Optimización de la técnica Inmunohistoquímica

FOR DUMMIES®

A Reference for the Rest of Us!®

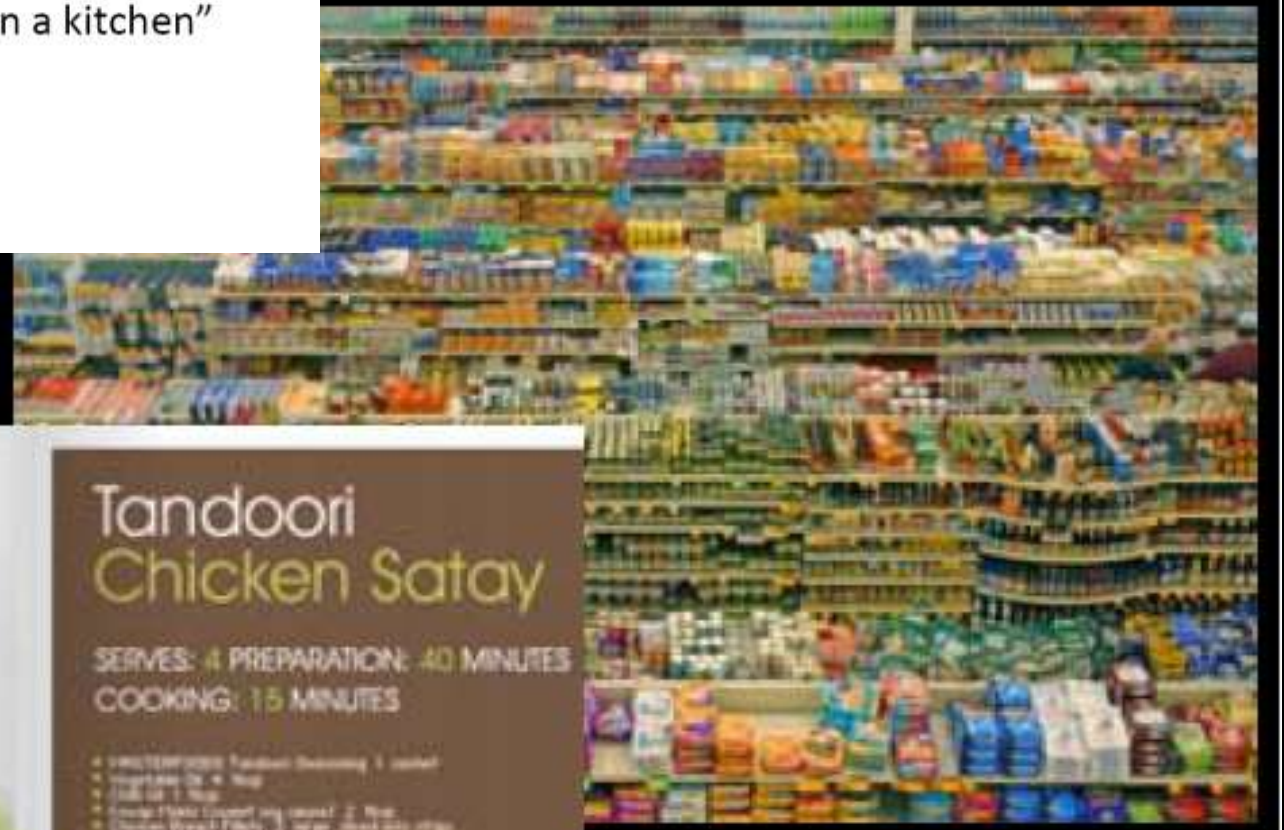
Optimiza tu técnica IHQ

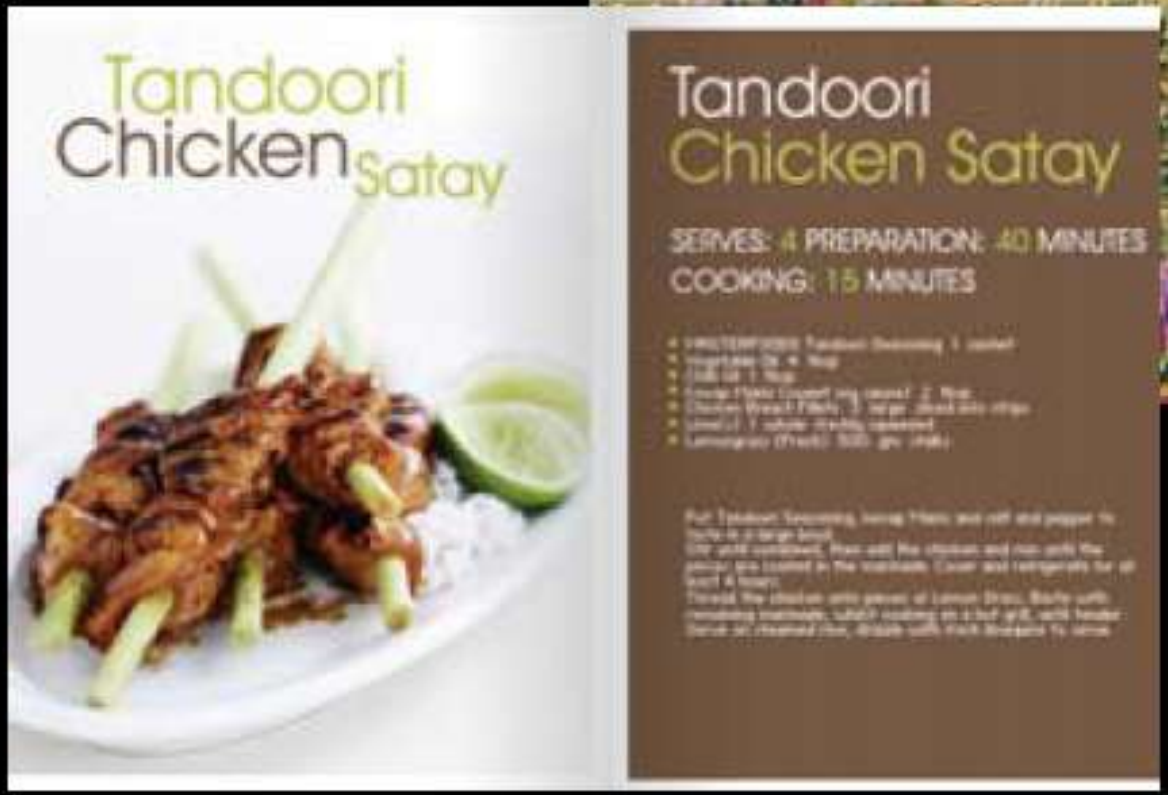
Dr. Angel Panizo

Complejo Hospitalario de Navarra

- **Optimizar (RAE): “Buscar la mejor manera de realizar una actividad”.**
- **Optimizar en IHQ:**
  - **Optimizar la técnica**
  - **Optimizar tiempos de ejecución**
  - **Optimizar costes y recursos**

- Immunohistochemistry is sometimes referred to as analogous to “cooking in a kitchen”
- The right mix of ingredients
- The right temperatures
- The right “incubation”





Tandoori
Chicken Satay

SERVES: 4 PREPARATION: 40 MINUTES
COOKING: 15 MINUTES

Rabbit anti-Claudin 3

For In Vitro Diagnostic Use
Lot No.

CE

[X] 18-7340 0.5 mL Concentrate Antibody

INTENDED USE

For In Vitro Diagnostic Use

Zymed's polyclonal Rabbit anti-Claudin 3 antibody is intended to qualitatively detect Claudin 3 protein in frozen and formalin-fixed, paraffin-embedded tissue sections. Interpretation must be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

BACKGROUND

Tight junctions are specialized regions of cell-cell contact that are particularly abundant in luminal epithelial cell sheets. Claudin-3 is part of the 20 member claudin family that share several essential features. Claudin-3 was found to be present or secreted by many of the genes up-regulated in ovarian cancer[1]. Furthermore, in a recent study the expression of Claudin-3 was shown capable of mediating Clostridium perfringens enterotoxin (CPE) binding and cytolysis; this suggests that Claudin-3 could be a potential therapeutic strategy for prostate cancer[2].

REAGENT PROVIDED

Rabbit anti-Claudin 3 Antibody is purified from rabbit antisera and diluted in phosphate buffered saline (PBS), pH 7.4, and 1% bovine serum albumin (BSA) with 0.1% sodium azide (NaN_3) as a preservative.

Immunogen: Synthetic peptide derived from C-Terminal of claudin-3
Clone: Z23.JM

Total protein concentration: ??? g/L
Antibody concentration: ??? mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Colon

EXPECTED STAINING PATTERN: Predominately membrane staining. Certain cancer tissues show cytoplasmic staining in addition to membrane staining.

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

Epitope Retrieval: Required (Citrate buffer, pH 6.0)(See page 2 for protocol)

Enzyme Digestion: Not Required (See page 2 for protocol)

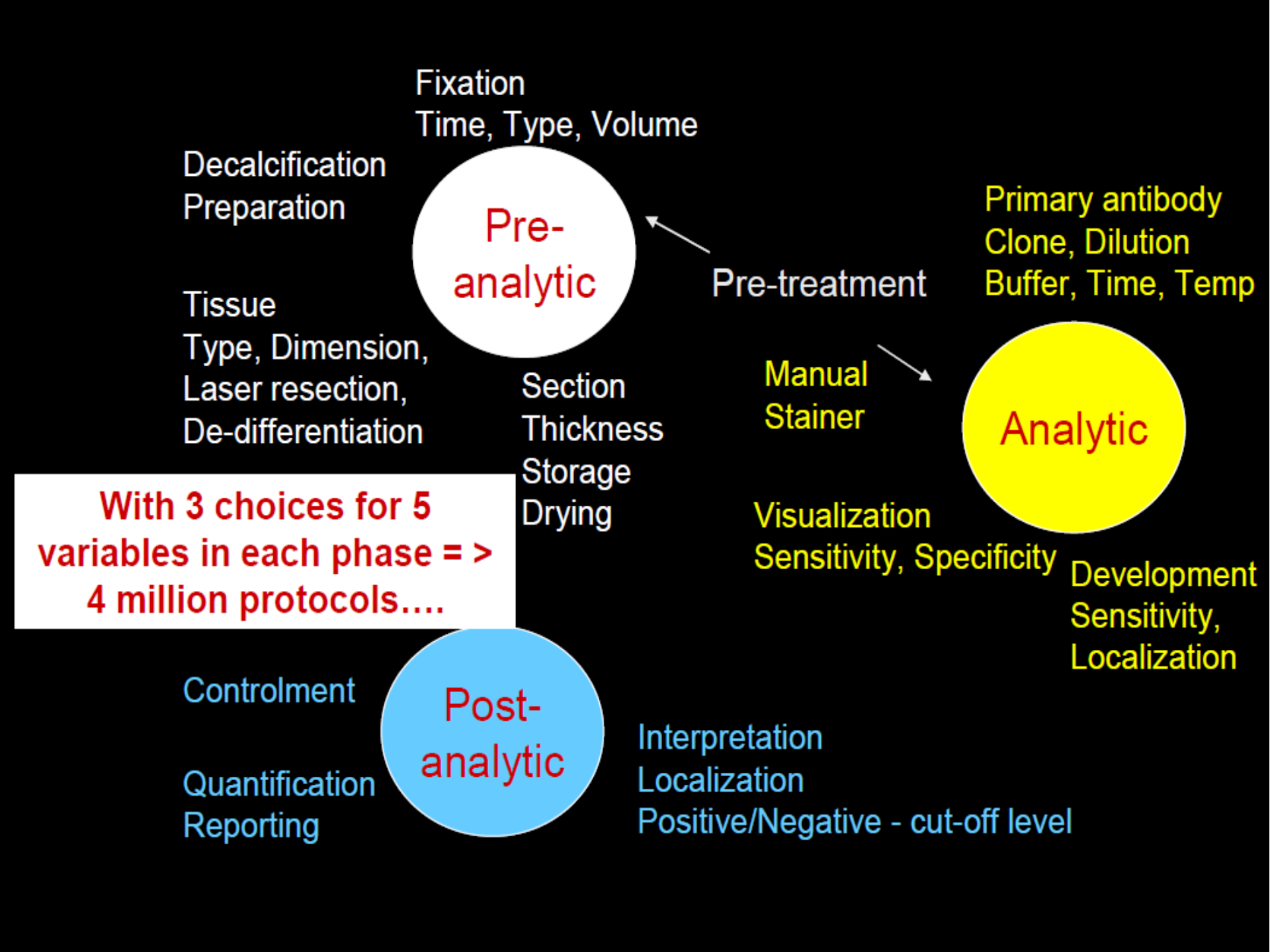
Rabbit anti-Claudin 3 may be diluted according to Table 1 when using the Zymed detection systems below.

Table 1 Dilution Table

Zymed Kit	Predilute Antibody	Dilution for Concentrate	Incubation Time
Histostain-SP or SAP kits*	Ready-To-Use	1: 50 - 1: 100	60 min.
Histostain®-*Plus* Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
Non-Biotin Amplification (NBA)™ Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
PicTure™-*Plus* Polymer Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
Cap-*Plus*™ Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.

*** Use Histostain-SP or SAP kits only for Cat. No. 08-0XXX and 18-X001 to 18-X200 primary antibodies.**

This is a guideline only. Optimal antibody concentrations may vary based on specimen and preparation method used, and should be determined by each individual laboratory.


Fixation
Time, Type, Volume
Decalcification
Preparation
Pre-analytic
Primary antibody
Clone, Dilution
Buffer, Time, Temp
Pre-treatment
Tissue
Type, Dimension,
Laser resection,
De-differentiation
Section
Thickness
Storage
Drying
Manual
Stainer
Analytic
With 3 choices for 5 variables in each phase = > 4 million protocols….
Visualization
Sensitivity, Specificity
Development
Sensitivity,
Localization
Controlment
Post-analytic
Interpretation
Localization
Positive/Negative - cut-off level
Quantification
Reporting

Pilares básicos en la optimizacion de IHQ

1. Elección del Ac y clon apropiado.
2. Elección de un control positivo apropiado.
3. Fijación y procesamiento adecuado del control.
4. Recuperación antigénica apropiada y eficiente.
5. Sistema de detección robusto, específico y con adecuada sensibilidad.

Selección del anticuerpo

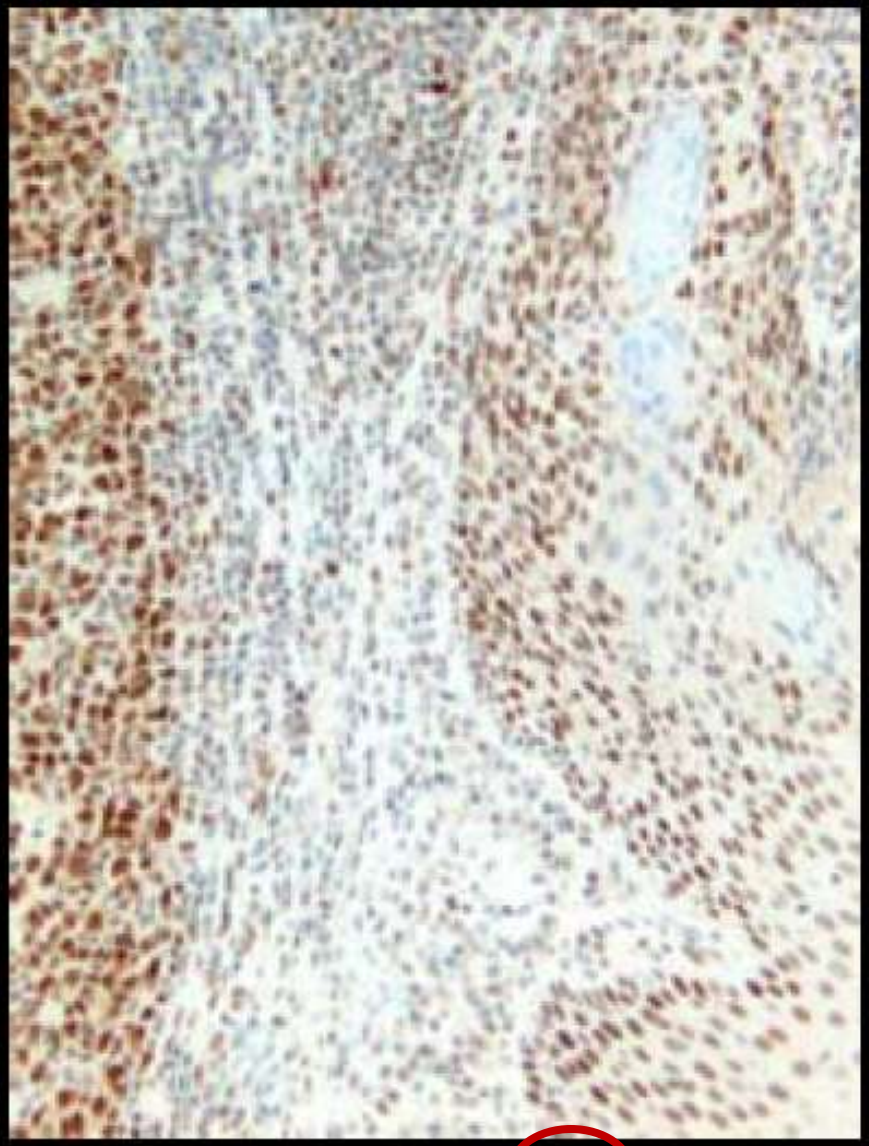
- **Problema 1:**
  - **¿qué Ac compro?**
- **Problema 2:**
  - **¿qué dilución empleo? ¿tiempo de incubación?**
- **Problema 3:**
  - **proporciona baja sensibilidad**
- **Problema 4:**
  - **proporciona baja especificidad**

Falsos negativos y falsos positivos

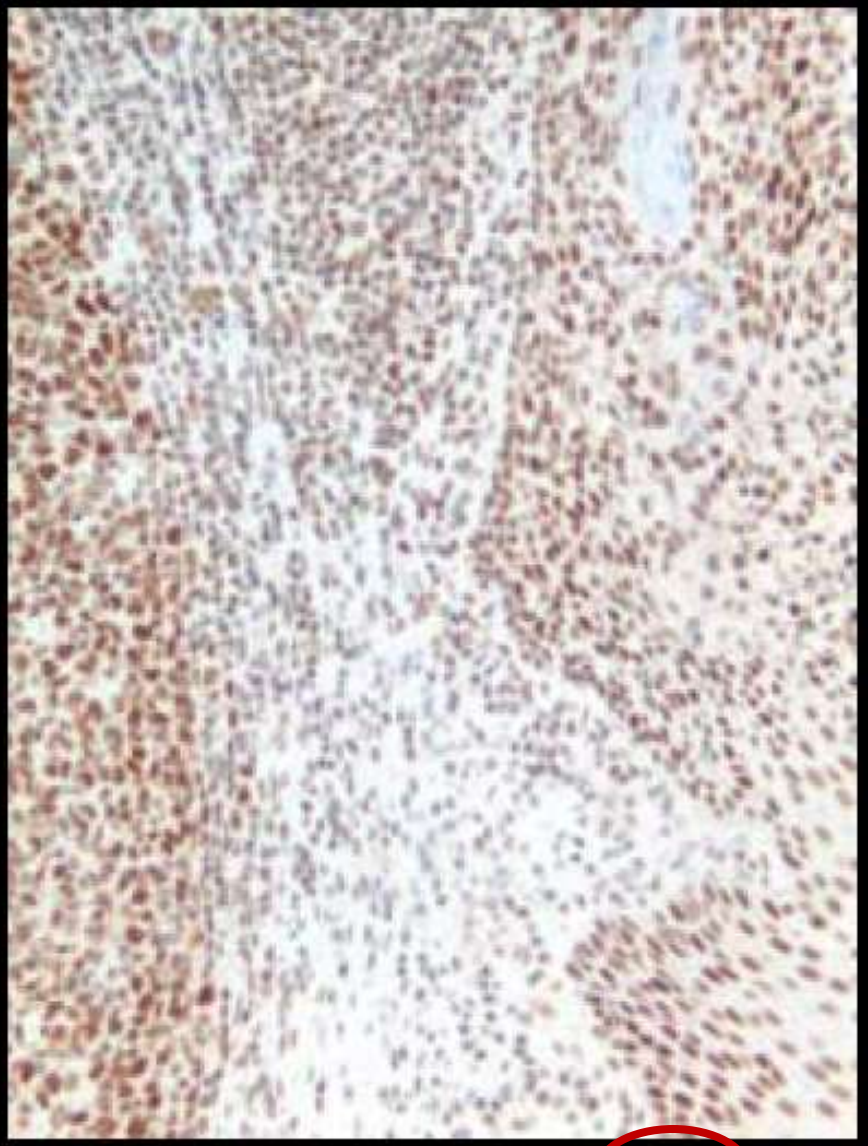
Selección del anticuerpo

- ¿Qué proteína queremos detectar?
- Busqueda de Ac:
  - Referencia de trabajos publicados (PubMed)
  - Webs Programas control Calidad IHQ:
    - www.seap.es/index.asp
    - www.nordiqc.org/
    - www.ciqc.ca/
    - www.ukneqas.org.uk
  - Buscadores de Ac en internet:
    - www.labome.com
    - www.antibodybeyond.com/
- ¿Ac policlonal o monoclonal? ¿cocktail de Acs?
- Valorar especificidad y sensibilidad del Ac

MLH1 test – Tonsil fixed 4 h NBF

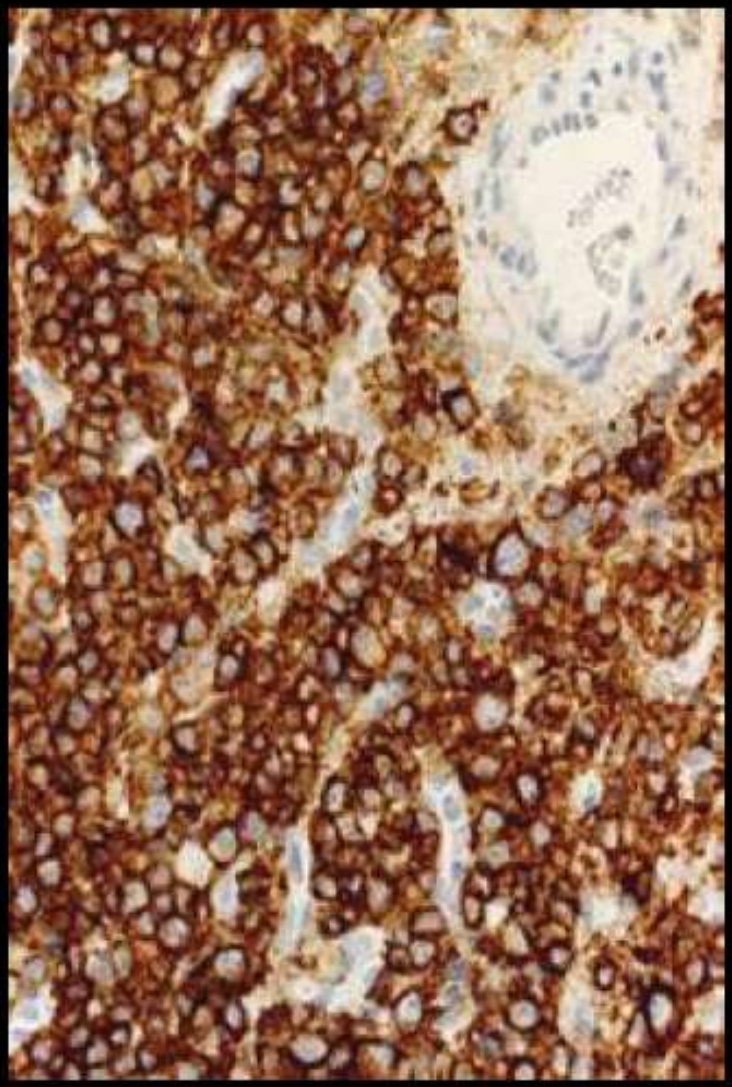


MLH1 clone ES05, 1:20 (Ref.)

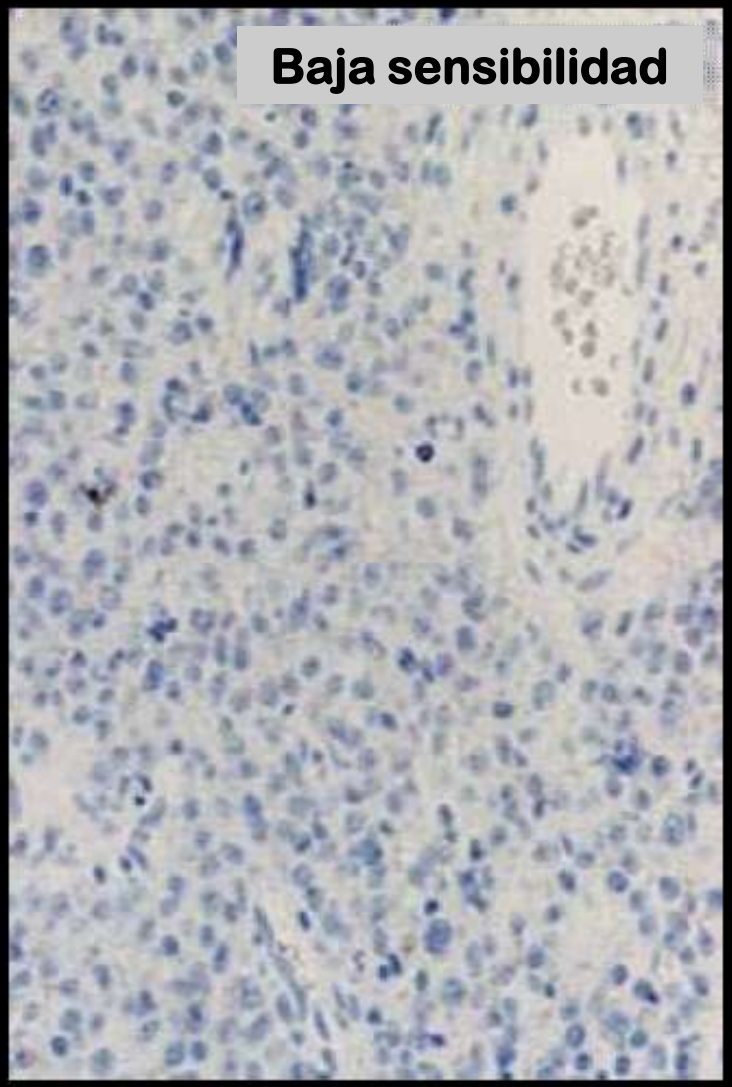


MLH1 clone EPR3894, 1:250

CD138 test – Plasma cytoma fixed 24 h NBF

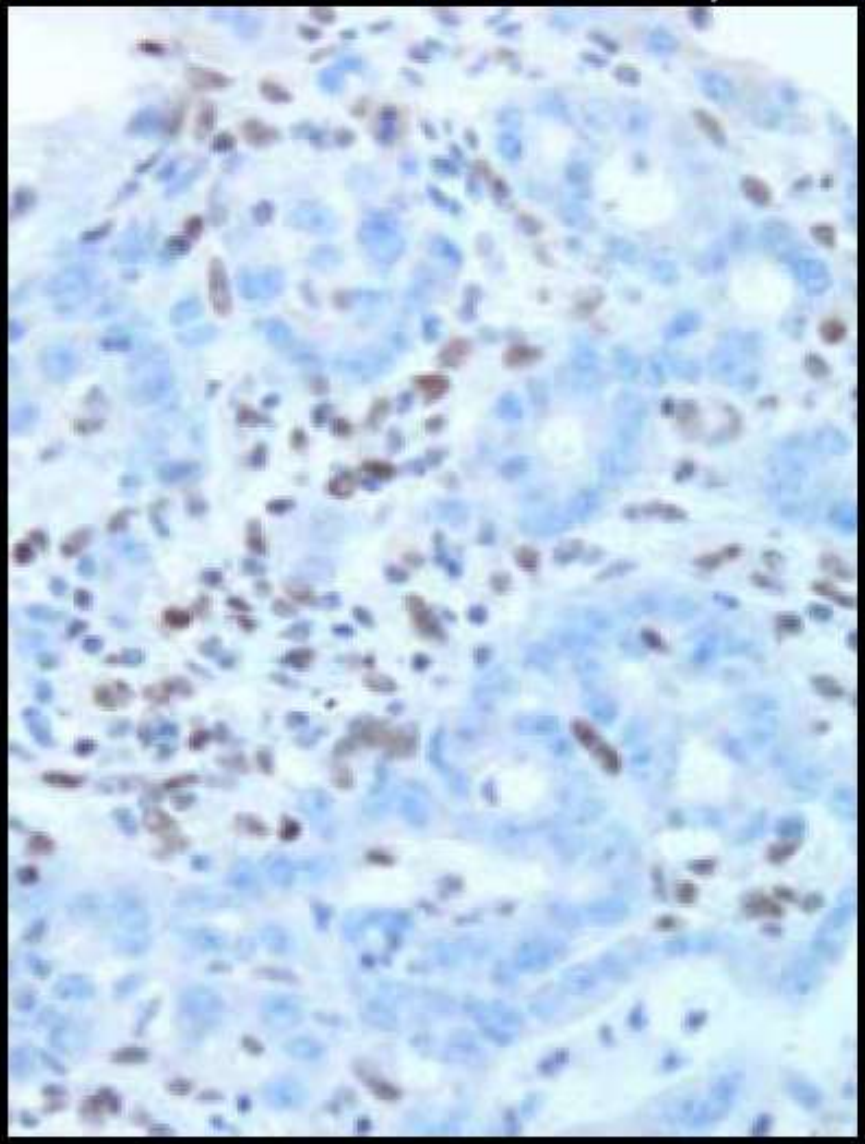


CD138 clone MI15, 1:100 TE

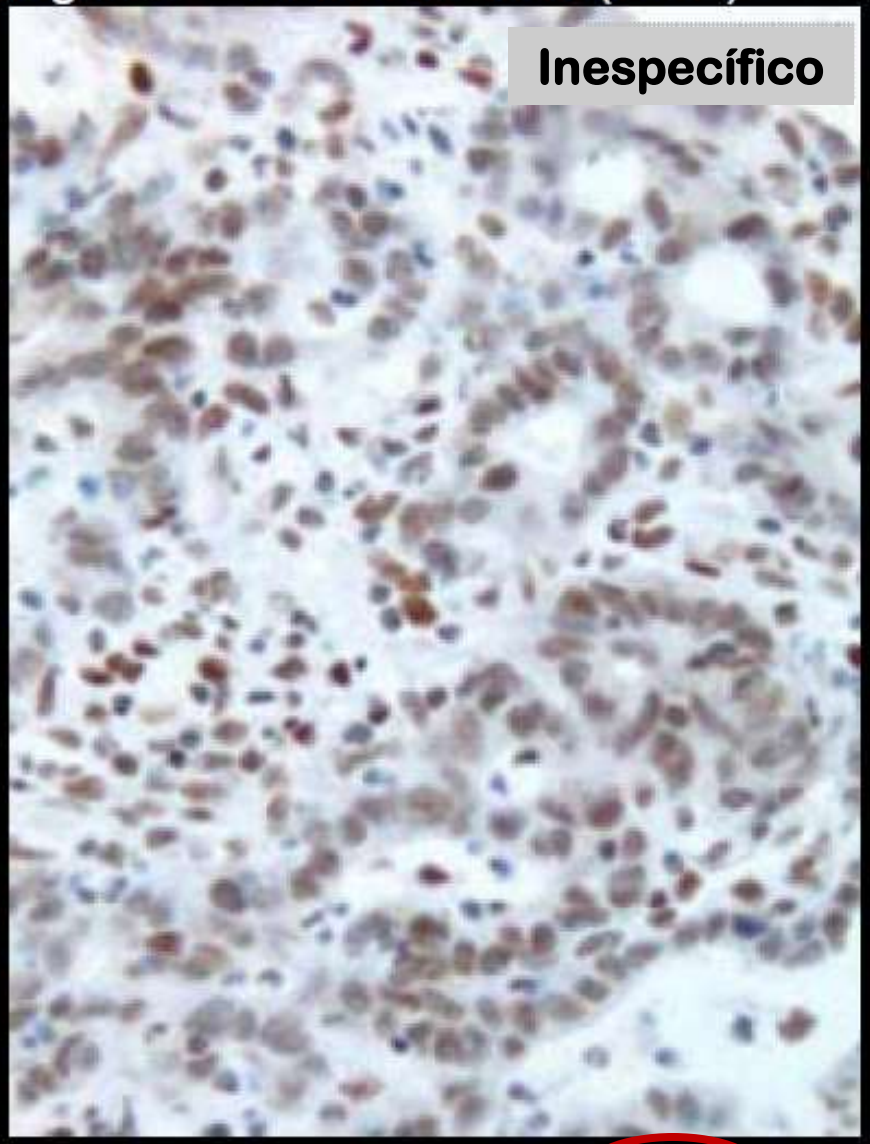


CD138 clone 5F7, 1:50 TE

MLH1 test – Colon ad. carc. 1, MLH1 negative – DNA mutation (PCR)



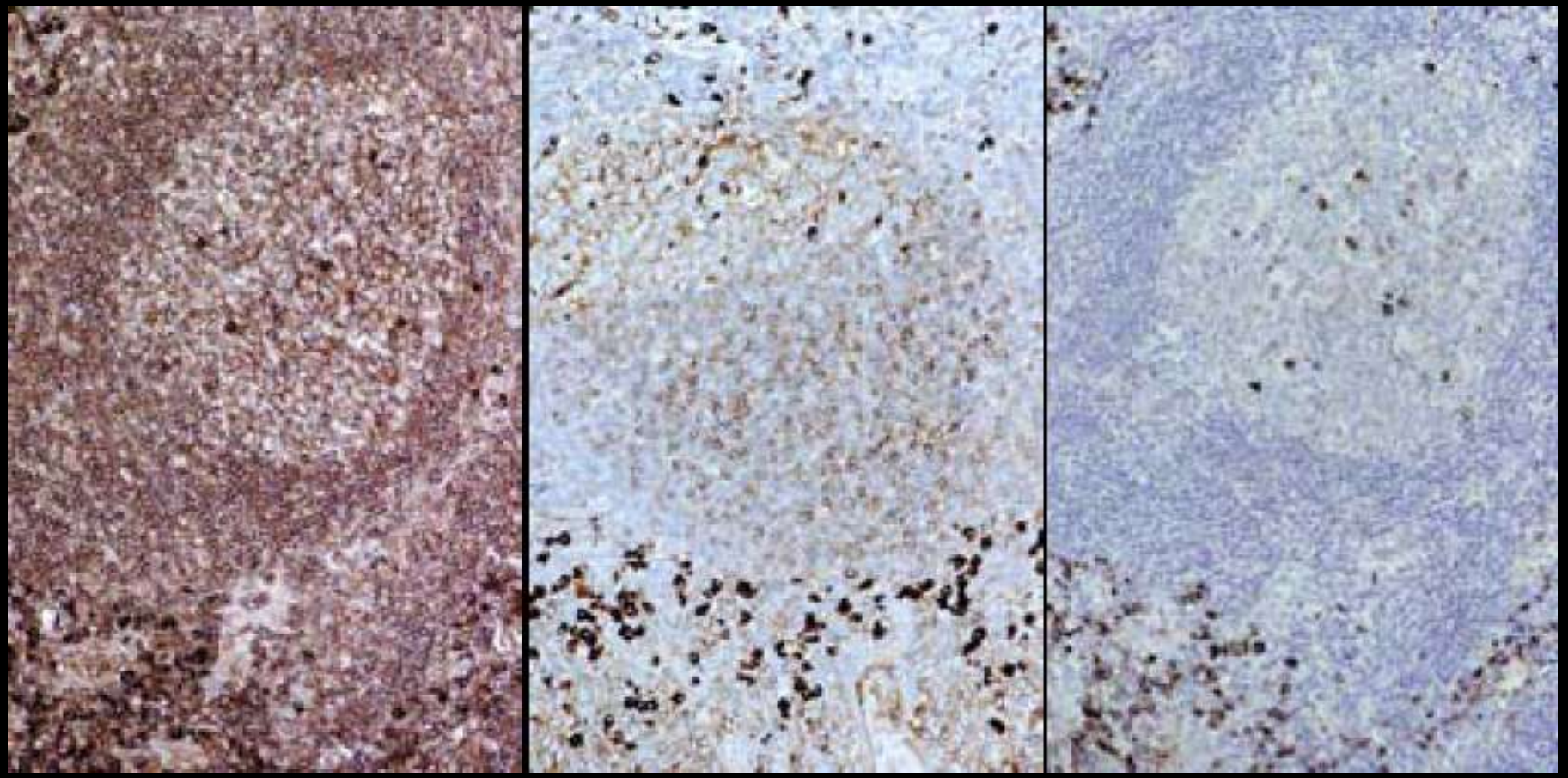
MLH1 clone ES05, 1:20 (Ref.)



MLH1 clone EPR3894, 1:250

IgK: Dako pAb A0191

Cadenas ligeras Ig

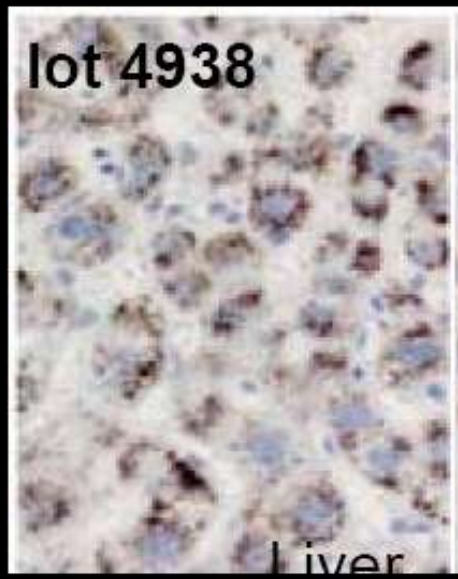


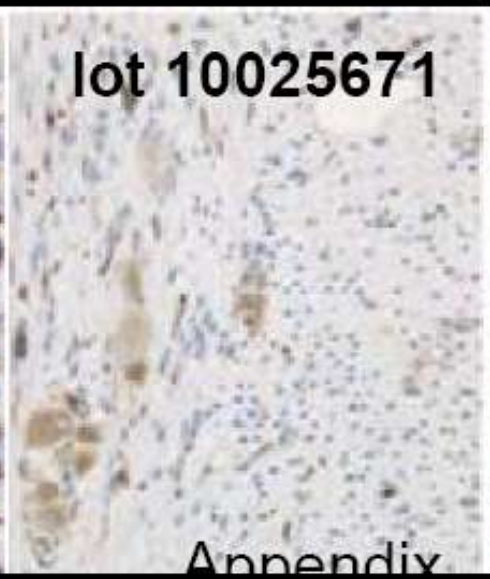
~1:300 ~1:3.000 ~1:30.000

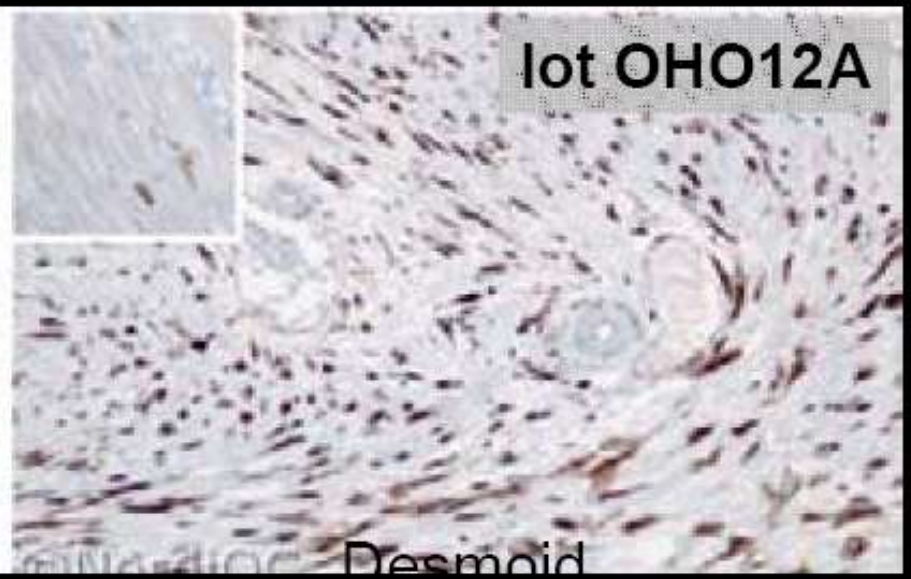
Efecto de la dilución del Ac

Variaciones de lote a lote:

CD117 pAb A4502







Elección control positivo apropiado

- **Problema 1:**
  - **¿qué control positivo utilizo?**
  - **¿tejido normal o tumor?**
- **Problema 2:**
  - **¿se tiñen las células que deberían teñirse?**
- **Problema 3:**
  - **¿qué patrón de tinción debo esperar?**
- **Problema 4:**
  - **¿es adecuada la intensidad de señal?**

SEAP-IAP

Garantía de Calidad en Patología de la Sociedad Española de Anatomía Patológica y
división española de International Academy of Pathology
GCP

Inicio | Participantes | Informes | Anticuerpos | Rondas | Técnicas | Enlaces

INICIO DE SESION

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¿Olvidó sus datos?

- timas noticias
- tividades científicas
- ganización de la Fundación de la Sociedad Española de atomía Patológica
- SEAP

PROGRAMAS DE EVALUACIÓN DE LA CALIDAD EN
INMUNOHISTOQUÍMICA Y PATOLOGÍA MOLECULAR

Leyenda: Preparaciones Virtuales disponibles. Fotos disponibles. Informes en PDF

ANTICUERPOS

ANTICUERPO	Ronda	Módulo	Año	TEJIDO
Bcl2	9	IHQ Linfoide	2007	Amígdala
Bcl2	8	IHQ Linfoide	2007	Amígdala
Bcl2	7	IHQ Linfoide	2007	Amígdala
Bcl2	2	IHQ Linfoide	2005	amígdala
Bcl6	14	IHQ Linfoide	2009	matriz de tejido
Bcl6	11	IHQ Linfoide	2008	matriz linfoide
Bcl6	5	IHQ Linfoide	2006	Amígdala
BhCG	13	IHQ General	2008	placenta
BhCG	6	IHQ General	2005	placenta
Calcitonina	8	IHQ General	2006	tiroides

Recommended control tissue

Multitissue controlblock

On the basis of the observations generated during the NordiQC assessments concerning identification of recommended control tissue, NordiQC and Aalborg Sygehus DK, Department of Pathology have established a database with suggestions how to design multitissue controlblocks and interpret the immunohistochemical reactions in these.

The database is based on 4 controlblocks consisting of preferable normal tissue. For each marker the reaction pattern is described and if possible quality indicators (text marked with red) either identified by NordiQC or personal experiences serve as quality indicator for the diagnostic use of the marker.

Multitissue controlblock 1
Appendix, tonsil, pancreas and liver

Multitissue controlblock 2
Brain, striated muscle, skin and melanoma

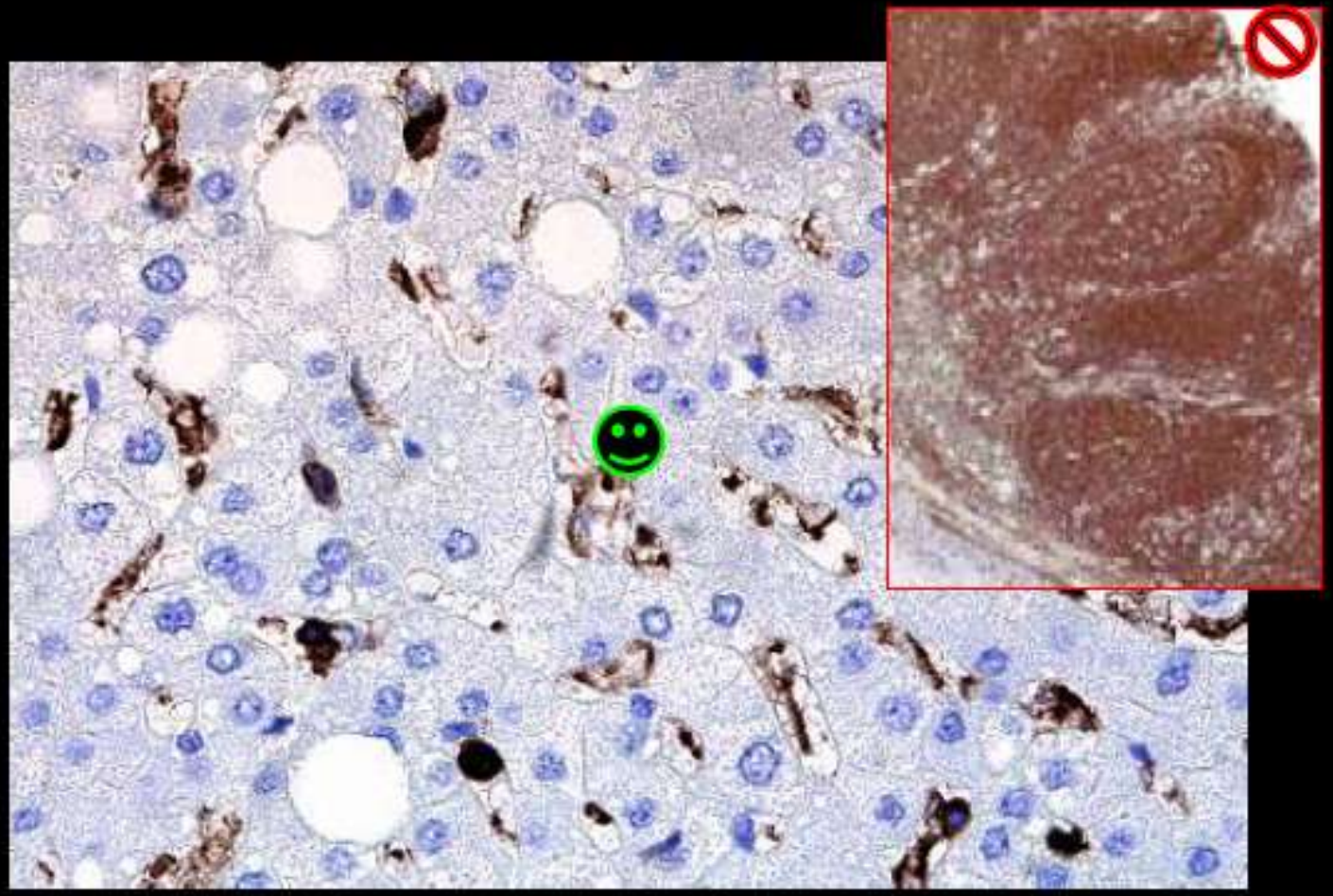
Multitissue controlblock 3
Lung, thyroid, prostate and placenta

Multitissue controlblock 4
Thymus, bone marrow, Hodgkin lymphoma and tonsil

The design of the multitissue controlblocks is only a guideline and can be modified to the individual laboratory.

MB K1	APPENDIX	TONSIL	PANCREAS	HEPAR
AAT **Alfa-1-antitrypsin** **(Cytopl)**	Macrophages & villi of columnar epithelial cells	Macrophages	0	Kupfer cells & hepatocytes (globuli)
ASMA **Alfa-smooth muscle actin** **(Cytopl)**	Smooth muscle cells in vessels and in muscle layers. Myofibroblasts lining the epithelial surface	Smooth muscle cells in vessels	Smooth muscle cells in vessels	Smooth muscle cells in sinusoids
B-CATENIN **Beta-catenin** **(Membrane)**	Membranes of columnar epithelial cells. Endothelial and follicular denritic cells	Membranes of squamous epithelial cells	Membranes of acinar epithelial cells (ducts) and endocrine cells	Hepatocytes - weak membranous
BCL2 **Bcl2-oncoprotein** **(Cytopl)**	All peripheral lymphocytes incl T-cells in germinal centres - Germinal centre B-cells are negative.	All peripheral lymphocytes incl T-cells in germinal centres - Germinal centre B-cells are negative.	Weak reaction of epithelial cells.	0
BCL6 **Bcl6-protein** **(Nuclear)**	Germinal centre B-cells	Germinal centre B-cells and basal squamous cells	0	0
BOB.1 **BOB.1-protein** **(Nuclear)**	Strong reaction in germinal centre B-cells - weak reaction in mantle zone B-cells and plasma cells	Strong reaction in germinal centre B-cells - weak reaction in mantle zone B-cells and plasma cells	0	0
CD2 **(Membrane)**	All T-cells - Isolated T-cell in germinal centres	All T-cells - Isolated T-cells in germinal centres	T-cells	T-cells
CD3 **(Membrane)**	All T-cells - Isolated T-cell in germinal centres	All T-cells - Isolated T-cell in germinal centres	T-cells	T-cells

Control recomendado para CD45



Hígado

Control fijado y procesado adecuadamente

- Adecuada **fijación** del tejido:
  - Problema 1: retraso en iniciar la fijación
  - Problema 2: fijación excesivamente corta
  - Problema 3: exceso de fijación
- Adecuado **procesamiento** del tejido:
  - Problema 1: decalcificación agresiva
  - Problema 2: grosor del corte
  - Problema 3: cortes almacenados

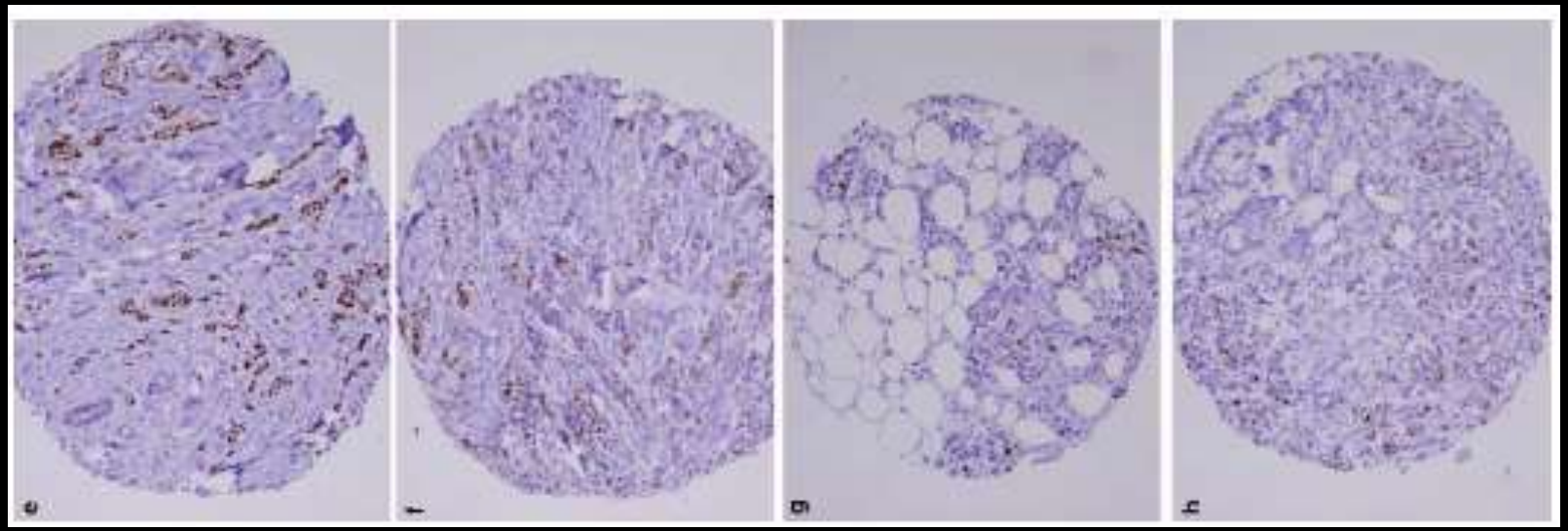
Falsos negativos y falsos positivos

Delay to formalin fixation effect on breast biomarkers

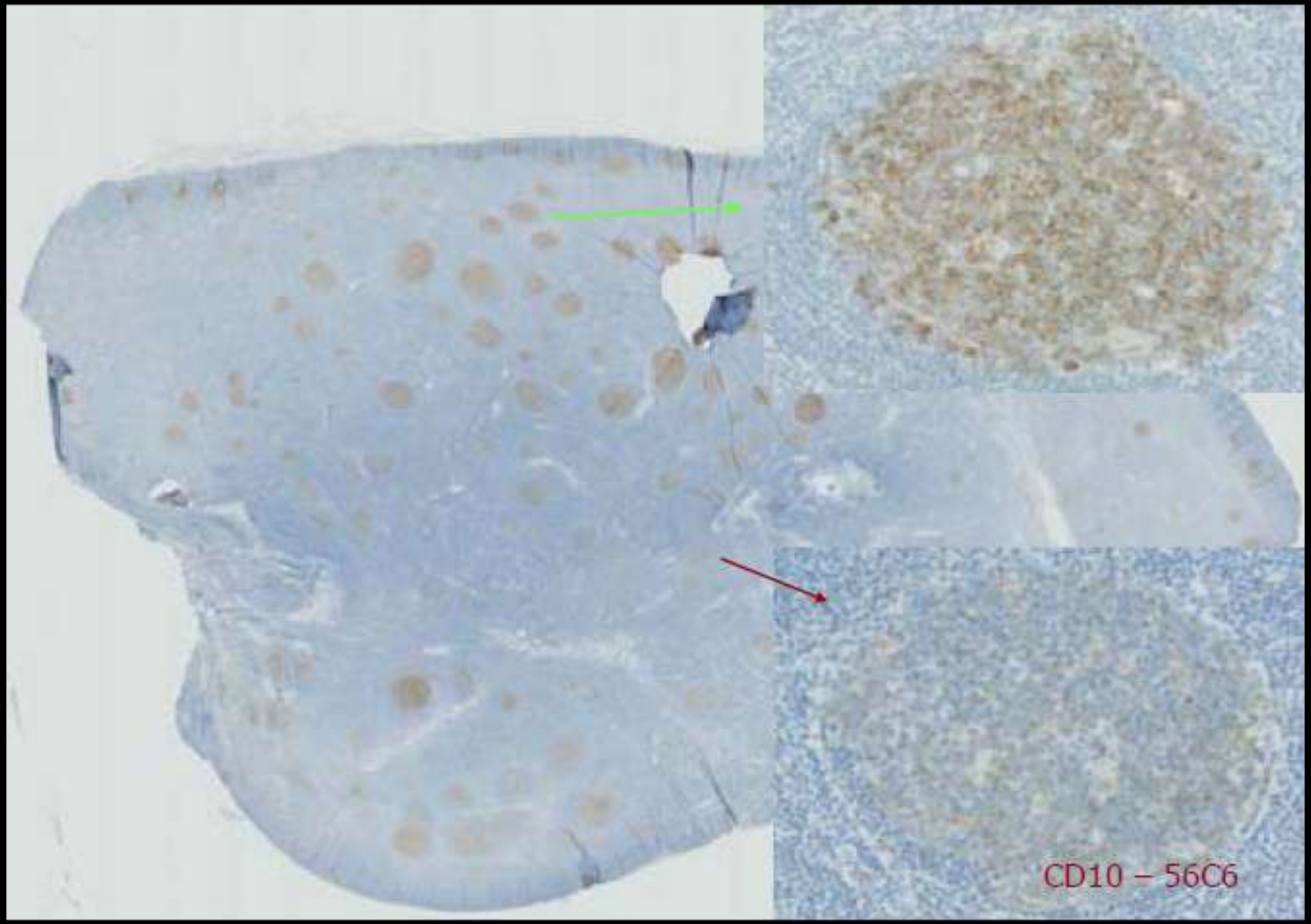
Thaer Khoury[1], Sheila Sait[2], Helena Hwang[1], Rameela Chandrasekhar[3], Gregory Wilding[3], Dongfeng Tan[4] and Swati Kulkarni[5]

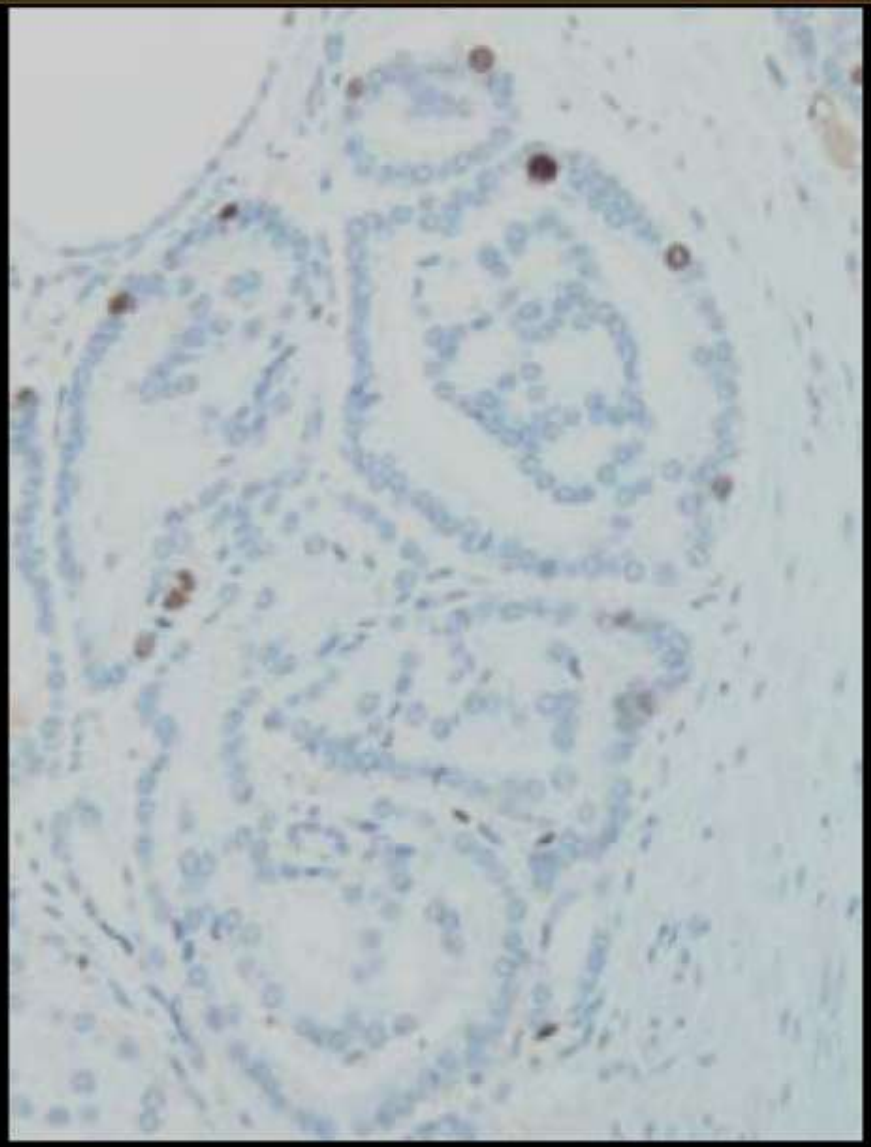
Delay to formalin fixation may invalidate hormone receptors and HER2 analyses. Invalid results of tumor markers could significantly alter the type of adjuvant therapy a patient receives and potentially impact outcome. The purpose of this study was to determine the effects of progressive delay to formalin fixation on breast cancer biomarkers. Ten palpable invasive breast cancers were resected and underwent immediate gross evaluation. For each case, the procured tumor was divided into eight parts and consecutively fixed after 0, 10, 30 min, 1, 2, 4, and 8 h; one section was kept in saline and stored overnight at 4°C. Two tissue microarray blocks were constructed. Estrogen and progesterone receptors and HER2 immunohistochemistry and fluorescence *in situ* hybridization were carried out. Statistical analyses including non-parametric sign test, exact McNemar's test and Page's L test were used. All 10 cases were invasive ductal carcinomas. Q score ⩾6 was identified in five cases for estrogen receptor and four for progesterone receptor. Mean Q score started to decline at the 2 h mark for estrogen receptor and 1 h mark for progesterone receptor. Lowest score was at 8 h mark for estrogen receptor and overnight for progesterone receptor. HER2 fluorescence *in situ* hybridization started to be compromised for interpretation at the 1 h mark and became statistically significant at the 2 h mark ($P<0.03$). To avoid delay to formalin fixation as a factor negatively affecting on breast biomarkers, we recommend not to delay formalin fixation for more than 1 h and not to store specimens overnight.

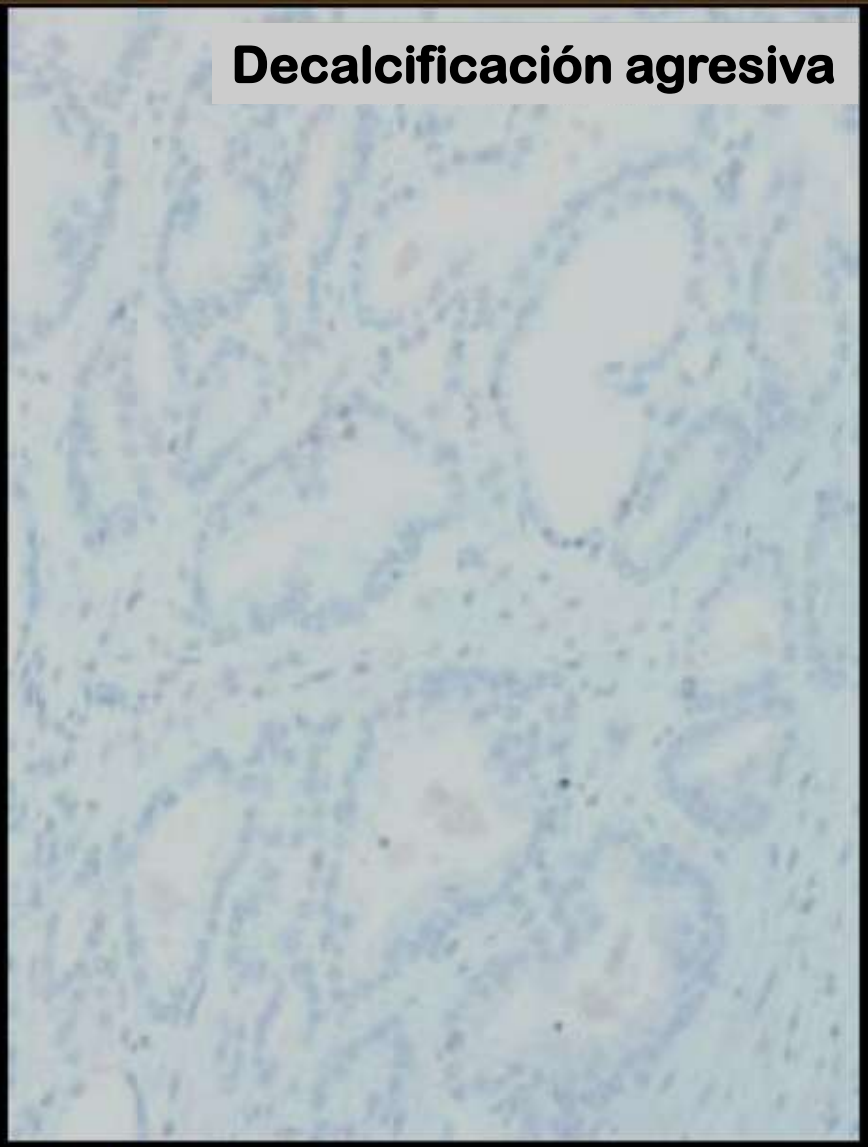
Modern Pathology (2009) **22,** 1457–1467; doi:10.1038/modpathol.2009.117; published online 4 September 2009




CD4 – SP35


CD10 – 56C6





Decalcificación agresiva

Prostate – Ki67, rmAb clone 30.9

10 % NBF 24h → 24h 10 % form. acid

10 % NBF + 10 % form. acid 24h

Immunocytochemistry Volume 6 Issue 3 (Run 76) Technical Article

Immunocytochemistry 2008; Volume 6 Issue 3

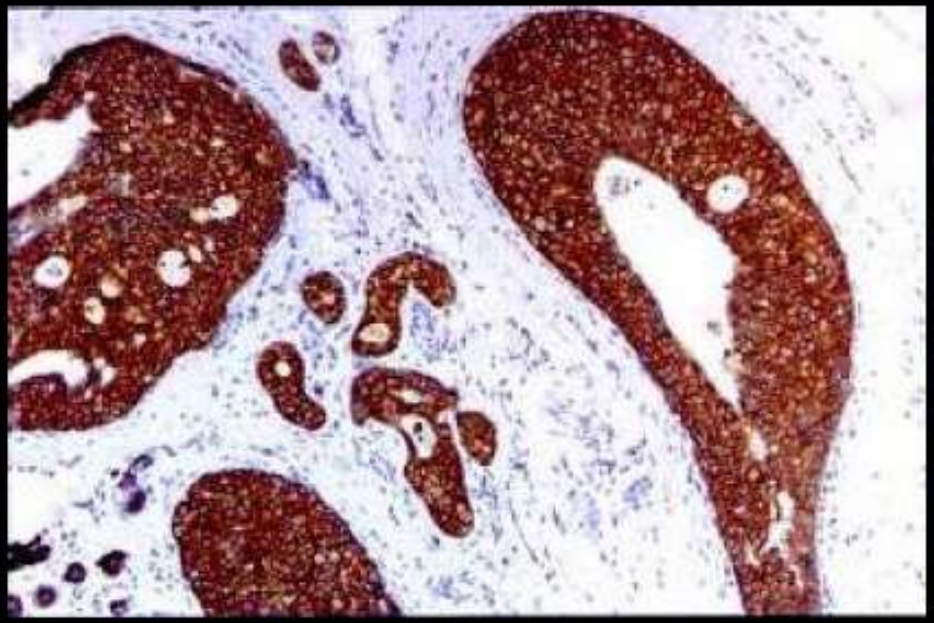
TECHNICAL ARTICLE

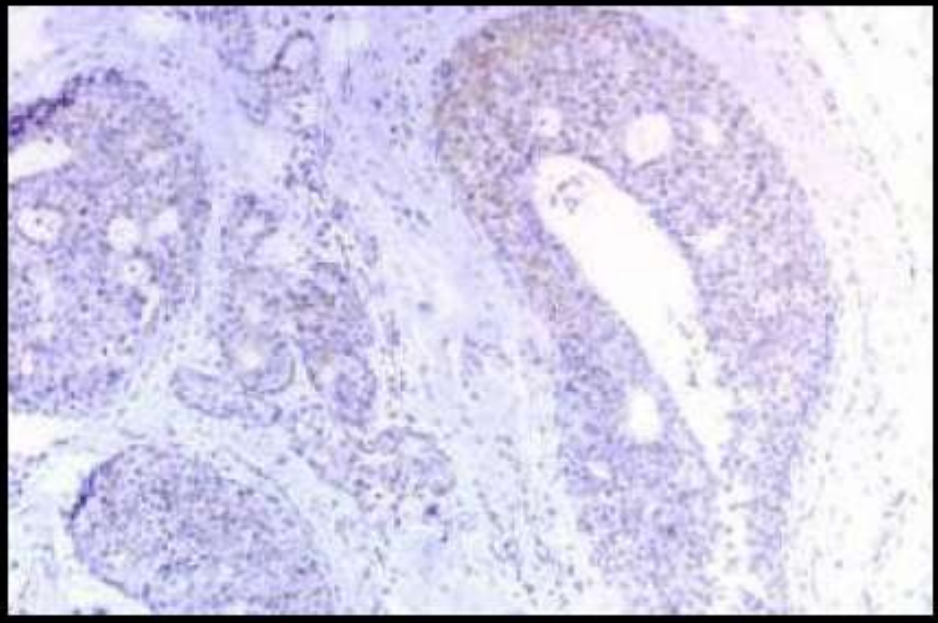
EXCESSIVE SECTION DRYING OF BREAST CANCER TISSUE PRIOR TO DEPARAFFINISATION AND ANTIGEN RETRIEVAL CAUSES A LOSS IN HER2-IMMUNO-REACTIVITY

Bent Lundgaard Hansen, Henrik Winther and Kristian Moller

Dako A/S, DK-2600 Glostrup, Denmark

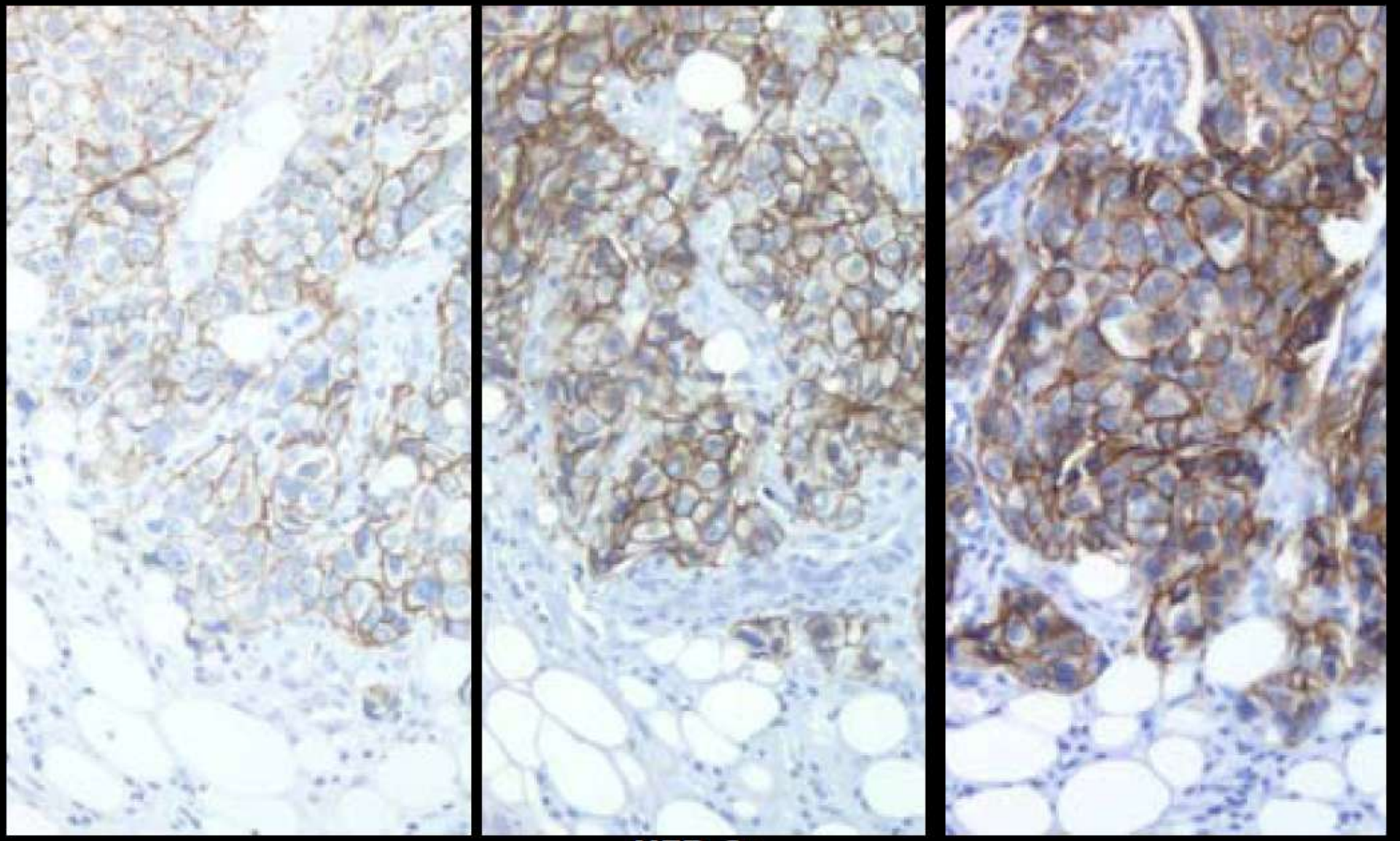
Correspondence: Kristian.Moller@dako.com





60°C 1h. HER-2: 3+

80°C 16h. HER-2: 1+



HER-2;

1 µm 3 µm 8 µm

Recuperación antigénica apropiada y eficiente

- **Problema 1:**
  - **¿necesito recuperación Ag?**
  - **¿qué tipo de recuperación Ag empleo?**
- **Problema 2:**
  - **¿qué buffer uso?**
  - **¿a que pH?**
  - **¿cuánto tiempo?**

Falsos negativos y falsos positivos

Heat Induced Epitope Retrieval

Optimized temperature-time-pH-buffer system

'Heating condition' = temperature × time:

121ºC/1 min 100ºC/20 min 95ºC/40 min 60ºC/24 h.

Device:
Water bath
MWO
Pressure cooker
Pressure cooker & MWO
Autoclave
Steam

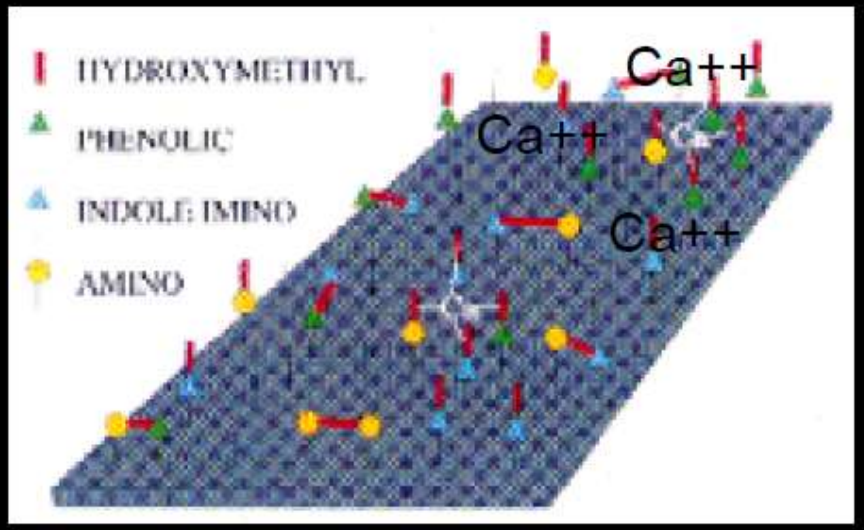
Considerations:
Efficiency
Standardization
Tissue damage
Performance

Para el 95 % de los epítopes es preferible el pH 8-9 al pH6

The heat induced antigen rerieval effect is related to the capacity of binding and removing Ca++ ions after formaldehyde fixation

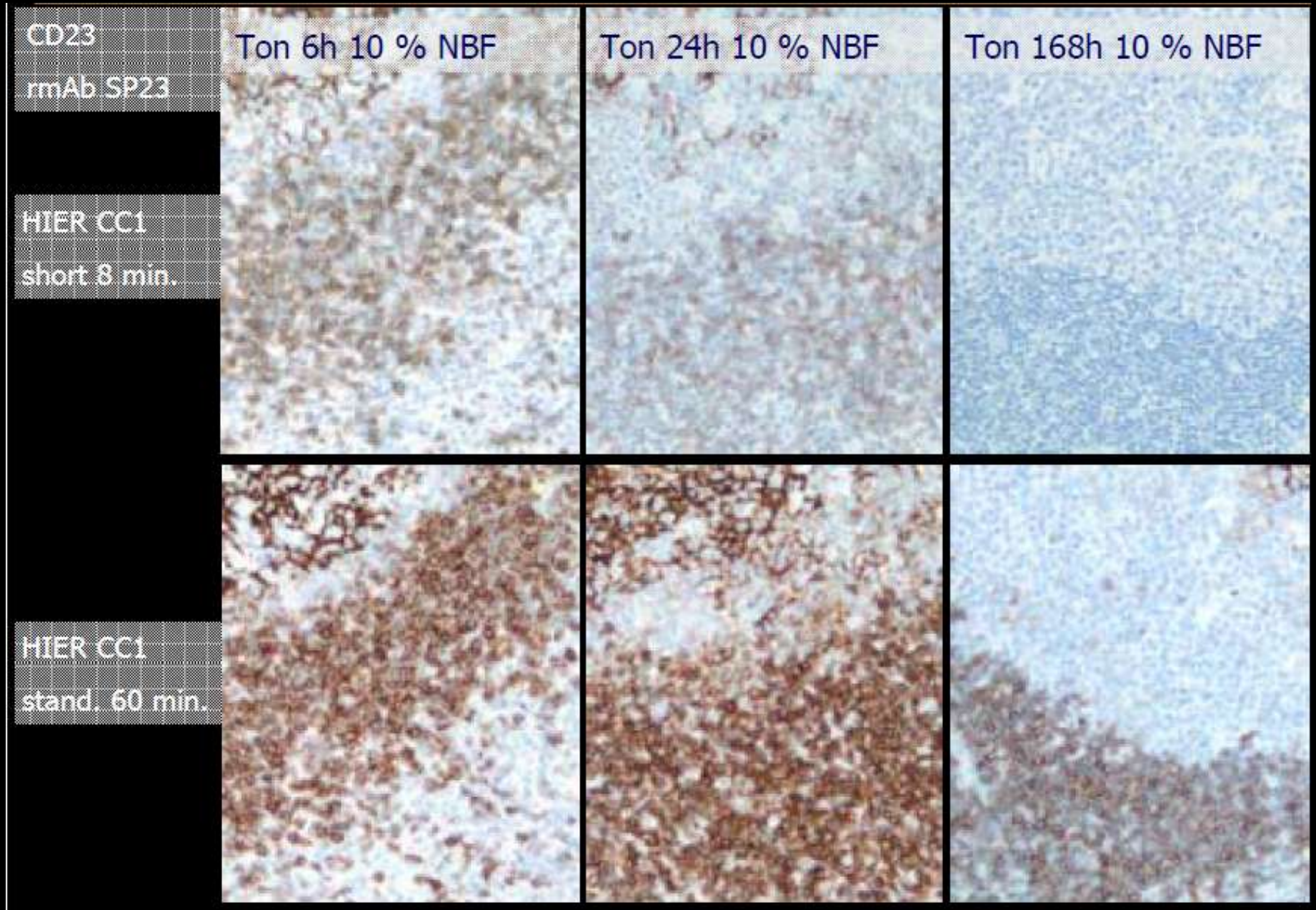
Calcium chelating agent

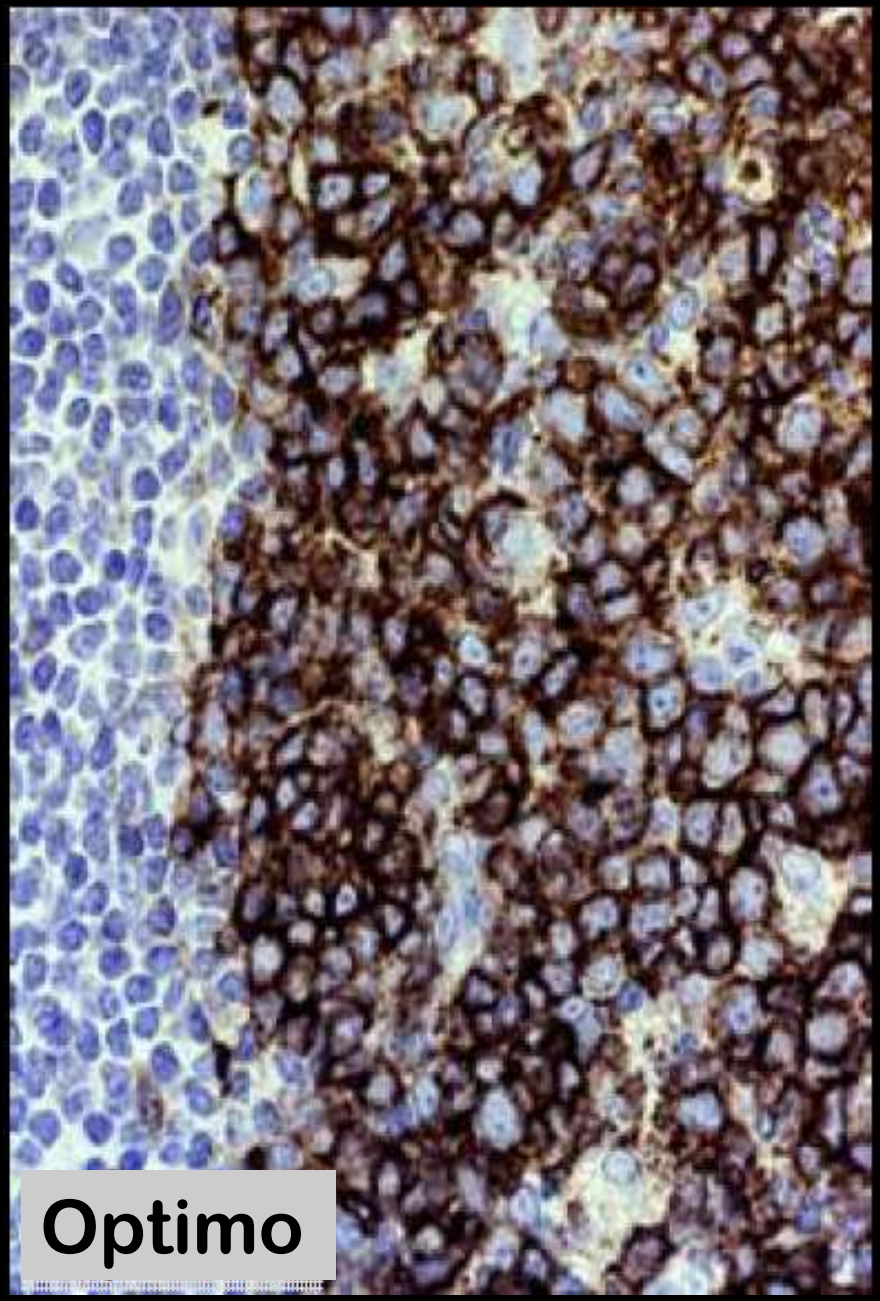
Citrate pH 6	++
EDTA pH 6	(+)
EDTA pH 8	+++
Tris/EDTA pH 9*	++++

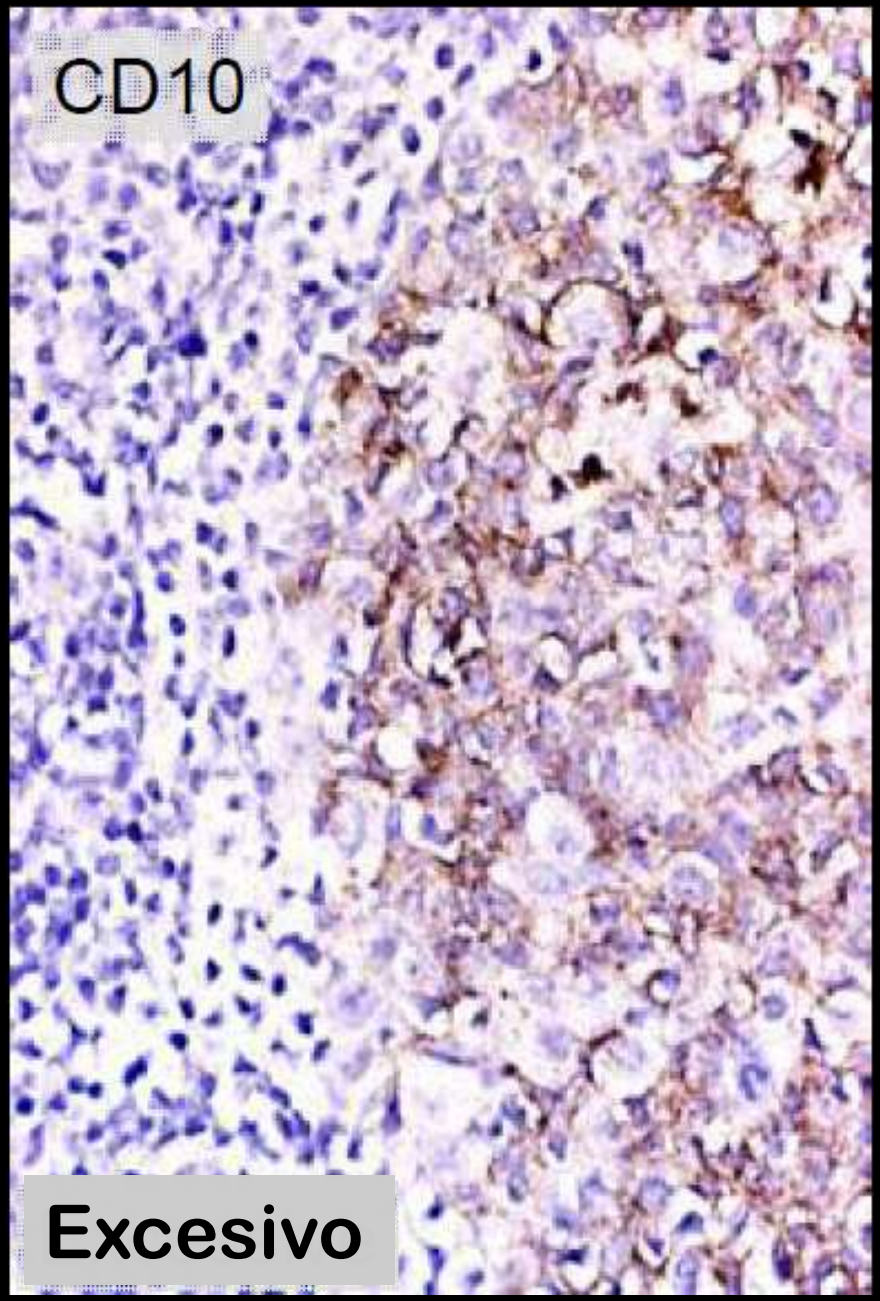


Morgan et al. J. Pathol. 182 233-237 1997 * own

Tiempo de recuperación Ag



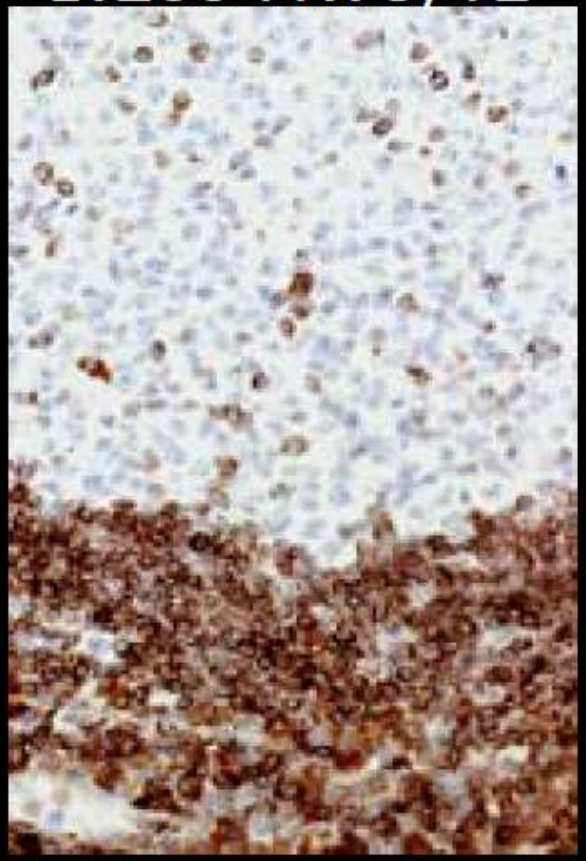

Optimo


CD10
Excesivo

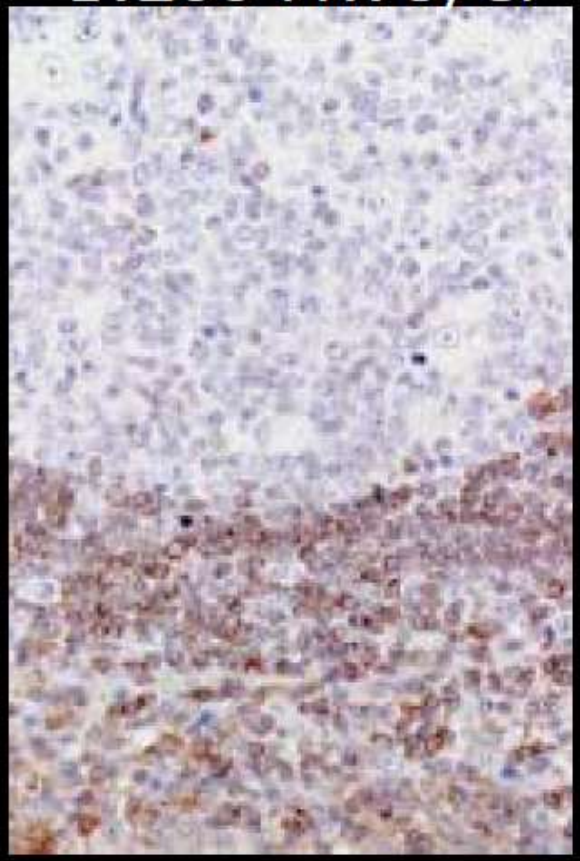
Tipo de Buffer

BCL2 clone 124

1:200 MWO/TE



1:200 MWO/Ci



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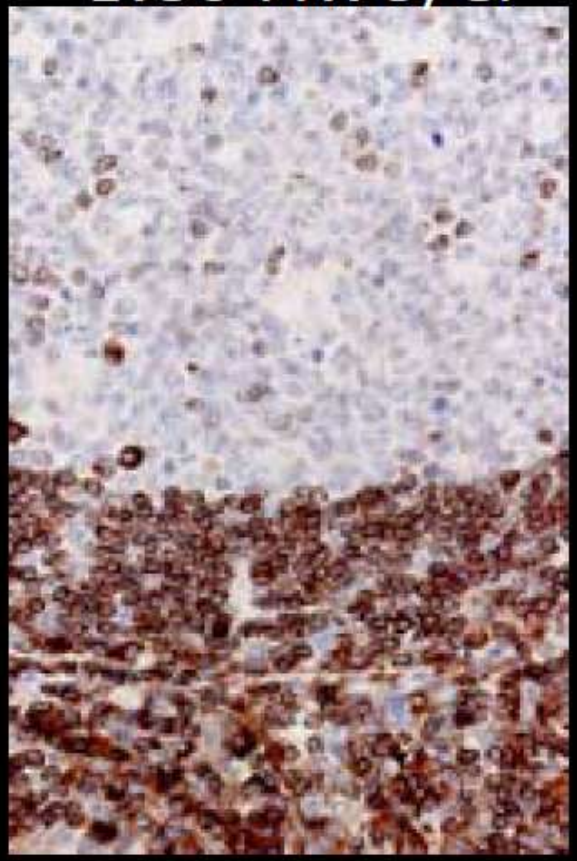
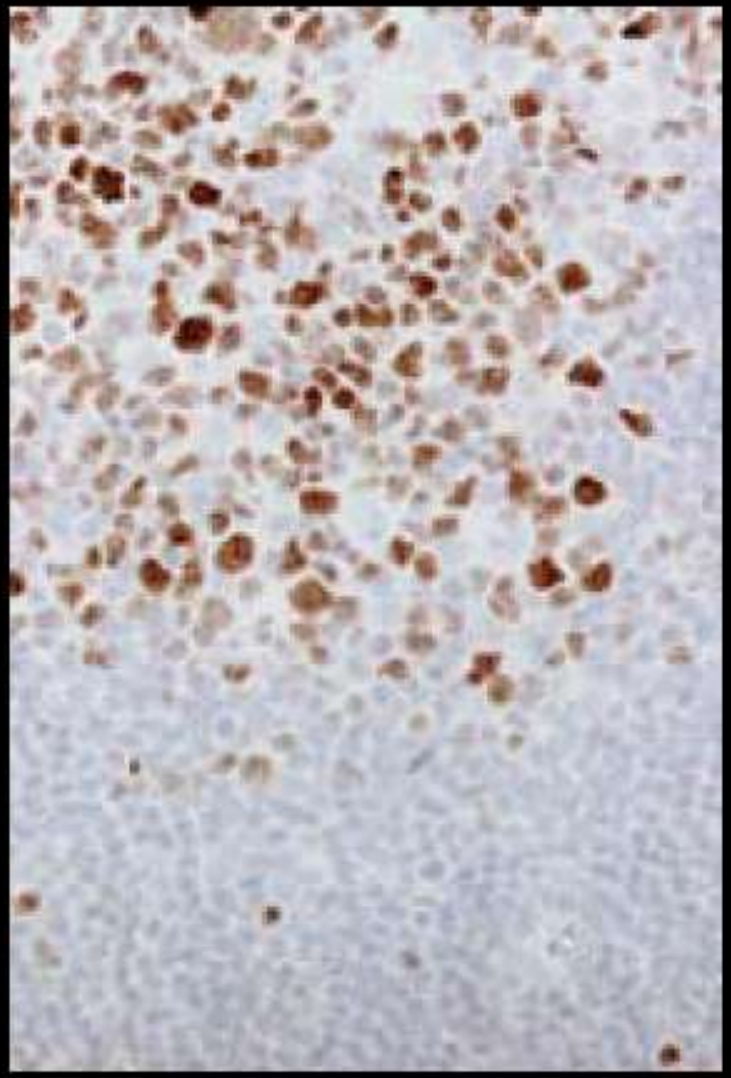
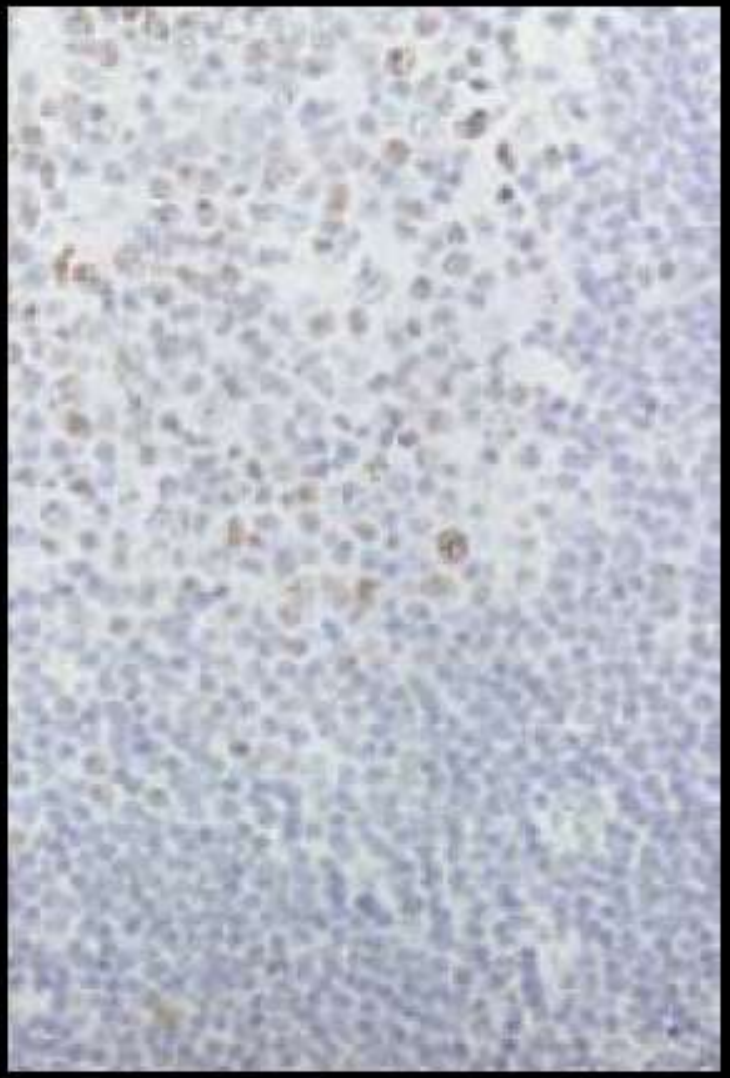


BCL6 clone PG-B6p

1:40 MWO/TE



1:10 MWO/Ci



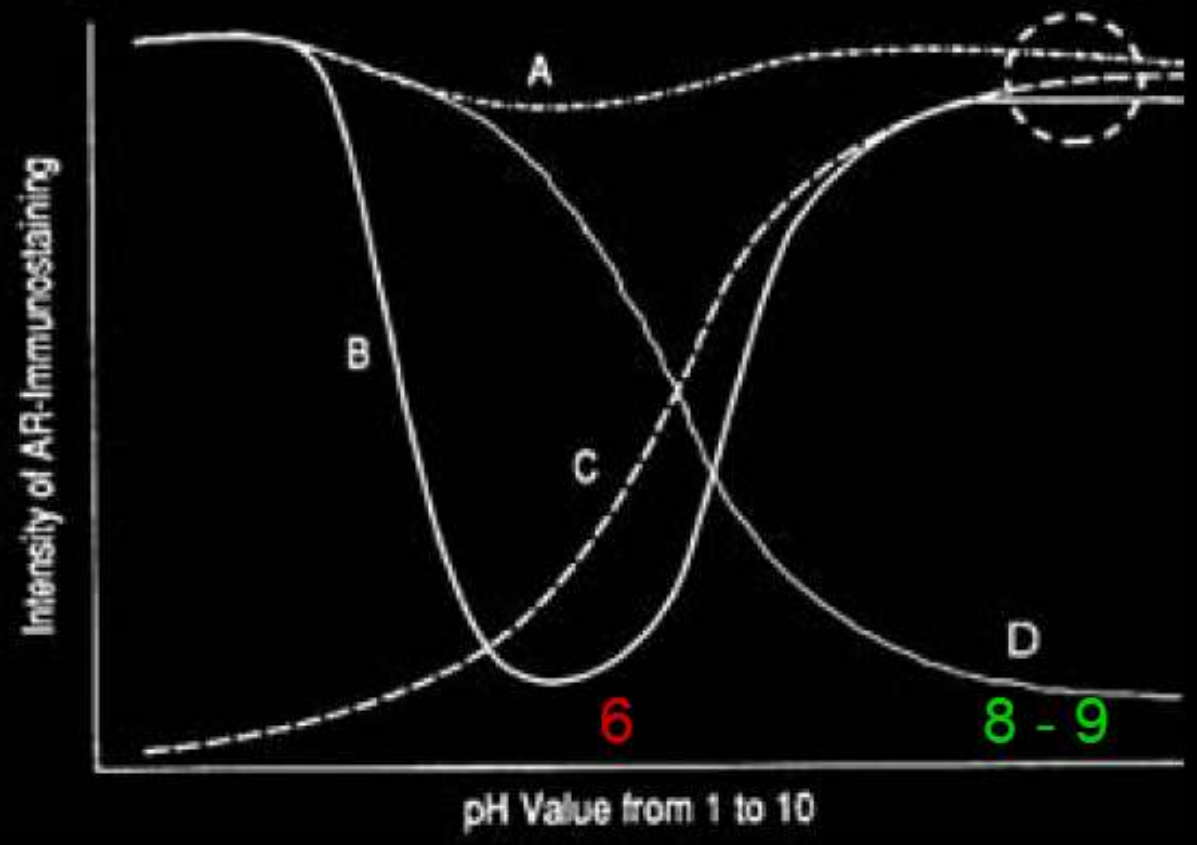
pH del Buffer

A: CD20, cl. L26

B: Ki67, cl. MIB1

C: HMB45

(D: MOC31)



Modified from: Shi et al. J Histochem Cytochem 1995 43:193-201

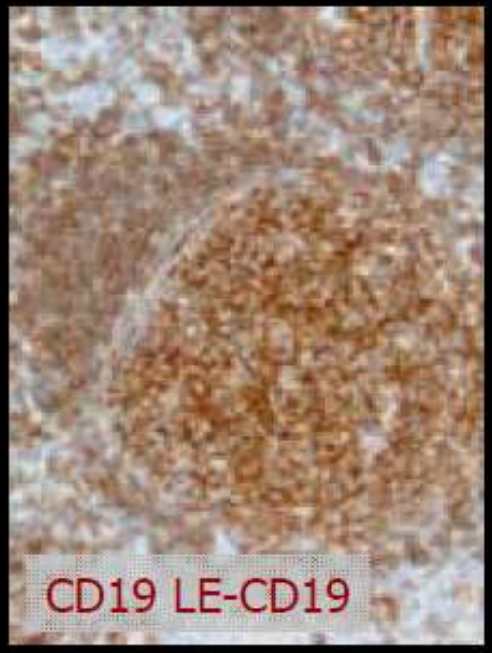
Tonsil

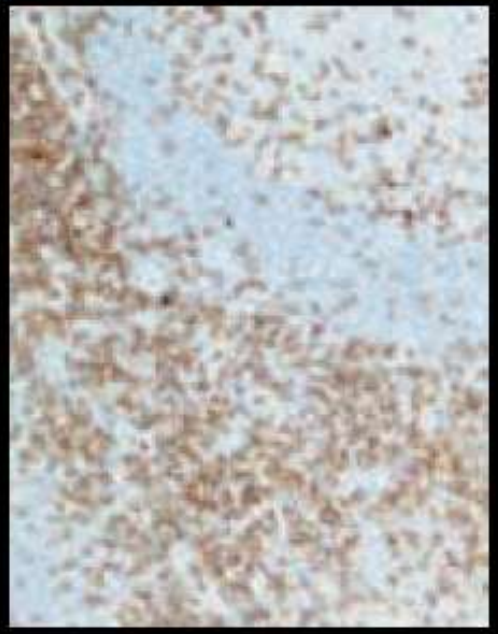
24 h NBF

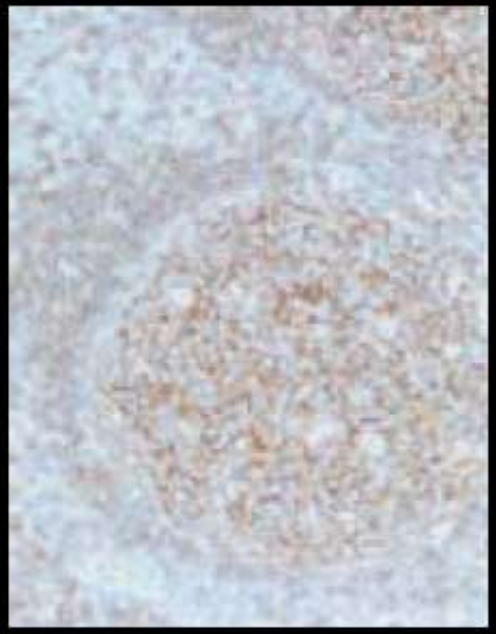
HIER

TE pH 9

Ci pH 6





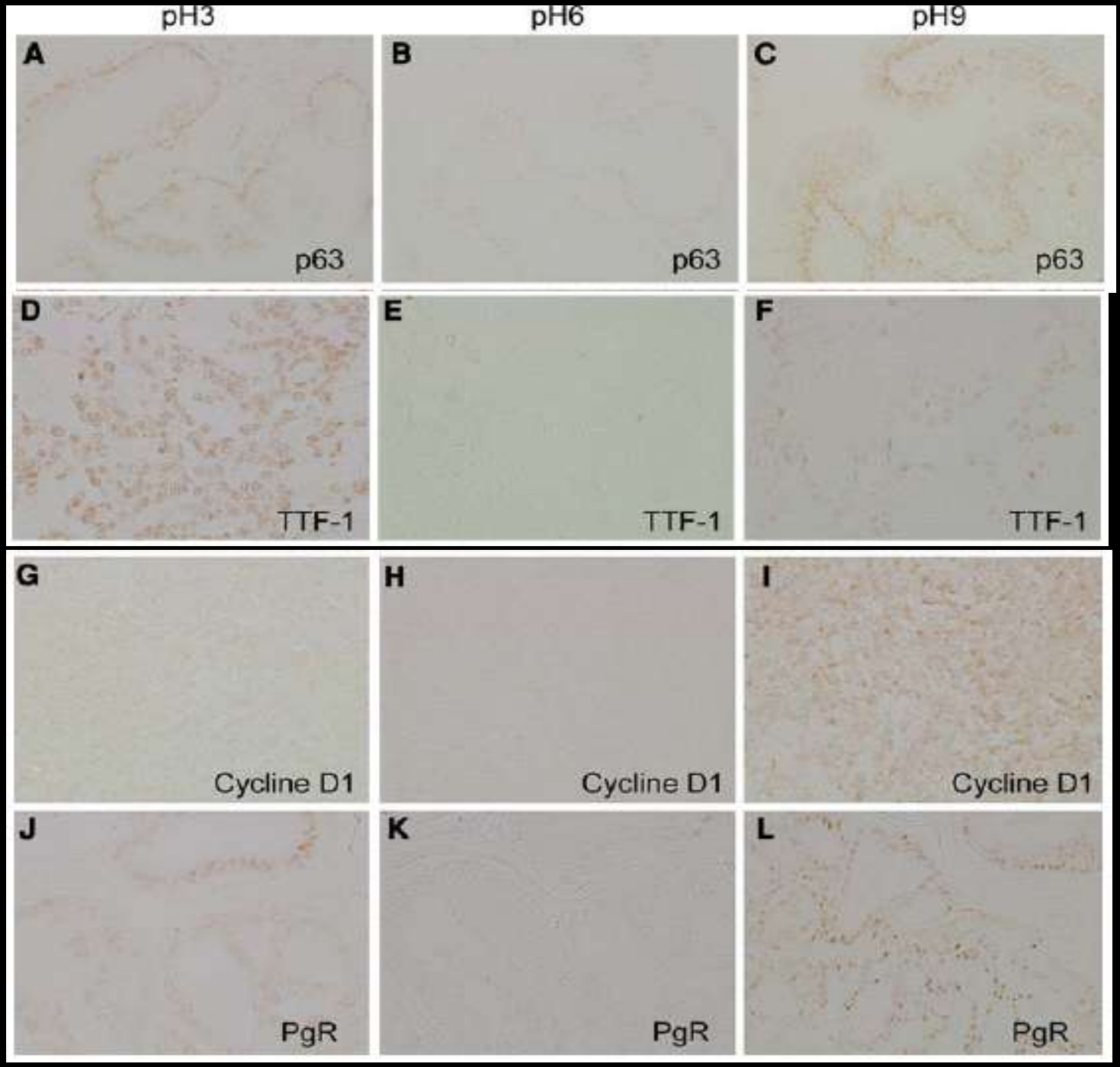


Selection of buffer pH by the isoelectric point of the antigen for the efficient heat-induced epitope retrieval: re-appraisal for nuclear protein pathobiology

Hanako Kajiya · Susumu Takekoshi · Mao Takei · Noboru Egashira · Takashi Miyakoshi · Akihito Serizawa · Akira Teramoto · Robert Y. Osamura

Histochem Cell Biol (2009) 132:659–667

Antigens	Amino sequence of epitopes	pI	Tissues	Heating procedures			
				Non-heating	pH 3.0	pH 6.0	pH 9.0
Neuro D1	Amino terminal (sequence not announced)	4.53	GHoma (1)	−	−	−	++
			GNoma (2)	−	±	±	+++
			ACTHoma (3)	−	±	±	+++
Ptx1	31–50	4.67	GNoma (1)	−	+	−	+++
			GHoma (2)	−	+	+	++
			GHoma (3)	−	+	±	+++
Cyclin D1	1–295	4.97	Lymphoma	−	−	−	++
PgR	164–534	5.00	Breast cancer	−	+	−	++
P63	1–205	5.63	Normal prostate	−	+	−	+++
DAX1	1–300	7.96	GNoma (1)	−	++	±	++
			GNoma (2)	−	+++	−	++
			GNoma (3)	−	+++	±	++
TBX19	Internal region (sequence not announced)	9.31	ACTHoma (1)	−	++	±	−
			ACTHoma (2)	−	+++	±	±
			ACTHoma (3)	−	+	+	±
TTF-1	1–123	10.00	Lung adenocarcinoma	−	++	−	+
Prop1	60–80	13.20	GNoma (1)	−	+++	−	−
			GNoma (2)	−	+++	−	+

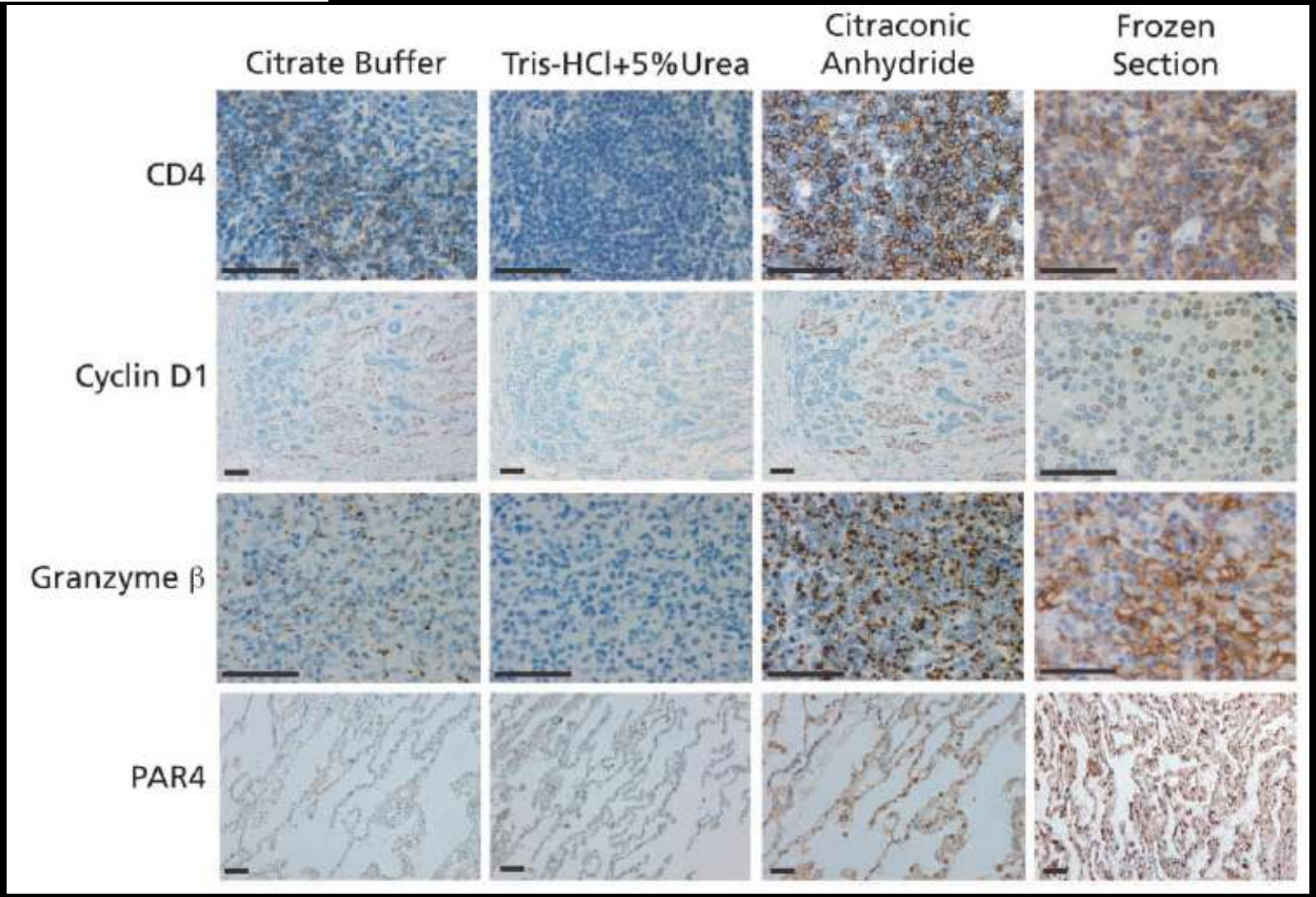

pH3
pH6
pH9
pI 5,63
pI 10,00
pI 4,97
pI 5,00
A
p63
B
p63
C
p63
D
TTF-1
E
TTF-1
F
TTF-1
G
Cycline D1
H
Cycline D1
I
Cycline D1
J
PgR
K
PgR
L
PgR

Reversing the Effects of Formalin Fixation with Citraconic Anhydride and Heat: A Universal Antigen Retrieval Method

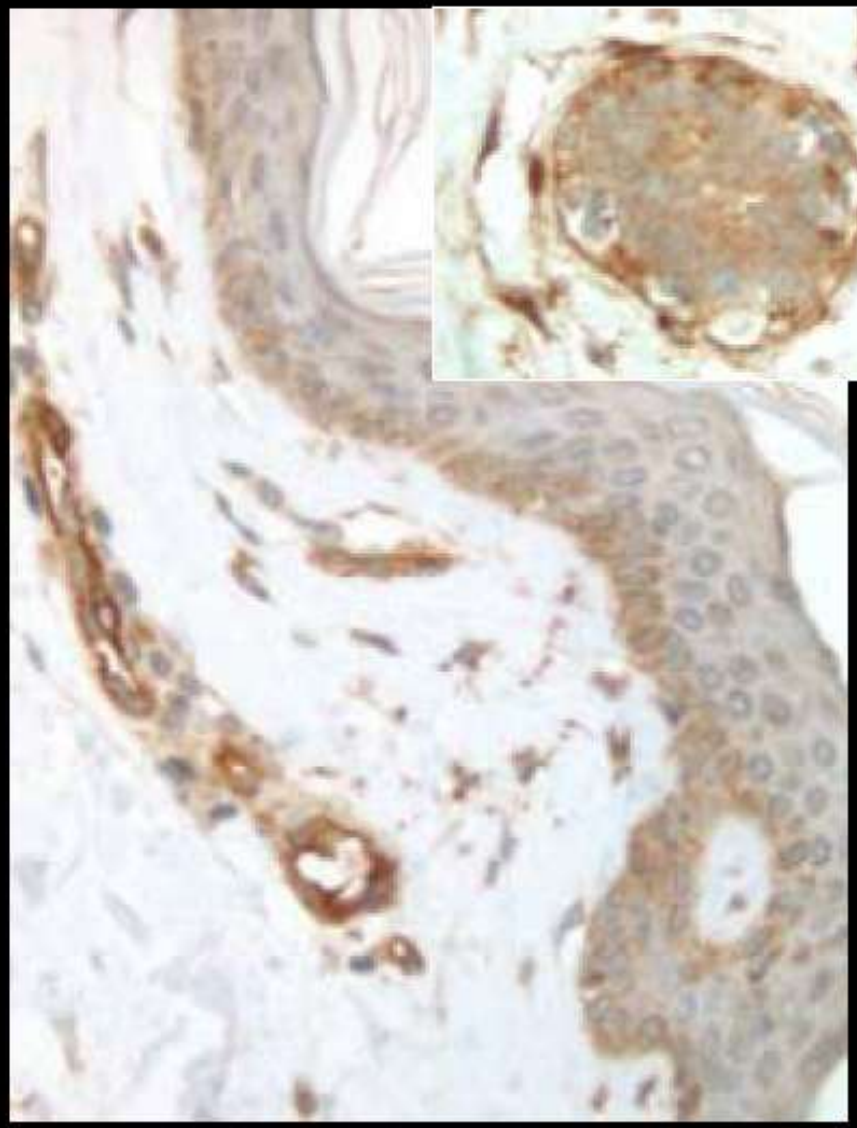
Shigeki Namimatsu, Mohammad Ghazizadeh, and Yuichi Sugisaki

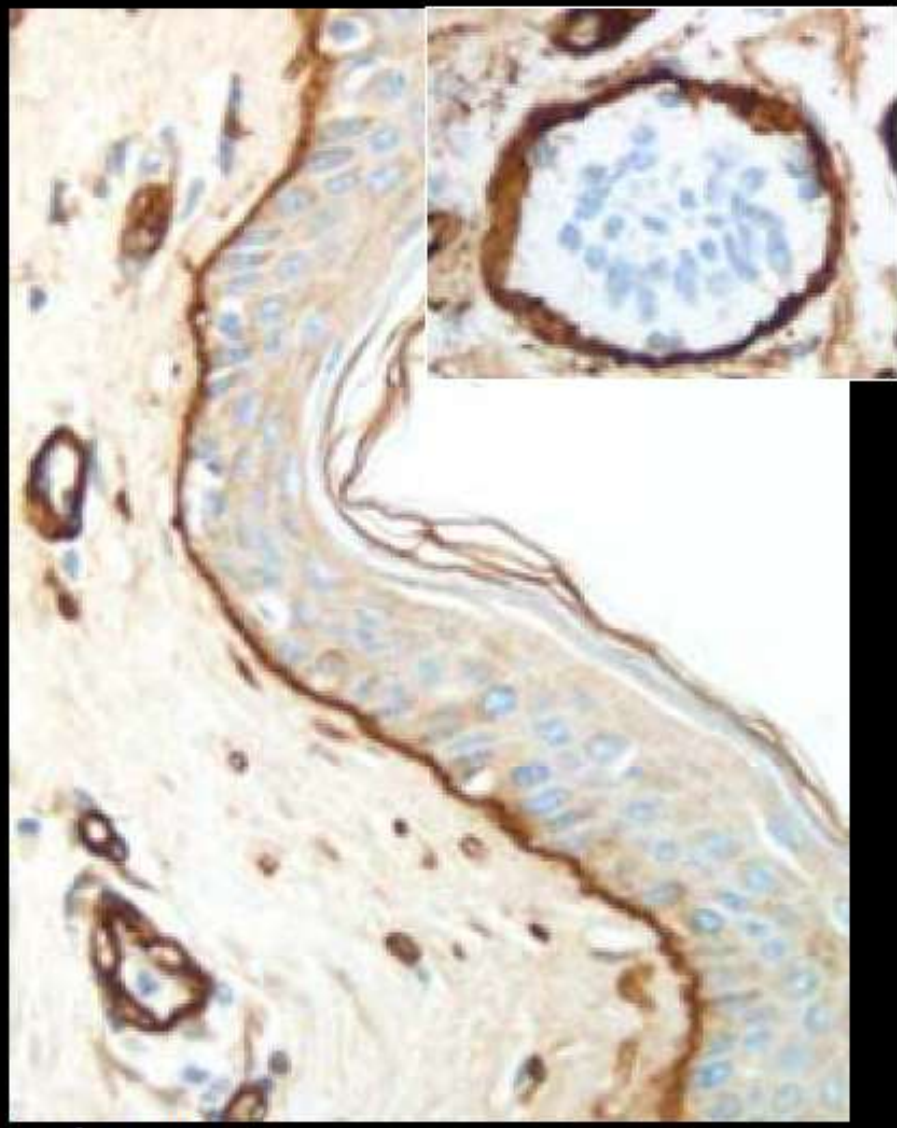
Division of Surgical Pathology, Nippon Medical School Hospital, Tokyo, Japan (SN,YS), and Department of Molecular Pathology, Institute of Gerontology, Nippon Medical School, Kawasaki, Japan (MG)

(J Histochem Cytochem 53:3–11, 2005)



Recuperación Ag enzimática





Laminin polyclonal Z0097

HIER TE / CC1

Proteolysis Pepsin / P1

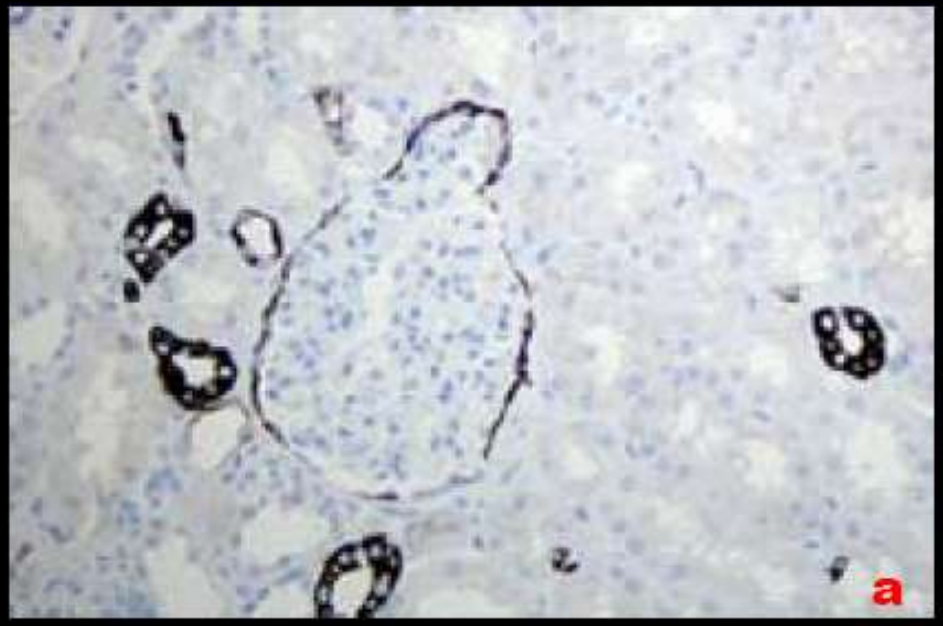
Primary Antibody Dilution	1:100	1:200	1:400	Negative Control
Enzyme of Choice	A	B	C	D
HIER pH 6.0	E	F	G	H
HIER pH 9.0	I	J	K	L
No Pretreat	M	N	O	P

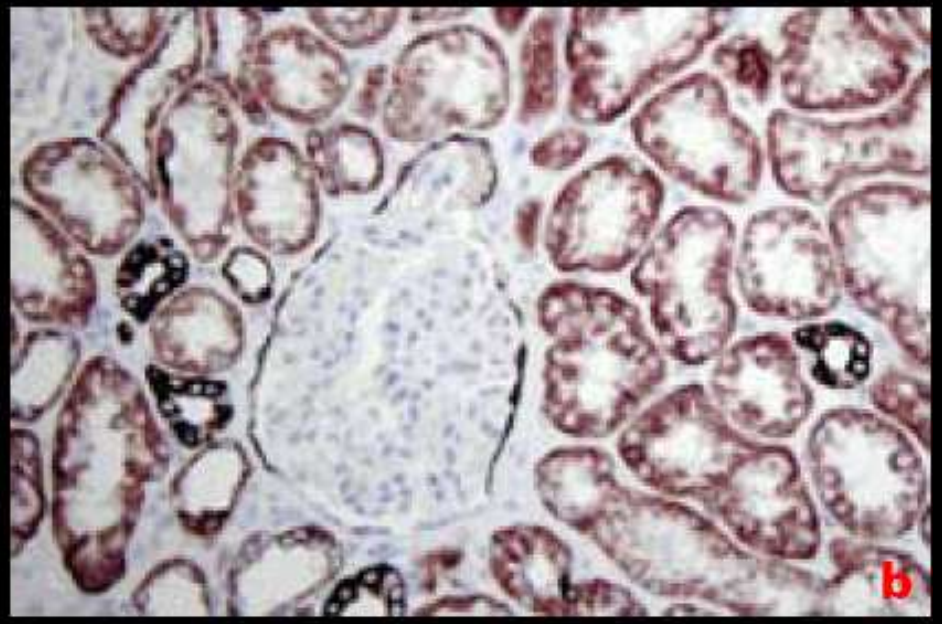
Sistema de detección

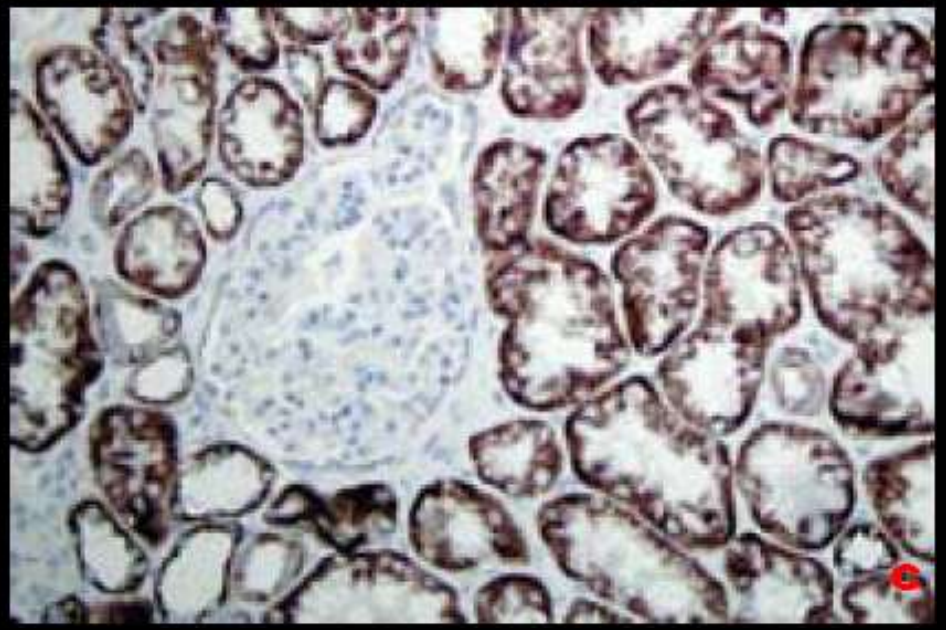
- **Problema 1:**
  - **¿qué sistema empleo?**
- **Problema 2:**
  - **Sistemas de detección basados en biotina**
- **Problema 3:**
  - **Peroxidasa endógena**
- **Problema 4:**
  - **Sensibilidad de los sistemas de detección**

Falsos negativos y falsos positivos

Biotina endógena







CK19 - Normal kidney
HIER: Tris/EDTA pH9

a. EnVision+
b. ABC
c. ABC negative control

Biotina endógena!!

BUSSOLATI G., GUGLIOTTA P., VOLANTE M., PACE M. & PAPOTTI M.

(1997) *Histopathology* 31, 400–407

Retrieved endogenous biotin: a novel marker and a potential pitfall in diagnostic immunohistochemistry

Aim: Antigen retrieval (AR) procedures are based on the effect of heating (by either microwave or pressure cooking treatments) on routinely fixed and paraffin embedded tissues. We observed that AR procedures restore the reactivity of endogenous biotin (EB) and report on the distribution of EB following AR in a series of routinely fixed and embedded tissues.

Methods and results: Following pressure cooking or microwave treatments, a simple streptavidin–peroxidase staining revealed retrieved endogenous biotin (REB) in normal tissues (such as liver, kidney and adrenal cortex), in oxyphylic cells and in some tumours, especially in carcinomas of the kidney and of the adrenal cortex. In formalin-fixed (but not in alcohol-fixed) tissue sections, the heating procedures caused an intense and finely granular cytoplasmic reaction, following a routine streptavidin-conjugated peroxidase treatment. The staining was prevented by blocking of EB by a sequential avidin–biotin treatment.

Conclusions: Retrieval of EB reactivity can cause pitfalls in diagnostic immunohistochemistry but, alternatively, it might also constitute a useful and novel diagnostic marker.

Tissue	Number of cases	Expression	Intensity/localization
Liver	5/5	+++	Intense, diffuse staining, negative in portal spaces
Kidney	7/7	+++	Intense in tubular epithelia, negative in the glomeruli
Pancreas	3/3	+	Focal acinar cells
Adrenal cortex	5/5	+++	Intense in the cortex, negative in the medulla
Adipose tissue	5/5	++	Weak
Brown fat	3/3	+++	Intense
Skeletal muscle	0/5		
Myocardium	0/3		
Stomach	1/4	+	In oxyphylic cells
Large bowel	0/5		
Ovary	2/2	+	Weak in the corpus luteum
Salpinx	2/2	+	Single cells
Endometrium	2/4	+	Weak in the proliferative phase
Testis	5/5	++	Intense in Leydig cells
Lung	2/2	+	Weak in peribronchial glands
Pituitary gland	3/3	+	Single cells
Salivary gland	3/3	+	Weak in ductal epithelium

Histopathology 1997, 30, 518–522

Immunohistochemical staining of hepatocellular carcinoma with monoclonal antibody against inhibin

W.G.McCLUGGAGE, P.MAXWELL, A.PATTERSON & J.M.SLOAN*
*Department of Pathology, Royal Group of Hospitals Trust, *The Queen's University of Belfast, Belfast, UK*

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UROLOGIC ONCOLOGY

Urologic Oncology: Seminars and Original Investigations 21 (2003) 191–196

Original article

Caveolin expression in adult renal tumors

Rafael Carrion[a], Beale E. Morgan[b], Myron Tannenbaum[a,c], Raoul Salup[a,b], Michael B. Morgan[a,b,c]

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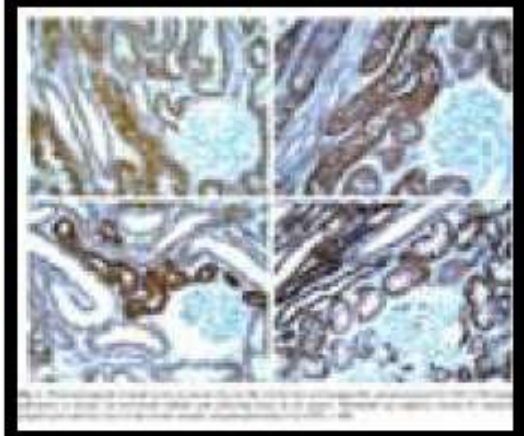
Pathology - Research and Practice 201 (2005) 801–808
PATHOLOGY RESEARCH AND PRACTICE
www.elsevier.de/prp

ORIGINAL ARTICLE

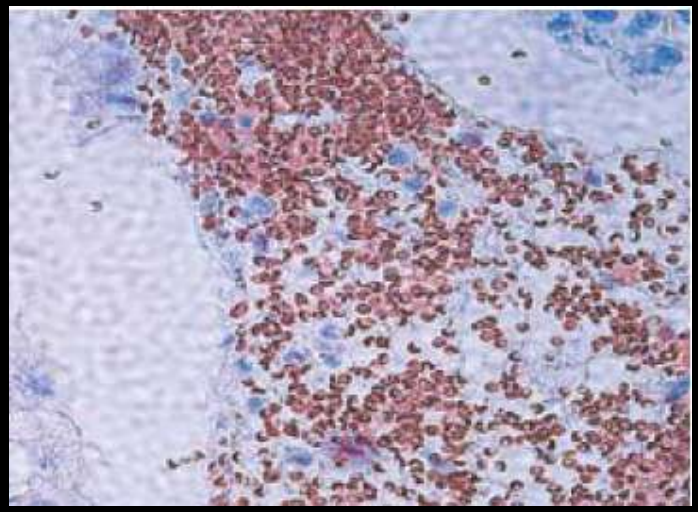
Expression of CD3 antigens in renal tubule epithelium and renal oncocytomas

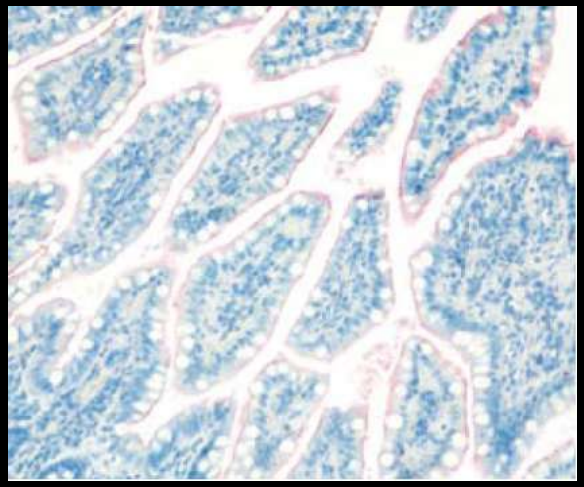
Joseph Alroy[a,b,*], Angelo A. Ucci[b], Gisso Azabdoaftan[b], Barbara F. Banner[c], John C. Cheville[d]

No deberían usarse en IHQ sistemas de detección basados en biotina



Enzyme: Peroxidase	Enzyme: Alkaline Phosphatase*
Red Blood Cells	Placenta Intestine — situated between cellular components of mucosa
Granulocytes	Proximal tubules of kidney
Eosinophils	Osteoblast in bone
Hepatocytes	Arterial & capillary endothelial cell surfaces
Muscle	Stromal reticulum cells
Kidney	Neutrophils
Monocytes	Follicle and mantle zones in most lymphoid tissue
*Alkaline Phosphatase is destroyed by routine fixation and paraffin-embedding procedures	





Biotin-free systems provide stronger immunohistochemical signal in oestrogen receptor evaluation of breast cancer

J Clin Pathol 2009;62:699–704.

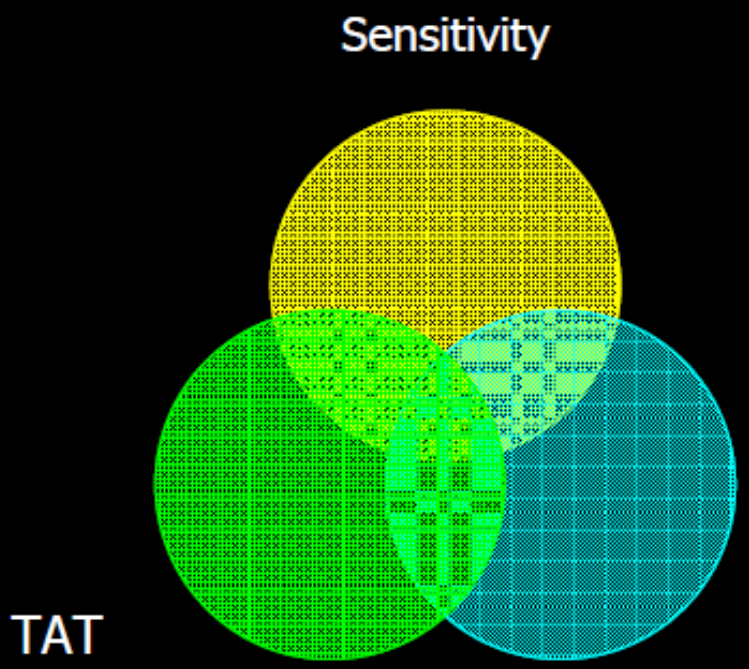
R M Rocha,[1,2,3] K Miller,[2] F Soares,[3] N Schenka,[4] J Vassallo,[3,4] H Gobbi[1]

Visualisation system	Type	Supplier*
EnVision+	Biotin-free polymer, first generation	Dako
Advance	Biotin-free polymer, second generation	Dako
NovoLink	Biotin-free polymer, second generation	Leica
SuperPicTure	Biotin-free polymer, second generation	Zymed
PicTure Max	Biotin-free polymer, second generation	Zymed
Super Sensitive non-biotin HRP	Biotin-free polymer, second generation 2nd generation	Biogenex
Mouse/Rabbit Polydetector HRP/DAB	Biotin-free polymer, second generation	Cell Marque
LSAB+	Streptavidin–biotin based system	Dako
EasyPath	Streptavidin–biotin based system	Signet
Super Sensitive	Streptavidin-biotin based system	Biogenex
Mouse/Rabbit Immunodetector HRP/DAB	Streptavidin–biotin based system	Cell Marque

Table 2 Comparison of 11 different visualisation systems grouped according to their staining intensities

Staining intensity level	Biotin-free polymer systems	Streptavidin–biotin systems
Strong	Advance (49.55 (16.69); $p = 0.0034$)	LSAB+ (45.3 (8.98; $p = 0.03$)
	NovoLink (43.8 (10.17); $p = 0.0061$)	
High intermediate	Super Sensitive non-biotin HRP (39.5 (9.34); $p = 0.01$)	Super Sensitive (37.7 (13.81); $p = 0.02$)
	PicTure Max (34.8 (13.37); $p = 0.01$)	
Low intermediate	SuperPicTure (31.6 (10.1); $p = 0.01$)	Mouse/Rabbit immunodetector (31.7 (7.1))
	Mouse/Rabbit Polydetector (31.2 (5.89); $p = 0.01$)	EasyPath (31.8 (11.67))
Weak	EnVision+ (29.5 (5.43))	–

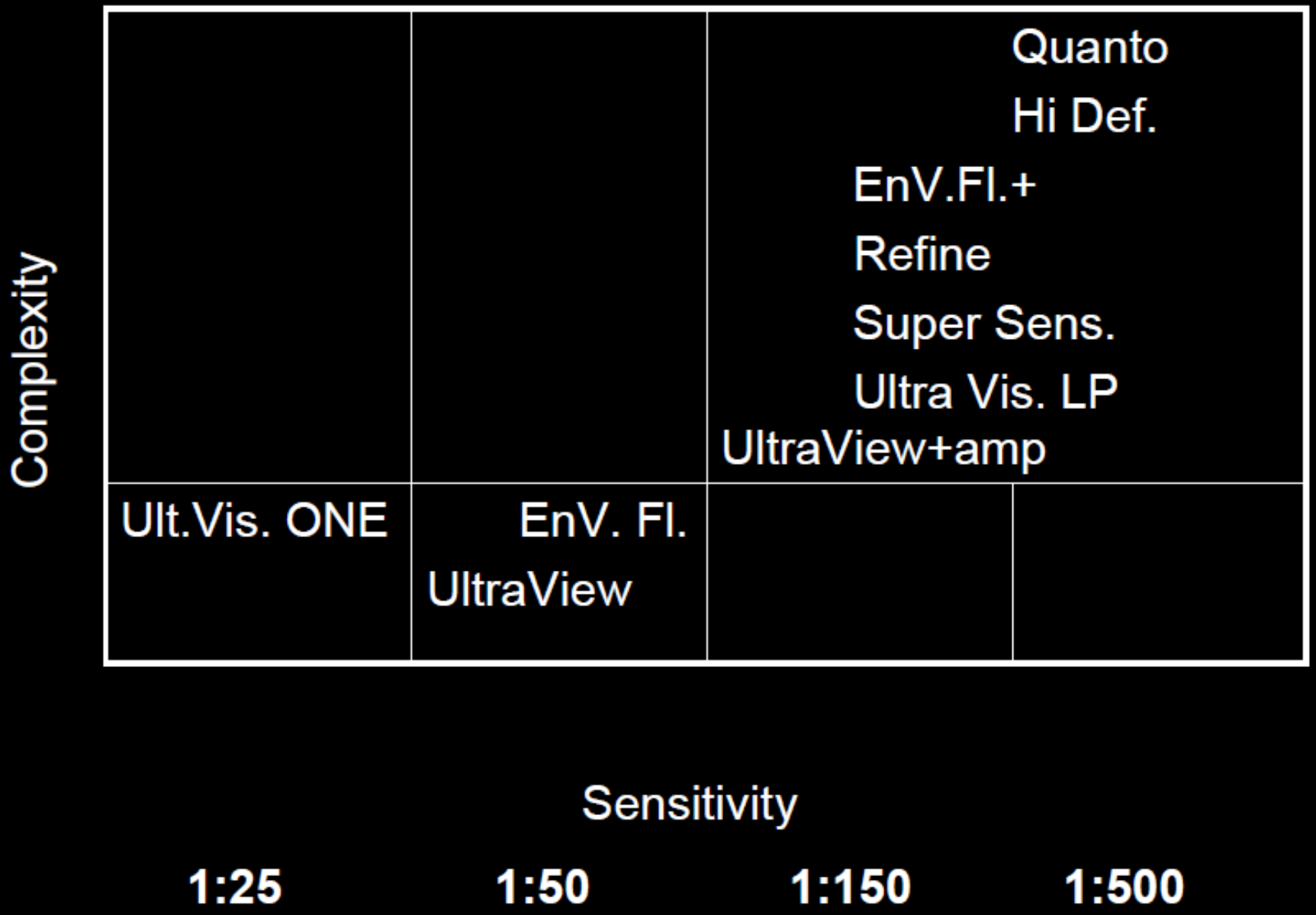
- EnVision
- UltraView
- UltraVision LP
- UltraVision One
- Refine
- ImPress
- Super Sensitive
- Super Picture
- Hi Def
- Quanto
- Advance
- MACH 3
- .....

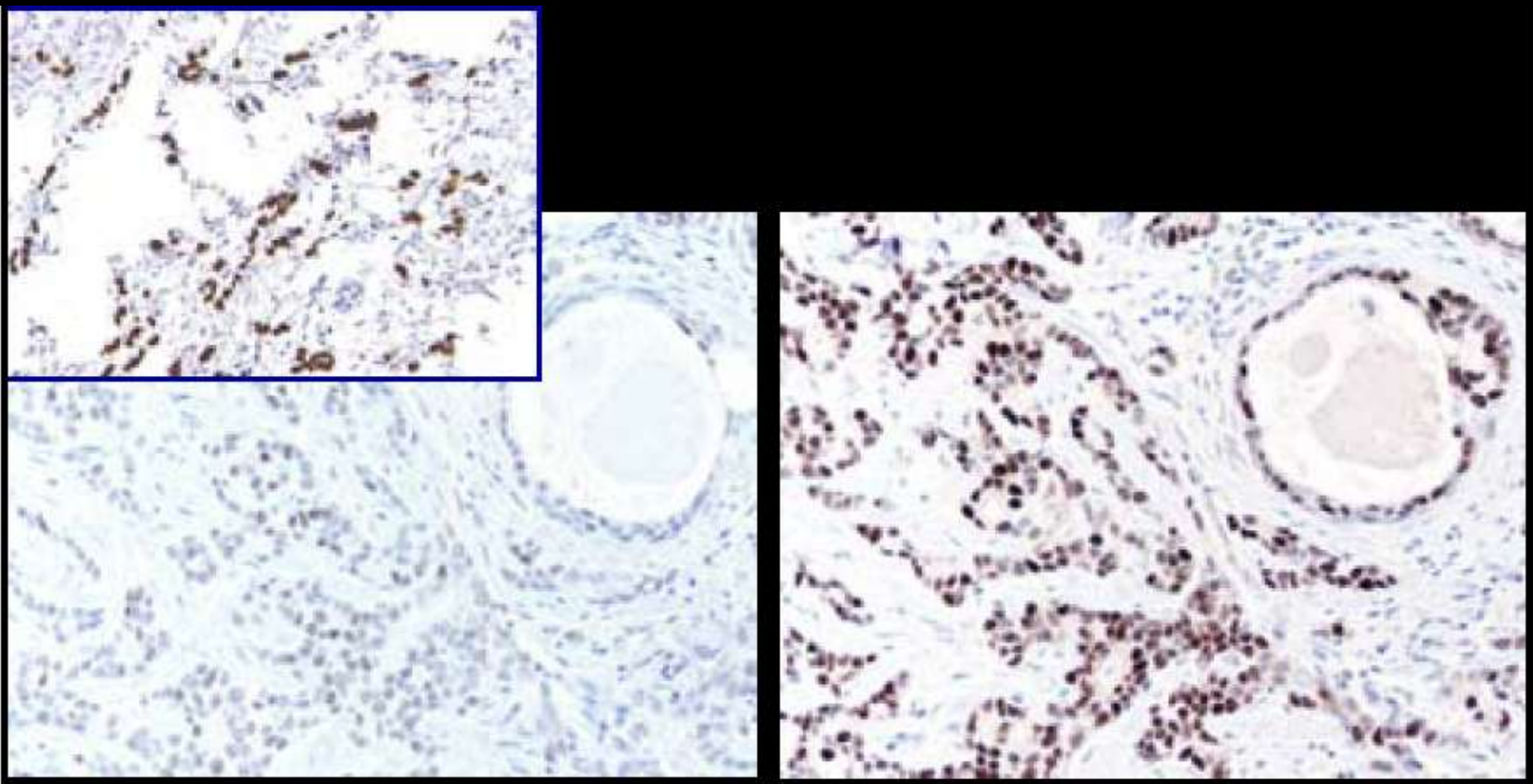


The choice of a polymer / multimer based system

Qué sistema de detección/amplificación emplear en cada plataforma?

Ventana XT/Ultra	→	UltraView + amplification
Dako Autostainer	→	EnVision Flex+
Leica BondMax	→	Refine
BioGenex i6000	→	Super-Sensitive polymer
Thermo Autostainer	→	UltraVision LP (or Quanto)
Manual	→	EnVision Flex+, Quanto, Hi Def, etc


Complexity
Quanto
Hi Def.
EnV.Fl.+
Refine
Super Sens.
Ultra Vis. LP
UltraView+amp
Ult.Vis. ONE
EnV. Fl.
UltraView
Sensitivity
1:25
1:50
1:150
1:500

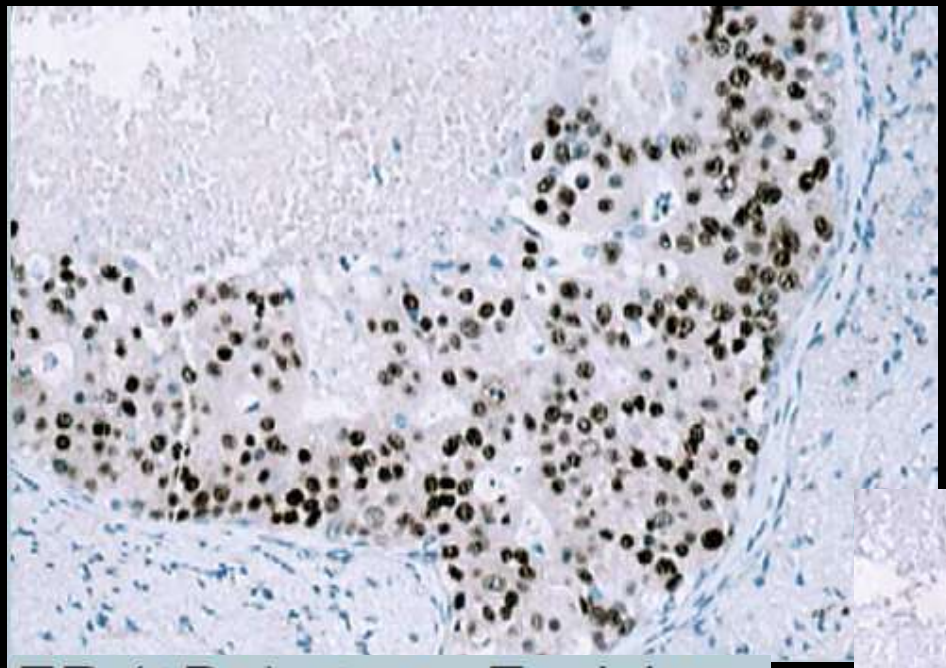


2-step polymer EnV. Flex

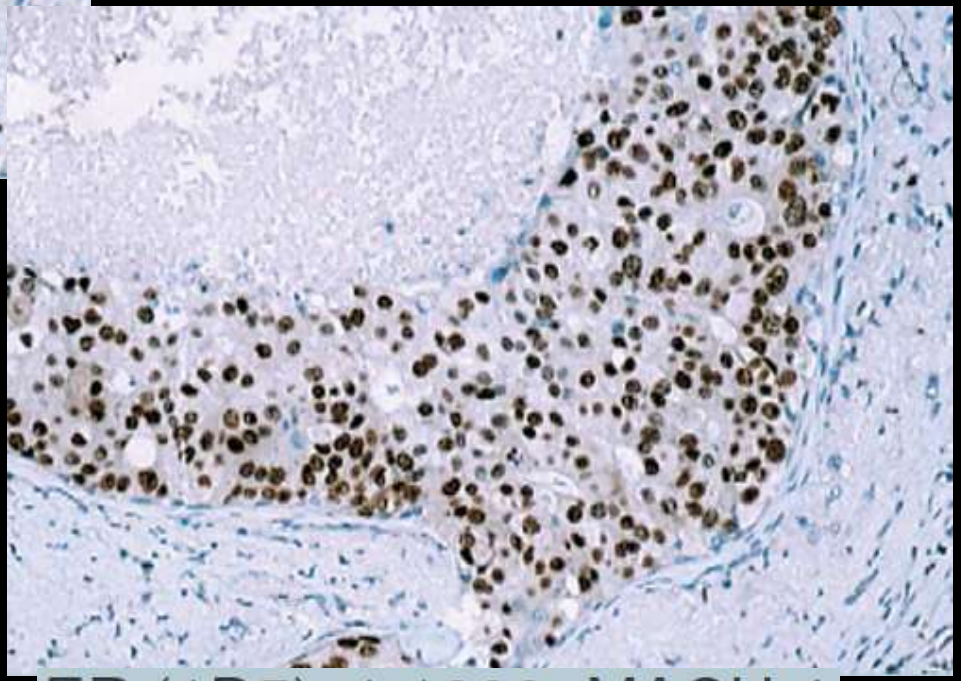
3-step polymer EnV. Flex+

ER 1D5 1:100

HIER Ci pH 6



ER (1D5), 1:25, Envision+

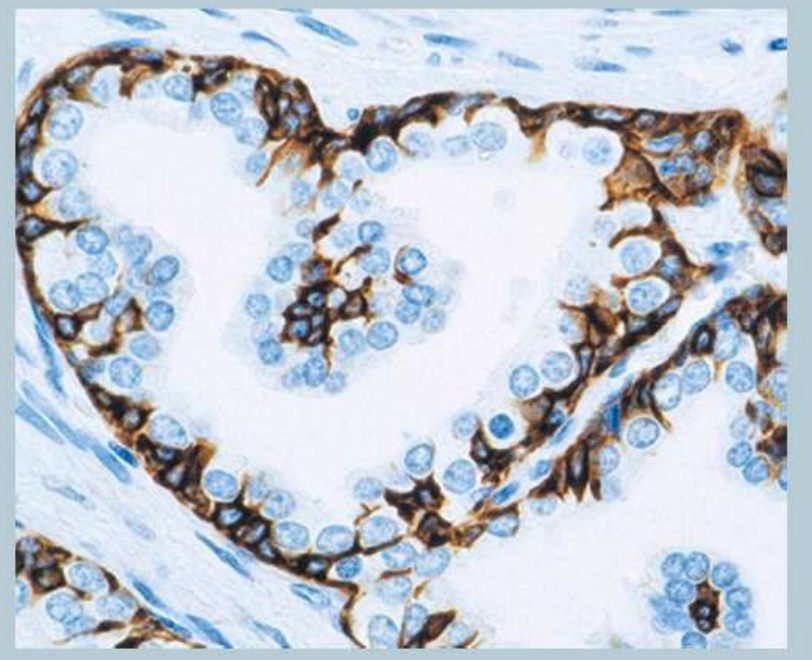


ER (1D5), 1:1000, MACH 4

Excellent immunohistochemistry begins with proper fixation. 24 hours in NBF is the golden standard.

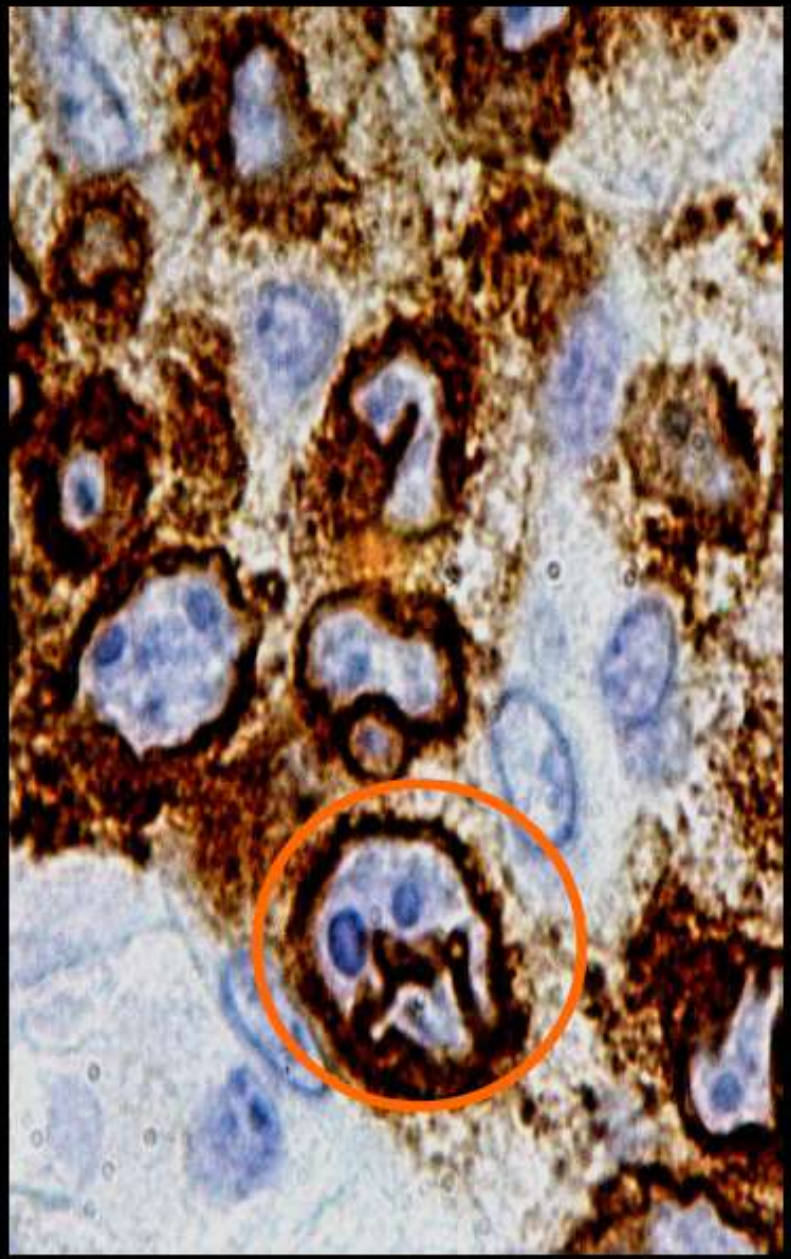
Good housekeeping practices such as tissue grossing, processing, deparaffinization and clean reagents is essential.

A proper antigen retrieval protocol is one of the most important factors for optimum IHC staining.



La optimización de un nuevo Ac para IHQ es un desafío, técnicamente complejo, pero NO MISION IMPOSIBLE!!

- Elección adecuada del Ac primario.
- Elección de un adecuado control positivo.
- Adecuada fijación y estandarización del procesamiento del tejido.
- Eficiente recuperación Ag.
- Sistema de detección/amplificación robusto, específico y sensible.




GRACIAS!!