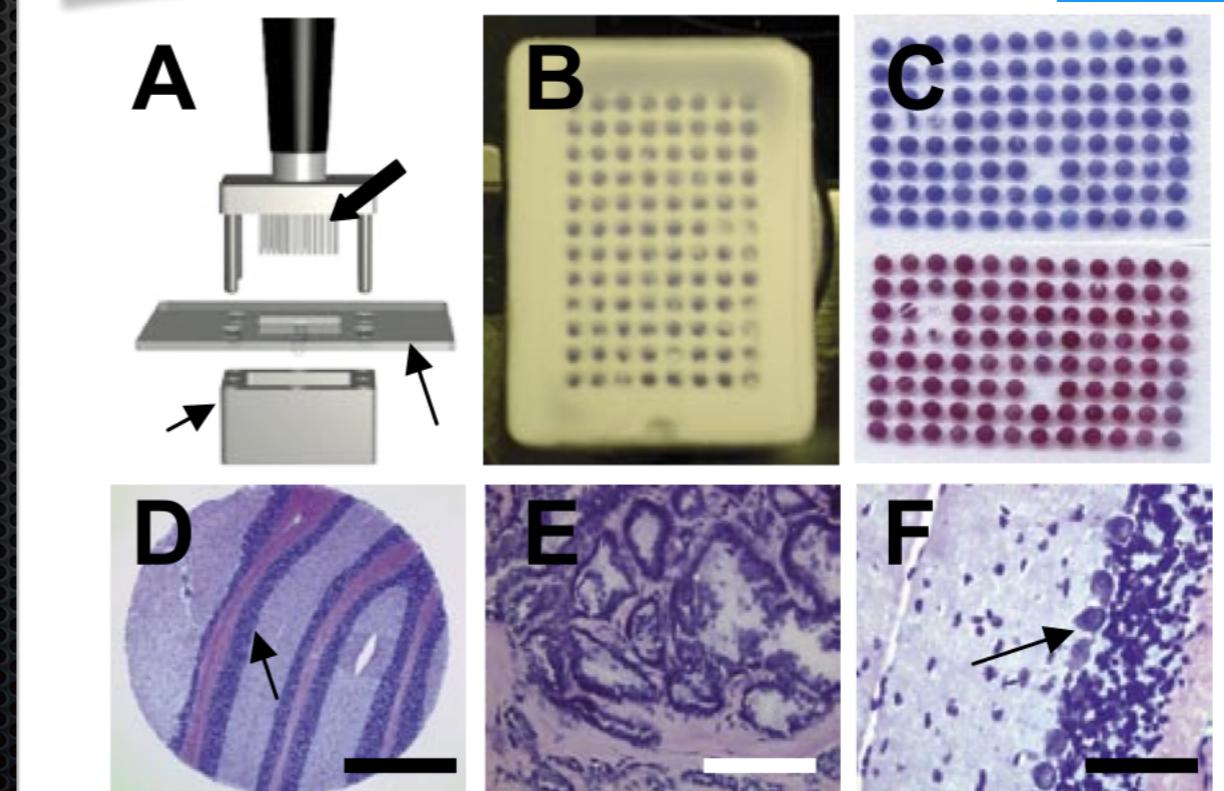
- Designed TMAs:
- Tissue with high expressors
- Tissue with low expressors
- Tissue with non-expressors

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

Lei Zhou<sup>1</sup>, Melissa Hodeib<sup>2</sup>, Joseph D. Abad<sup>2</sup>, Leopoldo Mendoza<sup>1</sup>, Anilkumar R. Kore<sup>1</sup>, and Zhongting Hu<sup>2</sup>  
<sup>1</sup>Ambion, Inc., Austin, TX and <sup>2</sup>Western University of Health Sciences, P. O. Box 30107, Pomona, CA, USA

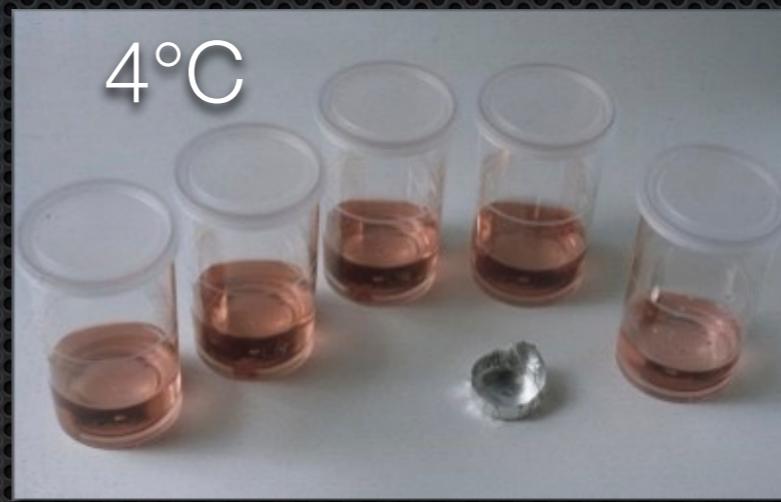
BioTechniques 43:101-105 (July 2007)  
doi 10.2144/000112498

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



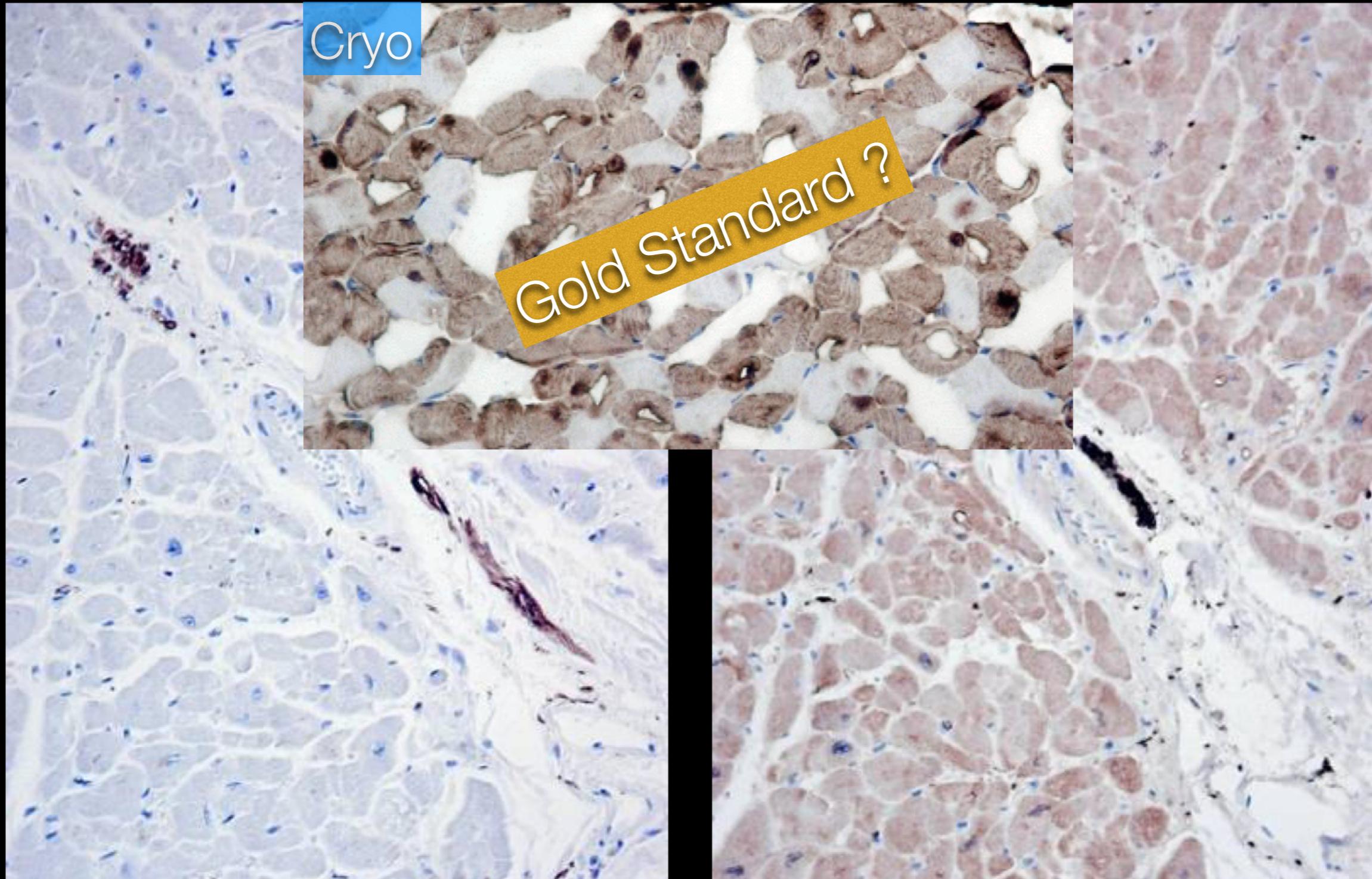
# Optimizing biomarker-protocols (IHC-protocols on frozen sections)

- "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+

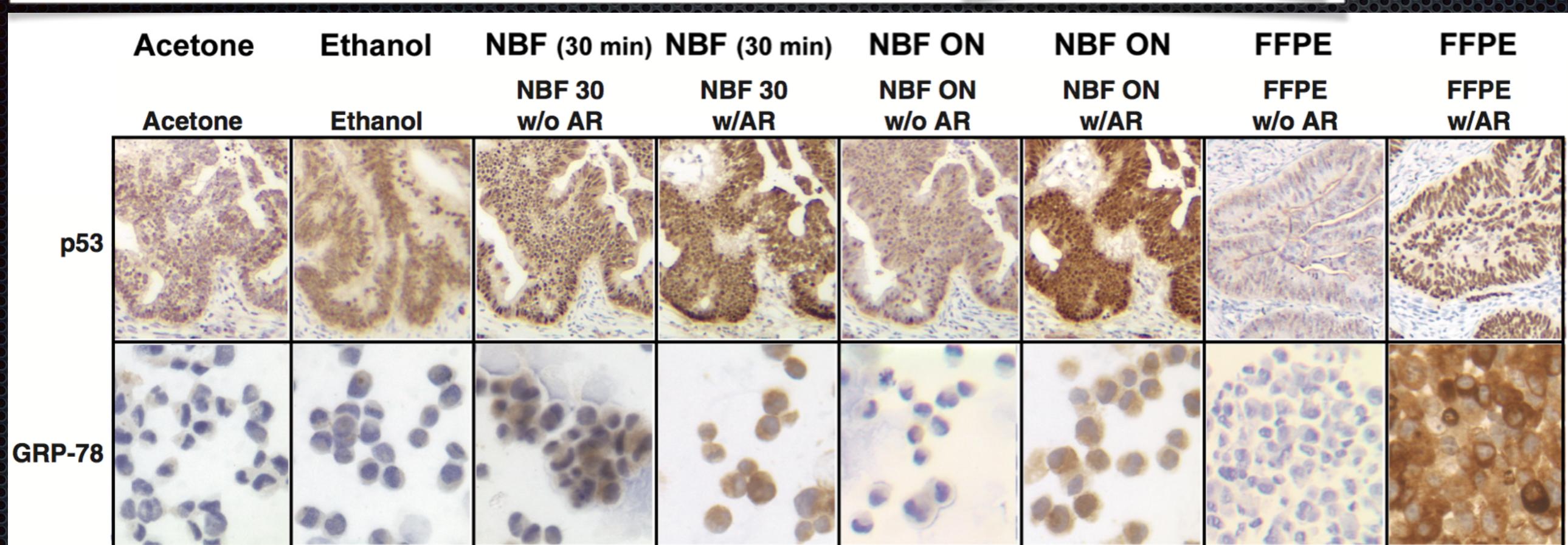
AR, antigen retrieval; CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; FFPE, formalin-fixed, paraffin-embedded; GRP, glucose-regulated protein; NBF, neutral buffered formalin; Rb, retinoblastoma; w/AR, use of the AR pretreatment before the immunohistochemical staining procedure;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

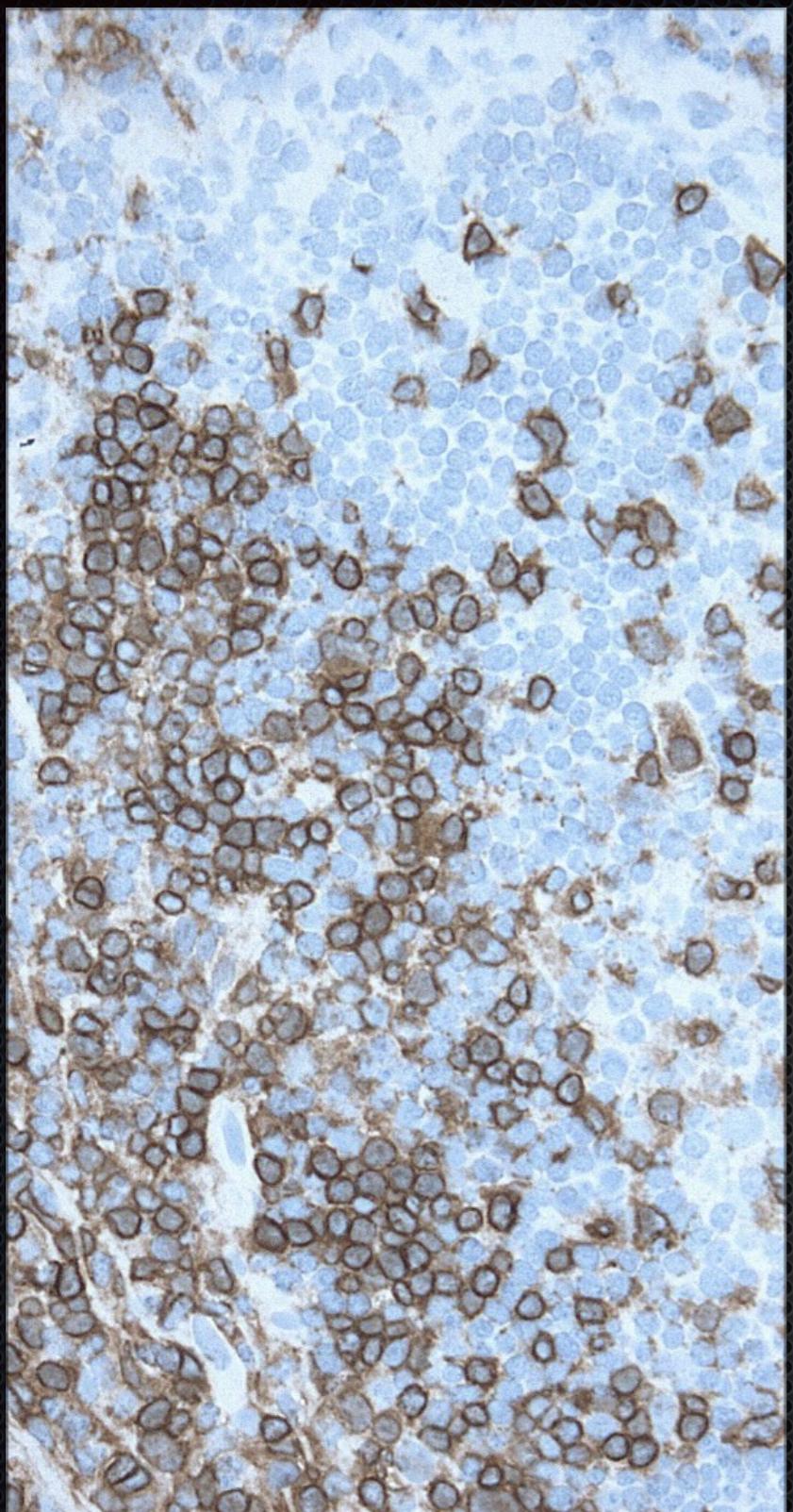
Am J Clin Pathol 2008;129:358-366



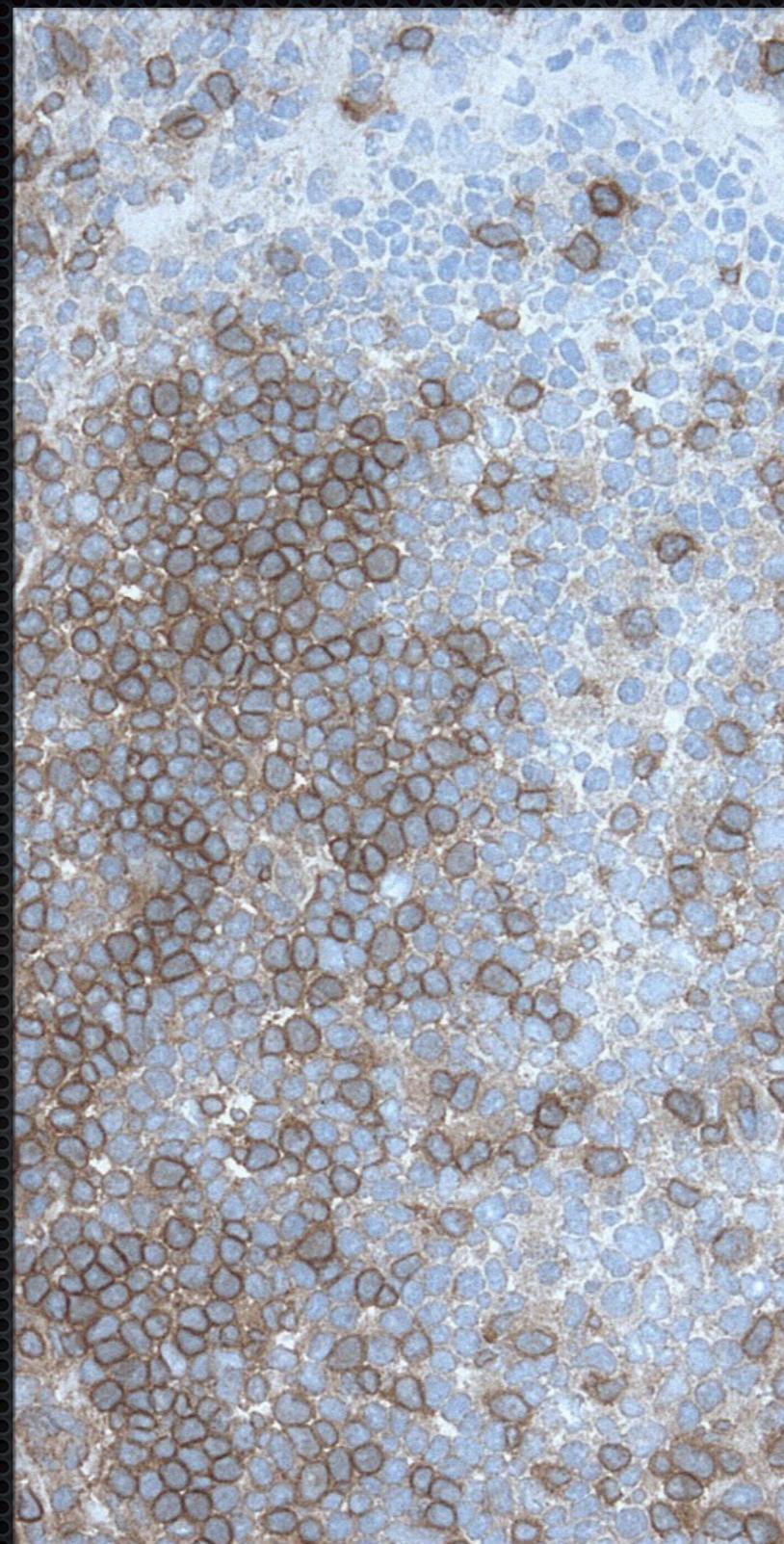
## Results:

- ★ 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.

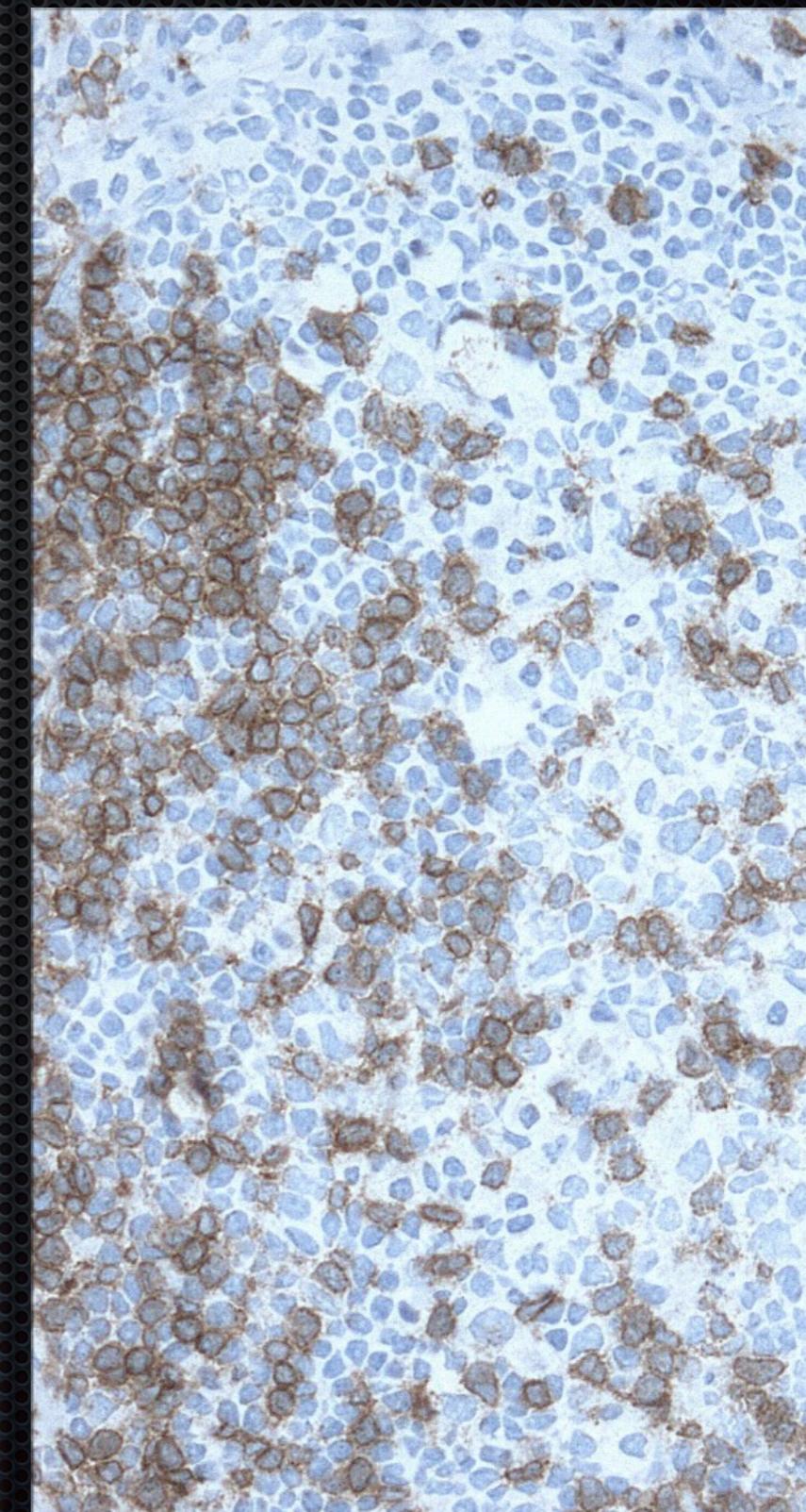
## CD3, F7.2.38 (Tonsil) various fixatives



Acetone 10'

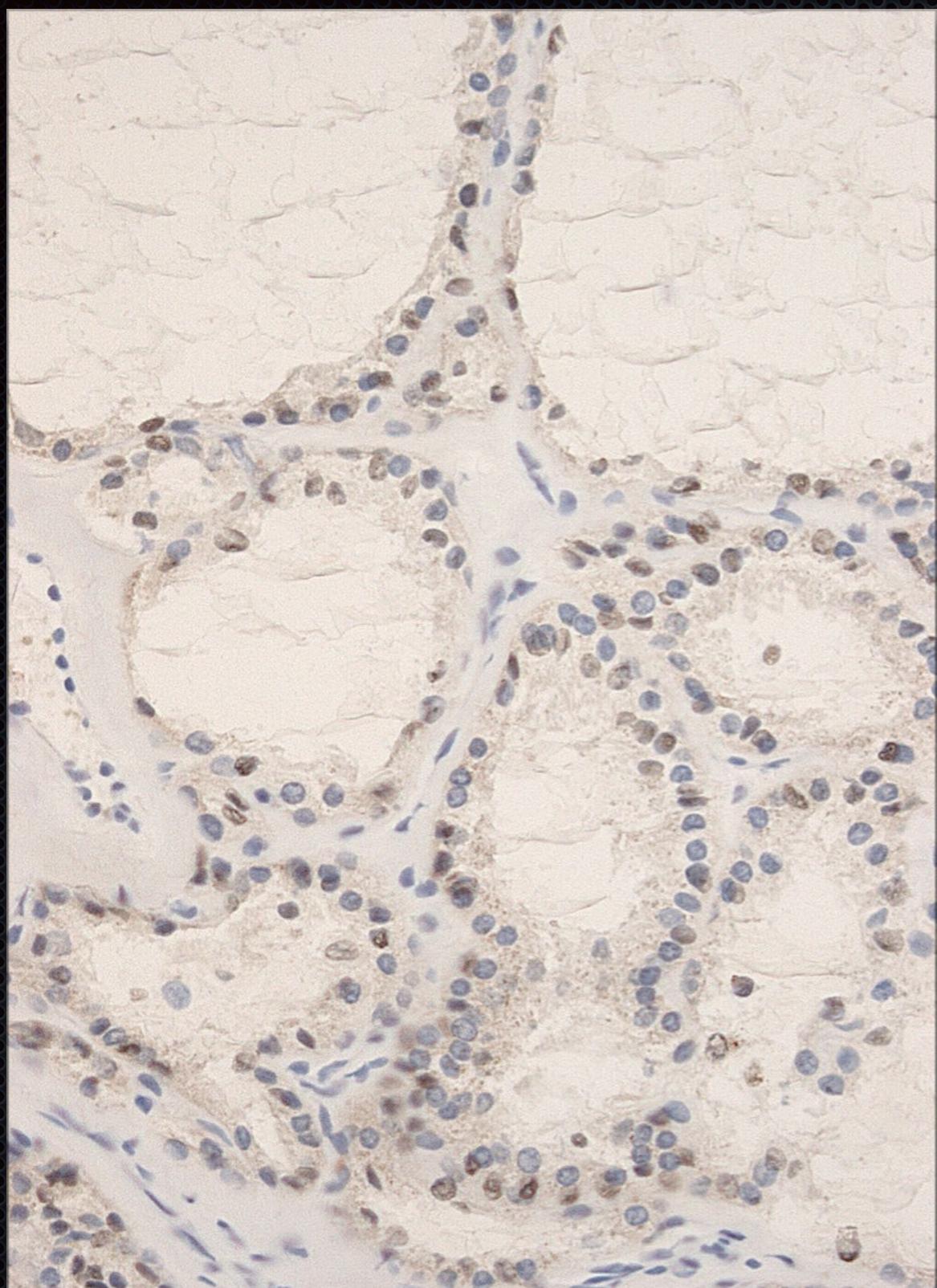


4% NBF 5'

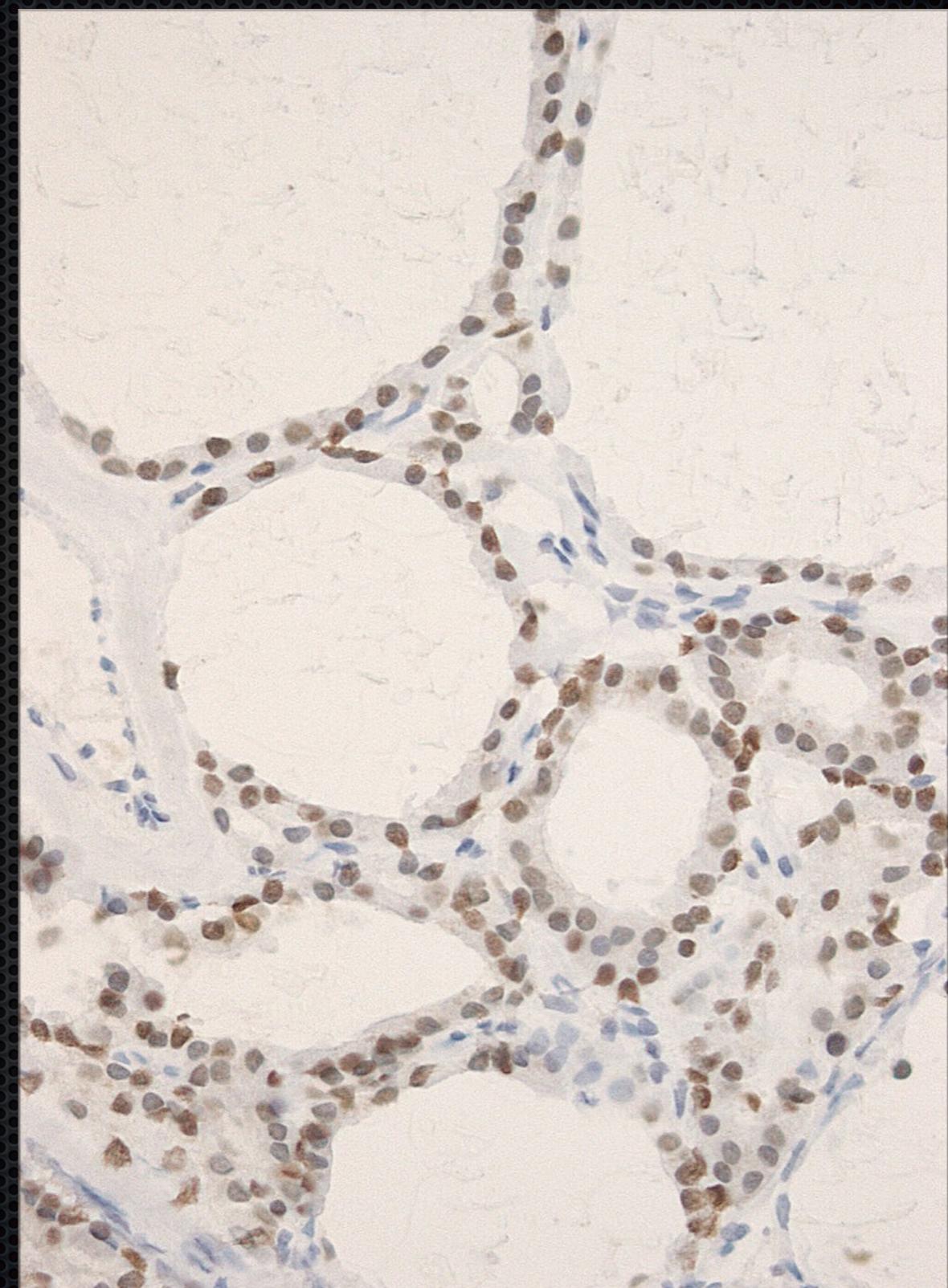


NBF 2' - TEG95° 30s

# TTF-1, SPT24 (Thyroid) various fixatives



Acetone 10'

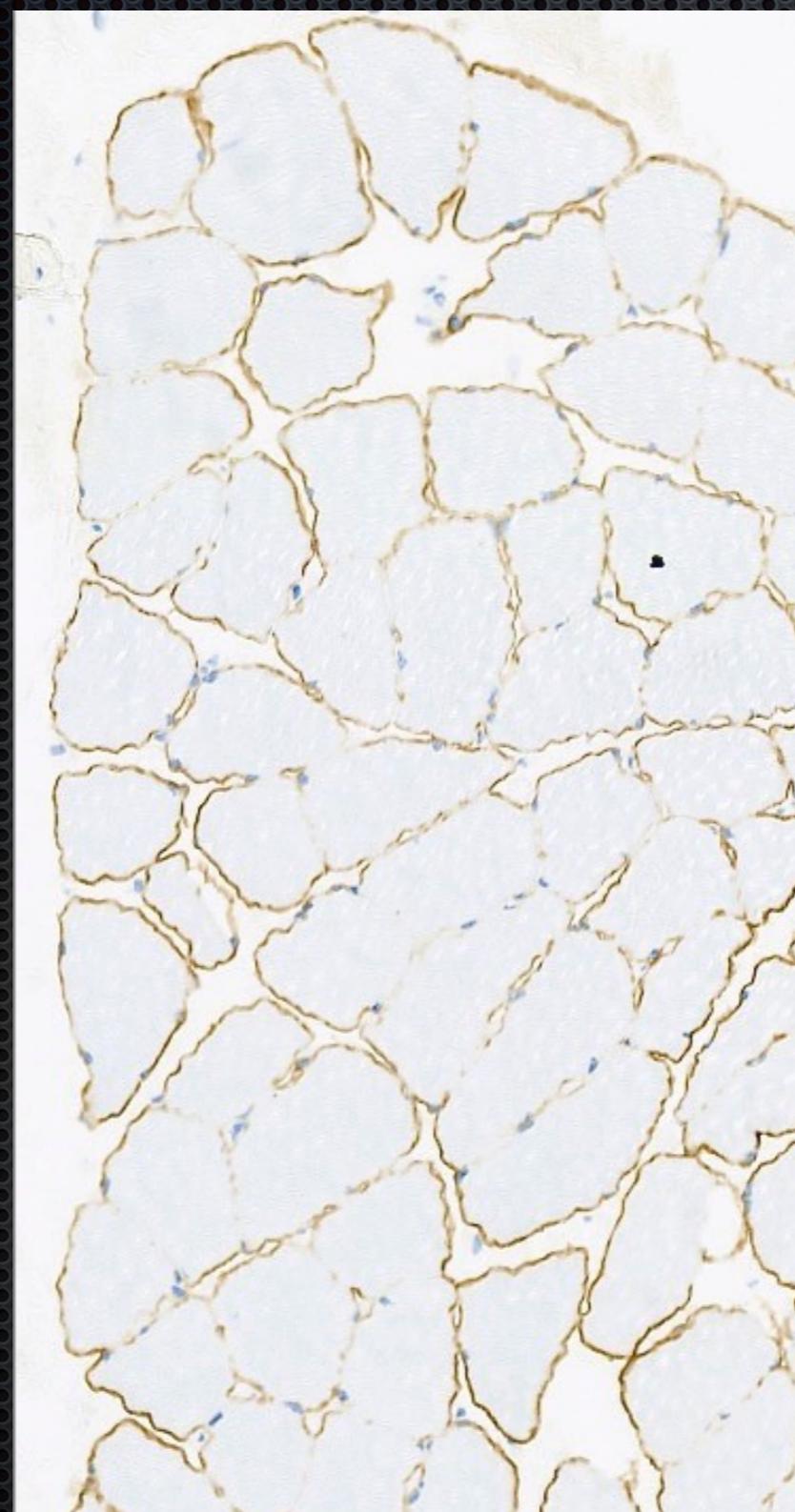


NBF 2' - TEG95° 30s

# LAM-A2, Mer3/22B2 - various fixatives



No fixation (muscle)

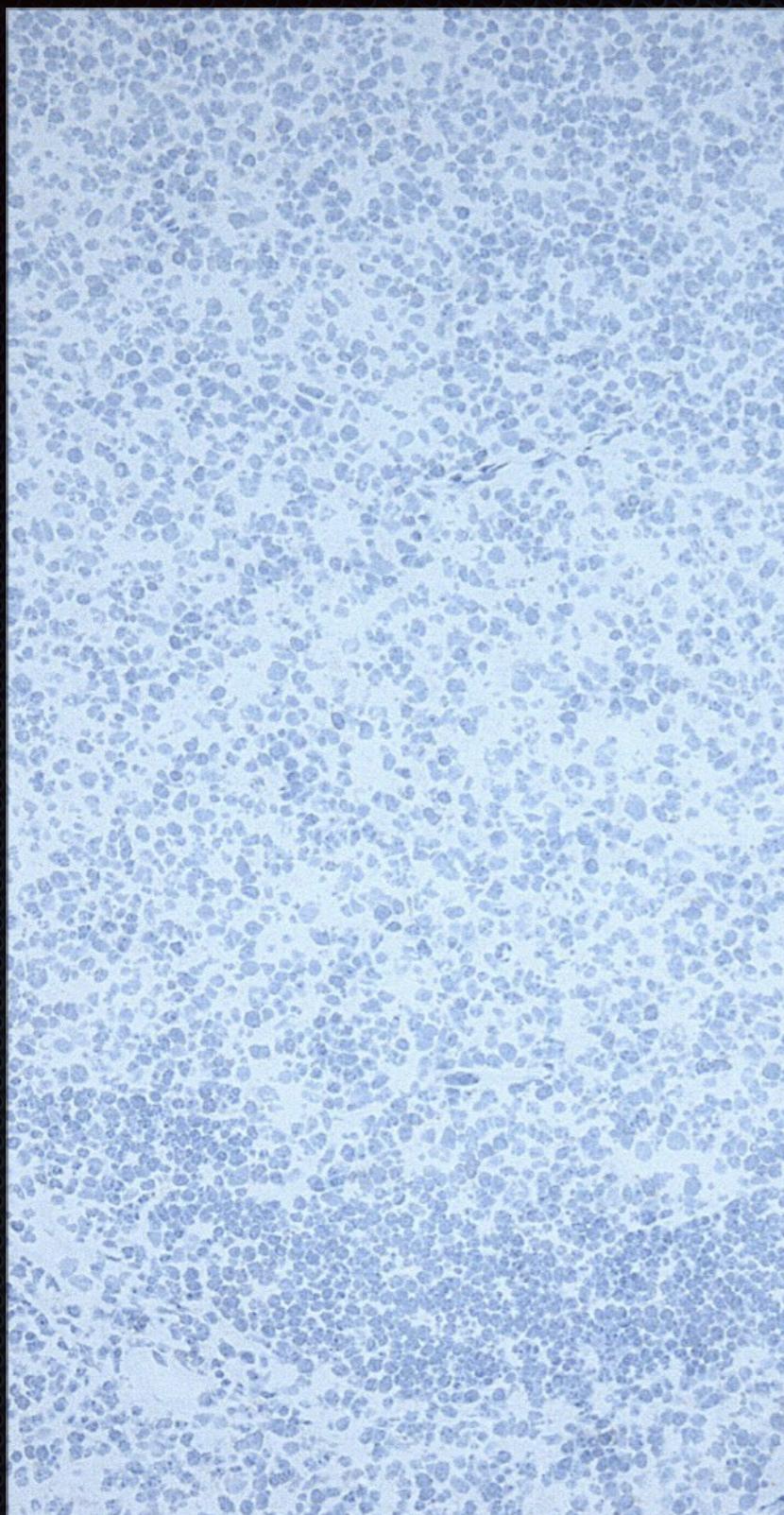


4% NBF 5' (muscle)

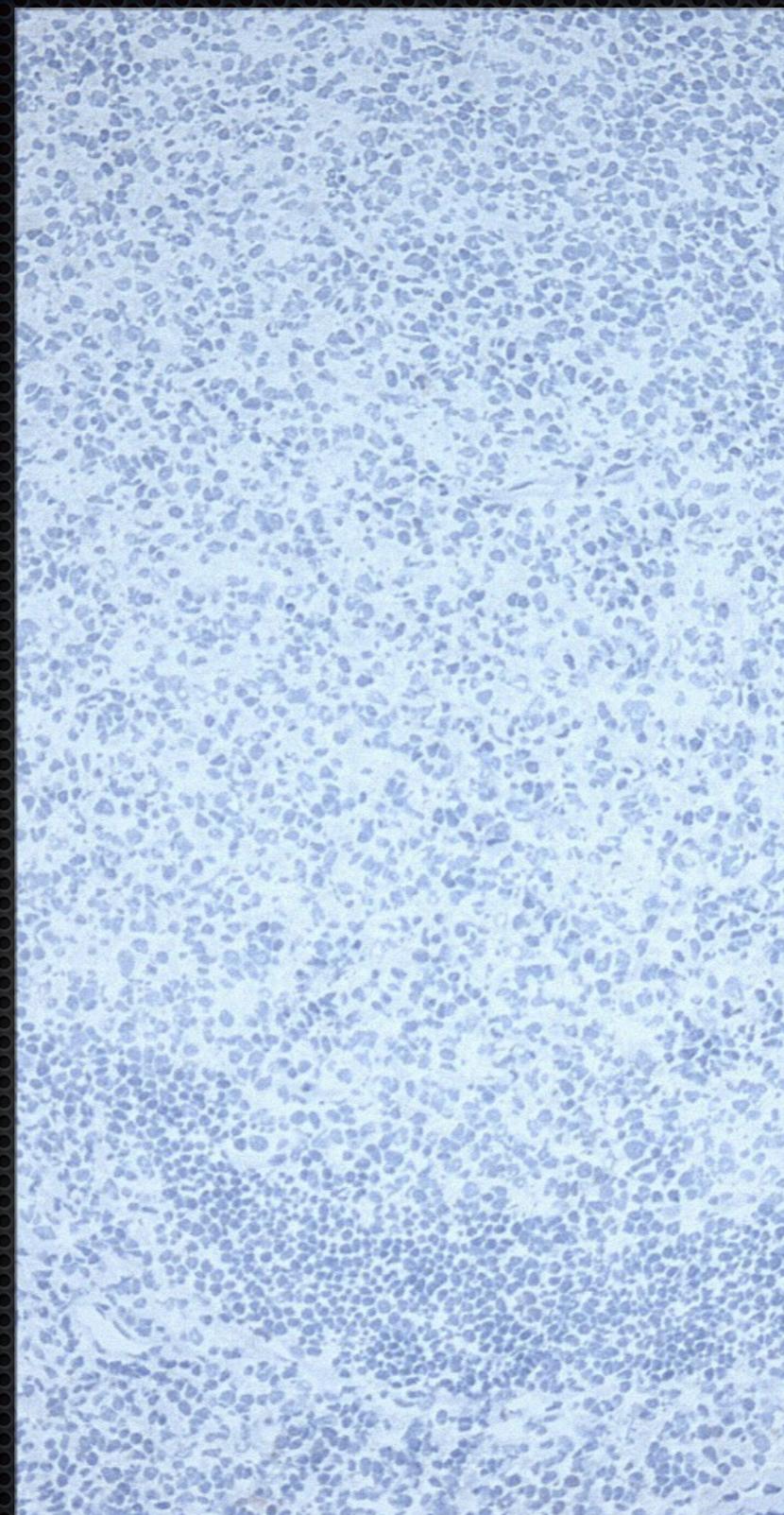


Acetone 10' (muscle)

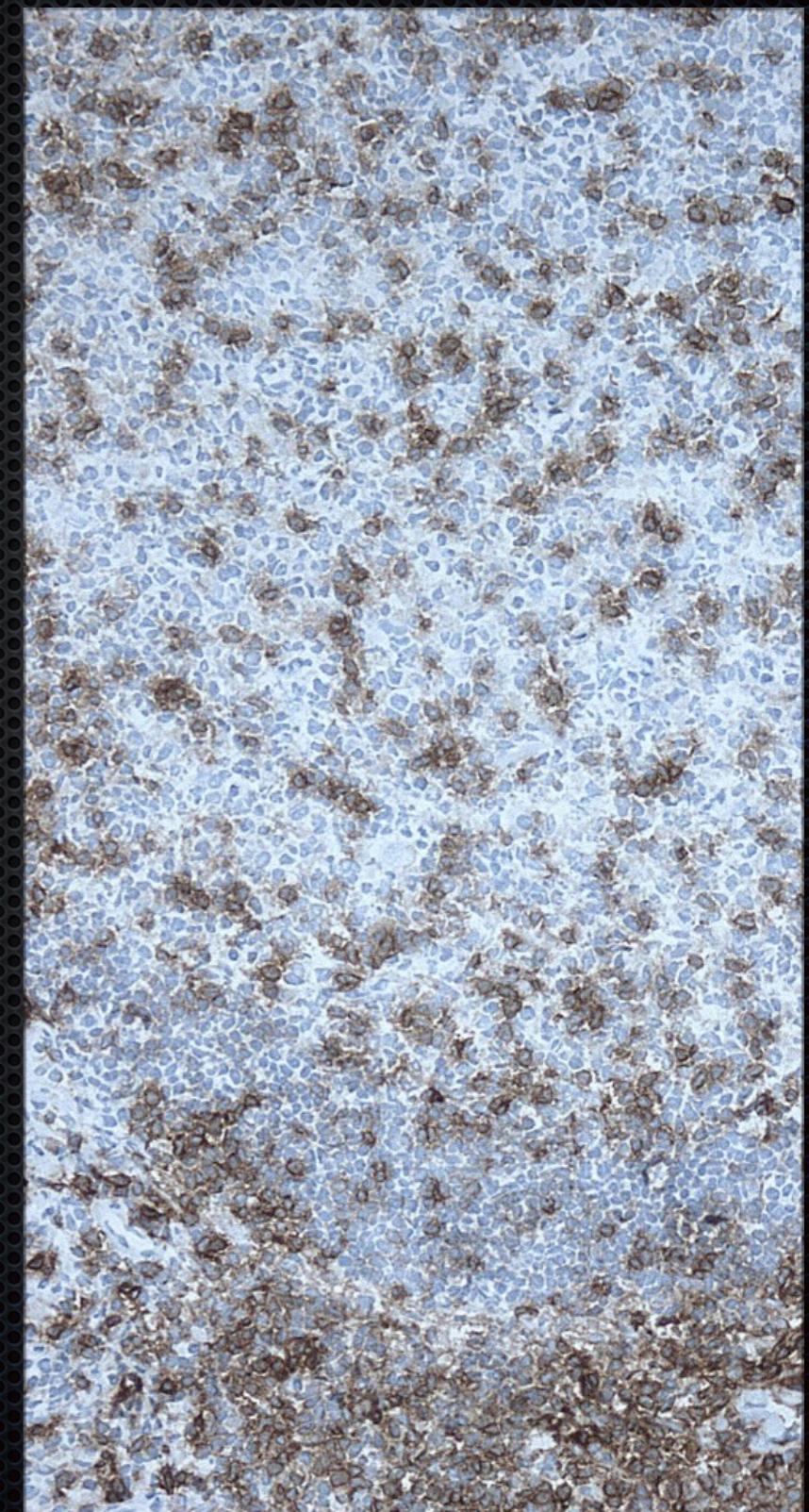
## CD5, 4C7 (Tonsil) various fixatives



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	<b>2-30 min &gt;&gt;&gt;</b>	TEG, pH9 (95°C)      30 sec
5	The vendors recommendations		

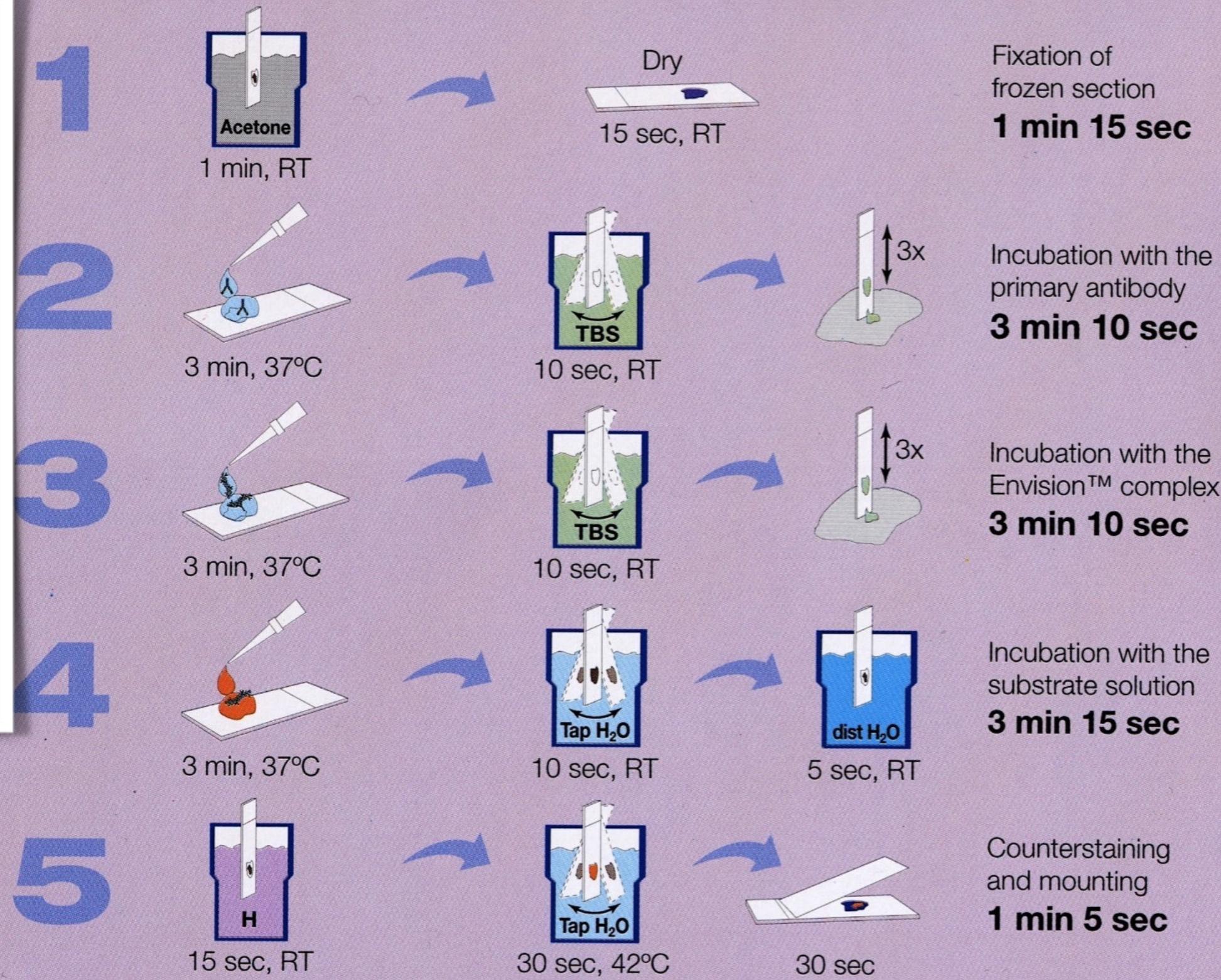
TEG: Tris-EGTA based buffer pH 9.0

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

Kämmerer et al

**ARTICLE**  
**A New Rapid Immunohistochemical Staining Technique Using  
 the EnVision Antibody Complex**

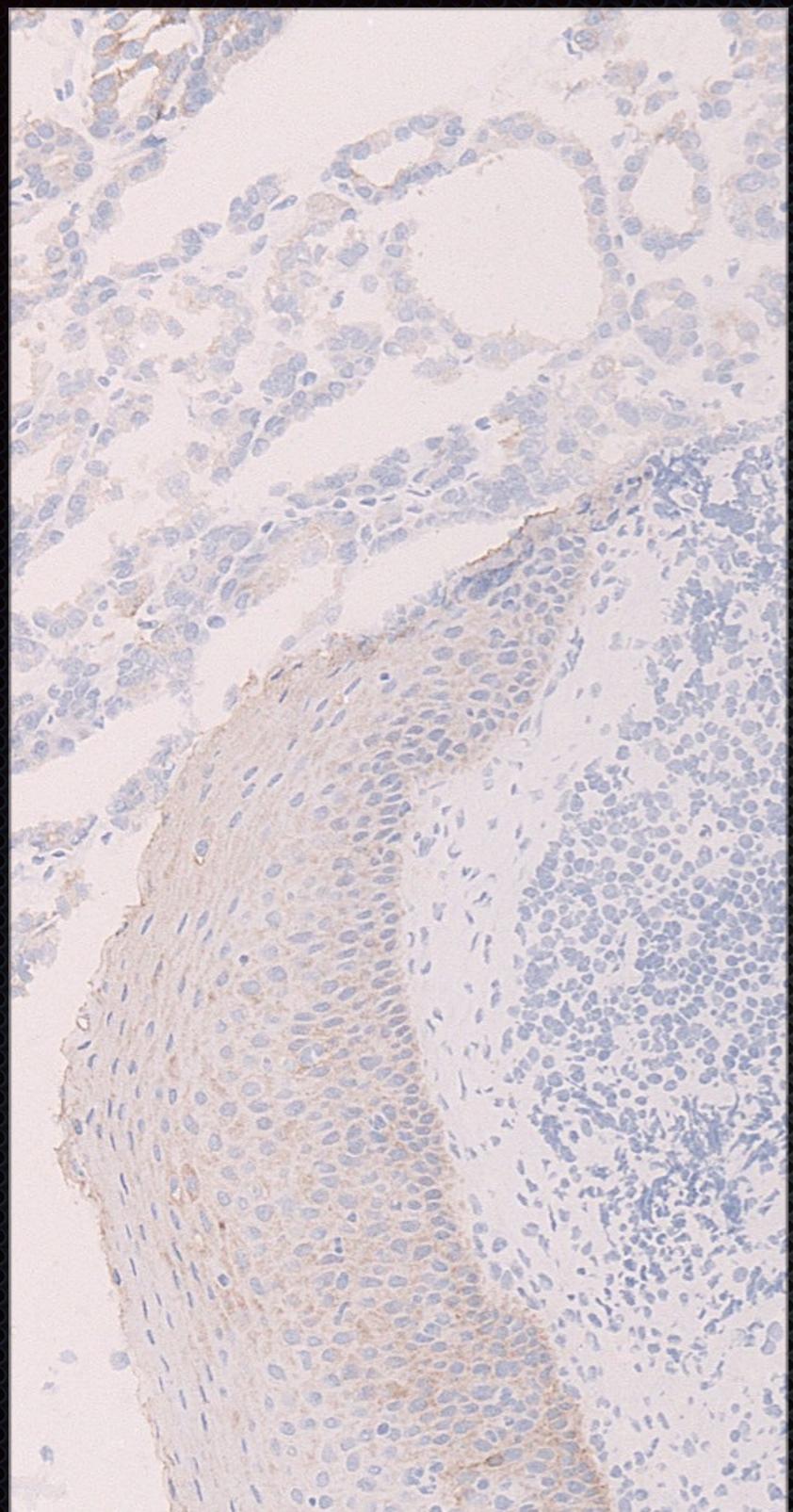
Ulrike Kämmerer, Michaela Kapp, Andrea Maria Gassel, Thomas Richter, Christian Tank,  
 Johannes Dietl, and Peter Ruck



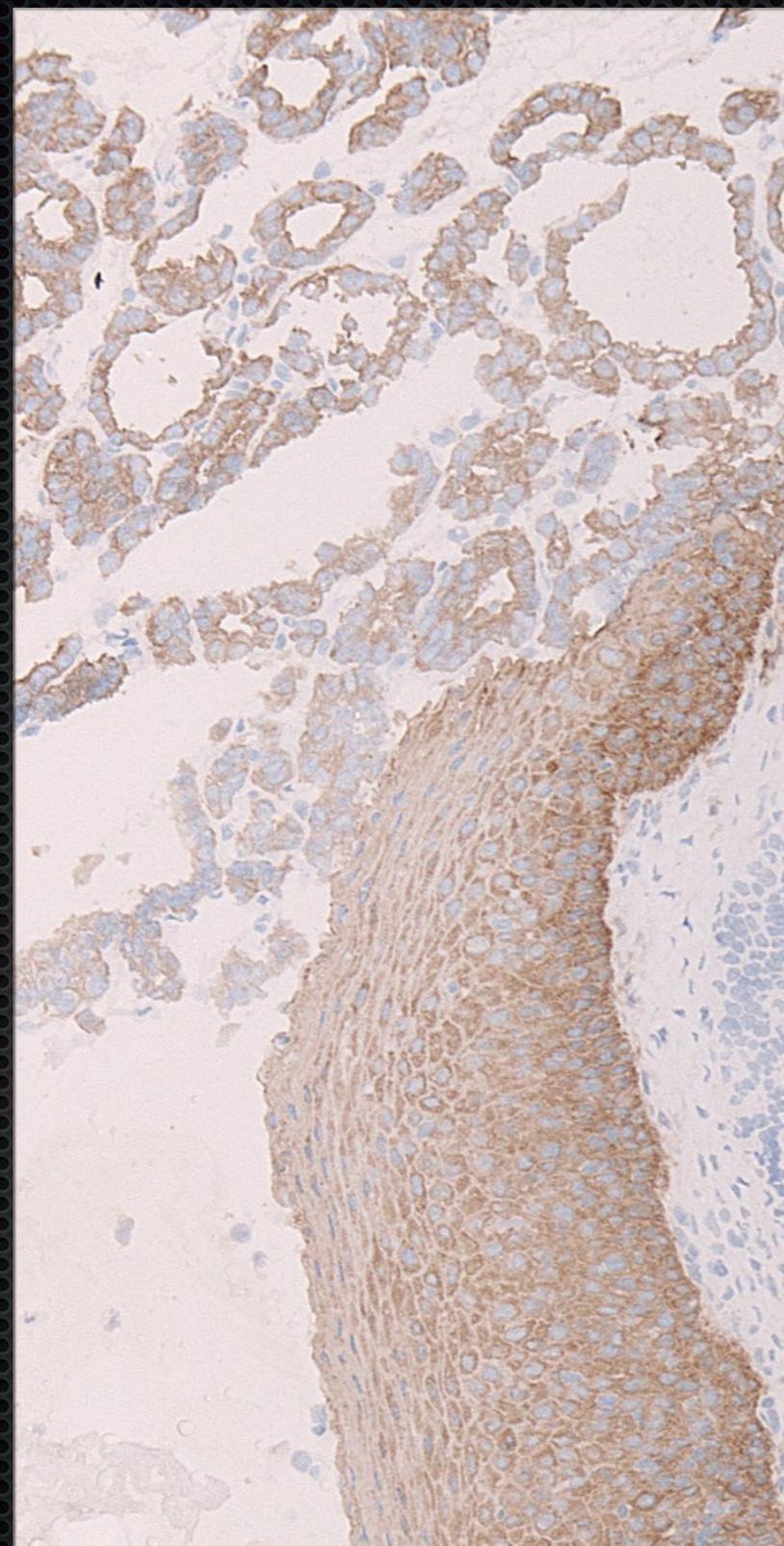
Step-by-step schematic of the rapid EnVision procedure.

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

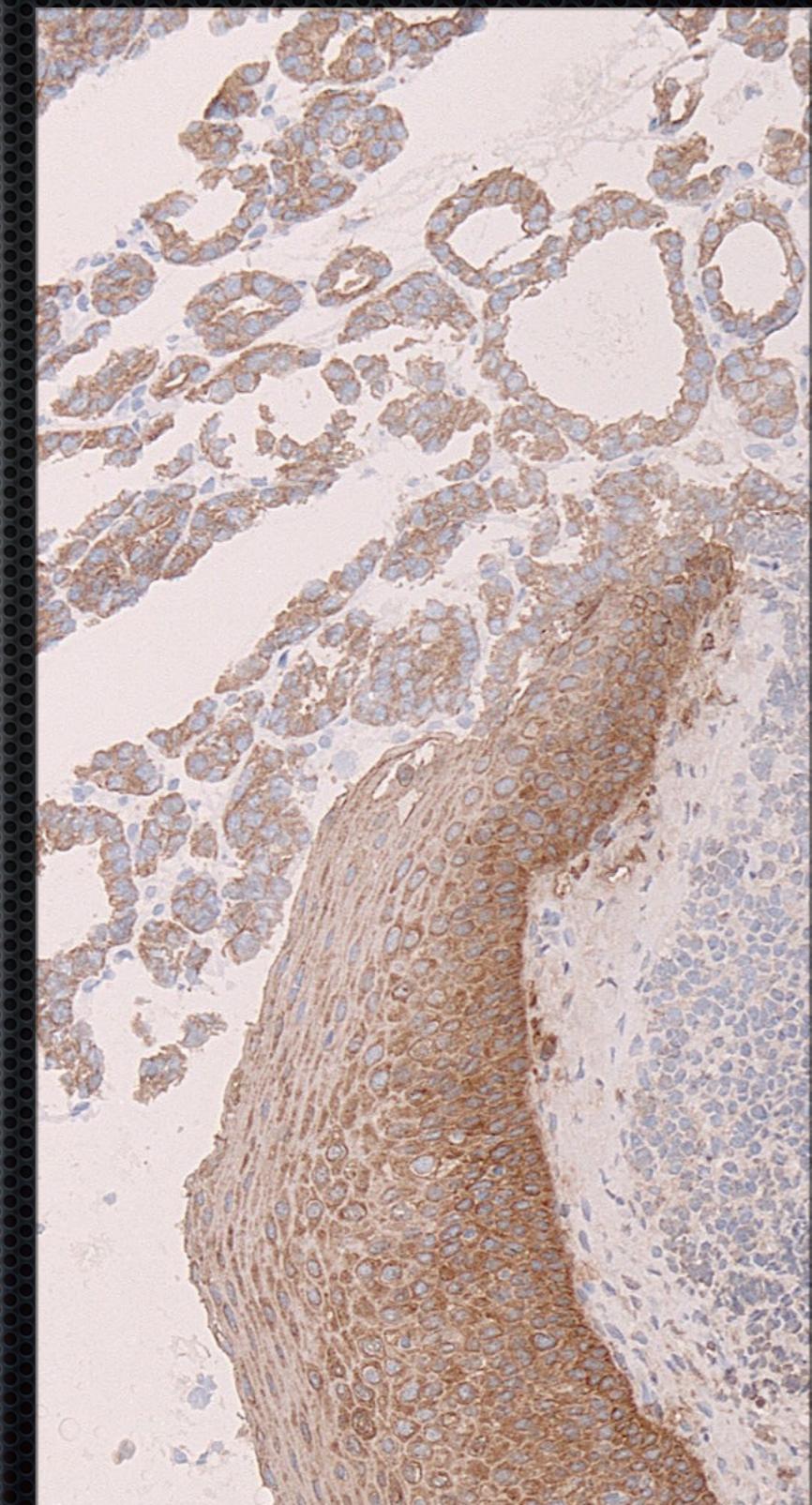
# Rapid-IHC - Pan-CK, AE1/AE3



NBF - TEG 60°C



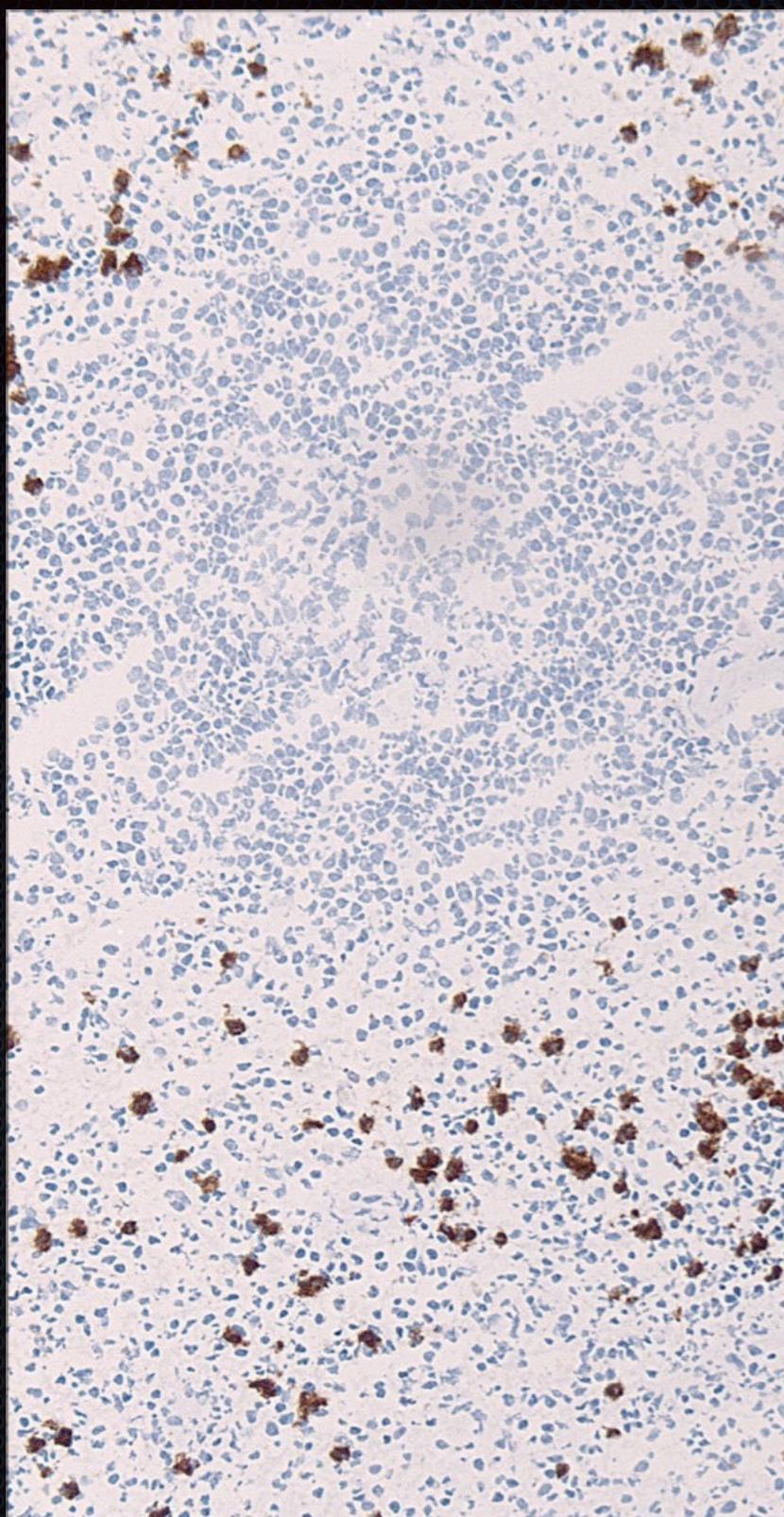
NBF - TEG 80°C



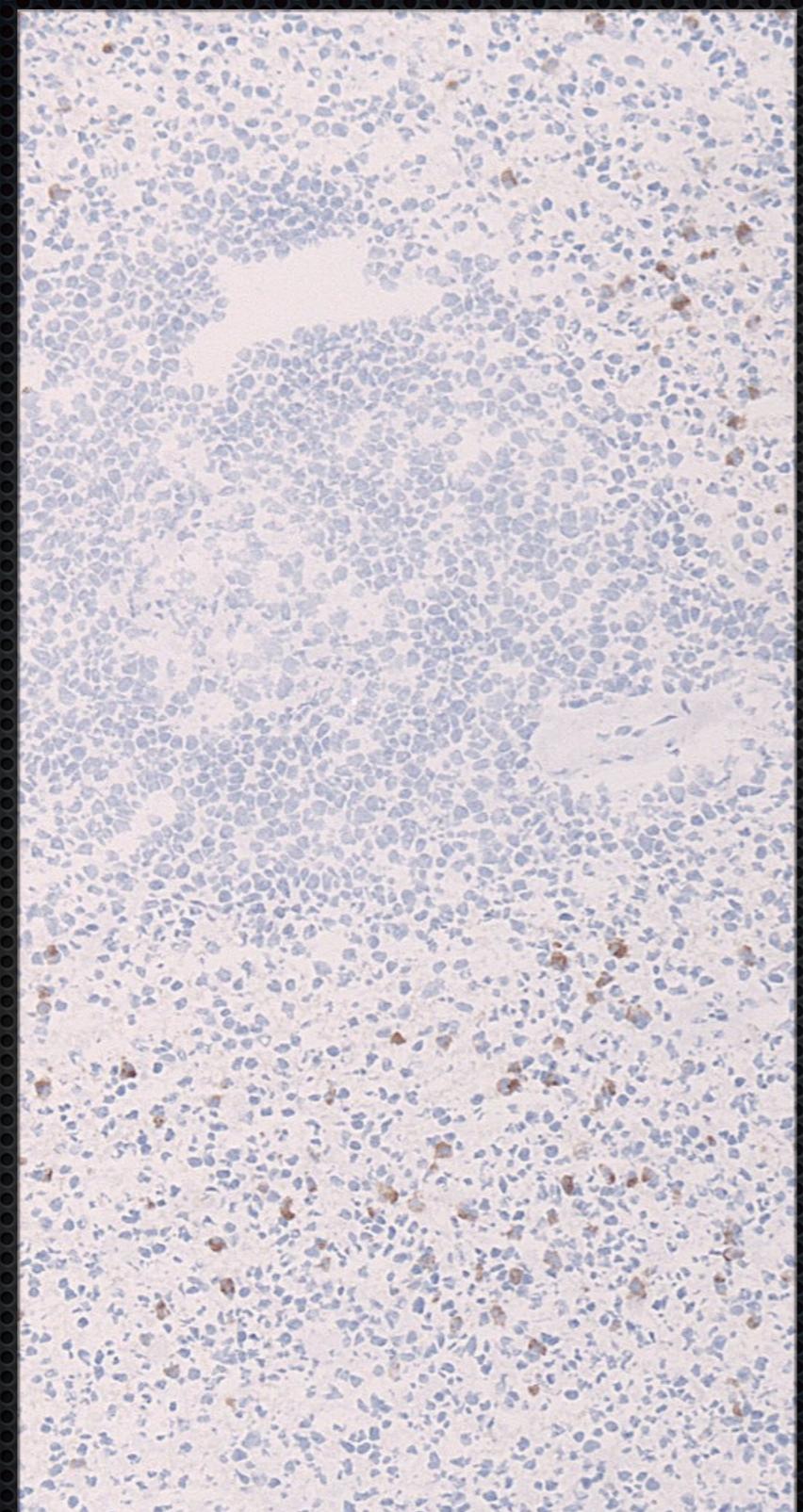
NBF - TEG 95°C

# Endogenous peroxidase

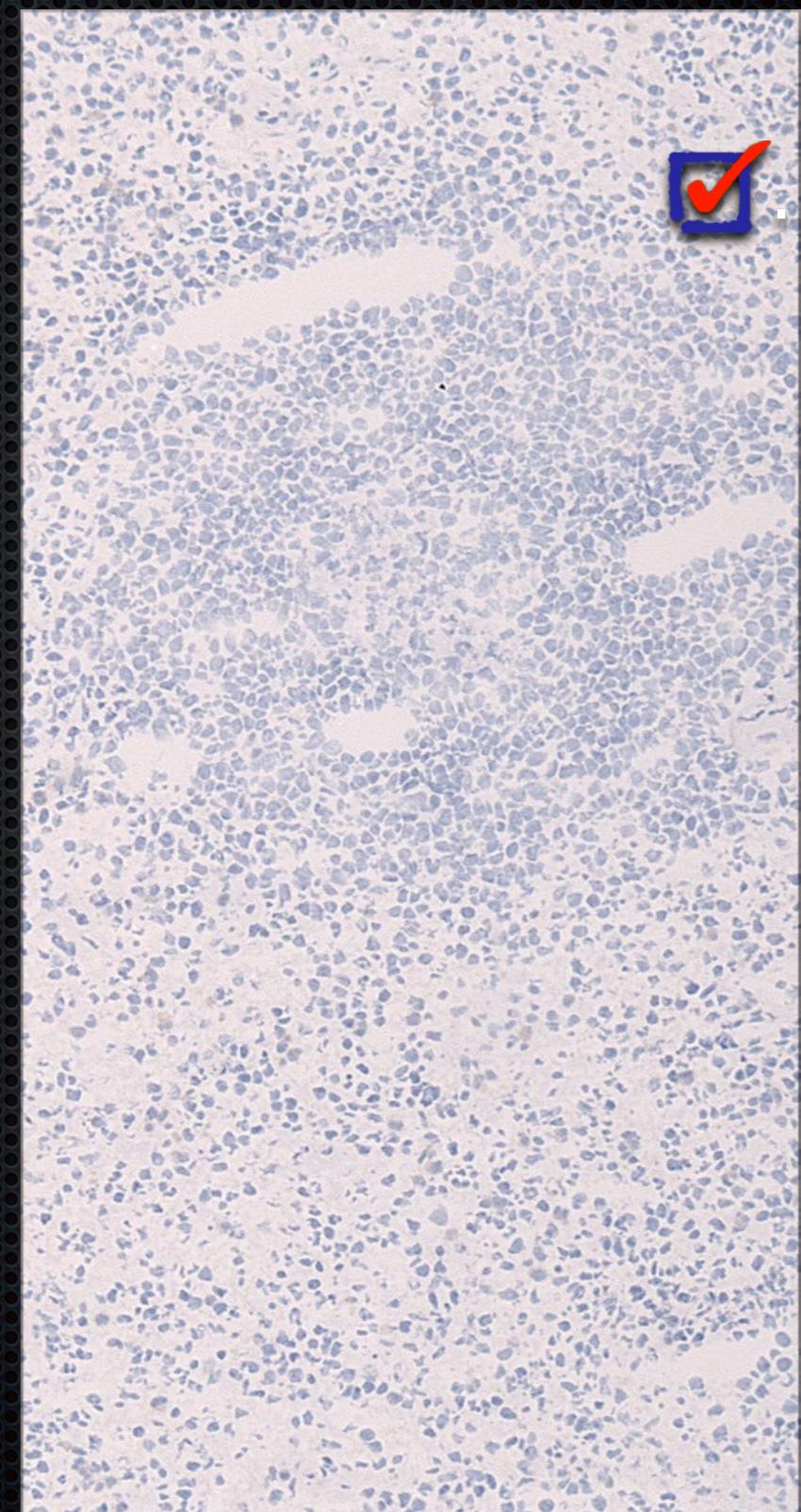
Spleen



NBF - TEG 60°C

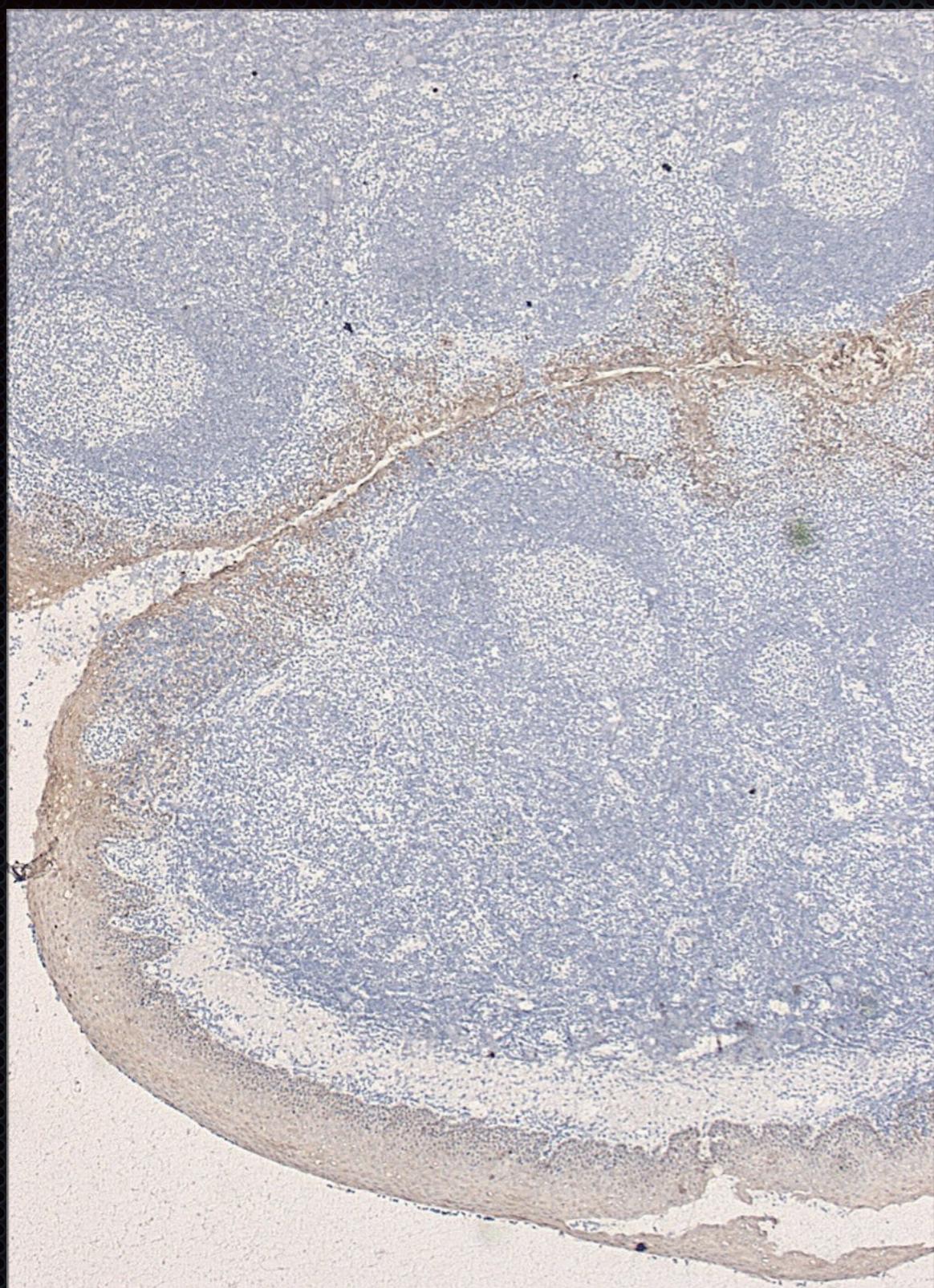


NBF - TEG 90°C

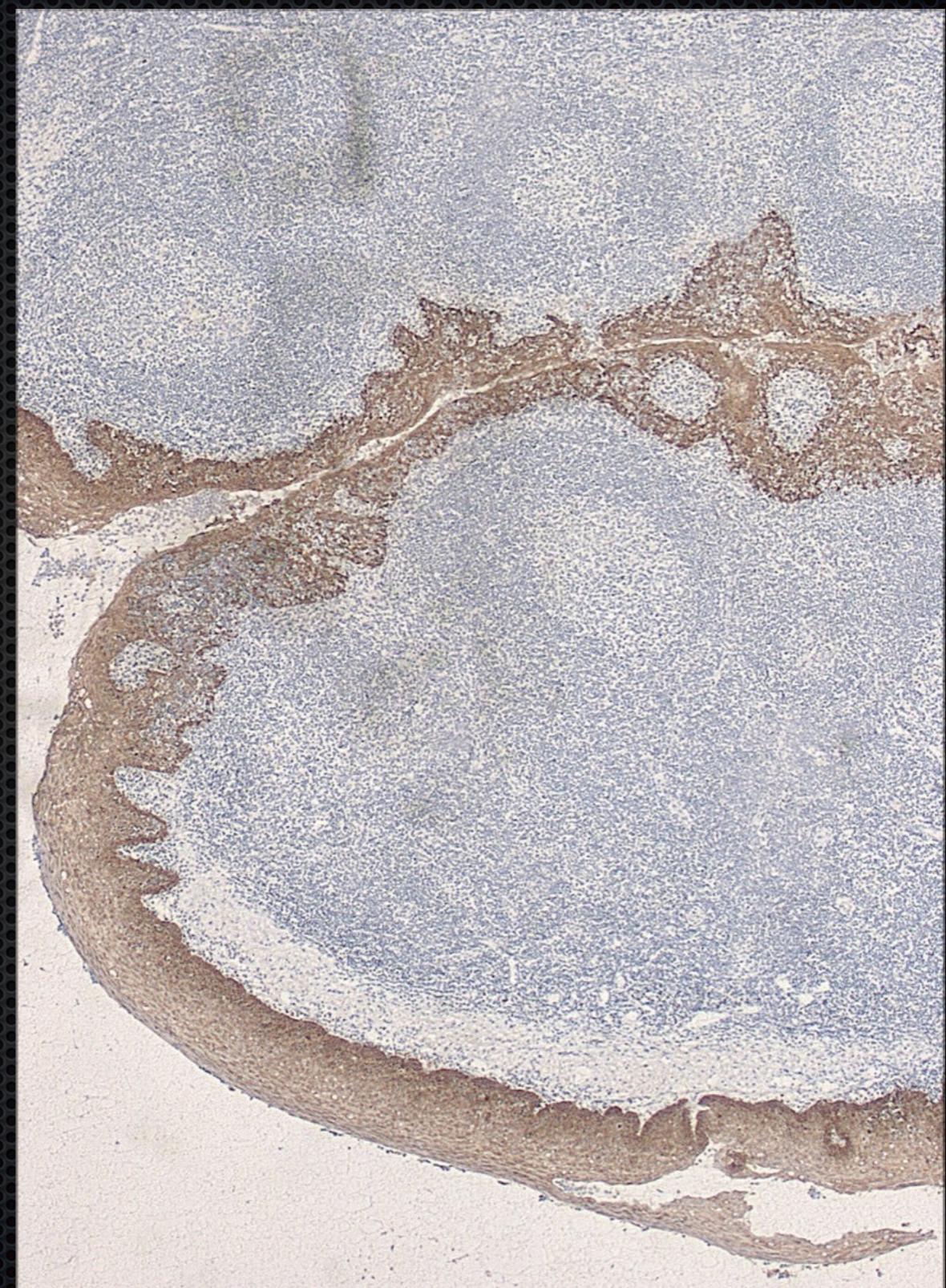


NBF - TEG 95°C

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

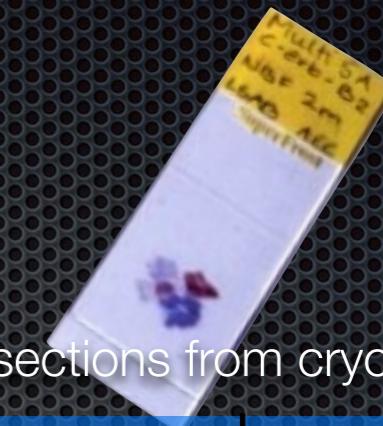


Acetone



NBF-TEG95

# Rapid-IHC fixation: Acetone vs NBF-MBO/TEG



Experiment performed on sections from cryo-multiblocks

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. / + Weak. / 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	+++	+++

# LabSat™ Frozen

An ultra-rapid automated staining instrument based on an innovative microfluidic technology

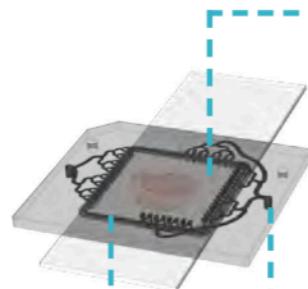


## A unique microfluidic tissue processor

### Fast Fluidic Exchange Technology

The staining chip, core of the Fast Fluidic Exchange Technology (FFeX), creates a chamber over the tissue sample where the staining takes place.

In LabSat™ a pressurized system moves reagents through a network of microfluidic channels and delivers them into this closed chamber almost instantaneously.



#### PRESSURE CONTROLLED

Reagents are delivered to the tissue through active flow

#### ULTRA-RAPID

#### CLOSED CHAMBER

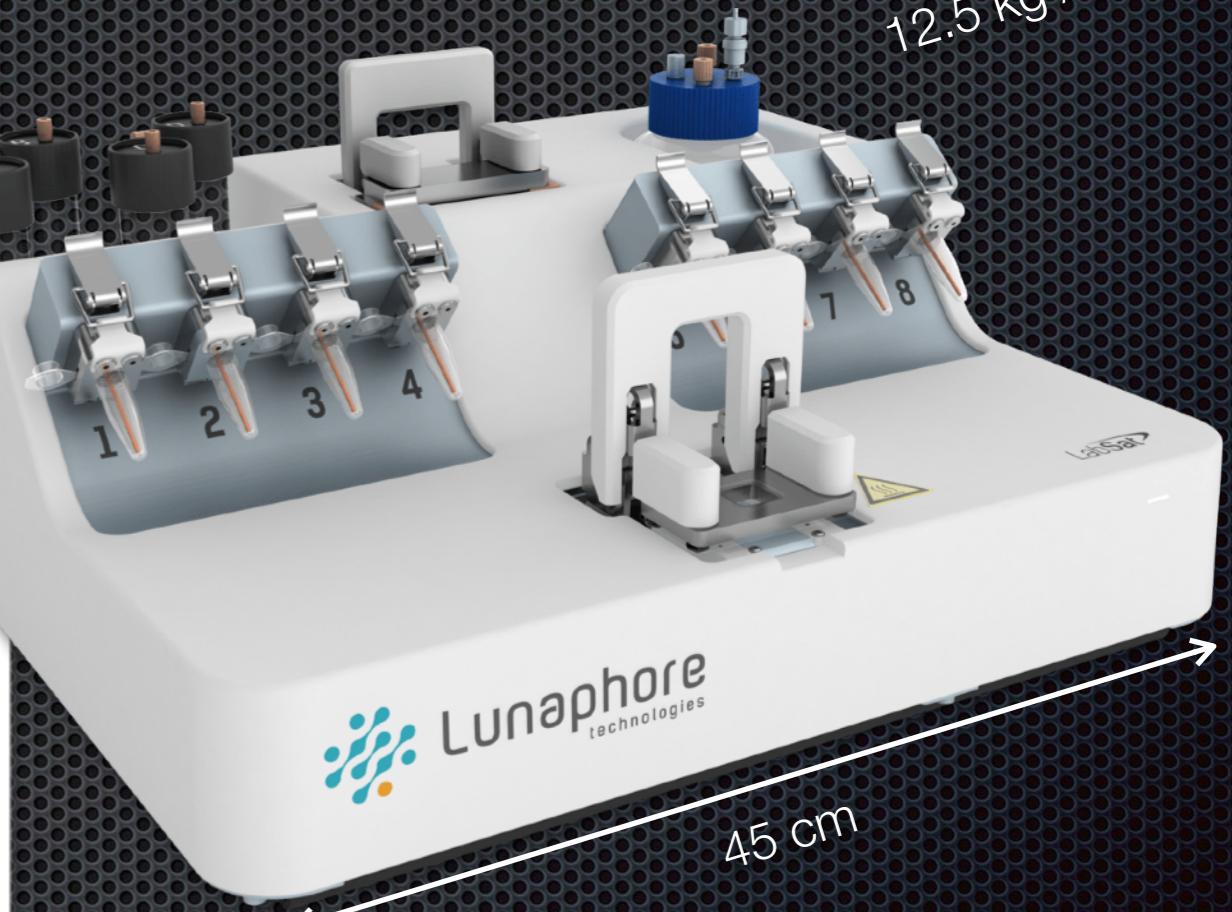
This micro-chamber enables the homogenous incubation of reagents on the tissue

#### HIGH STAINING PRECISION

#### TEMPERATURE CONTROLLED

Fine-tuning of temperature conditions is key to achieve a high performance of stainings

#### OPTIMIZED

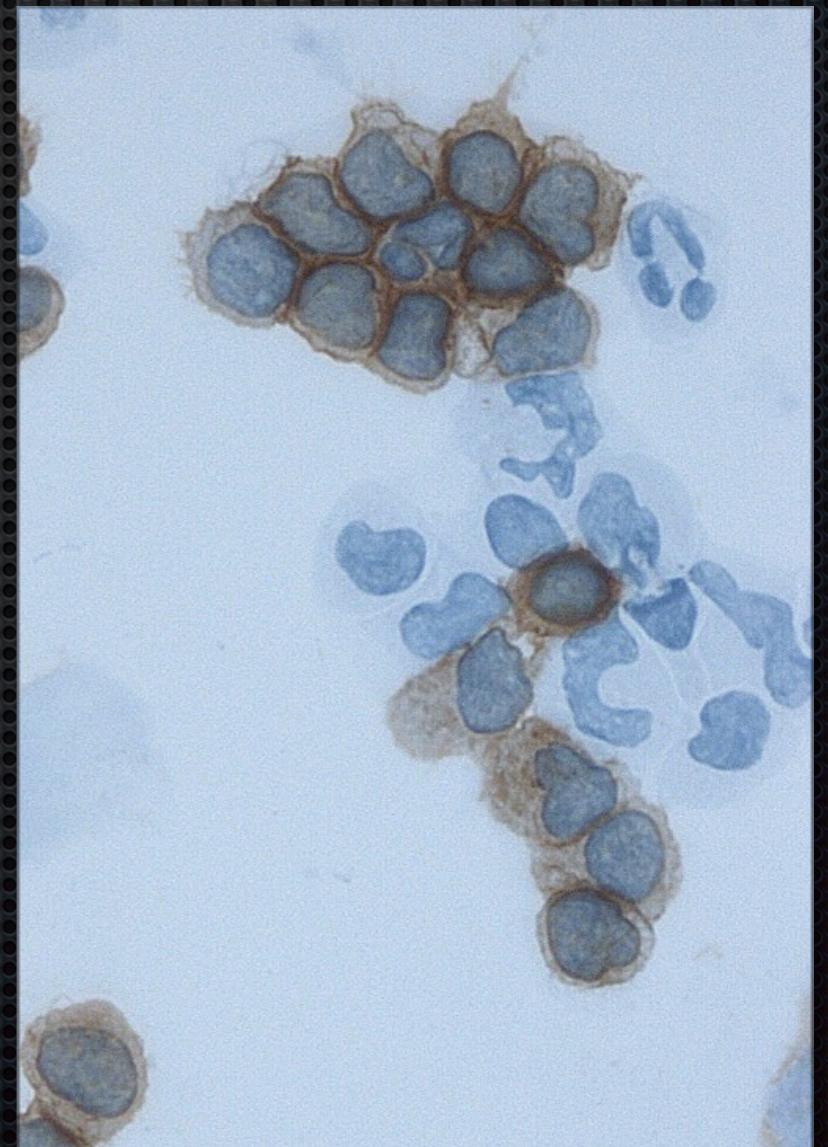


## LabSat™ Frozen

LabSat™ Frozen is an automated staining instrument, based on a cutting-edge microfluidic technology, capable of performing IHC assays on frozen sections in as little as 12 minutes, while maintaining high staining quality and reproducibility.

# ICC on cytological material

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



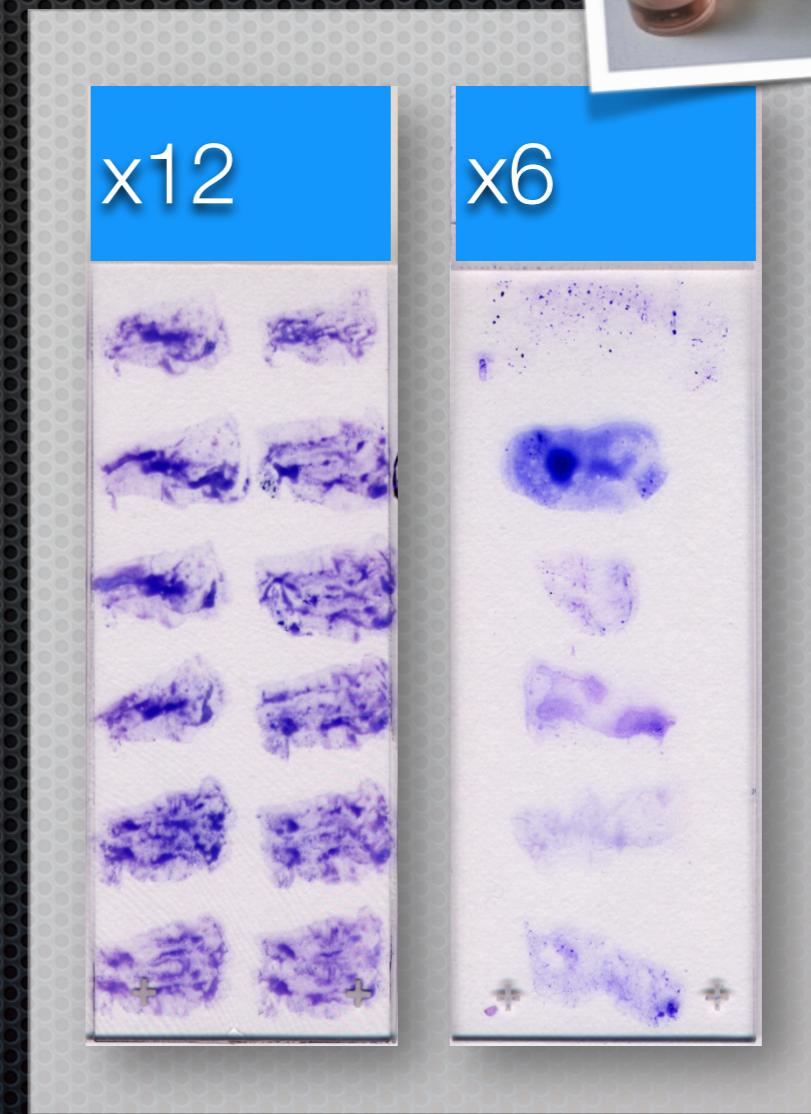
# Optimizing biomarker-protocols (ICC-protocols on **cells**)

## 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

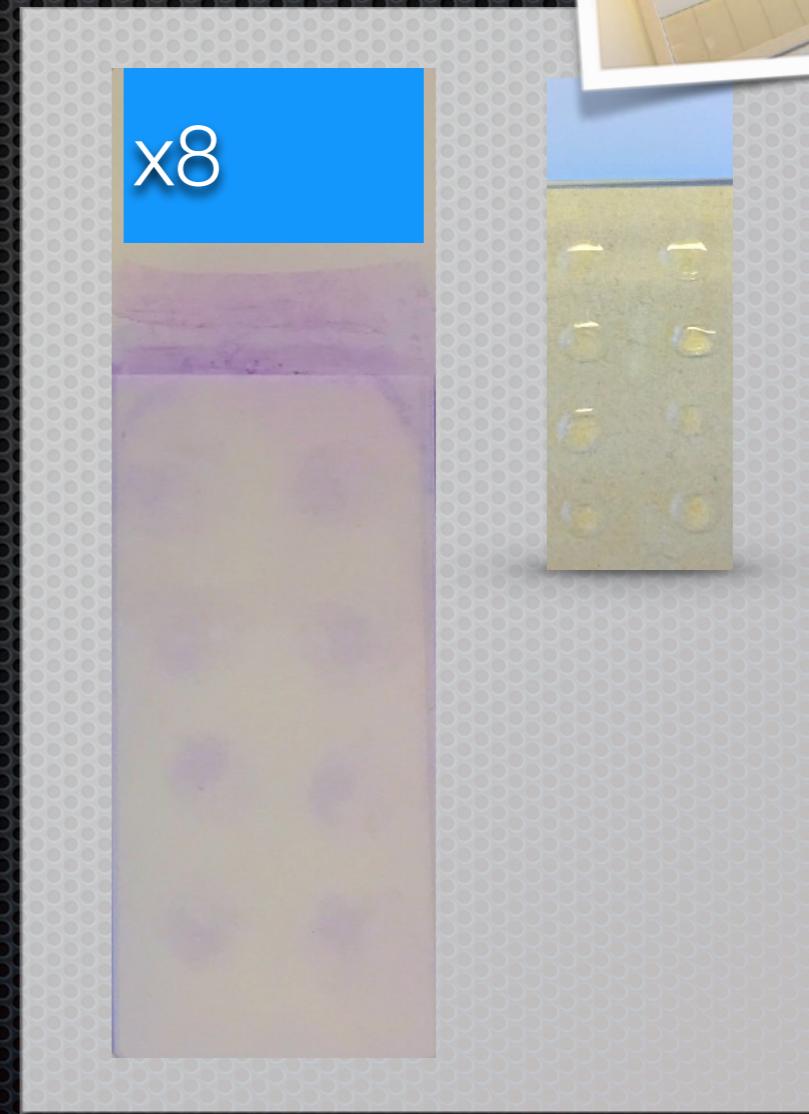
# Optimizing biomarker-protocols (ICC-protocols on cells)

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

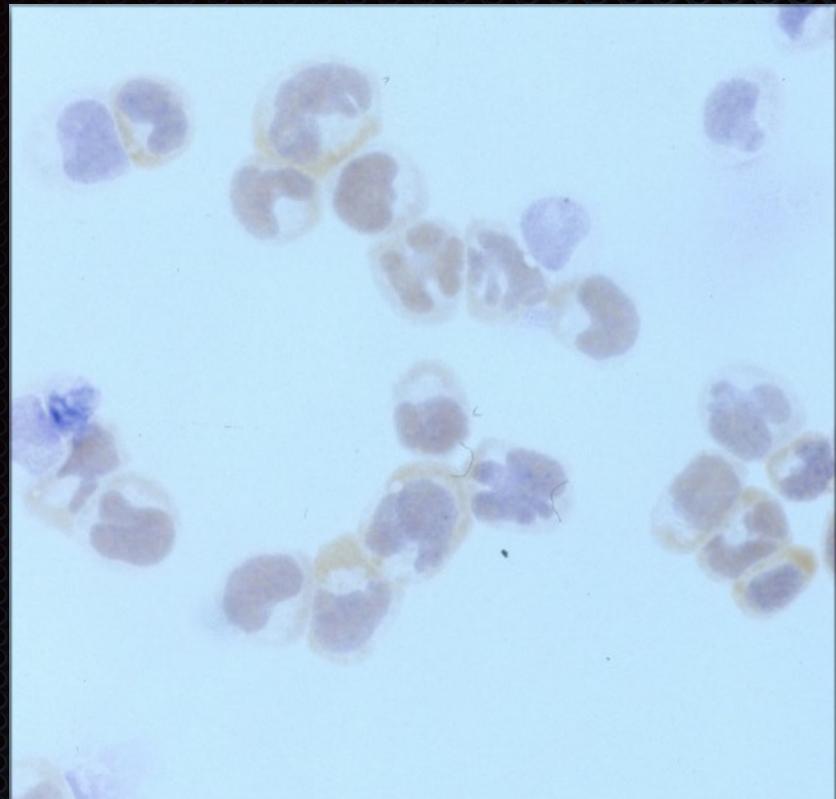


# Optimizing biomarker-protocols (ICC-protocols on cells)

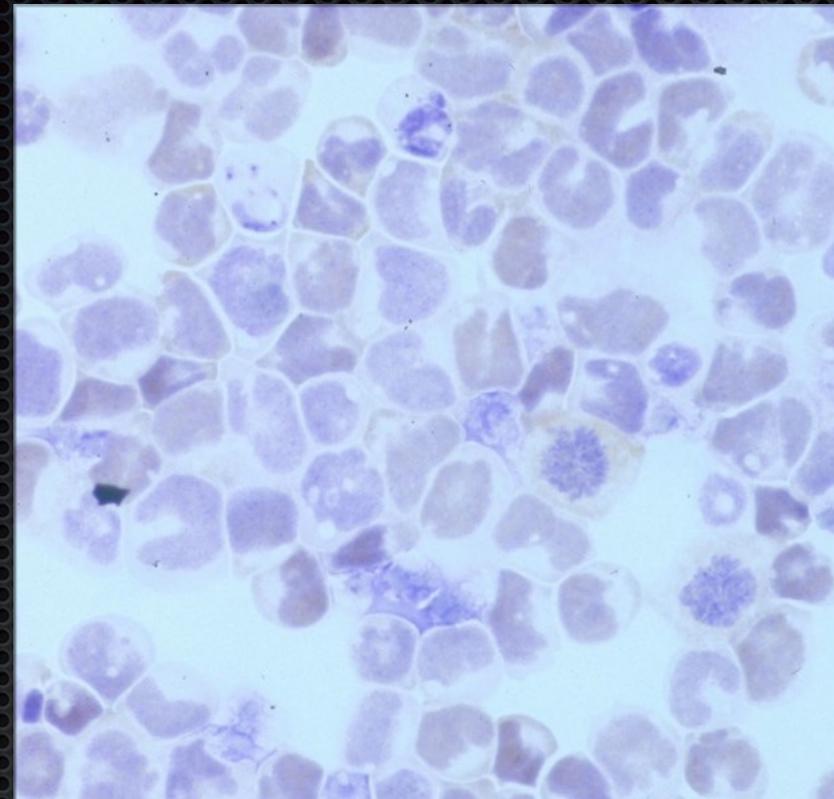
- Designed “Multi-cell cultures”:
  - “High expresser” cell lines
  - “Low expresser” cell lines
  - “Non expresser” cell lines



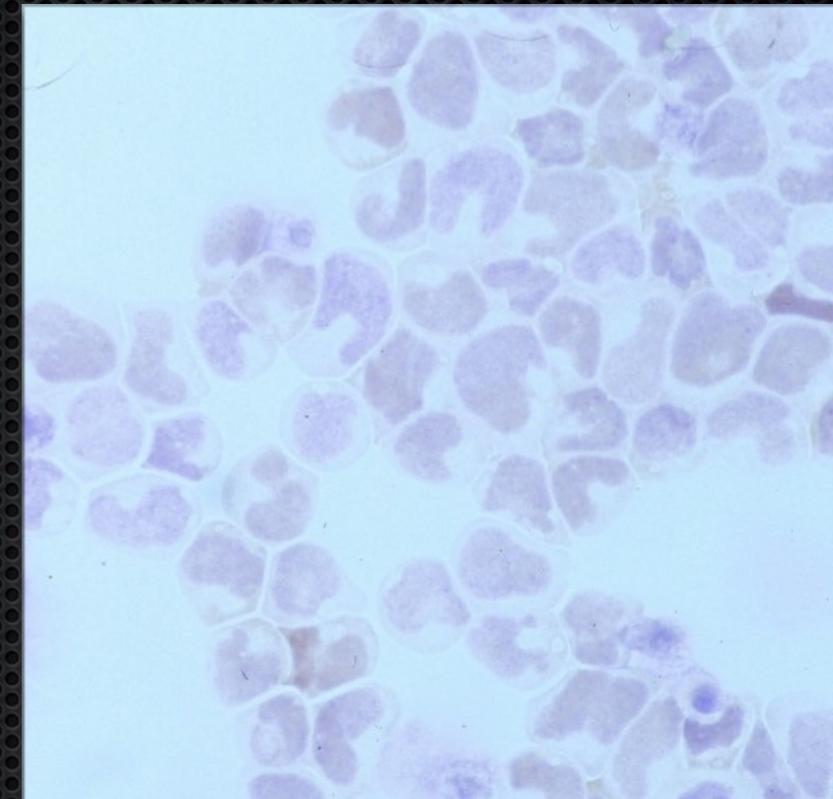
# TdT, poly (MOLT4 cells) and various fixatives



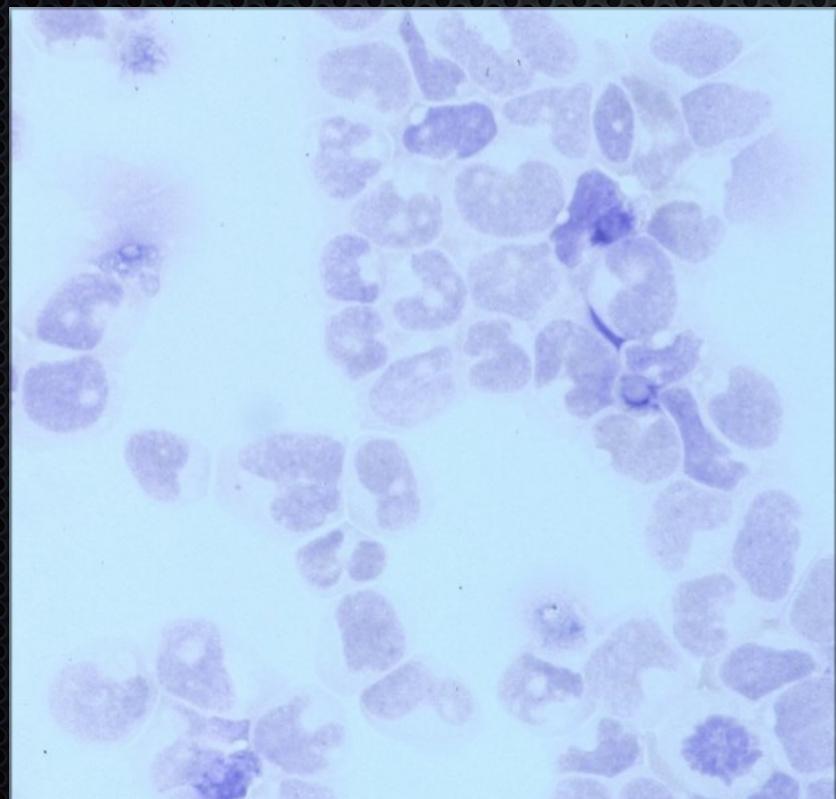
Acetone 5'



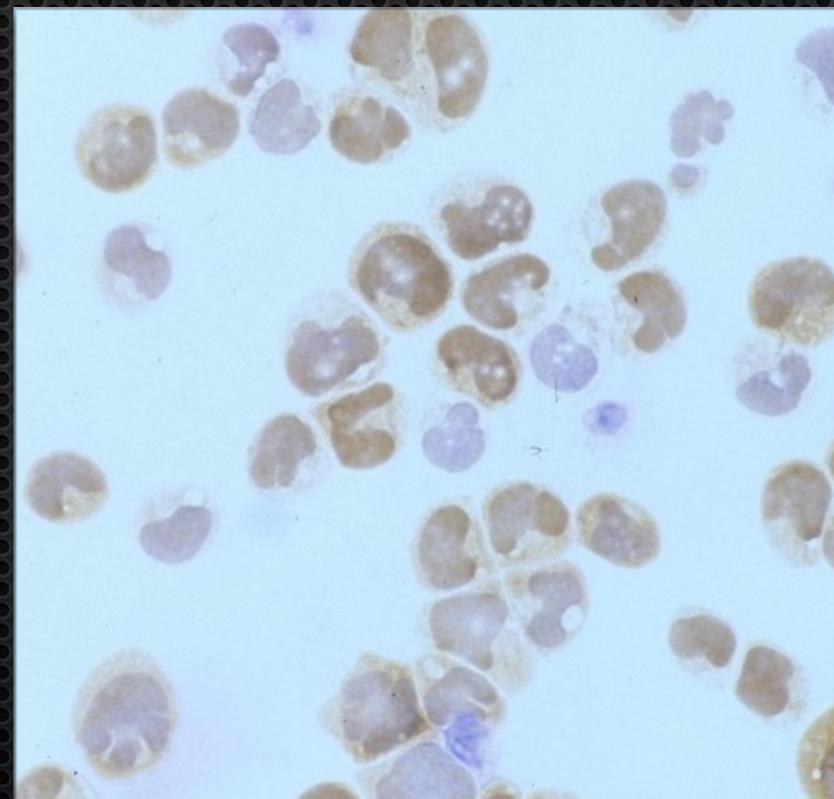
Acetone/Methanol 40s



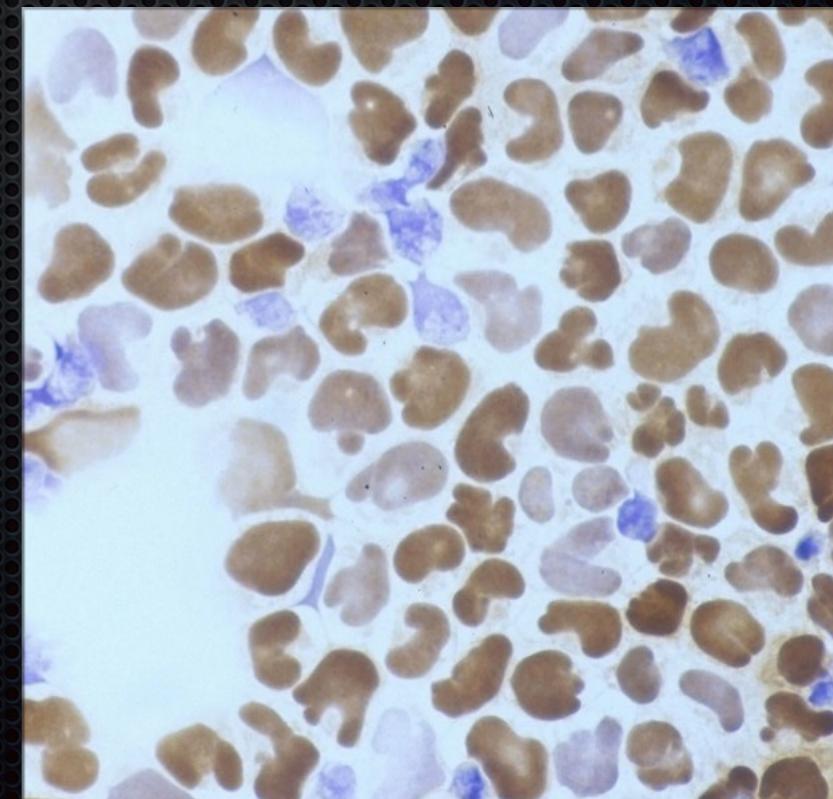
Methanol 5'



Ethanol 5'



NBF 5'



NBF 15' - TritonX100 5'

# Optimizing biomarker-protocols (ICC-protocols on cells)

## Fixation/Epitope retrieval “Test Battery”

*Manual*

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 Retrieval 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”



No	Method			
1	No fixation			
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)
6 ★	4% NBF	30 min	>>>	CC1, pH8.5 (95°C)
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”



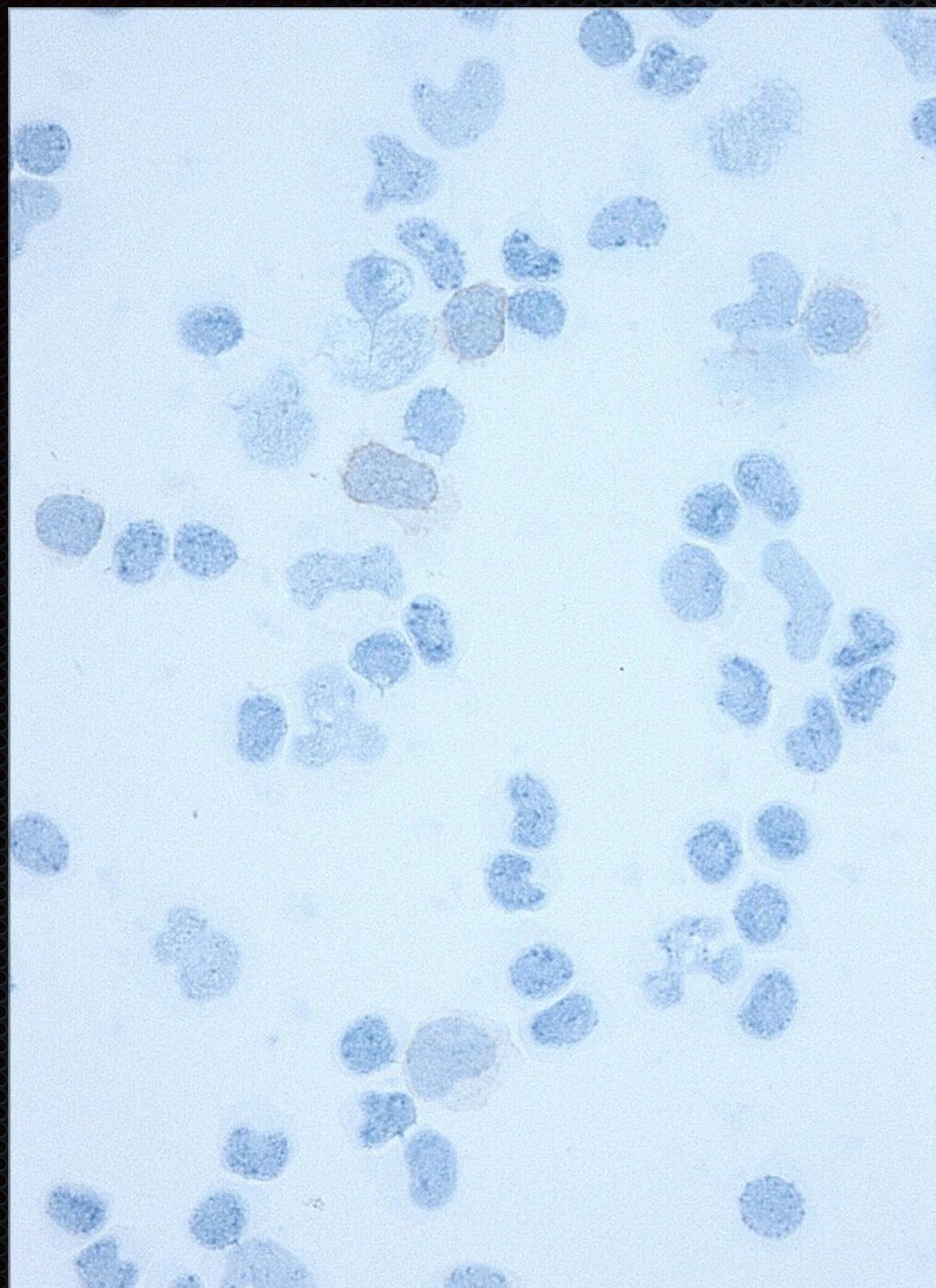
Omnis

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) 10 min
5 ★	4% NBF	30 min	>>>	TRS-L, pH6.1 (97°C) 10 min
7	The vendors recommendations			

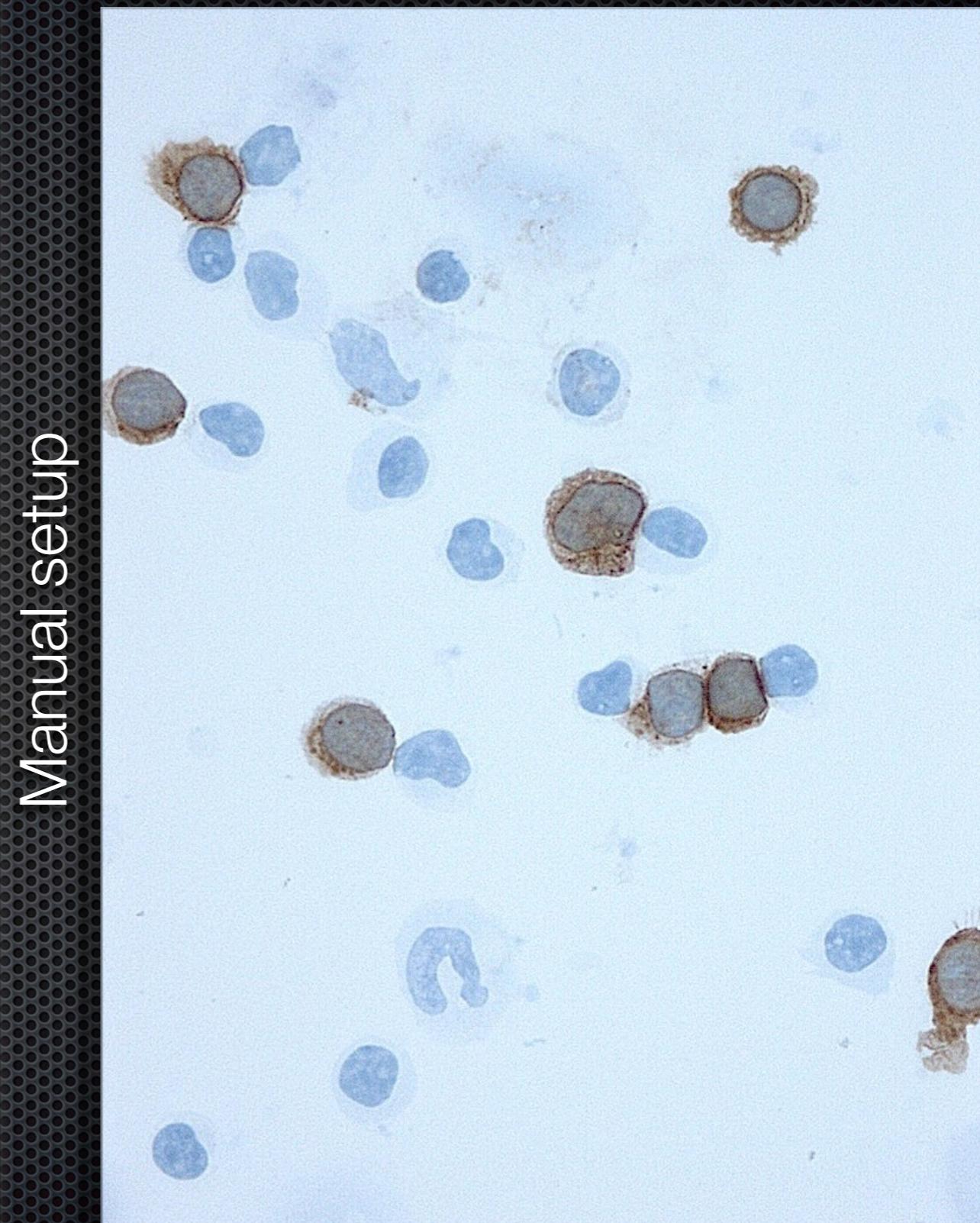
TRS-H: Tris-EDTA based buffer pH9.0

TRS-L: Citrate based buffer pH6.1

# CD79a, JCB117 (Buffy coat cytospin) Fixation



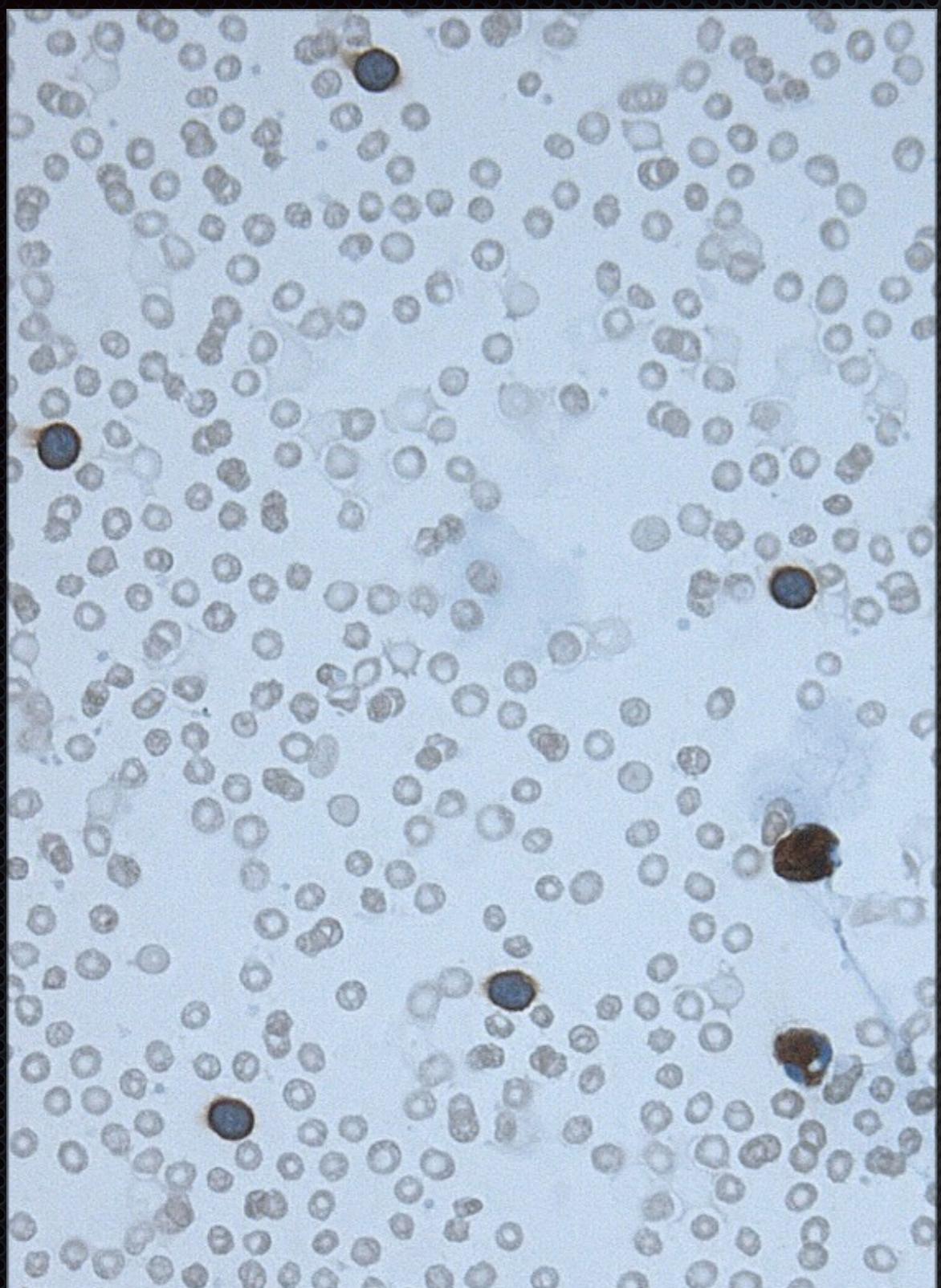
Acetone 10'



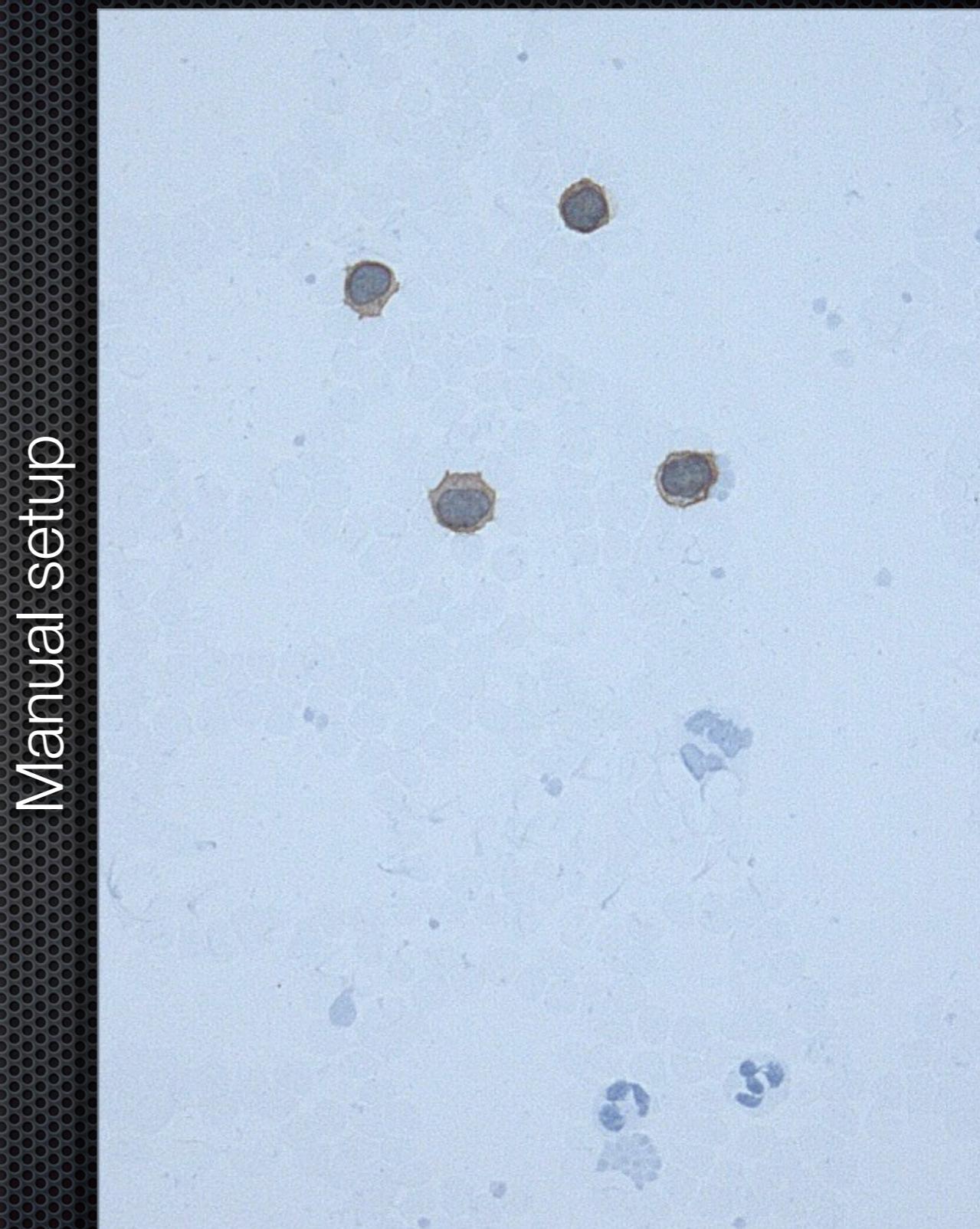
NBF 15' - TEG95° 15'

Manual setup

# CD79a, JCB117 (PB) Various fixatives



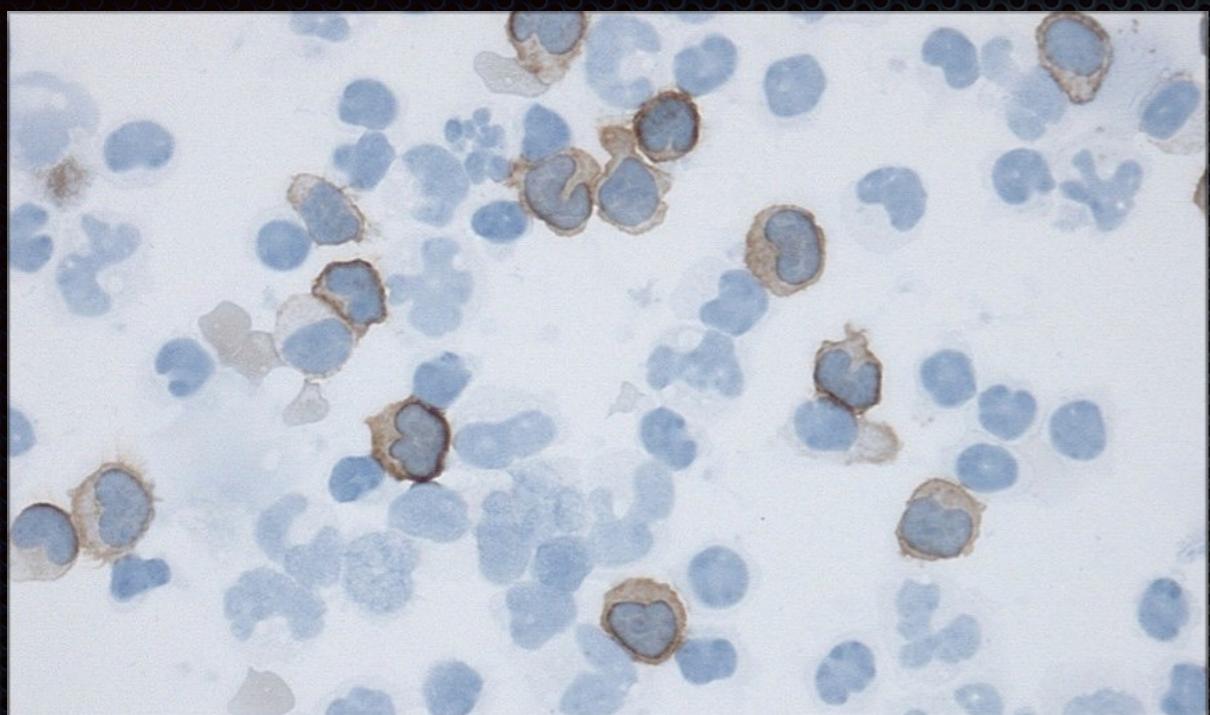
Acetone 10'



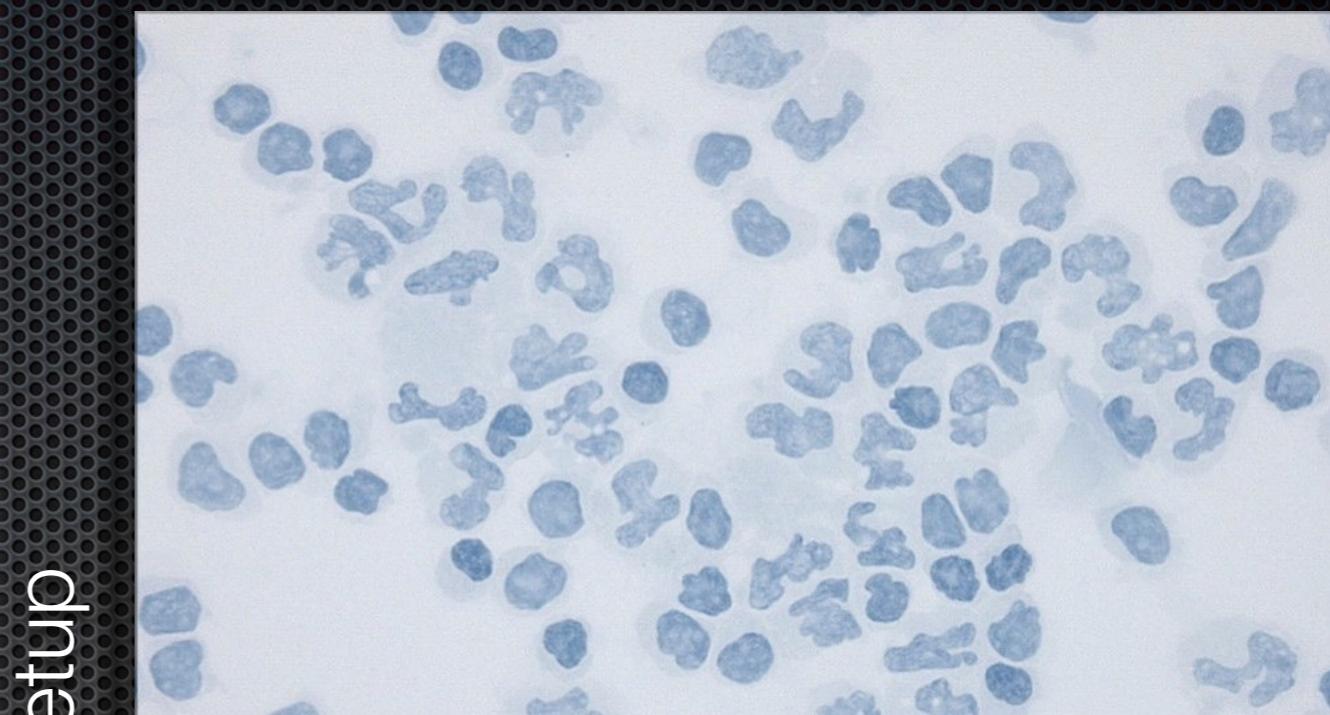
NBF 15' - TEG95° 15'

Manual setup

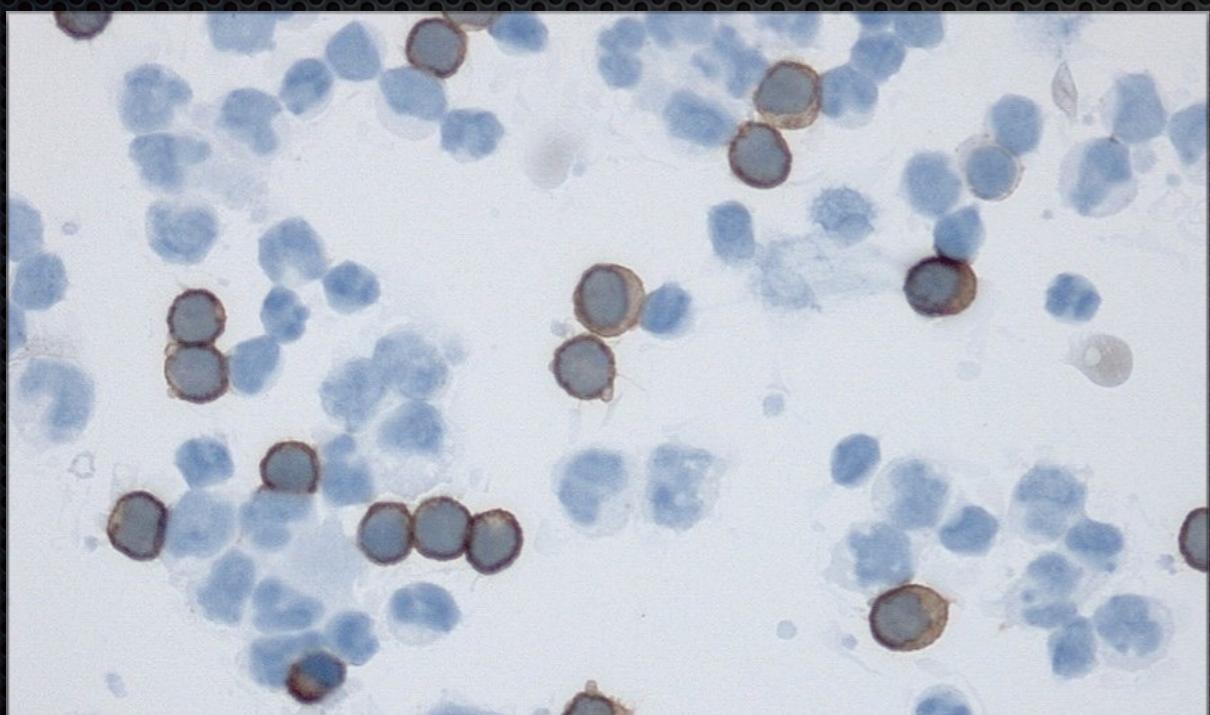
# CD8 Abs (Buffy coat PB) various fixatives



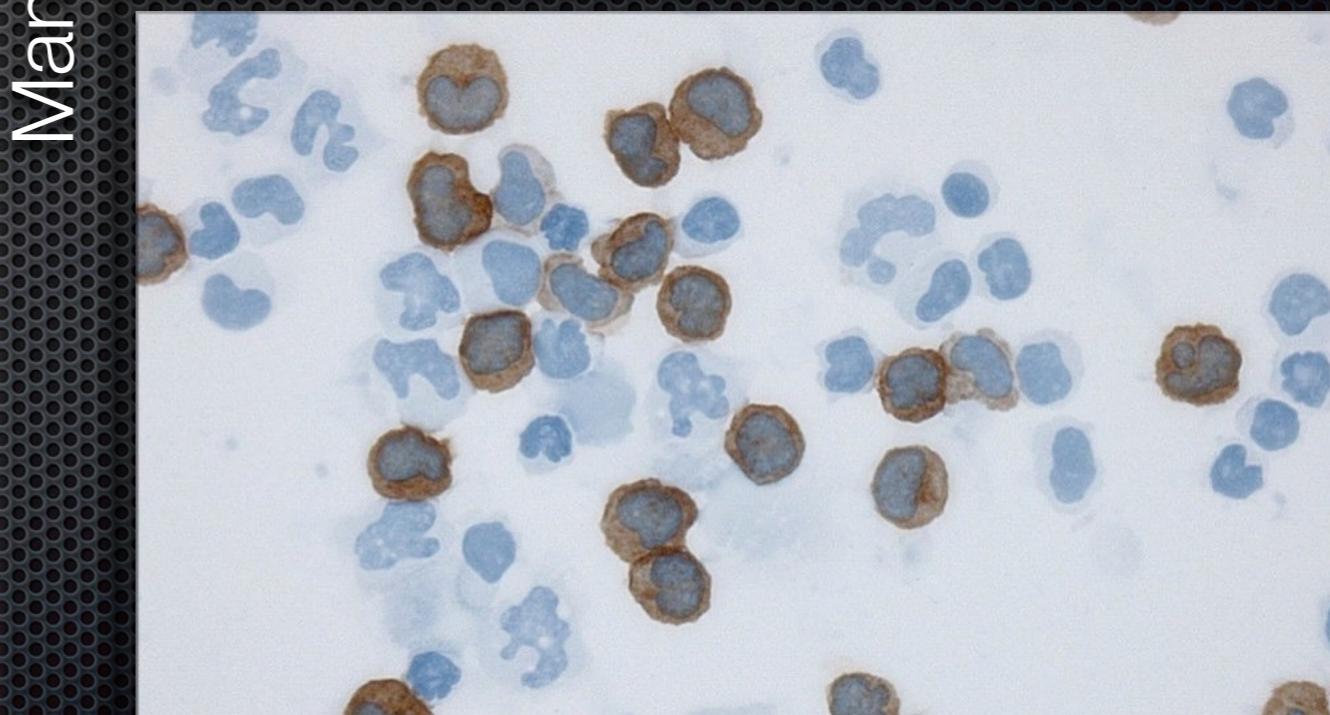
CD8, DK25 - Acetone 10'



CD8, DK25 - NBF 2' - TEG95° 30s



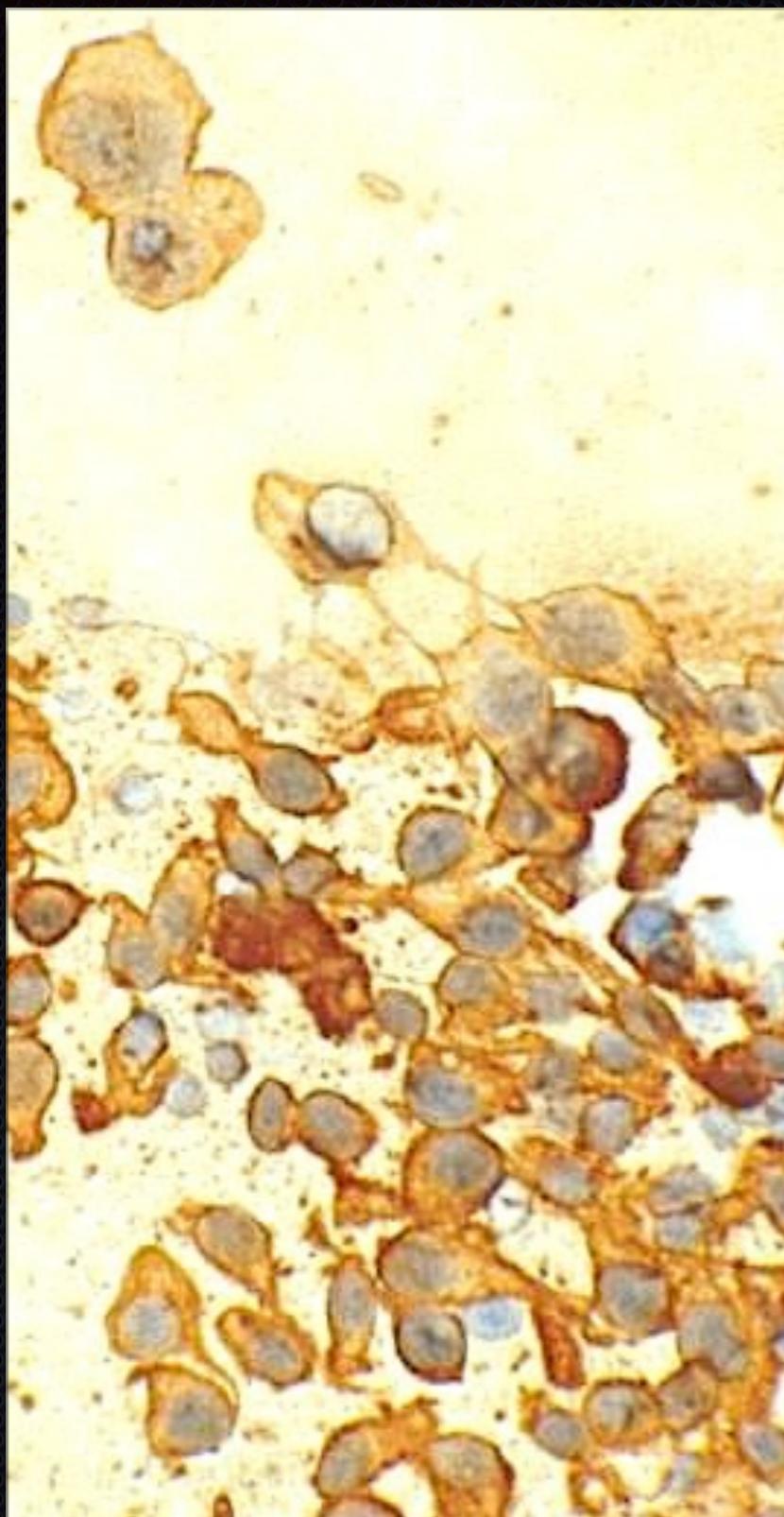
CD8, C8/144B - Acetone 10'



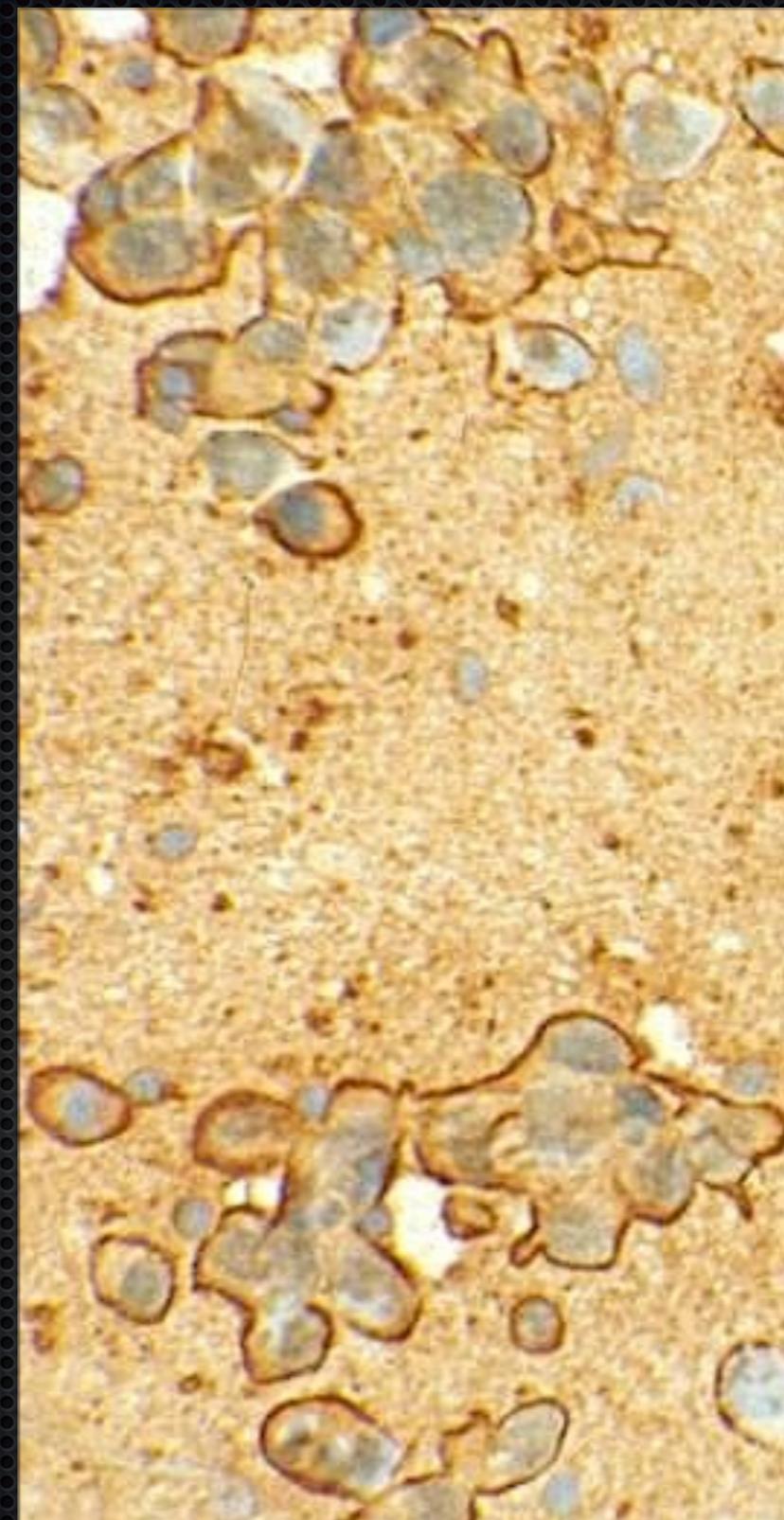
CD8, C8/144B NBF 2' - TEG95° 30s

Manual setup

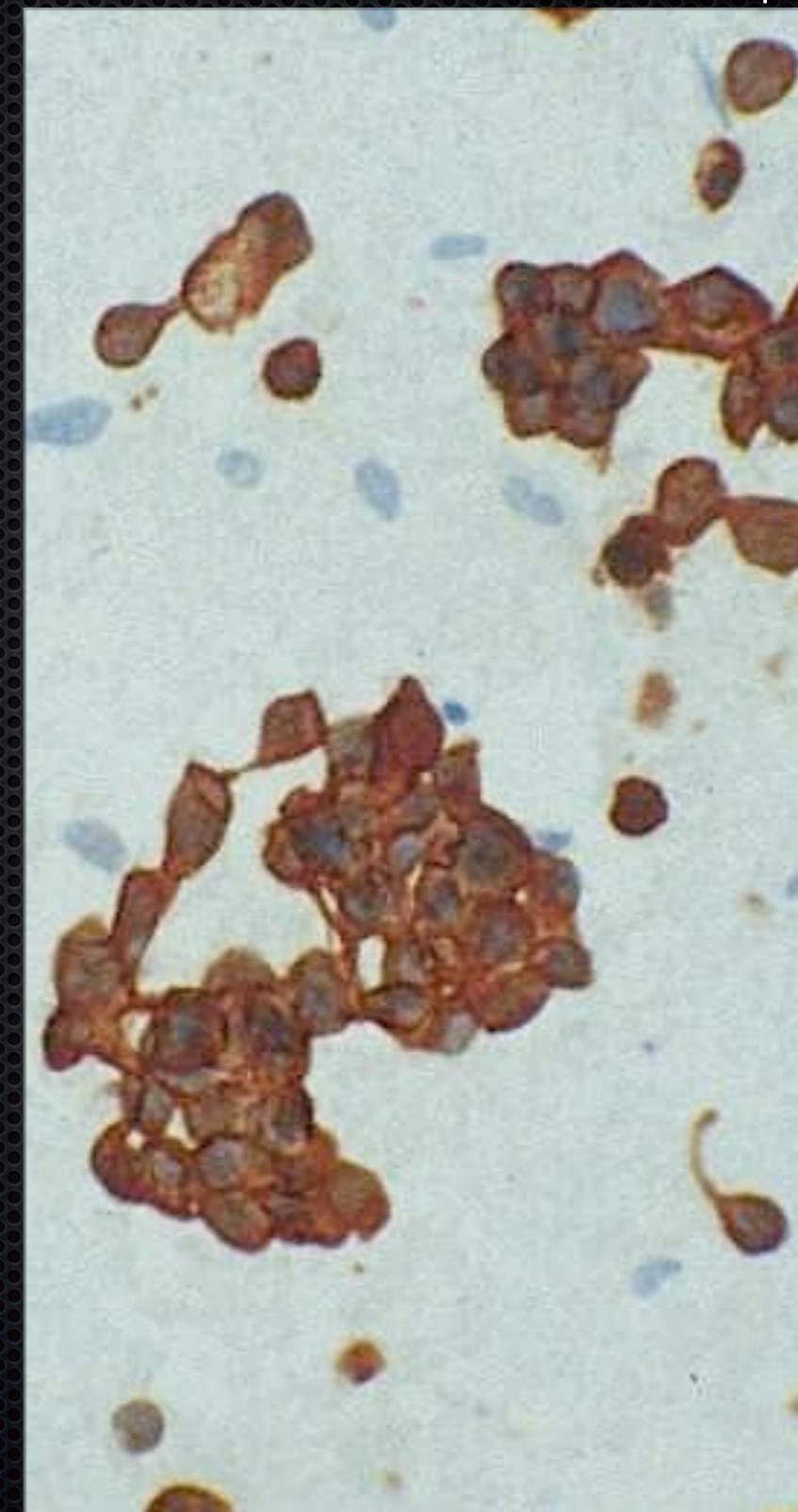
# CK7, OV-TL12/30 (Imprint) various fixatives



Acetone 10'

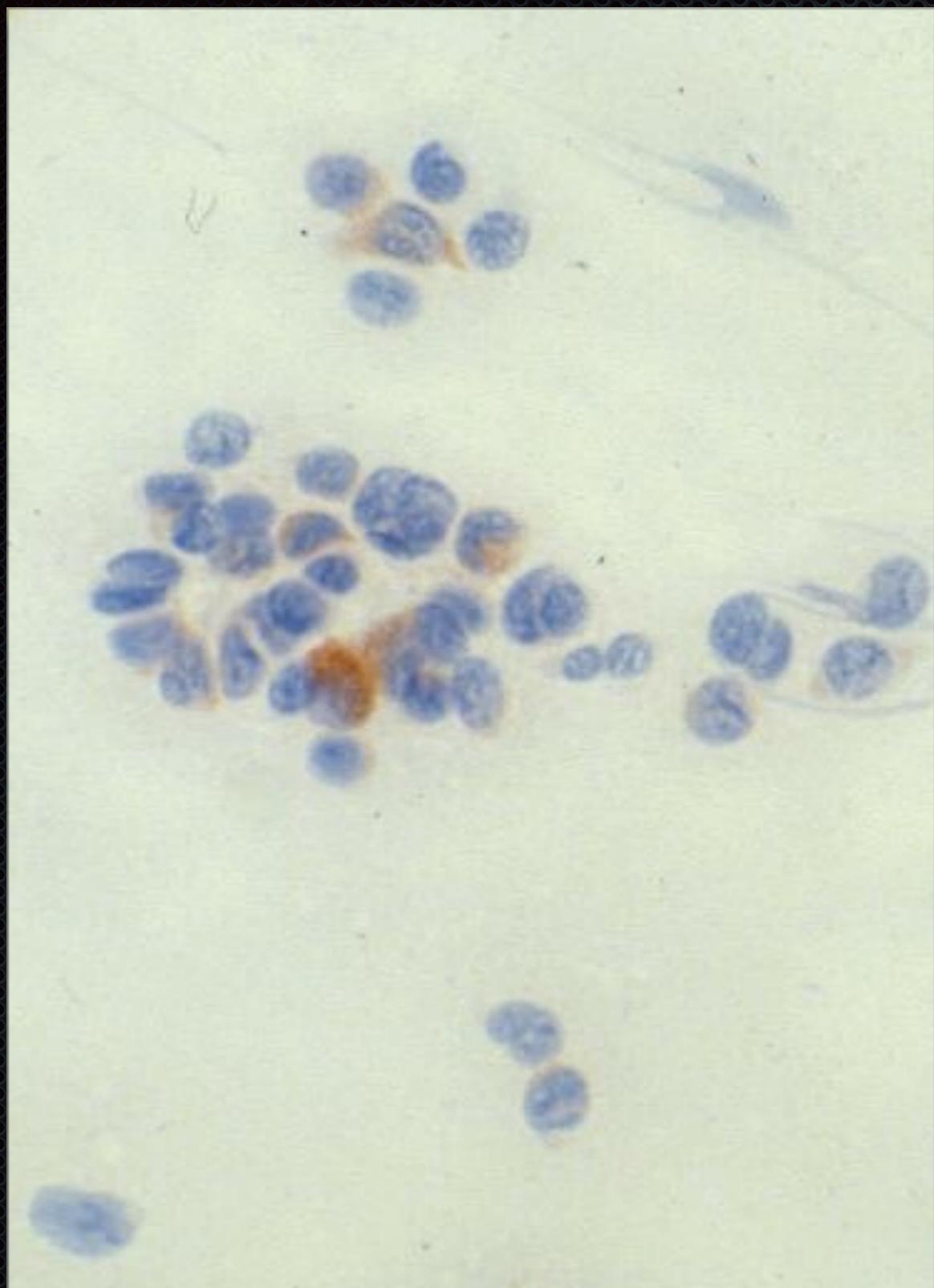


4% NBF 5'

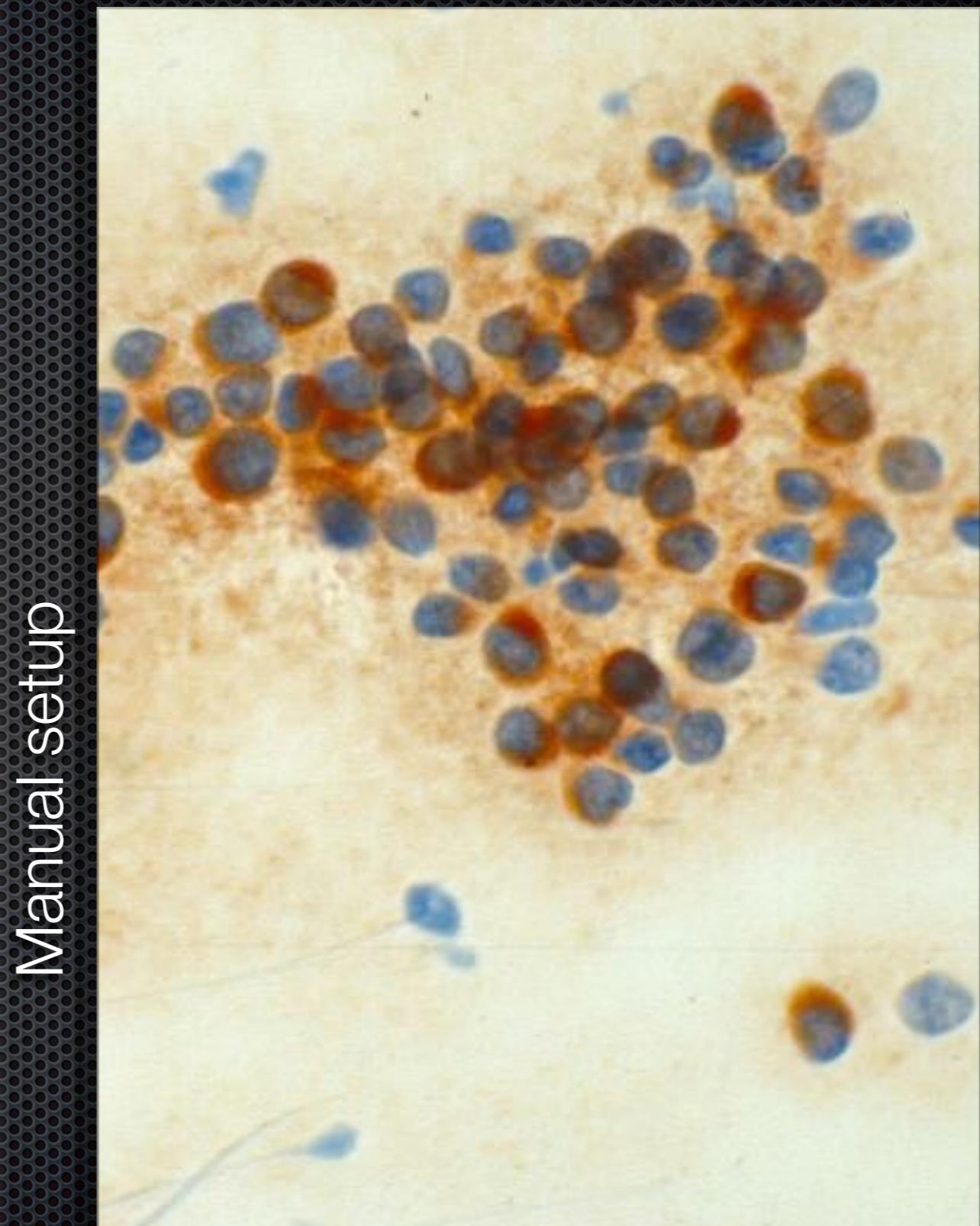


NBF 15' - TEG95° 15

# Tyrosinase, T311 (Imprint) Various fixatives



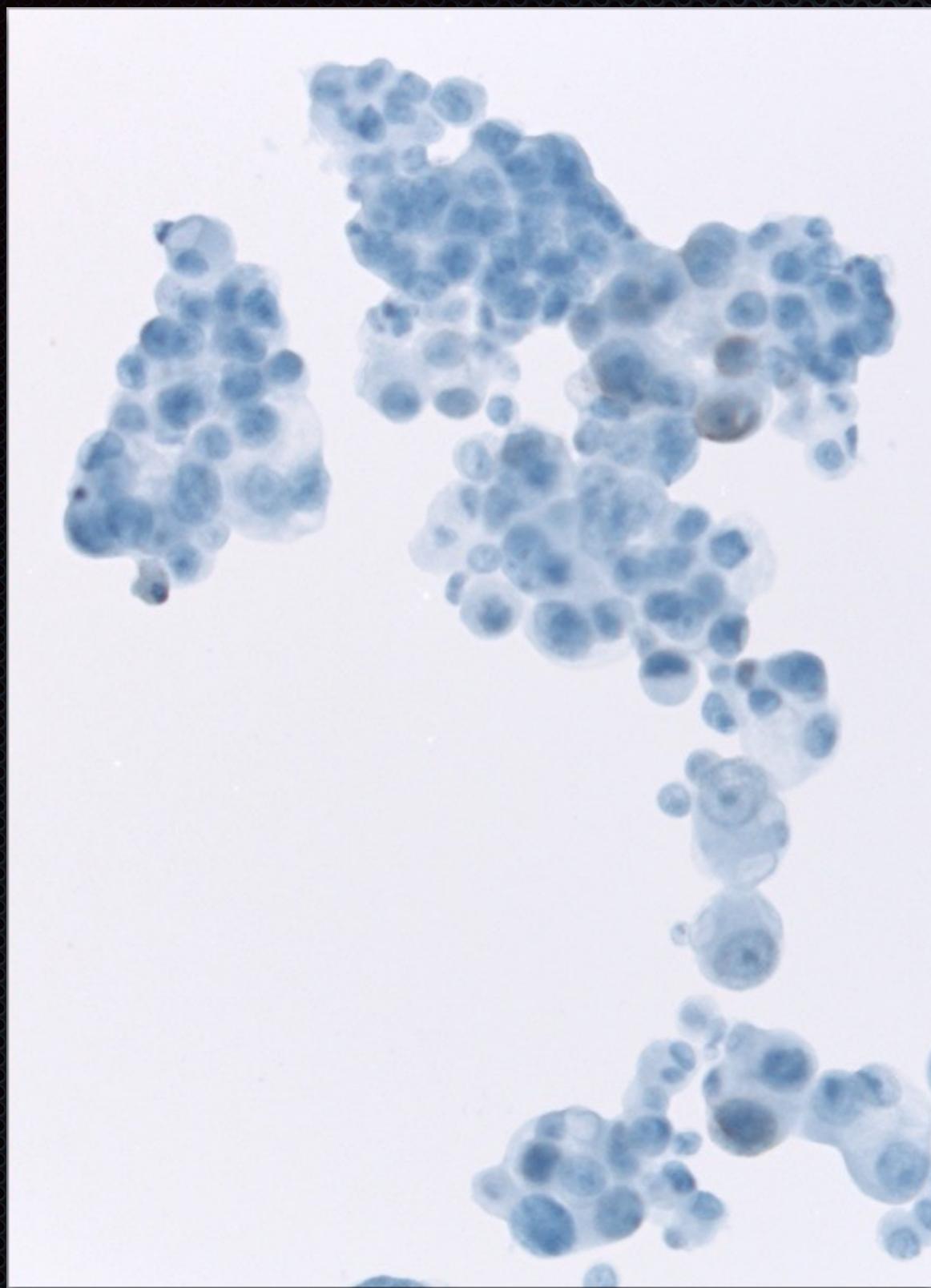
Acetone 10'



NBF 15' - TEG95° 15'

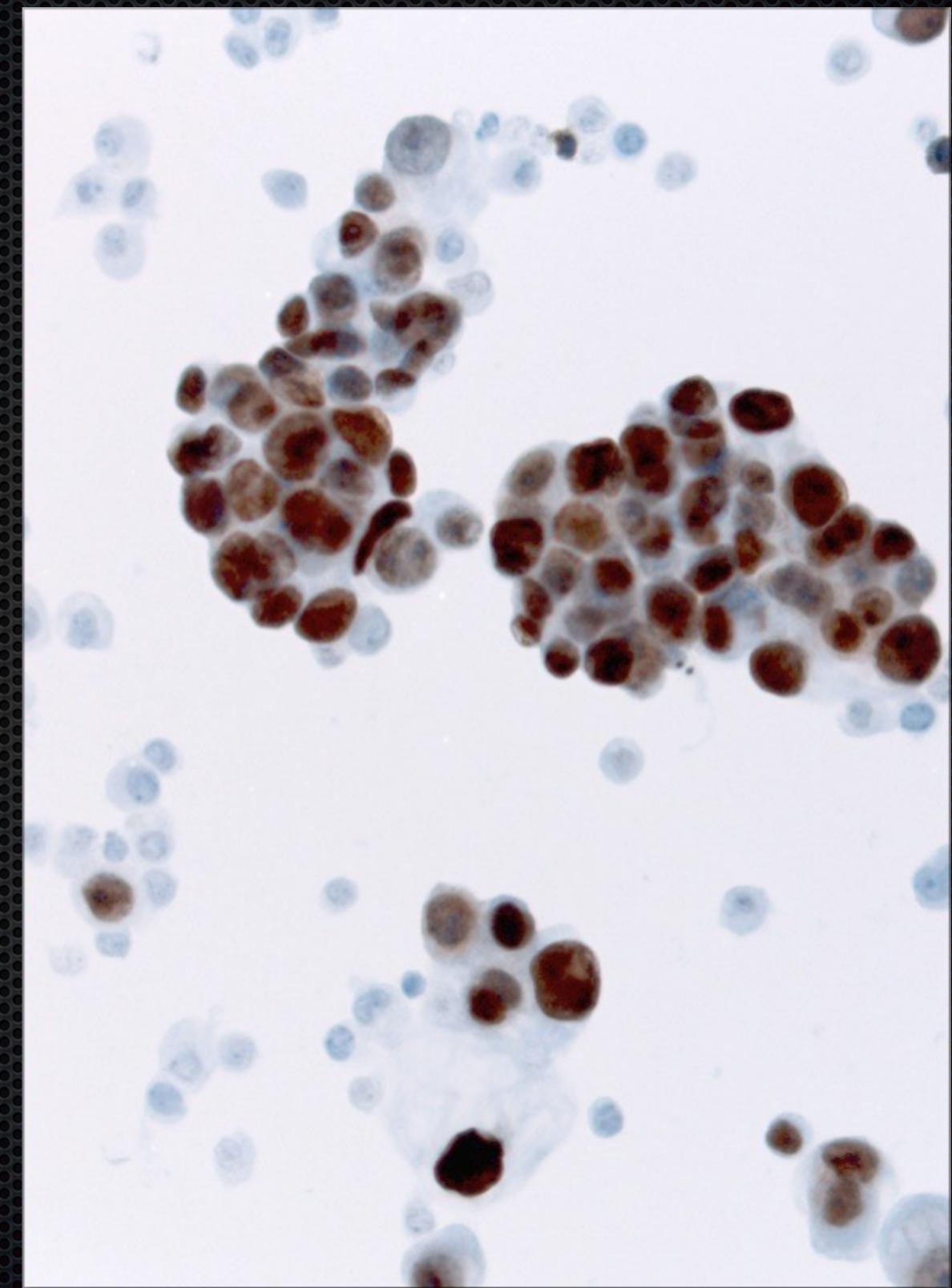
Manual setup

# TTF1, SPT24 (ThinPrep) Various fixatives



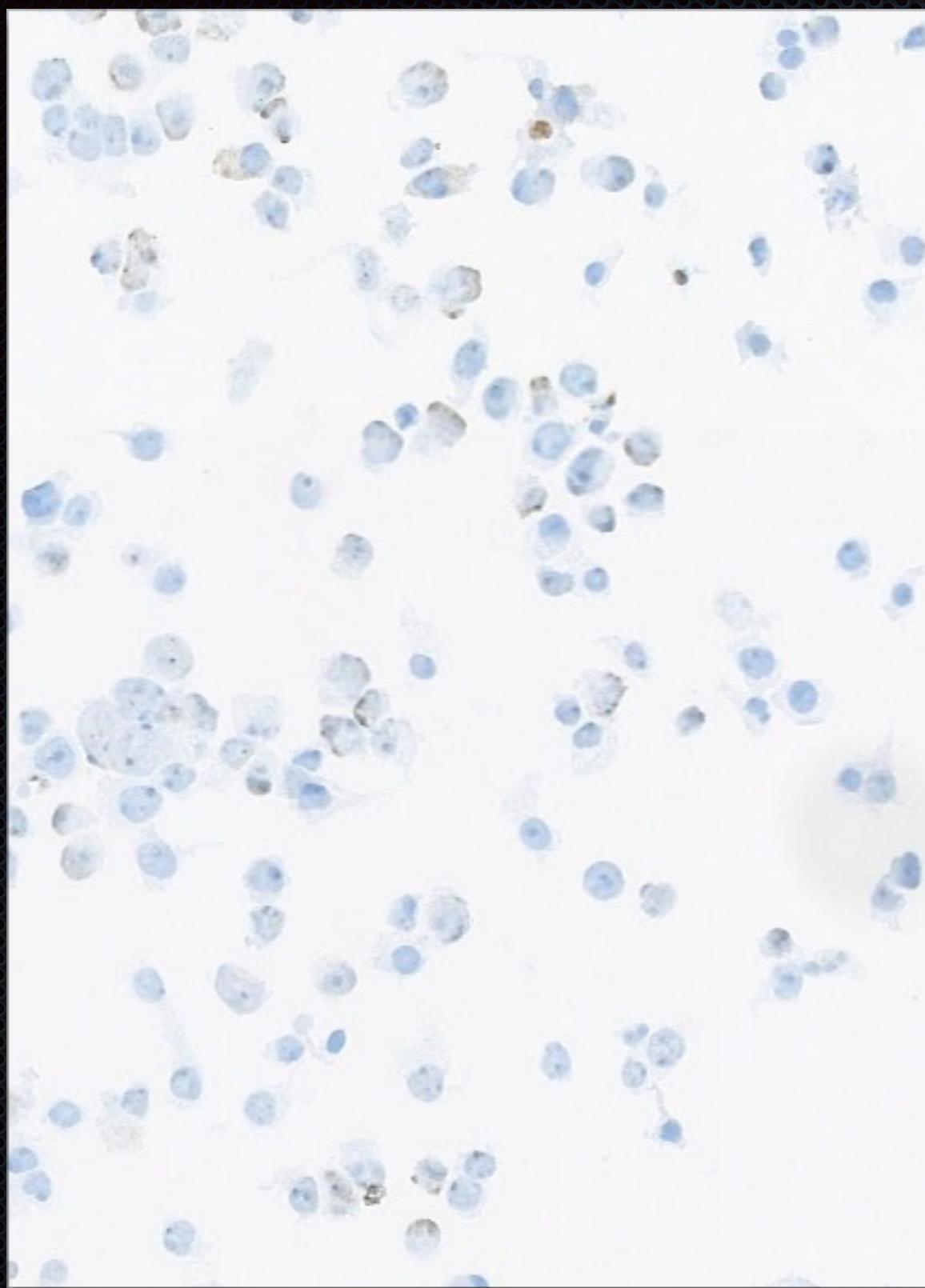
Manual setup

NBF 15' - TEG95° 15'

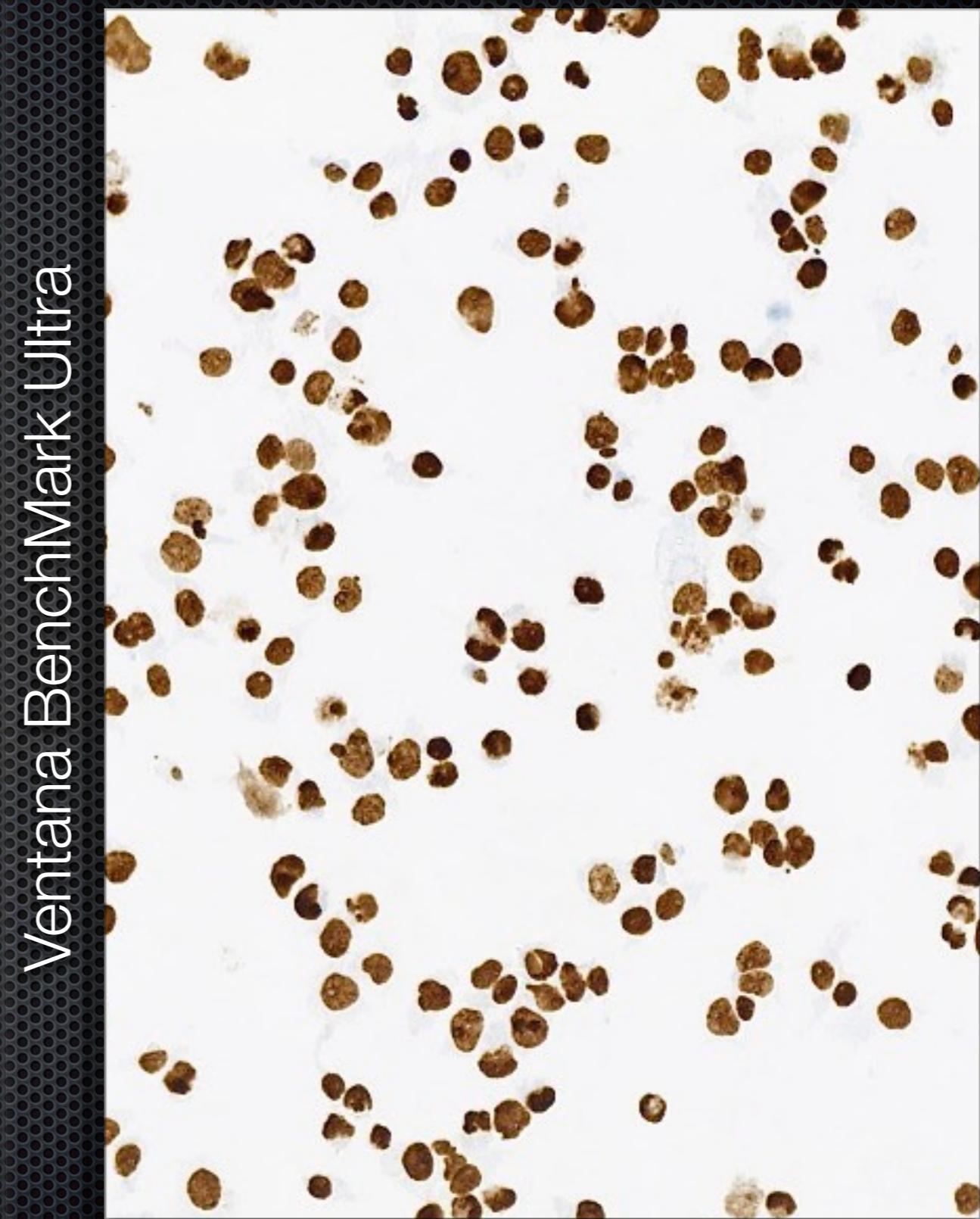


NBF 15' - MW/TEG 15'

# p40, BC28 (LBC - A431 cells) Various fixatives



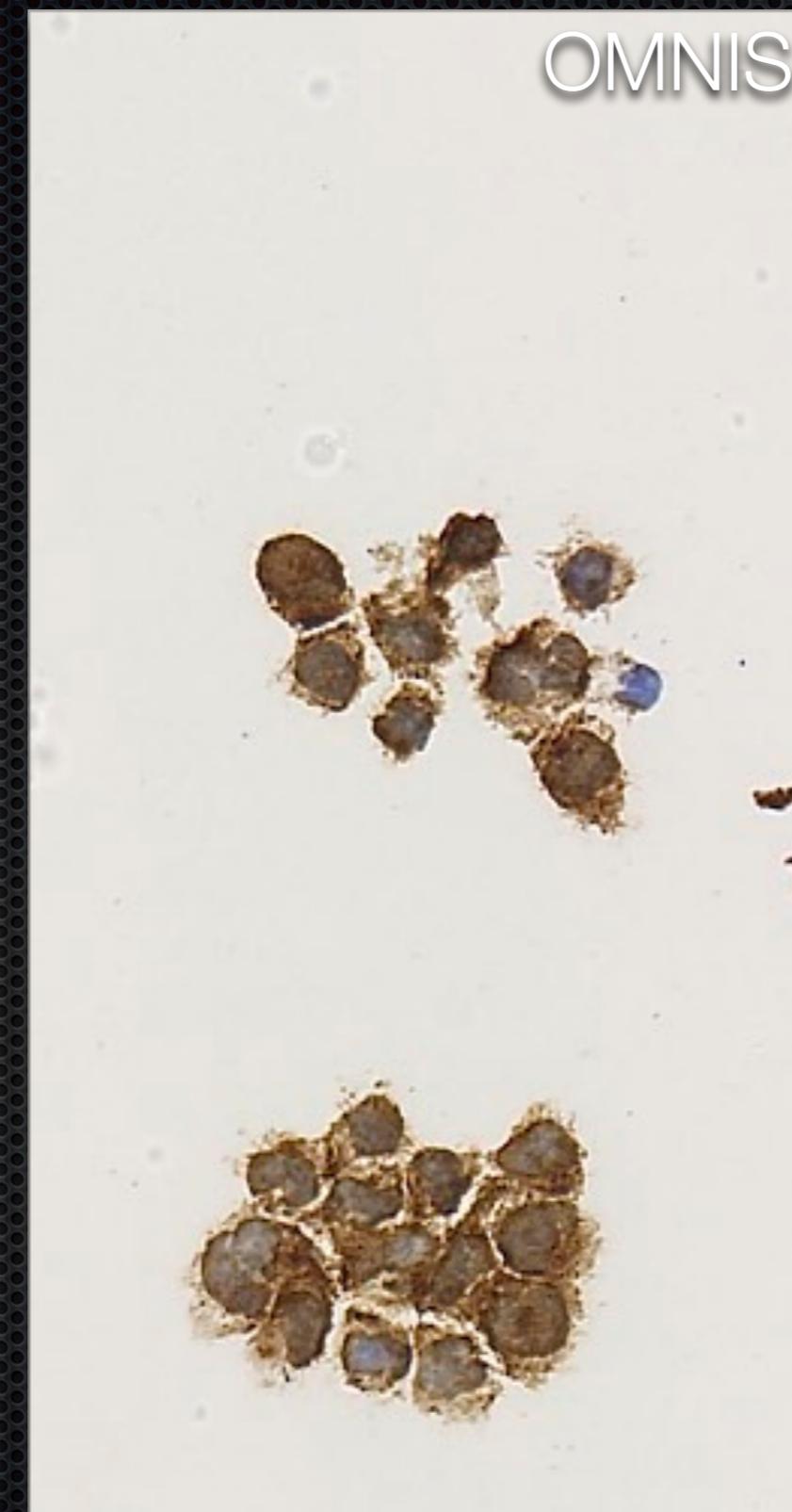
NBF 5'



NBF 5' - CC1\_32'\_95°

Ventana BenchMark Ultra

# CD34, EP88 (Kasumi-1 AML cell line)



NBF 30' - CC1\_8'\_95°

NBF 30' - TRS-H\_10'\_95°

NBF 30' - TRS-L\_10'\_95°

# TTF1, SPT24 (Thyroid imprint)

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NBF 30' - TRS-L\_10'\_95°



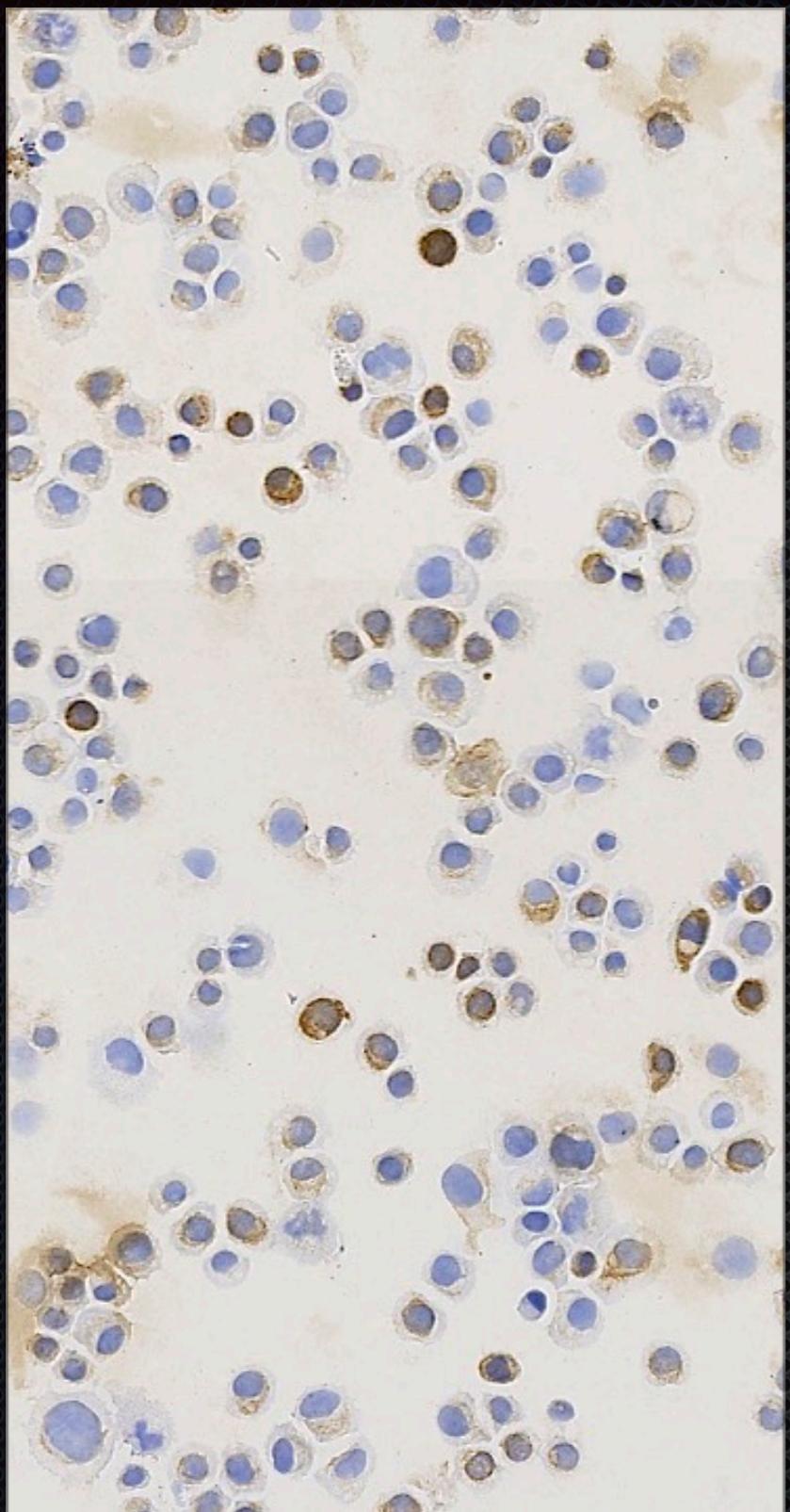
NBF 30' - TRS-H\_10'\_95°



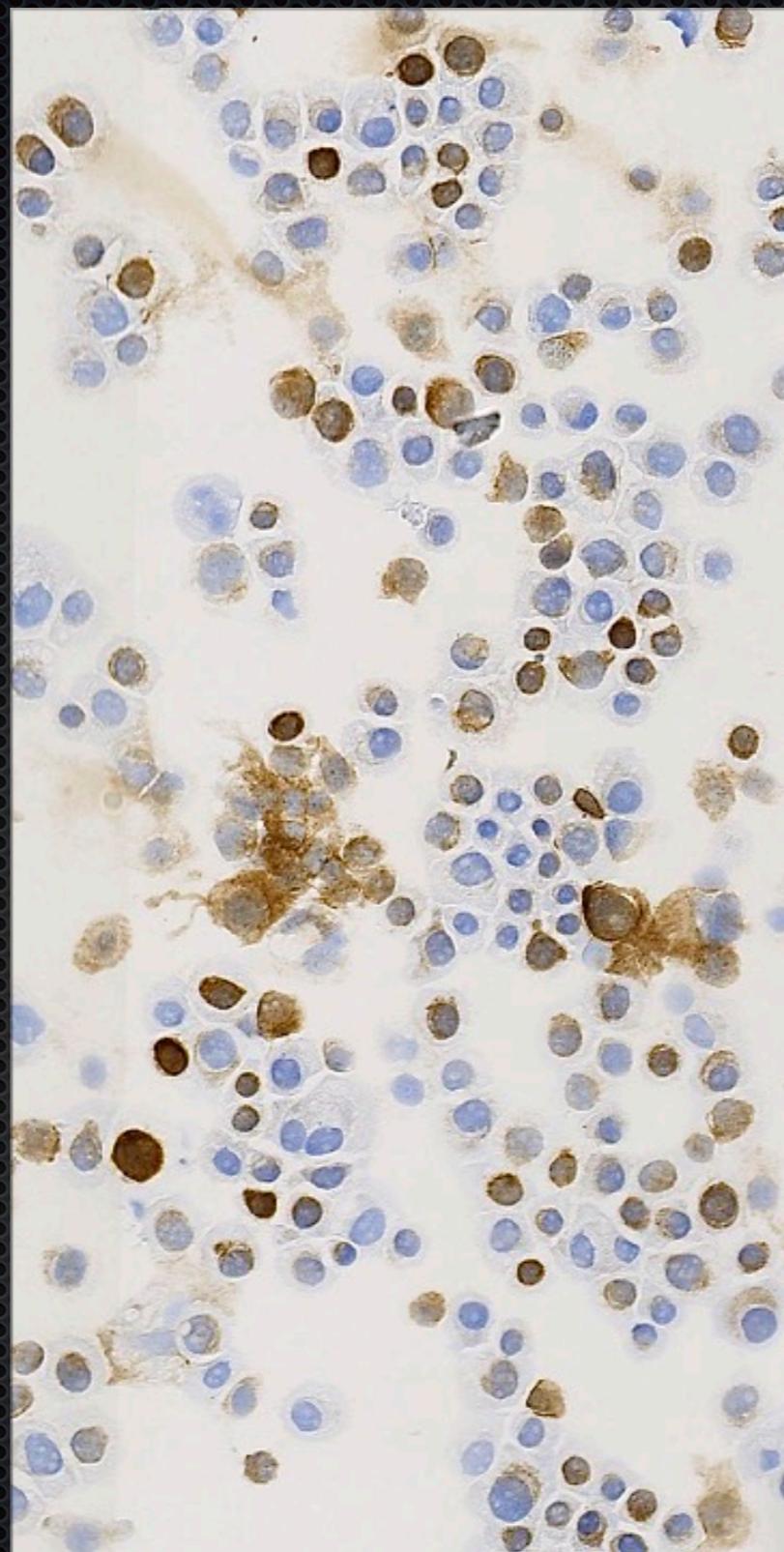
NBF 30' - TRS-H\_5'\_95°

# CK5, XM26 (BT20 cell line)

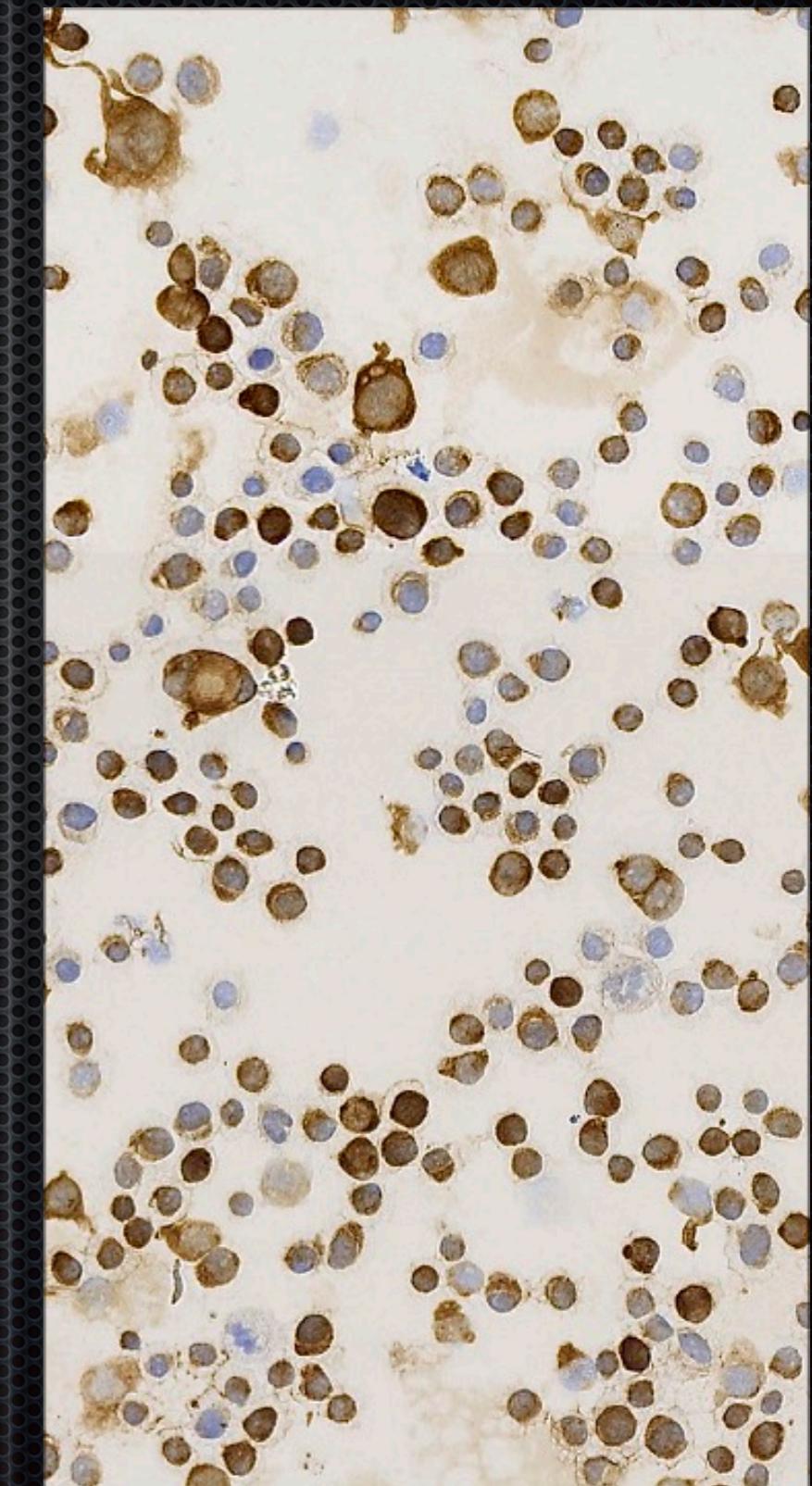
OMNIS



NBF 5'



NBF 30' - TRS-L\_10'\_95°



NBF 30' - TRS-H\_10'\_95°

# Cytology: Fixation and retrieval (Odense)

Antibody	Fixation	Retrieval
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
PR, 1E2	4% NBF 30 min	CC1_8_95
Desmin, H110	4% NBF 30 min	CC1_8_95
CD56, 56C04	4% NBF 30 min	CC1_8_95
Podoplanin, D240	4% NBF 30 min	CC1_8_95
MITF, 21CA5	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
PAX-8, ZR-1 (Zeta)	4% NBF 15 min	CC1_32_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
Calretinin, SP65	4% NBF 5 min	None
CD15, MMA	4% NBF 5 min	None
CD30, Ber-H2	4% NBF 5 min	None
CD61, 2f2	4% NBF 5 min	None
CD68, EBM11	4% NBF 5 min	None
CEA, Col-1	4% NBF 5 min	None
CK, AE1/AE3	4% NBF 5 min	None
CK, CAM 5.2	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
GATA3, L50-823	4% NBF 5 min	None
Melan-A, A103	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
S-100, p	4% NBF 5 min	None
Synaptophysin,	4% NBF 5 min	None
TdT, SEN28	4% NBF 5 min	None
TPO, MoAb47	4% NBF 5 min	None
Vimentin, V9	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'

11 Abs: Omnis

Antibody	Fixation	Retrieval
CD34, EP88	4% NBF 30 min	TRS-L_10_95
CDX2, EPR2764Y	4% NBF 30 min	TRS-L_10_95
CEA-M, COL-1	4% NBF 30 min	TRS-L_10_95
CK5, XM26	4% NBF 30 min	TRS-H_10_95
EMA, E29	4% NBF 30 min	TRS-L_10_95
EP-CAM, BS14	4% NBF 30 min	TRS-L_10_95
GATA3, EP368	4% NBF 30 min	TRS-L_10_95
P40, BC28	4% NBF 30 min	TRS-L_10_95
PAX-8, SPT24	4% NBF 30 min	TRS-H_10_95
TTF1, EP122	4% NBF 30 min	TRS-L_10_95
WT1, EP122	4% NBF 30 min	TRS-L_10_95

# ThinPrep (LBC): Fixation and retrieval (Odense)



Antibody	Fixation	Retrieval
CD3, 2GV6	4% NBF 30 min	CC1_32_100
CD1a, EP3622	4% NBF 30 min	CC1_32_100
CD5, SP19	4% NBF 30 min	CC1_32_100
CD56, 56C04	4% NBF 30 min	CC1_32_100
CD56, MRQ-42	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
CK19, A53-B/A2.26	4% NBF 30 min	CC1_32_100
CK7, SP52	4% NBF 30 min	CC1_32_100
E-Cadherin, 36	4% NBF 30 min	CC1_32_100
Ki67, 30-9	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

Antibody	Fixation	Retrieval
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

2 Abs: Omnis

Antibody	Fixation	Retrieval
p40, BC28	4% NBF 30 min	TRS-H_30_97
TTF-1, SPT24	4% NBF 30 min	TRS-H_30_97

# Cryo section: Fixation and retrieval (Odense)

Antibody	Fixation	Retrieval	Antibody	Fixation	Retrieval	Antibody	Fixation	Retrieval
MyoD1, EP212	None	None	Dysferlin, Ham1/7B6	None	None	Cranin, VIA4-1	4% NBF 15 min	CC1_8_90
CD56, MRQ-42	None	None	Sarcoglycan g, 35DAG/21B5	None	None	IgG4, EP138	4% NBF 30 min	CC1_8_90
Serca1, VE121G9	None	None	Sarcoglycan delta, δ-Sarc/12C1	None	None	CD4, SP35	4% NBF 5 min	None
C1q-FITC	None	None	Sarcoglycan beta, β-SARC/5B1	None	None	CD45, 2B11 & PD7/26	4% NBF 5 min	None
Albumin-FITC	None	None	Laminin alfa5, 4C7	None	None	CD56, 56C04	4% NBF 5 min	None
Fibrinogen-FITC	None	None	Caveolin 3, 26	None	None	C5b-9, aE11	4% NBF 5 min	None
C3-FITC	None	None	Emerin, 4G5	None	None	CD57, HNK-1	4% NBF 5 min	None
Lambda-FITC	None	None	Utrophin, DRP2/20C5	None	None	CD8, C8/144B	4% NBF 5 min	None
Kappa-FITC	None	None	Serca2, IID8	None	None	p63, A4A	4% NBF 5 min	None
IgA-FITC	None	None	Myosin neonatal, WB-MHCn	None	None	CD20cy, L26	Acetone 10 min	None
IgM-FITC	None	None	Myosin slow, WB-MHCs	None	None	Collagen IV, MAB3	Acetone 10 min	None
IgG-FITC	None	None	Myosin -Fast, MY32	None	None	Collagen IV, MAB1	Acetone 10 min	None
Dystrophin, 34C5	None	None	Laminin beta2, C4	None	None	GFAP, p	Acetone 10 min	None
Desmin, DE-R-11	None	None	Laminin beta1, 4E10	None	None	HLA-DR, CR3/43	Acetone 10 min	None
Collagen IV, CIV22	None	None	Dystrophin, DY8/6C5	None	None	HLA-ABC, W6/32	Acetone 10 min	None
Collagen VI, VI-26	None	None	Dystrophin, DY4/6D3	None	None	CD68, EBM11	Acetone 10 min	None
Pax-7, P3U1	None	None	Dystroglycan beta, NCL-43DAG	None	None	Collagen IV, MAB5	Acetone 10 min	Glycin/Urea
Laminin alfa2, 4H8-2	None	None	Actinin alfa, RBC2/1B6	None	None			
Laminin alfa2, Mer3/22B2	None	None	Sarcoglycan alfa, Ad1/20A6	None	None			
Calpain, 12A2	None	None						

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 !

Coffee.....

