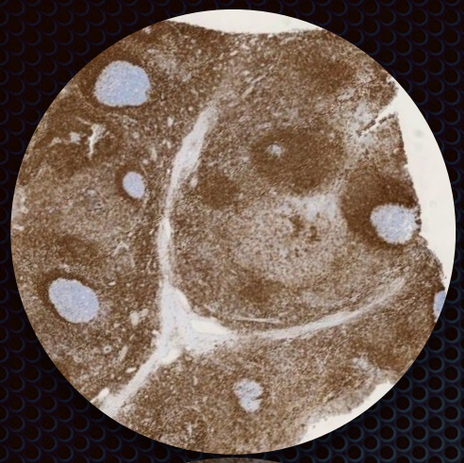


# Immunhistochemical principles

## The technical test approach
## Pre-analytical phase (I-II)

NQC Workshop 2019

Ole Nielsen,
Dept. of Pathology
Odense University Hospital

# The total test paradigm

"Immunohistochemistry is technically complex, and no aspect of this complexity can be ignored, from the moment of collecting the specimen to issuance of the final report "
Taylor CR. Arch Pathol Lab Med 2000; 124:945

## Preanalytic

Prefixation
Fixative
Fixation
Postfixation
Processing
Dehydration and clearing
Paraffin impregnation
Paraffin sectioning
Storage

## Analytic

Epitope retrieval
Blocking
Primary Antibody
Dilutent
Detection system
Chromogen
Counter stain
Mounting


## Interpretive

Design of controls
Positive controls
Negative controls
Interpretation
Critical Stain Indicator

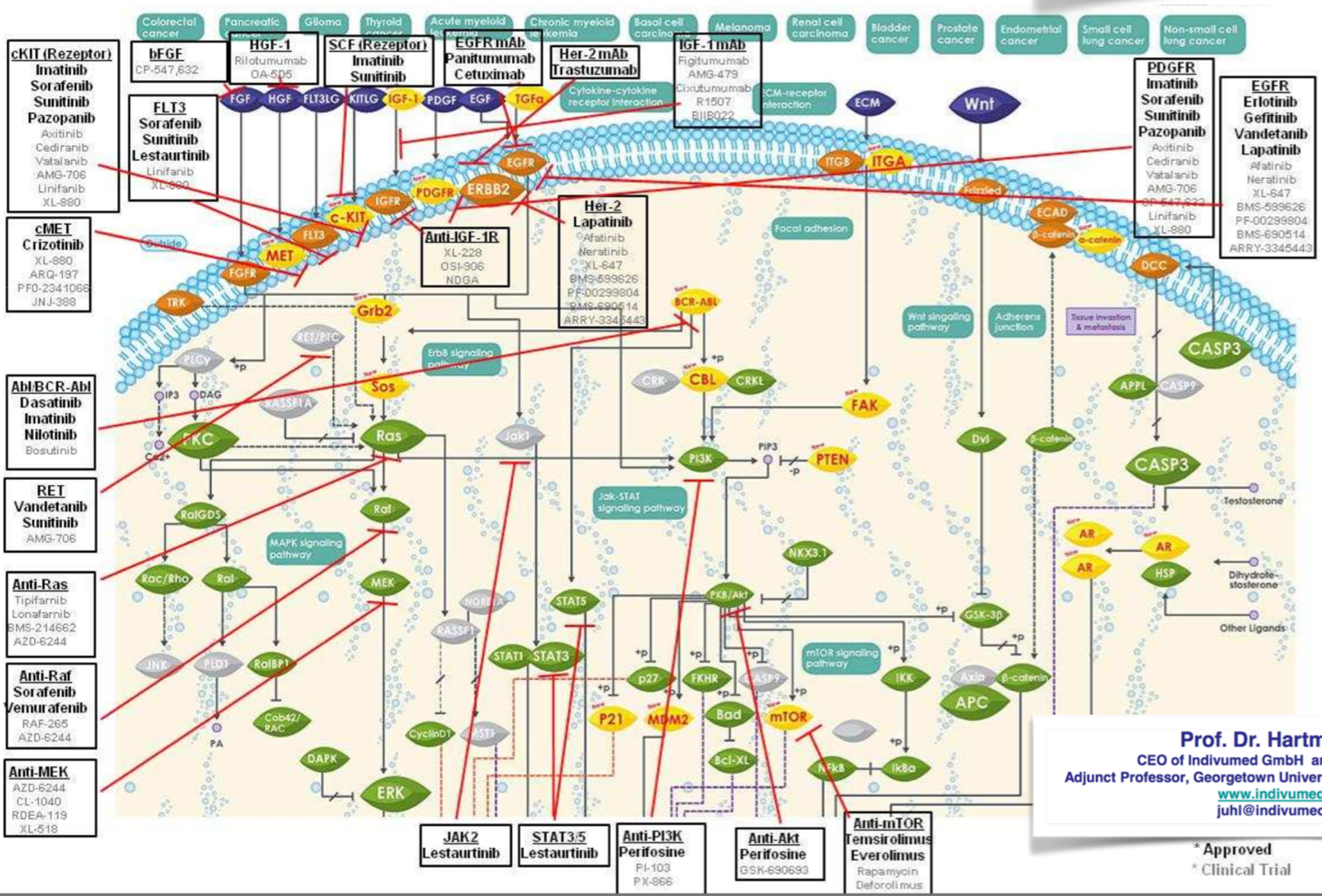
indivumed

NordiQC

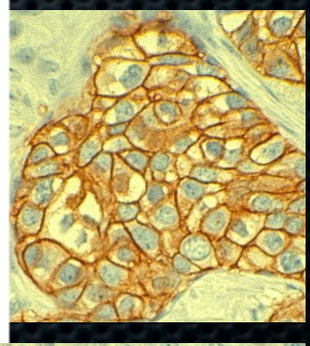
# Ca. 60 „Targeted Therapies“ are Approved and >800 Compounds are in Clinical Trial

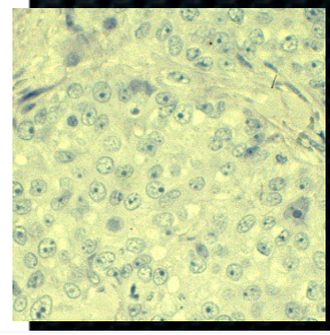
(Status 2012/01)

**Some require IHC-based Companion Diagnostics!**



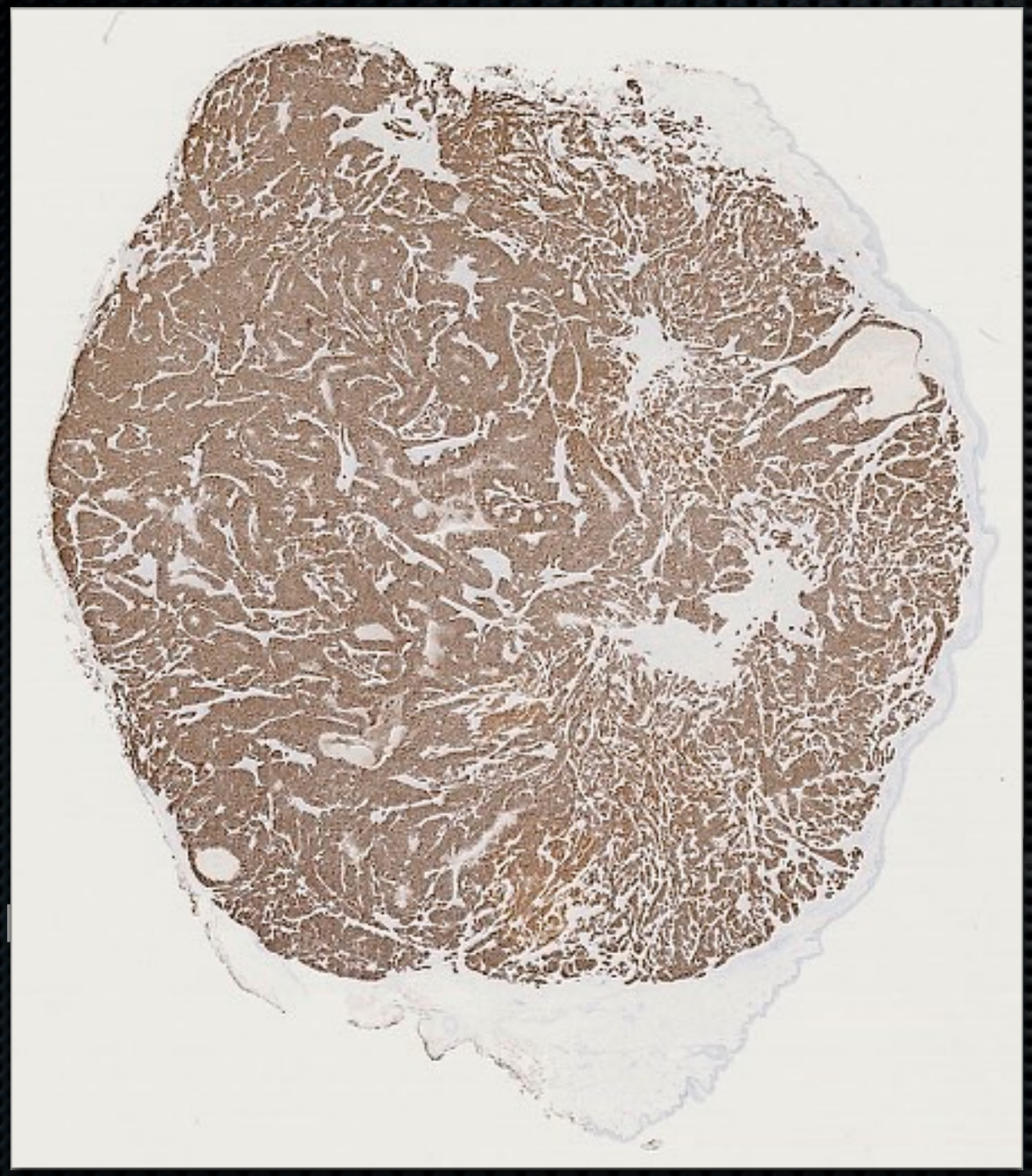

HER2






**Prof. Dr. Hartmut Juhl**
CEO of Indivumed GmbH and Inostics GmbH
Adjunct Professor, Georgetown University and Hamburg University
www.indivumed.com
juhl@indivumed.com

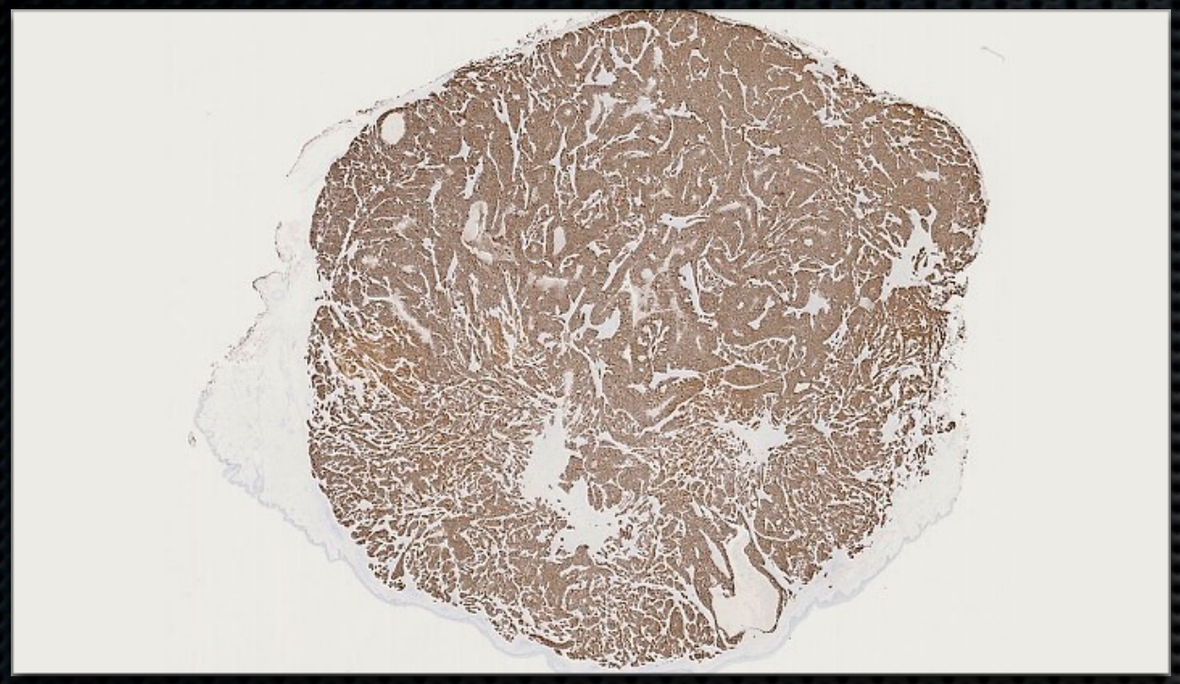
# Thyroid carcinoma: Biology or artefact?



TTF-1, SPT24

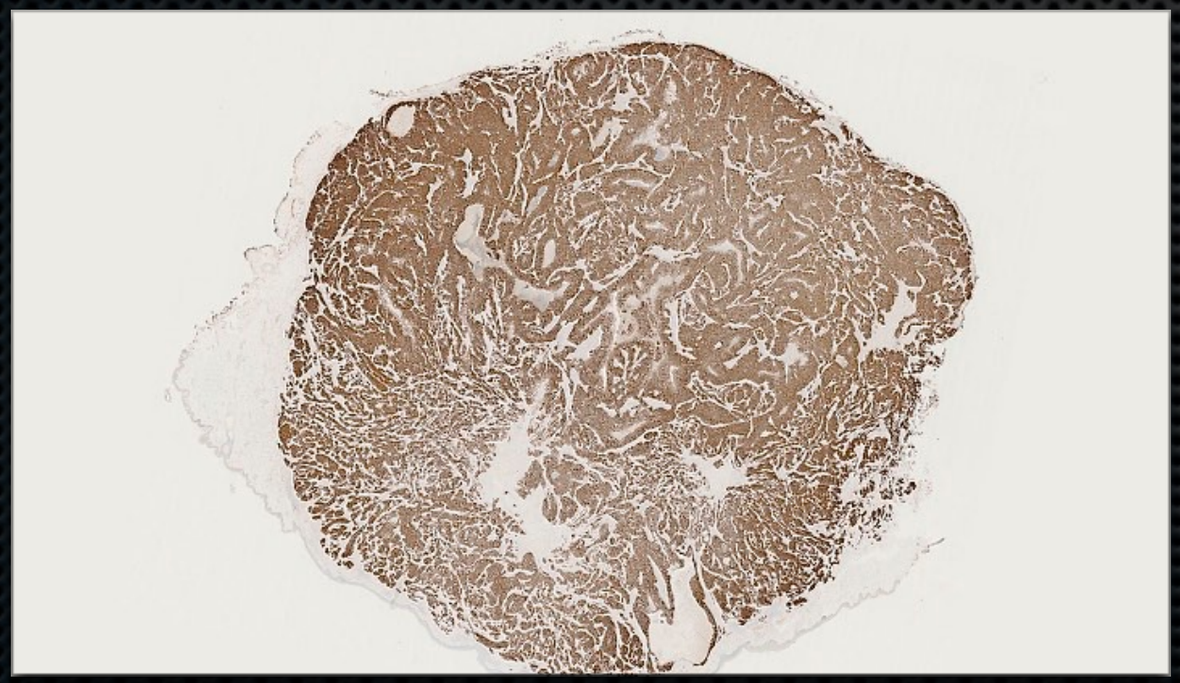

CD138, B-A38

# Thyroid carcinoma: Biology or artefact?



TTF-1, SPT24


CD138, B-A38



PAX-8, ZR-1


PAX-8, pAb (CM363A)

# A tissue quality index: an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue

Veronique M Neumeister[1], Fabio Parisi[1], Allison M England[1], Summar Siddiqui[1], Valsamo Anagnostou[1], Elizabeth Zarrella[1], Maria Vassilakopolou[1], Yalai Bai[1], Sasha Saylor[1], Anna Sapino[2], Yuval Kluger[1,2], David G Hicks[3], Gianni Bussolati[2], Stephanie Kwei[4] and David L Rimm[1]

**Laboratory Investigation (2014) 94,** 467–474


If we cannot control pre-analytical variables can we quantify the damage or tissue degradation caused by them?

# Effects of Preanalytical Variables on the Detection of Proteins by Immunohistochemistry in Formalin-Fixed, Paraffin-Embedded Tissue

*Kelly B. Engel, PhD; Helen M. Moore, PhD*

Arch Pathol Lab Med—Vol 135, May 2011

**Table 1. Potential Sources of Preanalytic Variation During Specimen Fixation and Processing**

- Prefixation
  - Duration and delay of temperature
  - Specimen size
  - Specimen manipulation (pathology ink)
- Fixative
  - Formula
  - Concentration
  - pH
  - Age of reagent
  - Preparation source
- Fixation
  - Tissue to fixative volume ratio
  - Method (immersion, injection, and sonication or microwave acceleration)
  - Conditions of primary and secondary fixation
    - Movement
    - Light exposure
    - Primary container
    - No. and position of cofixed specimens
- Postfixation
  - Washing conditions and duration
  - Storage reagent and duration
- Processing
  - Type of processor, frequency of servicing and reagent replacement
  - Tissue to reagent volume ratio
  - No. and position of coprocessed specimens
- Dehydration and clearing
  - Reagent
  - Temperature
  - No. of changes
  - Duration (total and change-specific)
- Paraffin impregnation
  - Type and melting point of wax
  - No. of changes
  - Duration (total and change-specific)
  - Method (immersion and sonication or microwave acceleration)
- Paraffin sectioning
  - Type of blade and frequency of replacement
  - Frequency of servicing and wax replacement
  - Temperature of block during sectioning
  - Slide pretreatment
  - Water bath conditions, if used
  - Chemical adhesives, if used
  - Temperature and duration of slide drying
- Storage
  - Temperature and duration of paraffin block storage
  - Temperature, duration, and manipulation of slide-mounted tissue sections

Decalcification:
Type, Time, Temperature

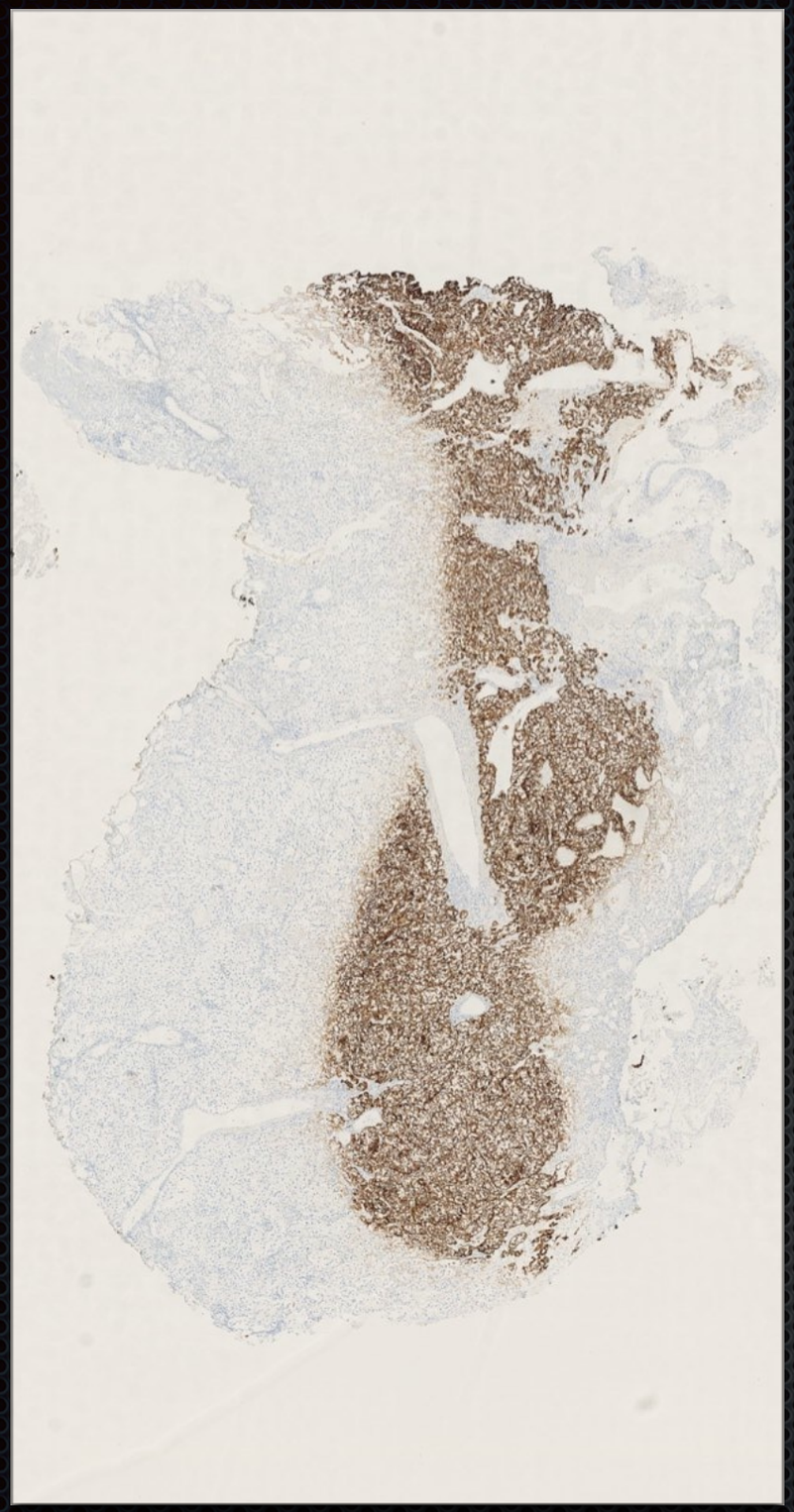
# Prefixation

- Surgical procedures
- Fixation delay / ischemia (time and temperature)
- Specimen size
- Specimen manipulation (pathology ink)

# Surgical procedures - Impact on IHC

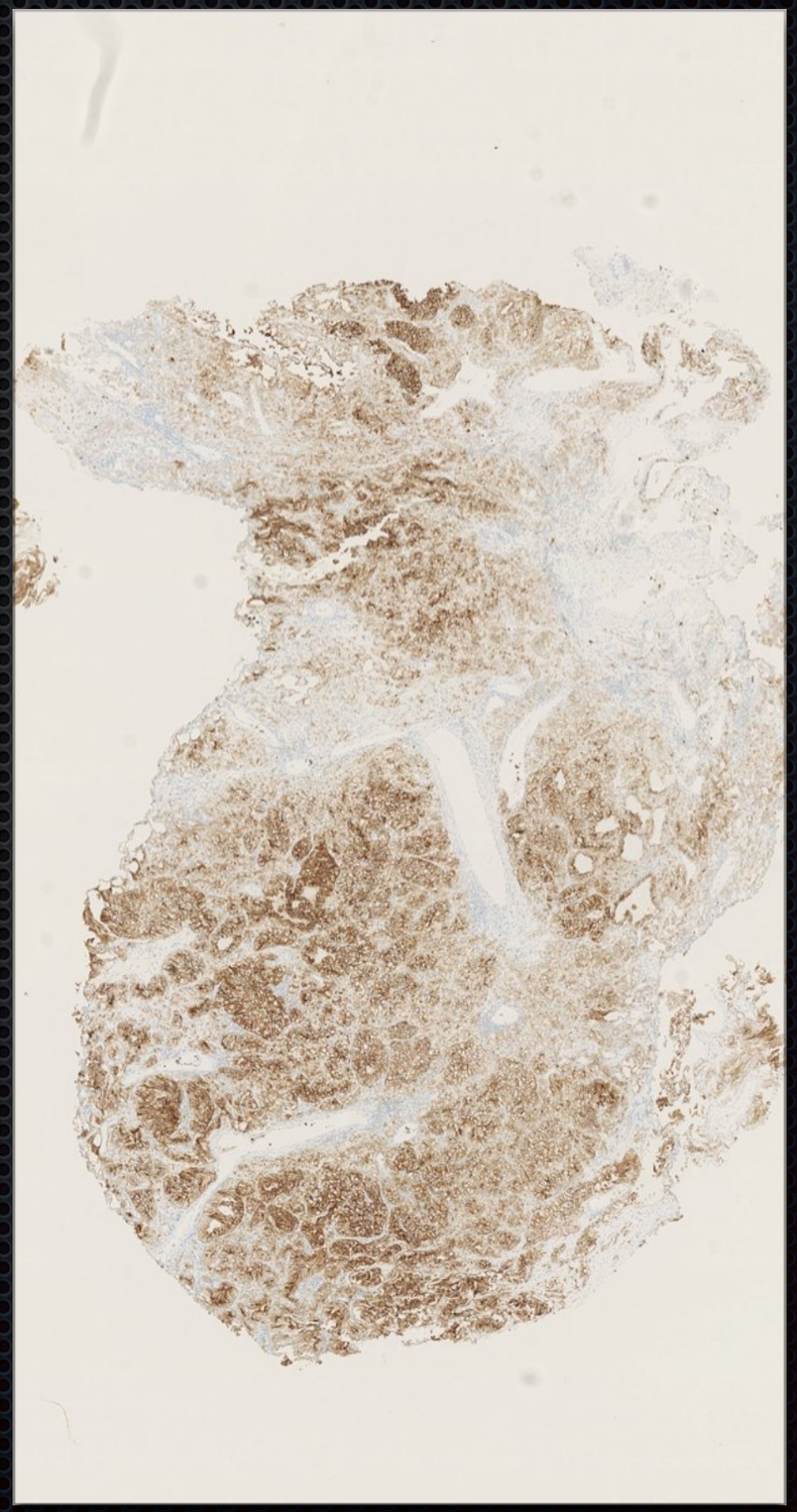
RCC

NordiQC



CK7/8, CAM5.2

CK8, EP17



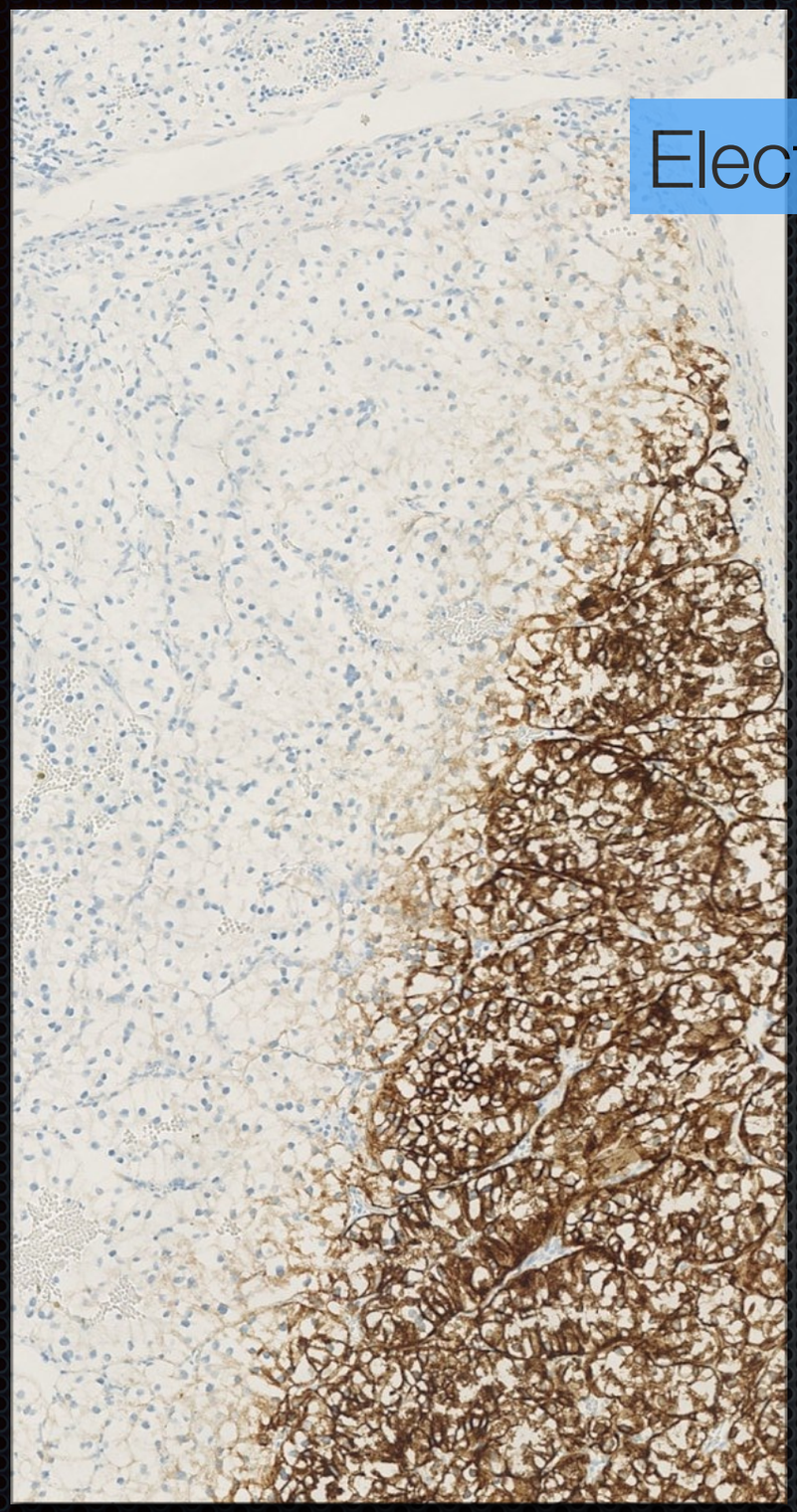
CD10, 56C6

# Surgical procedures - Impact on IHC

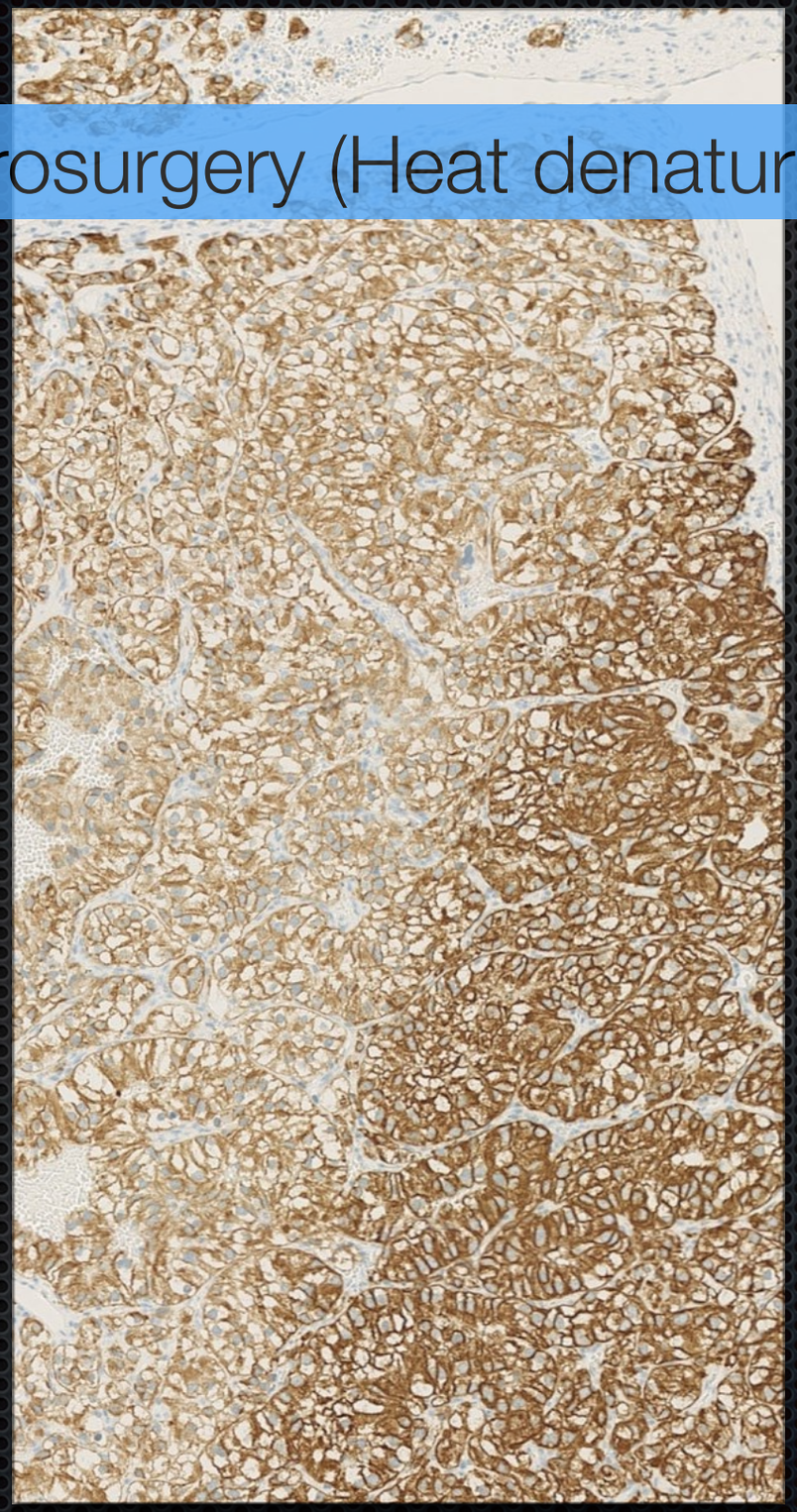
RCC

NordiQC

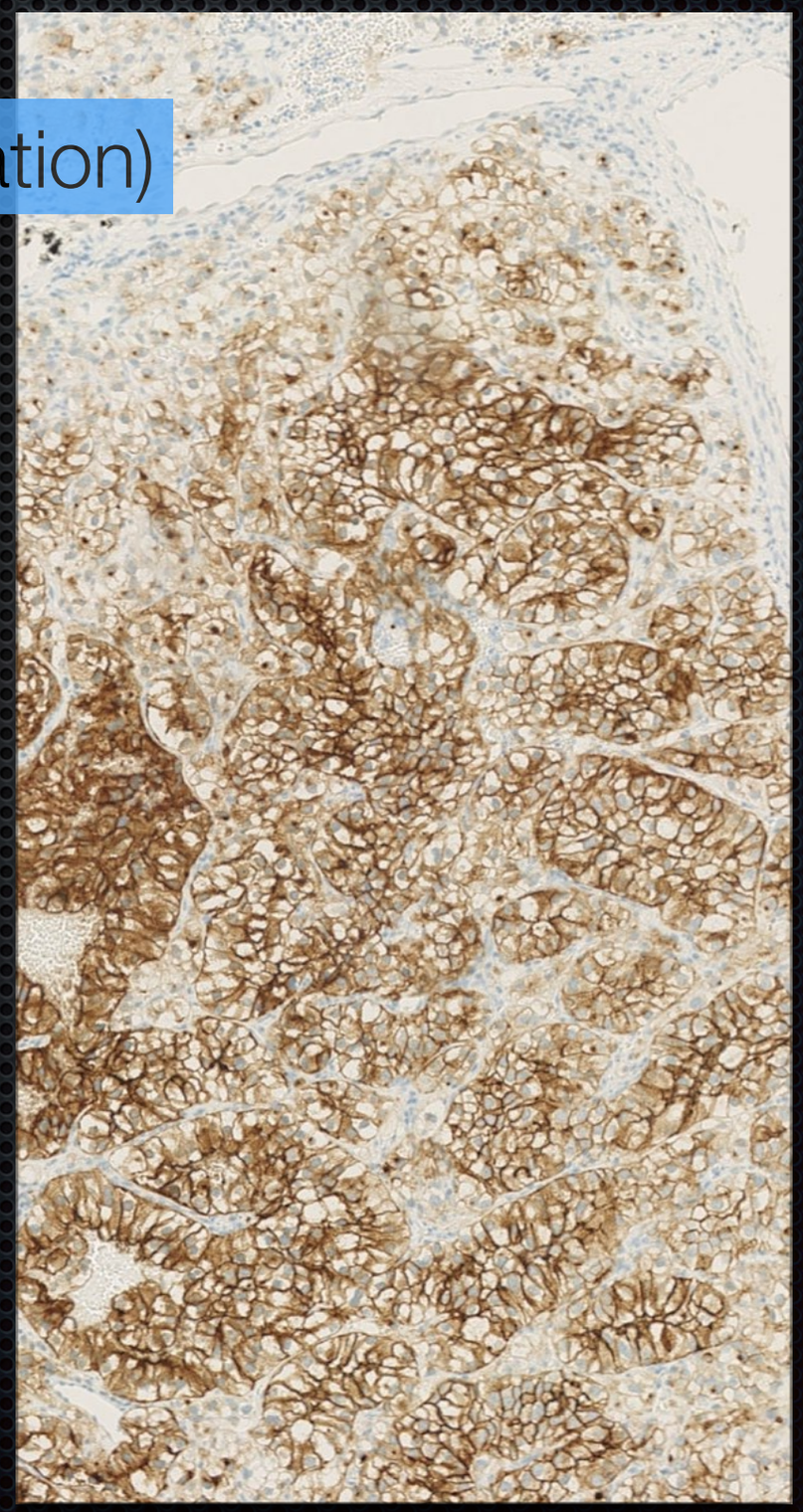
Electrosurgery (Heat denaturation)



CK7/8, CAM5.2

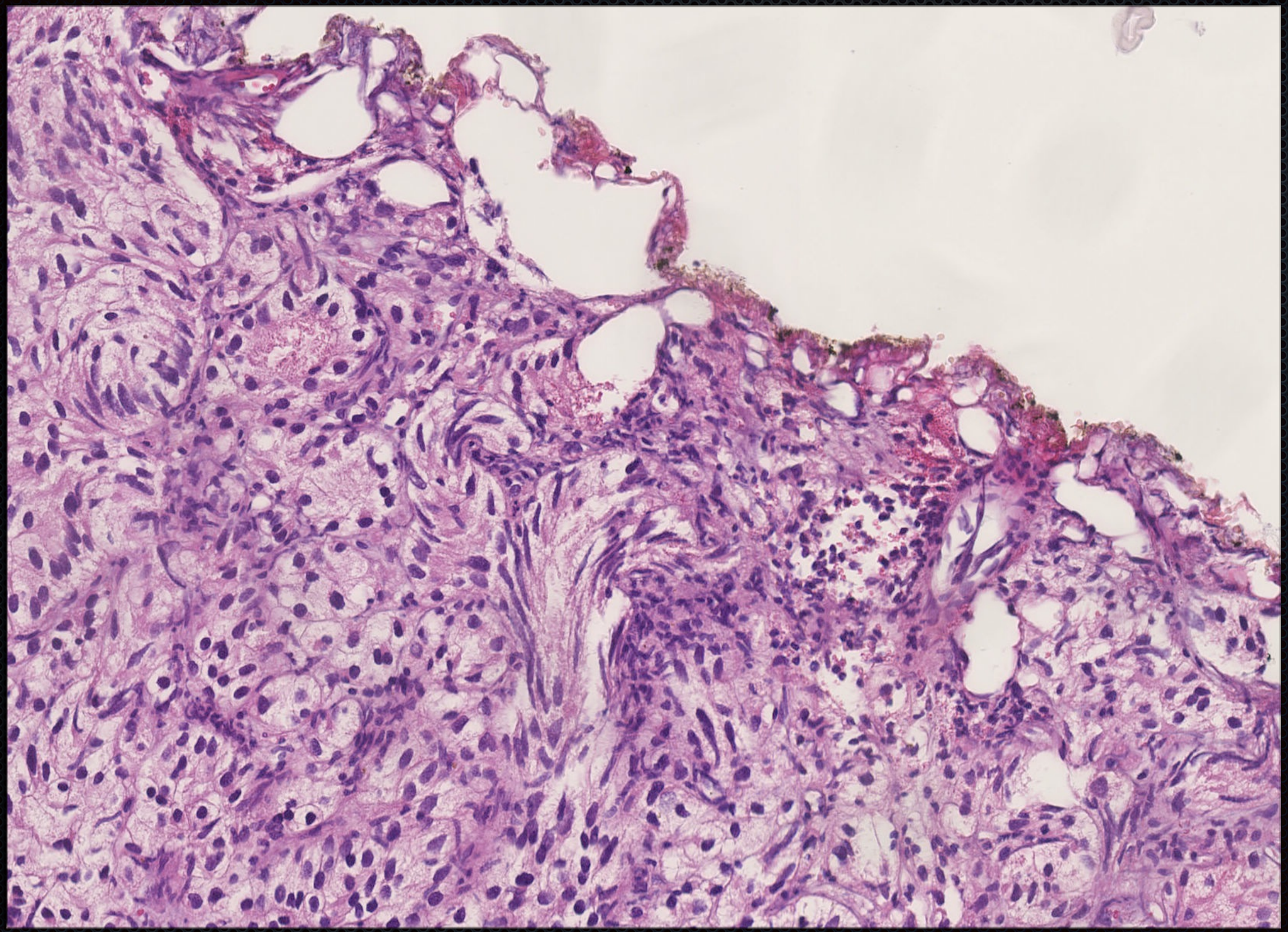


CK8, EP17

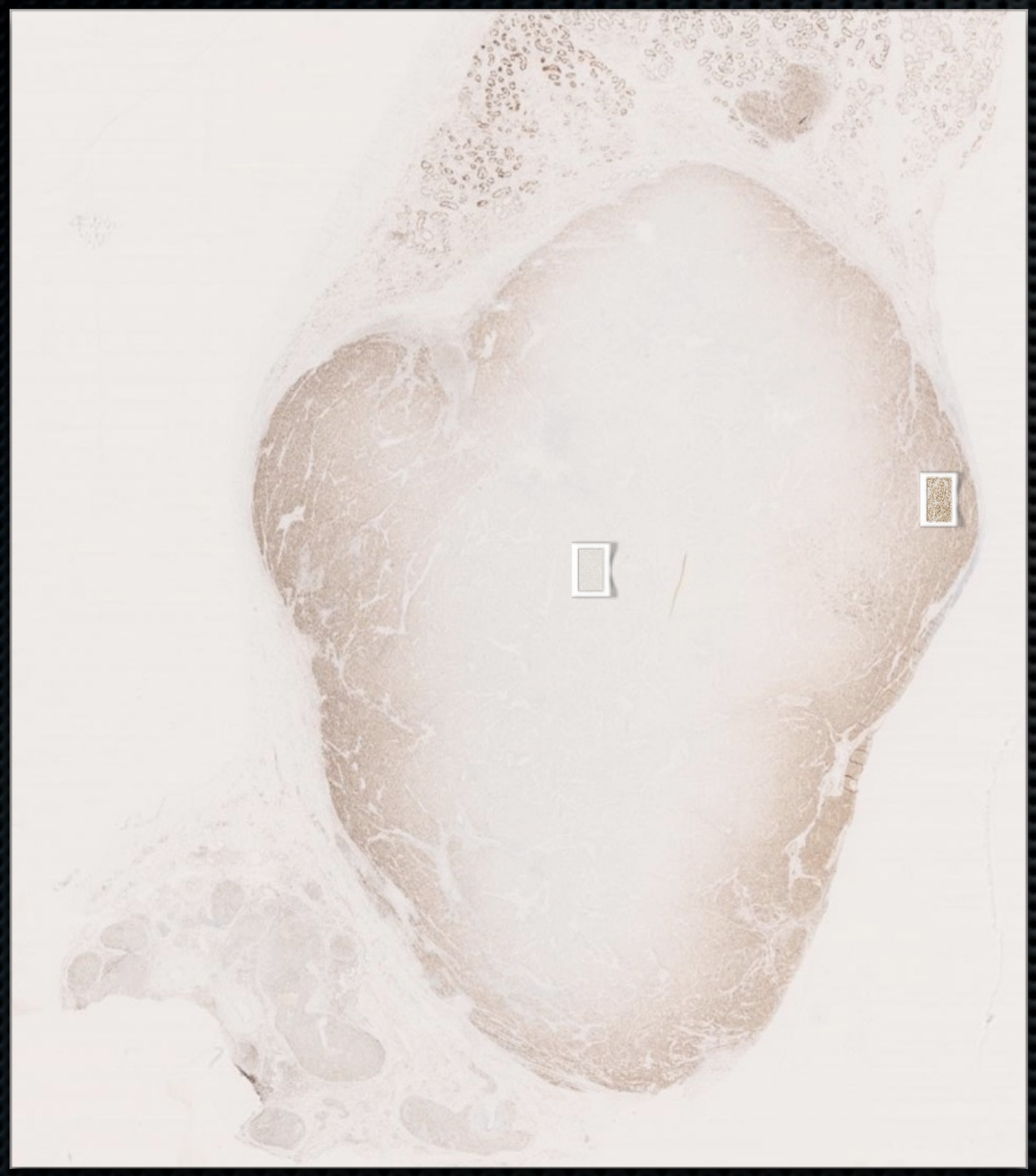


CD10, 56C6

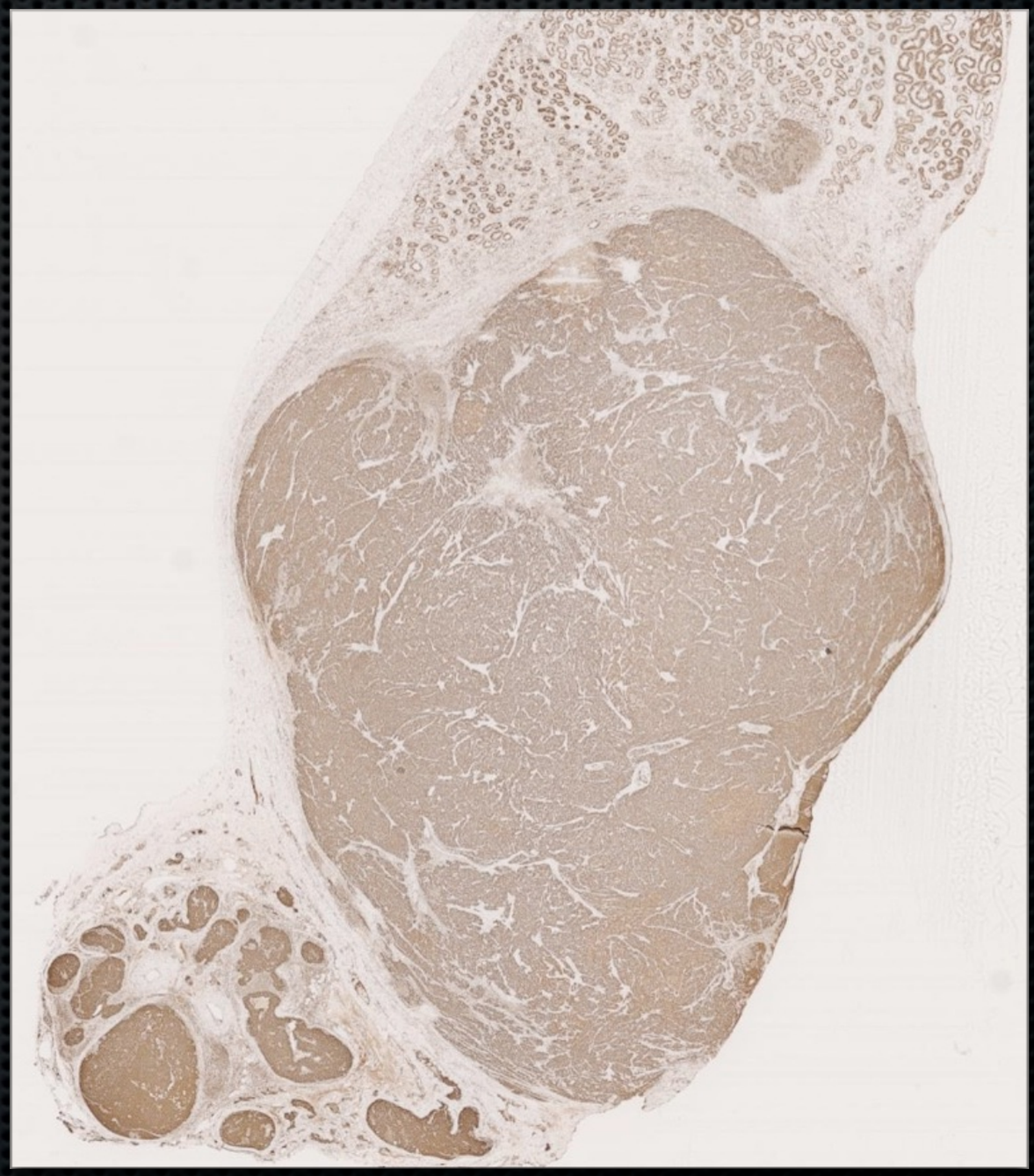
# “Electrosurgery” (Heat)  RCC



# Seminoma: Biology or Artefact?



PMS2, EPR3947



MSH6, EP49

# Seminoma: Biology or Artefact?

Delay of fixation?

Fixation procedure?


PMS2, EPR3947

# Effect of Delayed Formalin Fixation on Estrogen and Progesterone Receptors in Breast Cancer

## A Study of Three Different Clones

*Am J Clin Pathol* 2010;134:813-819

*Jingxin Qiu, MD, PhD,[1] Swati Kulkarni, MD,[2] Rameela Chandrasekhar,[3] Mark Rees, PhD,[4,6] Kathryn Hyde,[5] Gregory Wilding, PhD,[3] Dongfeng Tan, MD,[6] and Thaer Khoury, MD[1]*

**Figure 1** Mean Q score decline for estro
clones 1D5 (DAKO), 6F11 (Leica), and SP
relation to time of fixation.

# Effect of Delayed Formalin Fixation on Estrogen and Progesterone Receptors in Breast Cancer

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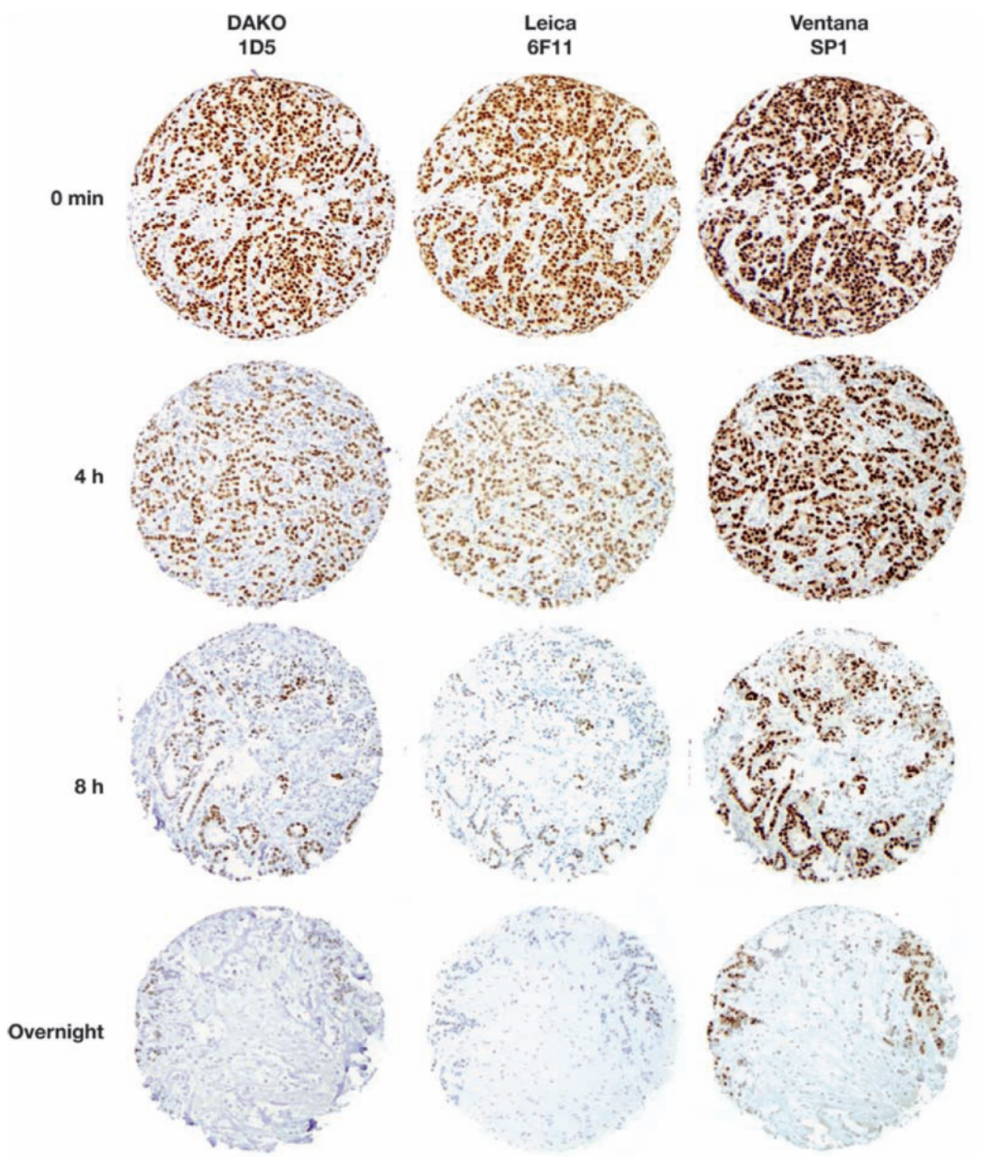



**Image 1** (Case 9) Estrogen receptor expression by clones 1D5 (DAKO), 6F11 (Leica), and SP1 (Ventana) at different delayed formalin fixation times (0 minutes, 4 and 8 hours, and overnight). Note the decreased number/percentage of positive cells and the intensity of the stain with increased time of delayed fixation.

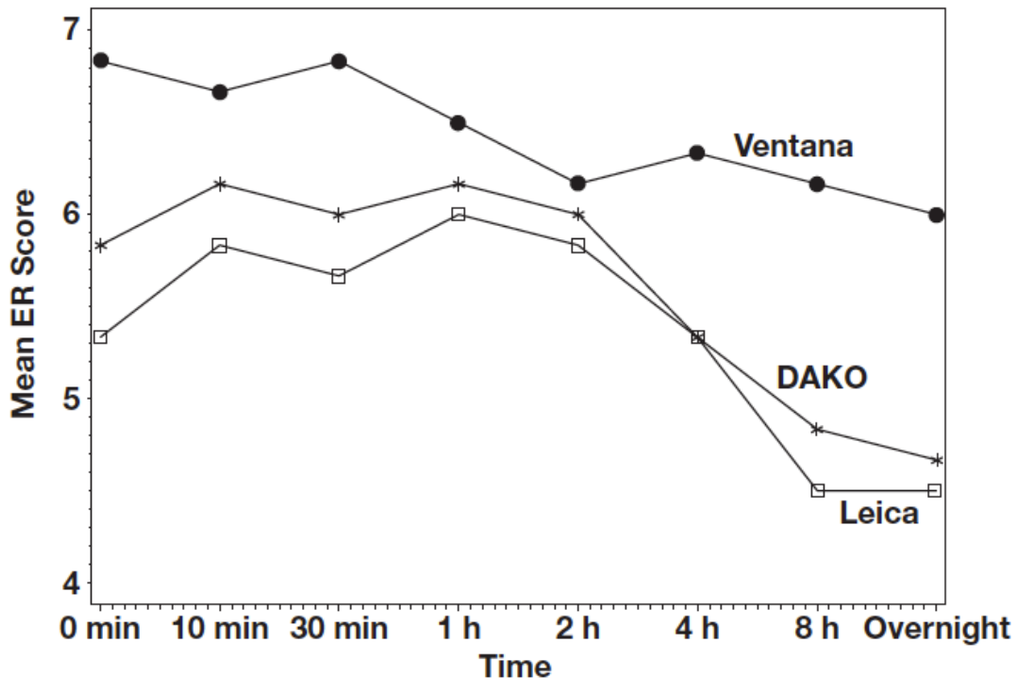



**Figure 1** Mean Q score decline for estrogen receptor by clones 1D5 (DAKO), 6F11 (Leica), and SP1 (Ventana) in relation to time of fixation.

# Effect of Delayed Formalin Fixation on Estrogen and Progesterone Receptors in Breast Cancer

## A Study of Three Different Clones

*Am J Clin Pathol* 2010;134:813-819

*Jingxin Qiu, MD, PhD,[1] Swati Kulkarni, MD,[2] Rameela Chandrasekhar,[3] Mark Rees, PhD,[4,6] Kathryn Hyde,[5] Gregory Wilding, PhD,[3] Dongfeng Tan, MD,[6] and Thaer Khoury, MD[1]*

***Based on our findings, it appears that regardless of the antibody clones evaluated, delayed formalin fixation has a negative effect on hormone receptors.***

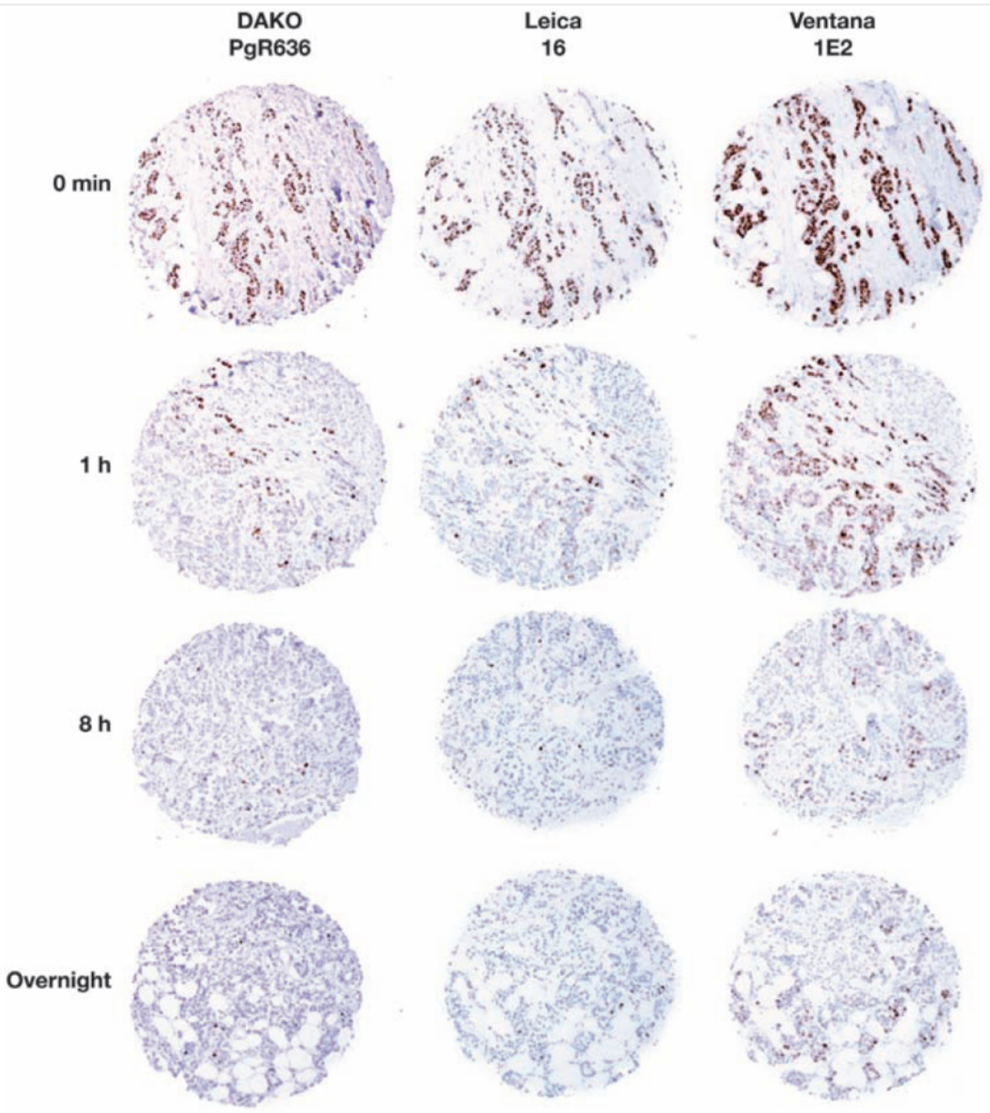



**Image 3** (Case 8) Progesterone receptor expression by clones PgR636 (DAKO), 16 (Leica), and 1E (Ventana) at different delayed formalin fixation times (0 minutes, 1 and 8 hours, and overnight).

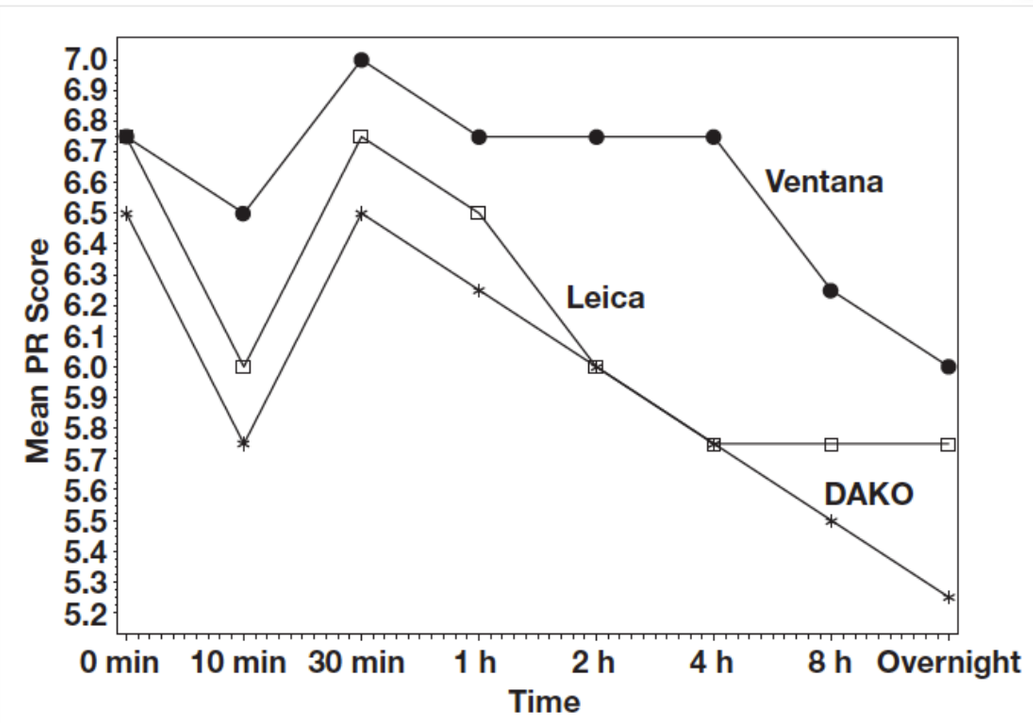
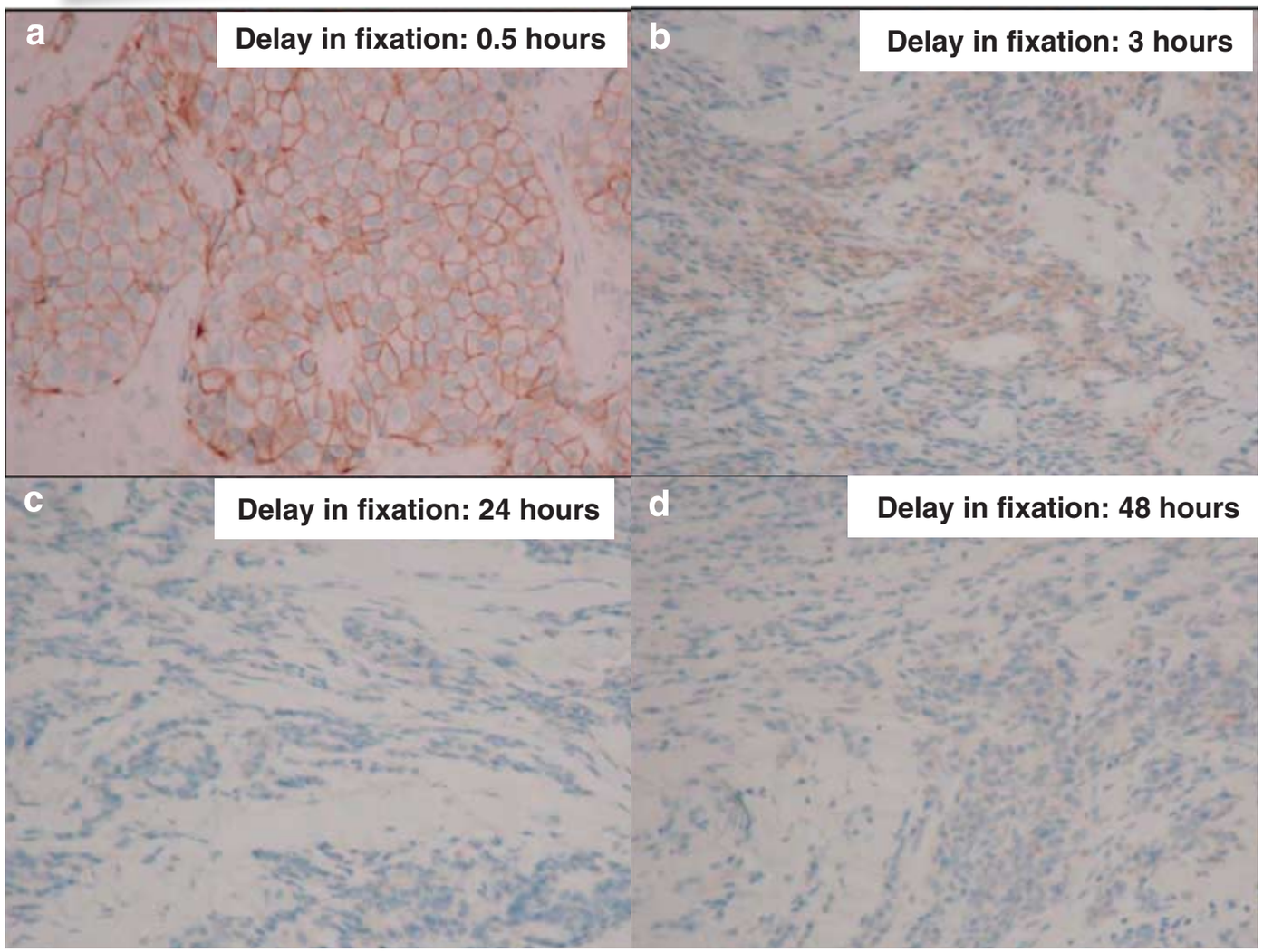



**Figure 2** Mean Q score decline for progesterone receptor (clones PgR636, 16 and 1E2) in relation to time of fixation. Values were not statistically significant by the Page L test.

# The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma

Isil Z Yildiz-Aktas, David J Dabbs and Rohit Bhargava

MODERN PATHOLOGY (2012) 25, 1098–1105




*"Non-refrigerated samples are affected more by prolonged cold ischemic time than refrigerated samples. Cold ischemic time period of as short as one-half hour may occasionally impact the immunohistochemical (IHC) staining for progesterone receptor. Significant reduction in IHC staining for hormone receptors, and HER2, however, generally does not result until 4 h for refrigerated samples and 2 h for non-refrigerated samples. The ASCO/CAP guideline of cold ischemic time period of 1 h is a prudent guideline to follow".*

# Quantitative assessment shows loss of antigenic epitopes as a function of pre-analytic variables

Yalai Bai[1], Juliana Tolles[2], Huan Cheng[1], Summar Siddiqui[1], Arun Gopinath[1], Eirini Pectasides[1], Robert L Camp[1], David L Rimm[1] and Annette M Molinaro[2]

**Laboratory Investigation (2011) 91,** 1253–1261

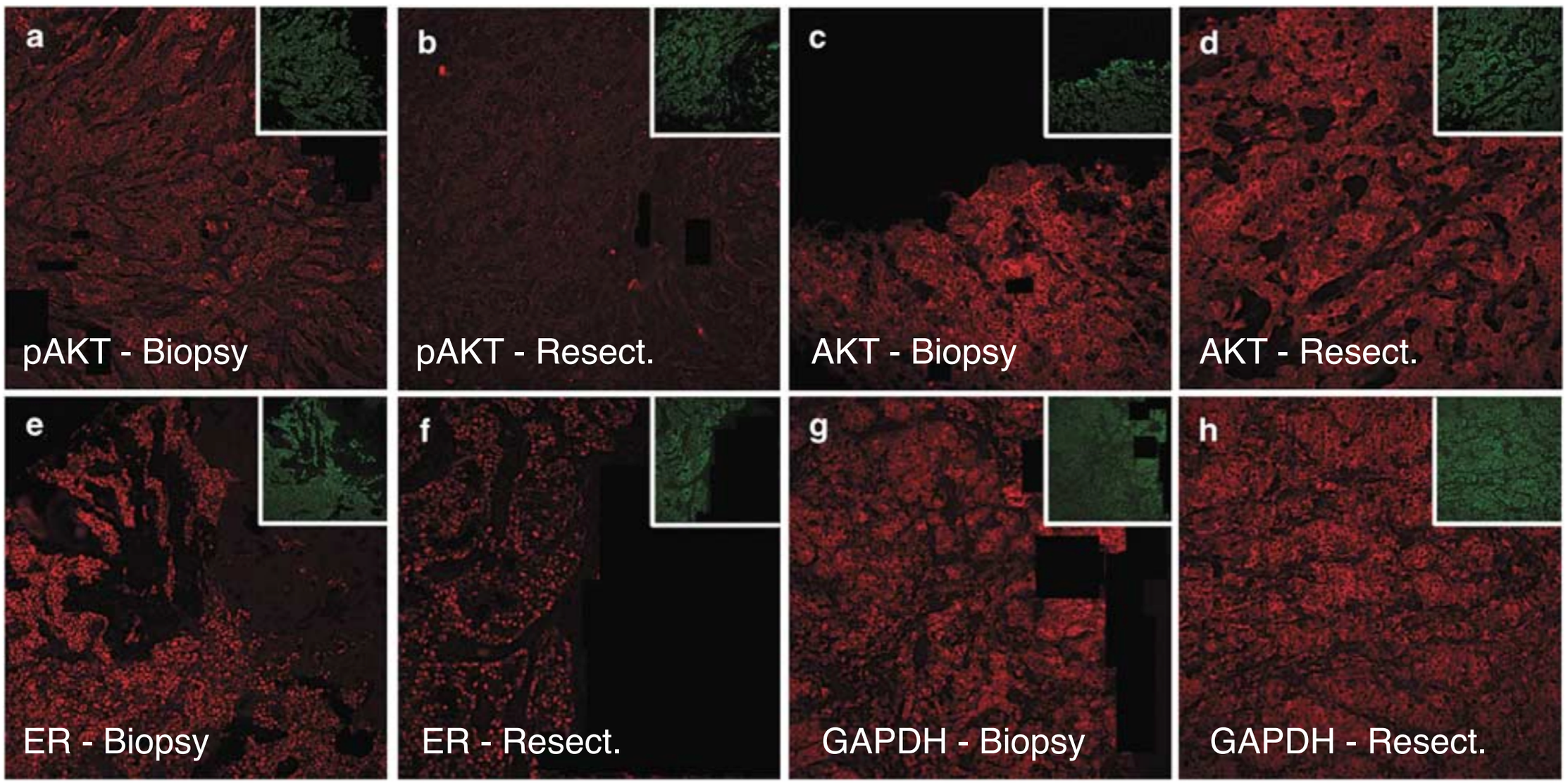



**Figure 2** Comparison of biomarker staining images between biopsies *vs* tumor resections on whole tissue section slides. Representative immunofluorescence staining of pAKT (red) in CNB (**a**) and tumor resection (**b**), AKT (red) in CNB (**c**) and tumor resection (**d**), ER (red) in CNB (**e**) and tumor resection (**f**), and GAPDH (red) in biopsy (**g**) and tumor resection (**h**) was illustrated. Each corresponding cytokeratin staining is shown as inset (green). Photographs are shown at magnification of $\times 20$.

# Quantitative assessment shows loss of antigenic epitopes as a function of pre-analytic variables

Yalai Bai[1], Juliana Tolles[2], Huan Cheng[1], Summar Siddiqui[1], Arun Gopinath[1], Eirini Pectasides[1], Robert L Camp[1], David L Rimm[1] and Annette M Molinaro[2]

**Laboratory Investigation (2011) 91,** 1253–1261

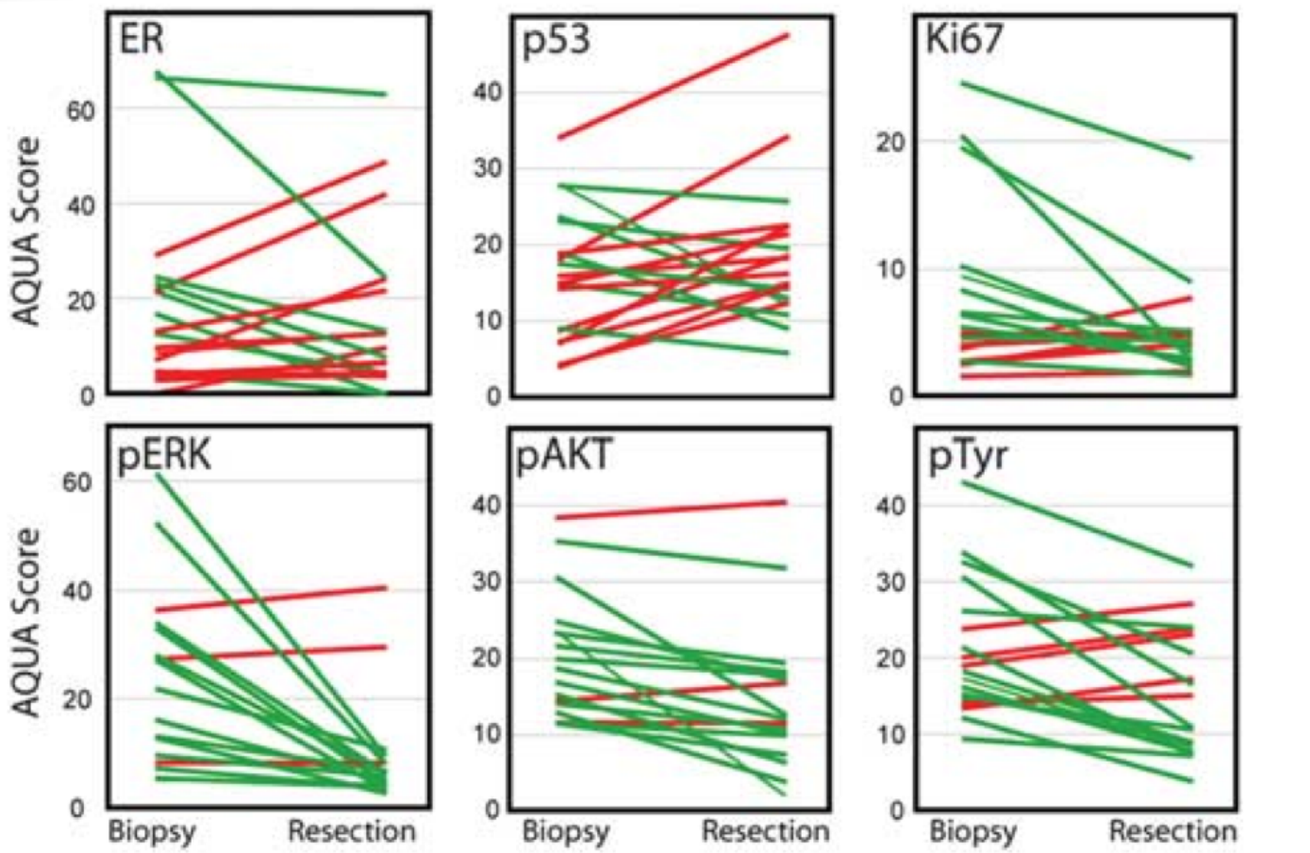



**Figure 1** Differences in biomarker expression in core needle biopsies *vs* tumor resections. Twenty core needle biopsies and matched tumor resections were arrayed in TMA with two-fold redundancy. In all, 1.5 mm core from each tumor block was arrayed in a recipient block. The TMA was immunohistochemically stained with ER, p53, Ki67, pERK, pAKT and pTyr and the results were quantified using AQUA. Scores represent the average of two cores. Specimens that showed decreased staining in the resection relative to biopsy are shown in green; those with higher resection levels are shown in red.

# Quantitative assessment shows loss of antigenic epitopes as a function of pre-analytic variables

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**Laboratory Investigation (2011) 91,** 1253–1261

- Detection levels for all phospho-epitopes were significantly decreased in tumor resections compared with biopsies while no significant change was seen in the corresponding total proteins.
- ER and cytokeratin showed significant loss of antigenicity.
- This data suggest that measurement of phospho-protein antigenicity in formalin-fixed tissue by immunological methods is dramatically affected by pre-analytic variables.
- This study suggests that core needle biopsies are more accurate for assessment of tissue biomarkers.

# Vacuum Sealing and Cooling as Methods to Preserve Surgical Specimens

*Thomas Kristensen, PhD,* Birte Engvad* Torsten Pless, MD,†*
*Steen Walter, MD, DMSc ak, MD**

Appl Immunohistochem Mol Morphol. 2011 Oct;19(

Liver, kid nd breast

**TABLE 1.** Antibody Clones and Dilutions and Epitope Retrieval Procedures

| Antibody | Clone | Source | Dilution | Retrieval |
|---|---|---|---|---|
| CD4 | SP35 | Ventana | RTU | CC1mild |
| CD8 | C8/144B | Ventana | RTU | CC1std |
| CD10 | 56C6 | Leica | 1:10 | CC1std |
| CD13 | 38C12 | Leica | 1:25 | CC1mild |
| CD14 | 7 | Leica | 1:25 | CC1std |
| CD34 | QBEnd/10 | Ventana | RTU | CC1mild |
| CD68 | PG-M1 | Dako | 1:50 | CC1std |
| CD138 | B-A38 | Ventana | RTU | CC1mild |
| CD138 | BC/B-B4 | Biocare | 1:500 | CC1mild |
| CDX2 | EPR2764Y | Ventana | RTU | CC1std |
| CK7+8 | CAM 5.2 | BD | 1:10 | Protease 1: 8 min |
| CK7 | SP52 | Ventana | RTU | CC1std |
| CK7 | OV-TL12/30 | Ventana | RTU | CC1std |
| CK18 | DC10 | Dako | 1:25 | CC1std |
| CK20 | SP33 | Ventana | RTU | CC1std |
| E-Cad | ECH-6 | Ventana | RTU | CC1std |
| E-Cad | HECD-1 | Abcam | 1:50 | CC1std |
| ER | SP1 | Ventana | RTU | CC1std |
| HEPA | OCH1E5 | Ventana | RTU | CC1mild |
| HLA-DR | CR3/43 | Dako | 1:200 | CC1mild |
| Ki67 | 30-9 | Ventana | RTU | CC1std |
| Lamins A/C | EPR4100 | Epitomics | 1:4000 | CC1std |
| PgR | 1E2 | Ventana | RTU | CC1mild |
| Villin | CWWB1 | Ventana | RTU | CC1std |


| | 2 | 4 | 8 | 20 | 44 | 92 |
|---|---|---|---|---|---|---|
| Treatment | | | | | | |
| Vacu (n=14 | IM R | IM R | IM R | IM R | IM R | IM R |
| Vacu (n=14 | IM R | IM R | IM R | IM R | IM R | IM R |
| RT (n=14) | IM R | IM R | IM R | IM R | IM R | IM R |
| 4°C (n=14) | IM R | IM R | IM R | IM R | IM R | IM R |
| Forma (n=7) | R | R | R | R | R | R |
| FineFi (n=7) | R | R | R | R | R | R |
| No trea (n=6) | | | | | | |

**FIGURE 1.** A t samples were included for each of the 5 organ types included in the study. The nental time = 0 hour. Experimental samples were collected after 1, 2, 4, 8, 20, 44, n fixed for approximately 48 hours before further processing and paraffin embed chemical and morphologic endpoints. Samples labeled "R" were snap frozen and n and analysis of RNA integrity. Samples labeled "R" were not analyzed with r ssue morphology or epitope T indicates room temperature.

# Vacuum Sealing and Cooling as Methods to Preserve Surgical Specimens

*Thomas Kristensen, PhD,* Birte Engvad, MD,* Ole Nielsen, MT,* Torsten Pless, MD,† Steen Walter, MD, DMSc, FEBU,‡ and Martin Bak, MD**

Appl Immunohistochem Mol Morphol. 2011 Oct;19(5):460-9.

Liver, kidney, spleen, colon and breast

**TABLE 1.** Antibody Clones and Dilutions and Epitope Retrieval Procedures

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| CD138 | B-A38 | Ventana | RTU | CC1mild |
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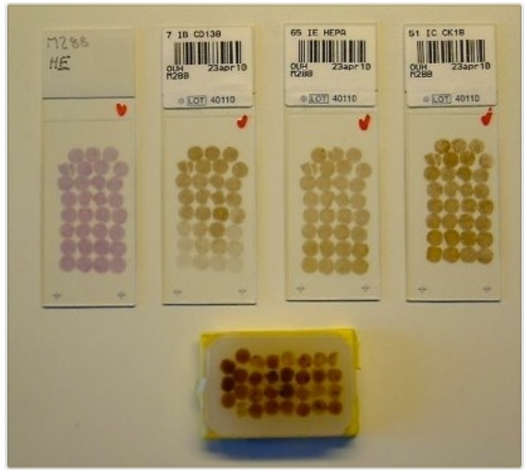
| CK18 | DC10 | Dako | 1:25 | CC1std |
| CK20 | SP33 | Ventana | RTU | CC1std |
| E-Cad | ECH-6 | Ventana | RTU | CC1std |
| E-Cad | HECD-1 | Abcam | 1:50 | CC1std |
| ER | SP1 | Ventana | RTU | CC1std |
| HEPA | OCH1E5 | Ventana | RTU | CC1mild |
| HLA-DR | CR3/43 | Dako | 1:200 | CC1mild |
| Ki67 | 30-9 | Ventana | RTU | CC1std |
| Lamins A/C | EPR4100 | Epitomics | 1:4000 | CC1std |
| PgR | 1E2 | Ventana | RTU | CC1mild |
| Villin | CWWB1 | Ventana | RTU | CC1std |



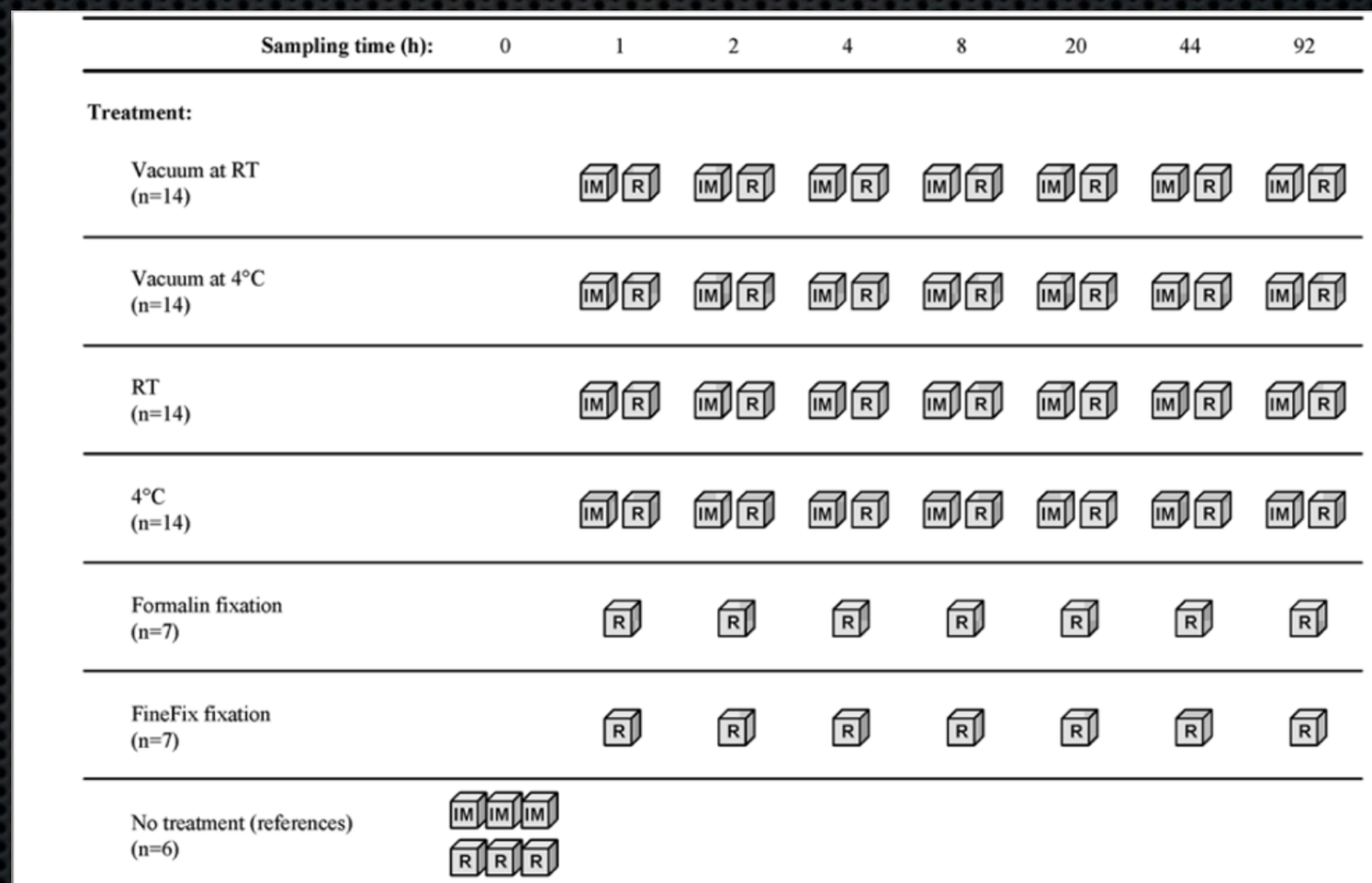




**FIGURE 1.** A total of 70 experimental samples and 6 reference samples were included for each of the 5 organ types included in the study. The 6 reference samples were collected at the experimental time = 0 hour. Experimental samples were collected after 1, 2, 4, 8, 20, 44, or 92 hours. Samples labeled "IM" were formalin fixed for approximately 48 hours before further processing and paraffin embedding and analyzed with respect to immunohistochemical and morphologic endpoints. Samples labeled "R" were snap frozen and stored at −80°C until subsequent RNA extraction and analysis of RNA integrity. Samples labeled "R" were not analyzed with respect to tissue morphology or epitope integrity. RT indicates room temperature.

# Results - Morphology and IHC

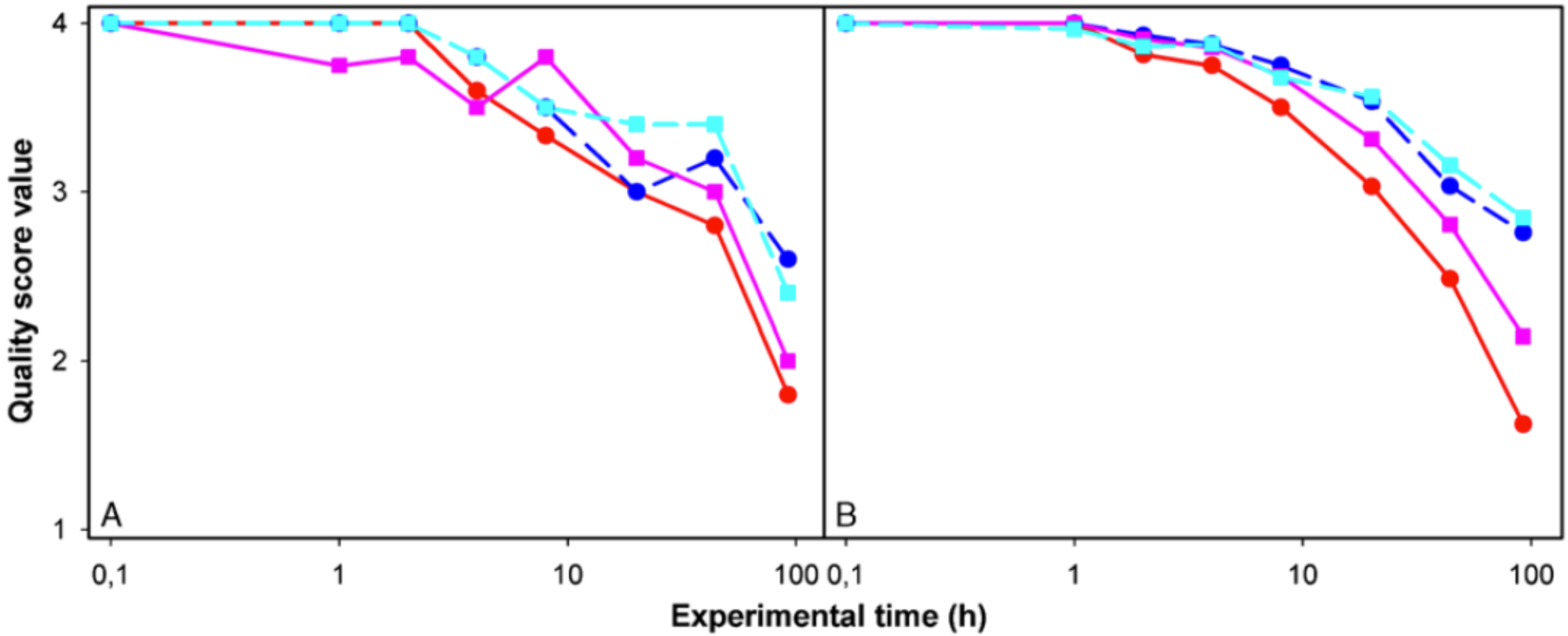
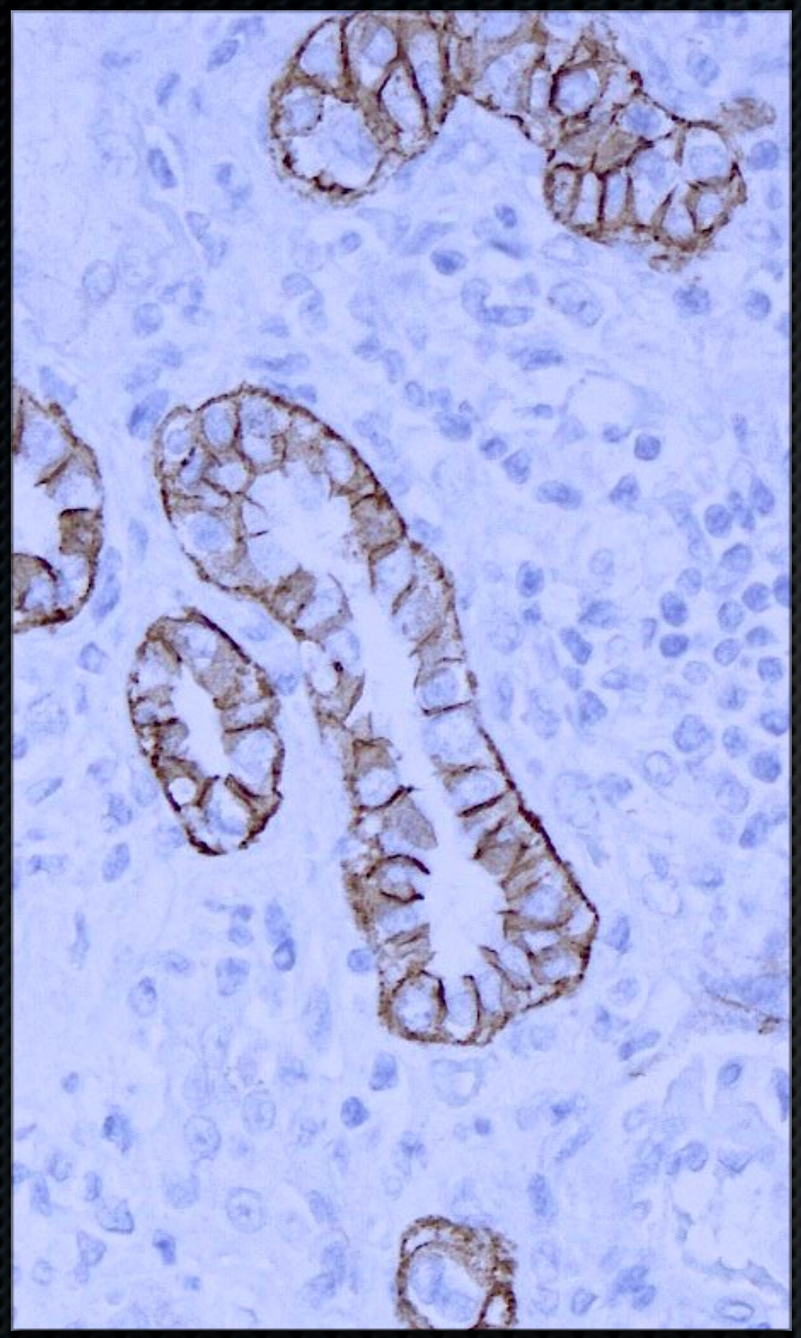
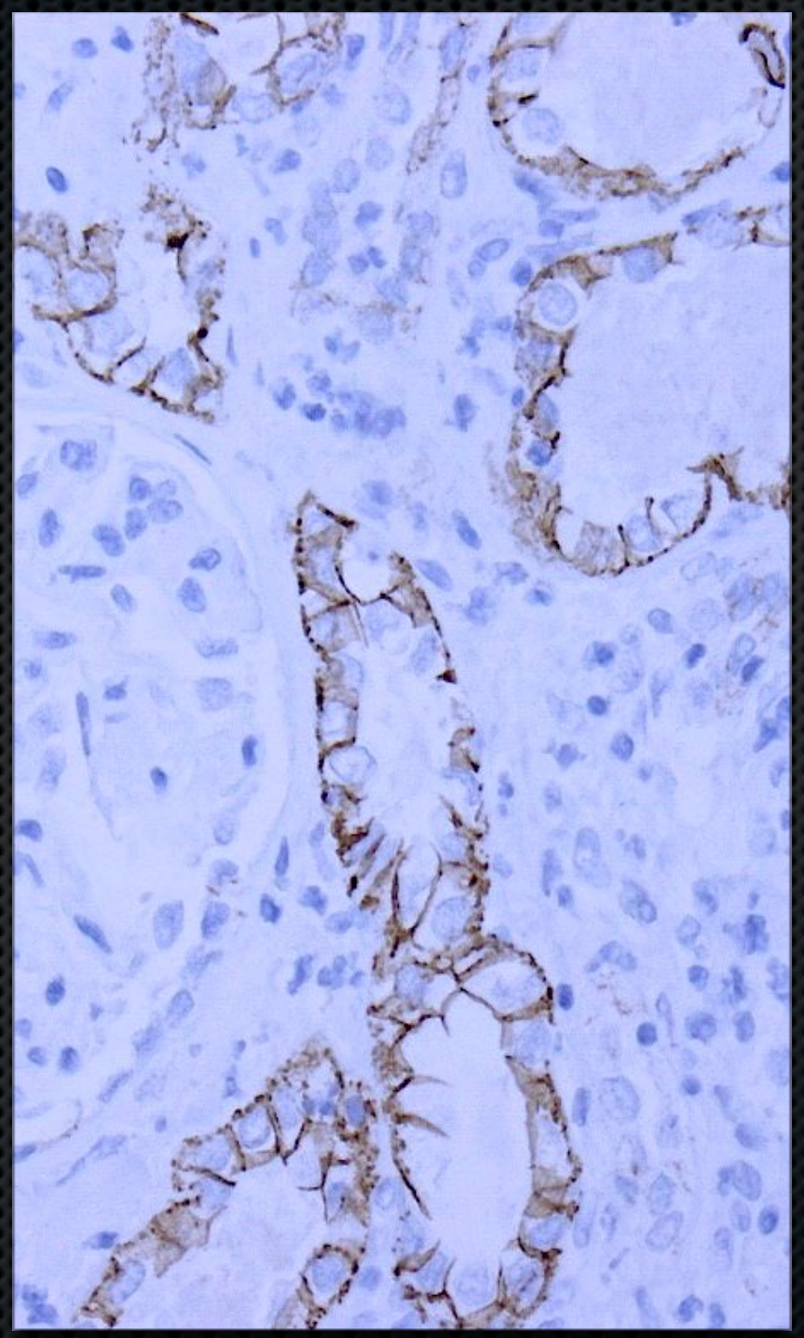



**FIGURE 4.** A, Morphologic integrity scores as a function of time in the 4 treatment groups: room temperature with vacuum ((—●—)), room temperature without vacuum ((—■—)), 4°C with vacuum ((- - ● - -)), and 4°C without vacuum ((- - ■ - -)). Each data point represents the mean of the score values in the 5 tissues. B, IHC staining quality scores as a function of time in the 4 treatment groups. Each data point represents the mean of all score values from all antibodies in all 5 tissues. Quality score value 4 corresponds to optimal, 3 corresponds to good, 2 corresponds to borderline, and 1 corresponds to poor morphologic integrity or IHC staining quality. Experimental time = 0 hour is depicted as 0.1 hour in both panels. IHC indicates immunohistochemical.

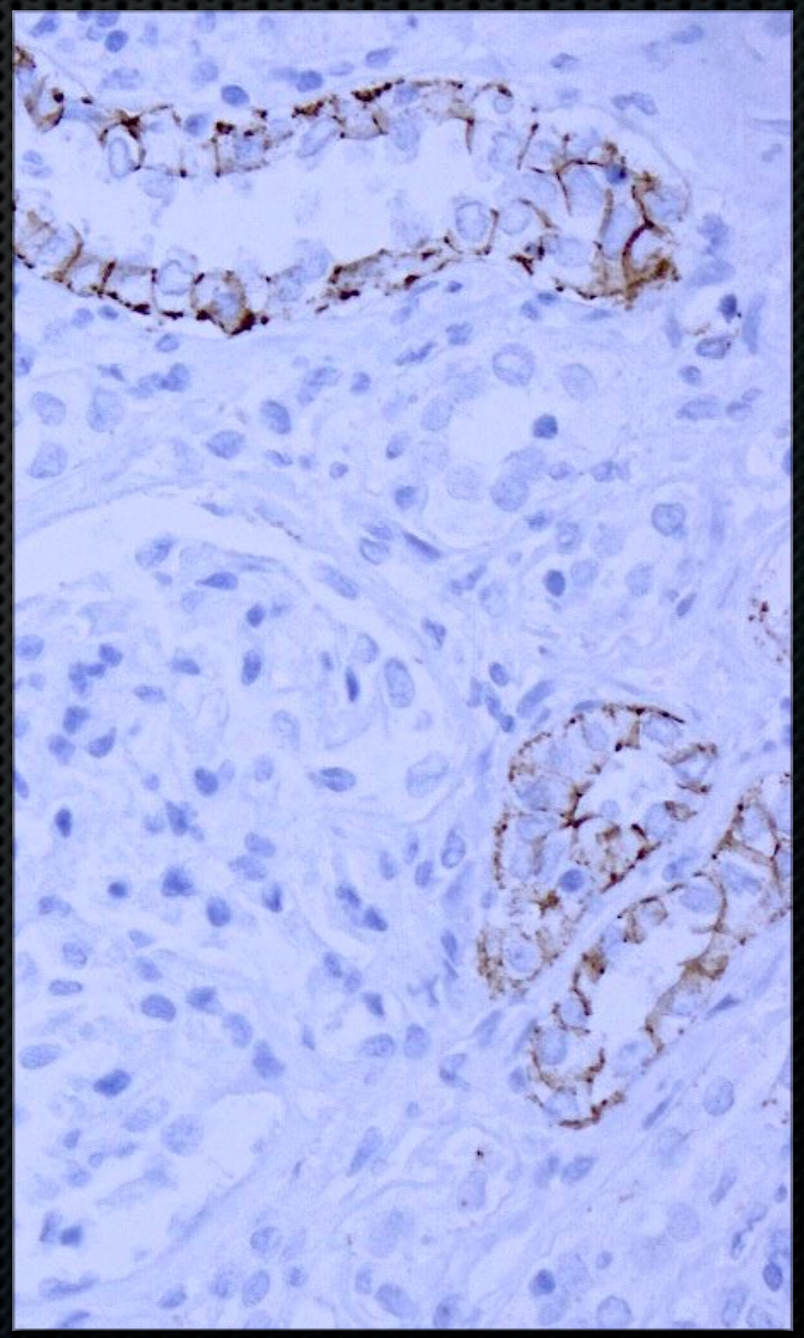
# E-Cadherin, HECD1 - Kidney



Ref. No delay

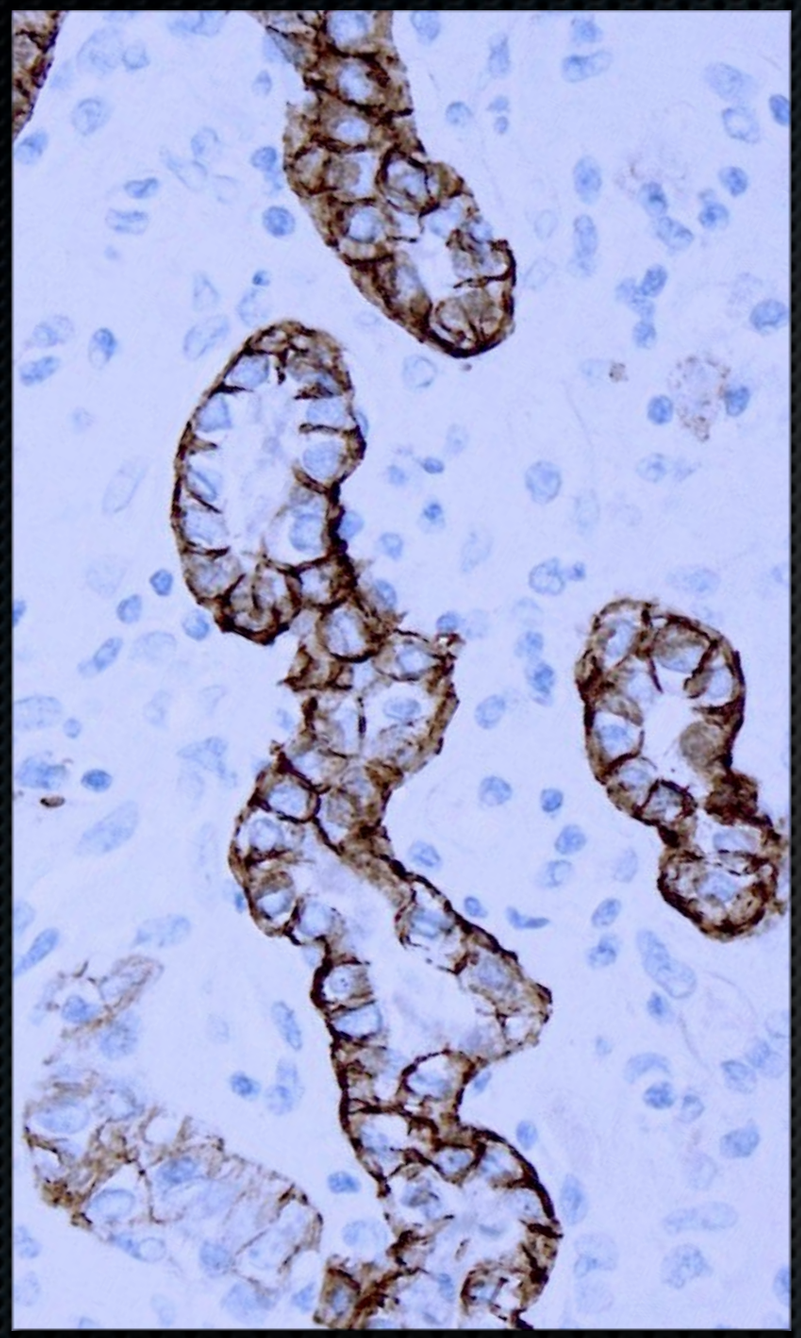


92hrs at 4°C/no vac

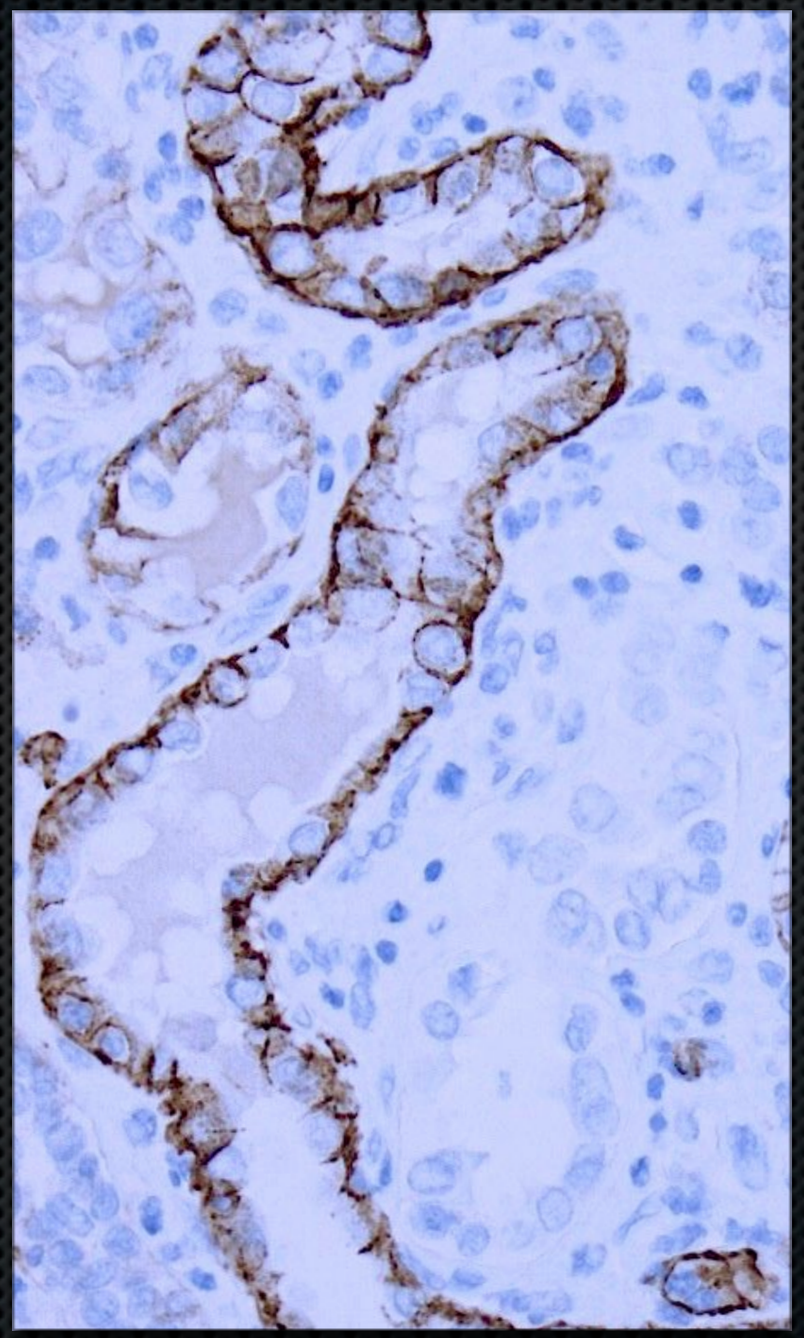


92hrs at 4°C/vac

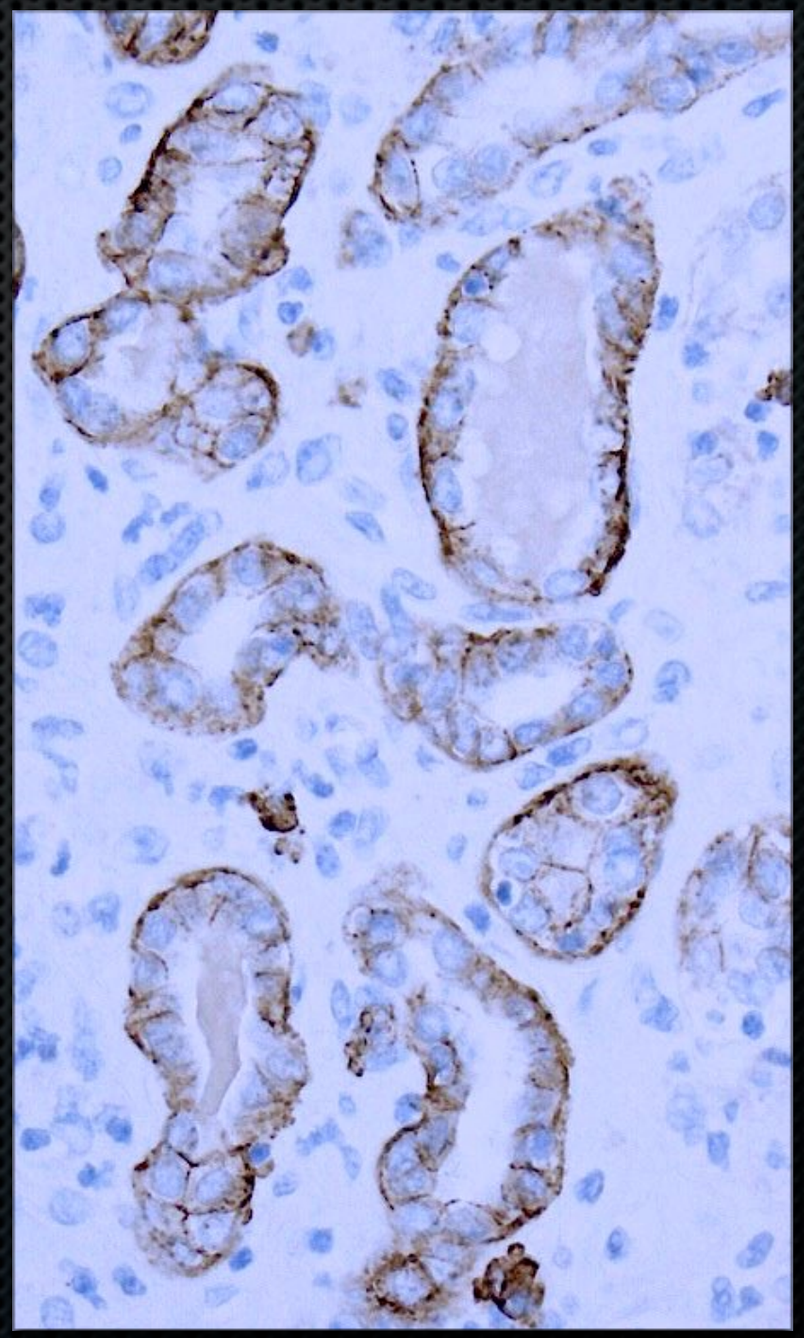
# CD138, B-A38 - Kidney



Ref. No delay

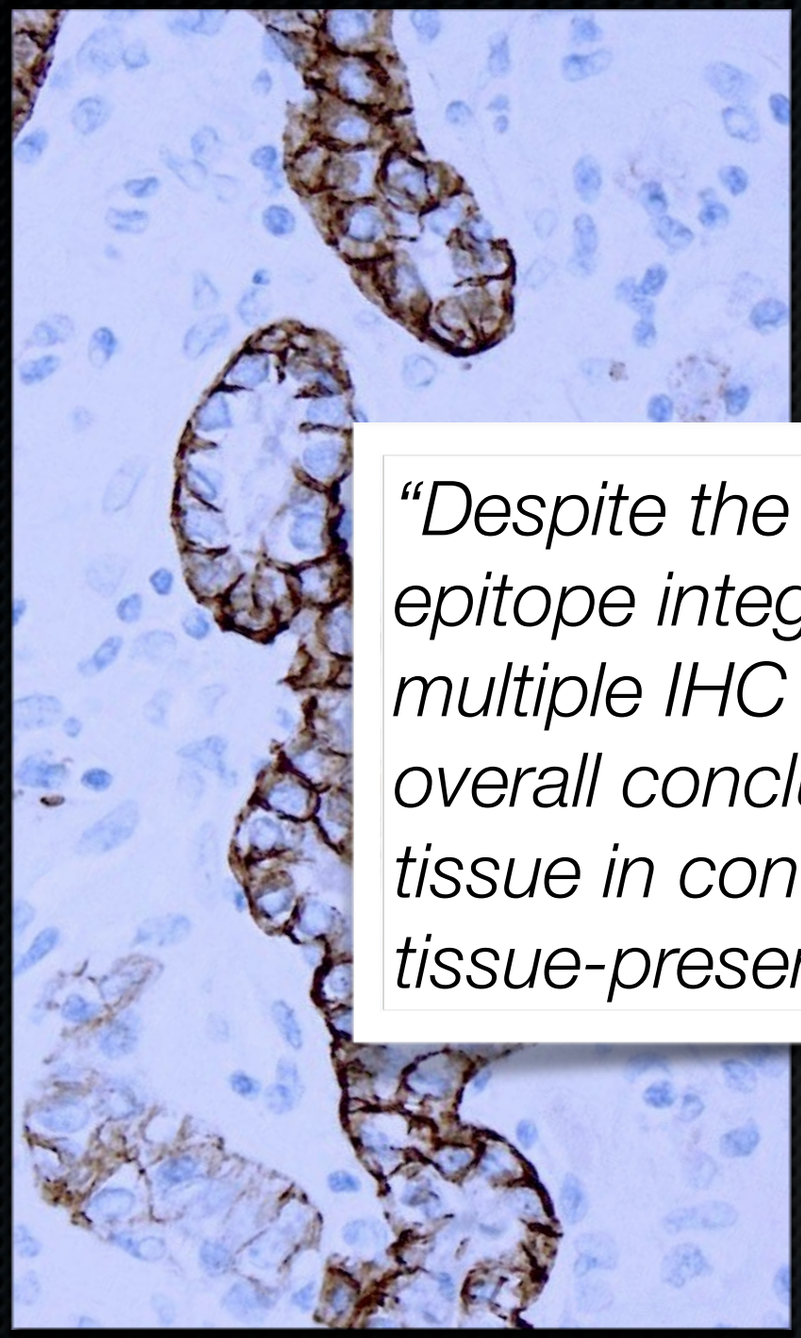


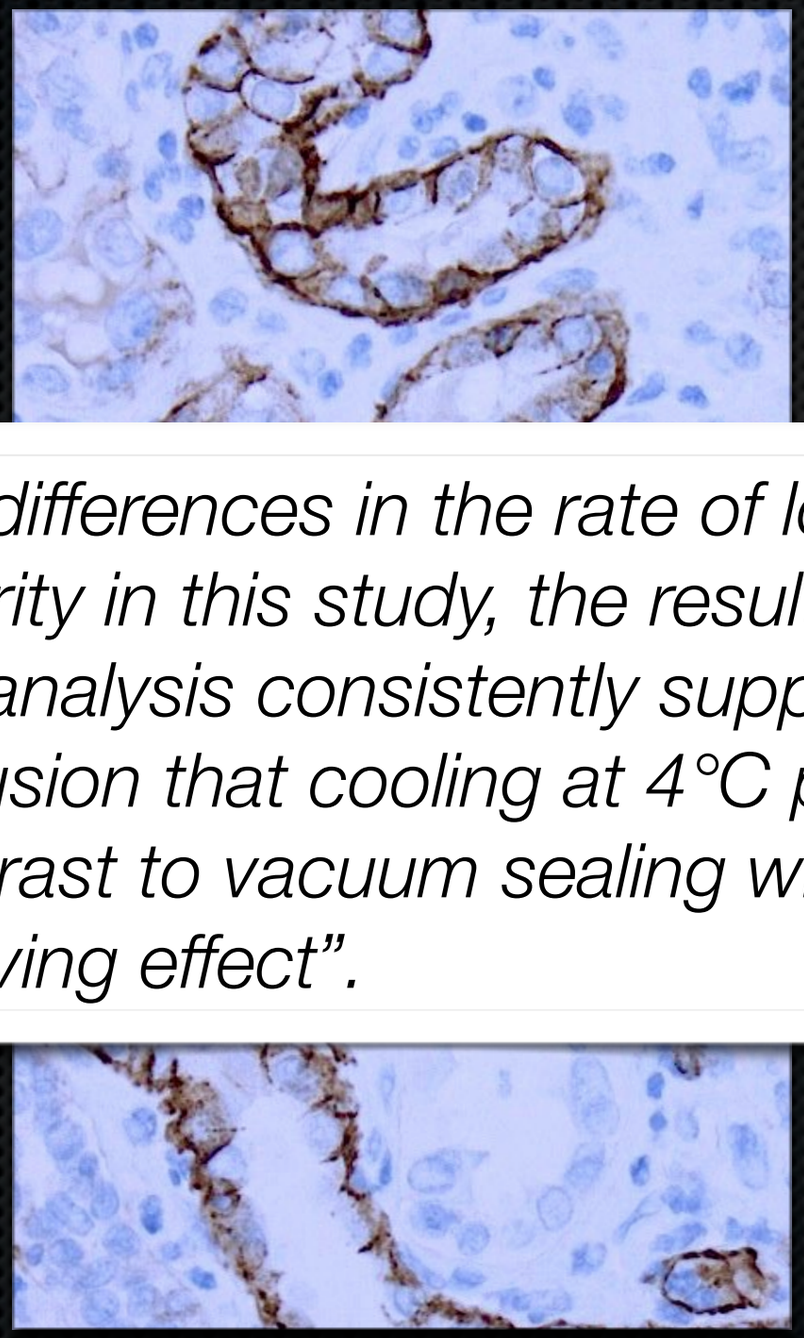
8hrs at 4°C/no vac

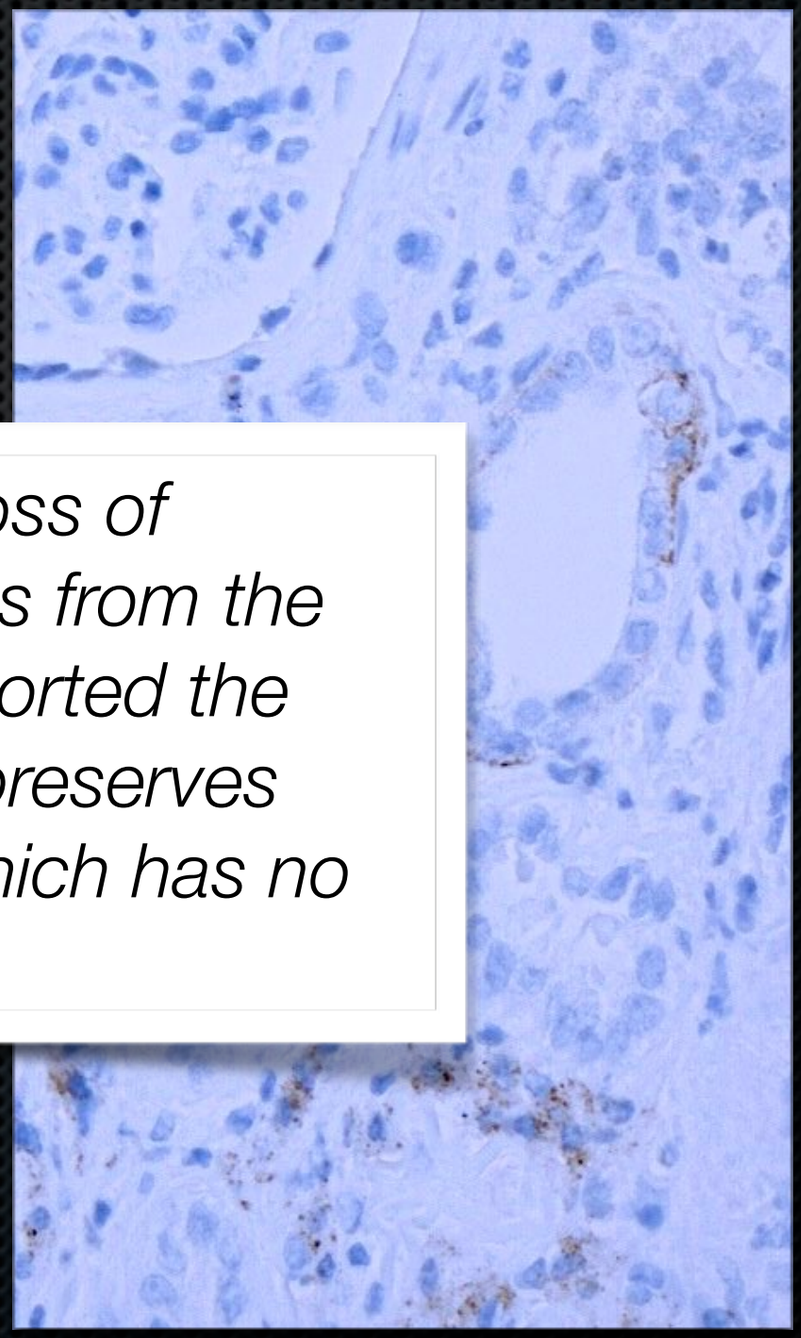


8hrs at 4°C/vac

# CD138, B-A38 - Kidney







*"Despite the differences in the rate of loss of epitope integrity in this study, the results from the multiple IHC analysis consistently supported the overall conclusion that cooling at 4°C preserves tissue in contrast to vacuum sealing which has no tissue-preserving effect".*

Ref. No delay

8hrs at 4°C/no vac

92hrs at 4°C/vac

# Under-Vacuum Sealed specimens and temperature

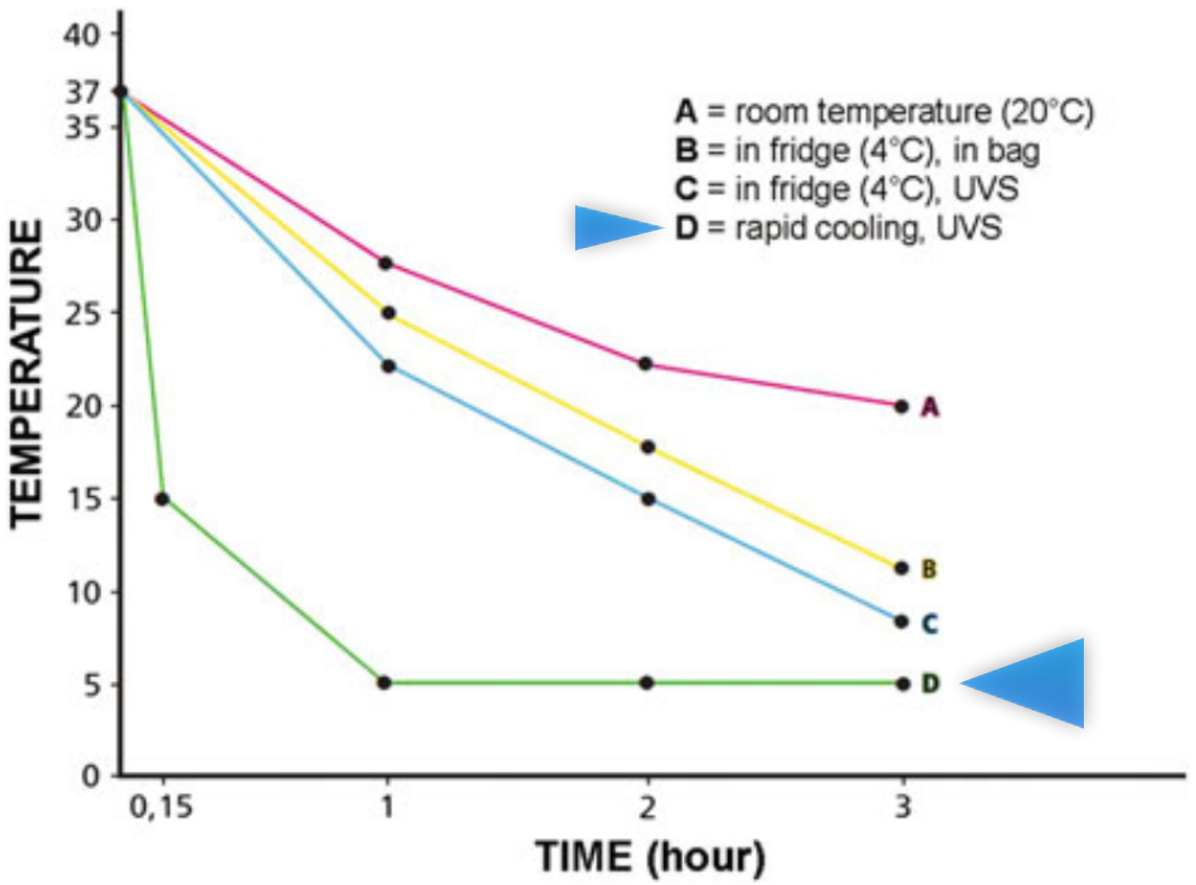



**Fig. 3** Variations of the inner temperature in a surgical specimen (approximately 7 cm in diameter) in different conditions. From the body temperature, on surgical removal, if left at room temperature (*A*), it takes approximately 3 h for the specimen to stabilize at the external temperature. When the specimen is left, free, in a fridge (*B*), the inner temperature will gradually go down. The cooling process in Under-Vacuum Sealed (UVS) specimens (*C*) is facilitated by the lack of insulating air. By using cooling (-20°C) elements (*D*), the cooling of the UVS specimens is very rapid

# Fixation delay

| Preanalytic variable | Published Guidelines and Recommendations<br>ASCO/CAP \| CLSI | Literature-Based Recommendations |
|---|---|---|
| Fixation delay | **Less than 1 hr** | Less than 12 hrs<br>**4°C is better than RT** |

Engel KB, Moore HM. Arch Pathol Lab Med. 2011;135:537-543

**ASCO/CAP**: American Society of Clinical Oncology/College of American Pathologists

I/LA28-A2
Vol. 31 No. 4
Replaces MM04-A
Vol. 19 No. 26

# Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition

Stephen M. Hewitt, MD, PhD
Max Robinowitz, MD
Steven A. Bogen, MD, PhD
Allen M. Gown, MD
Krishan L. Kalra, PhD
Christopher N. Otis, MD
Betsy Spaulding
Clive R. Taylor, MD, DPhil

+ A long list of experts and advisors

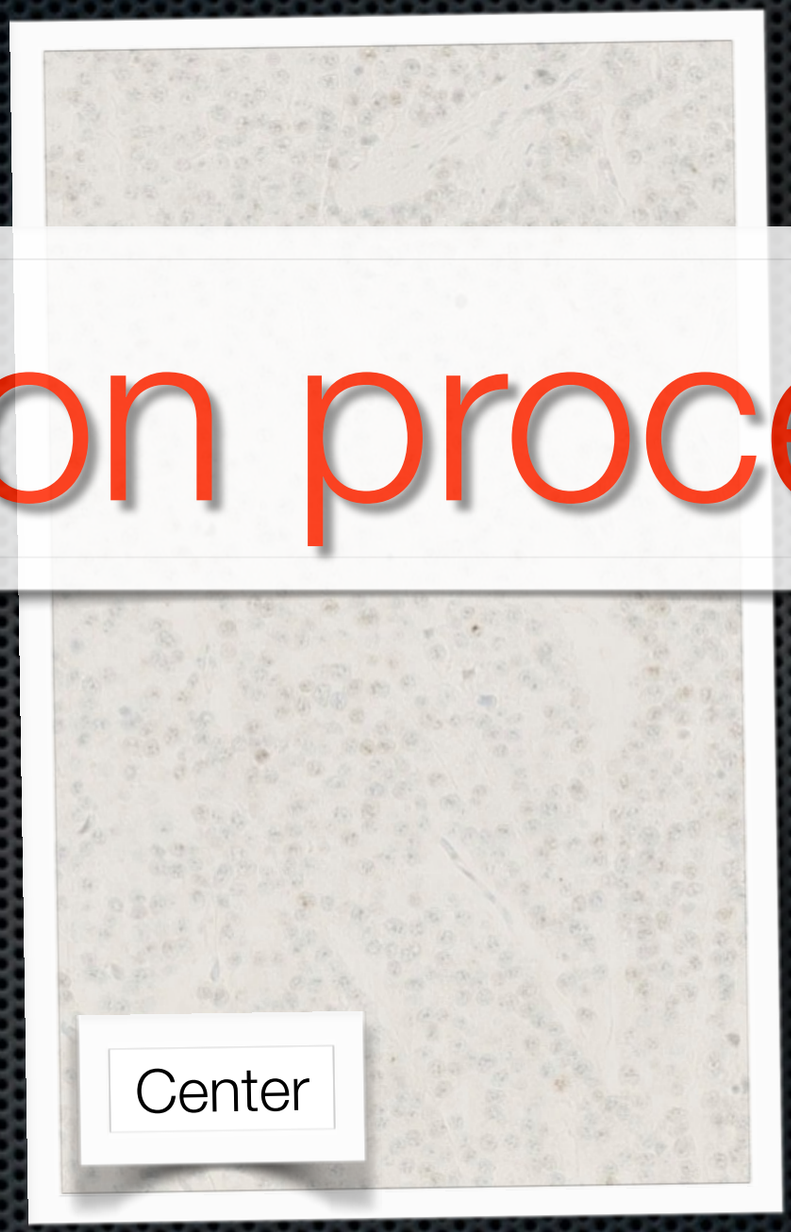
This document provides guidelines for the development of validated diagnostic, prognostic, and predictive immunohistochemical assays.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

( CLSI )

# Seminoma: Biology or Artefact?





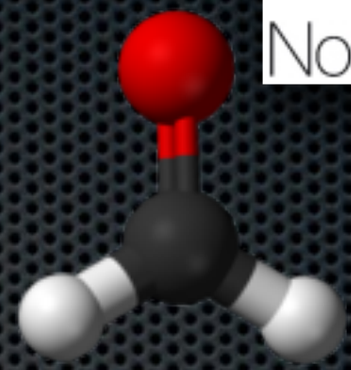
## Fixation procedure?

PMS2, EPR3947

# Fixation procedure

- Fixative
  - Formula
  - Concentation
  - pH
- Fixation
  - Tissue to fixative ratio
  - Method (Immersion, MWO, sonication, movement etc)
  - Time
  - Temperatur
- Postfixation
  - Washing conditions and duration
  - Storage reagent and duration

# Formaldehyde fixation



| | | |
|---|---|---|
| Phase 1 | Penetration | Very fast |
| Phase 2 | Binding | Very slow |
| Phase 3 | Cross-linking | Slow |

Formaldehyde obey the diffusion laws, that is, the depth penetrated is proportional to the square root of time.

## Penetration rate can be determind using the equation:

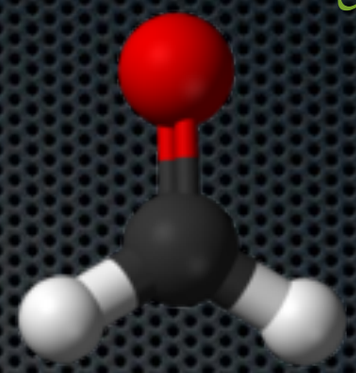
$$d = K\sqrt{t}$$

d = Distance penetrated in mm
K = Medawar's coefficient of diffusibility
t = Time in hours

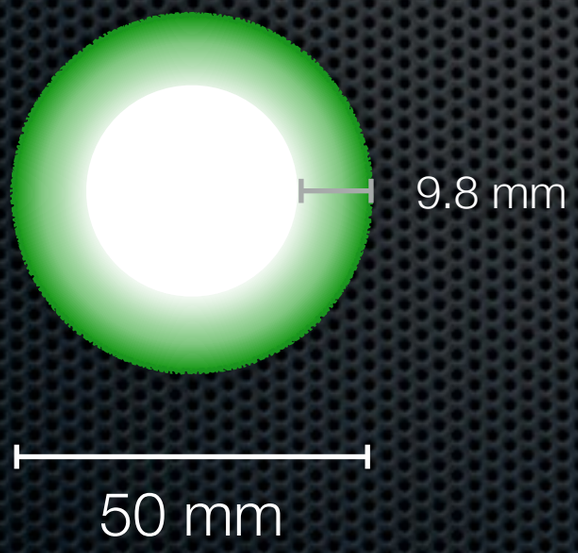
Medawar's K = 5,5
Alternative:
Baker's K = 3,6
Hewletts K = 2,0

# Formaldehyde fixation

Region Syddanmark
OUH
Afd. f. Klinisk Patologi
*O. Nielsen*




Fixation:
NBF 24 hrs




Penetration rate can be determind using the equation: $d = K\sqrt{t}$

Medawar's K = **5,5**
Baker's K = **3,6**

Hewletts K = **2,0**:

1 second d = 0.033 mm (124 mm/hr)
1 minute d = 0.26 mm (15.5 mm/hr)
4 minutes d = 0.52 mm (7.8 mm/hr)
16 minutes d = 1.04 mm (3.9 mm/hr)
**1 hour d = 2.0 mm (2.0 mm/hr)**
4 hours d = 4.0 mm (averages to 1.0mm/hr),
8 hours d = 5.66 mm (averages to 0.7mm/hr),
16 hours d = 8.0 mm (averages to 0.5mm/hr),
24 hours d = 9.8 mm (averages to 0.41mm/hr),
96 hours d = 19.6 mm (averages to 0.2mm/hr).

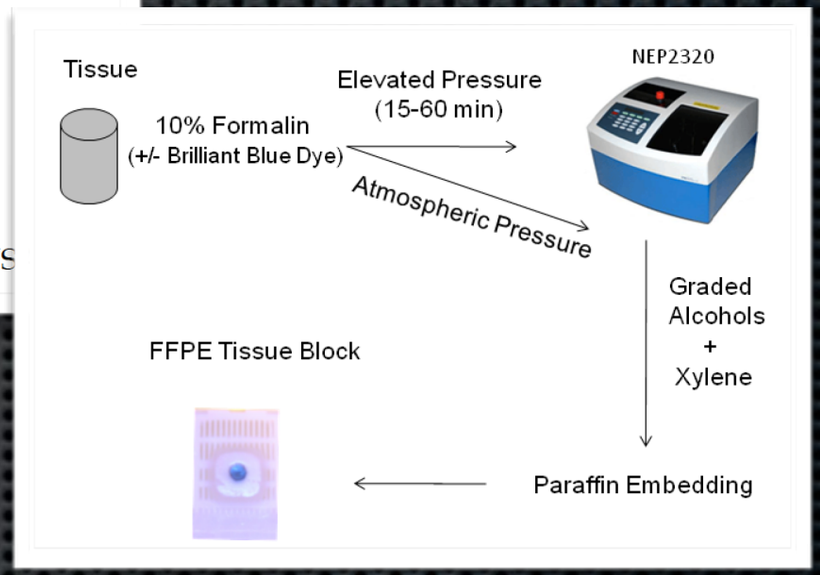
Journal of Cancer
2010; 1:178-183

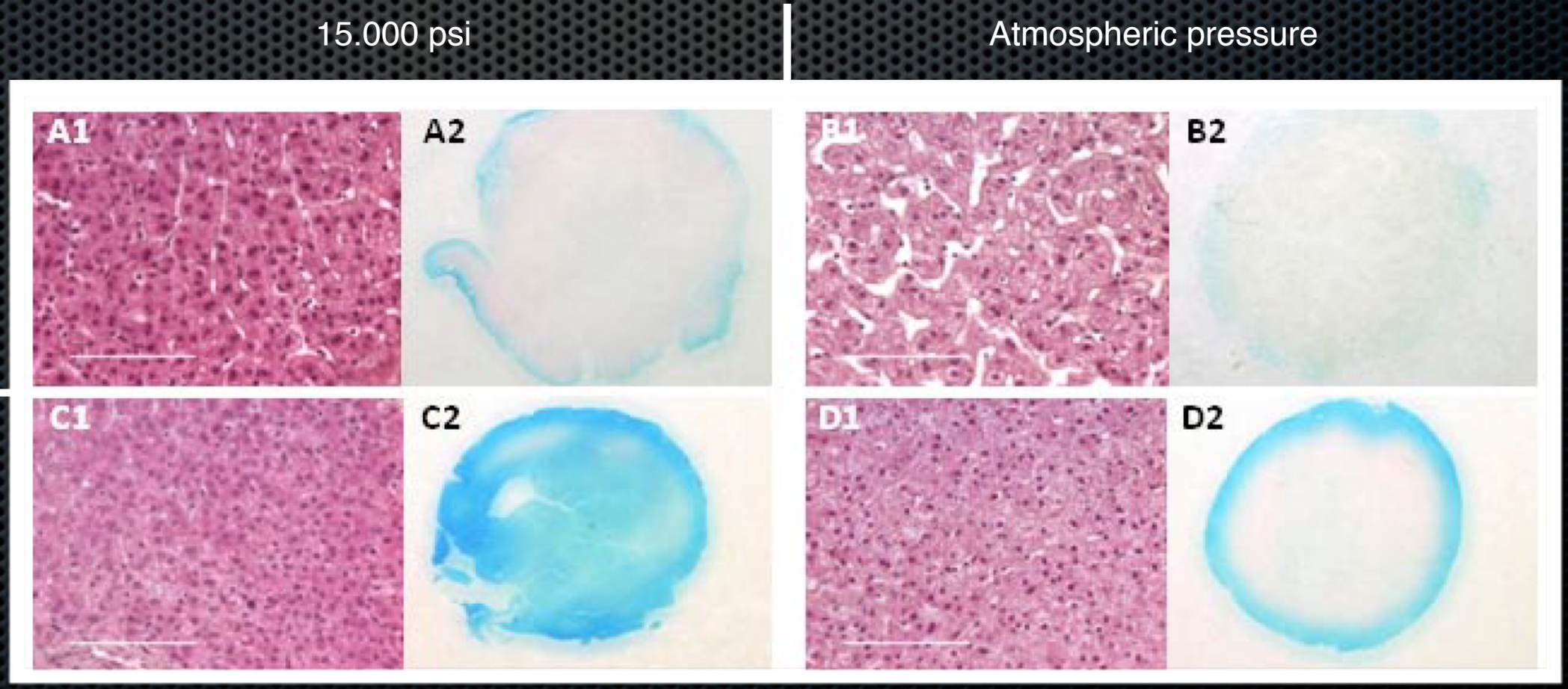

Short Research Communication

# Elevated Pressure Improves the Rate of Formalin Penetration while Preserving Tissue Morphology

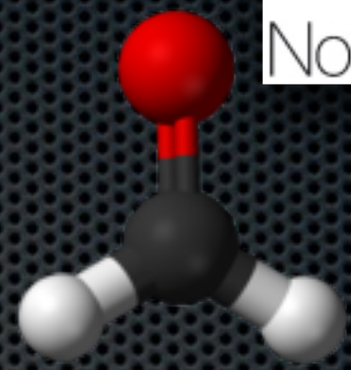
**Ingrid E. Chesnick[1], Jeffrey T. Mason[1], Timothy J. O'Leary[2], Carol B. Fowler[1,2] ✉**

1. Department of Biophysics, Armed Forces Institute of Pathology, Rockville, Maryland, USA;
2. Biomedical Laboratory Research and Development Service, Veterans Health Administration, Washington, DC, US



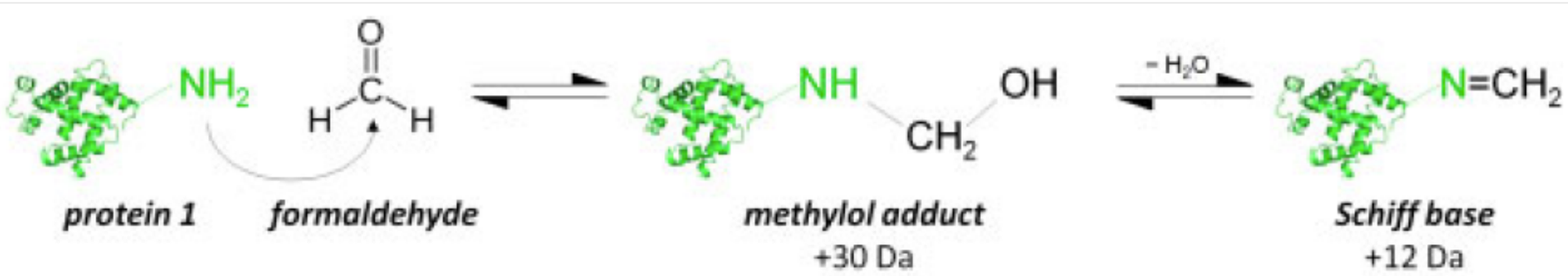



# Formaldehyde fixation



| | | |
|---|---|---|
| Phase 1 | Penetration | Very fast |
| Phase 2 | Binding | Very slow |
| Phase 3 | Cross-linking | Slow |

**Phase 2**

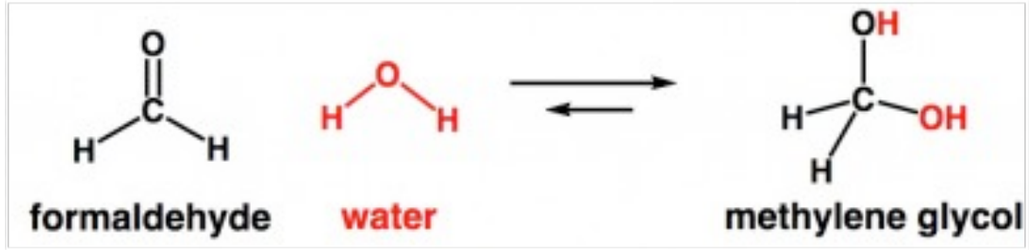



**Phase 3**

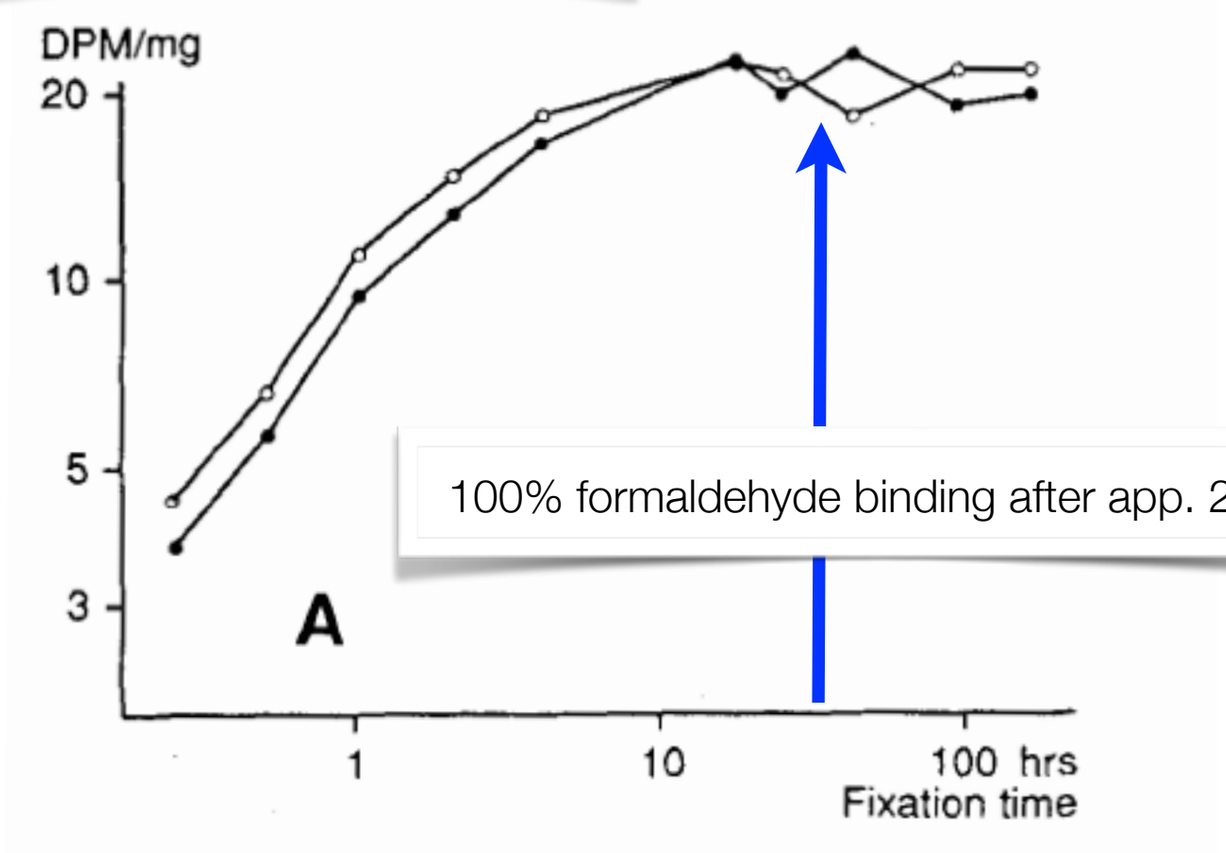
# Kinetic Studies of Formaldehyde Binding in Tissue

Biotechnique and Histochemistry. 1994; **69**, 177-179

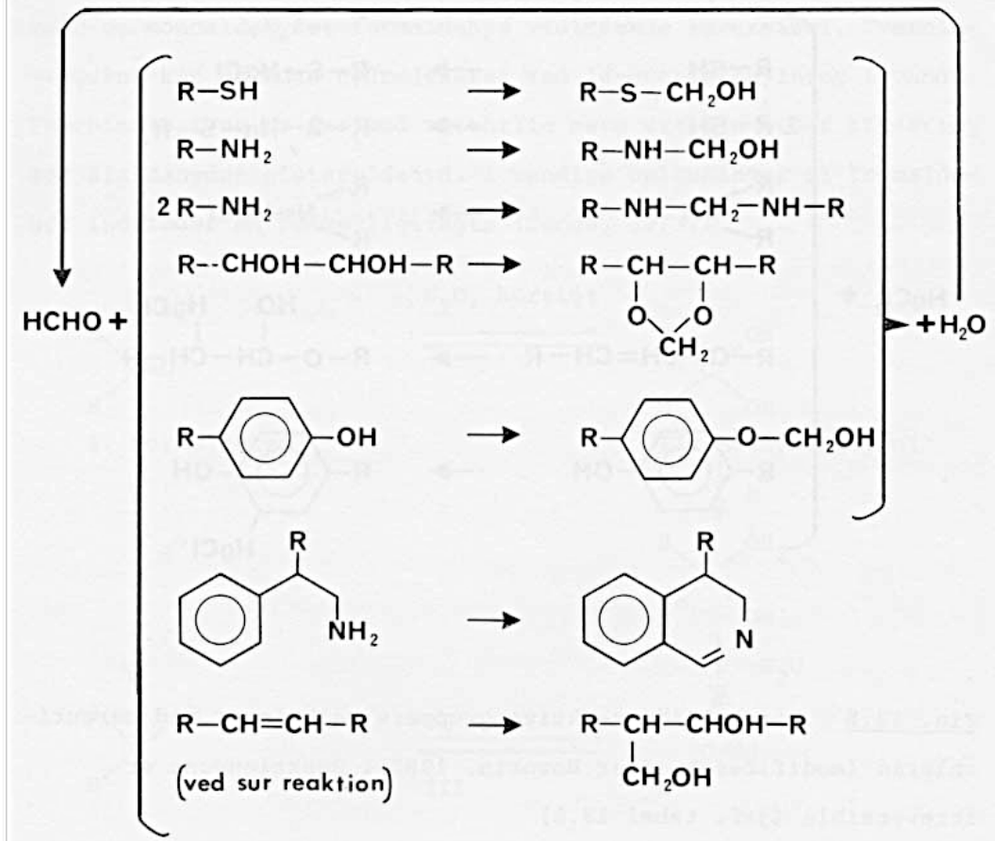
**Kerstin G. Helander**
*Laboratory of Membrane Biology, Center for Ulcer Research and Education, University of California, Los Angeles, California 90073*




4x4x4 mm liver tissue




100% formaldehyde binding after app. 25 hrs



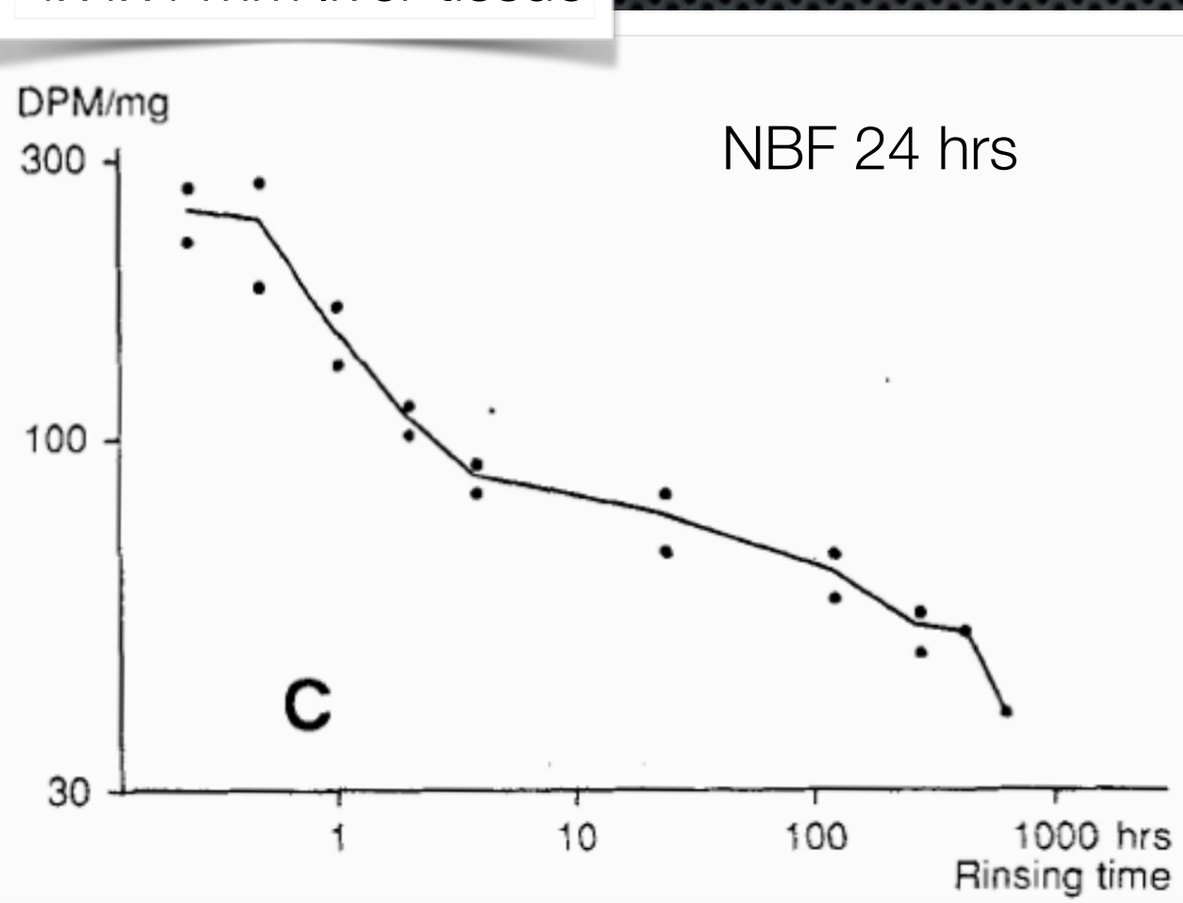
# Kinetic Studies of Formaldehyde Binding in Tissue

**Kerstin G. Helander**
*Laboratory of Membrane Biology, Center for Ulcer Research and Education, University of California, Los Angeles, California 90073*

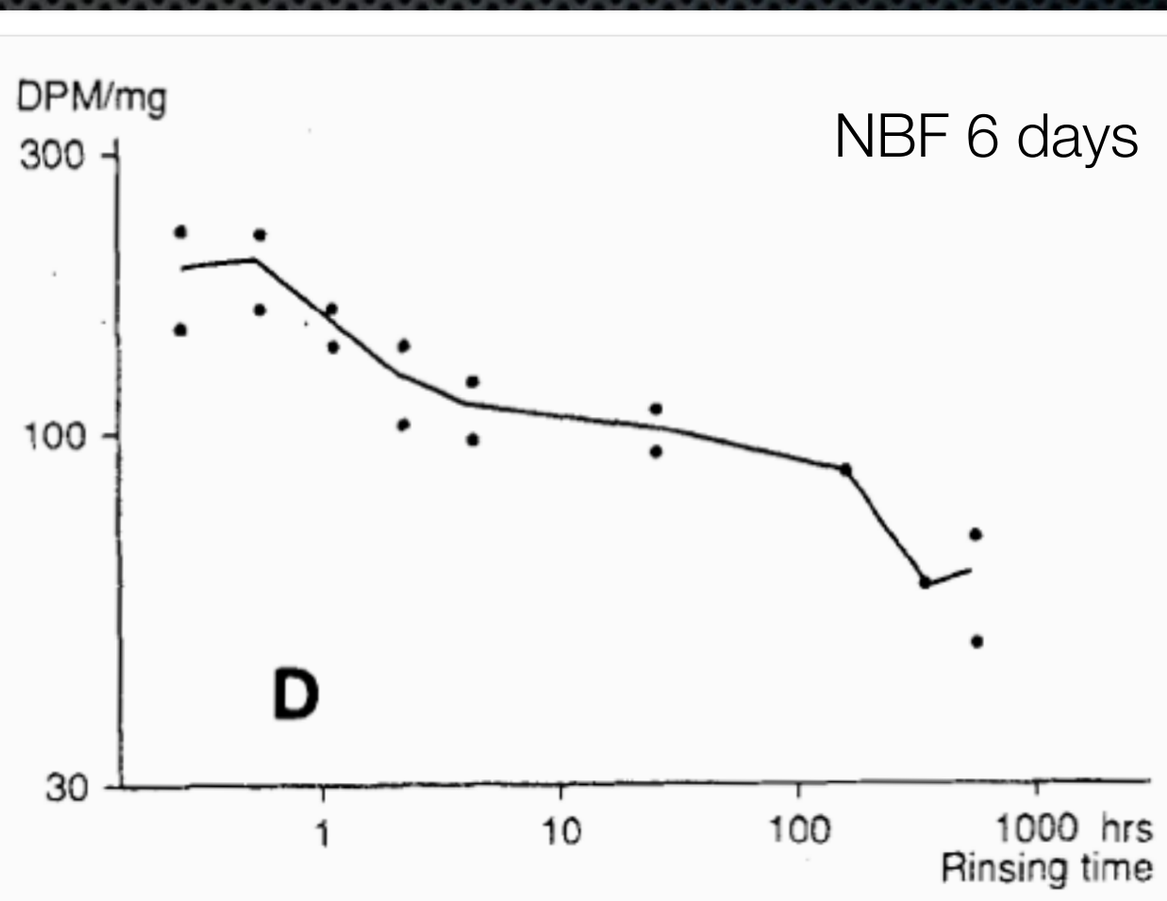
Biotechnique and Histochemistry. 1994; **69**, 177-179

# Formaldehyde binding is reversible:

4x4x4 mm liver tissue



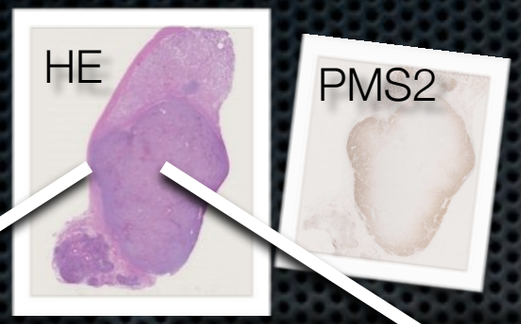

NBF 24 hrs

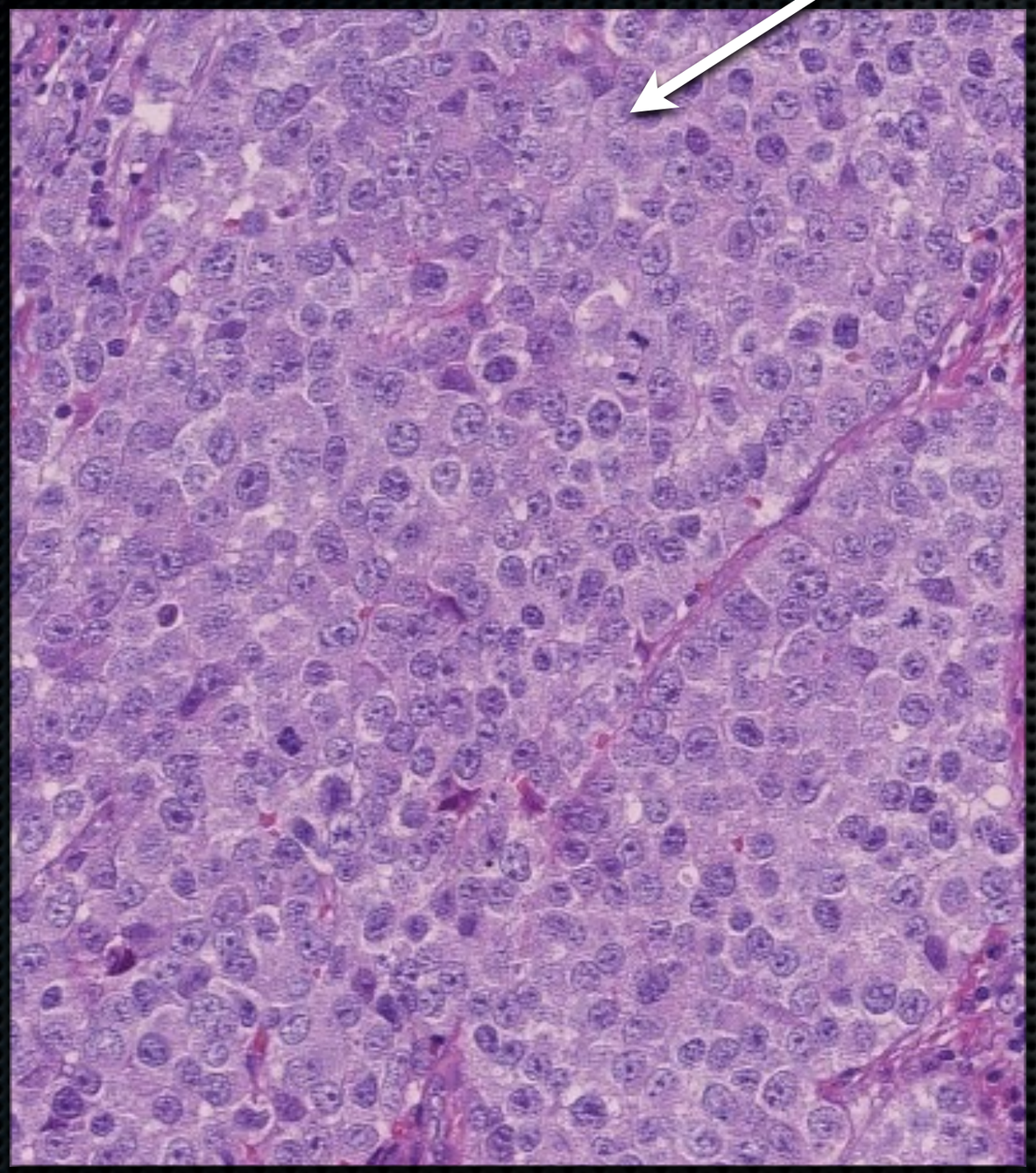



NBF 6 days

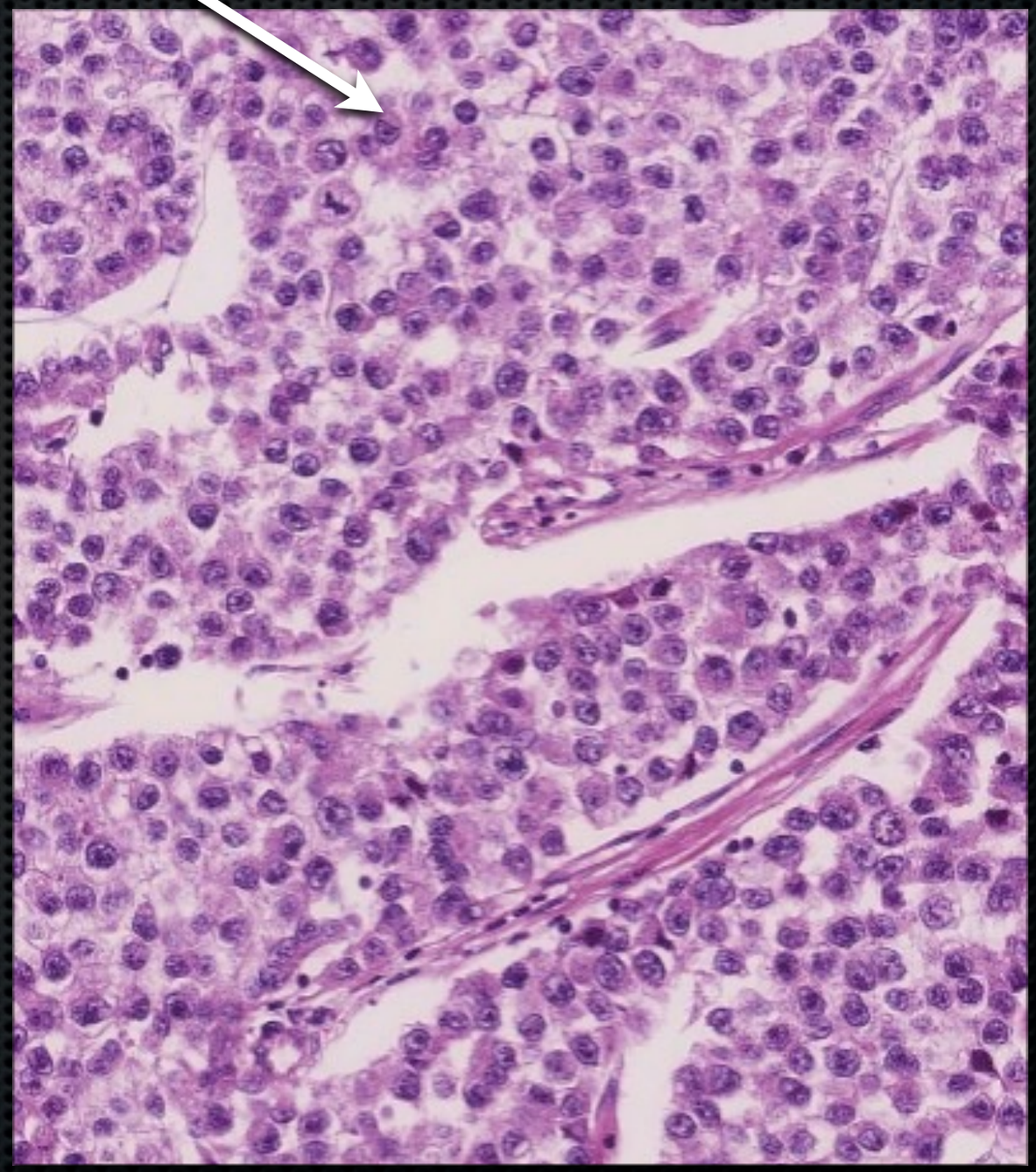
Rinsing with $dH_2O$

# Seminoma



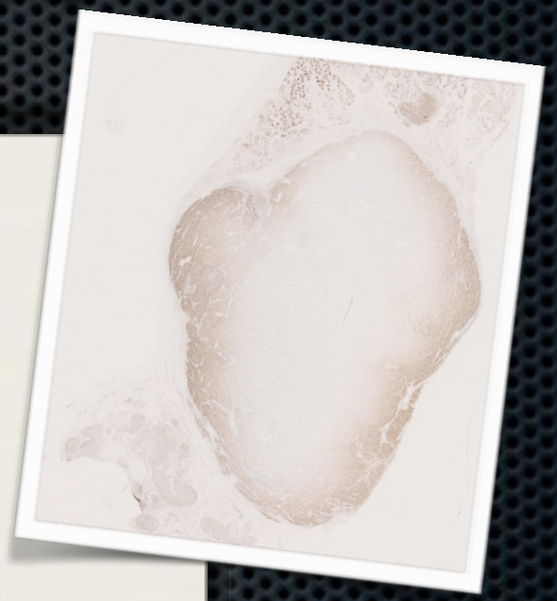
Edge



Center

# PMS2, EPR3947 and fixatives

Clone EPR3947 can not be used on alcohol-fixed tissue



Bouin 24 hrs

Zamboni 24 hrs

Clarke 24 hrs

Carnoy 24 hrs

Methacarn 24 hrs

Form/Zn 24 hrs

Ethanol 24 hrs

NBF168 hrs

NBF 48 hrs

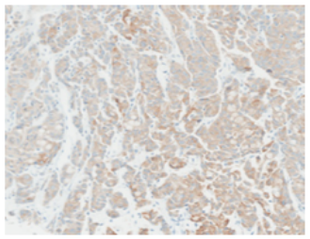
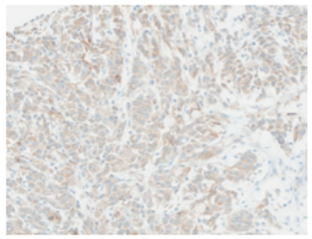
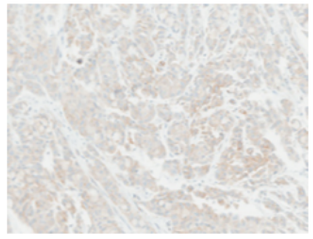
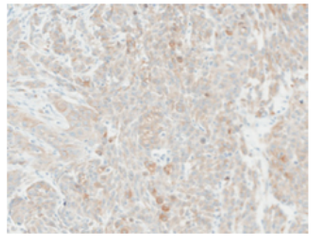
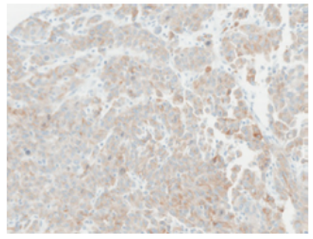
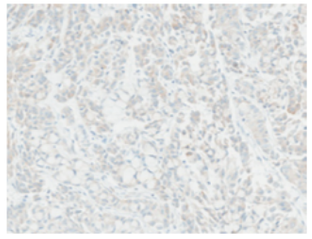
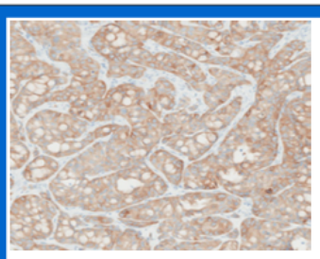
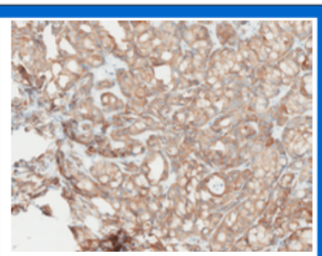
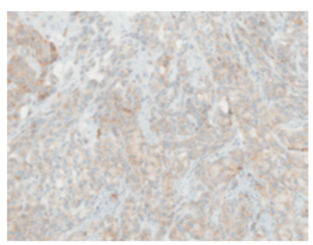
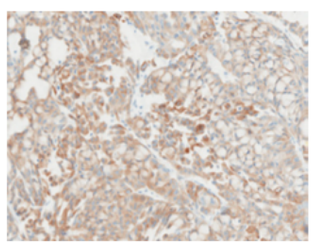
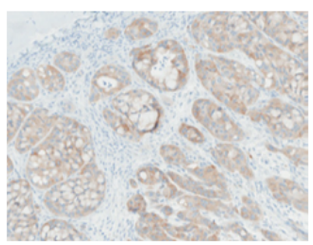
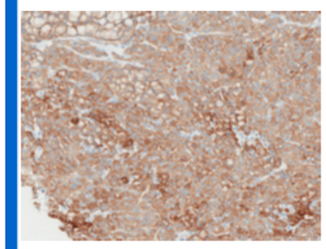
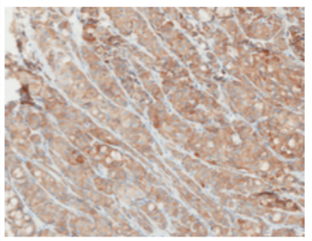
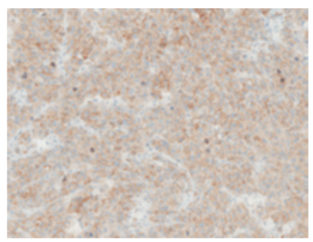
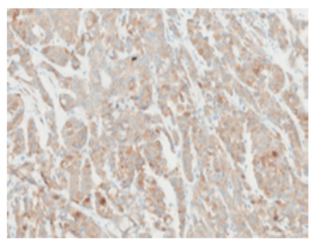
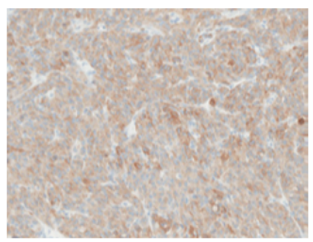
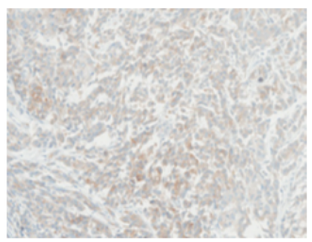
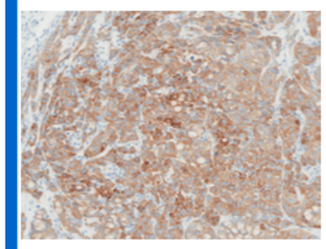
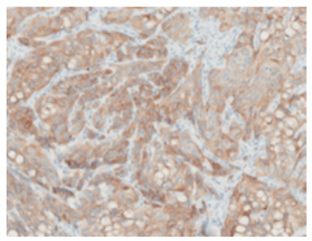
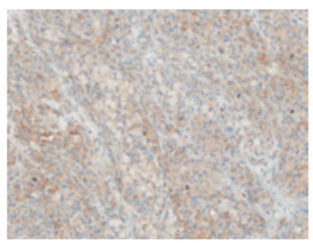
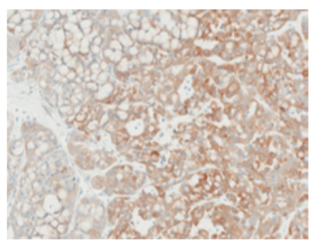
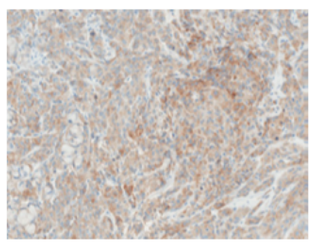
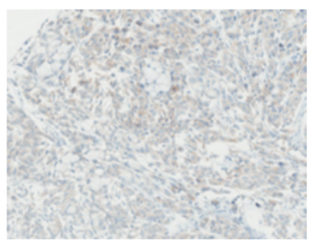
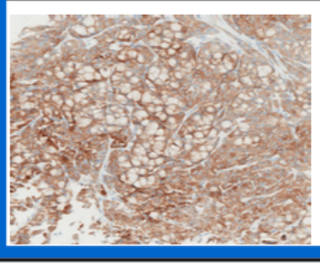
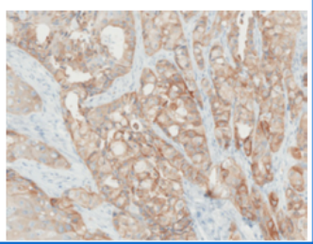
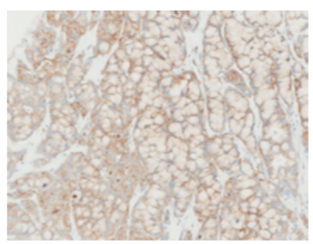
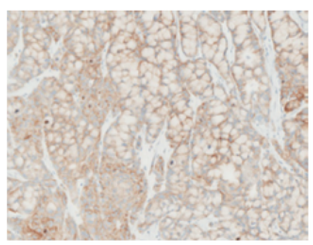
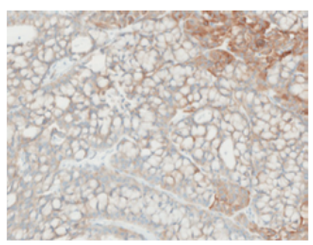
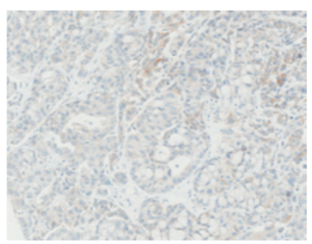
NBF 24 hrs

NBF 6 hrs

# Impact of Pre-Analytical Conditions on VENTANA anti-ALK (D5F3) IHC Assay Staining

## Fixative Recommendations to Achieve Optimal Staining Results with VENTANA anti-ALK (D5F3) IHC Assay

**VENTANA anti-ALK (D5F3) IHC Assay Staining of Tissue Across Fixatives and Fixation Times**

| Fixation Time (Hrs) | Fixative | | | | | |
|---|---|---|---|---|---|---|
| | 10% NBF | Zinc Formalin | PREFER | B5 | AFA | 95% Ethanol |
| 1* |  |  |  |  |  |  |
| 8 |  |  |  |  |  | Tumor cut through. No image available. |
| 12 |  |  |  |  |  |  |
| 24 |  |  |  |  |  |  |
| 72 |  |  |  |  |  |  |

# Minimum Formalin Fixation Time for Consistent Estrogen Receptor Immunohistochemical Staining of Invasive Breast Carcinoma

*Neal S. Goldstein, MD, Monica Ferkowicz, MT(ASCP), PathA(AAPA), Eva Odish, HTL(IHQ), Anju Mani, MD, and Farnaz Hastah, MD*

Am J Clin Pathol 2003;120:86-92

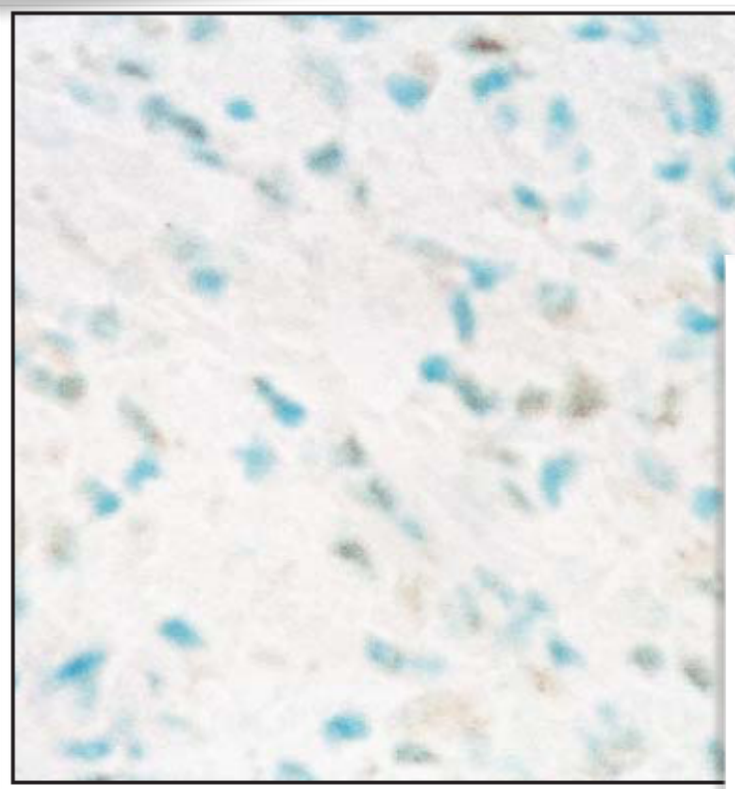


**Image 1** Fixation, 3 h; antigen retrieval, 40 min.

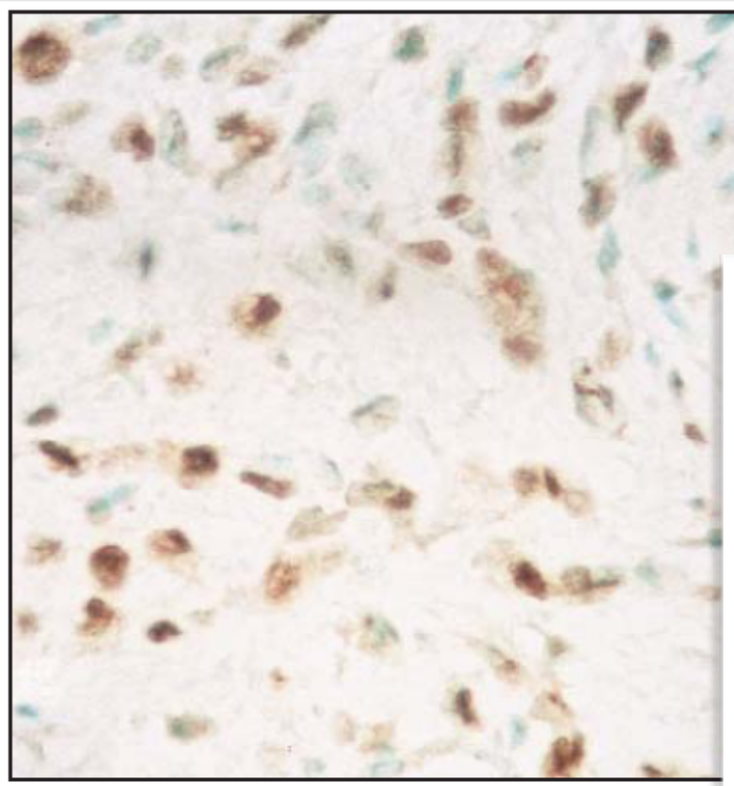


**Image 2** Fixation, 6 h; antigen retrieval, 40 min.

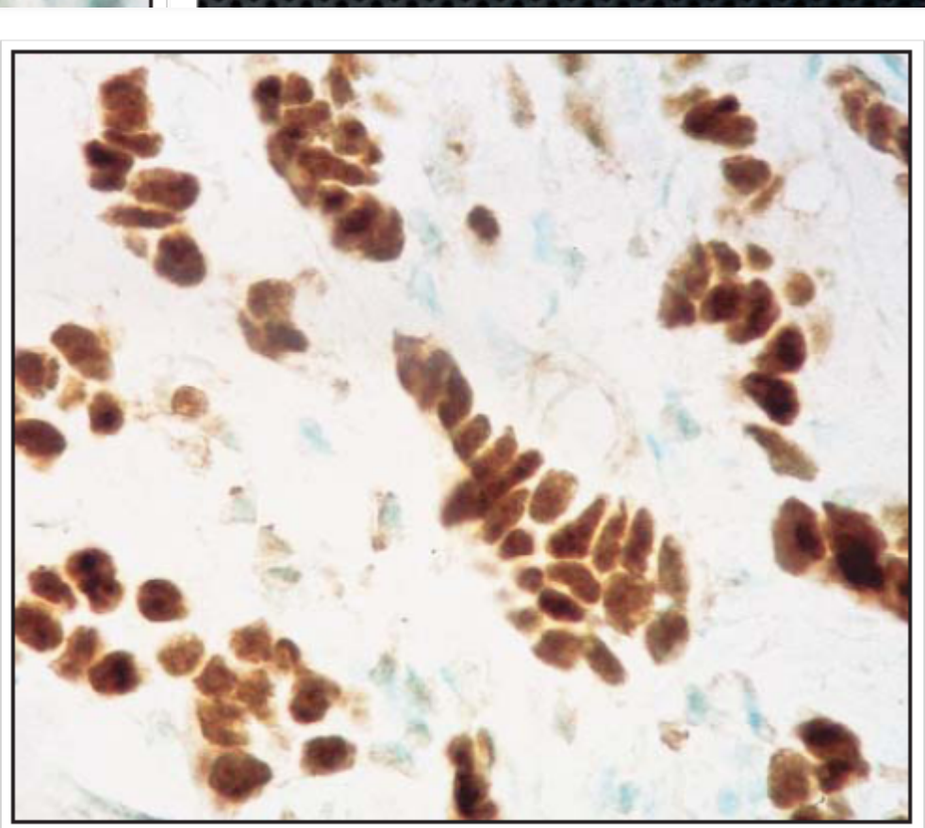


**Image 3** Fixation, 8 h; antigen retrieval, 40 min.

*"The minimum formalin fixation time for reliable immunohistochemical ER results is 6 to 8 hours in our laboratory, regardless of the type or size of specimen".*

(*Am J Surg Pathol* 2011;35:545–552)

# The Effect of Prolonged Fixation on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Cancer: A Prospective Study

*Leung Chu Tong, BA, MD,* Nahid Nelson, BSc, PhD,† Jim Tsourigiannis, BSc, M1 and Anna Marie Mulligan, MB, MSc, FRCPath*†*

**TABLE 1.** Antibodies and Conditions of Use

| | Clone | Source | Antigen Retrieval Time |
|---|---|---|---|
| ER | SP1 (Monoclonal) | Ventana | 30 min |
| PR | 1E2 (Monoclonal) | Ventana | 60 min |
| HER2 | A0485 (Polyclonal) | DAKO | 30 min |

Fixation i 4% NBF for 13 hours versus 79 hours

Concordance between short fixation and long fixation:

99 % Concordance for ER

95 % Concordance for PR

98 % Concordance for HER2

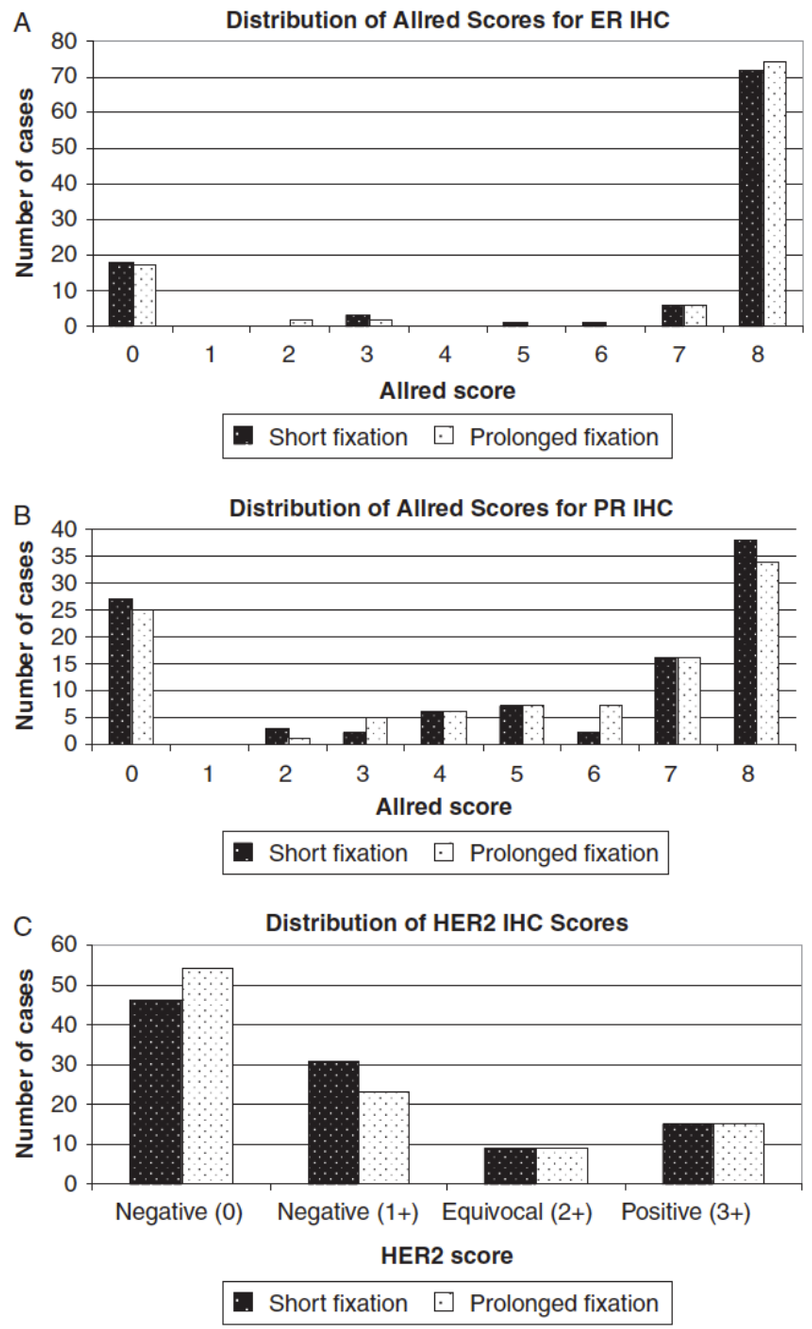
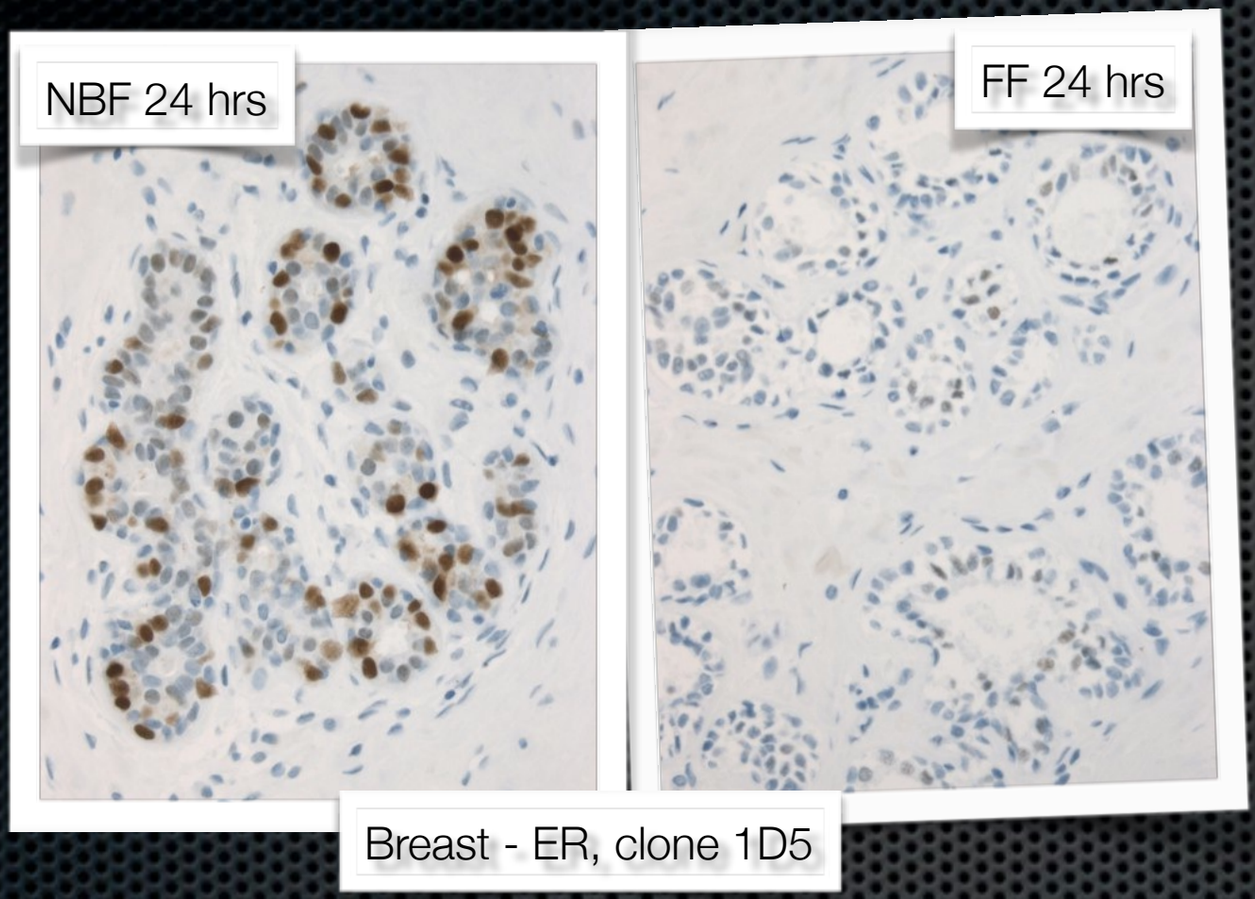
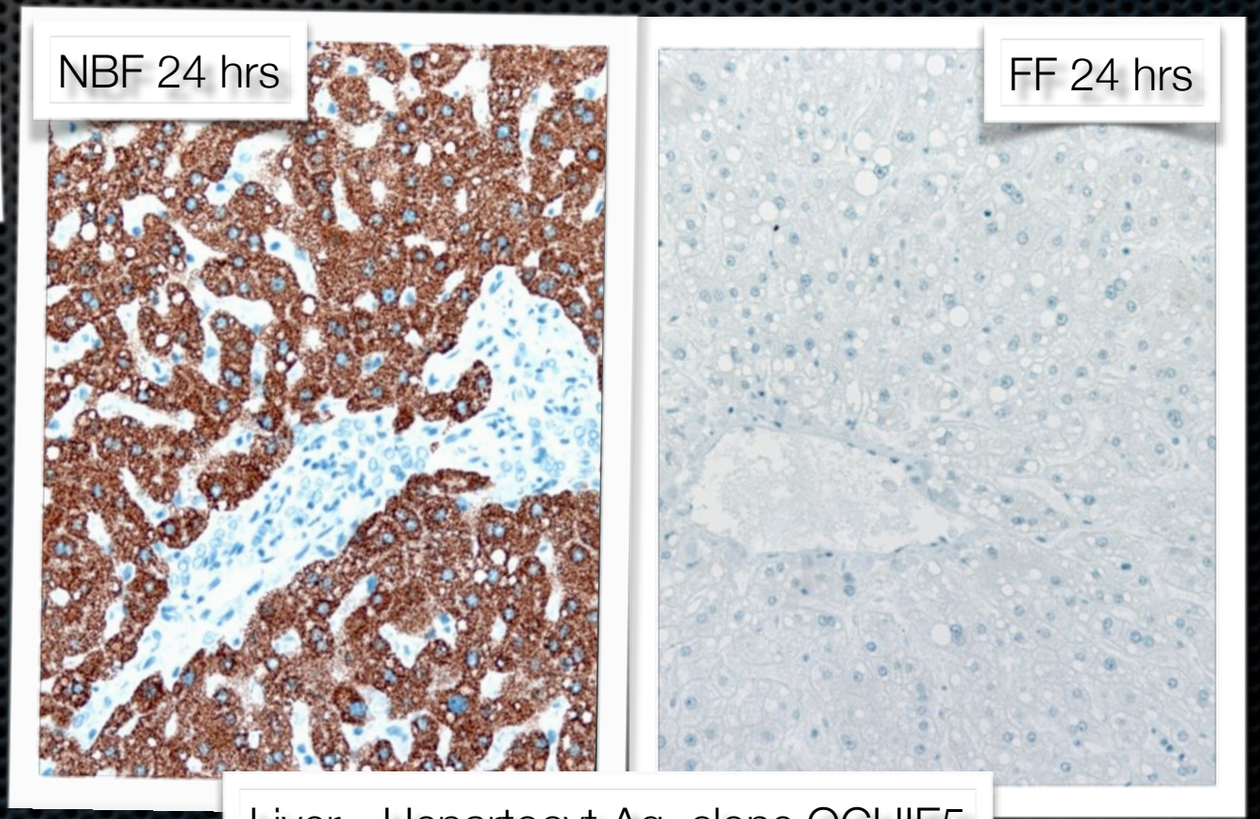



**FIGURE 1.** Distribution of (A) Allred scores for ER staining ($P = 1.0$), (B) Allred scores for PR staining ($P = 0.8625$), and (C) HER2 scores ($P = 1.0$) in the SF and PF groups.

# 4% NBF versus FineFix




Breast - ER, clone 1D5




Liver - Hepartocyt Ag, clone OCHIE5

*"With existing IHC-protocols 35% (9 of 26) of the antibodies gave poor or borderline reactions on tissues fixed in FineFix"*

*(Unpublished data)*

# Alternatives to 4% NBF...

| Name | Contains... | Company |
|---|---|---|
| F-solv | Denat. EtOH / Aldehyde derivate / Stabiliser | Yvsolab |
| UPM | Ethanol / Methanol / 2-Propanol / Formaldehyde | Copan |
| GreenFix | Ethandial / Ethanol | Diapath |
| CyMol | Ethanol / Methanol / 2-Propanol | Copan |
| RCL-2 | Ethanol / Acetic acid / Complex carbohydrates | Alphelys |
| FineFix | Ethanol / Glycerol / PVA / Simple carbohydrates | Milestone |
| Formaldehyde-EtOH | Formaldehyde / Ethanol / Buffer | BBC Biochemical |
| Zn-Formalin | Formaldehyde / Methanol / Zn-sulfate | Richard-Allen |
| Prefer | Glyoxal / Ethanol | Anatech |
| Davidson's AFA | Formaldehyde / Ethanol / Acetic acid | Electron Micr. Sci. |
| Molecular Fixativ | Methanol / Polyethylenglycol | Sakura |
| Pen-Fix | Formaldehyde / Ethanol / Buffer | Richard-Allen |
| Histochoice | Glyoxal / Zn-sulfate / Butandial | Ameresco-Inc. |
| O-Fix | Formaldehyd / Ethanol / Acetic acid | SurgiPath |
| GTF | Glyoxal / Ethanol | StatLab Medical |
| PAXgene Tissue-fix | Alcohols / Acid / A soluble organic compound | Qiagen- PreAnalytix |

# PAXgene Tissue
## New Tissue Fixation/Stabilization Technology

- Development began in 2007:
  - >1,500 compounds and combinations screened
  - >8,000 tissue samples tested to date
- Technology requirements
  - Histomorphology must be equivalent to FFPE tissue
  - RNA, DNA, miRNA must be preserved and of high quality
- Two-reagent system finalized in 2009
  - Fixation and stabilization reagents, both formalin-free
- First collection device
  - Container with two chamber one closure
- Under evaluation within SPIDIA

| | |
|---|---|
| Consortium | 7 public research organizations, 8 companies, 1 standards organization (CEN) |
| Coordinator | QIAGEN GmbH |

PreAnalytiX
A QIAGEN / BD Company

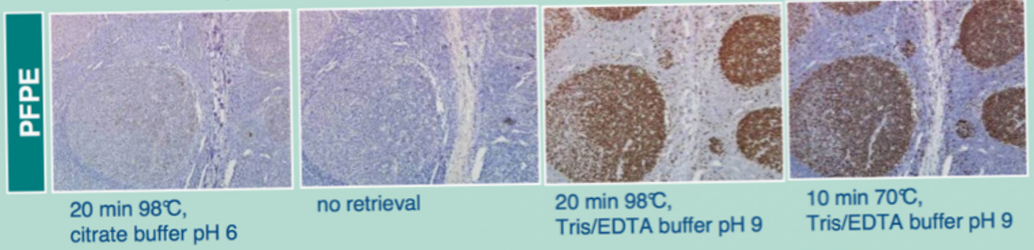
## Summary
PAXgene Tissue …

- … is a standardized system for tissue fixation, stabilization and biomolecule purification.
- … preserves histomorphology and biomolecules.
- … works without crosslinking and chemical modification.
- … treated tissue can be stored within the stabilization reagent, or after processing.
- … results in comparable morphology but superior molecular results
- … requires protocol adaptations for immunhistochemistry staining

PAXgene Tissue enables multimodal analysis of biomolecules from the same sample, which is used for morphological analysis

## Immunhistochemistry
Ki67 - Optimization of Epitope Retrieval

- Human tonsil
- Ki-67, clone MIB-1, Labelled streptavidin-biotin (LSAB) assay






PreAnalytiX
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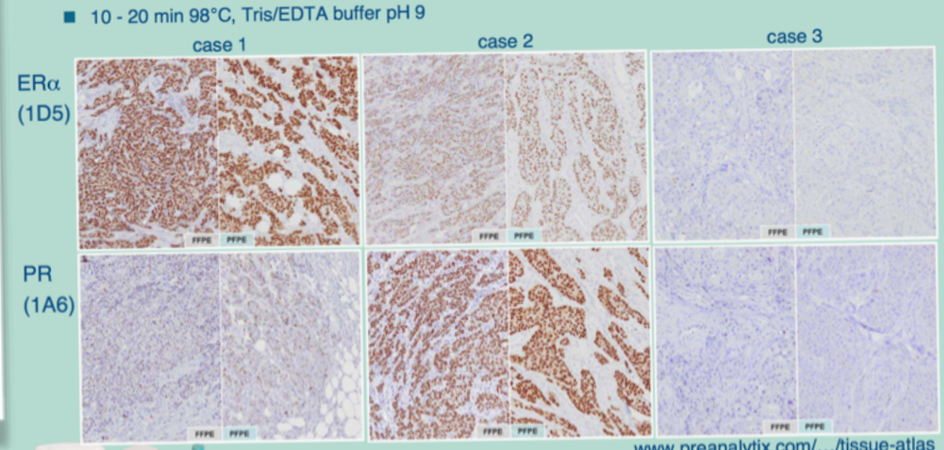
## Immunhistochemistry
Estrogen and Progesteron Receptor

- Human breast cancer cases (Cureline)
- Labelled streptavidin-biotin (LSAB) assays
- 10 - 20 min 98°C, Tris/EDTA buffer pH 9




www.preanalytix.com/…/tissue-atlas



PreAnalytiX
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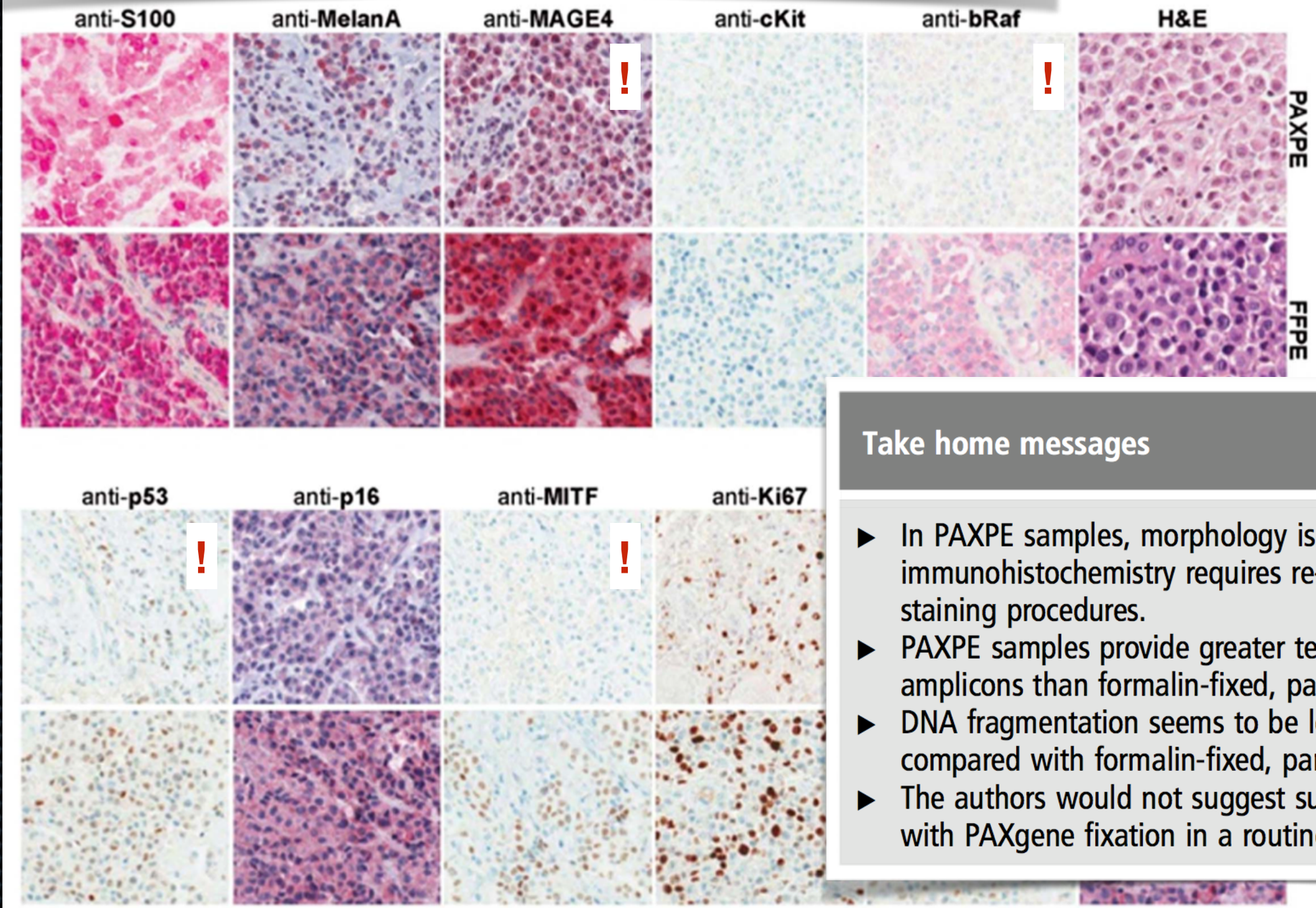
# Will PAXgene substitute formalin? A morphological and molecular comparative study using a new fixative system

Benedetta Belloni,[1] Chiara Lambertini,[2] Paolo Nuciforo,[2] Jay Phillips,[3] Eric Bruening,[3] Stephane Wong,[3] Reinhard Dummer[1]

*J Clin Pathol* 2013;**66**:124–135.




Morphology was well preserved in PAXPE samples. However, 5 out of 11 IHC markers showed significantly lower overall staining and staining intensity with PAXPE tissues in comparison with formalin-fixed, paraffin-embedded (FFPE).

## Take home messages

- In PAXPE samples, morphology is well preserved but immunohistochemistry requires re-evaluation of markers and staining procedures.
- PAXPE samples provide greater template integrity of mRNA amplicons than formalin-fixed, paraffin-embedded samples.
- DNA fragmentation seems to be lower in PAXPE samples compared with formalin-fixed, paraffin-embedded samples.
- The authors would not suggest substituting formalin fixation with PAXgene fixation in a routine pathology laboratory.

# Choice of Fixative Is Crucial to Successful Immunohistochemical Detection of Phosphoproteins in Paraffin-embedded Tumor Tissues

(J Histochem Cytochem 57:257–264, 2009)

Janine A. Burns, Yuan Li, Carol A. Cheney, Yangsi Ou, Laura L. Franlin-Pfeifer, Nelly Kuklin, and Zhi-Qiang Zhang

Department of Biologics Research, Merck Research Laboratories, West Point, Pennsylvania

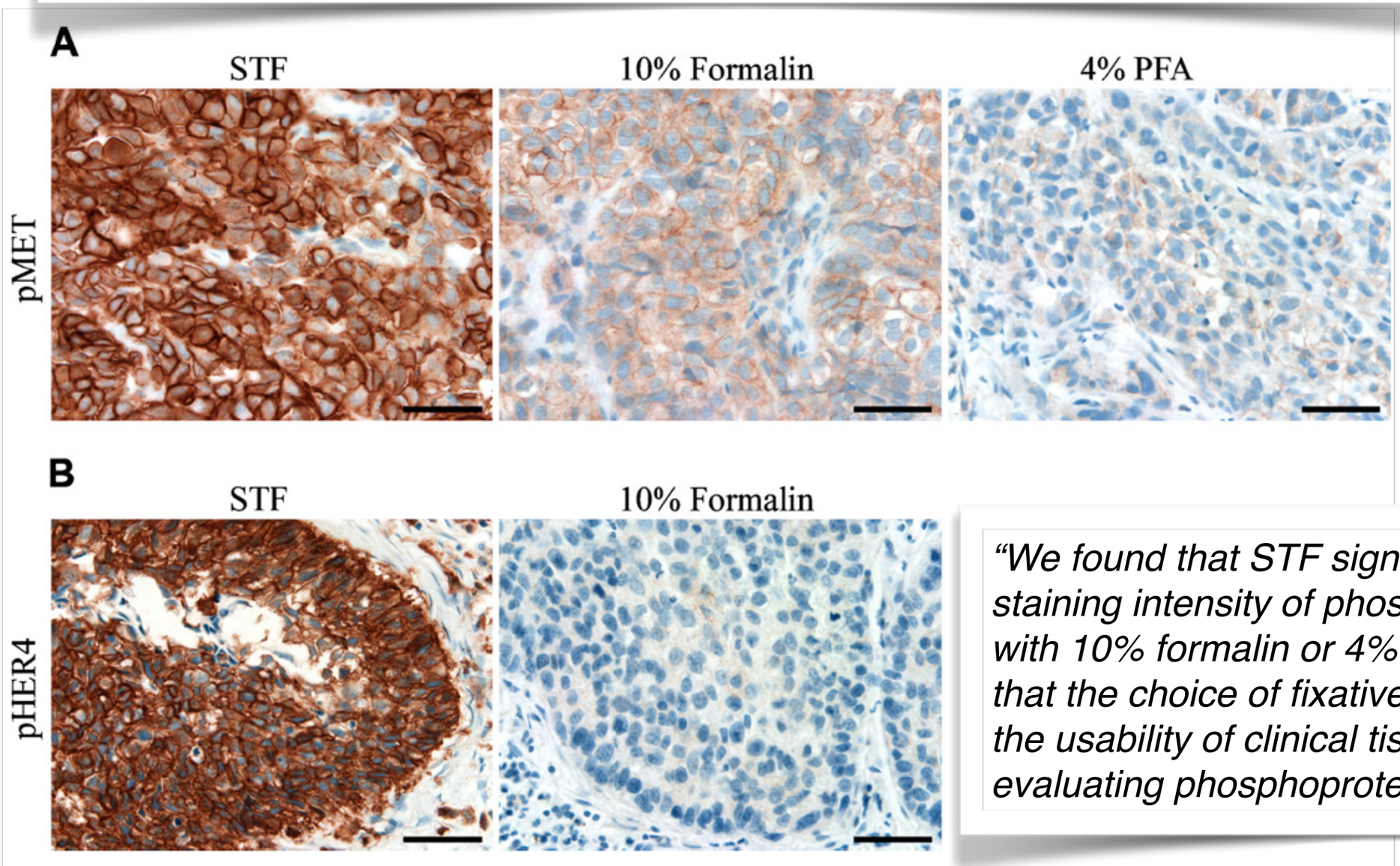



**Figure 2** IHC staining of phosphoproteins in xenograft and human clinical tumor tissues. (**A**) SKOV-3 xenograft tumor tissues fixed in Streck's tissue fixative (STF), 10% formalin, and 4% paraformaldehyde (PFA) were stained with anti-pMet antibody. (**B**) Human lung tumor tissue fixed in 10% formalin and STF were stained with anti-pHER4 antibody. Bar = 50 μm.

Streck's tissue fixative (STF)

*"We found that STF significantly enhanced the staining intensity of phosphoproteins compared with 10% formalin or 4% PFA. Our results indicate that the choice of fixative could significantly affect the usability of clinical tissue samples for evaluating phosphoprotein by IHC".*

OPEN ACCESS Freely available online

PLOS ONE

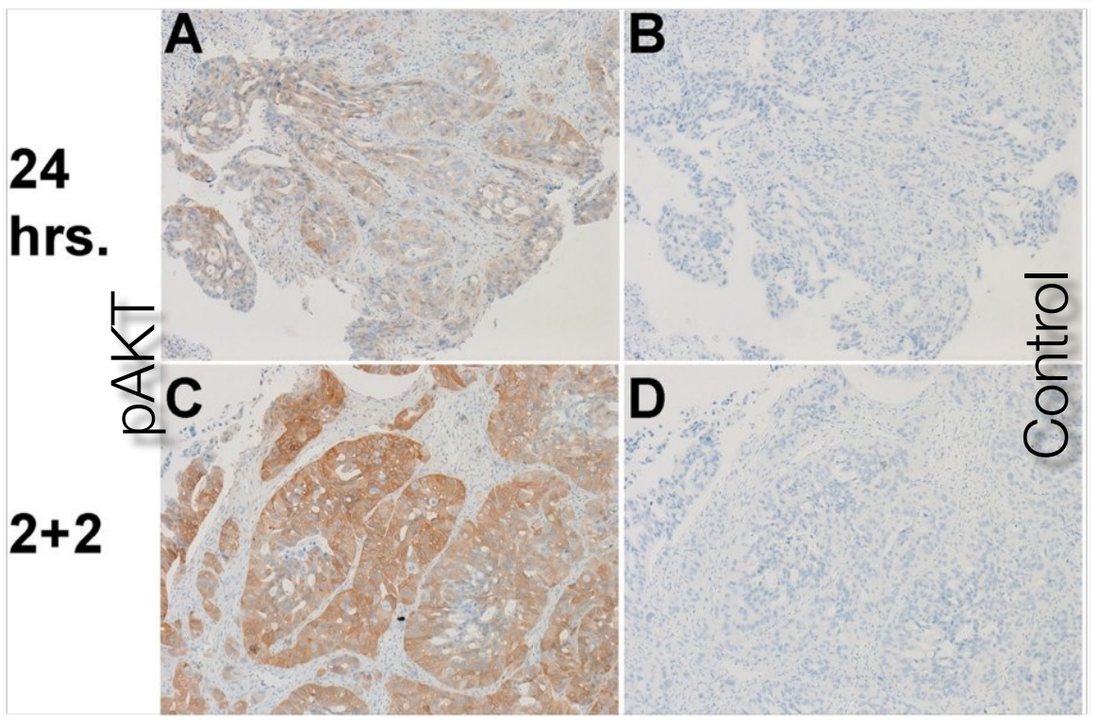
# Rapid Two-Temperature Formalin Fixation

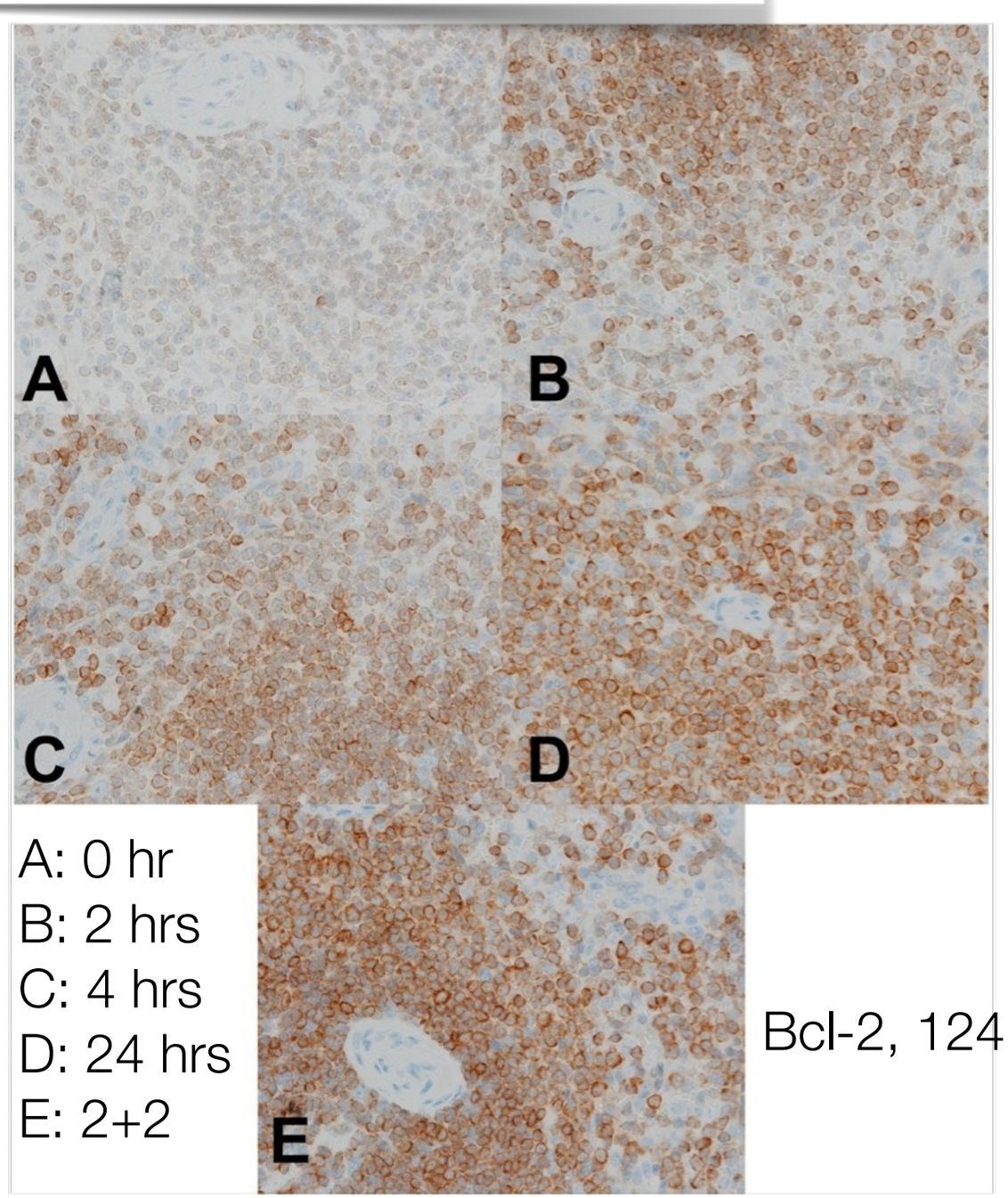
**David Chafin[1☯], Abbey Theiss[1☯], Esteban Roberts[1], Grace Borlee[2¤], Michael Otter[1], Geoffrey S. Baird[2,3*]**

**1** Ventana Medical Systems, Inc., Tucson, Arizona, United States of America, **2** Department of Laboratory Medicine, University of Washington, Seattle, Washington, United States of America, **3** Department of Pathology, University of Washington, Seattle, Washington, United States of America

bcl-2 IHC after different fixation conditions

| Control | No Pre-Soak | | | | 2 Hr Pre-Soak (4°C) | | | |
|---|---|---|---|---|---|---|---|---|
| RT 10% NBF | 35° 10% NBF | 40° 10% NBF | 45° 10% NBF | 50° 10% NBF | 35° 10% NBF | 40° 10% NBF | 45° 10% NBF | 50° 10% NBF |
| 0 hr | 0.5 hr | 0.5 hr | 0.5 hr | 0.5 hr | 0.5 hr | 0.5 hr | 0.5 hr | 0.5 hr |
| 2 hr | 1 hr | 1 hr | 1 hr | 1 hr | 1 hr | 1 hr | 1 hr | 1 hr |
| 4 hr | 2 hr | 2 hr | 2 hr | 2 hr | 2 hr | 2 hr | 2 hr | 2 hr |
| 8 hr | 4 hr | 4 hr | 4 hr | 4 hr | 4 hr | 4 hr | 4 hr | 4 hr |
| 24 hr | 6 hr | 6 hr | 6 hr | 6 hr | 6 hr | 6 hr | 6 hr | 6 hr |



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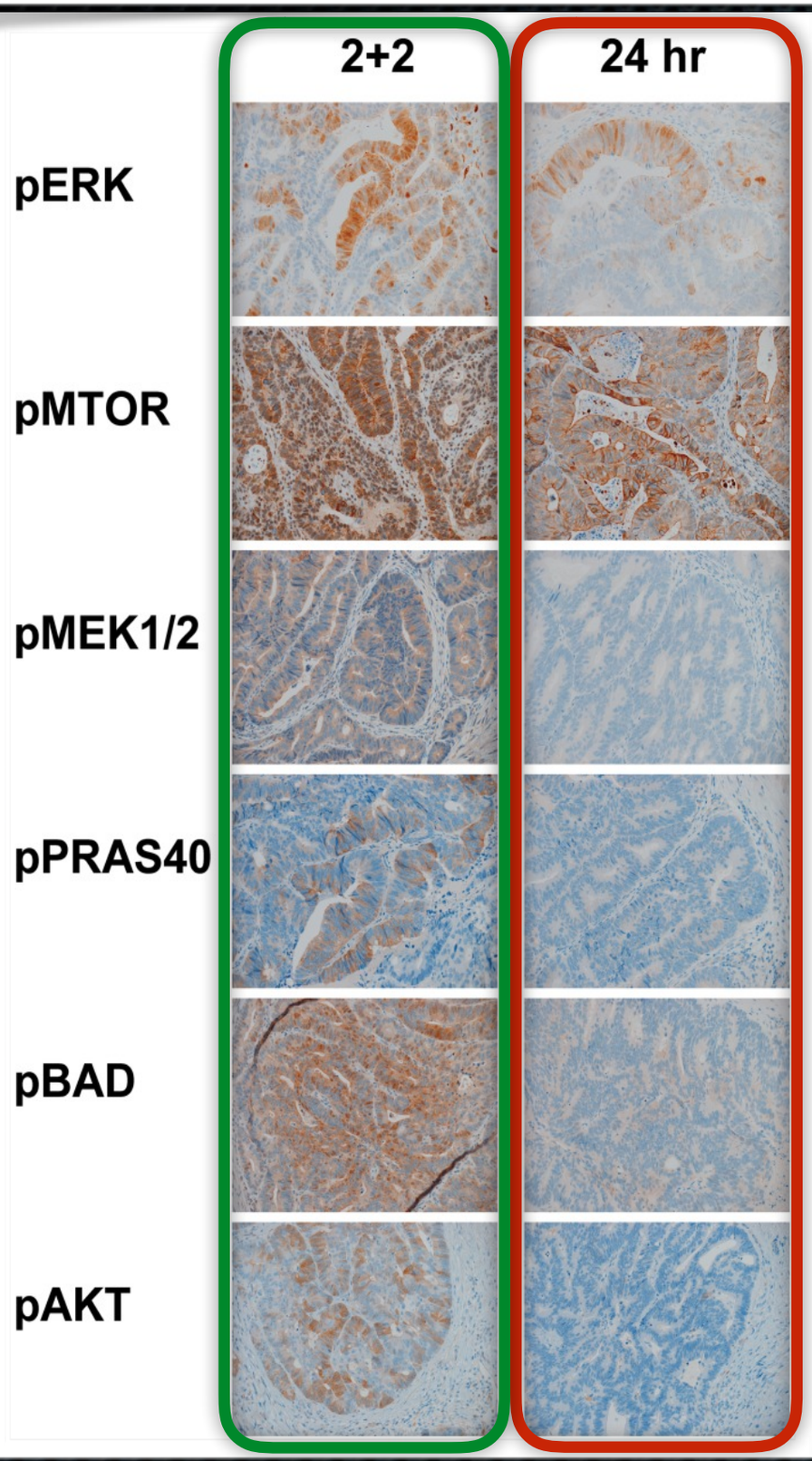
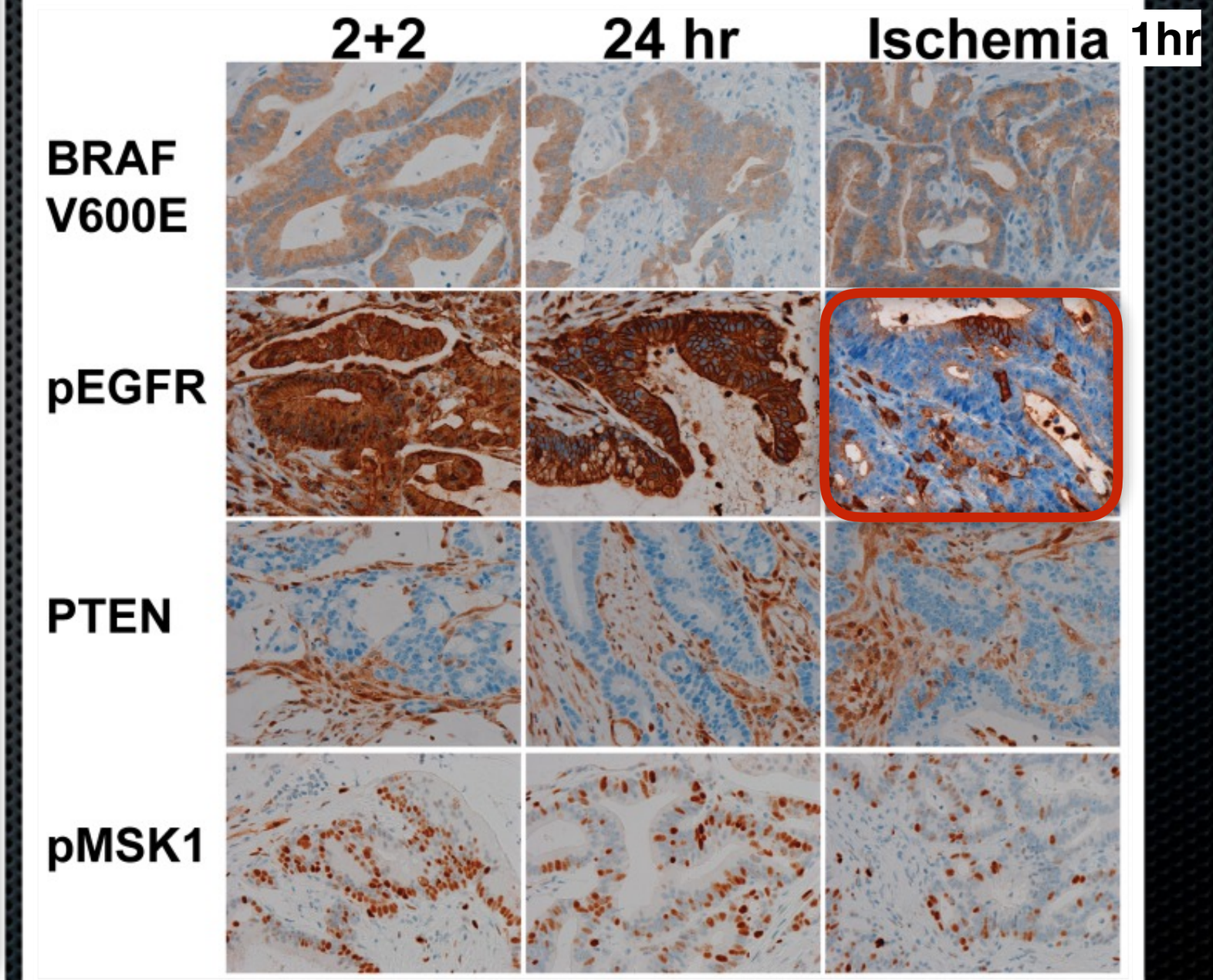
PLOS ONE

# Immunohistochemistry of Colorectal Cancer Biomarker Phosphorylation Requires Controlled Tissue Fixation

**Abbey P. Theiss[1☯], David Chafin[1☯], Daniel R. Bauer[1], Thomas M. Grogan[1], Geoffrey S. Baird[2,3]***

**1** Ventana Medical Systems Inc., Tucson, Arizona, United States of America, **2** Department of Laboratory Medicine, University of Washington, Seattle, Washington, United States of America, **3** Department of Pathology, University of Washington, Seattle, Washington, United States of America



# NBF fixation at 4°C ? - A practical solution ? 🤔

Evening Standard

LONDON, TUESDAY, 27 APRIL 2004 www.thisislondon.co.uk Incorporating THE EVENING NEWS 40p

## Self-chilling beer

### The can that turns into a mobile fridge

IT IS every beer lover's summer nightmare — stuck in the middle of a park with the sun warming your drink. Thankfully, scientists have come up with a solution: the self-cooling beer can.

Slightly longer than a normal drink can, it simply needs a twist to cool its content down. It can, its inventors claim, cool a beer to the perfect temperature of 3C within three minutes.

The I C (Instant Cool) Can works by using water evaporation. The top half is surrounded by a layer of watery gel. The base contains a water-absorbing material in a vacuum, and a special heat-absorbing chamber.

When the bottom is twisted, a seal between the two halves is broken. The vacuum draws the gel, and the heat, into the base. The gel is absorbed by the material, the heat is absorbed by the chamber — and the drink gets cold.

Trials showed that on average, the temperature of the liquid in the can fell 16C when the system was activated. Drinks stayed cool for up to an hour. The cans are already being tested by two large British brewers, who hope to have them on sale before the end of the summer.

According to Barney Guarino, chief executive of Tempra Technology, the company that developed the can,

By Mark Prigg
Science Correspondent

Britain is likely to get the te… first. "We are talking to seve… brewers, and we think we will … cans on sale before the end of … "We expect to do one final test … summers, then launch very soon …

The more expensive beers … tails are likely to be the first … from cooling cans, as — acco… Mr Guarino — consumers are … used to paying higher prices fo…

"All the market research we h… shows consumers don't mind … premium prices for this," he … "There simply aren't the econ… scale to make it cheap. But t… happen over time, and we ha… cans will become commonplace …

The system may also be devel… the hugely popular alcopop ma…

However one brewer was … technology could prove too e… for the average consumer.

A spokesman for Scottish … which brews beers including … Kronenbourg and John Smith's … would be unlikely to use the s… the present time. "The price p… is a problem, but this is an area … looking at for the future," he …

#### HOW IT WORKS

1. Twist can base to break seal
2. This causes sudden drop in pressure
3. That leads to evaporation of gel
4. The energy required results in rapid cooling of beer
5. Gel and heat absorbed in base



#### THE SELF-COOLING BEER CAN

**After the widget, the next smart package for beer could be this cooling can from Tempra Technology. It is currently being tested by two UK brewers**

The beer is surrounded by an outer compartment containing a watery gel

The bottom section, sealed from the top, contains a dessicant in a vacuum

The user twists the can to break the seal and the drop in pressure causes the water around the upper part to evaporate quickly, cooling the beer. The water is absorbed by the dessicant, and a sink absorbs the heat stopping the can feeling warm to the touch



The beer can be cooled by 16.7 °C in three minutes, the company claims

The beer can be cooled by 16.7 °C in three minutes, the company claims

# Fixation

| Preanalytic variable | Published Guidelines and Recommendations<br>ASCO/CAP \| CLSI | Literature-Based Recommendations |
|---|---|---|
| Fixative formula | **4% NBF** # | 4% NBF |
| Time in fixative | **24 hrs*** | 24 hrs |
| Tissue to fixative ratio | **1:10** | 1:1 to 1:20 **(1:2)** |

Engel KB, Moore HM. Arch Pathol Lab Med. 2011;135:537–543

Arch Pathol Lab Med—Vol 131, January 2007

**American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer**

*Appl Immunohistochem Mol Morphol* • Volume 16, Number 6, December 2008

Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry

*6-48 hrs

*8-72 hrs 4% phosphate buffered formaldehyde, pH 7,0 - 7,4

# **4% NBF = 4% neutral buffet formaldehyde = 10% neutral buffet formalin**

# Decalcification

- Type
  - Strong acid (e.g. HCl)
  - Weak organic acid (e.g. formic acid)
  - Chelating agents (e.g. EDTA)
- Time, Temperature
- Time in fixative before decalcification

# Call for a European programme in external quality assurance for bone marrow immunohistochemistry; report of a European Bone Marrow Working Group pilot study

*J Clin Pathol* 2009;**62**:547–551. doi:10.1136/jcp.2008.063446

E E Torlakovic,[1] K Naresh,[2] M Kremer,[3] J van der Walt,[4] E Hyjek,[5] A Porwit[6]

## Take-home messages

- Immunohistochemistry tests are commonly if not regularly used in bone marrow trephine biopsies (BMTB) obtained for both primary and secondary bone marrow diseases, with or without morphological evidence of disease.
- Proficiency testing for BMTB immunohistochemistry (IHC) by extralaboratory quality assurance (EQA) programmes is not possible if the number of methods for tissue processing is not markedly reduced.
- The survey determined that almost all participants believed that their results were either "good" or "optimal" (90%) and that their daily QC/QA programmes were either "good" or "optimal" (93%); however, only 21% of laboratories were found to produce no poor results. This discrepancy is particularly important because it was shown with most commonly used IHC tests.
- The European Bone Marrow Working Group IHC Group is calling for a reduction in the number of methods used for BMTB processing and establishment of a unified EQA programme for BMTB IHC for all European countries.

**Table 1** Tissue processing

| **Fixative** | **n (28)** |
|---|---|
| 10% NBF | 15 |
| 5% NBF | 1 |
| B5 | 5 |
| AZF | 4 |
| Schäffer's fixative | 2 |
| Burckhard fixative | 1 |
| F–G mixture | 1 |
| SUSA | 1 |
| **Fixation time** | **2 to >24 hours** |
| Exact and uniform | 5 |
| Variable | 23 |
| **Decalcification** | **n (28)** |
| None | 2 |
| EDTA | 16 |
| RDO (Rapid Decalcifier) | 1 |
| SUSA (acid) | 1 |
| 10% nitric acid | 1 |
| Stieve solution* | 1 |
| Gooding and Stewart's† | 4 |
| Zenker/glacial acetic acid solution | 1 |
| Osteosoft | 1 |
| **Decalcification time** | **45 minutes to 3 days‡** |
| Exact and uniform timing | 15 |
| Variable | 13 |

*Mercuric chloride/formaldehyde/acetic acid; †10% formic acid and 5% formaldehyde; ‡mostly depending of decalcifying reagent.
AZF, acetic acid–zinc–formalin; F–G mixture, formaldehyde–glutaraldehyde mixture; NBF, neutral buffered formalin; SUSA, sublimate mercury II chloride.

# IHC and decalcification

- ✓Decalc™ (HCl-based)
- ✓Buffet formic acid (4M formic acid + 0.5M Na-formiat)
- ✓10% EDTA, pH 7

# IHC and decalcification (2007)

24 hrs 4% NBF fixation prior to decalcification. 124 different antibodies on TMA's

| Method \ Intensity | 0/+ | ++ | +++ | ++++ |
|---|---|---|---|---|
| EDTA, 10% pH7 | 0 | 0 | 119 | 5 |
| Formic acid (BFA) | 2 | 13 | 103 | 6 |
| Decalc™ (HCl) | 101 | 21 | 2 | 0 |

Reference/No decalcification: ++++

Buffet formic acid (BFA): (4M formic acid + 0.5M Na-formiat)

# IHC and decalcification (2007)

| Antibody | Clone | Ref | Decalc | Formic | EDTA |
|---|---|---|---|---|---|
| Elastase, neutrophil , NP57 | NP57 | +++ | 0 | 0 | +++ |
| CD105, SN6h | SN6h | +++ | 0 | + | +++ |
| Bcl-2, 124 -Oncoprotein | 124 | +++ | 0 | ++ | +++ |
| Bcl-6, PG-B6p | PG-B6p | +++ | 0 | ++ | +++ |
| CD40, 11E9 | 1,1E+10 | +++ | 0 | ++ | +++ |
| Factor XIII-a, poly | | +++ | + | ++ | +++ |
| Oct-1, 12F11 | 12F11 | +++ | 0 | ++ | +++ |
| Oct-2 (C20), poly | | +++ | 0 | ++ | +++ |
| MUM1, MUM1p -Multiple Myeloma | MUM1p | +++ | + | ++ | +++ |
| Bob 1, TG14 | TG14 | +++ | 0 | ++ | +++ |
| CD4, 4B12 | 4B12 | +++ | 0 | ++ | +++ |
| CD43, MT1 | MT1 | +++ | 0 | ++ | +++ |
| TCAR, BF1 -T-Cell Antigen | ßF1 | +++ | 0 | ++ | +++ |
| CD16, 2H7- Fc Gamma Receptor | 2H7 | +++ | 0 | ++ | +++ |
| CD52, HI186 | HI186 | +++ | 0 | ++ | +++ |

# IHC and decalcification (2014)

24 hrs 4% NBF fixation prior to decalcification. 193 different antibodies on TMA's

| Method \ Intensity | 0/+ | ++ | ++(+) | +++ | ++++ |
|---|---|---|---|---|---|
| EDTA, 10% pH7 | 0 | 0 | 5 | 185 | 3 |
| Formic acid (BFA) | 1 | 15 | 8 | 163 | 6 |
| Decalc™ (HCl) | 159 | 23 | 1 | 8 | 2 |

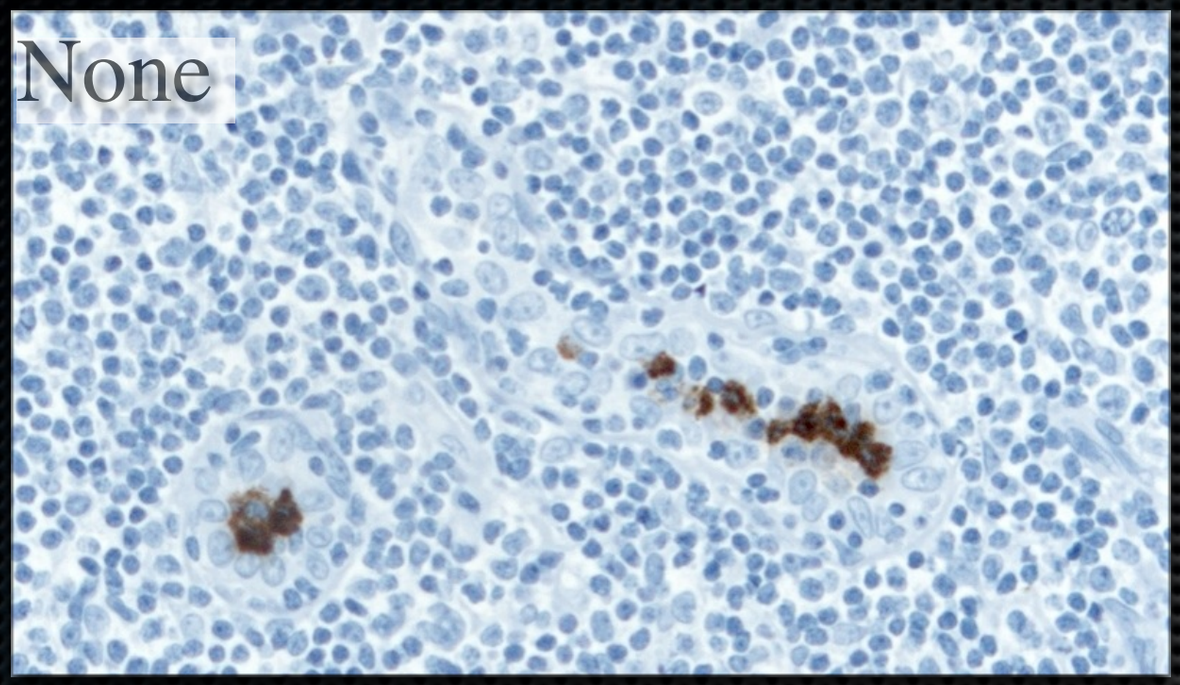
Reference/No decalcification: ++++

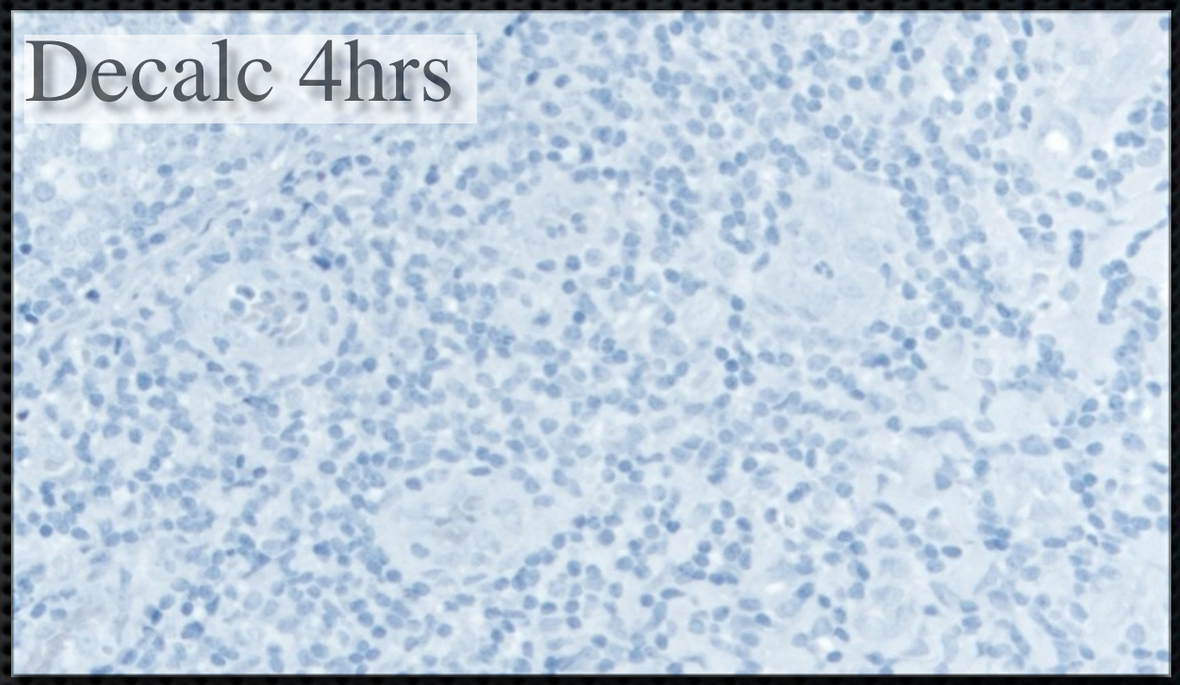
Buffet formic acid (BFA): (4M formic acid + 0.5M Na-formiat)

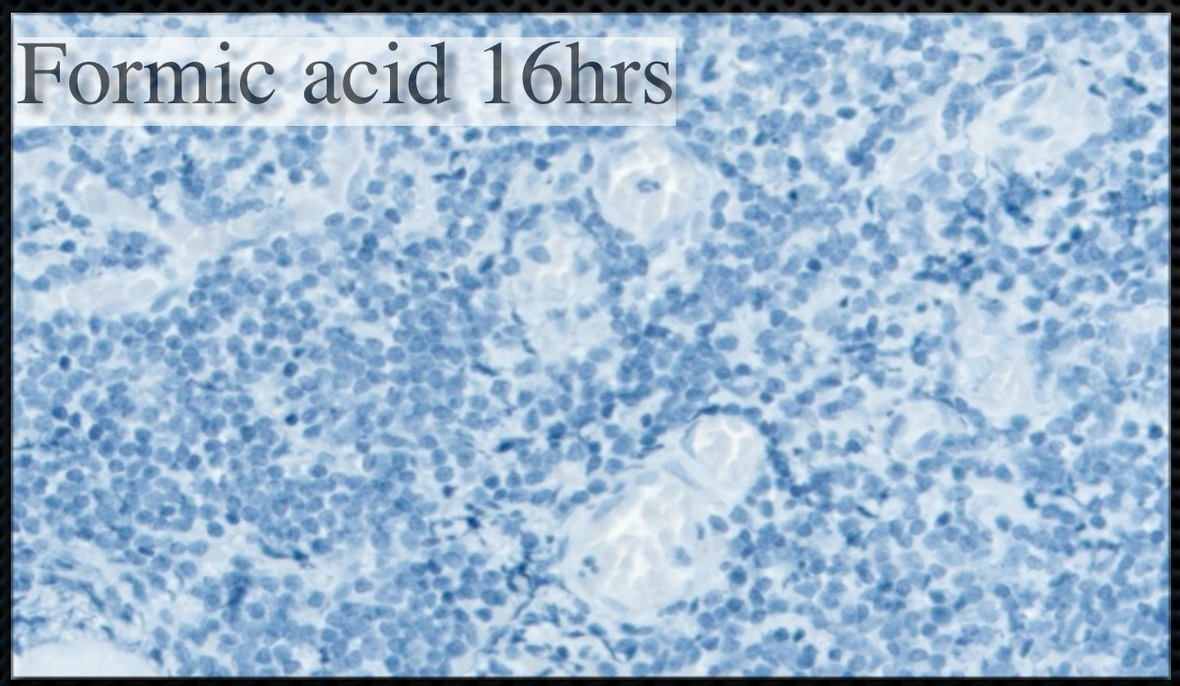
# IHC and decalcification (2014)

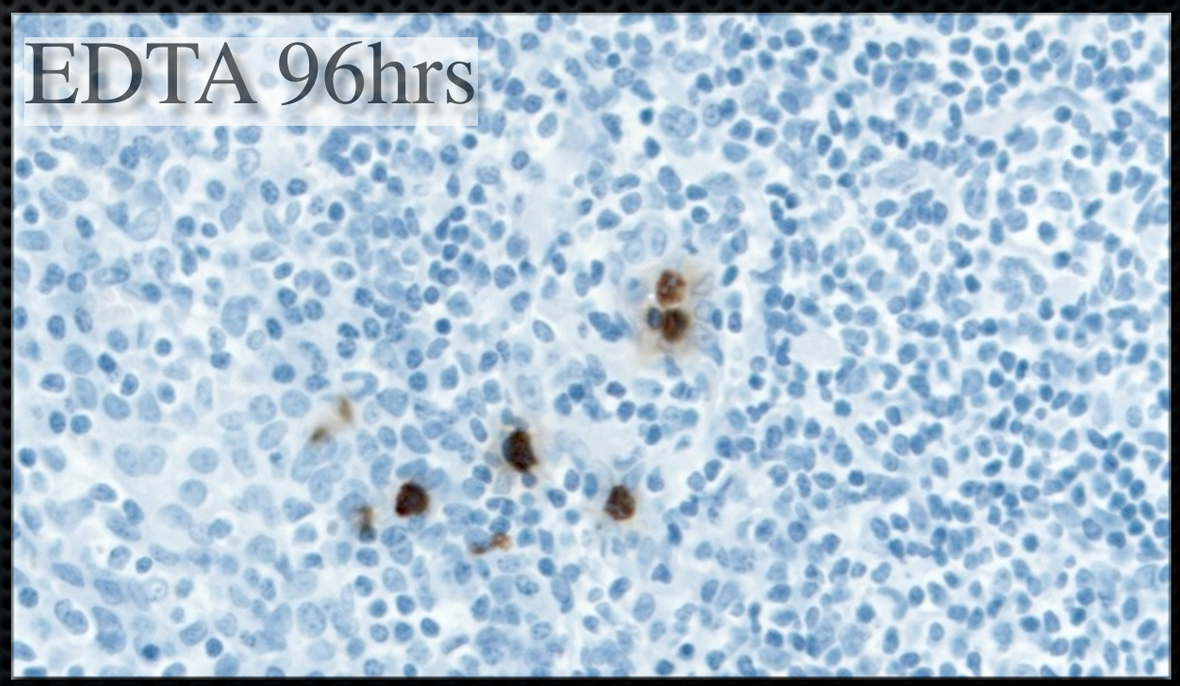
| Antibody | Reference | DECAL | Formic | EDTA |
|---|---|---|---|---|
| CD303, 124B3.13 | +++ | + | + | +++ |
| Makrofag, MAC 387 | +++ | 0 | ++ | ++(+) |
| Bcl-2, 124 * | +++ | 0 | ++ | +++ |
| TCAR, BF1 * | +++ | 0 | ++ | +++ |
| Galectin-3, 9C4 | +++ | 0 | ++ | +++ |
| Caveolin-1, 4D6 | +++ | 0 | ++ | +++ |
| CD279, NAT105 | +++ | 0 | ++ | +++ |
| Inhibin Alpha, R1 | +++ | 0 | ++ | +++ |
| Bcl-2, E17 | +++ | 0 | ++ | +++ |
| FOXP1, EPR4113 | +++ | 0 | ++ | +++ |
| pHH3, E173 | +++ | 0 | ++ | +++ |
| CD1a, EP3622 | +++ | 0 | ++ | +++ |
| CD19, SP110 | +++ | 0 | ++ | +++ |
| CD103, EPR4166(2) | +++ | 0 | ++ | +++ |
| CD123, 6H6 | +++ | 0 | ++ | ++++ |
| Neuroblastoma, NB84 | +++ | 0 | ++/+ | +++ |
| MUM1, MUM1p | +++ | + | ++(+) | ++(+) |
| Podoplanin. D2-40 | +++ | + | ++(+) | ++(+) |
| Hairy Cell, DBA.44 | +++ | 0 | ++(+) | +++ |
| Oct-2 (C20), poly | +++ | 0 | ++(+) | +++ |
| CD27, 137B4 | +++ | 0 | ++(+) | +++ |
| CEA, Col-1 | +++ | 0 | ++(+) | +++ |
| NSE, H14 | +++ | +(+) | ++(+) | +++ |
| CD117, YR145 | +++ | ++(+) | ++(+) | +++ |

# Decalcification and Elastase, neutrophilic, NP57

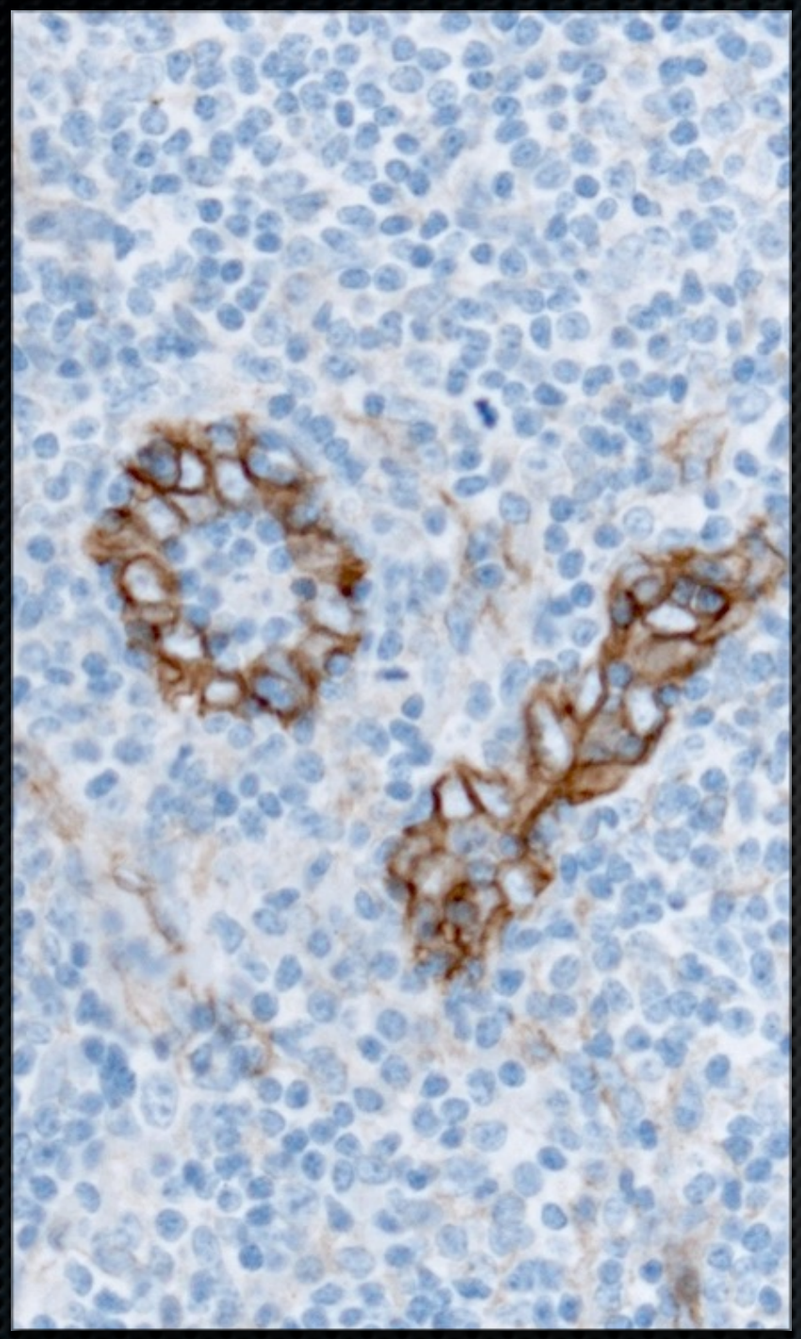


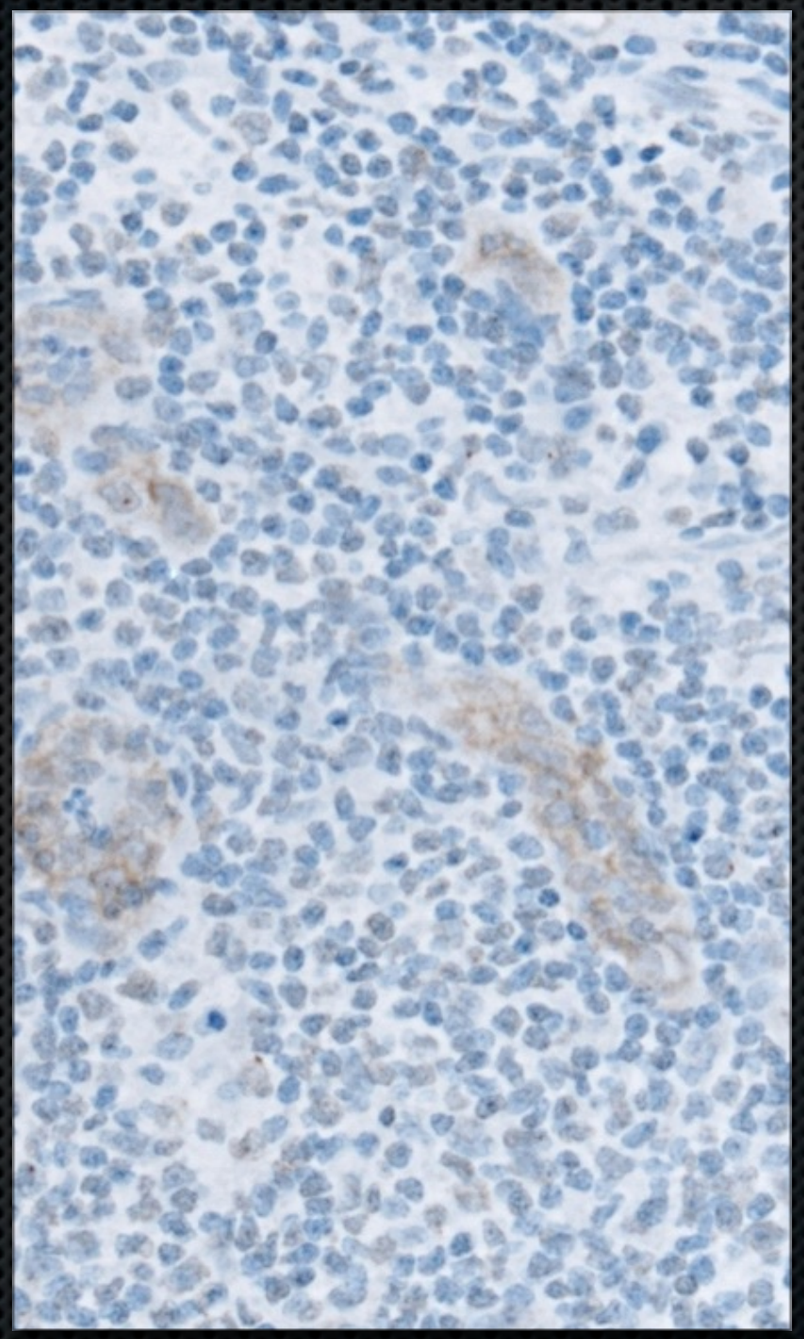



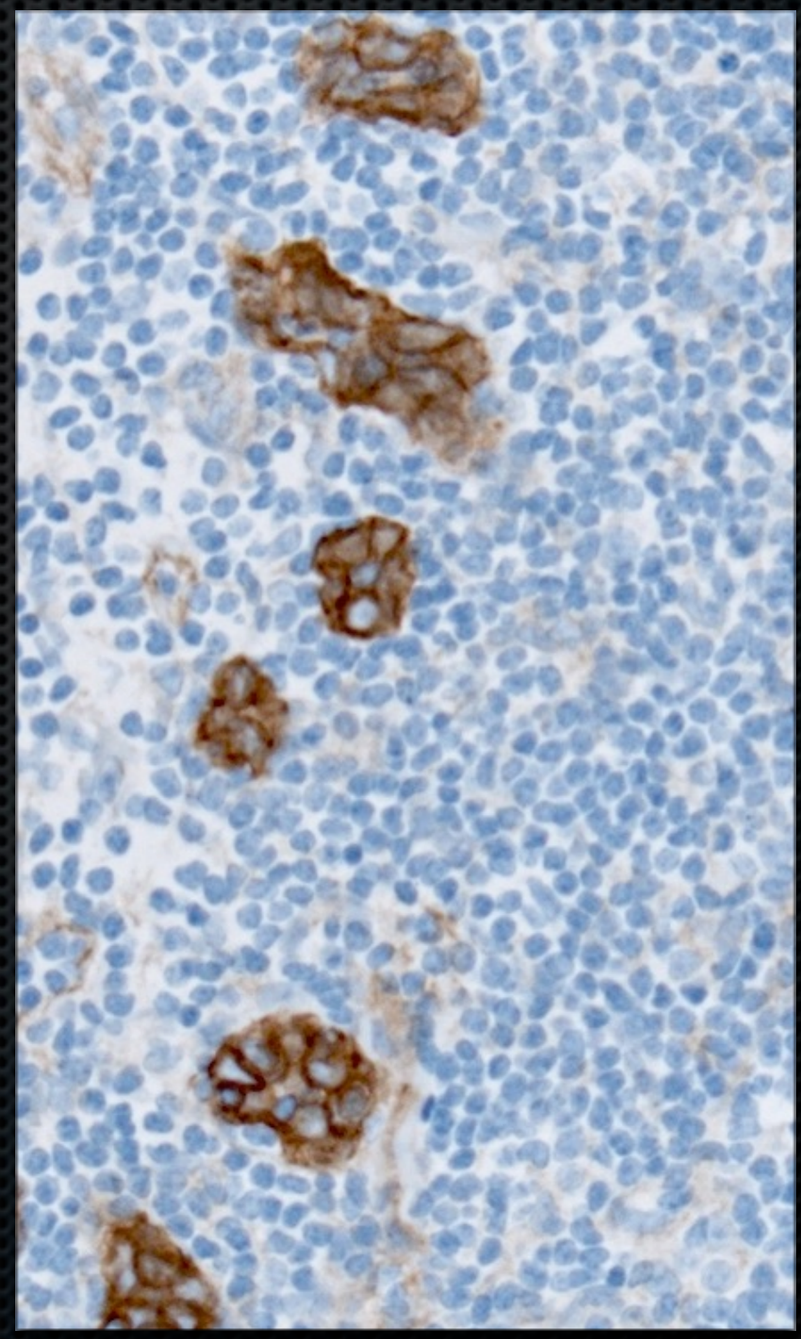
# Decalcification and CD105, SN6h



No decalcification

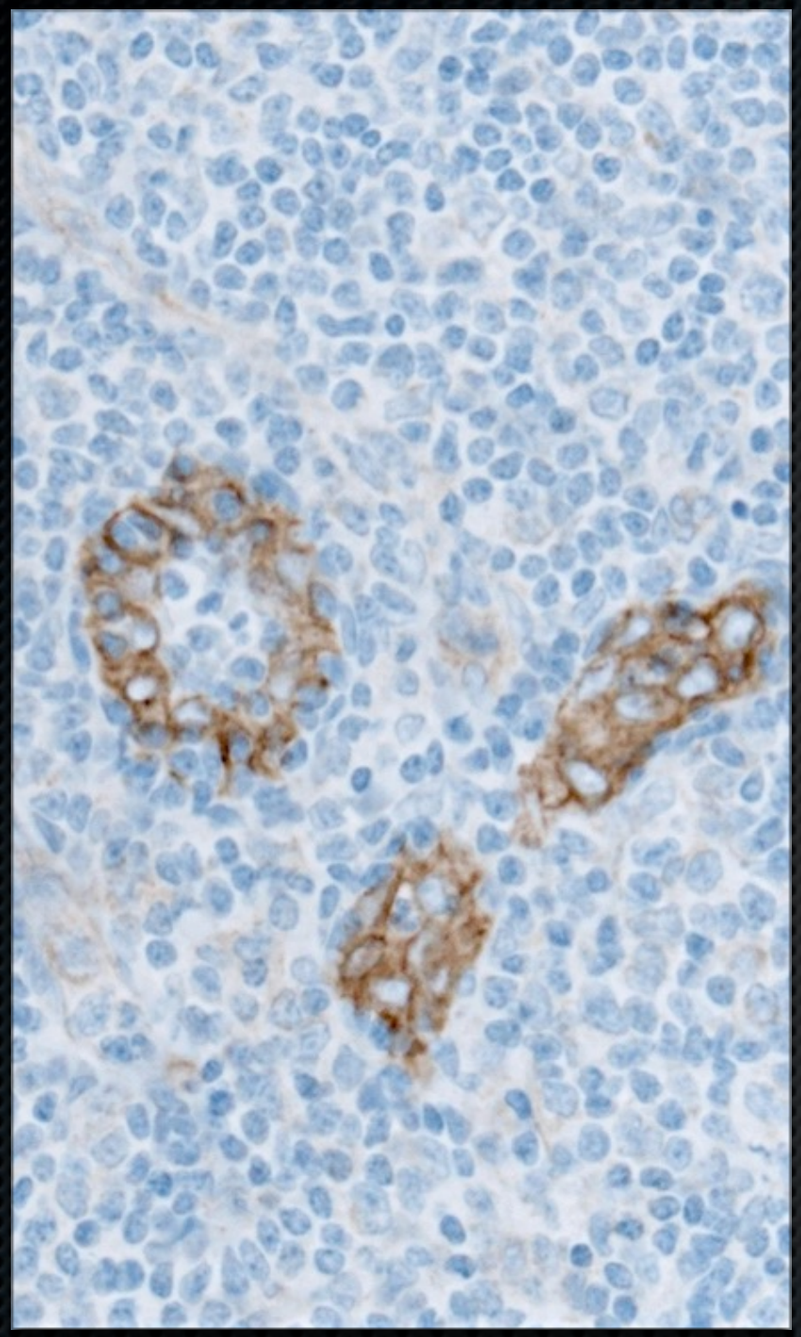


Formic acid 16hrs

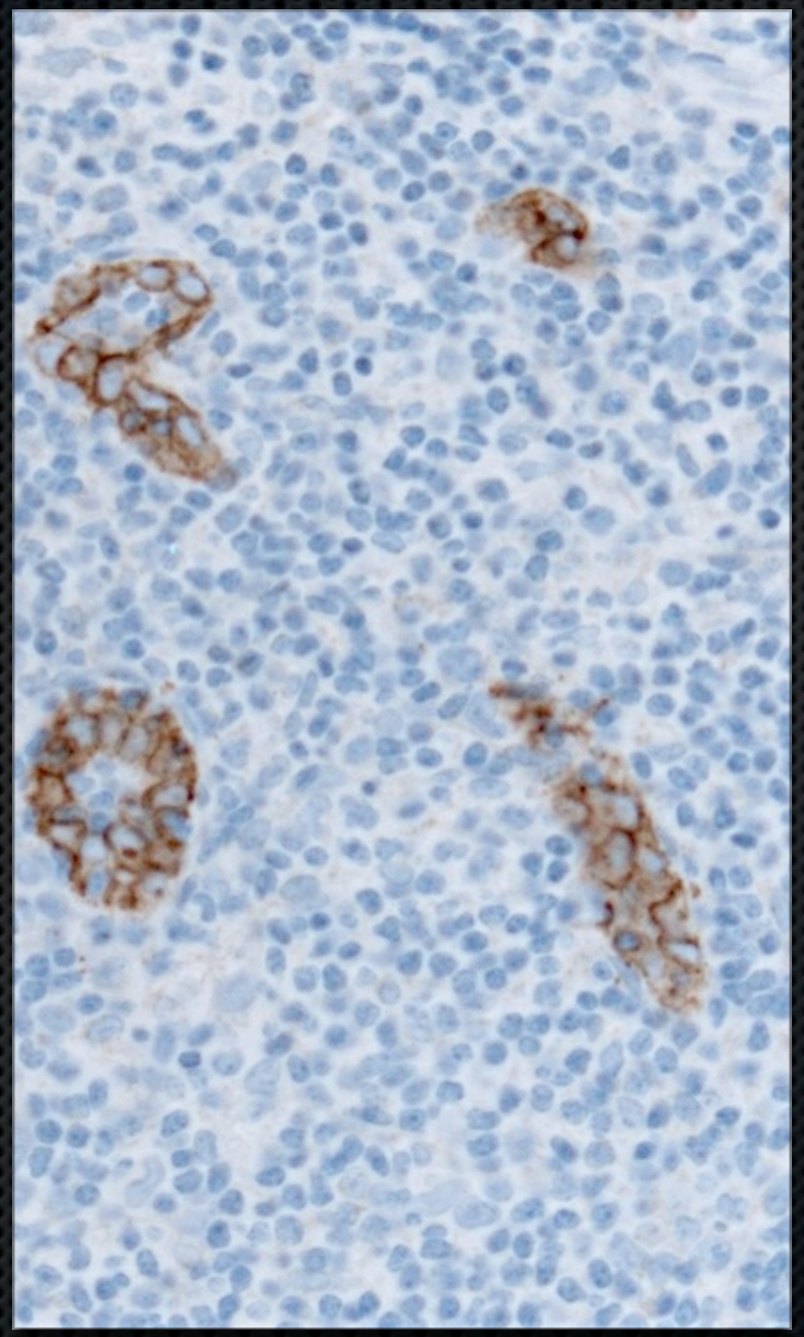


EDTA 96hrs

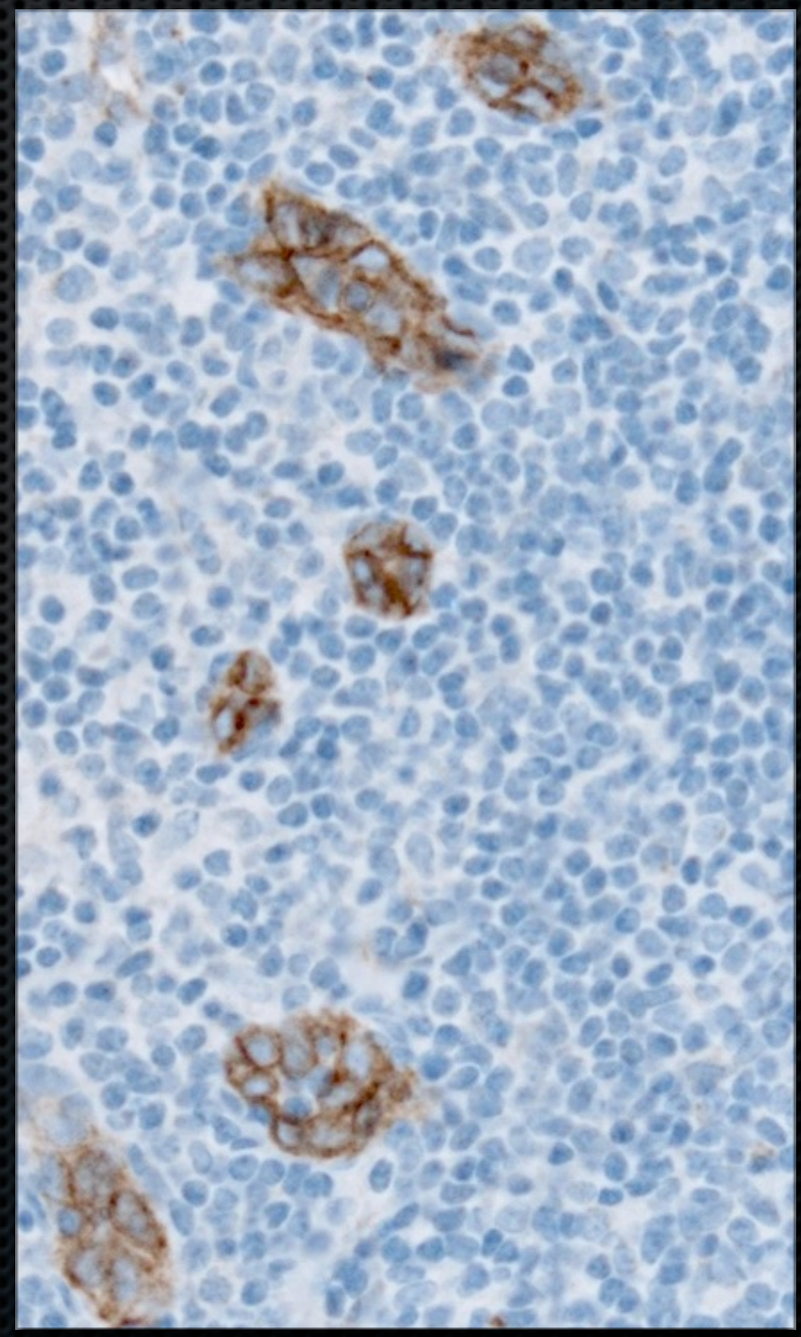
# Decalcification and CD105, 4G11



No decalcification

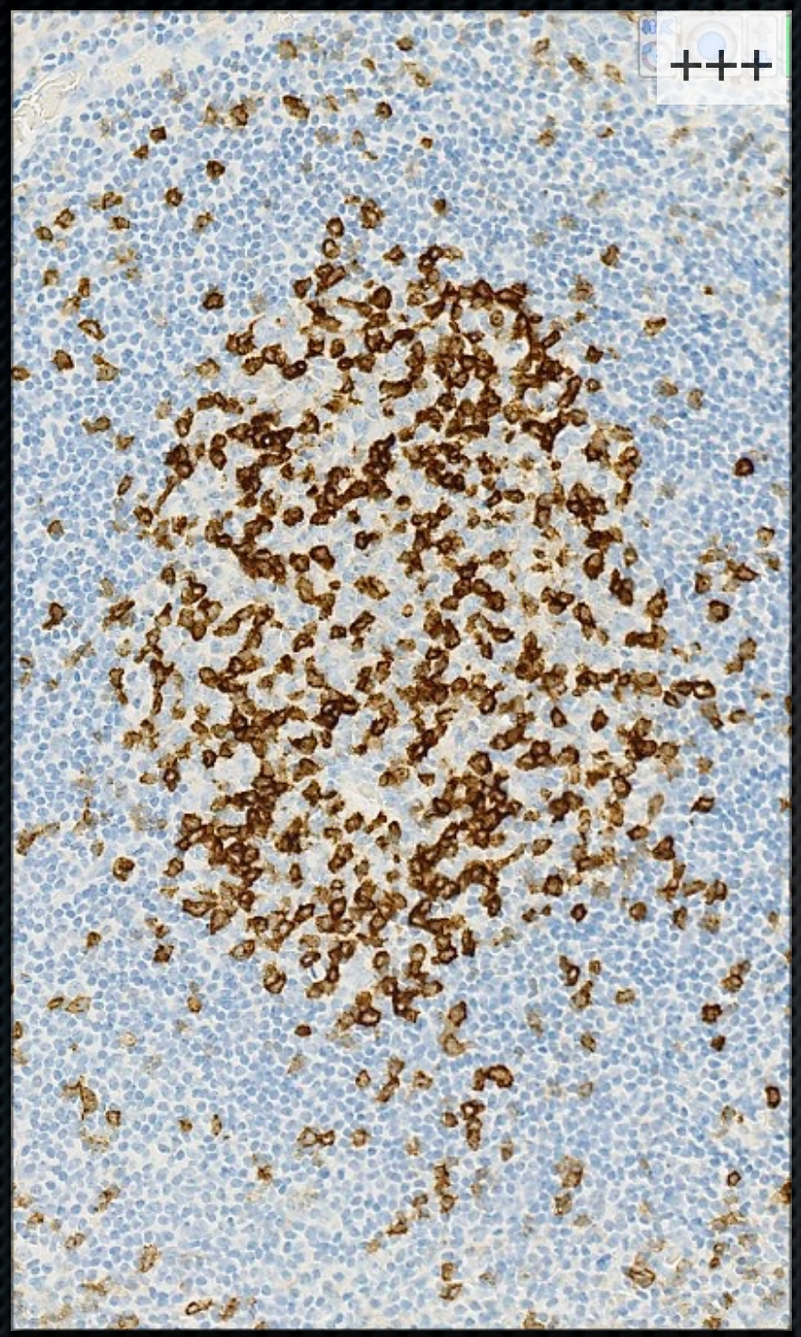


Formic acid 16hrs

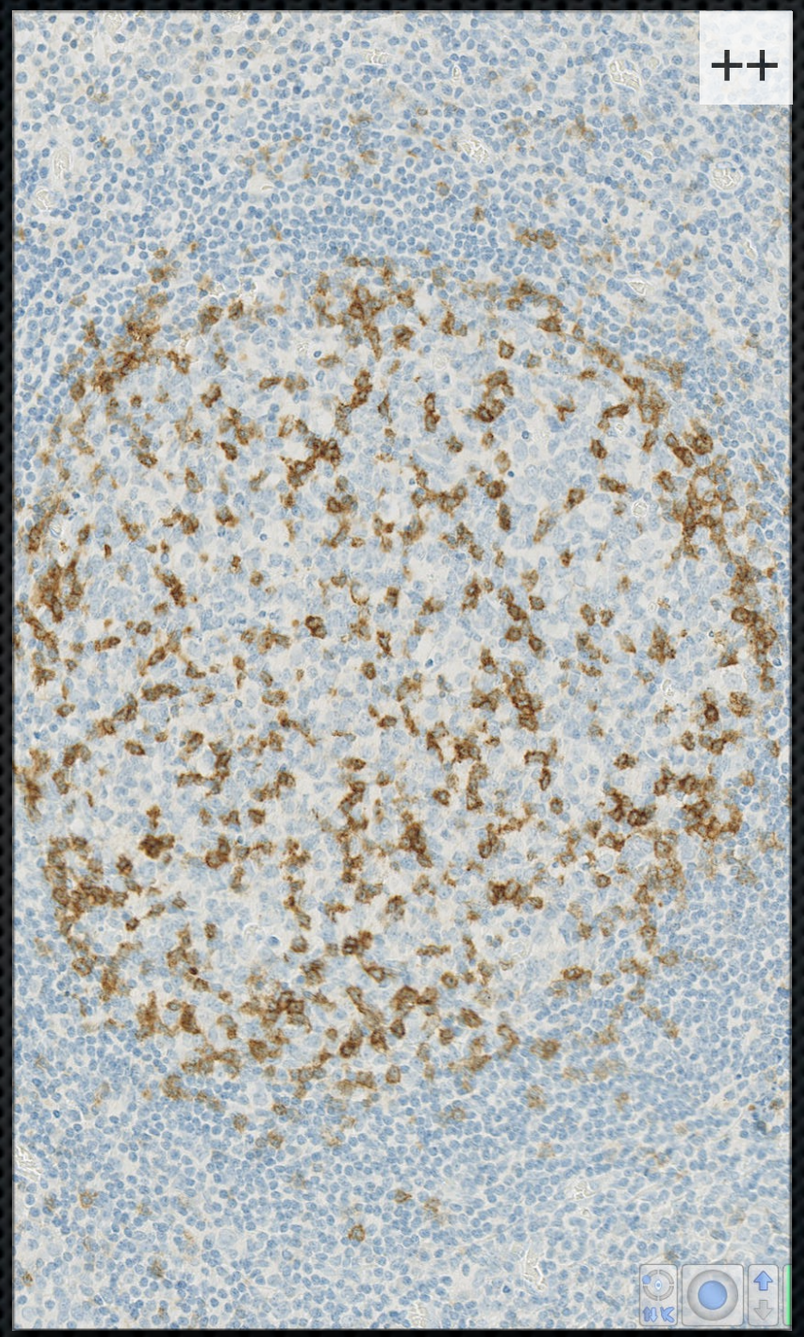


EDTA 96hrs

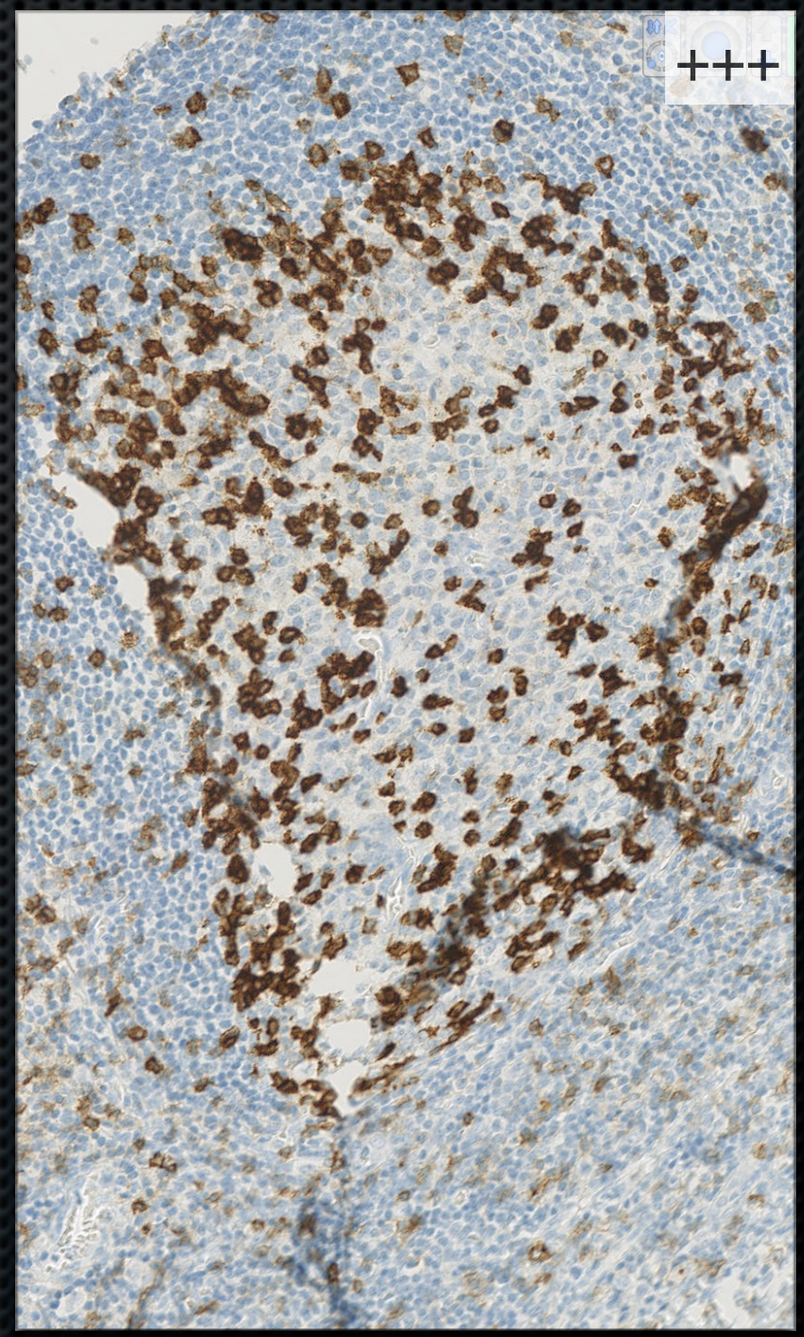
# Decalcification and CD279 (PD-1), NAT105




No decalcification



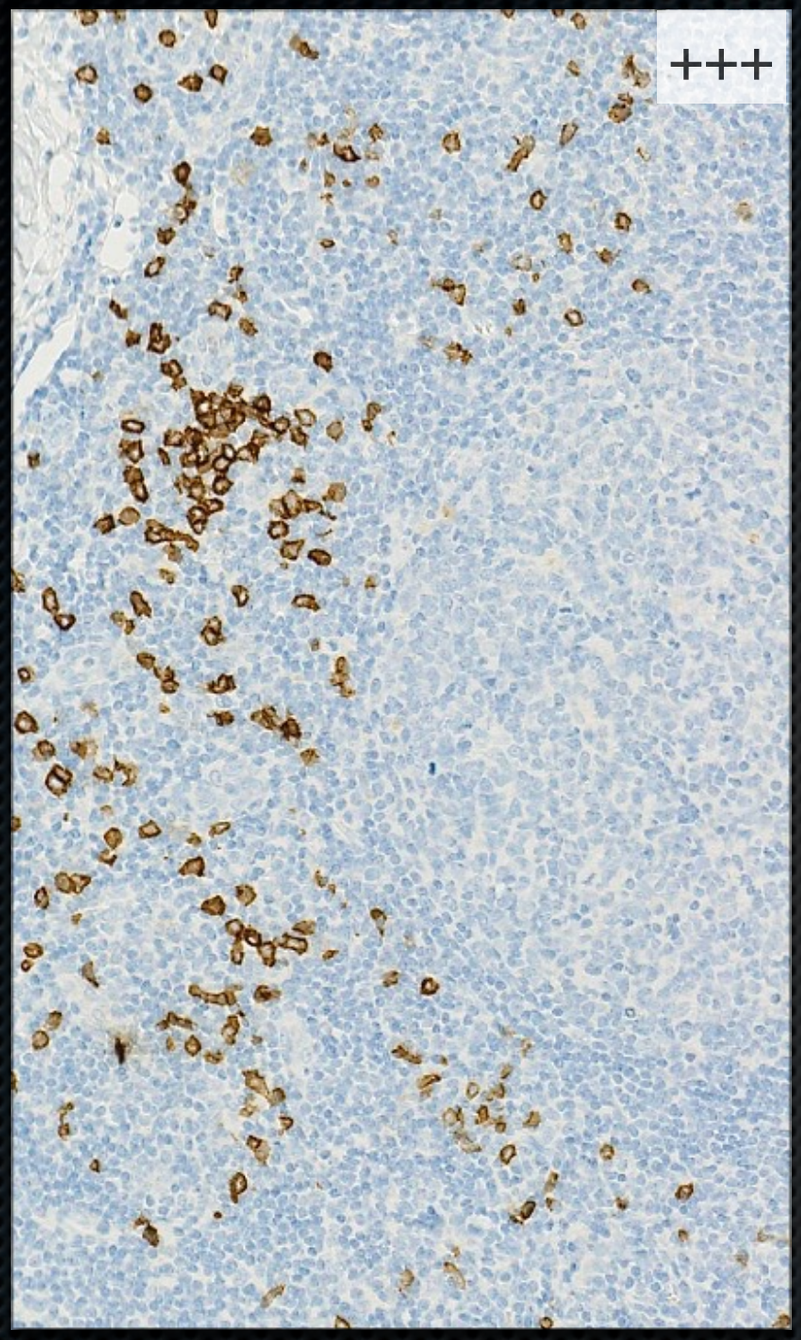

Formic acid 16hrs

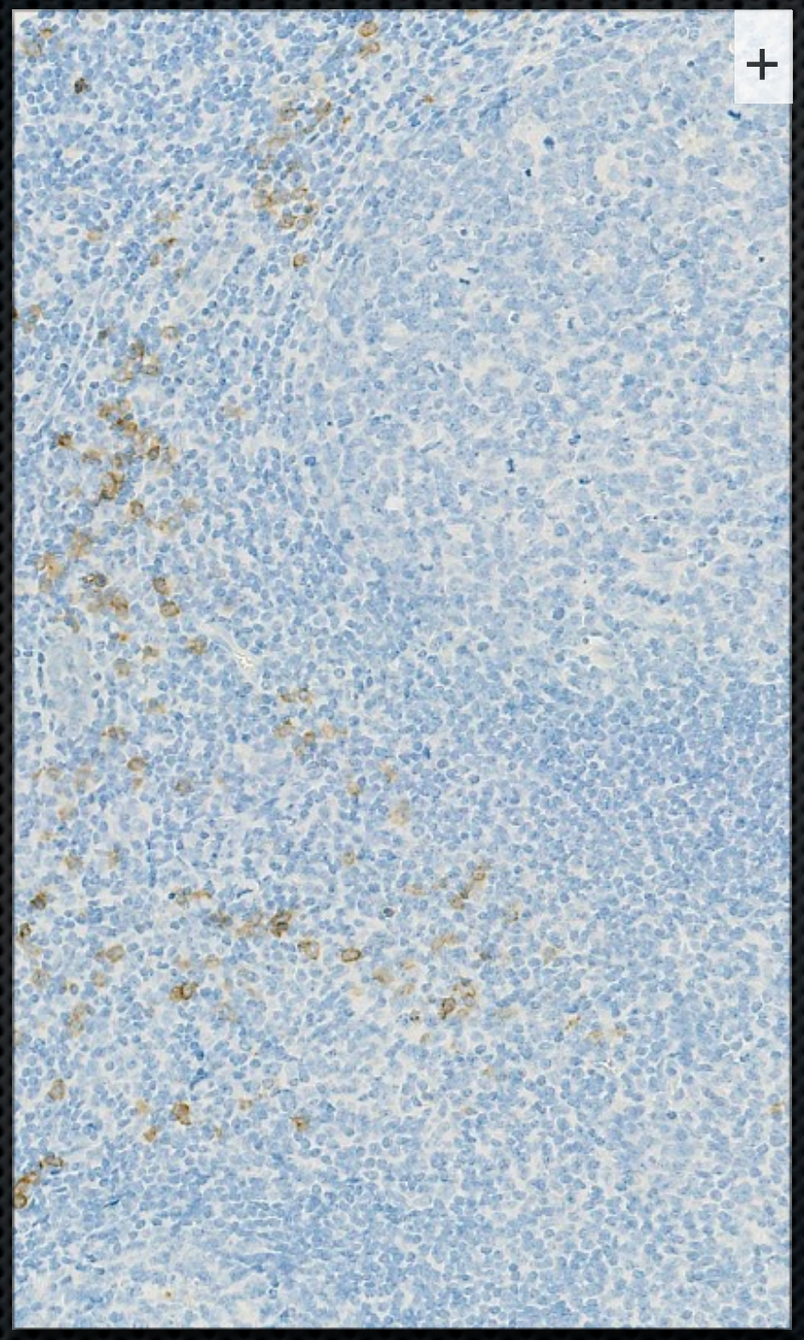


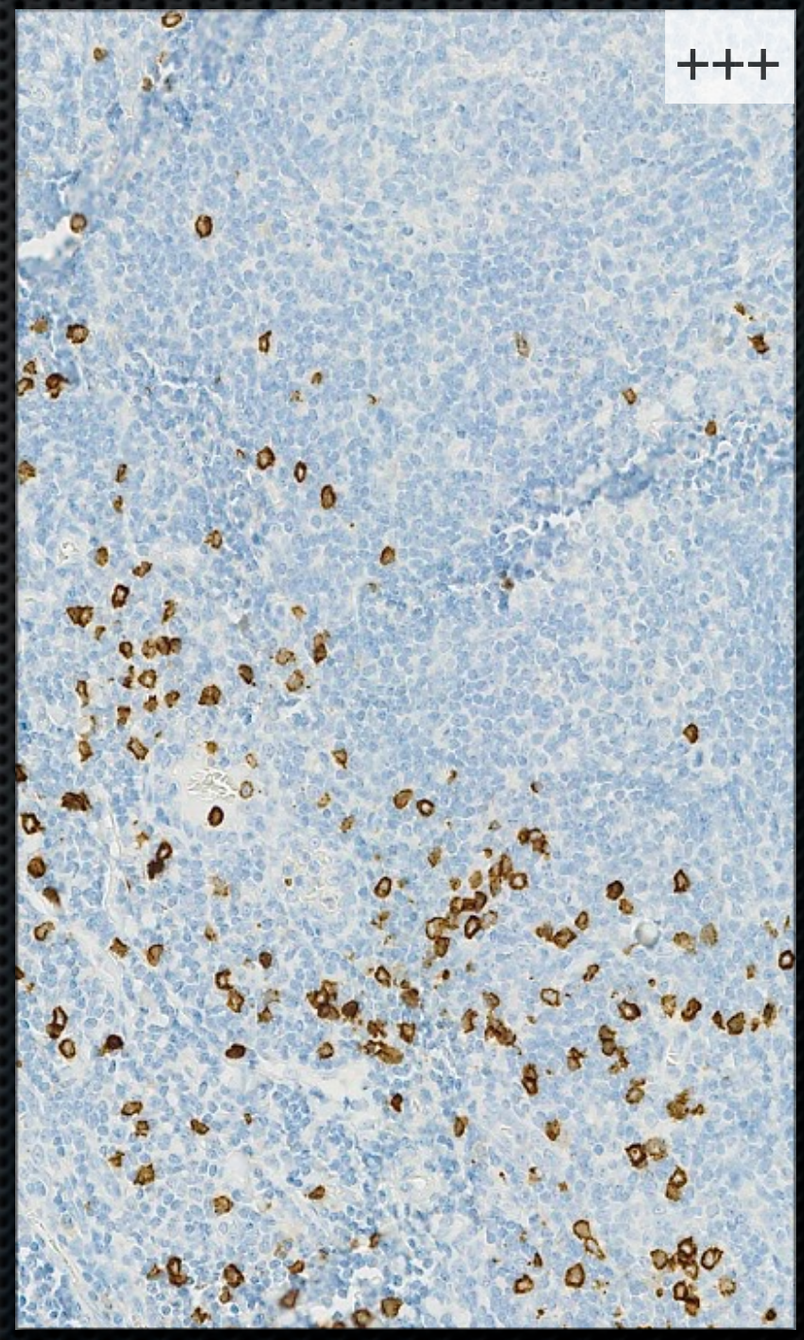

EDTA 96hrs

# Decalcification and CD303, 124B3.13

**CD303: Marker for Plasmacytoid dendritic cells**



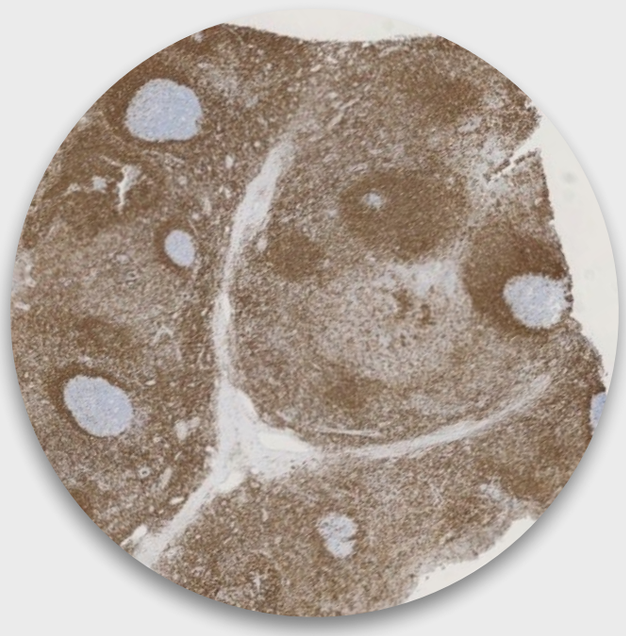
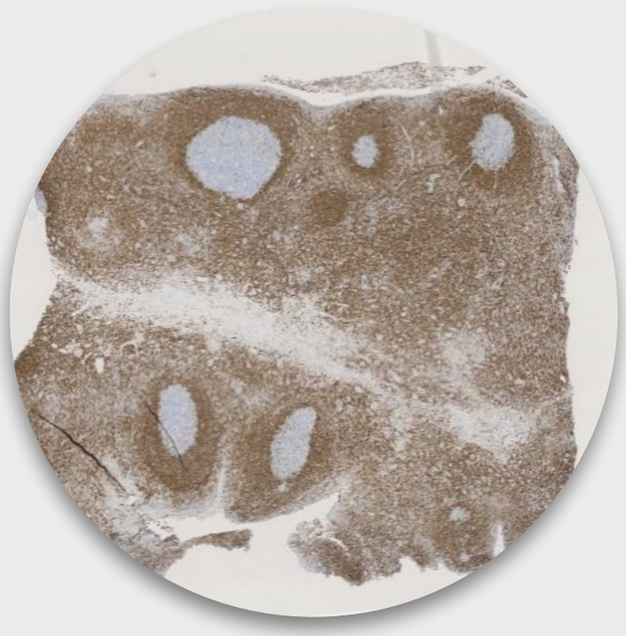
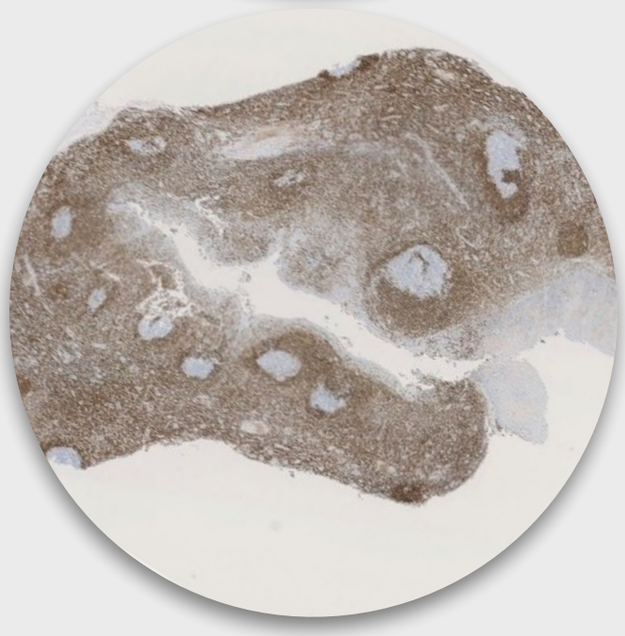
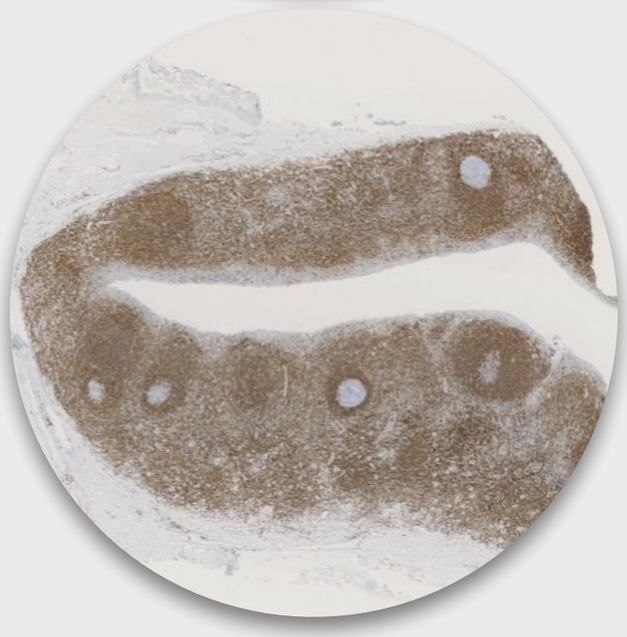




No decalcification  Formic acid 16hrs  EDTA 96hrs

# Fixation time and decalcification in buffet formic acid (BFA)

Bcl2, clone124

| | NBF 6 hrs | NBF 24 hrs | NBF 48 hrs |
|---|---|---|---|
| No decal |  |  |  |
| + | | | |
| BFA 16 hrs |  |  |  |

# Decalcification

- Most antigens don't survive decalcification in strong acid (e.g. Decal™)
- All tested antigens survive decalcification in EDTA and show no, or minimal reduction in staining intensity
- Only very few antigens don't survive decalcification in formic acid, but app. 10% show a slight reduction in staining intensity - learn!

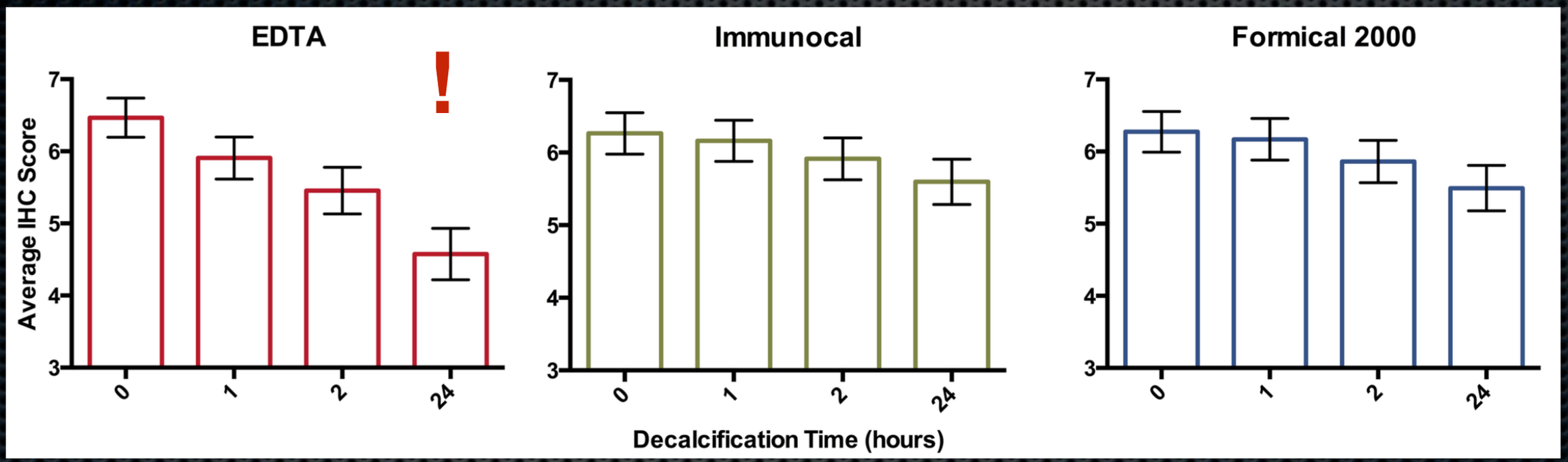
# Effects of Decalcification on Immunohistochemistry Comparing: Immunocal®, Formical2000®, and EDTA Stat®

Philip E. Ferguson, M.D.[1,2,3] & Yolanda Sanchez, MS-CRM[4]

[1]PathMD, LLC, [2]Doctors' Anatomic Pathology Services, [3]Saint Bernards Medical Center, and [4]Leica Biosystems

## Antibodies:

CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD31, CD34, CD45, CD79a, CD138, Bcl-2, Bcl-6, Ki-67, AE1/AE3, BerEP4, CDX-2, CAM5.2, CK7, CK20, Desmin, E-Cadherin, MOC-31, S-100, Smooth Muscle Myosin-HC (SMM-HC), and CEA.




Conclusions: As expected, decalcification has negative effects on IHC staining. Weak acid decalcification reagents (Formical2000 and Immunocal) showed better performance characteristics compared to EDTA Stat*, (in contrast to Odense findings!) and nuclear transcription markers appear to be more sensitive to the effects of decalcification.

** The exact formulation of EDTA Stat solution is unknown*

# Decalcification

| Preanalytic variable | Published Guidelines and Recommendations<br>CLSI | Literature-Based Recommendations |
|---|---|---|
| Decalcification | Interpret with caution - antigens could be lost! | **Tissue should be fixed 24 hrs in NBF prior to decalcification.**<br><br>**EDTA < Formic acid < Strong acid** |

International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology



REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

*Int. Jnl. Lab. Hem.* 2015, **37**, 431–449

# ICSH guidelines for the standardization of bone marrow immunohistochemistry

E. E. TORLAKOVIC*, R. K. BRYNES†, E. HYJEK‡, S.-H. LEE§, H. KREIPE¶, M. KREMER**, R. MCKENNA††, Y. SADAHIRA‡‡, A. TZANKOV§§, M. REIS¶¶, A. PORWIT*,***, FOR THE INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY

**Table 1.** Recommended protocols for bone marrow (BM) fixation and decalcification

| Turnaround time (TAT)* | Fixative | Fixation time | Decalcification | Decal time | Comments |
|---|---|---|---|---|---|
| Very short TAT | Acetic acid–zinc–formalin (AZF) | 2–72 h† | Shandon™ TBD-1™ Decalcifier | 30–40 min† | Whenever possible, longer fixation (within the range) is preferred |
| Intermediate TAT | AZF | Overnight | Gooding and Stewart's decalcification fluid (10% formic acid and 5% formaldehyde)‡ | 6 h | So-called 'Hammersmith Protocol' |
| Standard TAT | 10% buffered formalin = 3.7% formaldehyde | 8–72 h (overnight fixation is preferred)† | 14% EDTA | 16–24 h† | Preferred protocol for BM biopsy fixation and decalcification |

*Consideration of agitation and warming to 37 °C of the decalcifying solutions are recommended for each protocol. Ultrasonic decalcification may also be employed. These methods were shown to significantly shorten TAT.
†The timing may vary based on ancillary use of stirrers, ultrasound energization, microwave or other heating methods, or their combination.
‡Although decalcifying fixative is not recommended to be used alone, decalcifying fixative can produce superior results when used after the BM biopsy was already properly fixed in AZF or formalin.

# Tissue-processing

Region Syddanmark
OUH
Afd. f.
Klinisk
Patologi
*O. Nielsen*

# Implementation of a Microwave-assisted Tissue-processing System and an Automated Embedding System for Breast Needle Core Biopsy Samples: Morphology, Immunohistochemistry, and FISH Evaluation

*Enrico Pegolo, MD, Maura Pandolfi, BSc, and Carla Di Loreto, MD*

(*Appl Immunohistochem Mol Morphol* 2012;00:000–000)

***Material:*** *233 consecutive needle core breast biopsies.*
*The fixation time was strictly standardized, ranging from 18 to 24 hours.*
*After fixation, half of the core specimens from each case were randomly assigned to the conventional processing system (Leica ASP 300S 16-hrs program) and the other half to the MW-assisted tissue-processing system Sakura Tissue-Tek Xpress 120 (1-hr program).*

**TABLE 2.** Immunohistochemical Analysis

| Antibodies | Clone, Species | Manufacturer | Pretreatment | Dilution/Time |
|---|---|---|---|---|
| CK 5/6 | D5/16B4, mouse | Dako | Tris/EDTA buffer (pH 9) at 97°C for 15 min | 1:50/30 min |
| CK 19 | RCK108, mouse | Dako | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:50/20 min |
| E-cadherin | NCH-38, mouse | Dako | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:100/20 min |
| p63 | 4A4, mouse | Dako | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:100/20 min |
| SMA | 1A4, mouse | Dako | Tris/EDTA buffer (pH 9) at 97°C for 15 min | 1:200/20 min |
| ER | SP1, rabbit | Aczonpharma (Bologna, Italy) | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:200/40 min |
| PR | PgR 636, mouse | Dako | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:100/40 min |
| Ki-67 | Mib-1, mouse | Dako | Citrate buffer (pH 6.1) at 97°C for 20 min | 1:50/20 min |
| HercepTest | Polyclonal, rabbit | Dako | Dako Epitope Retrieval Solution | Predil/30 min |

CK indicates cytokeratin; ER, estrogen receptor; PR, progesterone receptor; Predil, prediluted; SMA, smooth muscle actin.

# Implementation of a Microwave-assisted Tissue-processing System and an Automated Embedding System for Breast Needle Core Biopsy Samples: Morphology, Immunohistochemistry, and FISH Evaluation

*Enrico Pegolo, MD, Maura Pandolfi, BSc, and Carla Di Loreto, MD*

(*Appl Immunohistochem Mol Morphol* 2012;00:000–000)

**TABLE 3.** Estrogen Receptor Status in the Conventionally Processed and in the Matched MW-assisted Processed NCBs of Breast Carcinomas

| | ER Status (Conventional) | | |
|---|---|---|---|
| **ER Status (MW)** | **Positive** | **Negative** | **Total** |
| Positive | 62 | 0 | 62 |
| Negative | 0 | 16 | 16 |
| Total | 62 | 16 | 78 |

Cohen κ test = 1.

ER indicates estrogen receptor; MW, microwave-assisted processing system; NCB, needle core biopsy.

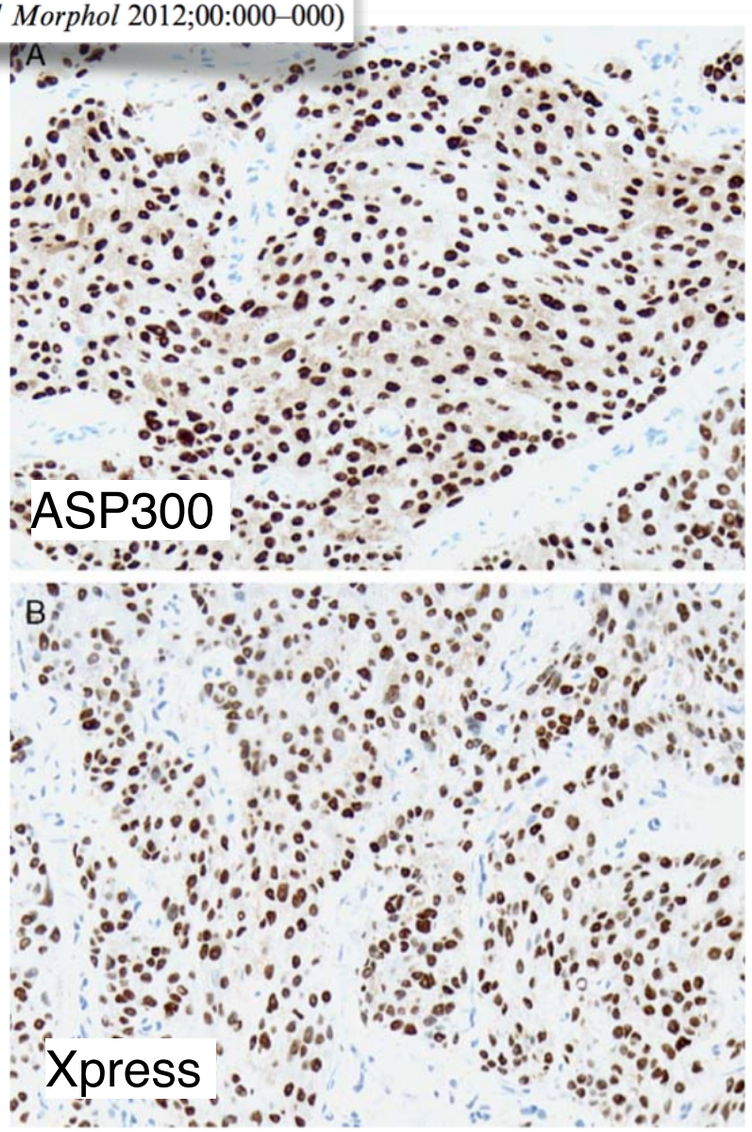



**FIGURE 2.** Needle core biopsy: invasive ductal carcinoma. Immunohistochemical reaction for estrogen receptor in the nuclei of tumor cells. The reaction is the same in the specimens prepared using the conventional processing method (A) and the microwave-assisted processing method (B) (A and B, immunoperoxydase for estrogen receptor, hematoxylin counterstain, original magnification ×200). full color online

# Implementation of a Microwave-assisted Tissue-processing System and an Automated Embedding System for Breast Needle Core Biopsy Samples: Morphology, Immunohistochemistry, and FISH Evaluation

*Enrico Pegolo, MD, Maura Pandolfi, BSc, and Carla Di Loreto, MD*

(*Appl Immunohistochem Mol Morphol* 2012;00:000–000)

**TABLE 6.** HER2 Immunohistochemical Results in the Conventionally Processed and in the Matched MW-assisted Processed NCBs of Breast Carcinomas

| | HER2 IHC (Conventional) | | | |
|---|---|---|---|---|
| **HER2 IHC (MW)** | **Negative** | **Equivocal** | **Positive** | **Total** |
| Negative | 50 | 0 | 0 | 50 |
| Equivocal | 2 | 11 | 0 | 13 |
| Positive | 0 | 0 | 8 | 8 |
| Total | 52 | 11 | 8 | 71 |

Cohen κ test = 0.93. $P = 0.88$, $\chi^2$ test.

IHC indicate immunohistochemistry; MW, microwave-assisted processing system; NCB, needle core biopsy.

The quality of H&E and immunohistochemical tissue sections provided by the new system is comparable to that obtained after the conventional processing method; this system also reduces the turnaround time for surgical pathology reports. Moreover, this is the first study that validates the assessment of the main prognostic and predictive biomarkers in breast NCBs processed by a MW-assisted system and automatically embedded.

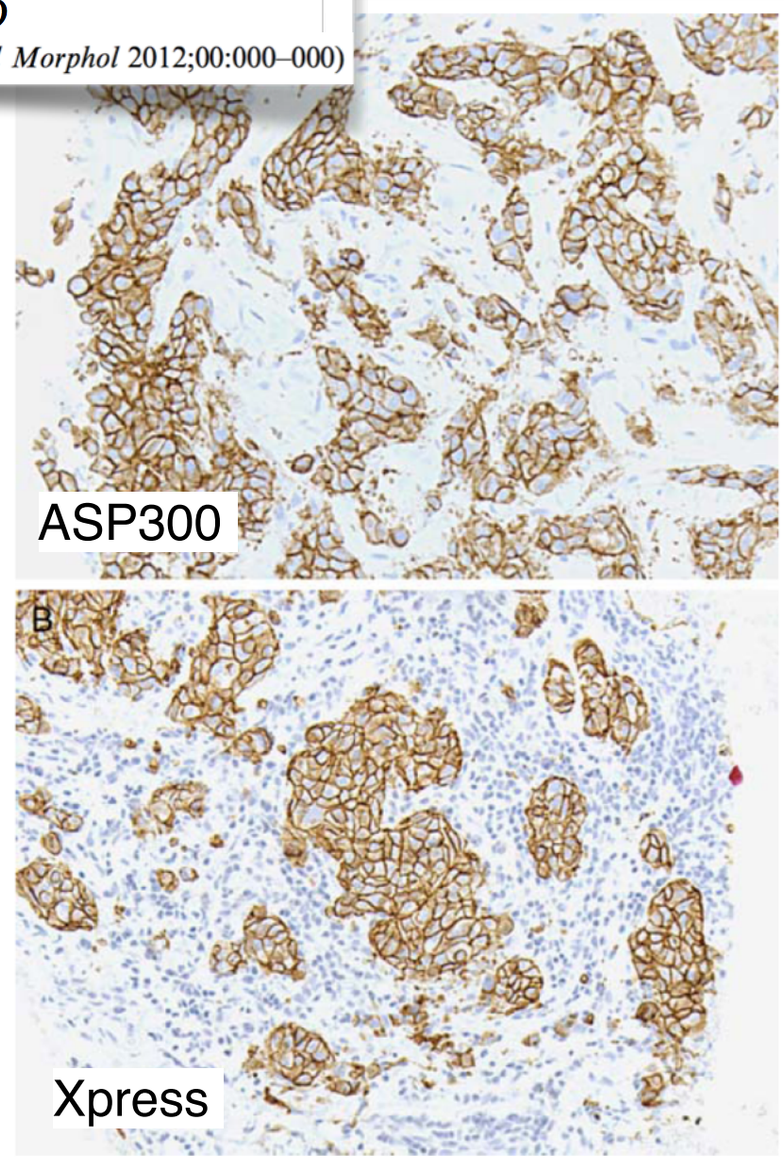



**FIGURE 5.** Needle core biopsy: invasive ductal carcinoma. Immunohistochemical reaction for HER2 (HercepTest) in the cell membranes of tumor cells. The same strong complete membrane staining (score 3+) is observed in the specimens prepared using the conventional processing method (A) and the microwave-assisted processing method (B) (A and B, HercepTest, original magnification ×200). full color online

# Processing

| Preanalytic variable | Published Guidelines and Recommendations<br>ASCO/CAP \| CLSI | Literature-Based Recommendations |
|---|---|---|
| Dehydration | 1.25 - 15 hrs | 10 hrs |
| Type of paraffin | Paraffin (55°C-58°C) | Paraffin (45°C) |
| Time in paraffin | 0.5 - 4.5 hrs | 1 - 2 hrs or 8 hrs |

Engel KB, Moore HM. Arch Pathol Lab Med. 2011;135:537–543

# Paraffin sectioning

- Type of blade and frequency of replacement
- Frequency of servicing and wax replacement
- Temperature of block during sectioning
- Slide pretreatment
- Water bath conditions, if used
- Chemical adhesives, if used
- Temperature and duration of slide drying

Immunocytochemistry 2008; Volume 6 Issue 3

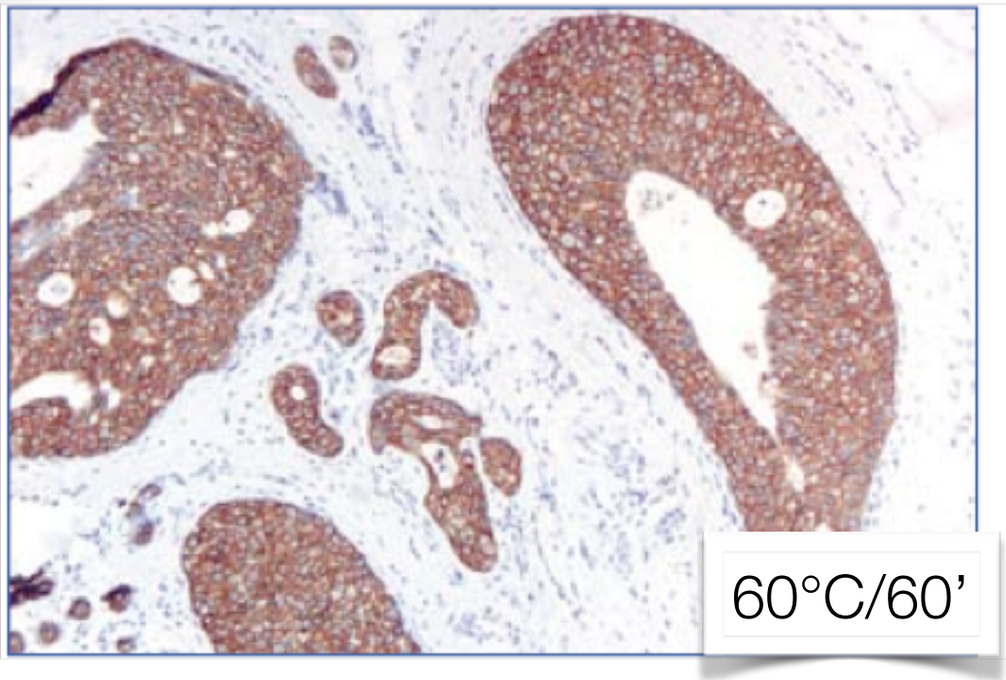

**TECHNICAL ARTICLE**

# EXCESSIVE SECTION DRYING OF BREAST CANCER TISSUE PRIOR TO DEPARAFFINISATION AND ANTIGEN RETRIEVAL CAUSES A LOSS IN HER2-IMMUNO-REACTIVITY

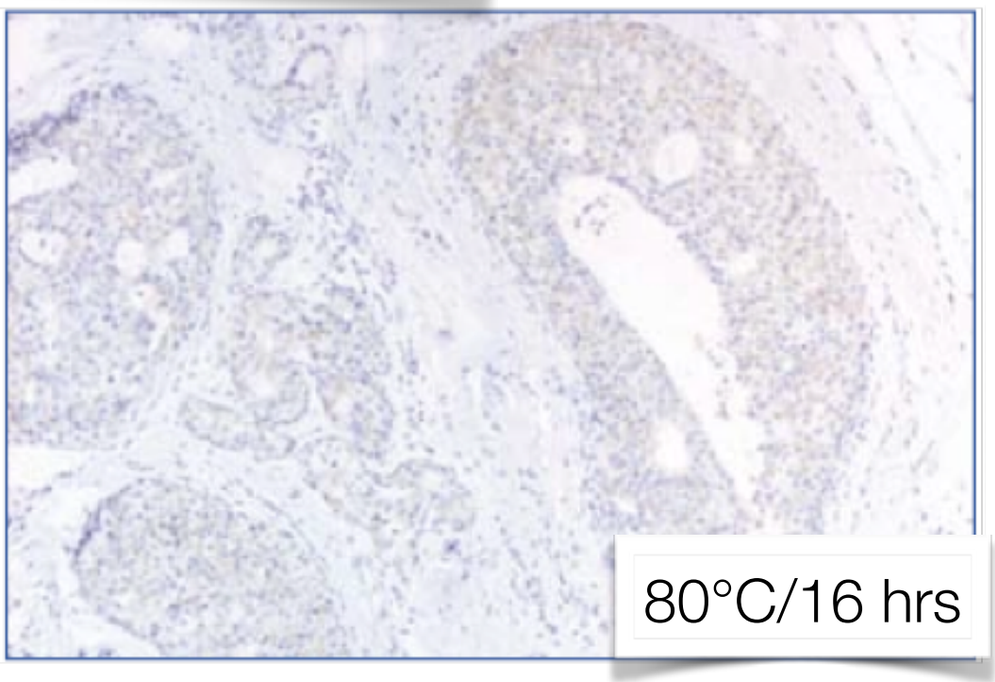
**Bent Lundgaard Hansen, Henrik Winther and Kristian Moller**

*Dako A/S, DK-2600, Glostrup, Denmark*

**Antibodies:**
a. HercepTest
b. Clone 4B5
c. Clone CB11

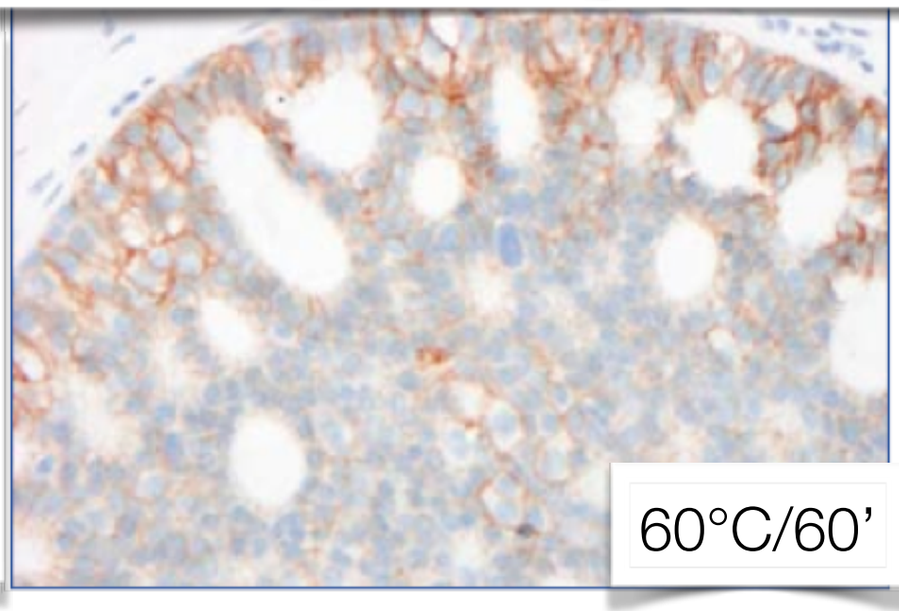


60°C/60’

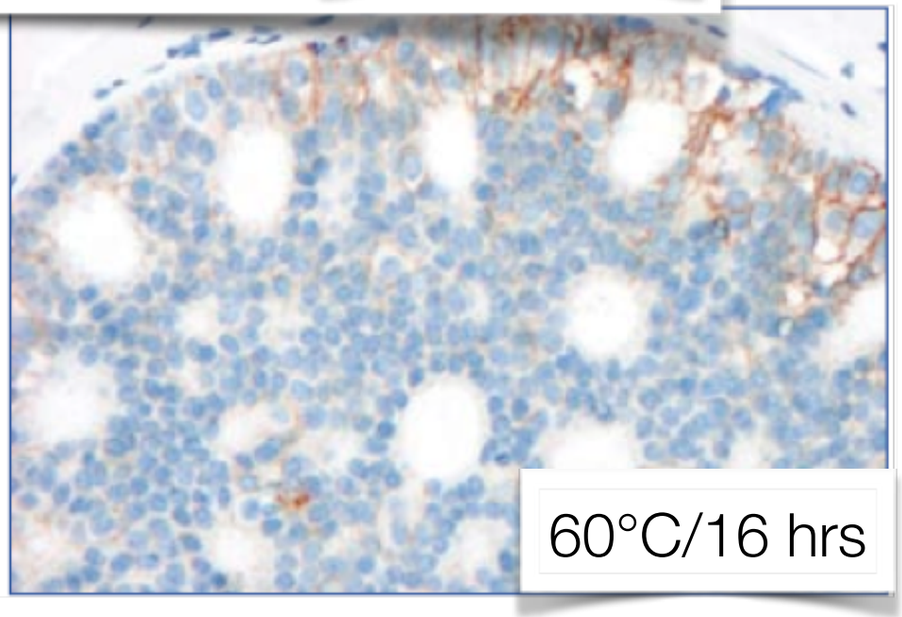


80°C/16 hrs

*“Procedure for drying of tissue prior to deparaffinization: The drying temperature should be 60°C for a maximum of one hour, 37 °C for a maximum of 24 hours, or ambient temperature for 24 hours or longer”.*

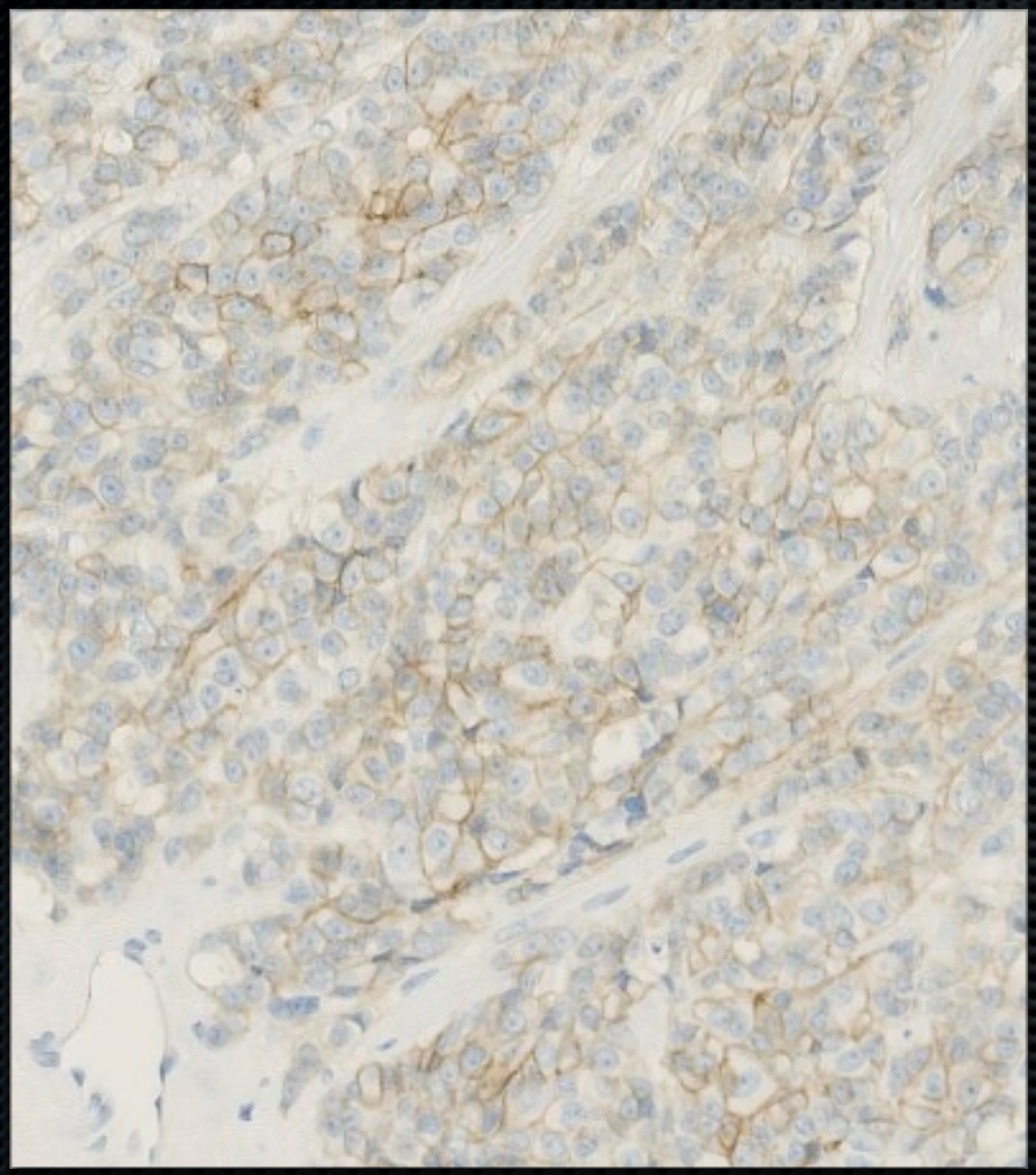


60°C/60’

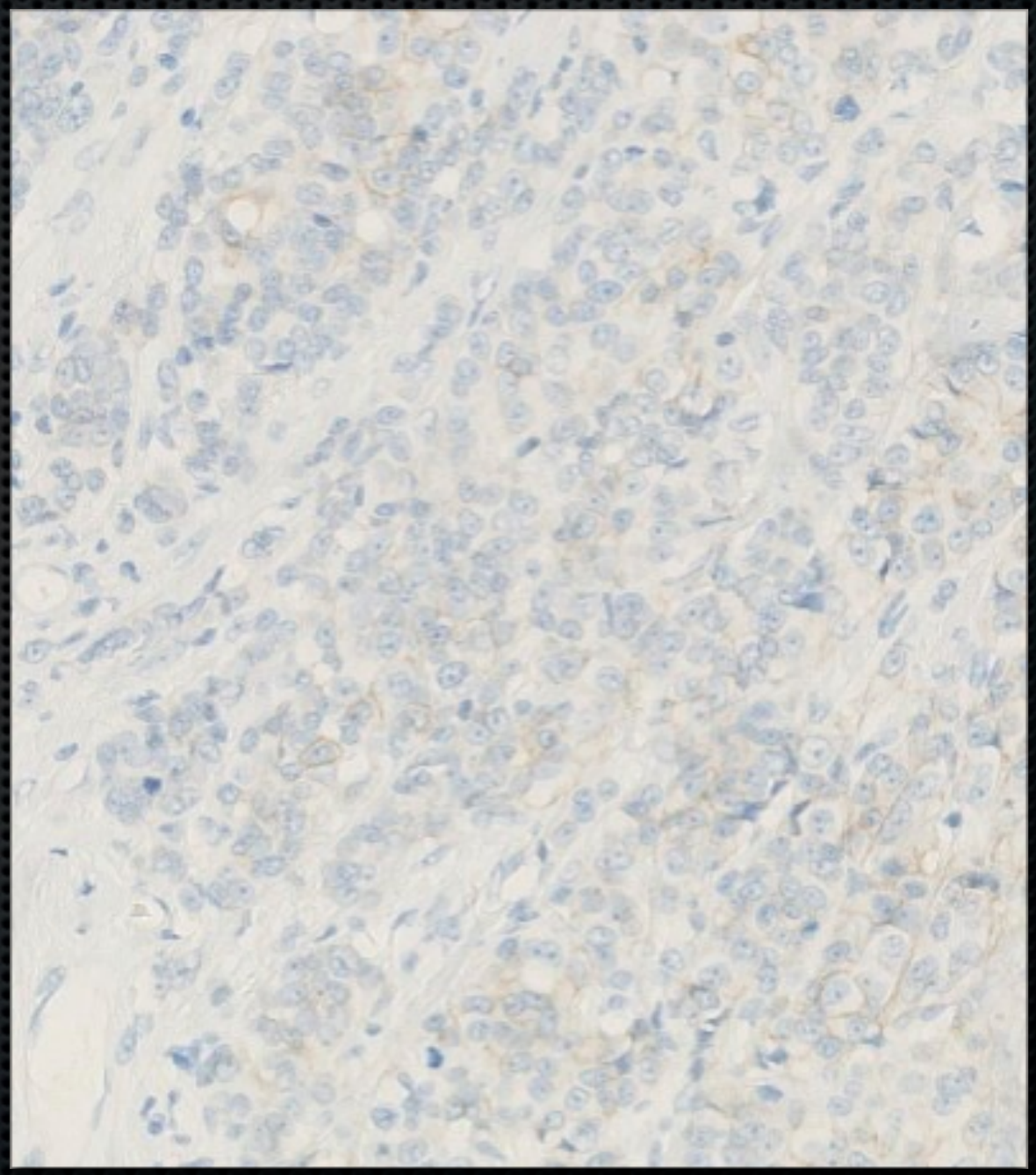


60°C/16 hrs

# Drying of sections - HER2, 4B5

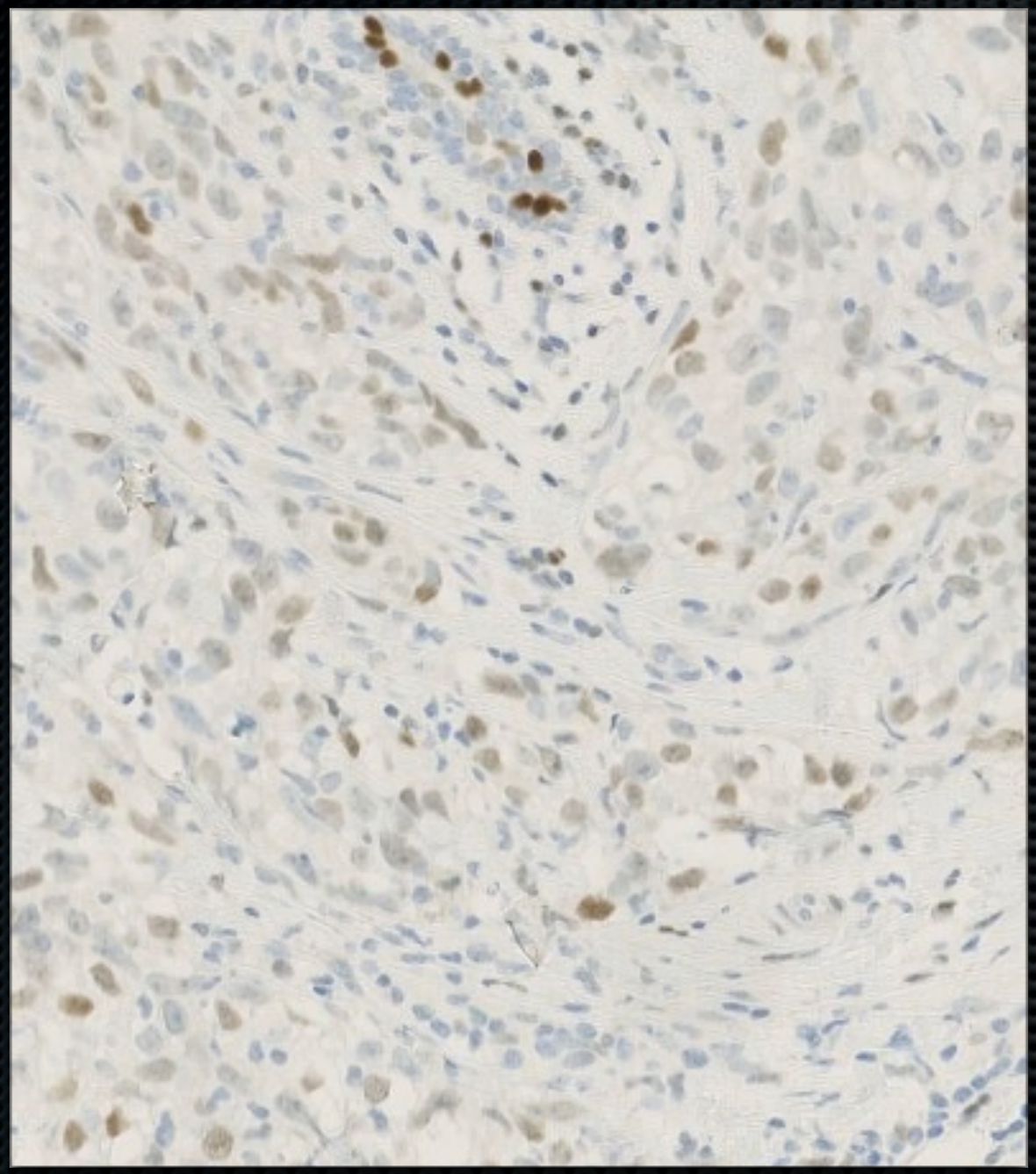


60 min at 60°C

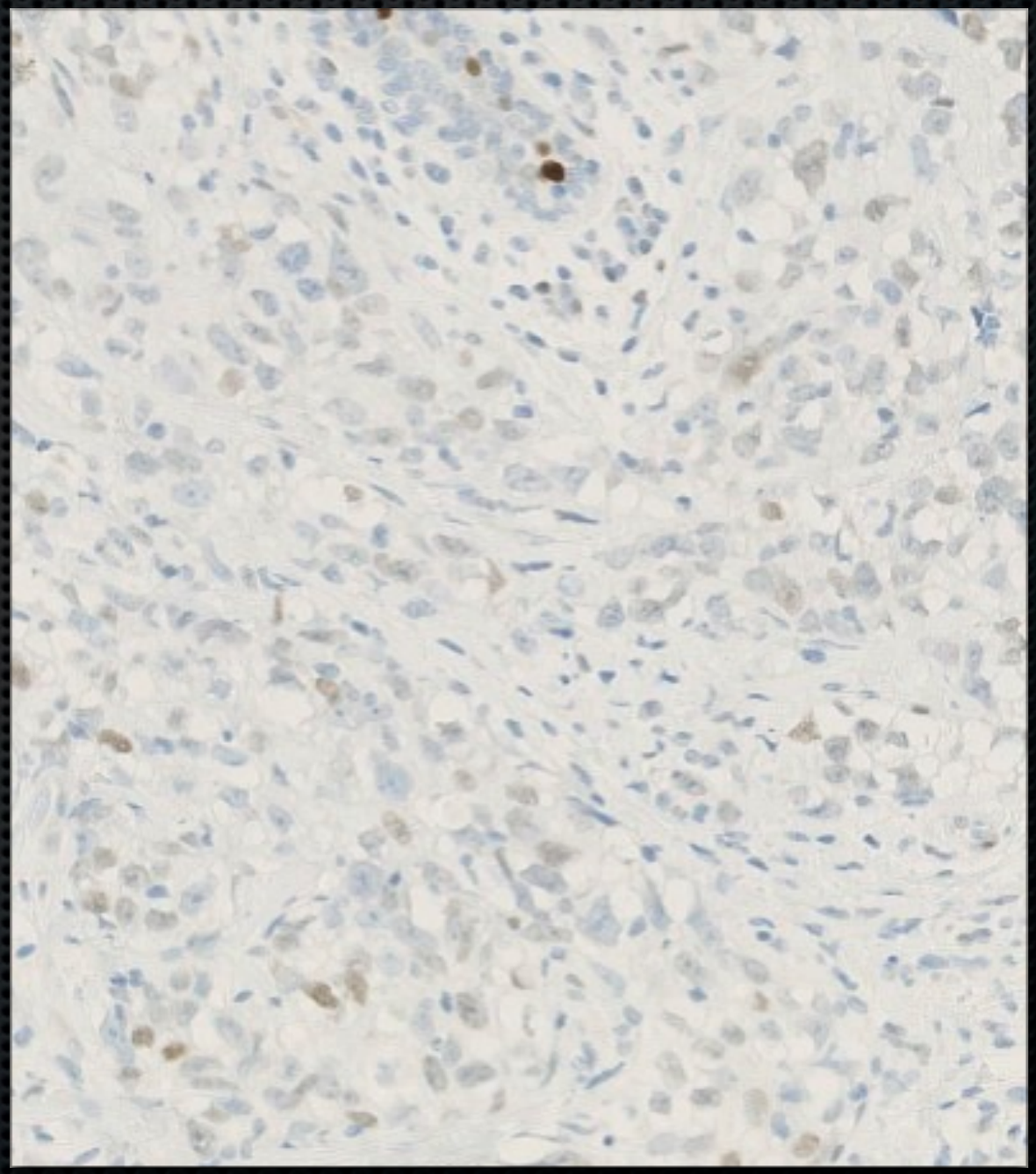


16 hrs at 80°C

# Drying of sections - ER, SP1



60 min at 60°C



16 hrs at 80°C

# Drying of sections (Baking)

| Preanalytic variable | Published Guidelines and Recommendations<br>ASCO/CAP \| CLSI | Literature-Based Recommendations |
|---|---|---|
| Drying of sections | **<u>24 hrs at RT or 1 hr at 50°C - 60°C</u>** | 24 hrs at RT or overnight at 37°C |

Engel KB, Moore HM. Arch Pathol Lab Med. 2011;135:537–543

# Storage

- Temperature and duration of paraffin block storage

- Temperature, duration, and manipulation of slide-mounted tissue sections

Modern Pathology (2004) 17, 1414–1420

www.modernpathology.org

# Influence of slide aging on results of translational research studies using immunohistochemistry

Martina Mirlacher, Marlis Kasper, Martina Storz, Yvonne Knecht, Ursula Dürmüller, Ronald Simon, Michael J Mihatsch and Guido Sauter

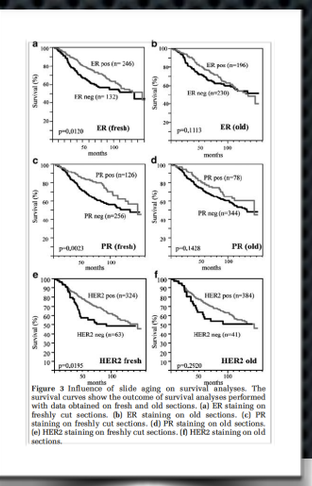


Figure 3 Influence of slide aging on survival analyses. The survival curves show the outcome of survival analyses performed with data obtained on fresh and old sections. (a) ER staining on freshly cut sections. (b) ER staining on old sections. (c) PR staining on freshly cut sections. (d) PR staining on old sections. (e) HER2 staining on freshly cut sections. (f) HER2 staining on old sections.

Fresh sections (F) vs. sections stored at 4°C for 6 months (O)

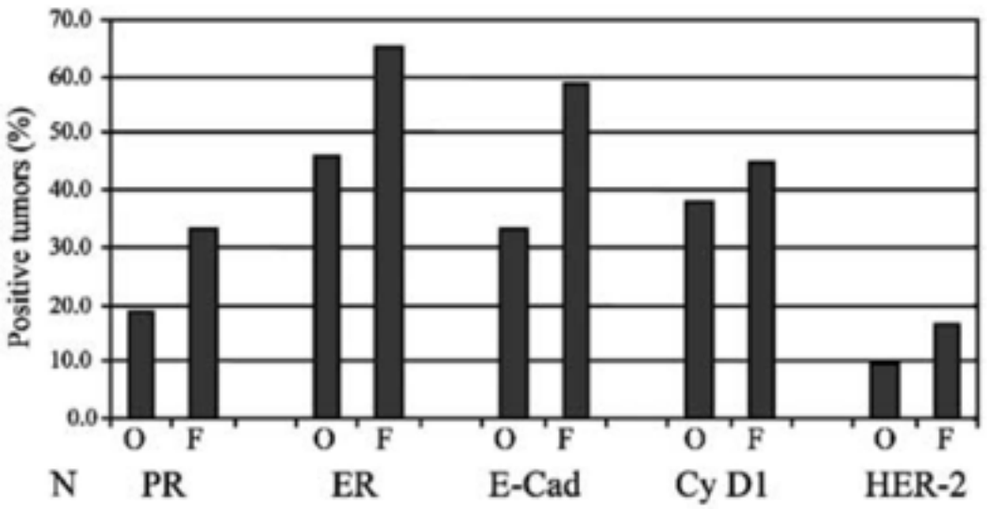
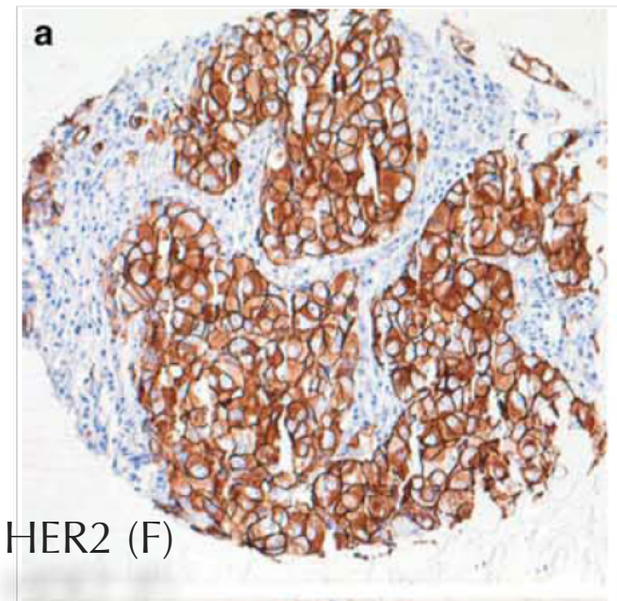
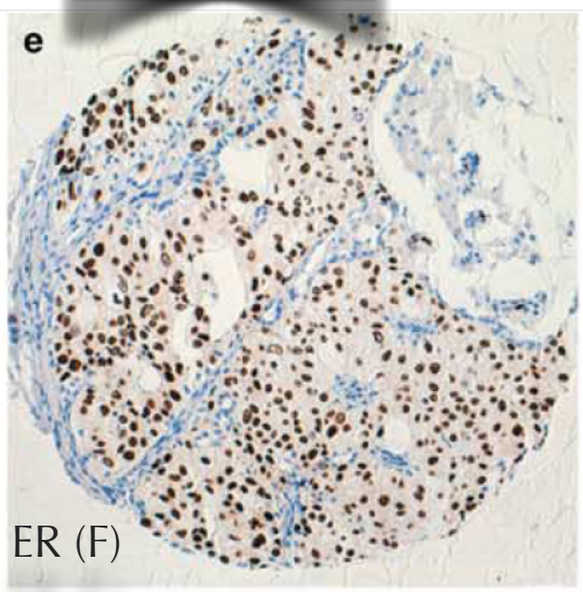


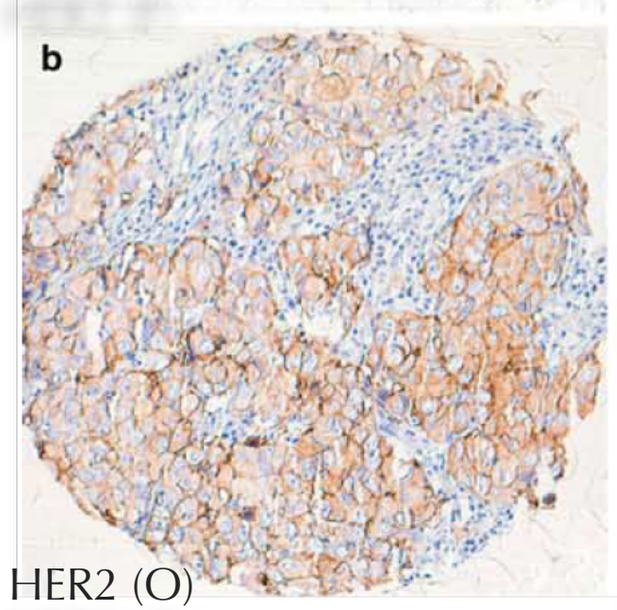
**Figure 2** Influence of slide aging on the fraction of positive cases. For each antibody, the frequency of positive cases is shown as separate bars for old (O) and fresh (F) sections.



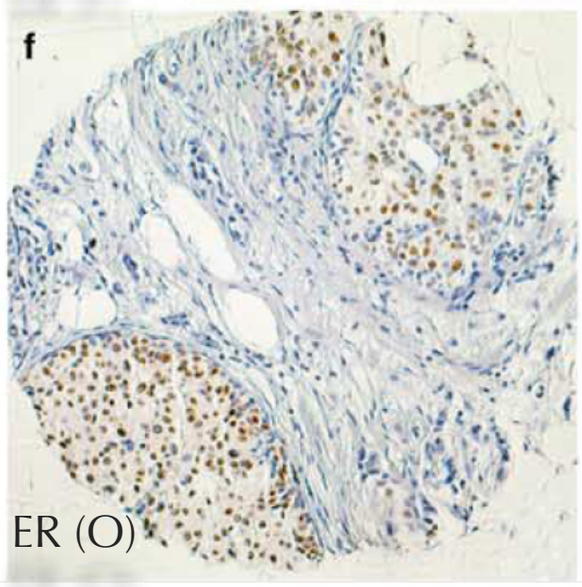
HER2 (F)



ER (F)



HER2 (O)

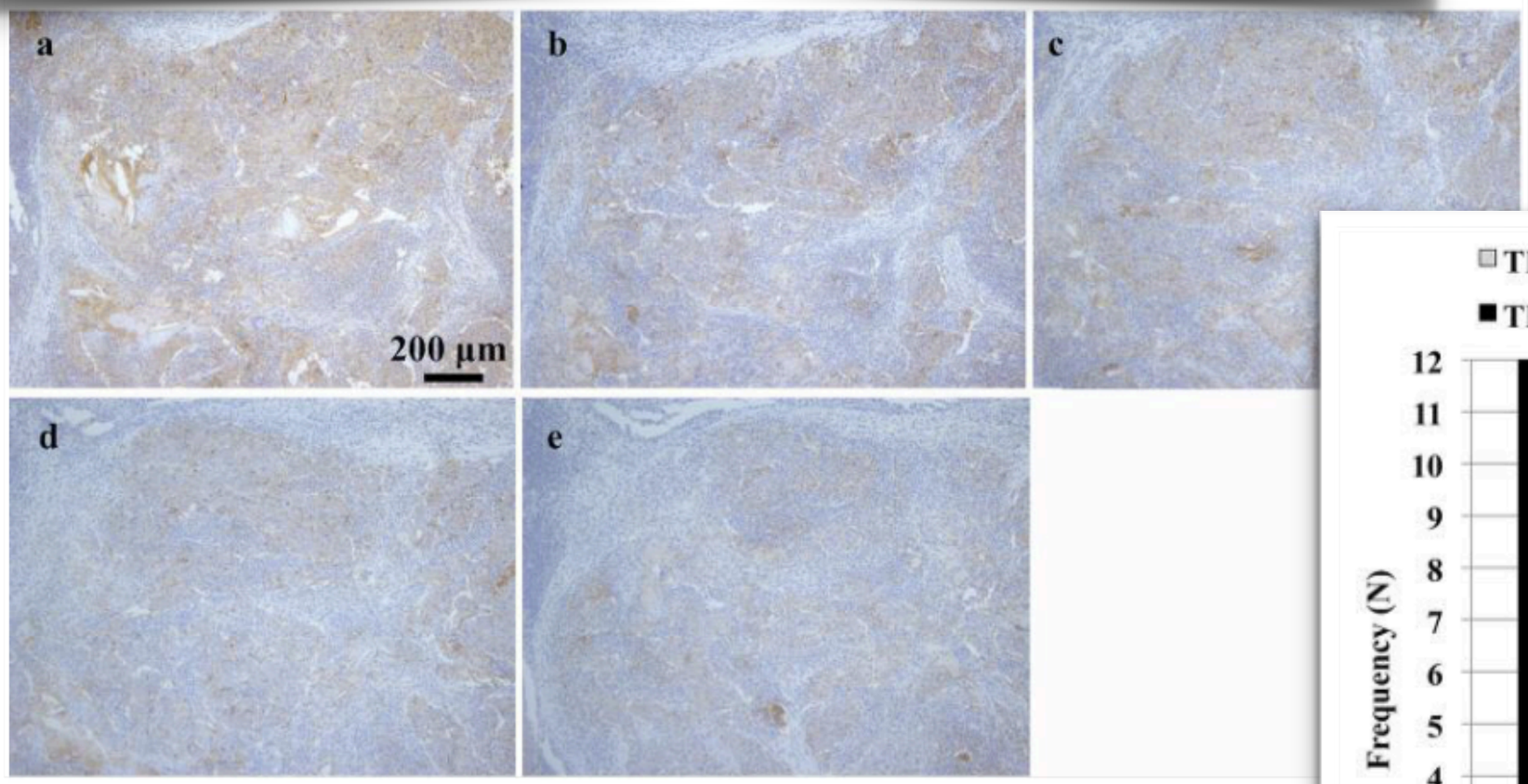


ER (O)

ANTICANCER RESEARCH *38*: 1401-1405 (2018)
doi:10.21873/anticanres.12363

# Reduced Tumour Proportion Scores for Programmed Cell Death Ligand 1 in Stored Paraffin Tissue Sections

YUKI SATO[1], DAICHI FUJIMOTO[1], KEIICHIRO UEHARA[2], HAYATO KAWACHI[1],




**Representative immunohistochemistry results for programmed cell death ligand 1 using clone 28-8 antibodies: a: freshly-stained specimen; b: 2 weeks of storage; c: 4 weeks of storage; d: 6 weeks of storage; e: 8 weeks of storage**

Fresh sections vs. sections stored at 4°C for 2-8 weeks

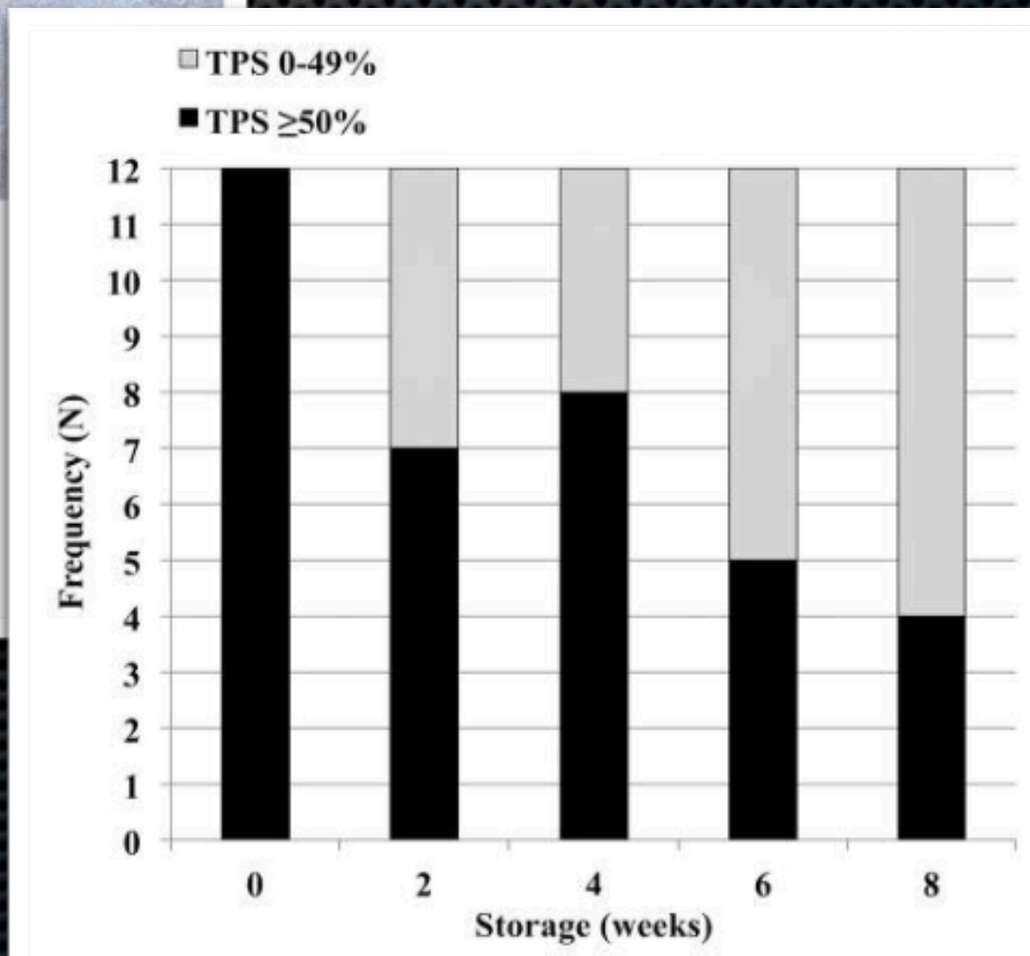



Figure 2. *Changes in distribution of the tumour proportion score (TPS) for programmed cell death ligand 1 (PD-L1), using a 50% cutoff. After 2, 4, 6, and 8 weeks of slide storage, false-negative results were observed in five (41%), four (33%), seven (58%), and eight (67%) patients, respectively.*

# Influence of Storage Temperature and High-Temperature Antigen Retrieval Buffers on Results of Immunohistochemical Staining in Sections Stored for Long Periods

Applied Immunohistochemistry *6(4): 209–213, 1998*

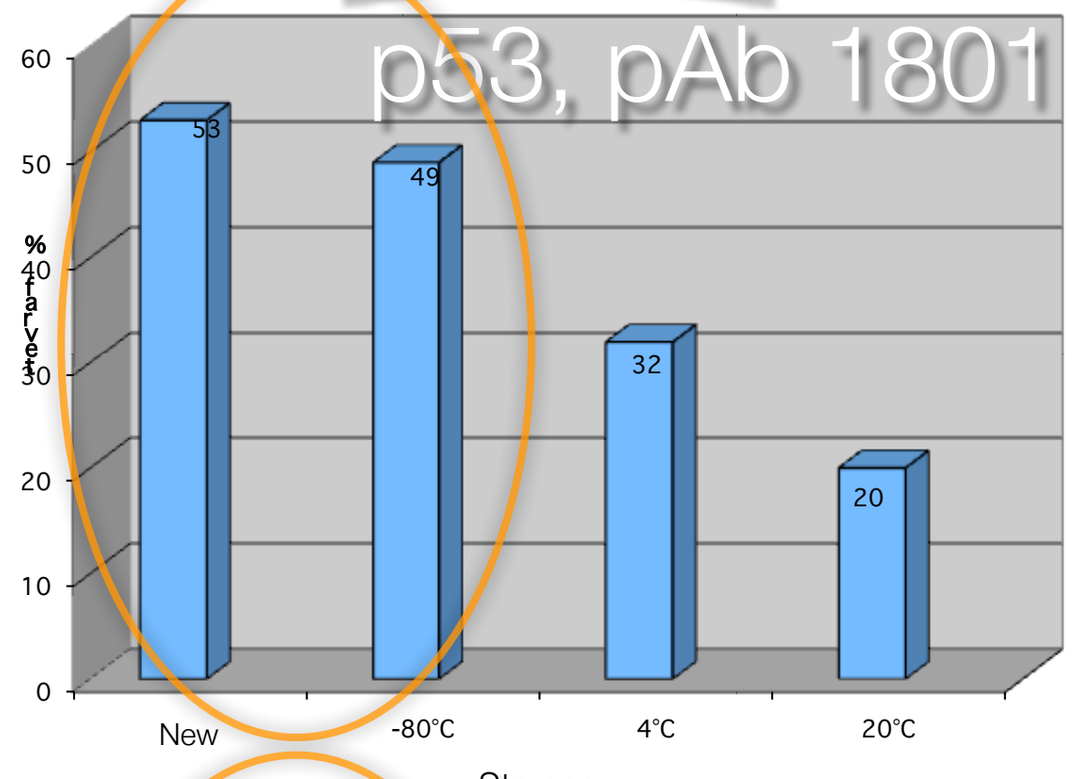
Dorthe A. Grabau, M.D., Ph.D., Ole Nielsen, H.T., Steinbjørn Hansen, M.D., Mette M. Nielsen, M.D., Anne-Vibeke Lænkholm, M.D., Ann Knoop, M.D., and Per Pfeiffer, M.D., Ph.D.

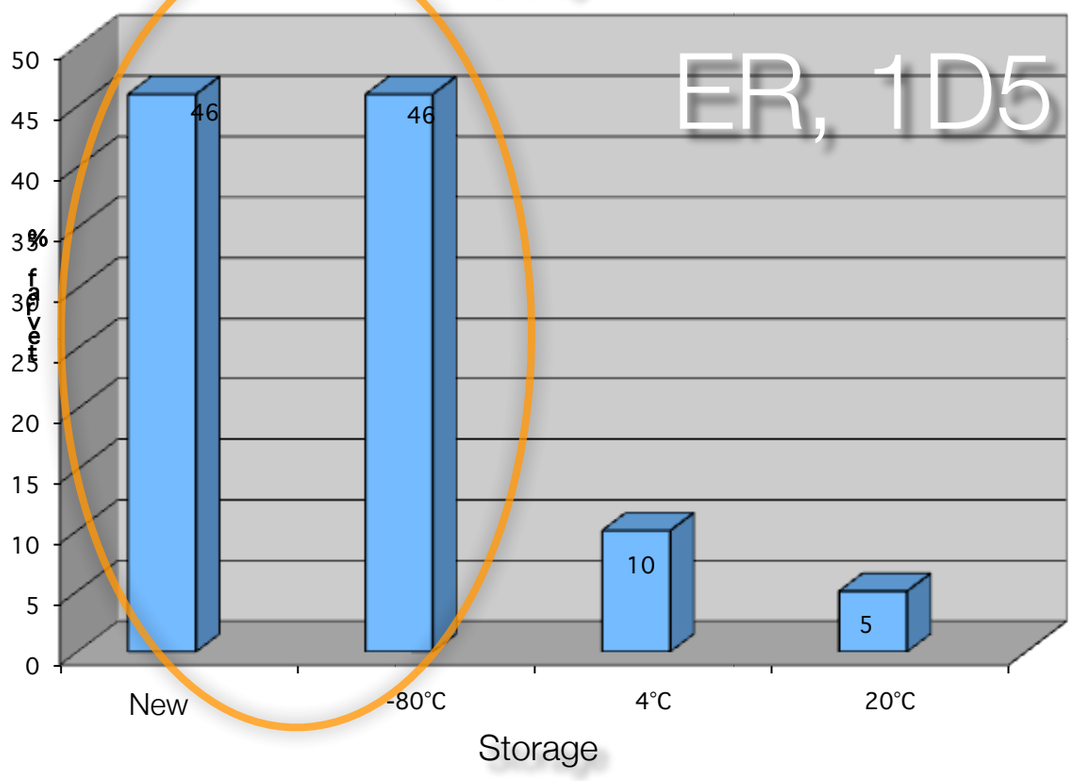
Estrogen Receptor, 1D5




Storage at -80°C gave superior results

NordiQC



# Factors Influencing the Degradation of Archival Formalin-Fixed Paraffin-Embedded Tissue Sections

**Ran Xie, Joon-Yong Chung, Kris Ylaya, Reginald L. Williams, Natalie Guerrero, Nathan Nakatsuka, Cortessia Badie, and Stephen M. Hewitt**

Tissue Array Research Program, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(J Histochem Cytochem 59:356–365, 2011)

# Water?

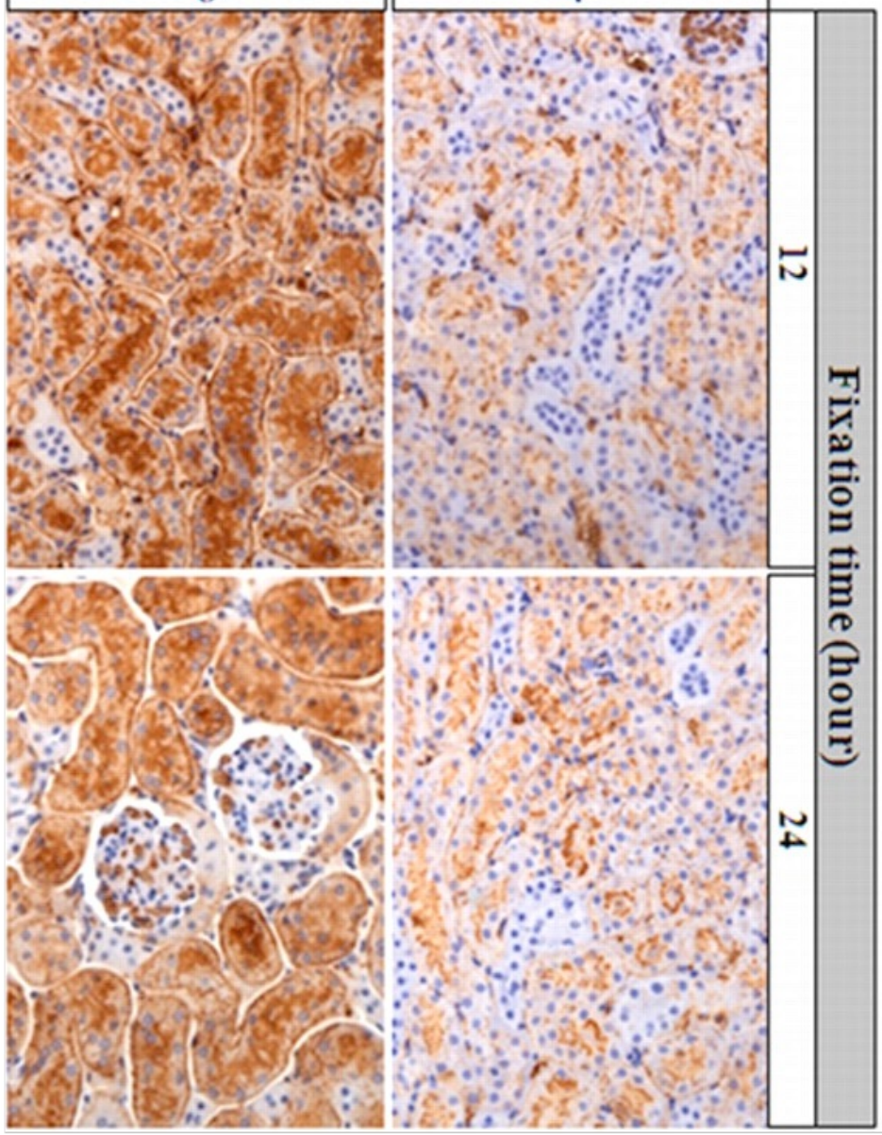
Aquaporin 1 (AQP1)

**Factors Influencing the Degradation of Archival Formalin-Fixed Paraffin-Embedded Tissue Sections**

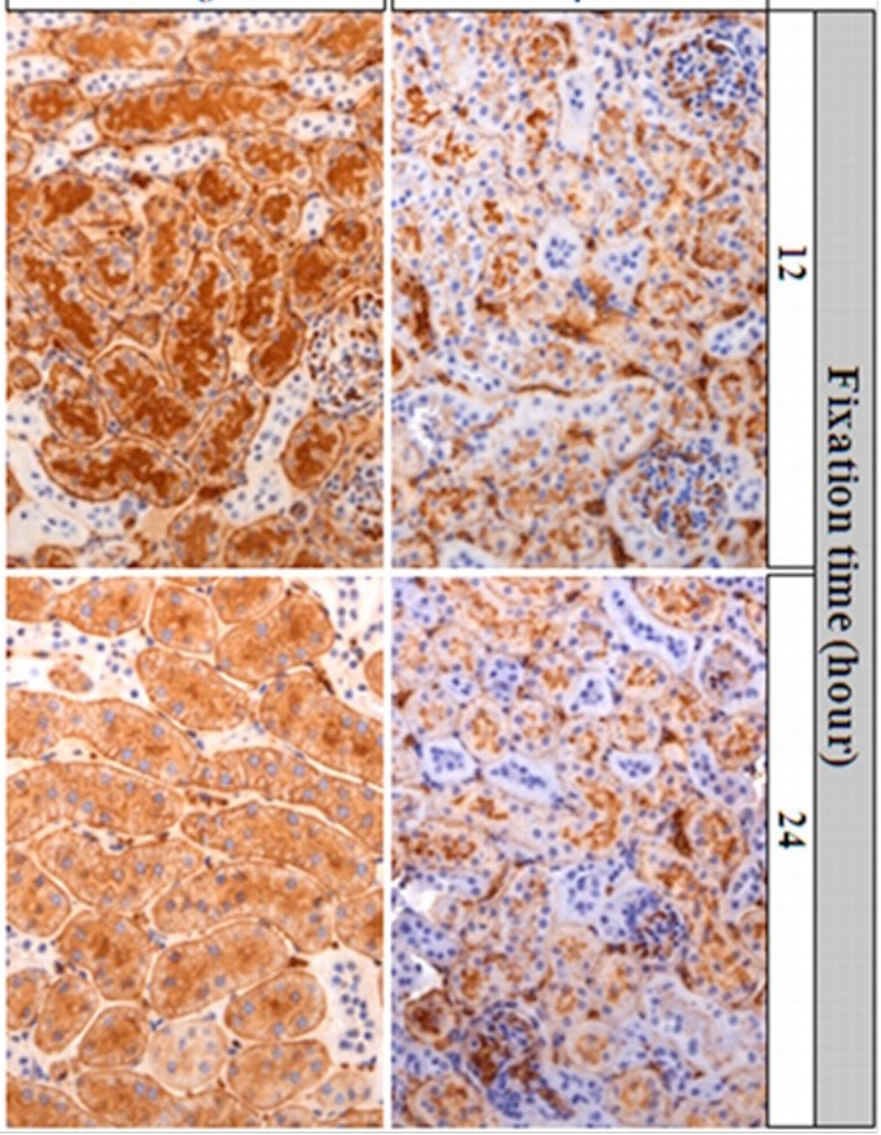
**Ran Xie, Joon-Yong Chung, Kris Ylaya, Reginald L. Williams, Natalie Guerrero, Nathan Nakatsuka, Cortessia Badie, and Stephen M. Hewitt**
Tissue Array Research Program, Laboratory of Pathology, Center for Cancer Research. National Cancer Institute. National Institutes of Health. Bethesda, Maryland.

(J Histochem Cytochem 59:356–365, 2011)

Slide storage 2-weeks




Slide storage 0




NordiQC

# Storage 3 months

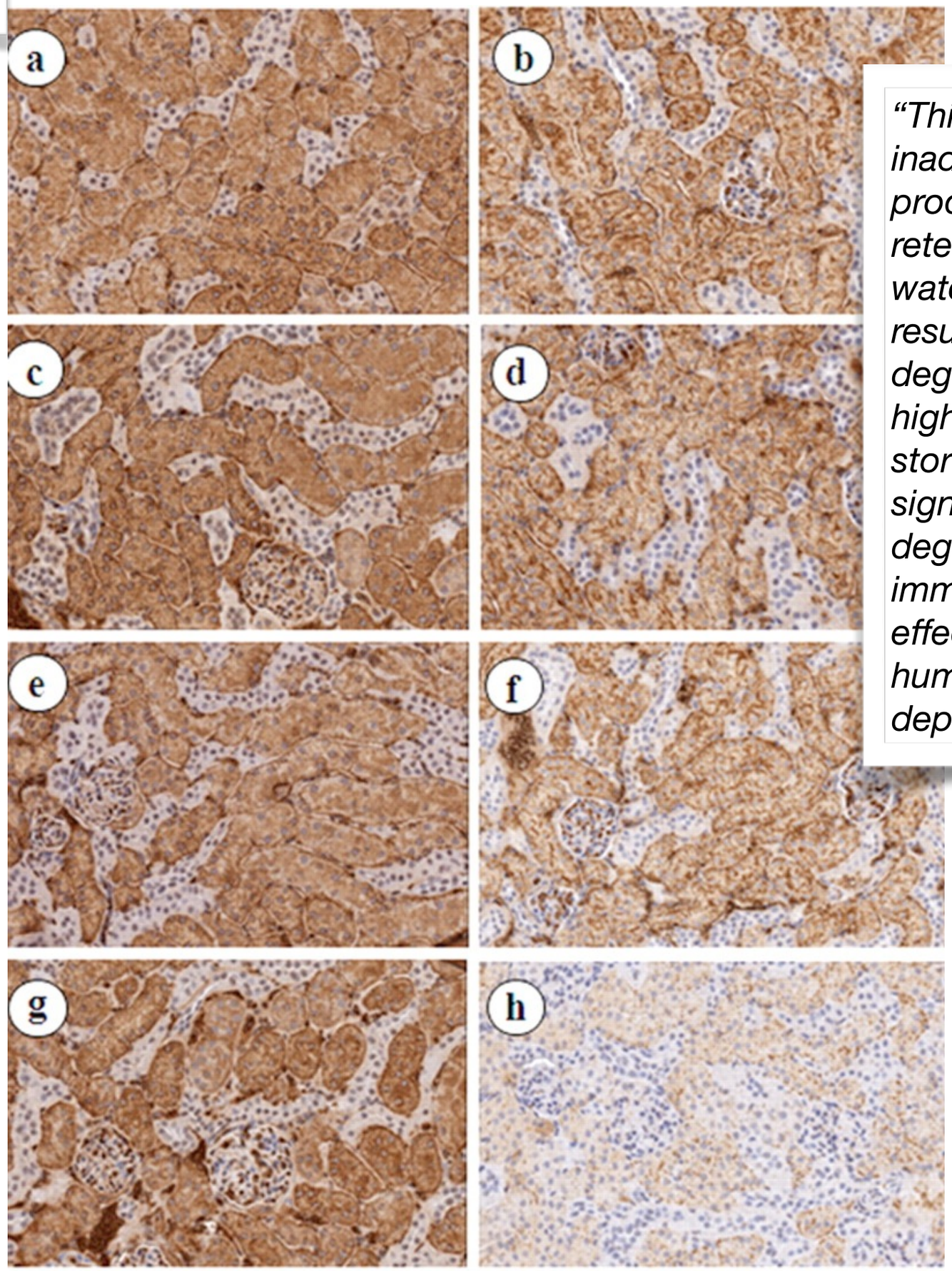
Dry Wet

RT

4°C

30°C

37°C




*"This study revealed that inadequate tissue processing, resulting in retention of endogenous water in tissue sections, results in antigen degradation. Exposure to high humidity during storage results in significant protein degradation and reduced immunoreactivity, and the effects of storage humidity are temperature dependent".*

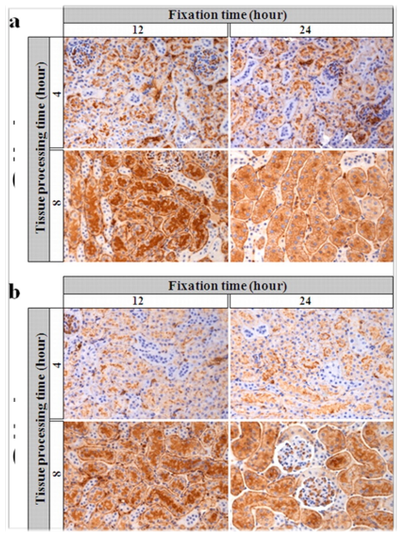
**Factors Influencing the Degradation of Archival Formalin-Fixed Paraffin-Embedded Tissue Sections**


**Ran Xie, Joon-Yong Chung, Kris Ylaya, Reginald L. Williams, Natalie Guerrero, Nathan Nakatsuka, Cortessia Badie, and Stephen M. Hewitt**

Tissue Array Research Program, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.



(J Histochem Cytochem 59:356–365, 2011)

# Loss of antigenicity with tissue age in breast cancer

Susan E Combs[1], Gang Han[1], Nikita Mani[1], Susan Beruti[2], Michael Nerenberg[3] and David L Rimm[1]

**Laboratory Investigation (2016) 96,** 264–269

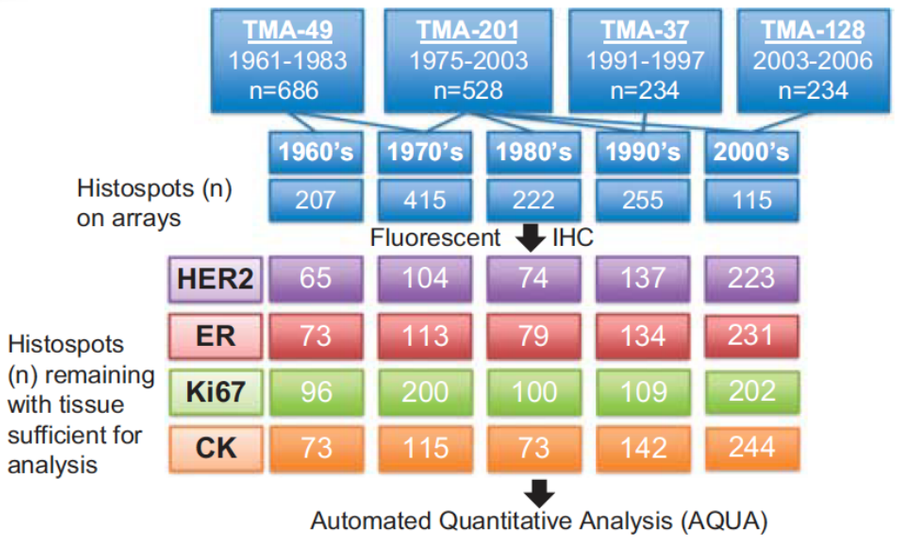




**Figure 1** A consort diagram showing the cohorts from which the tissues were derived and the date ranges for each followed by the number of cases analyzed for each biomarker. IHC, immunohistochemistry; TMA, tissue microarray.

The average signal decreased with preservation time for all biomarkers measured. For **ER** and **HER2**, there was an average of 10% signal loss after 9.9 years and 8.5 years, respectively, compared with the most recent tissue. Detection of **Ki67** expression was lost more rapidly, with 10% signal loss in just 4.5 years. Overall, these results demonstrate the need for adjustment of tissue age when studying FFPE biospecimens. The rate of antigenicity loss is biomarker specific and should be considered as an important variable for studies using archived tissues.

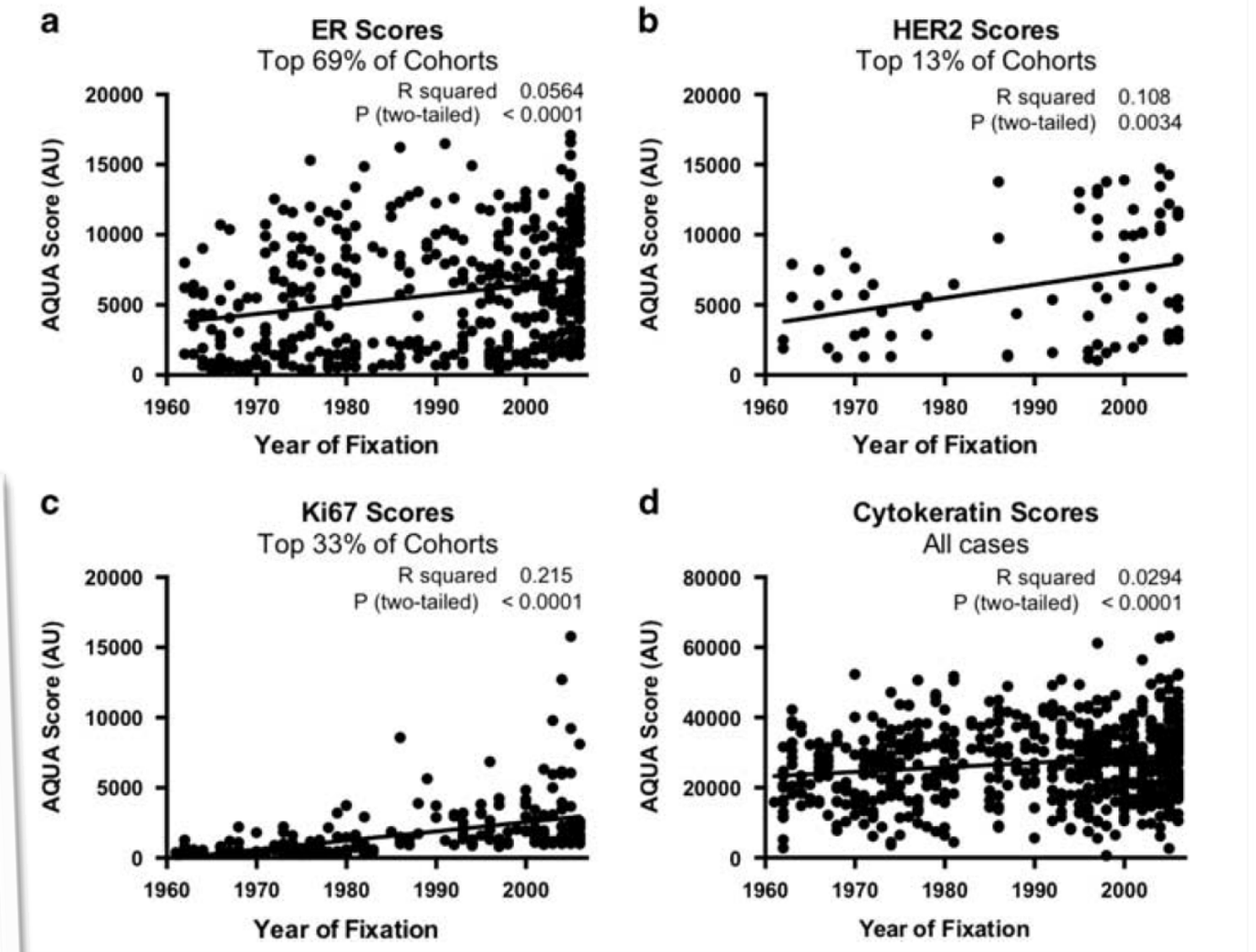



**Figure 2** The distribution of scores for each biomarker as a function of tissue age after omitting the fraction of expected negative cases. (**a**) ER, (**b**) HER2, (**c**) Ki67 and (**d**) cytokeratin. The fraction of positive cases is shown by percentage beneath the biomarker in the title. The regression value and *P*-value are presented in the insets. Au, arbitrary unit.

# Storage of specimen

| Preanalytic variable | Published Guidelines and Recommendations<br>ASCO/CAP \| CLSI | Literature-Based Recommendations |
|---|---|---|
| Storage of paraffin blocks | Indefinitely * | < 25 years * |
| Storage of sections (slide) | 7 days or < 6 weeks | < 6 days |

Engel KB, Moore HM. Arch Pathol Lab Med. 2011;135:537–543

* new data indicates up to 10% loss in 5 years

| | |
|---|---|
| Days | 20°C |
| Weeks | 4°C |
| Months | -20°C |
| Years | -80°C |

# Effects of Preanalytical Variables on the Detection of Proteins by Immunohistochemistry in Formalin-Fixed, Paraffin-Embedded Tissue

*Kelly B. Engel, PhD; Helen M. Moore, PhD*

Arch Pathol Lab Med—Vol 135, May 2011

**Table 1. Potential Sources of Preanalytic Variation During Specimen Fixation and Processing**

- Prefixation
  - Duration and delay of temperature
  - Specimen size
  - Specimen manipulation (pathology ink)
- Fixative
  - Formula
  - Concentration
  - pH
  - Age of reagent
  - Preparation source
- Fixation
  - Tissue to fixative volume ratio
  - Method (immersion, injection, and sonication or microwave acceleration)
  - Conditions of primary and secondary fixation
    - Movement
    - Light exposure
    - Primary container
    - No. and position of cofixed specimens
- Postfixation
  - Washing conditions and duration
  - Storage reagent and duration
- Processing
  - Type of processor, frequency of servicing and reagent replacement
  - Tissue to reagent volume ratio
  - No. and position of coprocessed specimens
- Dehydration and clearing
  - Reagent
  - Temperature
  - No. of changes
  - Duration (total and change-specific)
- Paraffin impregnation
  - Type and melting point of wax
  - No. of changes
  - Duration (total and change-specific)
  - Method (immersion and sonication or microwave acceleration)
- Paraffin sectioning
  - Type of blade and frequency of replacement
  - Frequency of servicing and wax replacement
  - Temperature of block during sectioning
  - Slide pretreatment
  - Water bath conditions, if used
  - Chemical adhesives, if used
  - Temperature and duration of slide drying
- Storage
  - Temperature and duration of paraffin block storage
  - Temperature, duration, and manipulation of slide-mounted tissue sections

Decalcification:
Type, Time, Temperature

# Effect of Preanalytic Variables on an Automated PTEN Immunohistochemistry Assay for Prostate Cancer

rmAb, clone D4.3

*Liana B. Guedes, MD; Carlos L. Morais, MD; Helen Fedor, BS; Jessica Hicks, MS; Bora Gurel, MD; Jonathan Melamed, MD; Peng Lee, MD; Anuradha Gopalan, MD; Beatrice S. Knudsen, MD, PhD; Lawrence D. True, MD; Howard I. Scher, MD; Samson W. Fine, MD; Bruce J. Trock, PhD; Angelo M. De Marzo, MD, PhD; Tamara L. Lotan, MD*

(*Arch Pathol Lab Med.* 2019;143:338–348;

## Variables tested:

- Effect of Duration of Cold Ischemia Prior to Fixation
- Effect of Duration of Formalin Fixation
- Effect of Different Fixatives
- Effect of Tissue Processing Protocol
- Effect of Block Age
- Effect of Unstained Slide Age
- Intermachine Reproducibility
- Effect of automated immunostaining platform

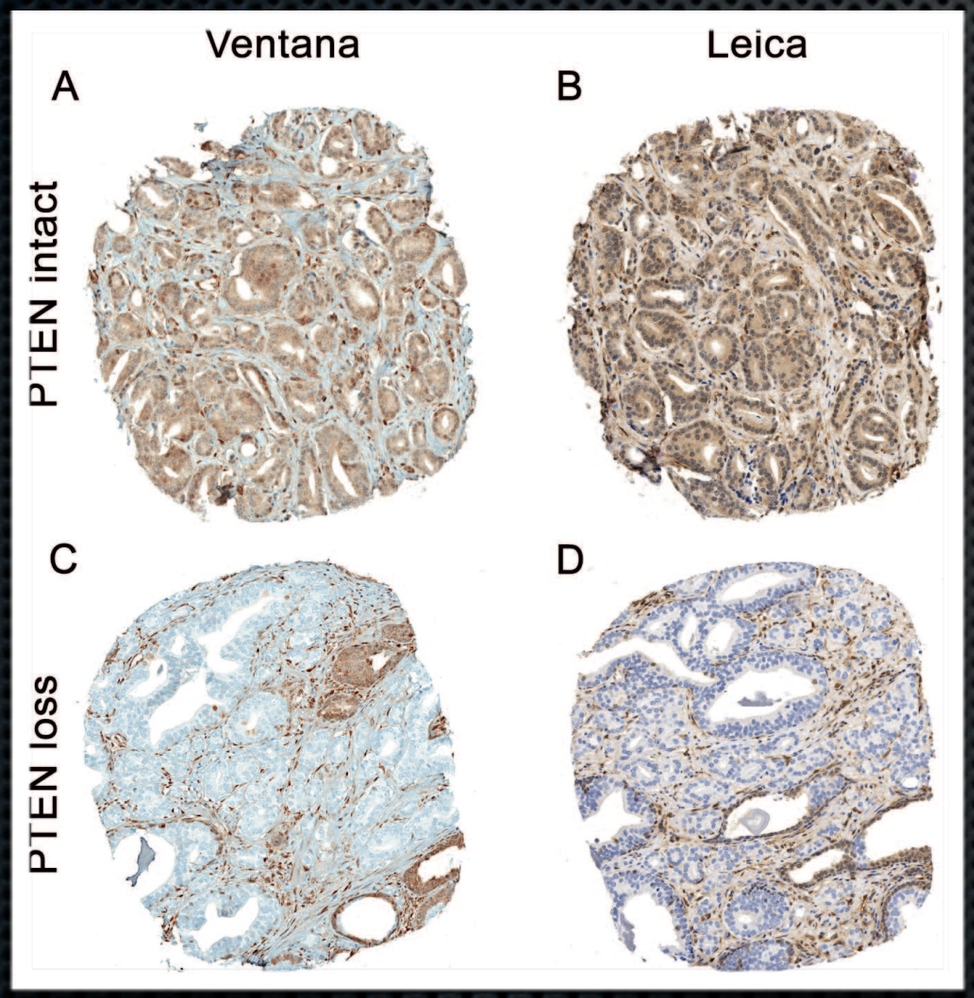
**A setup to follow for the analytic validation of new biomarker assays!**

# Effect of Preanalytic Variables on an Automated PTEN Immunohistochemistry Assay for Prostate Cancer

rmAb, clone D4.3

*Liana B. Guedes, MD; Carlos L. Morais, MD; Helen Fedor, BS; Jessica Hicks, MS; Bora Gurel, MD; Jonathan Melamed, MD; Peng Lee, MD; Anuradha Gopalan, MD; Beatrice S. Knudsen, MD, PhD; Lawrence D. True, MD; Howard I. Scher, MD; Samson W. Fine, MD; Bruce J. Trock, PhD; Angelo M. De Marzo, MD, PhD; Tamara L. Lotan, MD*

(*Arch Pathol Lab Med.* 2019;143:338–348;




## *Conclusions:*

Automated PTEN immunostaining is robust to most preanalytic variables in the prostate and may be performed on prostate tumor tissues subjected to a wide range of preanalytic conditions. These data may help guide assay development if PTEN becomes a key predictive biomarker.

# A tissue quality index: an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue

Veronique M Neumeister[1], Fabio Parisi[1], Allison M England[1], Summar Siddiqui[1], Valsamo Anagnostou[1], Elizabeth Zarrella[1], Maria Vassilakopolou[1], Yalai Bai[1], Sasha Saylor[1], Anna Sapino[2], Yuval Kluger[1,2], David G Hicks[3], Gianni Bussolati[2], Stephanie Kwei[4] and David L Rimm[1]

**Laboratory Investigation (2014) 94, 467–474**


With focus on delay of fixation

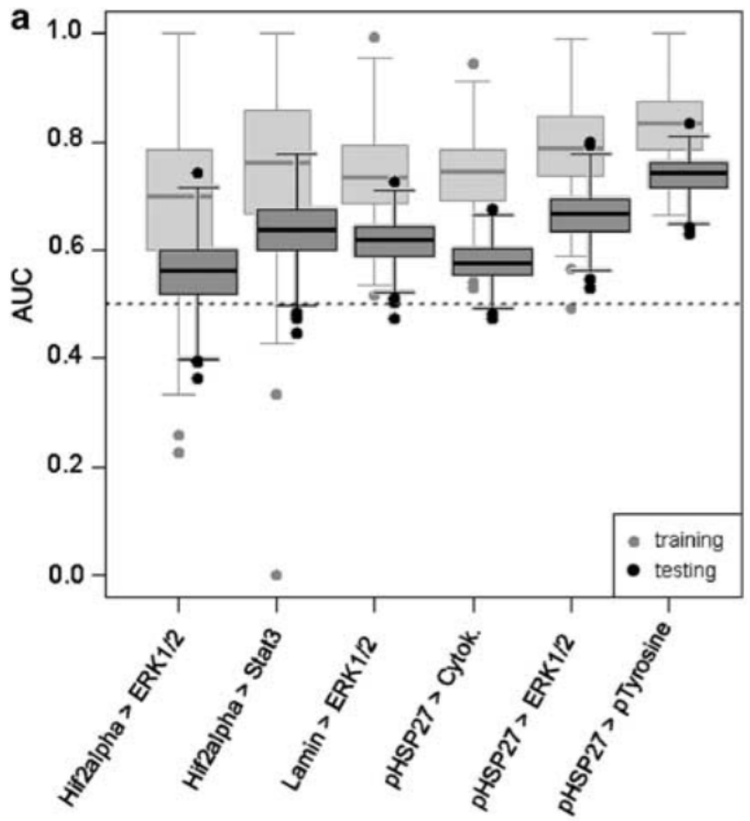
- Aim: Developing a quantitative intrinsic control that can measure the degree of degradation of any FFPE sample.
- If we cannot control pre-analytical variables can we quantify the damage or tissue degradation caused by them?
- Can we disqualify specimens for Companion dx testing?

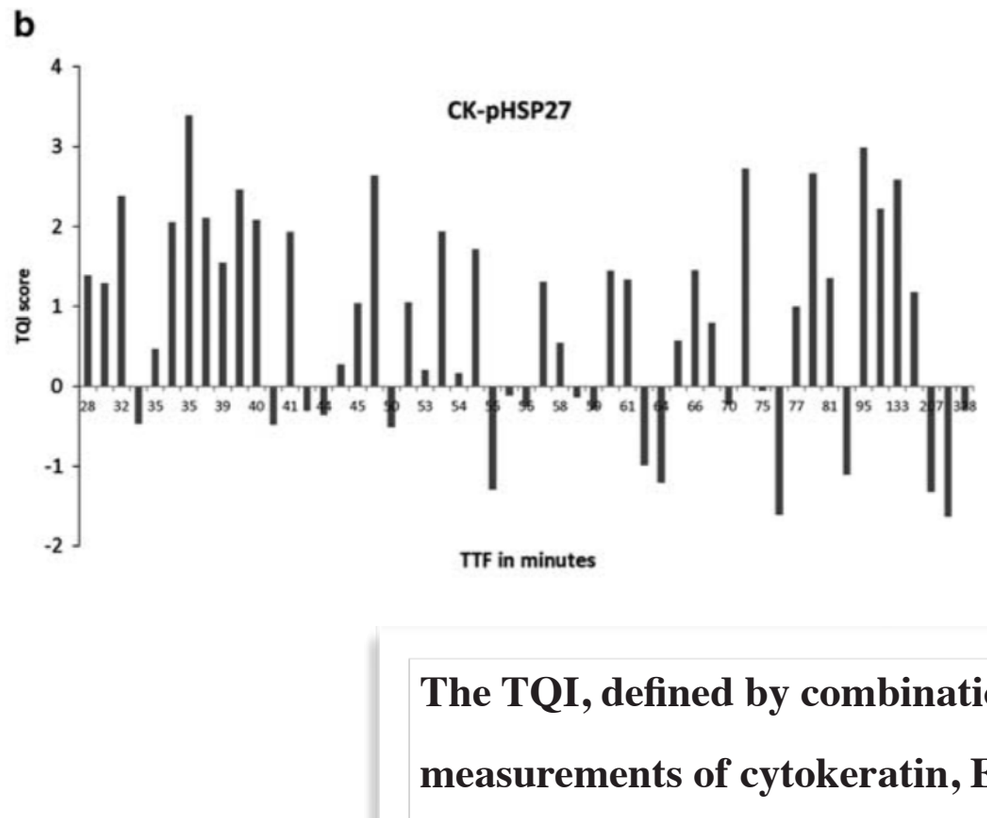
# TQI: Tissue Quality Index

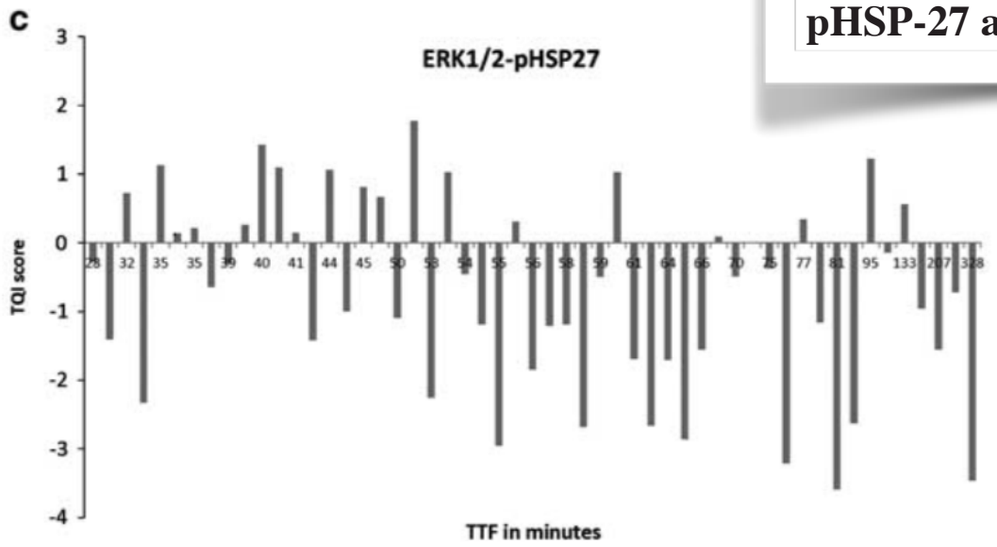
**Table 1 Antibodies tested for the TQI**

| Symbol | Description | Antibody Clone/Isotype | Supplier |
|---|---|---|---|
| *Markers of Cold Ischaemia* | | | |
| ACTB | Beta-Actin | 13E5/IgG | Cell Signaling Technology |
| TUBB | Beta-Tubulin | pF3/IgG | Cell Signaling Technology |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase | 14C10/IgG | Cell Signaling Technology |
| HIST4 | Histone 4 | L64C1 | Cell Signaling Technology |
| HIST3 | Histone 3 | 96C10/IgG1, kappa | Cell Signaling Technology |
| LMNA/C | Lamin A/C | Polyclonal | Cell Signaling Technology |
| LDHA | Lactat Dehydrogenase | IgG, C4B5 | Cell Signaling Technology |
| ERalpha | Estrogen Receptor alpha | SP1/IgG | Thermo Scientific |
| CK | Cytokeratin | AE1/AE3/IgG1 | DAKO |
| CK | Cytokeratin | Polyclonal | DAKO |
| ERK1/2 | P44/42MAPK (Erk1/2) | 137F5, IgG | Cell Signaling Technology |
| p53 | Anti-Human p53 protein | IgG2b. DO-7 | DAKO |
| *Markers of Hypoxia* | | | |
| CCND1 | Cyclin D1 | IgG/SP4 | Thermo Fisher Fremont |
| Caspase | Cleaved Caspase 3 (Asp175) | Polyclonal | Cell Signaling Technology |
| HIF1 | Hypoxia Inducible Factor 1 | Polyclonal | Novus Biological |
| AKAP13 | A-kinase anchoring protein 13 | IgG2a/ZX-18 | |
| CDC42 | | IgG3/B-8 | |
| CCNB1 | Cyclin B1 | GNS-11/IgG2 | |
| HIF-2alpha | Hypoxia inducible factor-2α | ep190b/IgG1 | |
| CA9 | Carbonic Anhydrase IX | Polyclonal(aa581-592 | |
| *Markers of phosphorylated proteins* | | | |
| pAKT 473 | phospho-Akt (ser473) | D9E/IgG | Cell Signaling Technology |
| ERK1/2 | Phospho-p44/43MAPK (Erk1/2) (Thr292/Tyr204) | IgG | Cell Signaling Technology |
| pER | Phospho-Estrogen Receptor alpha (Ser118) | 16J4/IgG2b | Cell Signaling Technology |
| Anti-Phosphotyrosine | 4G10 Anti-Phosphotyrosine | IgG2b | Millipore |
| Anti-Phosphotyrosine | | p-Tyr-100 | Cell Signaling Technology |
| pHSP27 (pS78) | Phosphorylated Heat Shock Protein 27 | Y175 | Epitomics |
| pHer2 (Tyr1248) | Phospho-Her2/ErbB2 (Tyr1248) | PN2A | Thermo Scientific |
| Phospho-Stat3 (Tyr705) | Phospho-Stat3 (Tyr705) | D3A7/IgG | Cell Signaling Technology |
| p-S6 Ribosomal Protein (Ser235/236) | Phospho-S6 Ribosomal Protein (Ser235/236) | D52.2.2E/IgG | Cell Signaling Technology |
| Phospho-Jak2 (Tyr1007/1008) | Phospho-Jak2 (Tyr1007/1008) | Polyclonal | Cell Signaling Technology |
| Phospho-Met (Tyr1234/1235) | Phospho-Met (Tyr1234/1235) | IgG | Cell Signaling Technology |
| Phospho-Sapk/Jnk | Phospho-Sapk/Jnk | IgG | Cell Signaling Technology |
| Phospho mTor (Ser2448) | Phospho mTor (Ser2448) | 49F9/IgG | Cell Signaling Technology |
| *Markers of posttranslational modification* | | | |
| Sumo1 | small ubiquitin related modifier 1 | Y299/IgG | Abcam |
| Acetylated-Lysine | Proteins posttranslat. Modified by acetylation | Polyclonal, purified | Cell Signaling Technology |
| NEDD8 | Neural precursor cell-expr. devel. Downreg. protein9 | IgG, 19E3 | Cell Signaling Technology |

# TQI: Tissue Quality Index




**The TQI, defined by combinations of measurements of cytokeratin, ERK1/2 and pHSP-27 and their relationship to cold ischemic**

**Figure 1** (**a**) The performance of six marker combinations on the testing and validation subgroup of the time to fixation breast cancer series as measured by receiver-operator characteristic (ROC) curves and area under the curve (AUC) values. The tissue quality index (TQI) was then calculated on the complete time to fixation breast cancer series. (**b**) TQI values of cytokeratin:pHSP27 and (**c**) ERK1/2:pHSP27 in relationship with increasing cold ischemic time.

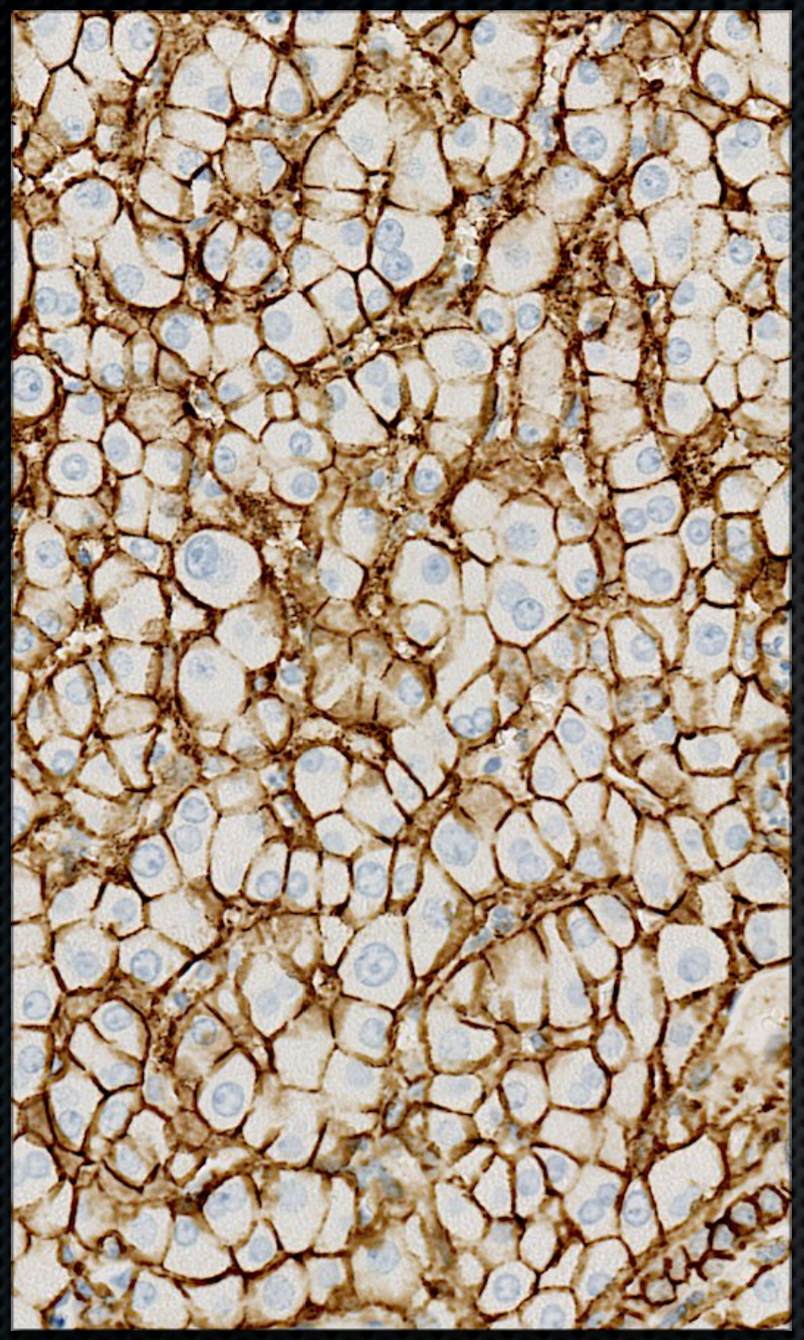
# “The poor man’s TQI”

“Damage Controls”

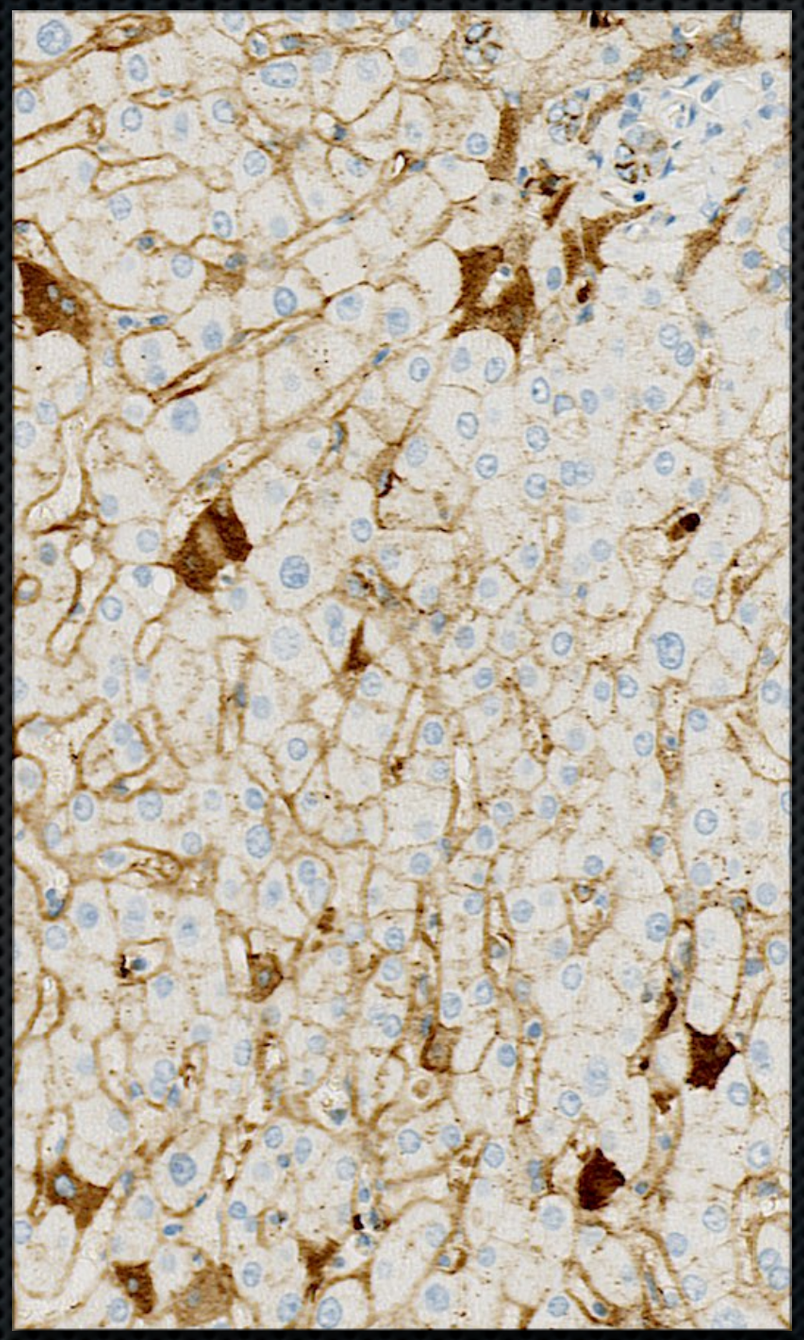
- **Fixation delay/Cold ischemia**
  - CD138, B-A38
- **Poor/short fixation in NBF**
  - MLH1, ES05
  - PMS2, EPR3947
  - BCL6, LN22
  - BCL2, 124
- **Electrosurgery**
  - CK, CAM5.2

# CD138: Simple marker of fixation delay

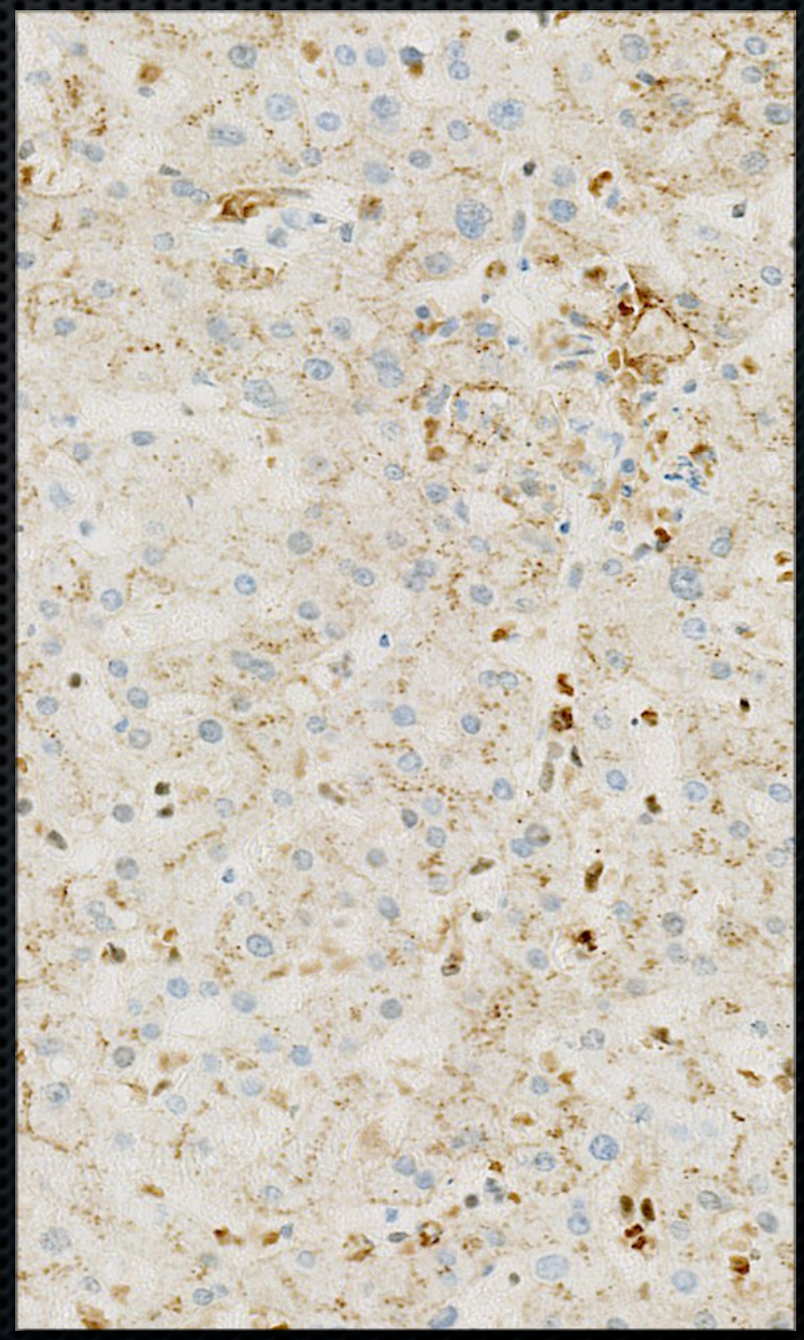
Odense data



Liver: No Fix delay



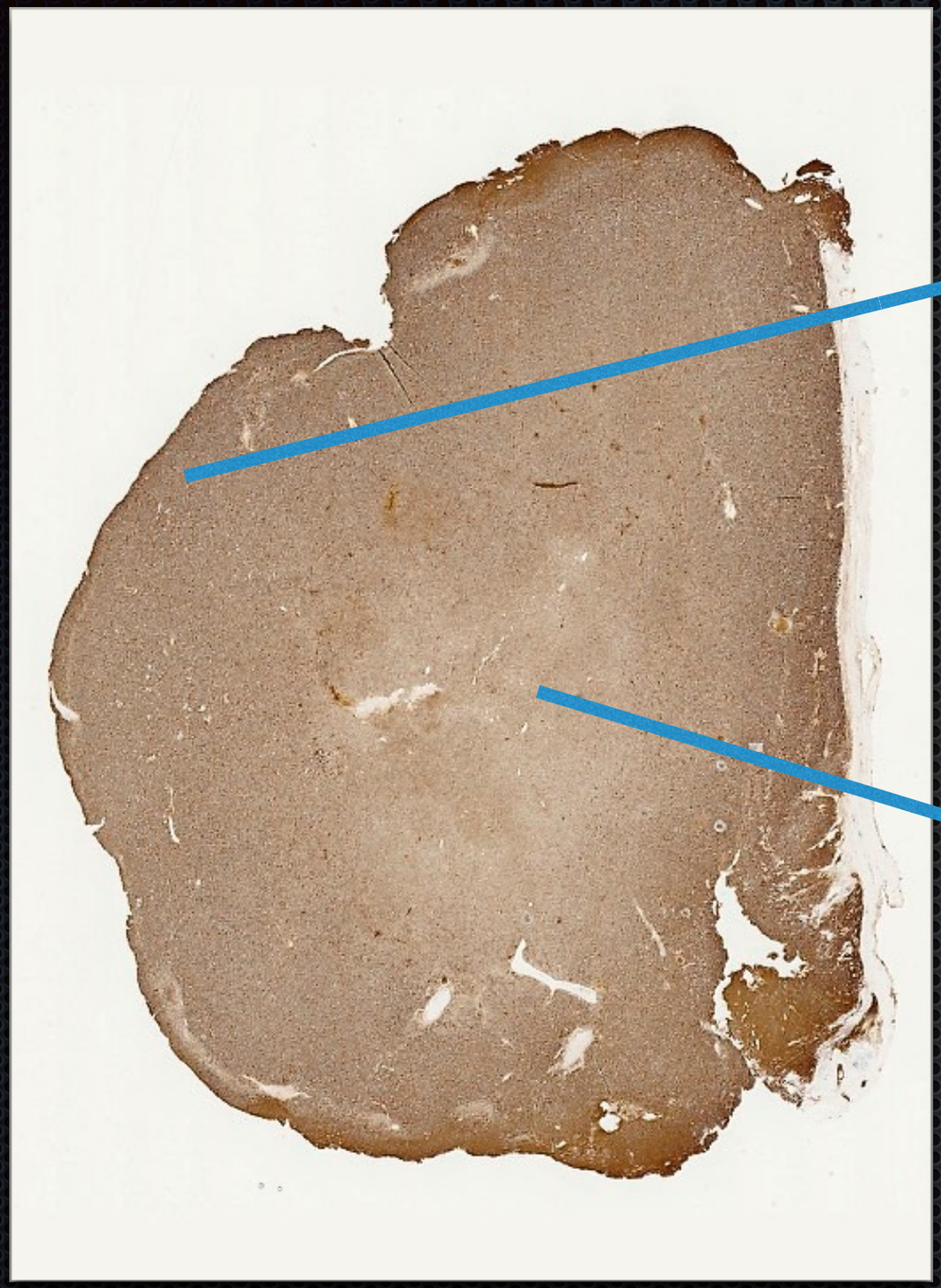
Liver 16 hrs delay

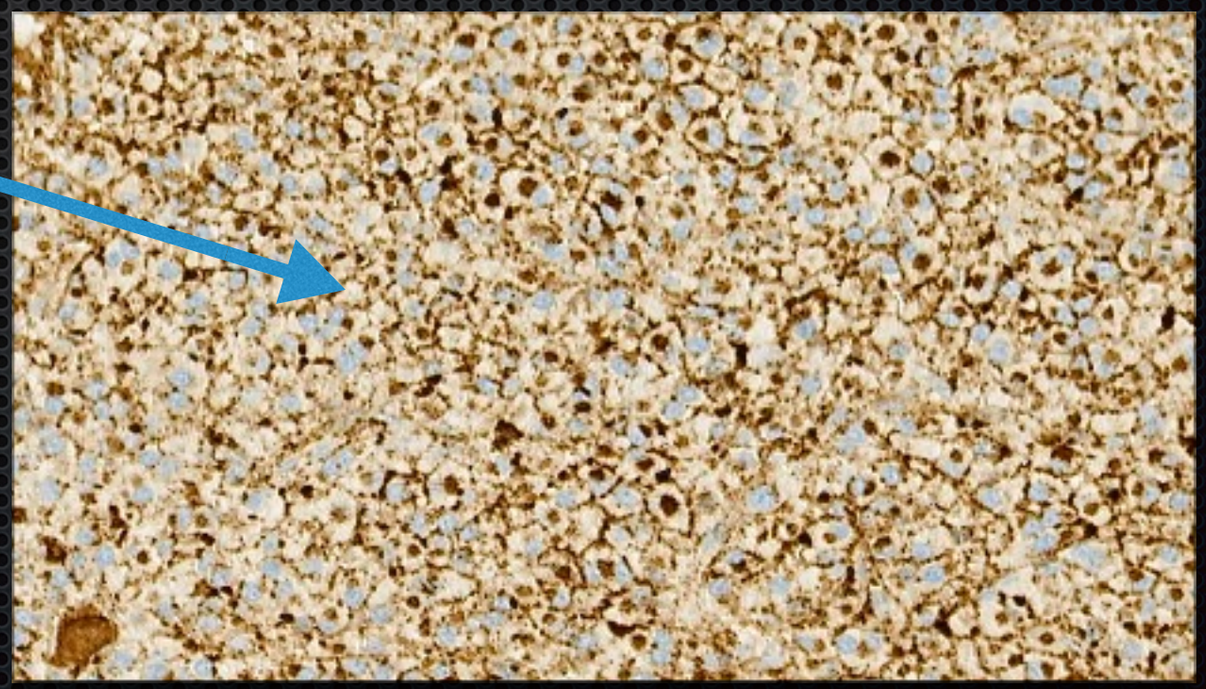


Liver 48 hrs delay

# CD138 (B-A38): Simple marker of fixation delay

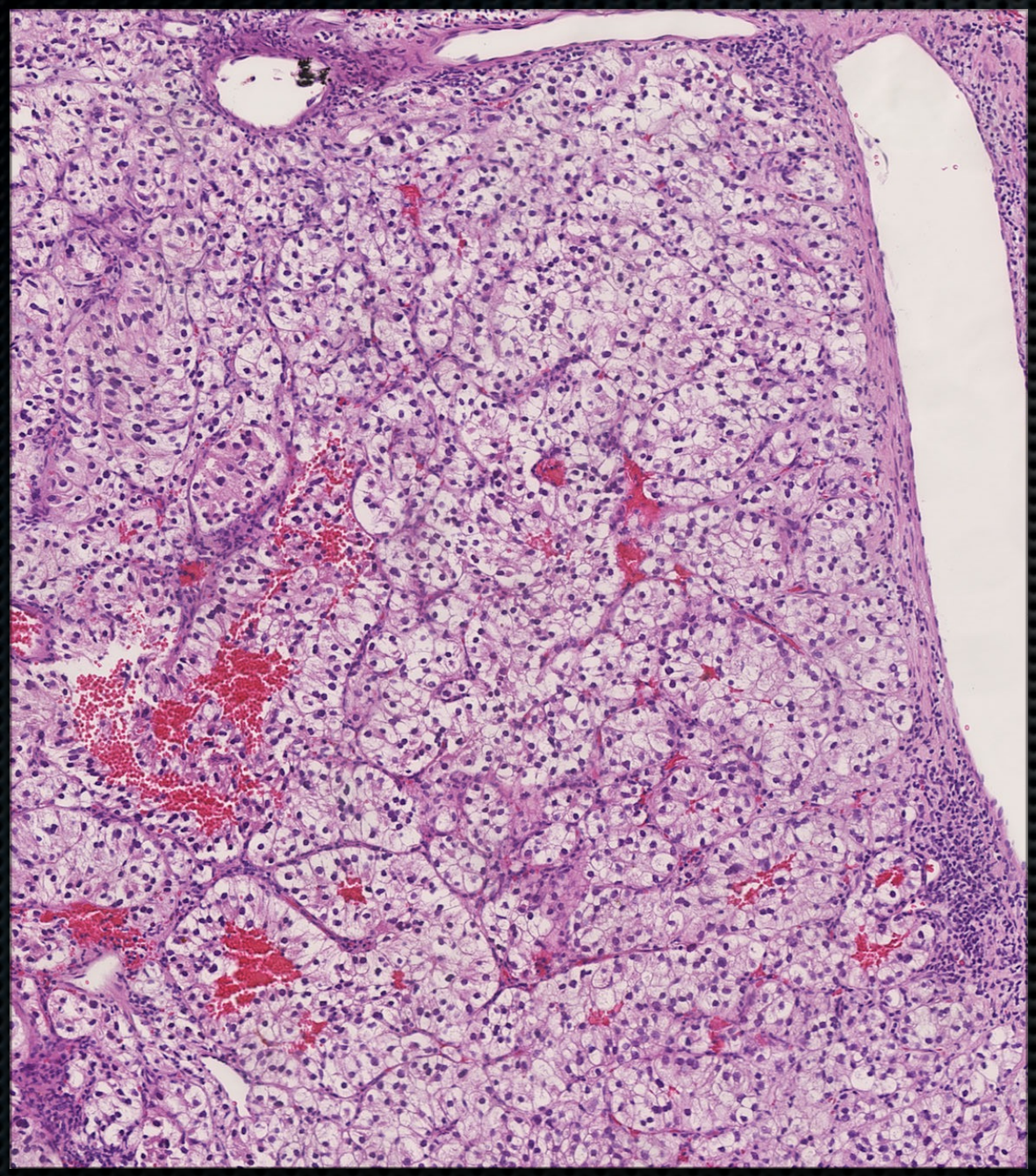
Odense data





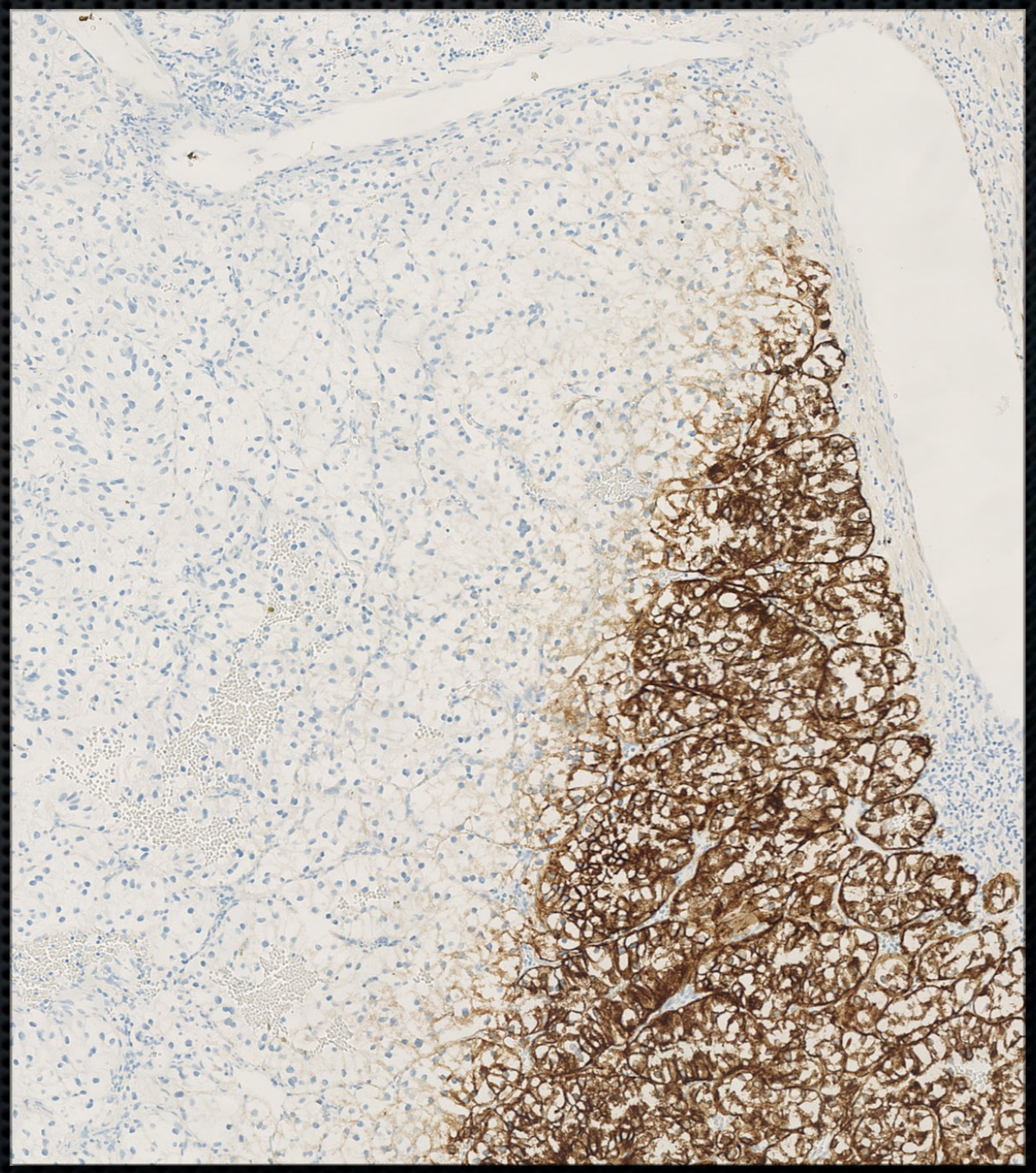
Plasmacytoma

# CK, CAM5.2 simple marker of electrosurgery



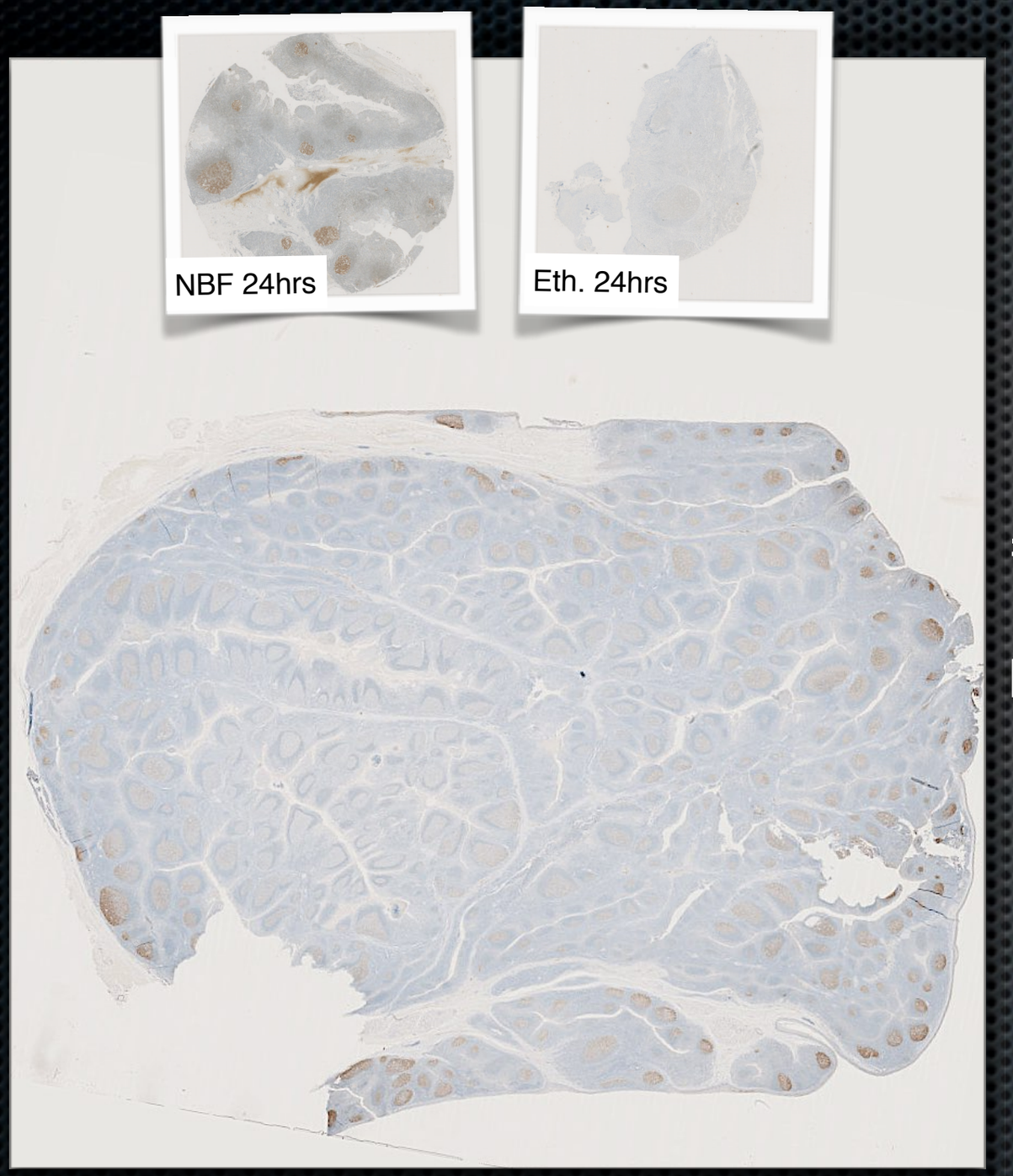
HE

RCC



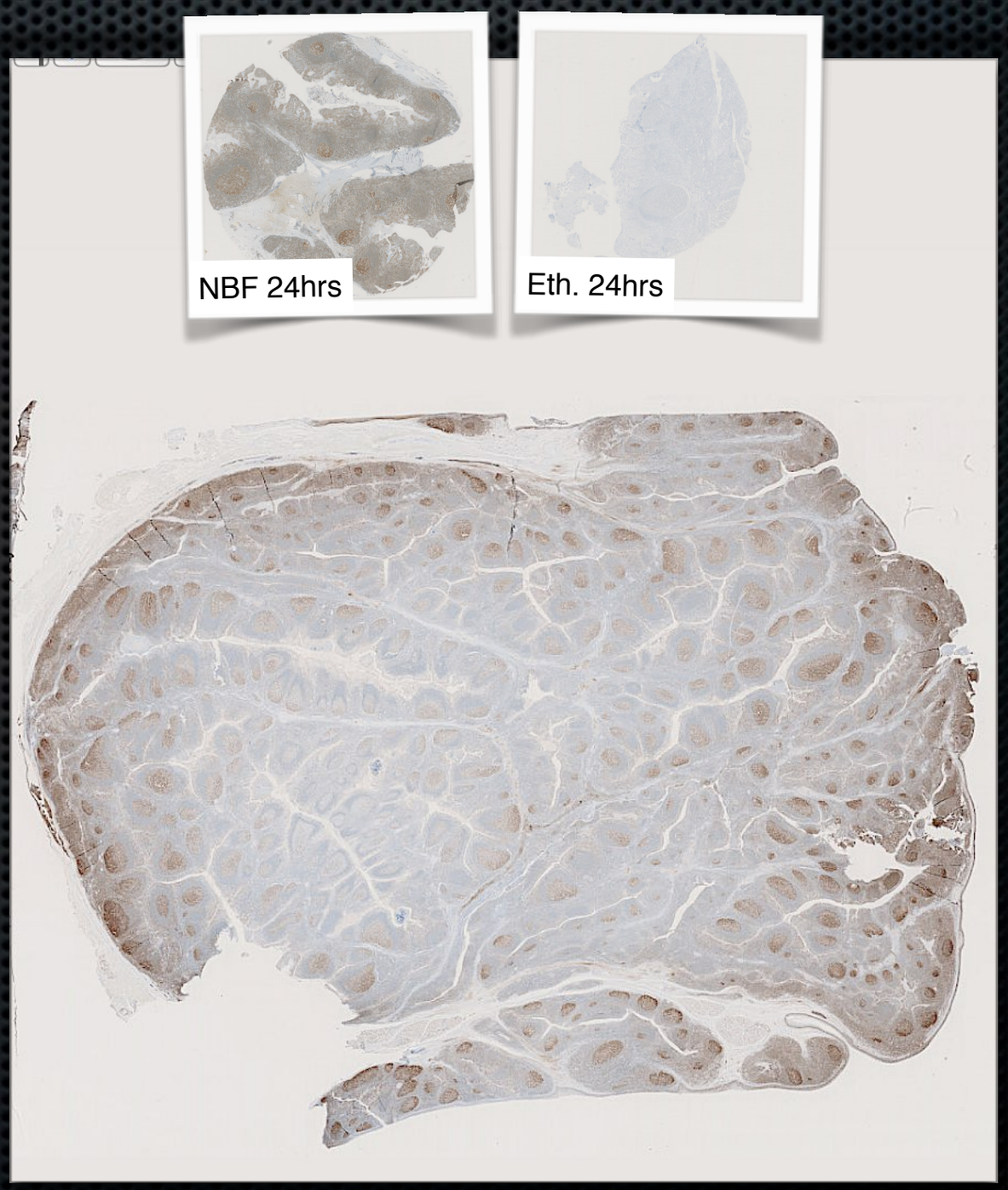
CK, CAM5.2

# Markers of poor/short NBF fixation




BCL6, LN22

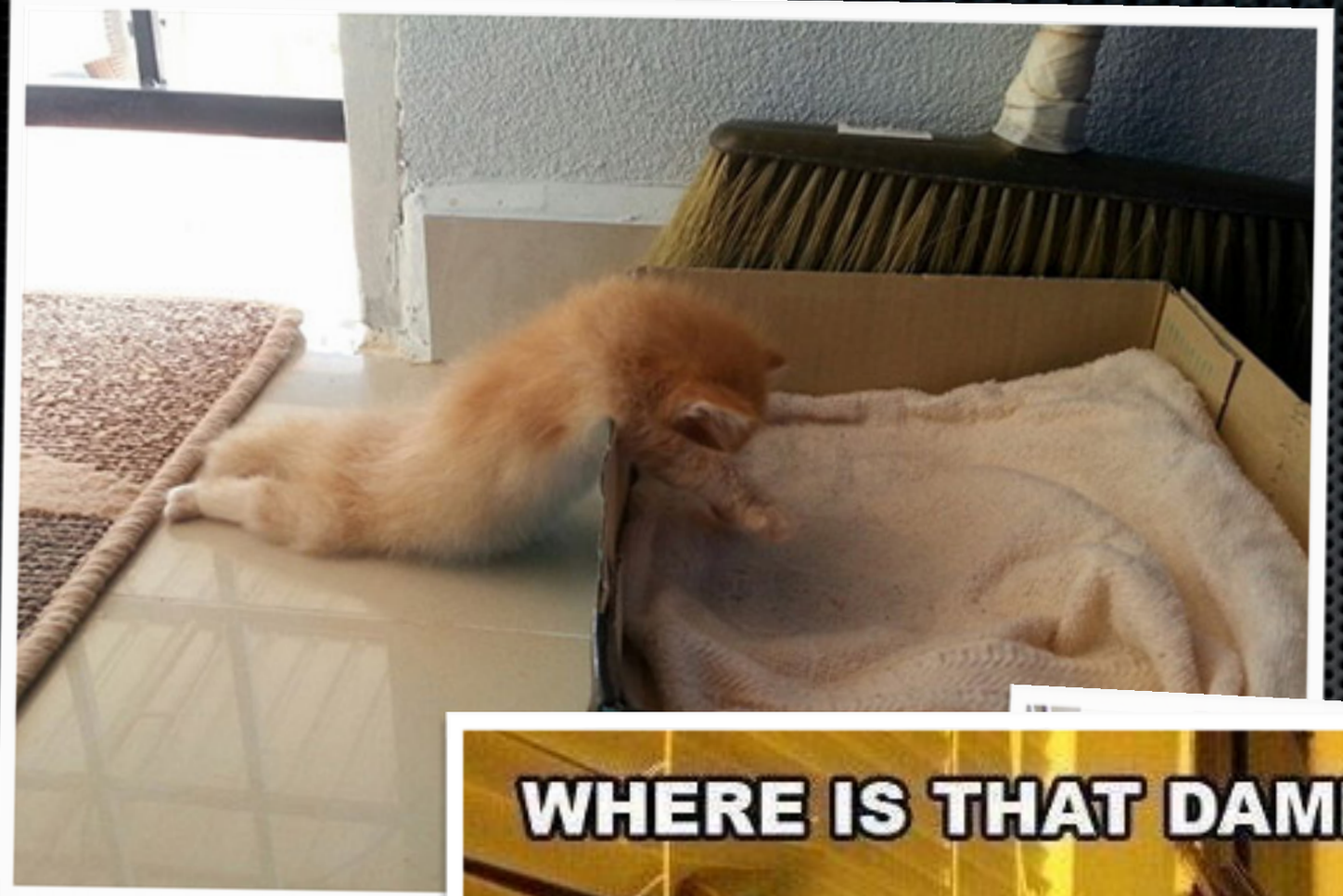
Tonsil




PMS2, EPR3947

# Conclusions

- Less than half of the identified preanalytical variables in IHC have been examined in published research
- The majority of tested preanalytical variables impact the final IHC results
- There is a continued need for rigorous research and comprehensive guidelines on specimen fixation, processing, and storage



NordiQC

# Thank you for your attention!