NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 19th–21st 2018

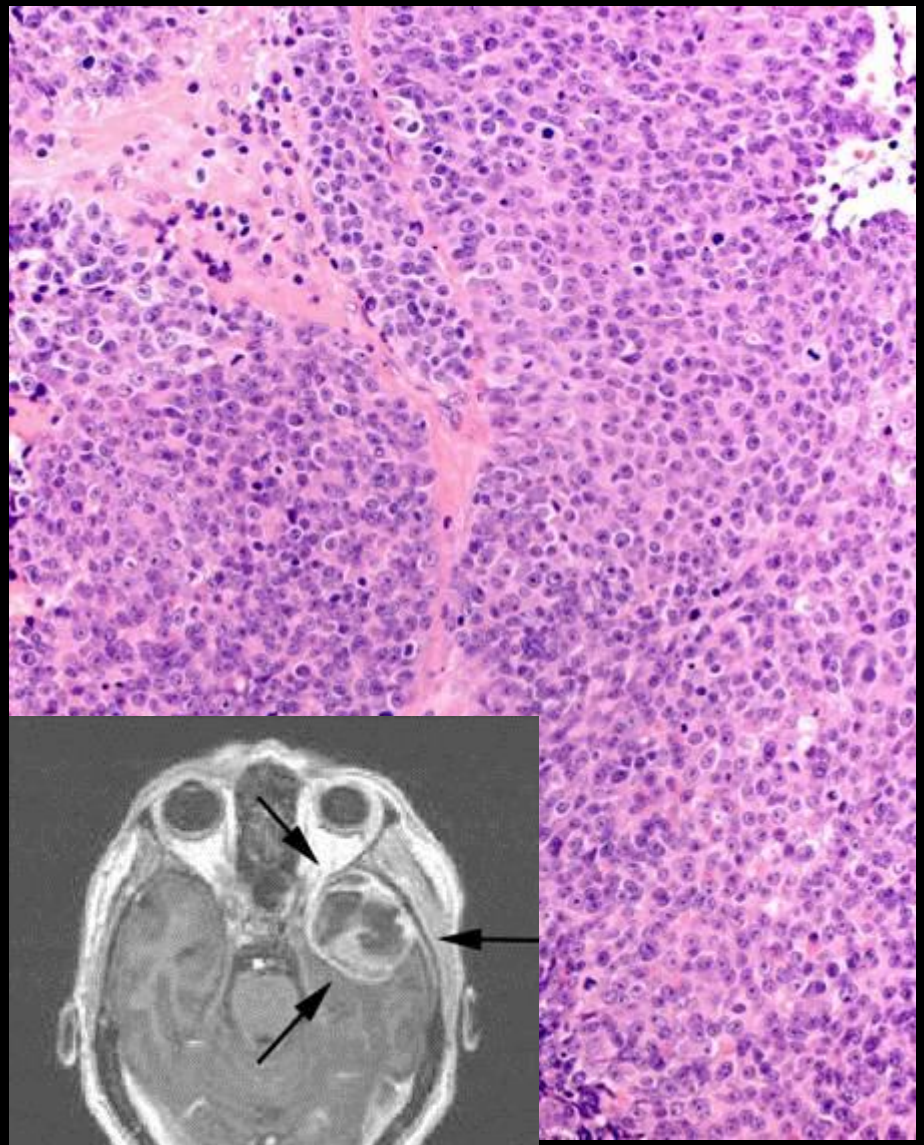
The unknown primary tumour: IHC classification – part I, the primary panel

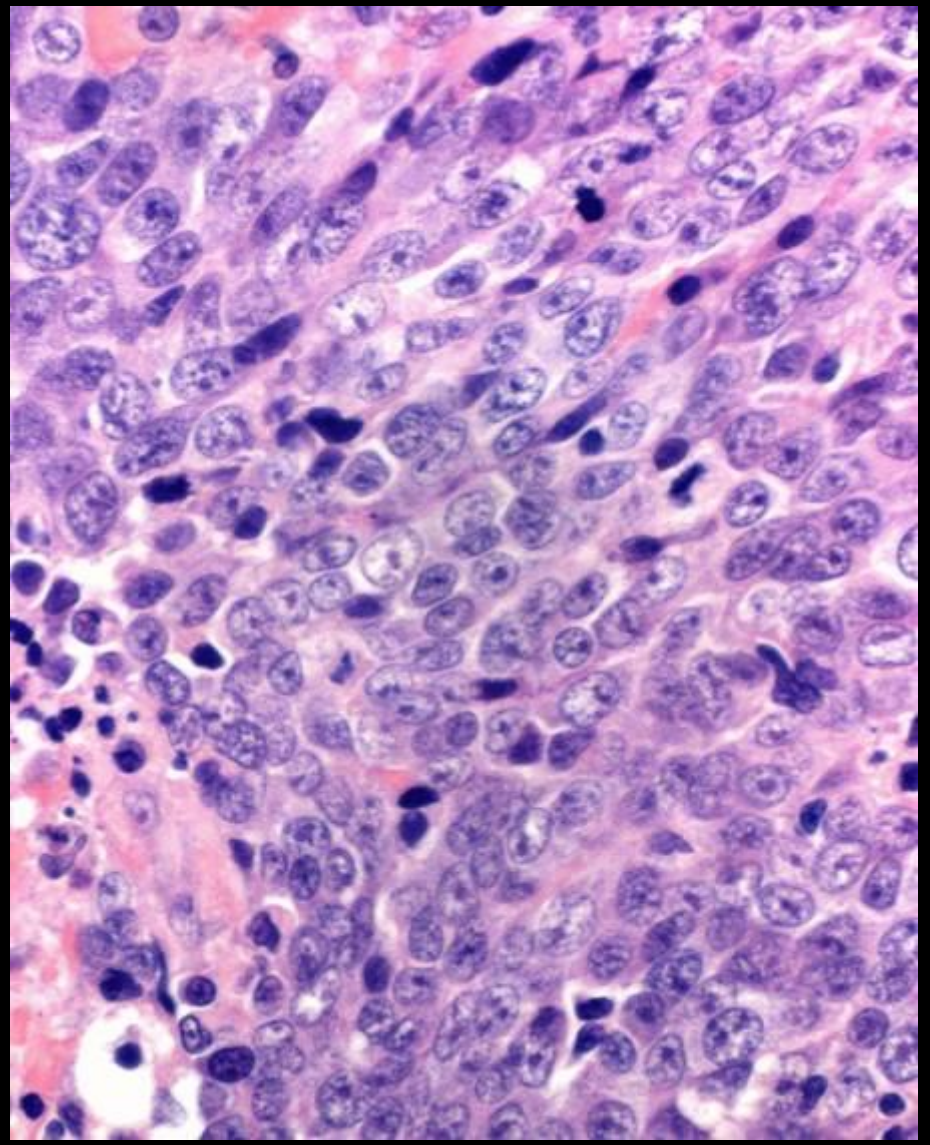
- Antibody selection, protocol optimization, controls and EQA

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark

NORTH DENMARK REGION

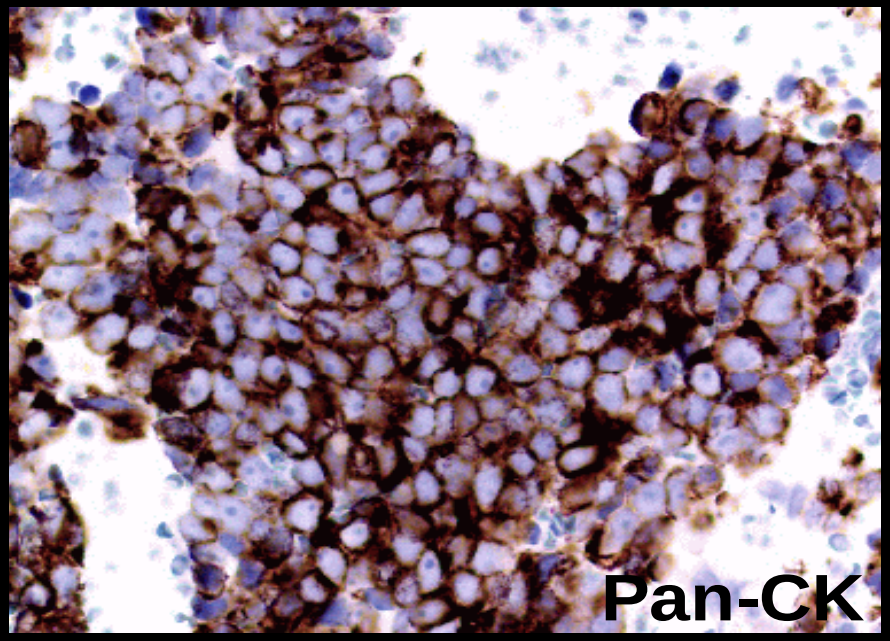
Tumours of unknown origin: Histology

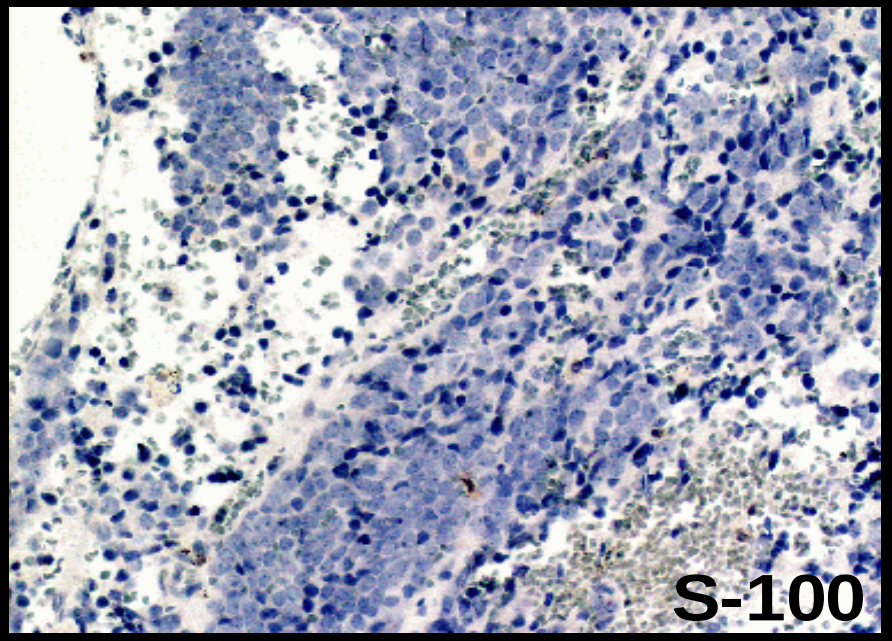


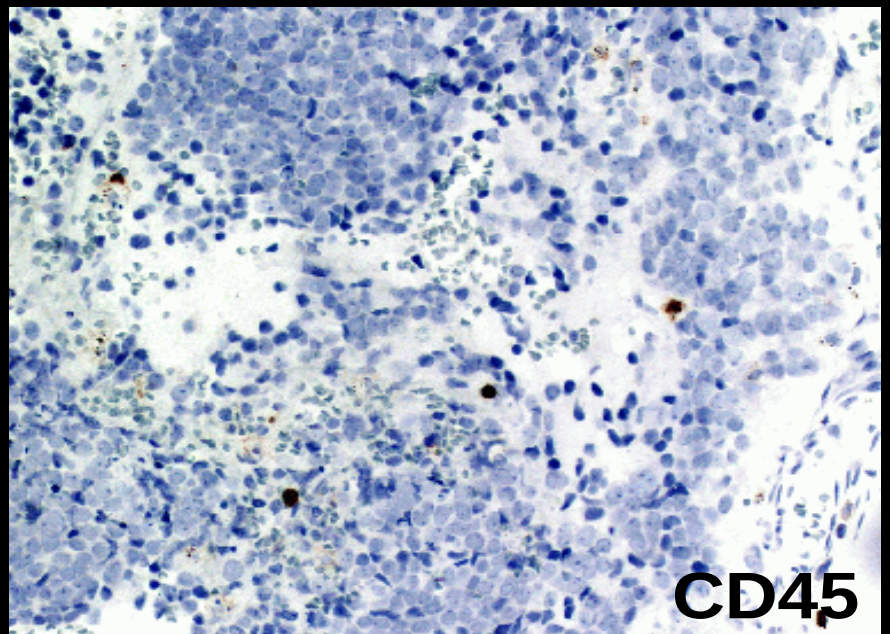


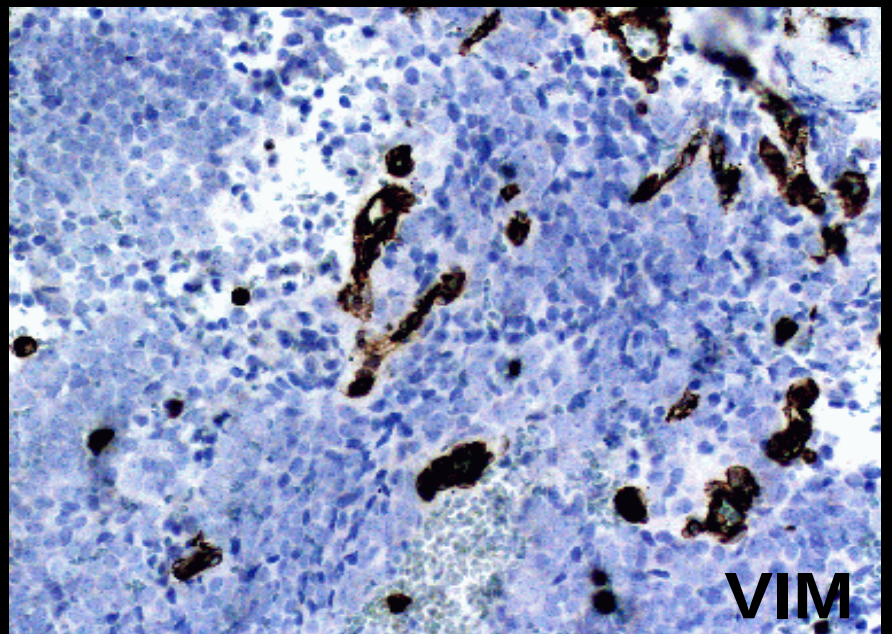
Brain tumour - biopsy

Tumours of unknown origin: Immunohistochemistry









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Dennis M. Frisman, M.D.
Associate Medical Editor, Amirsys Inc.
& Founder, ImmunoQuery

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Elizabeth Hammond, M.D.
Executive Editor for Pathology, Amirsys Inc.

Experts

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Joel Greenson, M.D.	Gastrointestinal
Mahul Amin, M.D.	Genitourinary
Bruce Wenig, M.D.	Head & Neck
Lester Thompson, M.D.	Head & Neck, Endocrine
Jeff Mederios, M.D.	Lymphoma
Angelica Putnam, M.D.	Pediatric
Jeremy Wallentine, M.D.	Pediatric
Cyril Fisher, M.D. D.Sc.	Soft Tissue
César Moran, M.D.	Thoracic
Elizabeth Hammond, M.D.	Undifferentiated Neoplasms, Gynecology

Coming Soon:

Kathy Foucar, M.D.	Blood & Bone Marrow
Susan Lester, M.D., Ph.D.	Breast
Peter Burger, M.D.	Neuropathology

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Build Dx Panel | Build Ab Panel | Analyze Results

Enter a search phrase to select a Diagnosis Group (and repeat for a 2 or 3 Dx Group search), set Sensitivity and Minimum Refs, then click Build Panel button.

meso | View All

- **Adenoca CK07 positive CK20 Negative**
  Mesothelioma, NOS
- **Mesothelioma, All**
  Mesothelioma, Biphasic; Proliferation, Mesothelial, NOS; Mesothelioma, Sarcomatoid; Mesothelioma, NOS; Mesothelioma, Epithelioid
- **Mesothelioma, benign proliferations**
  Proliferation, Mesothelial, NOS
- **Mesothelioma, lymphohistiocytoid**

Selected Dxs:

none selected

Set Sensitivity: 1 2 3

Set Minimum Refs: All > 1 > 5

Build Panel

Open Cases

Start date	Case Description

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Diagnosis Group and Antibody Education

Enter a Diagnosis Group or Antibody search phrase and select the desired item.

Learn About a Diagnosis Group:

Learn About an Antibody:

News:

PathIQ® ImmunoQuery® New Smart Search Accelerates Finding Diagnoses and Antibodies
01/08/2009
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Amirsys Streamlines User Navigation in ImmunoQuery Diagnosis Panels
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PathIQ® ImmunoQuery® Meta-Analysis Augmented with Expert-Selected References
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New Expert Diagnostic Panels for PathIQ® ImmunoQuery® v2.0
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Ask an Expert | Meta-analysis

Dx Panel for Mesoth...

Antibody				
G-GCS-H				
EPO				
CK 19				
CK 18				
C-MET				
AMAD-2				
AE1				
PKK1				
CAM 5.2				
35BH11				
H-CALDESMON				
AE1_AE3				
KERATIN-PAN				
CK 05				
CD44H				
MESOTHELIN				
CA 15-3	89%	19	76 - 100	1
PODOPLANIN	85%	503	[illegible]	6
CALRETININ	85%	1,345	83 - 87	31
CK 05_06	83%	646	80 - 86	[illegible]
34BE12	82%	82	73 - 90	3
N-CADHERIN	81%	242	76 - 86	6

References For CALRETININ: Close X

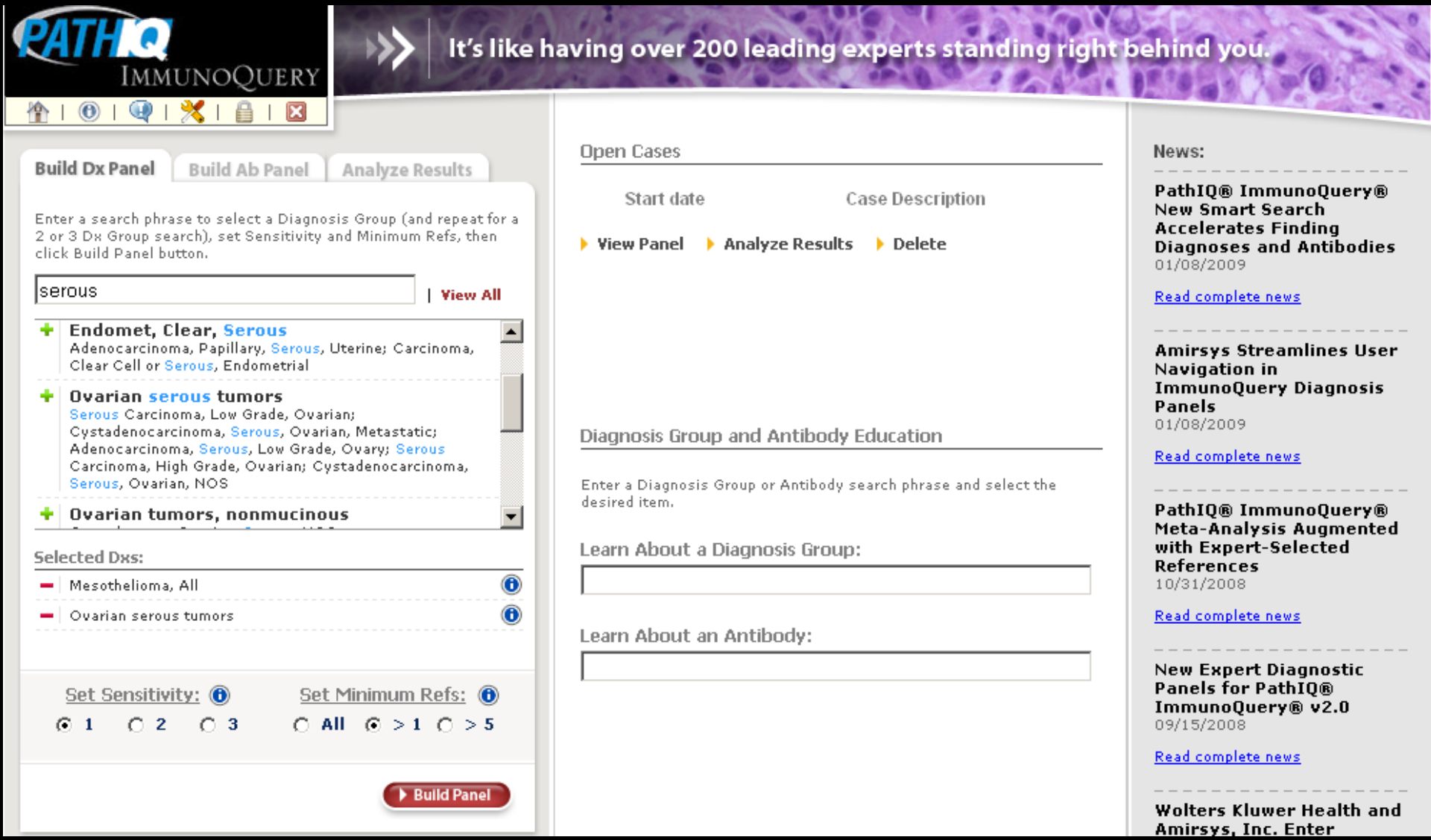
Articles Sorted by relevance: 31

Year Published: 2008
Author(s): Lyons-Boudreaux V, Mody DR, Zhai J, Coffey D
Article: Cytologic malignancy versus benignancy: how useful are the "newer" markers in body fluid cytology?
Publication: ARCH PATHOL LAB MED. 132:23-28

Year Published: 2006
Author(s): BARNETSON,R.J. , BURNETT,R.A. , DOWNIE,I. , HARPER,C.M. , ROBERTS,F.
Article: IMMUNOHSTIOCHEMICAL ANALYSIS OF PERITONEAL MESOTHELIOMA AND PRIMARY AND SECONDARY SEROUS CARCINOMA OF THE PERITONEUM. ANTIBODIES TO ESTROGEN AND PROGESTERONE RECEPTORS ARE USEFUL.
Publication: AM J CLIN PATHOL. 125 :67-76

Year Published: 2006
Author(s): WINSTANLEY,A.M. , LANDON,G. , BERNEY,D. , MINHAS,S. , FISHER,C. , PARKINSON,M.C.
Article: THE IMMUNOHISTOCHEMICAL PROFILE OF MALIGNANT MESOTHELIOMAS OF THE TUNICA VAGINALIS. A STUDY OF 20 CASES.
Publication: AM J SURG PATHOL. 30 :1-6

Year Published: 2003
Author(s): LUGLI,A. , FORSTER,Y. , HAAS,P. , NOCITO,A. , BUCHER,C. , BISSIG,H. , MIRLACHER,M. , STORZ,M. , MIHATSCH,M.J. , SAUTER,G.
Article: CALRETININ EXPRESSION IN HUMAN NORMAL AND NEOPLASTIC TISSUES: A TISSUE MICROARRAY ANALYSIS ON 5233 TISSUE SAMPLES.
Publication: HUM PATHOL. 34 :994-1000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Build Dx Panel
Build Ab Panel
Analyze Results
Enter a search phrase to select a Diagnosis Group (and repeat for a 2 or 3 Dx Group search), set Sensitivity and Minimum Refs, then click Build Panel button.
serous
View All
Endomet, Clear, Serous
Adenocarcinoma, Papillary, Serous, Uterine; Carcinoma, Clear Cell or Serous, Endometrial
Ovarian serous tumors
Serous Carcinoma, Low Grade, Ovarian; Cystadenocarcinoma, Serous, Ovarian, Metastatic; Adenocarcinoma, Serous, Low Grade, Ovary; Serous Carcinoma, High Grade, Ovarian; Cystadenocarcinoma, Serous, Ovarian, NOS
Ovarian tumors, nonmucinous
Selected Dxs:
Mesothelioma, All
Ovarian serous tumors
Set Sensitivity:
1 2 3
Set Minimum Refs:
All > 1 > 5
Build Panel
Open Cases
Start date
Case Description
View Panel
Analyze Results
Delete
Diagnosis Group and Antibody Education
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Learn About a Diagnosis Group:
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Amirsys Streamlines User Navigation in ImmunoQuery Diagnosis Panels
01/08/2009
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PathIQ® ImmunoQuery® Meta-Analysis Augmented with Expert-Selected References
10/31/2008
Read complete news
New Expert Diagnostic Panels for PathIQ® ImmunoQuery® v2.0
09/15/2008
Read complete news
Wolters Kluwer Health and Amirsys, Inc. Enter

	Mesothelioma, All			Ovarian Serous Tumors		
	Positive	Cases	vs2	Positive	Cases	vs1
ERP NUCLEAR	0%	71		95%	63	
H-CALDESMON	97%	70		5%	40	
MOC-31	8%	404		98%	62	
BER-EP4	10%	1,421		97%	99	
S-100 CYTOPLASMIC/NUCLEAR	5%	208		73%	52	
TAG-72	5%	1,545		73%	85	
LEWIS-Y	8%	266		73%	45	
E-CADHERIN MEMBRANE/CYTOPLASMIC	35%	265		100%	20	
CALRETININ Nucleus/Cytoplasm	85%	1,345		22%	232	
CA 19-9 CYTOPLASMIC	1%	152		64%	85	
PRP NUCLEAR	0%	22		62%	63	
THROMBOMOD CYTOPLASMIC	65%	1,039		5%	108	
PODOPLANIN MEMBRANE/CYTOPLASMIC	85%	503		28%	111	

Build Dx Panel | **Build Ab Panel** | Analyze Results

Enter a search phrase to select an Antibody (and repeat for a 2 or 3 Antibody search), then click Build Panel button.

kera | View All

- **KERATIN-HMW**
  KERATIN-HMW
- **KERATIN-LMW**
  KERATIN-LMW
- **KERATIN-PAN**
  KERATIN-PAN

Selected Abs:

- VIMENTIN
- KERATIN-PAN

Build Panel

PATHIQ IMMUNOQUERY

Discrete Diagnosis (15)	VIMENTIN			KERATIN-PAN			# of Refs
	Pos	Positive	Cases	Pos	Positive	Cases	
Ewing's Sarcoma, Atypical		44%	9		0%	5	2
Carcinoma, Small Cell, Breast		44%	9		0%	2 ?	2
Medulloblastoma, NOS		42%	57		0%	53	2
Pheochromocytoma, NOS		40%	63		16%	116	4
Stromal Sarcoma, Low Grade		38%	8 ?		0%	6	2
Askin Tumor		37%	19		0%	14	2
Seminoma, Testes		30%	96		21%	170	6
Clear Cell Tumor Of Lung		29%	17		0%	32	5
Alveolar Soft Part Sarcoma		25%	4 ?		0%	3	4
Leiomyoma, Epithelioid		20%	5		15%	13	2
Neuroblastoma, Olfactory		8%	13		8%	38	4
Thymic Carcinoma, Spindle Cell		0%	10		0%	10 ?	1
Solitary Fibrous Tumor, Malignant		0%	1 ?		0%	1	1
Seminoma, Spermatocytic		0%	7		0%	3	2
Sarcoma, Perivascular Epithelioid Cell		0%	4		0%	4	1

IHC classification of the Unknown Primary Tumour

Pathologist

- knowledge, acceptance, skill

Tumour material

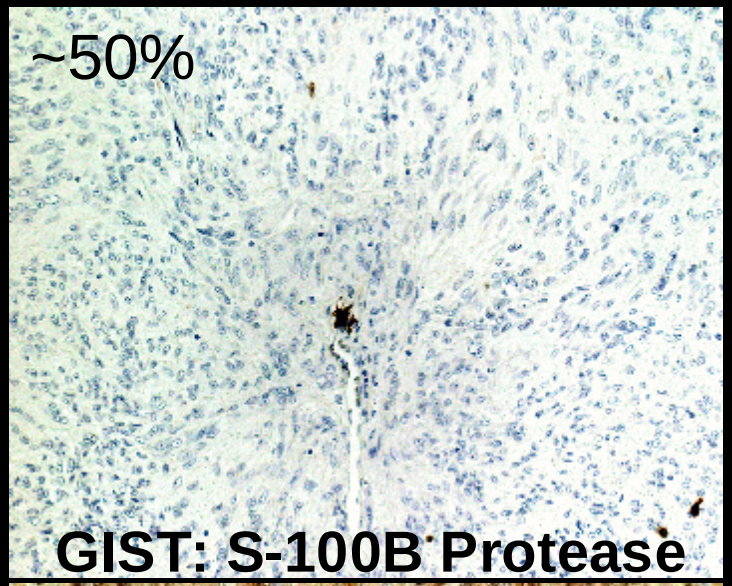
- diagnostic markers

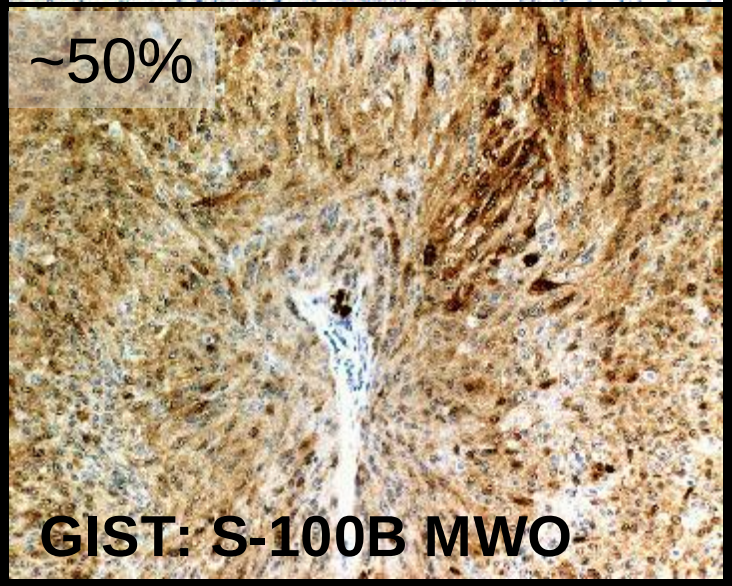
Antibodies available

- applic. in diagnostic algorithms

Methods

- protocol:
  sensitivity, specificity, reliability
- interpretation:
  cut-off level for positivity
  clinical relevance





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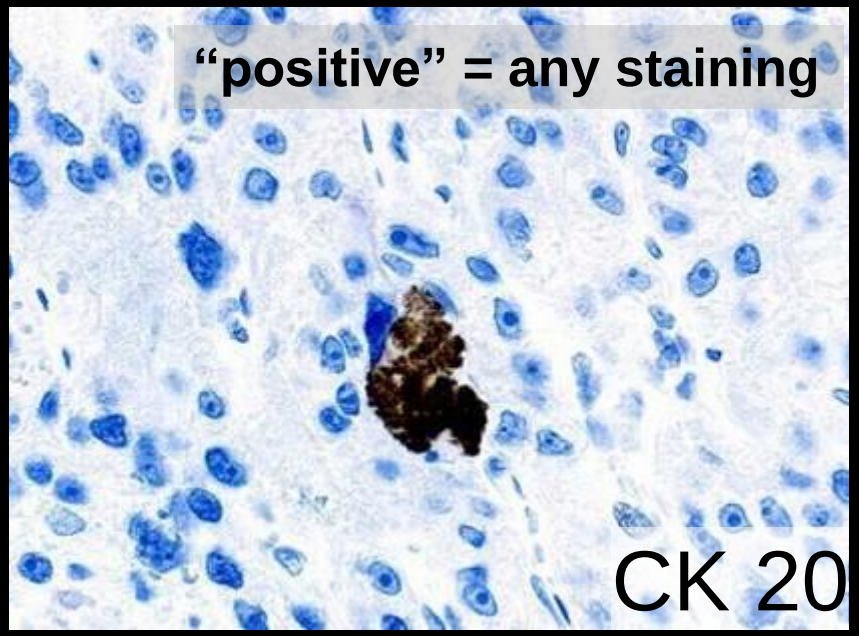
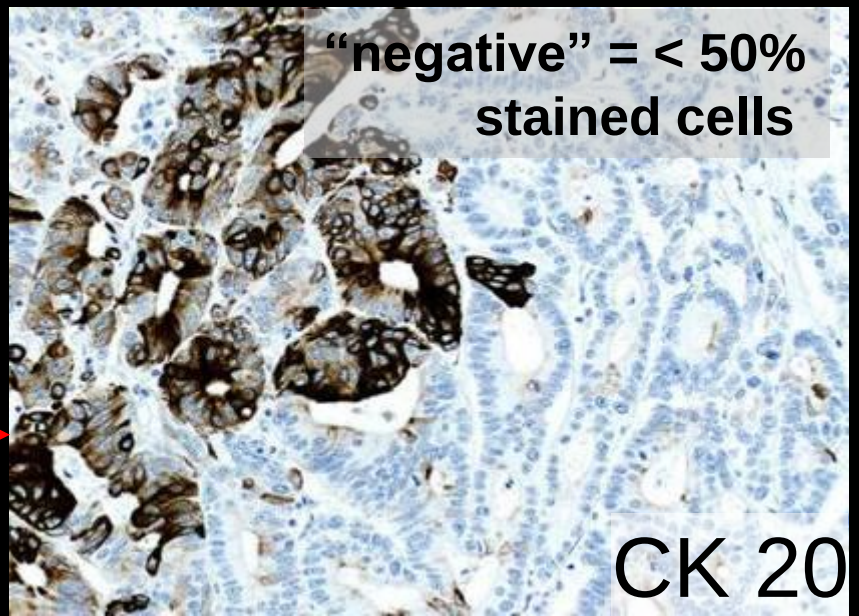


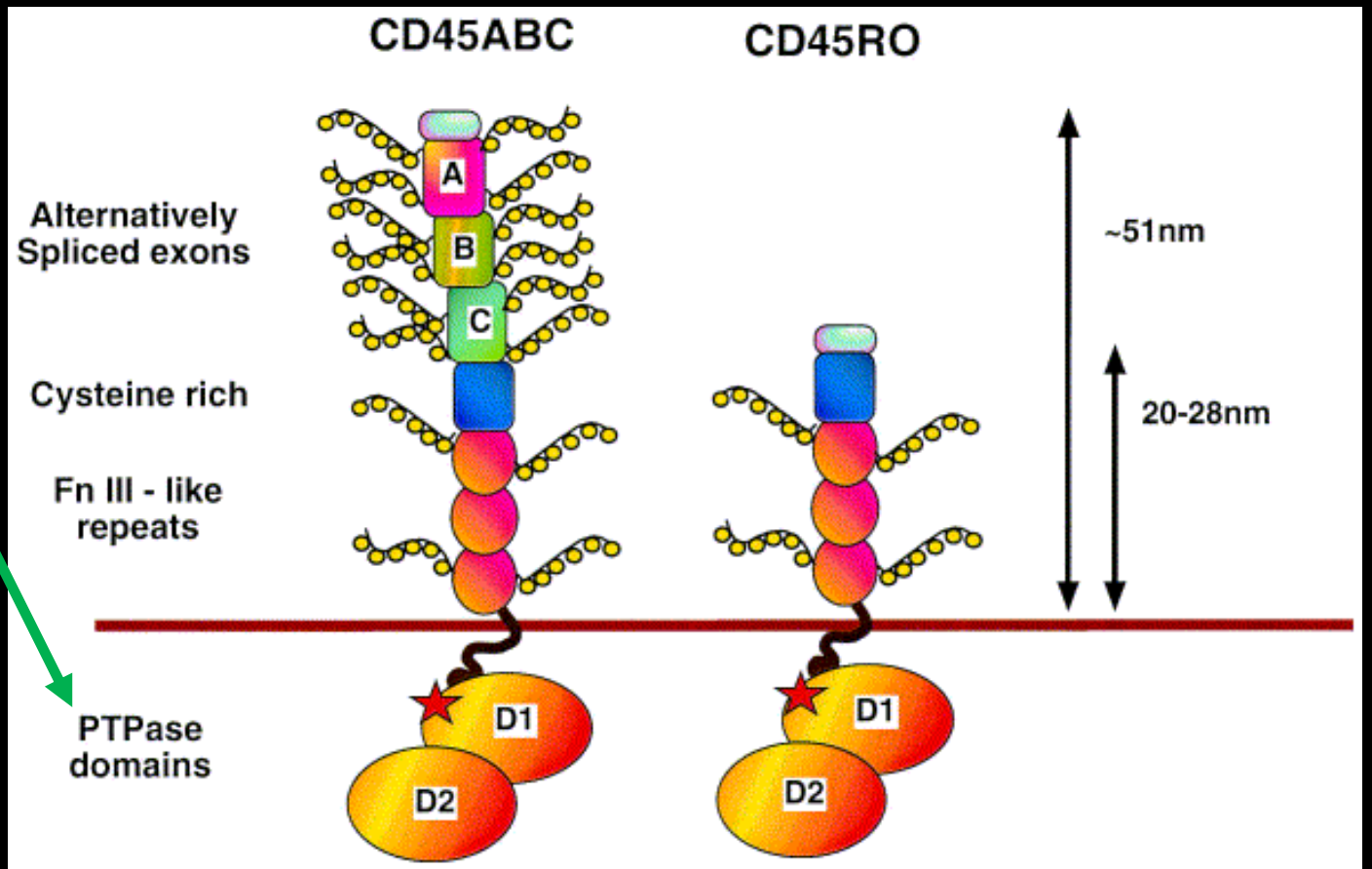


Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

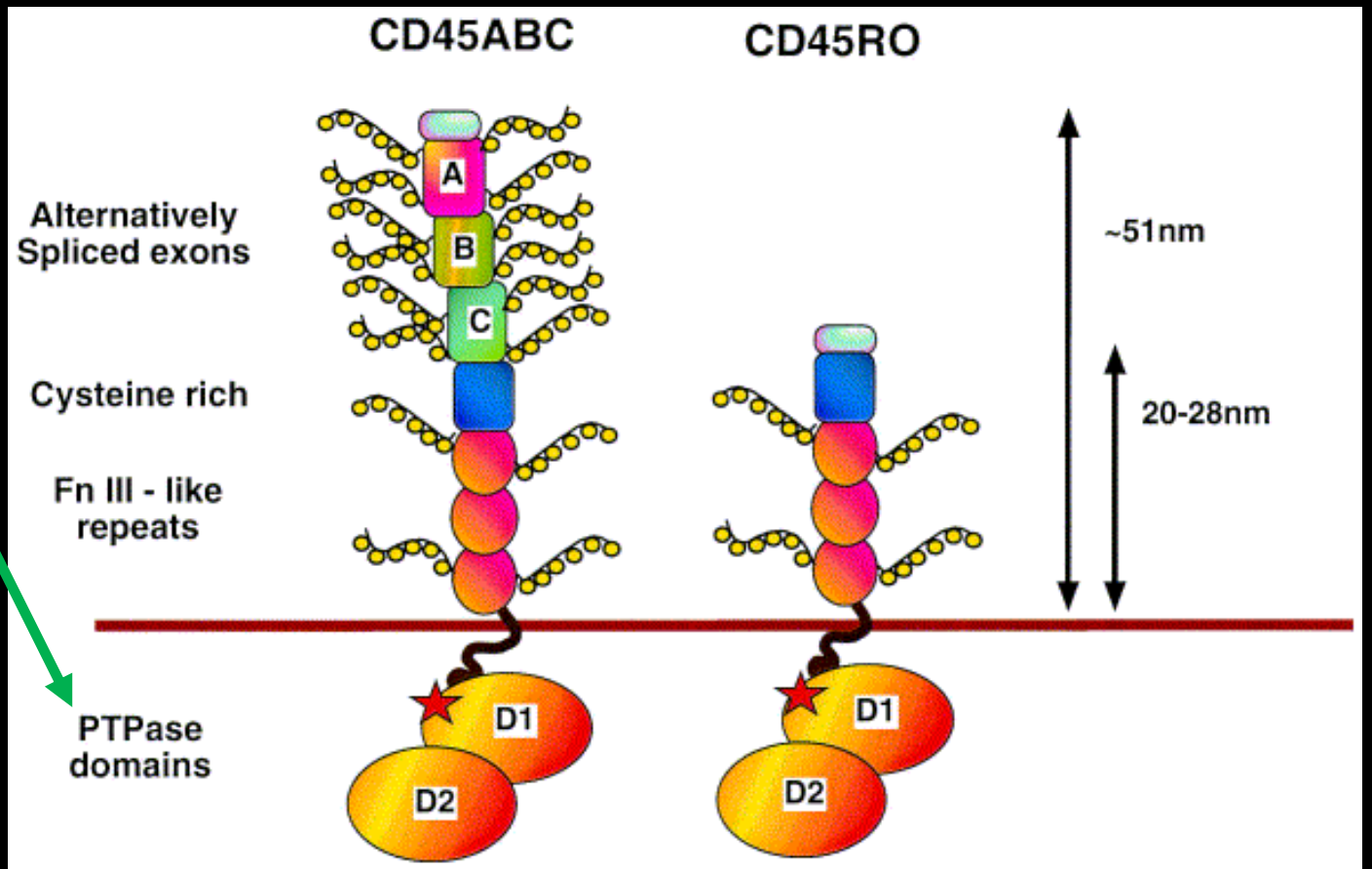
CD45 - Leucocyte common antigen (LCA)

- Transmembrane protein tyrosin phosphatase essential for **haematopoietic signal transduction and cell activation**
- Membrane associated component: 5 isotypes
- Intracellular component: one common type



CD45 - Leucocyte common antigen (LCA)

- Large majority of haematolymphoid cells and neoplasms
- Lost in *maturing erythocytes, megakaryocytes and plasmacells*
- ”Never” found in non-haematolymphoid cells and neoplasms



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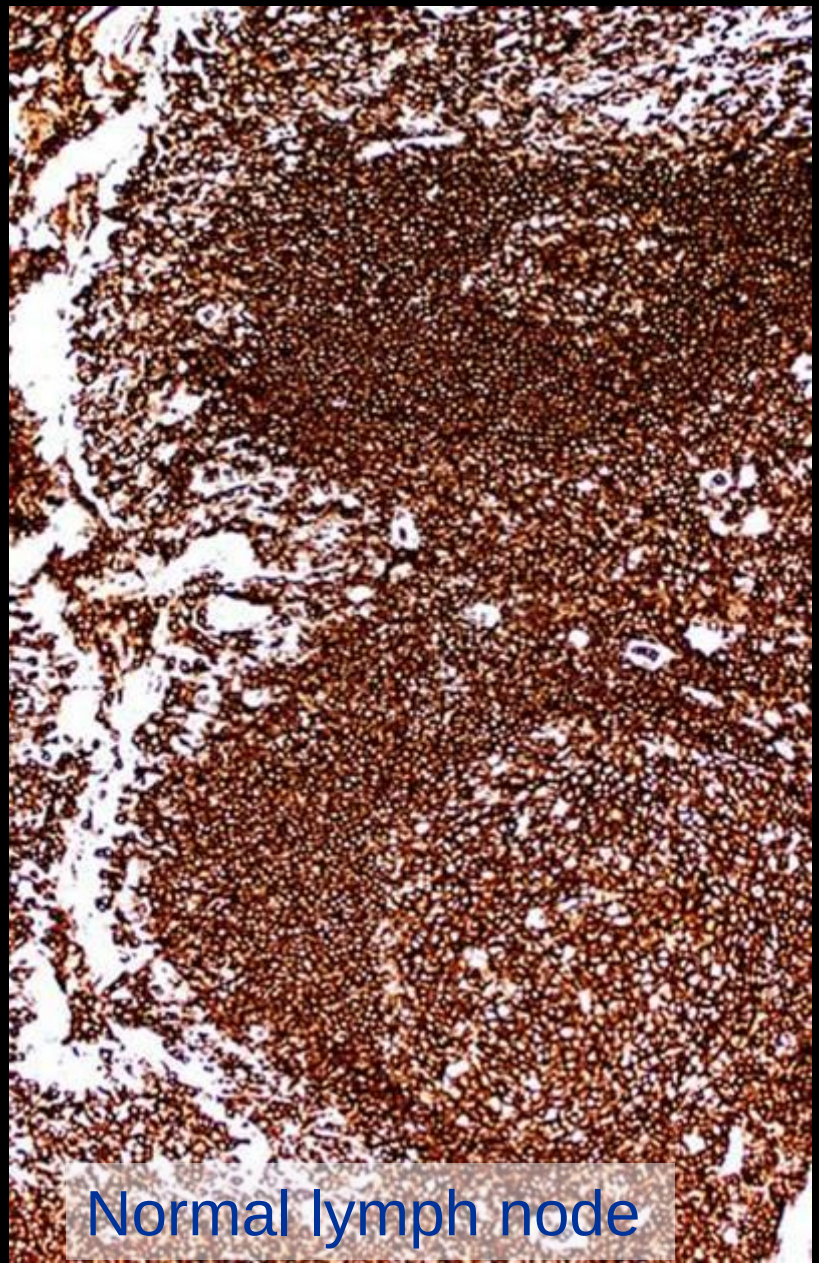
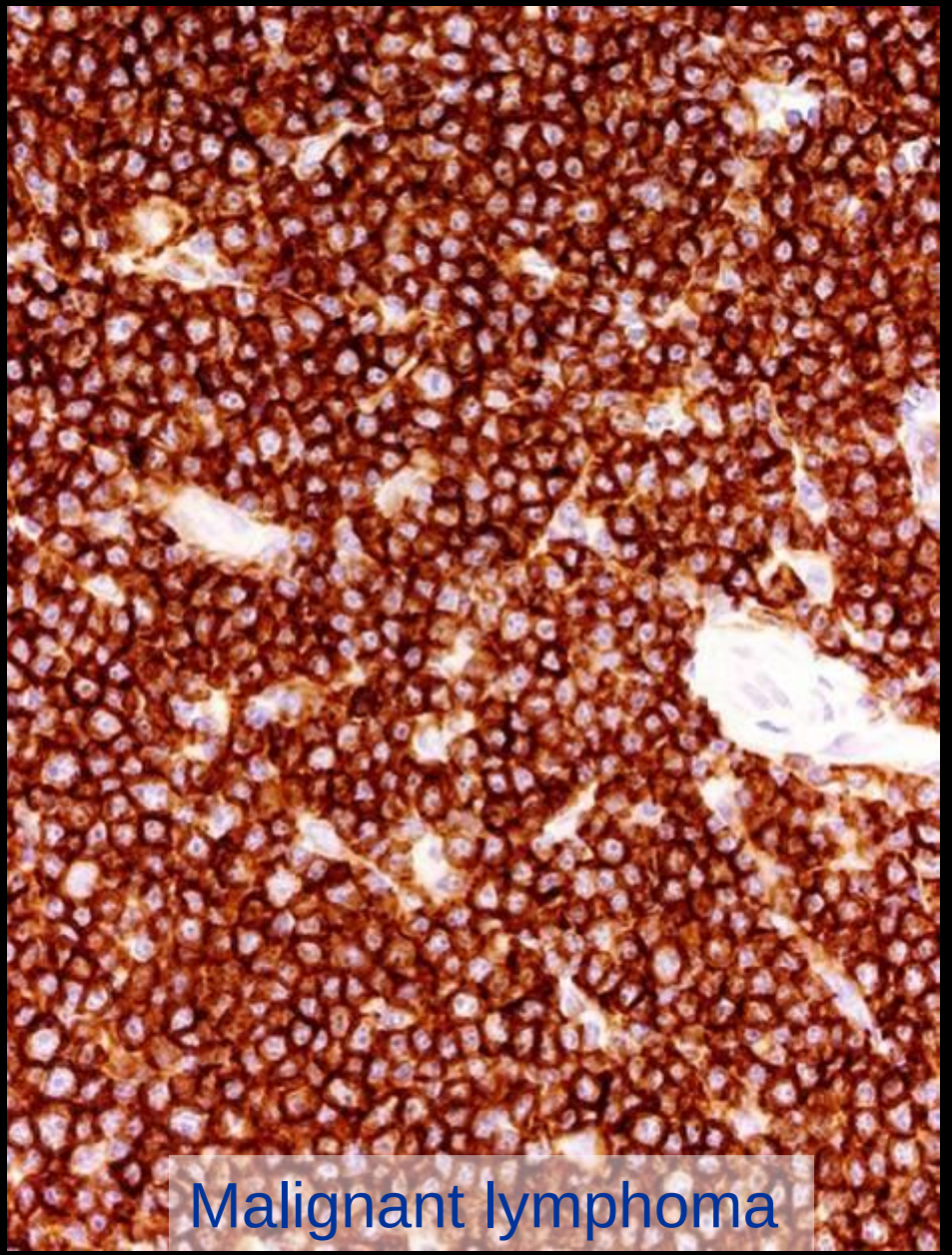
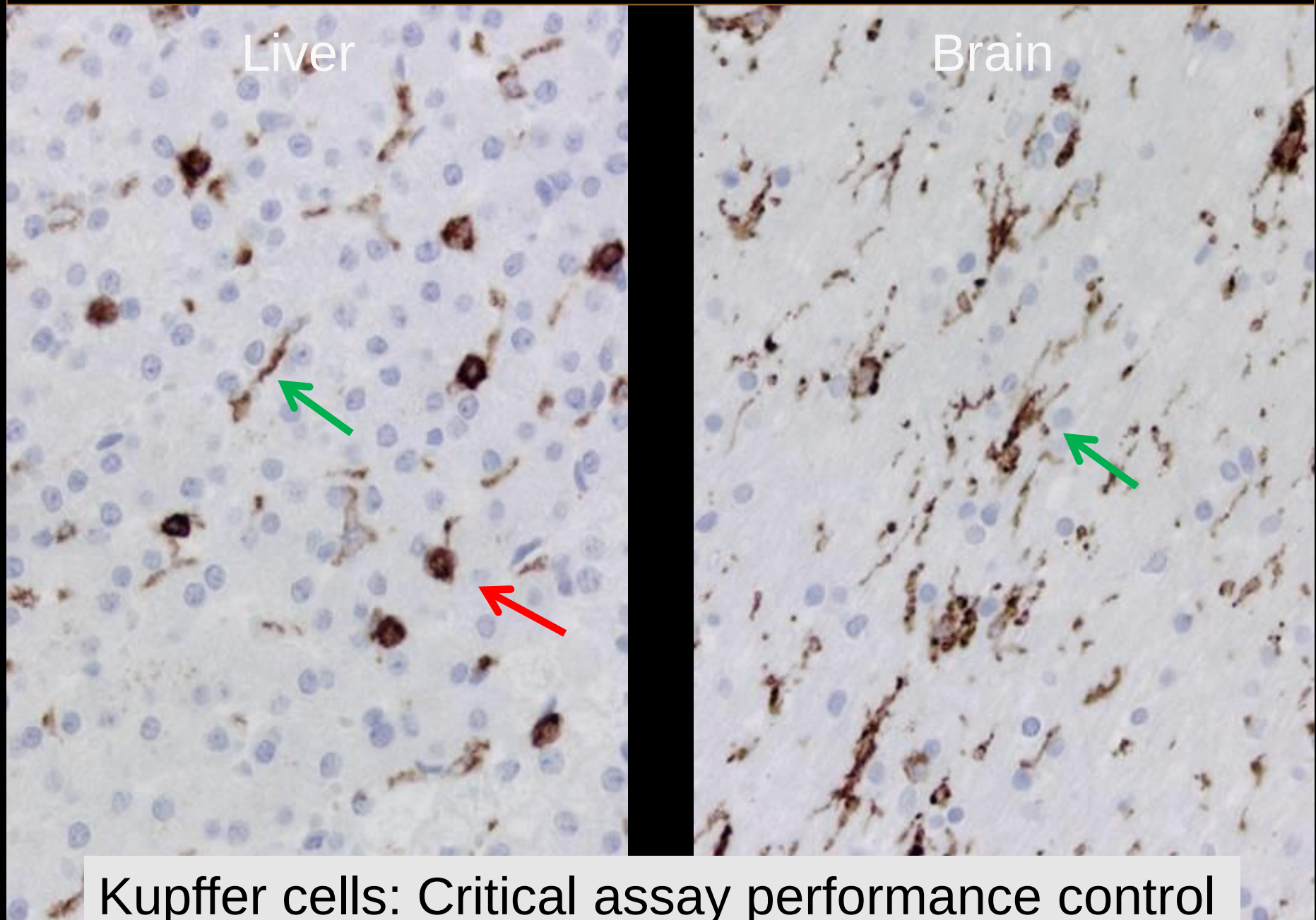


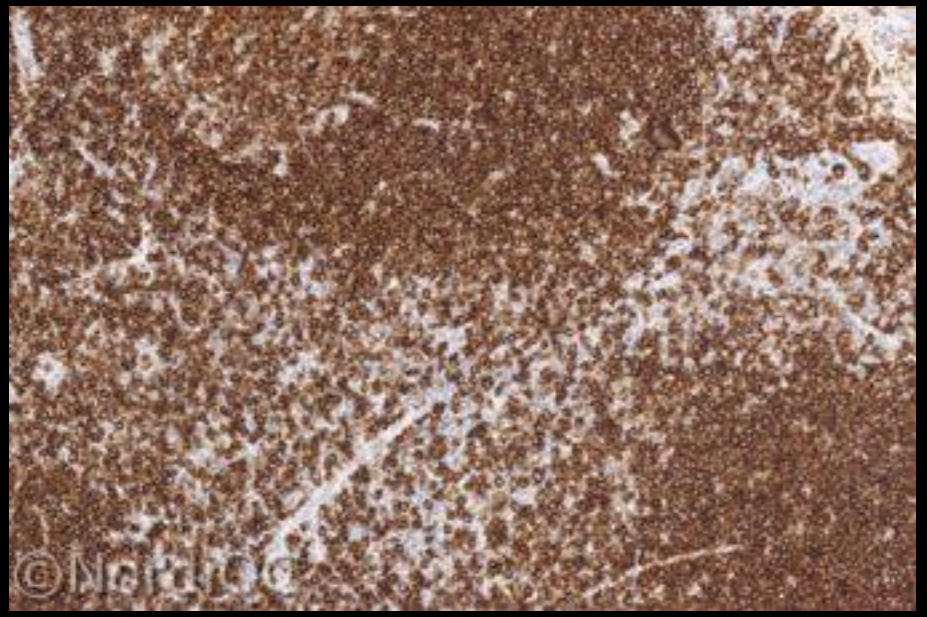


CD45 - Leucocyte common antigen (LCA)



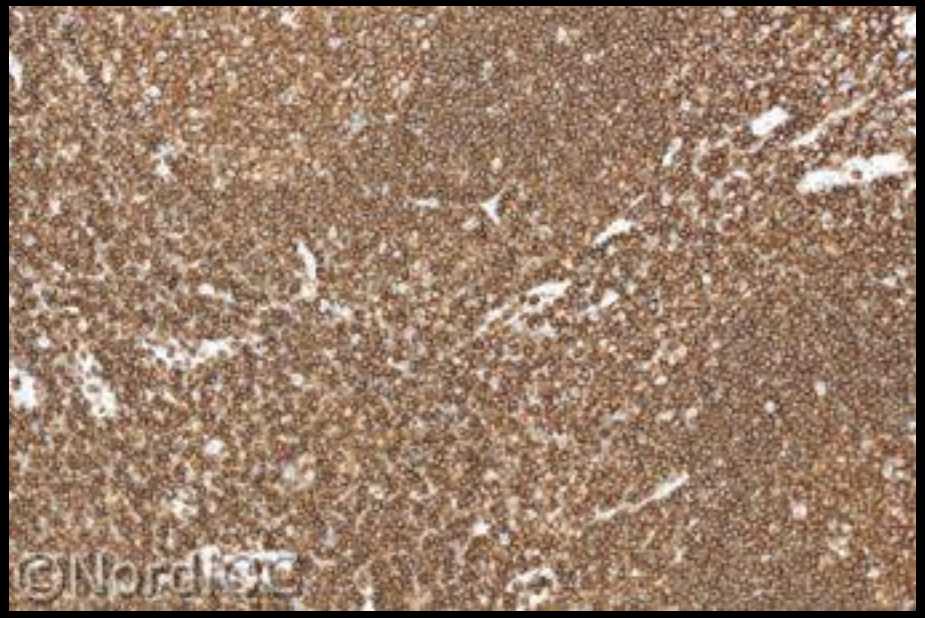
Kupffer cells: Critical assay performance control

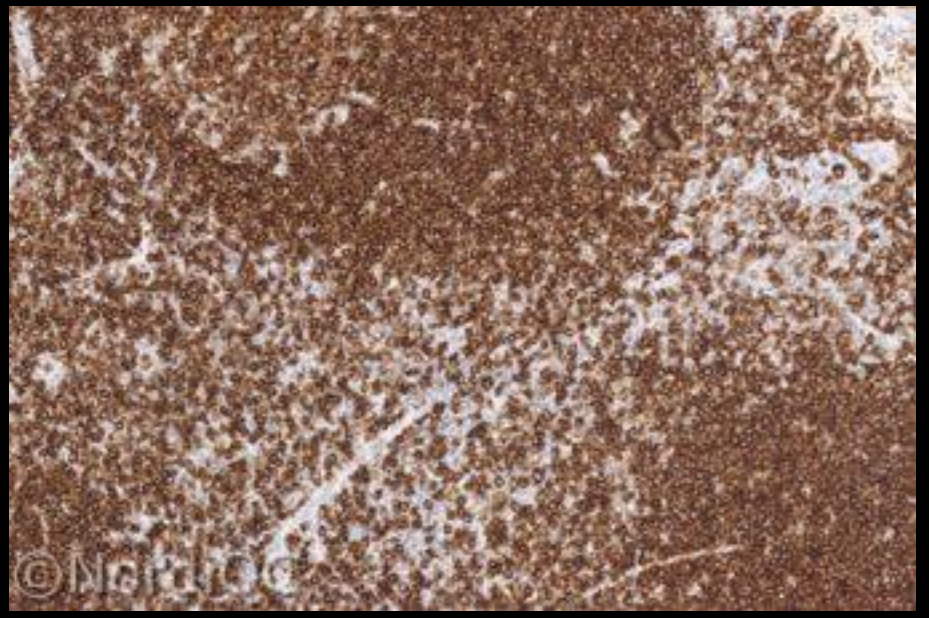
CD45 – NordiQC run 37 2013

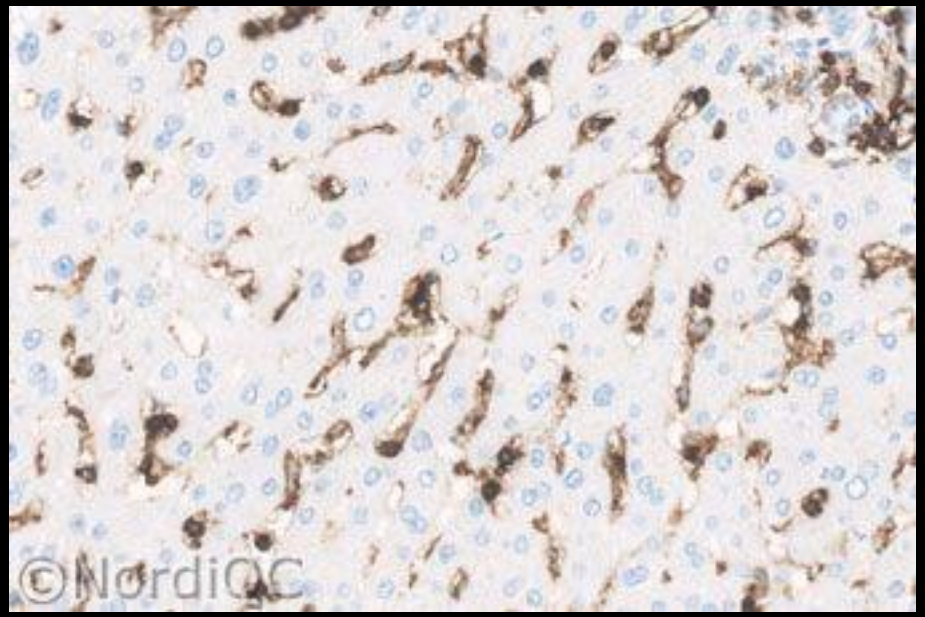


Which is best?

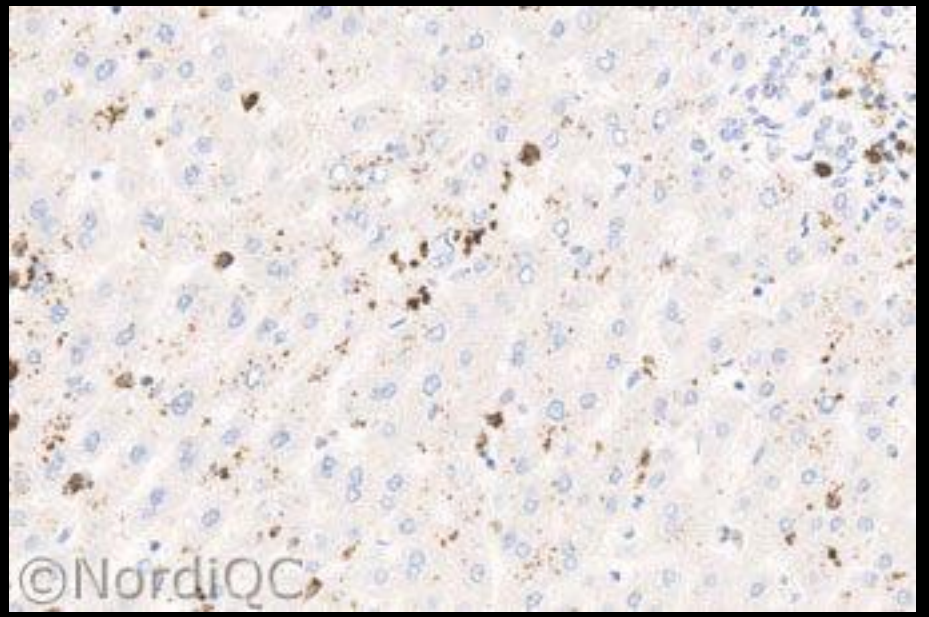
CD45 – NordiQC run 37 2013





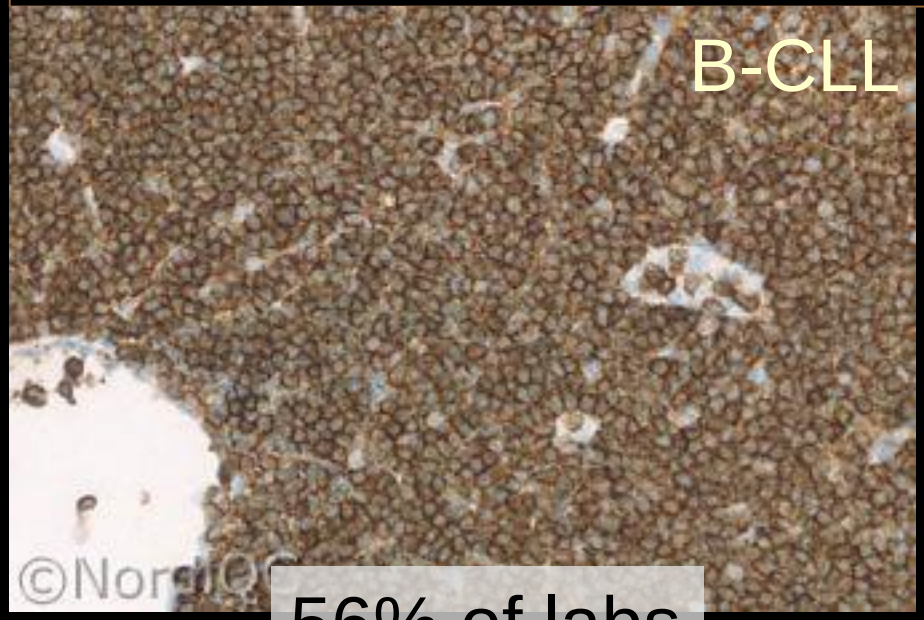


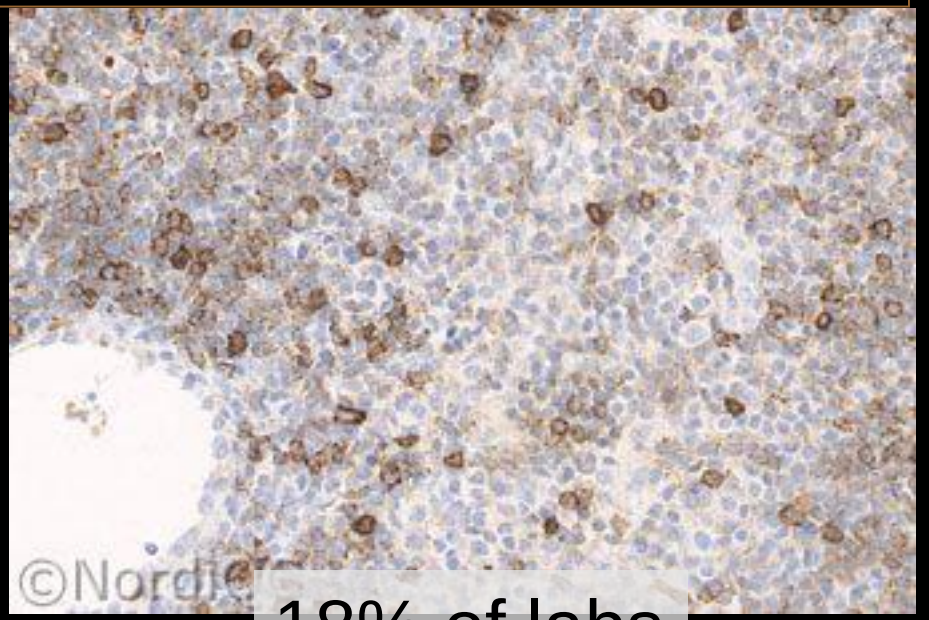
Optimal



Insufficient

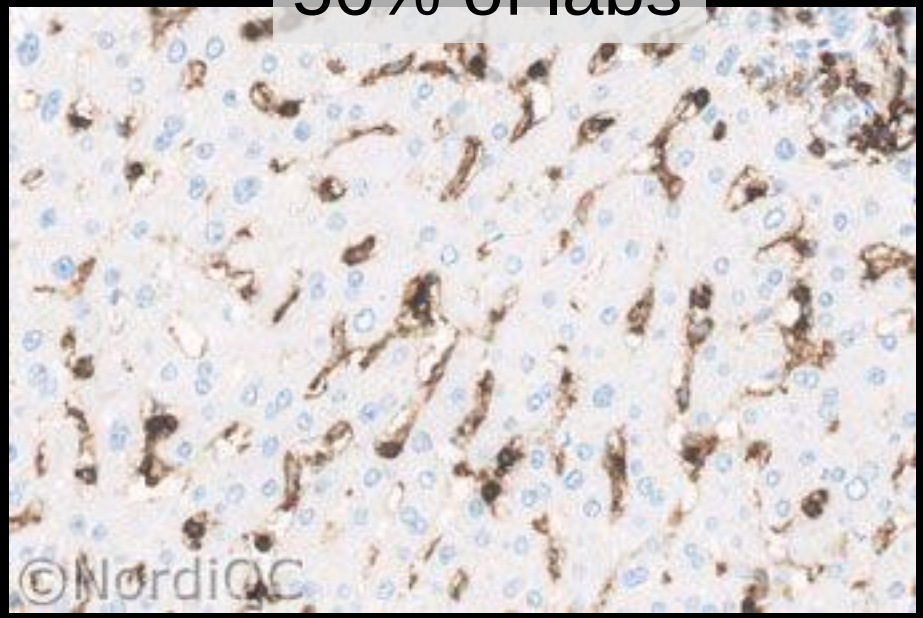
CD45 – NordiQC run 37

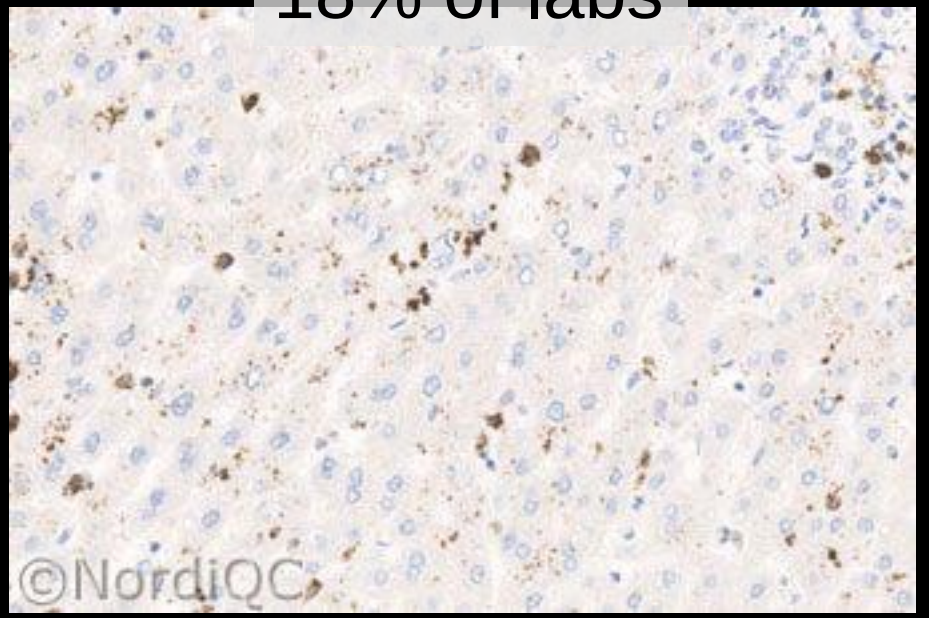




56% of labs

18% of labs





Optimal

Insufficient

CD45 – NordiQC run 37

Table 1. **Antibodies and assessment marks for CD45, run 37**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
Ready-To-Use Antibodies								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100%	100%
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	%

205 56% 82%

CD45 – NordiQC run 37

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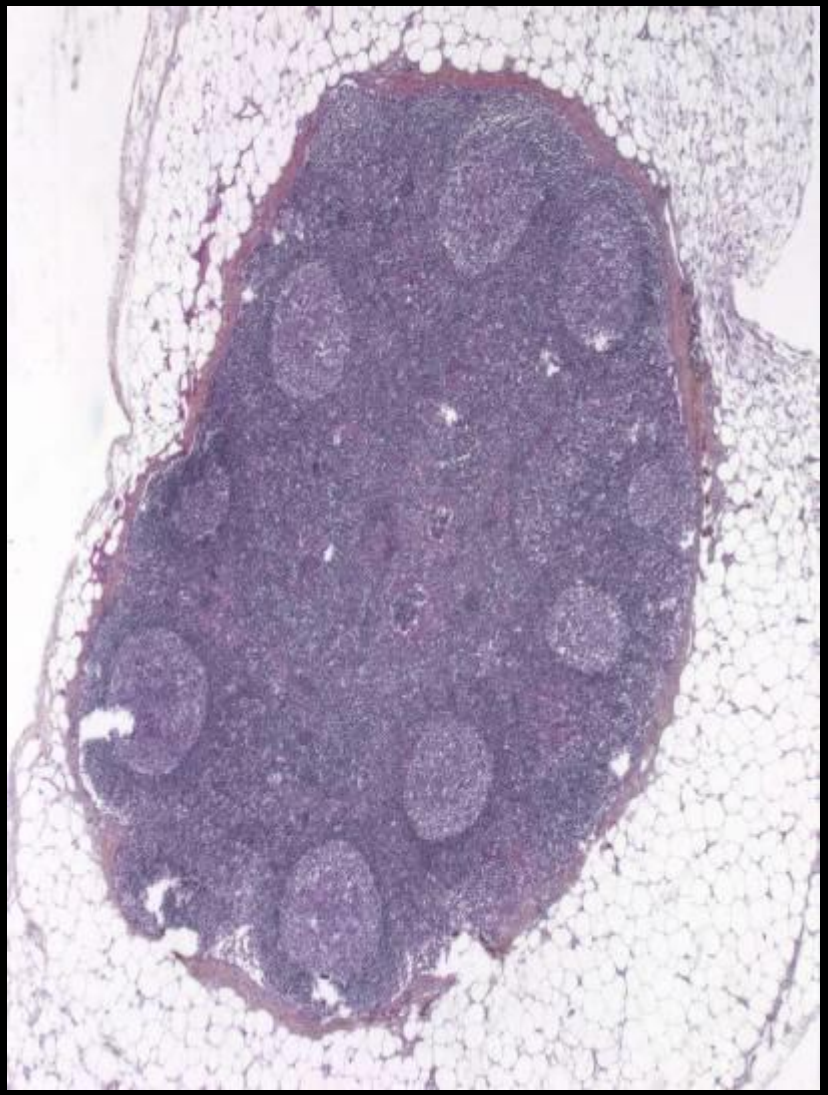
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	%

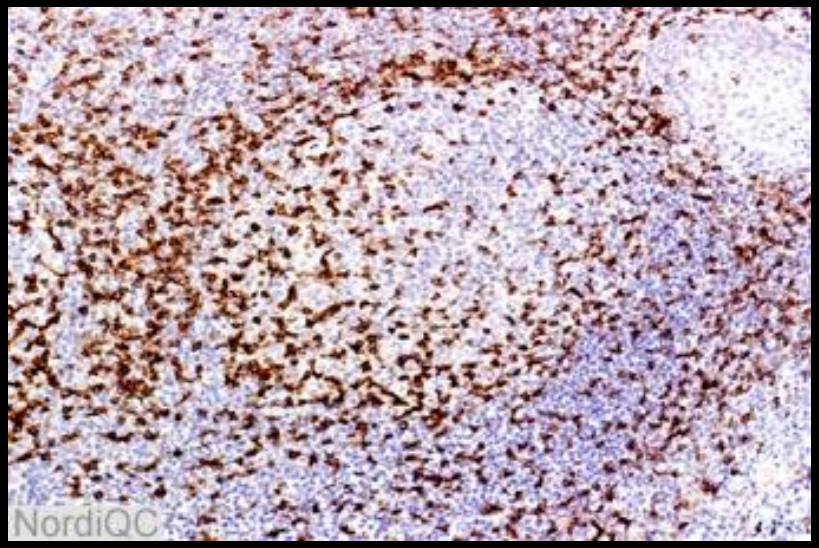
They followed the vendor's protocol recommendation: no HIER

205 56% 82%

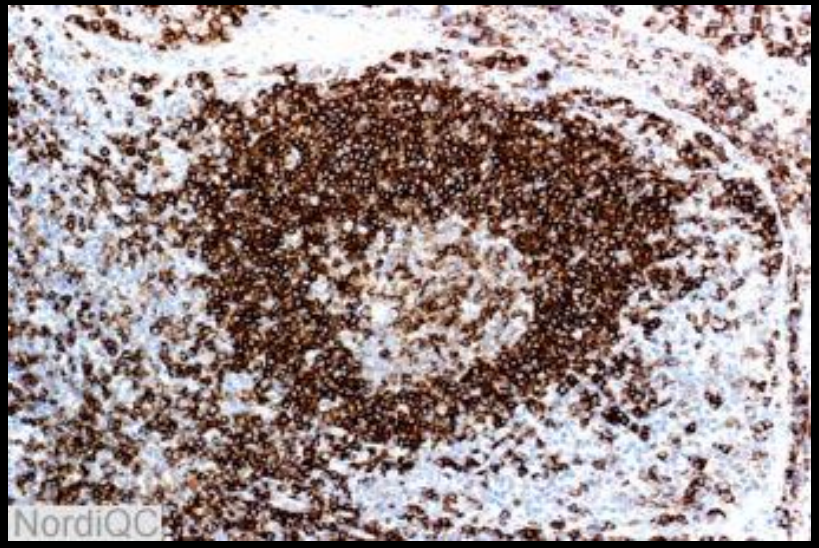
CD45 - Leucocyte common antigen (LCA)



Lymph node/Tonsil



- CD45 RO ~ T-cells



- CD45 RA ~ B-cells

CD45 - Leucocyte common antigen (LCA)

Cytokeratin-Positive, CD45-Negative Primary Centroblastic Lymphoma of the Adrenal Gland

A Potential for a Diagnostic Pitfall

Ludvik R. Donner, MD, PhD; Frank E. Mott, MD; Isaac Tafur, MD

● We report a case of cytokeratin-positive, CD45-negative primary polymorphic centroblastic lymphoma of the adrenal gland. Additional immunostaining, which demonstrated positivity for CD20 and κ light chain, as well as detection of the monoclonal rearrangement of the immunoglobulin heavy chain gene, helped to establish the diagnosis of lymphoma and to rule out an initially favored diagnosis of poorly differentiated carcinoma.

(*Arch Pathol Lab Med.* 2001;125:1104–1106)

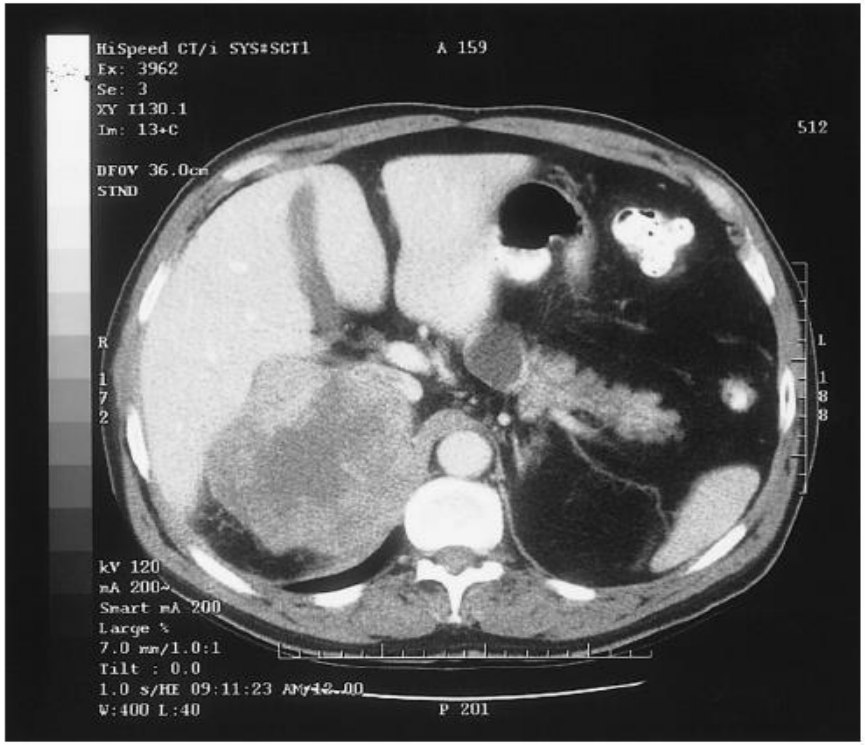


Figure 1. *Computed tomography of a large right suprarenal mass involving the liver.*

CD45 - Leucocyte common antigen (LCA)

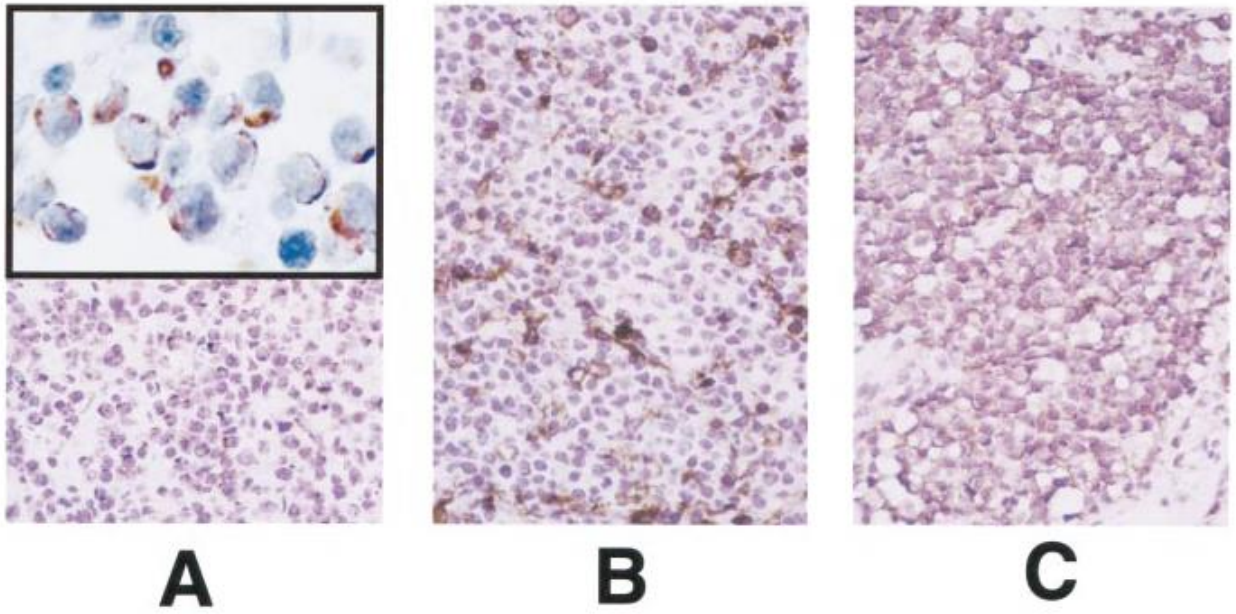


Figure 3. *Note immunoreactivity of the lymphoma cells for cytokeratin (A) and CD20 (C) but not CD45 (B) (original magnification ×100, inset ×250).*

Molecular Biologic Findings

Monoclonal rearrangement of the immunoglobulin heavy chain gene was identified by polymerase chain reaction (data not shown).

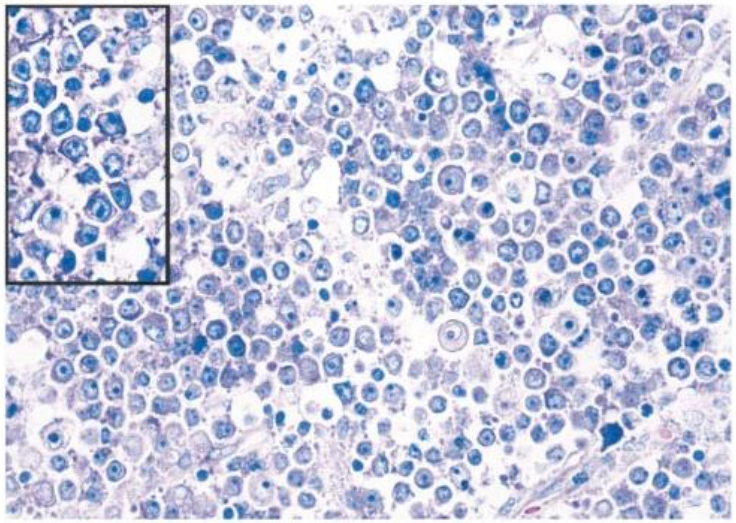


Figure 2. *Light microscopic appearance of the tumor (Giemsa stain, original magnification ×100, inset ×250).*

CD45 - Leucocyte common antigen (LCA)

MATERIALS AND METHODS

We performed immunohistochemical stains for cytokeratin (AE1/AE3. Cell Marque. Austin. Tex: CAM5.2. Becton Dickinson. San Jose, Calif; cytokeratins 5/6, Zymed, San Francisco, Calif; cytokeratin 7, Dako Corporation, Carpinteria, Calif; cytokeratin 20, Dako; 34βE12, Enzo, New York, NY), CD3, CD20, CD30, CD45RO, CD68, κ light chain, λ light chain, myeloperoxidase, epithelial membrane antigen, neuron-specific enolase, synaptophysin, S100 protein, HMB-45 (Dako), and chromogranin A (Cell Marque) on a TechMate 500 with a ChemMate Secondary Detection Kit–Peroxidase/DAB (Ventana Medical Systems, Tucson, Ariz). The histologic sections were pretreated by steaming in citrate buffer solution (Target Retrieval Solution, Dako) for 30 minutes at 99°C.

The monoclonal antibodies AE1/AE3 (working concentration, 0.4 μg of protein/mL) were applied for 25 minutes at room temperature. The immunostaining was repeated twice, each time with identical results.

Primary panel for the unknown primary tumour

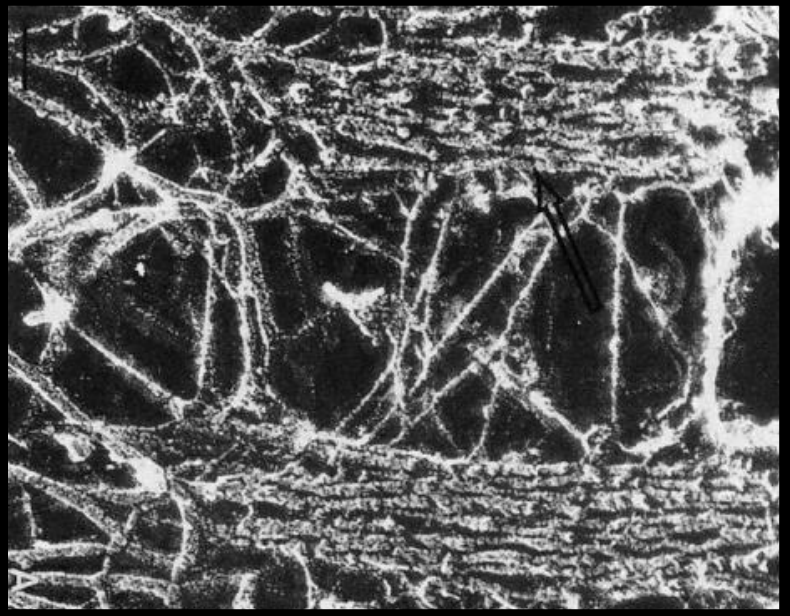
	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

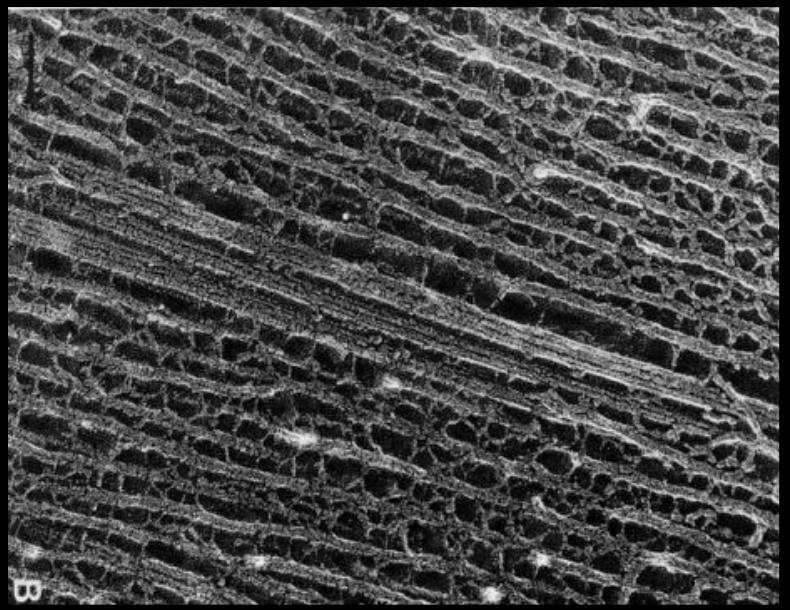
Cellular filaments

Microfilaments: (6 nm)

Intermediate filaments (7- 11 nm)

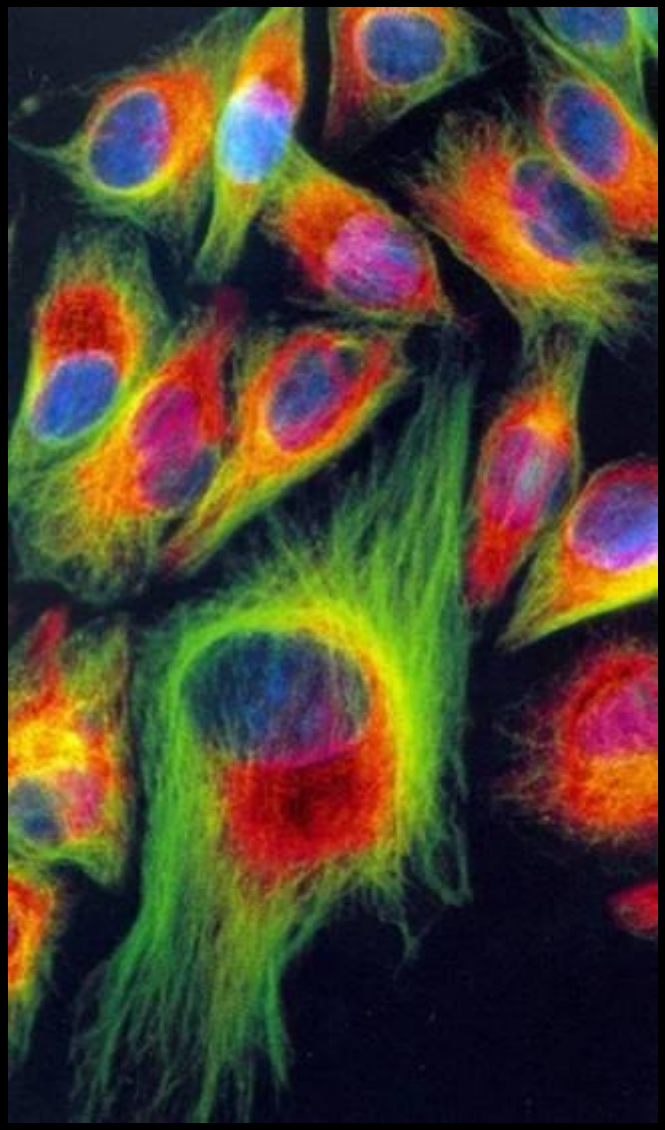
Microtubuli (23 nm)





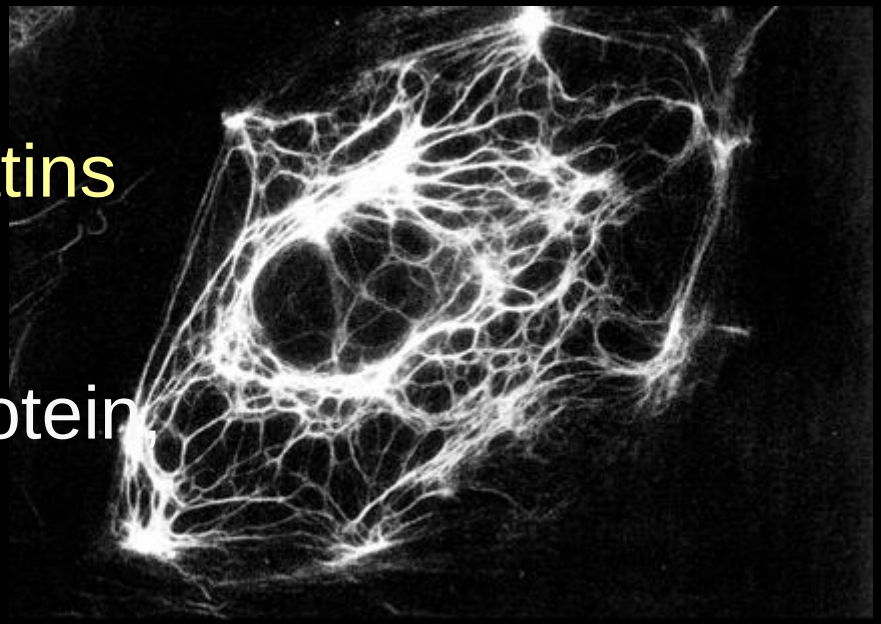
Intermediate filaments

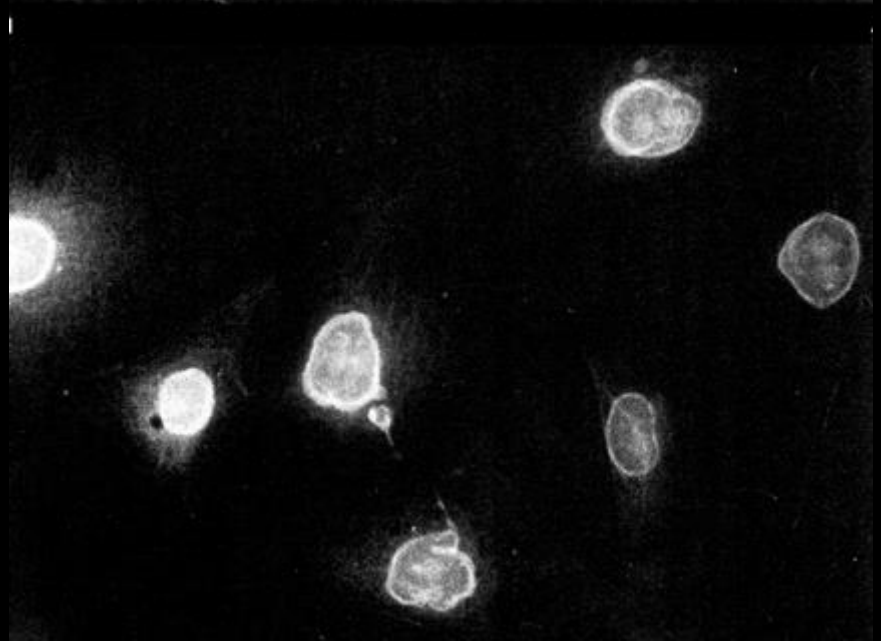
- Group of mainly cytoplasmic filaments 7 – 11 nm in diameter
- Part of the cytoskeleton in virtually all cells, creating as meshwork and connecting nuclear membrane with cell membrane
- Often associated with microfilaments (6 nm) and microtubules (23 nm)
- Important for mechanical strength and cellular functions



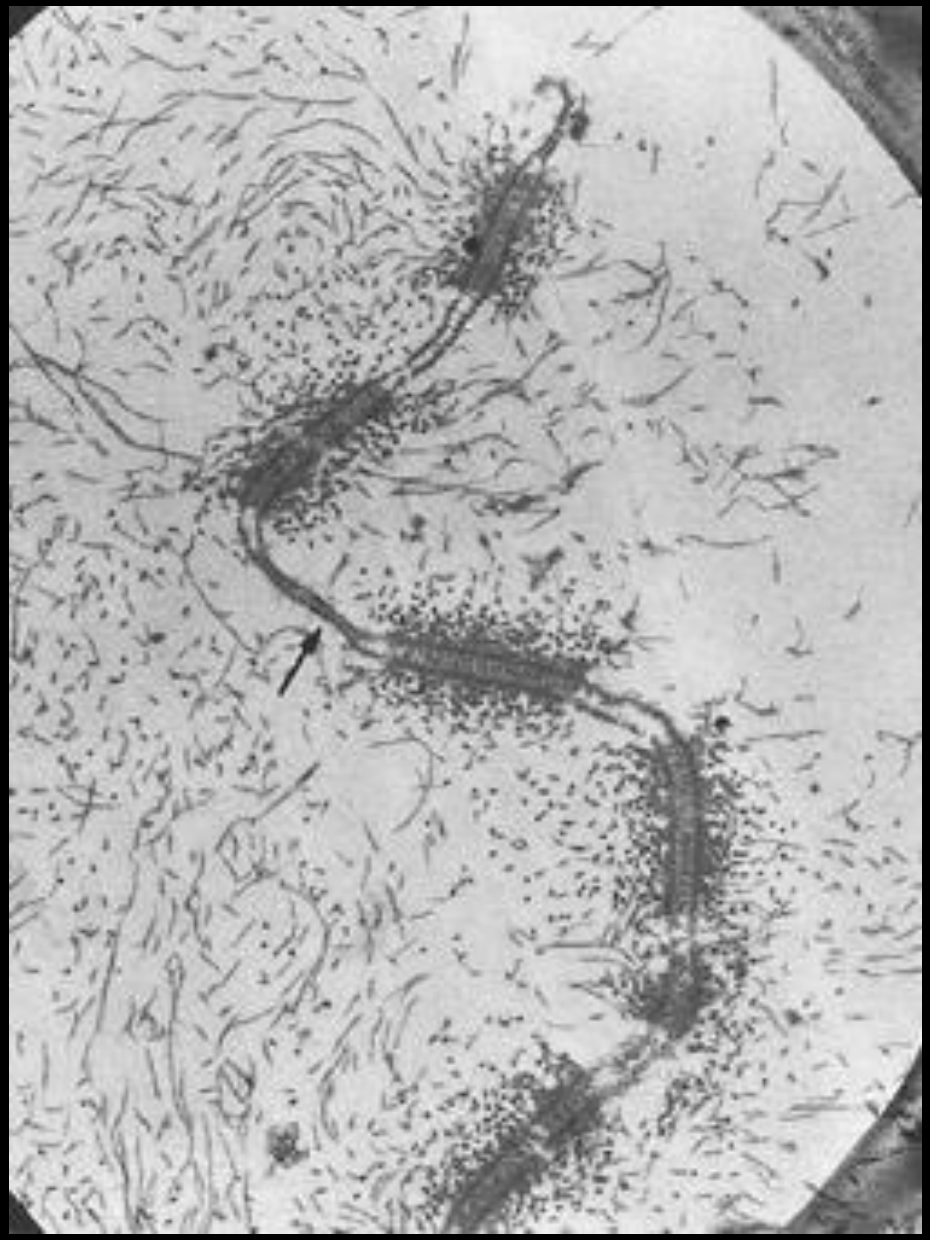
Intermediate filaments - 5 classes

- **I** acidic cytokeratins
- **II** basic-neutral cytokeratins
- III vimentin, desmin,
- glial fibrillary acidic protein
- peripherin
- IV neurofilament protein,
- α-internexin, nestin
- V lamins





Cytokeratins as tonofilaments



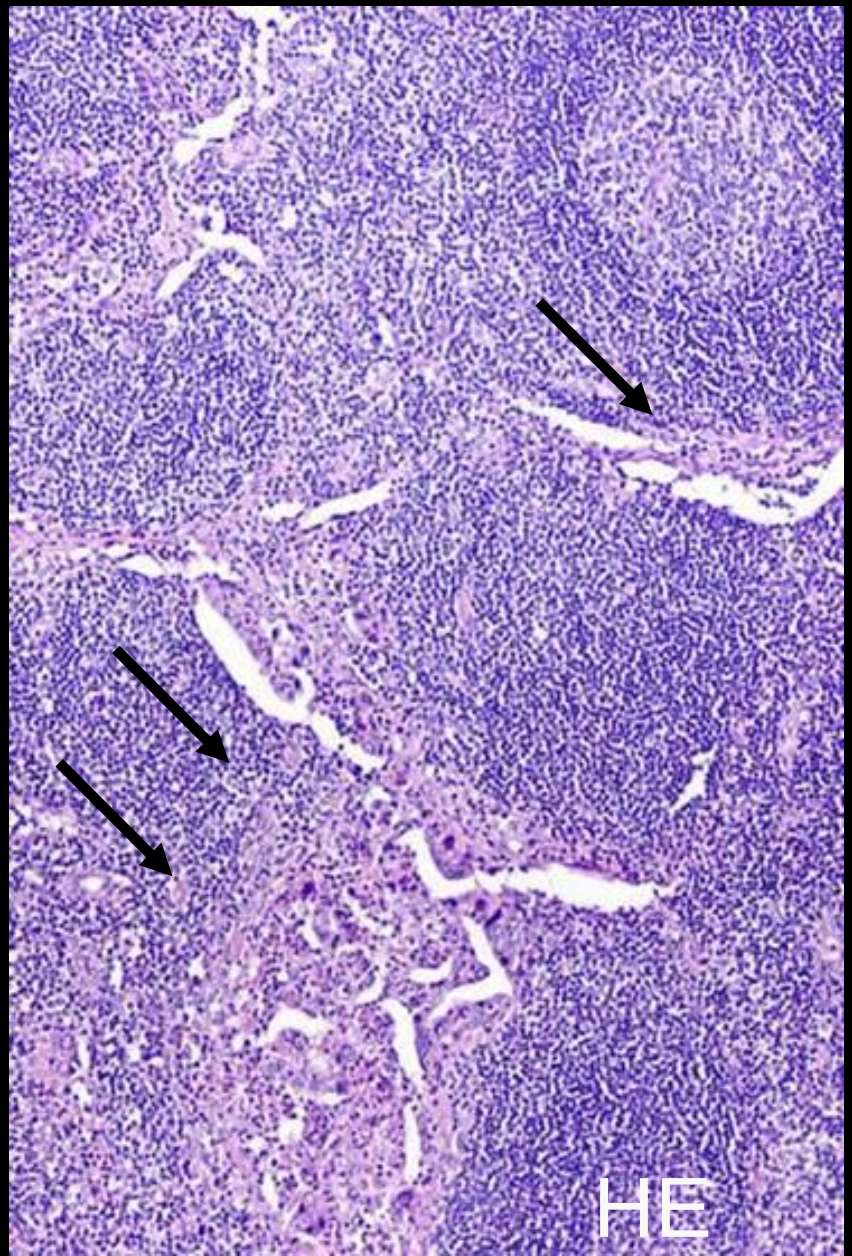
Cytokeratin intermediate filaments attached to desmosomes

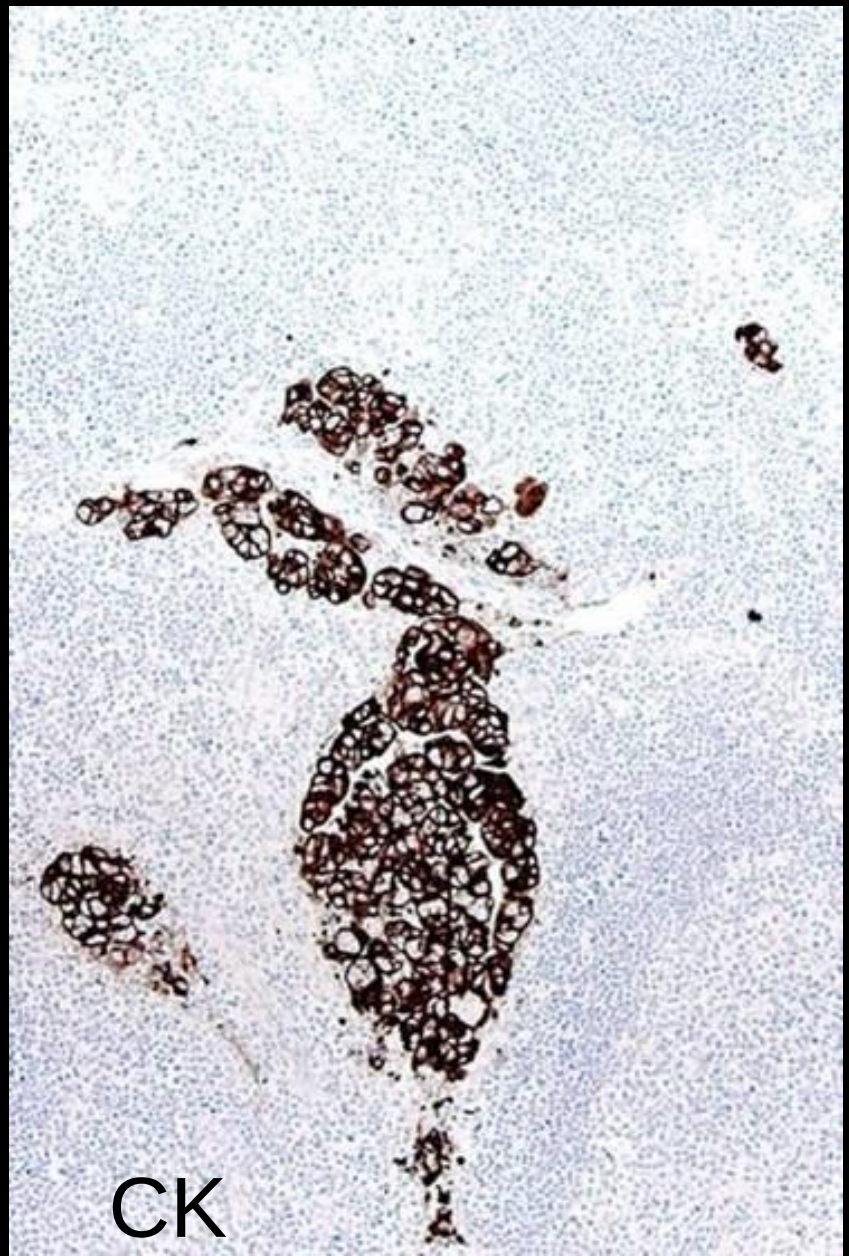
Drochmans et al.
J Cell Biol. 1978, 79:427

Cytokeratins in diagnostic pathology

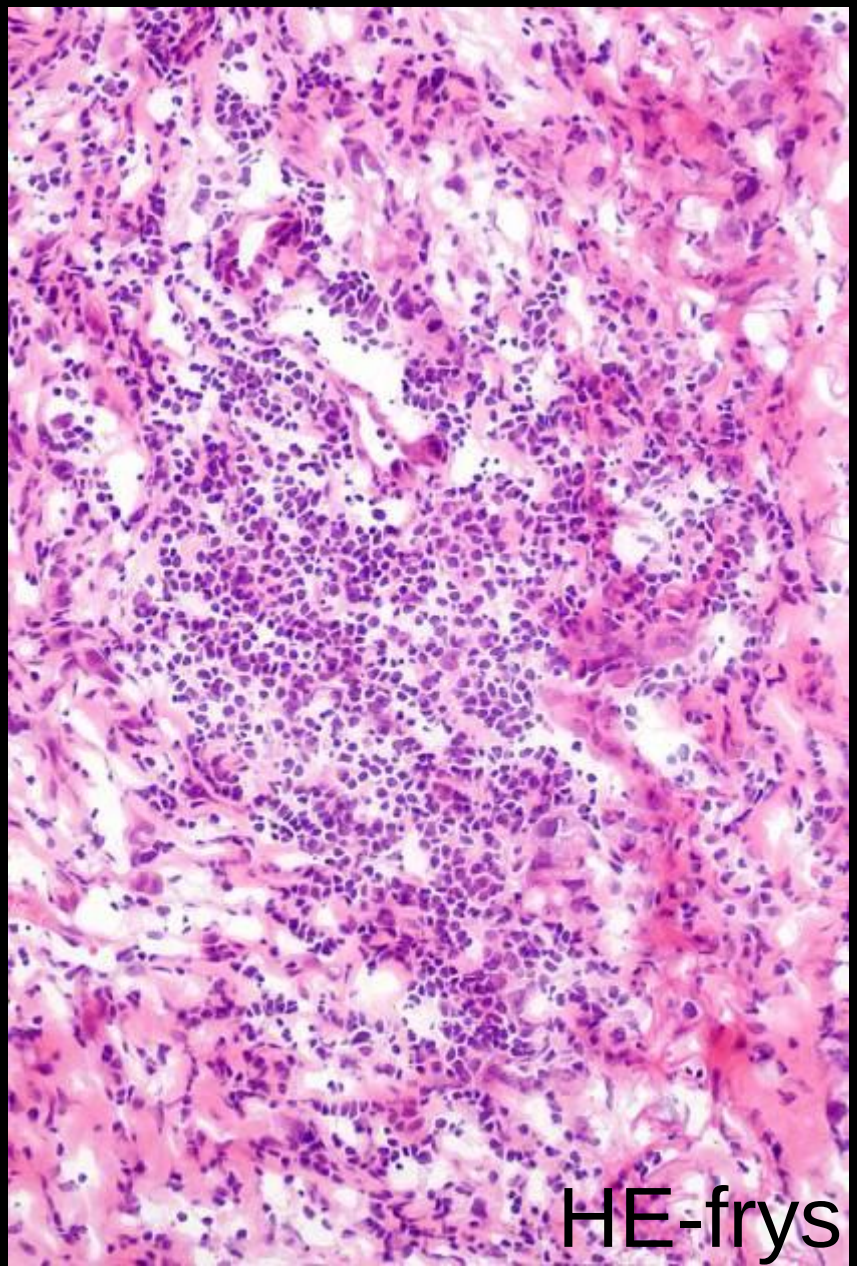
- Cytokeratins (CKs) belong to the most fundamental markers of epithelial differentiation
- CKs comprise a large family of subtypes. Different cell types express different patterns of CK subtypes
- Cancers generally express CK patterns that at least in part represent the pattern of the putative cell of origin
- Metastases express CK patterns fairly concordant with those of the primary tumours

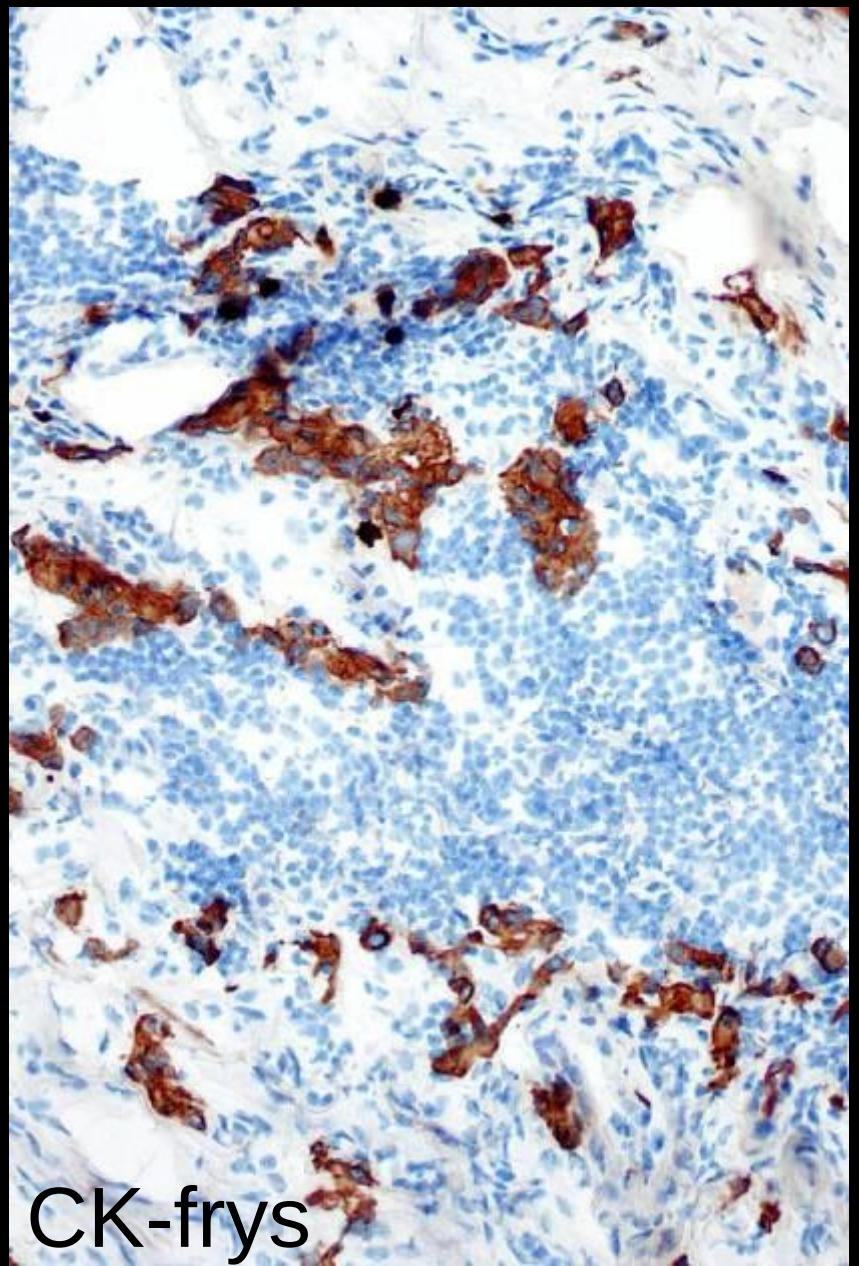
Micrometastases identified by cytokeratin





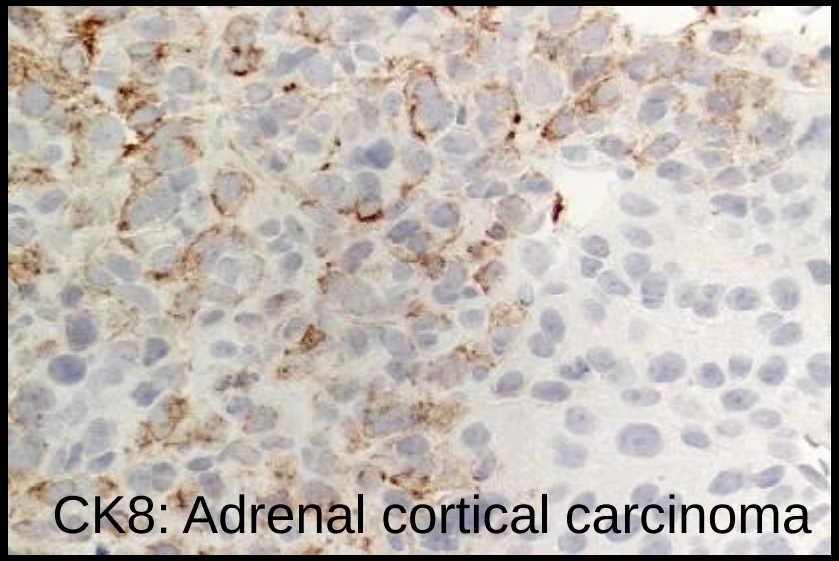
Carcinoma in frosen section identified by cytokeratin



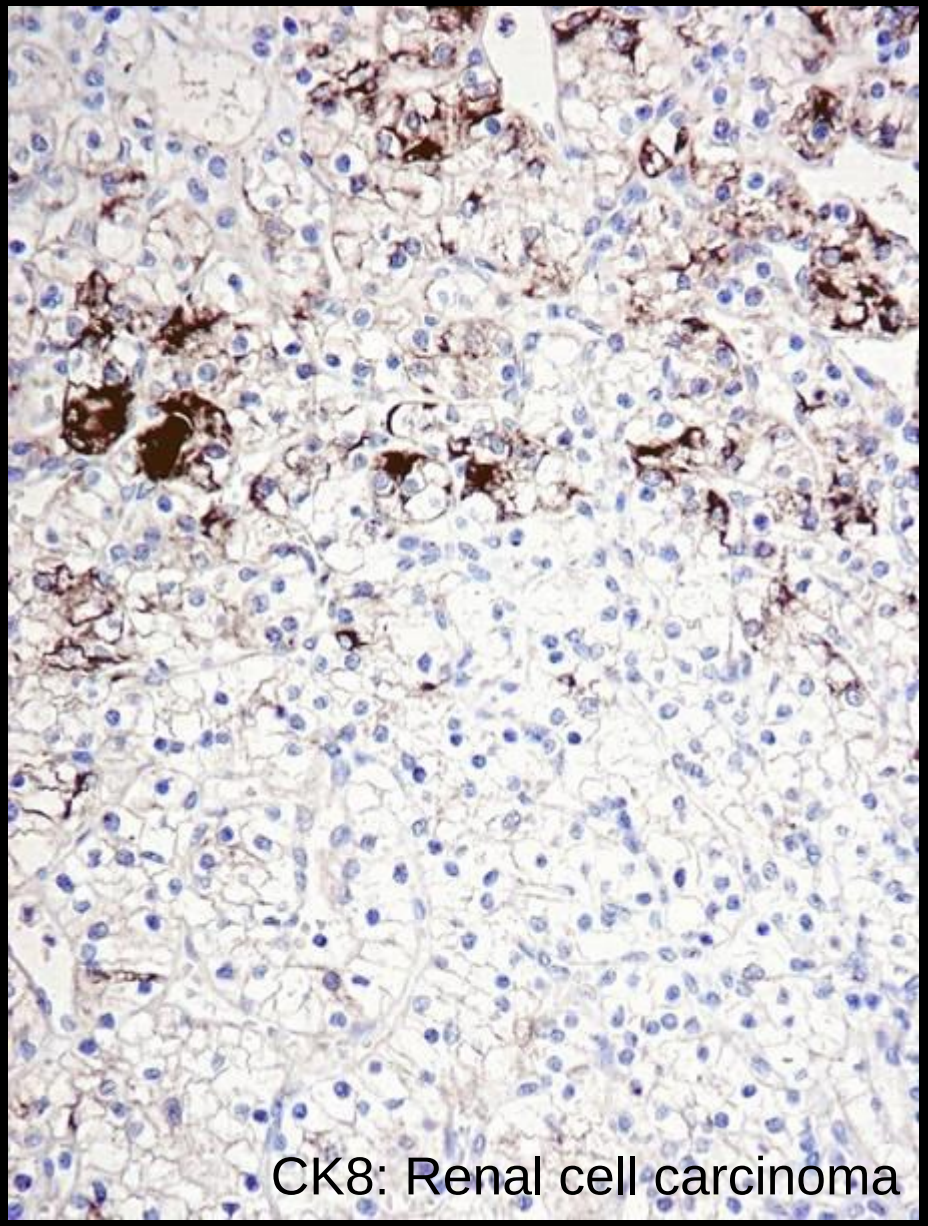


Low molecular weight cytokeratins in carcinomas

- Carcinomas “always” LMW-CK-positive, except some cases of
  - Renal cell carcinoma
  - Adrenal cortical carcinoma
  - Small cell carcinoma



CK8: Adrenal cortical carcinoma

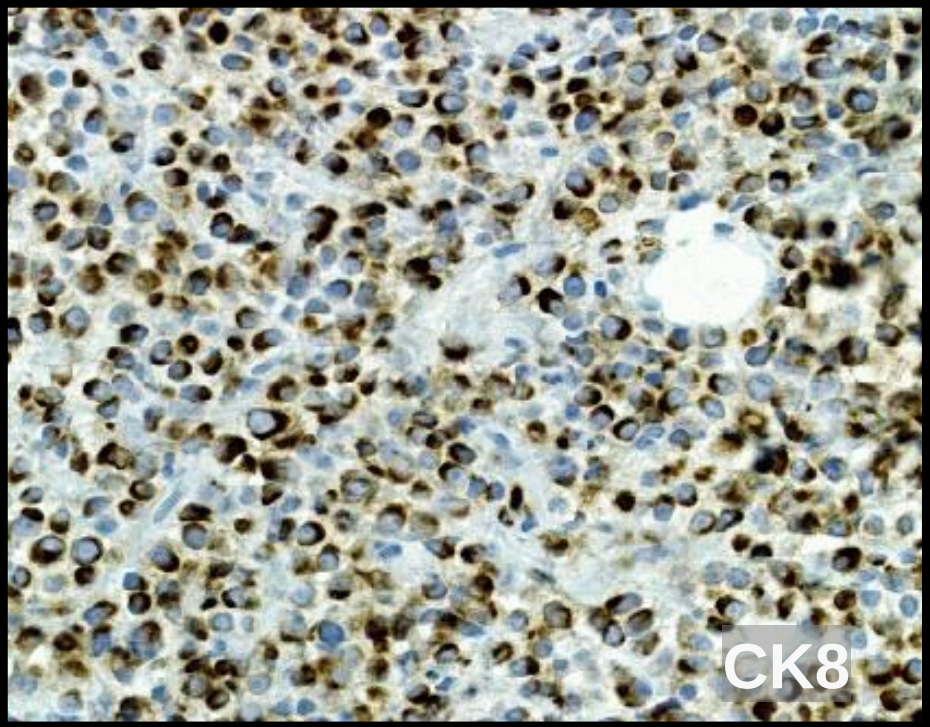


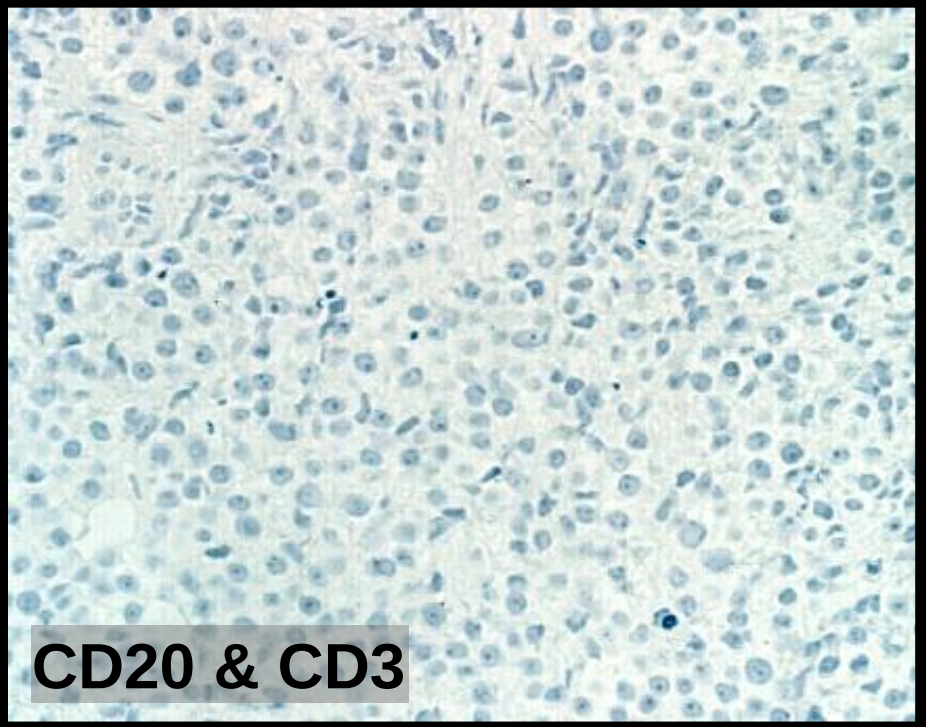
CK8: Renal cell carcinoma

Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

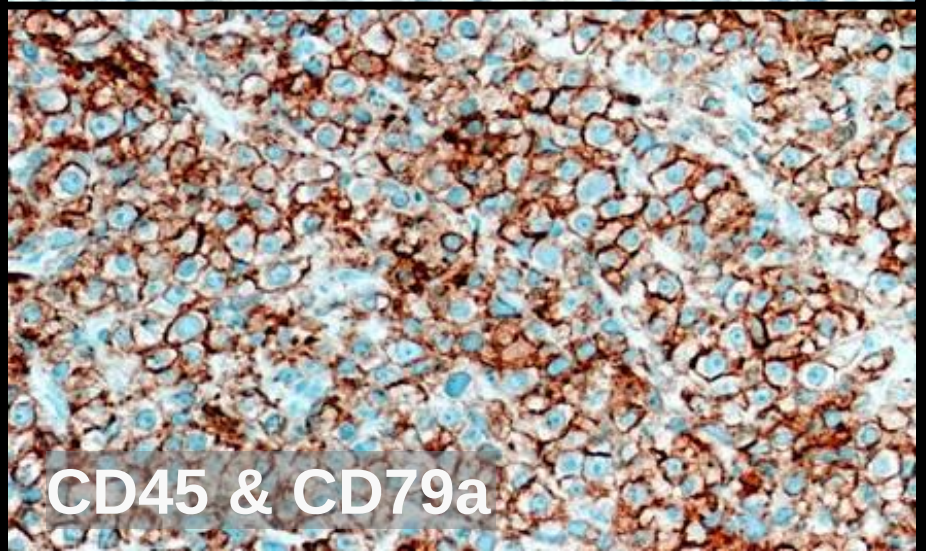
Cytokeratins in non-epithelial tumours





♀ 42 y, tumour infiltrating retroperitoneum

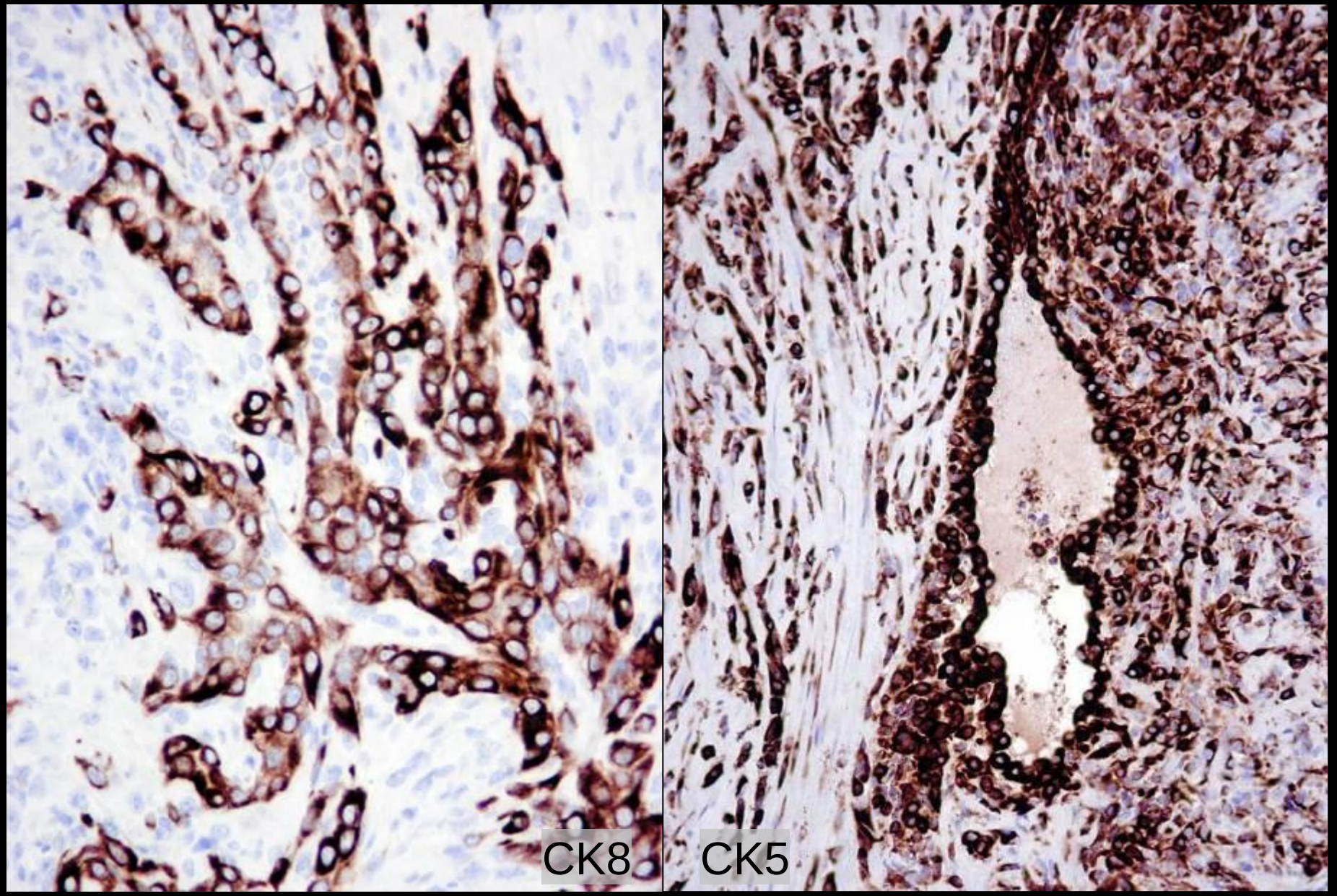
Malignant lymphoma !



Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

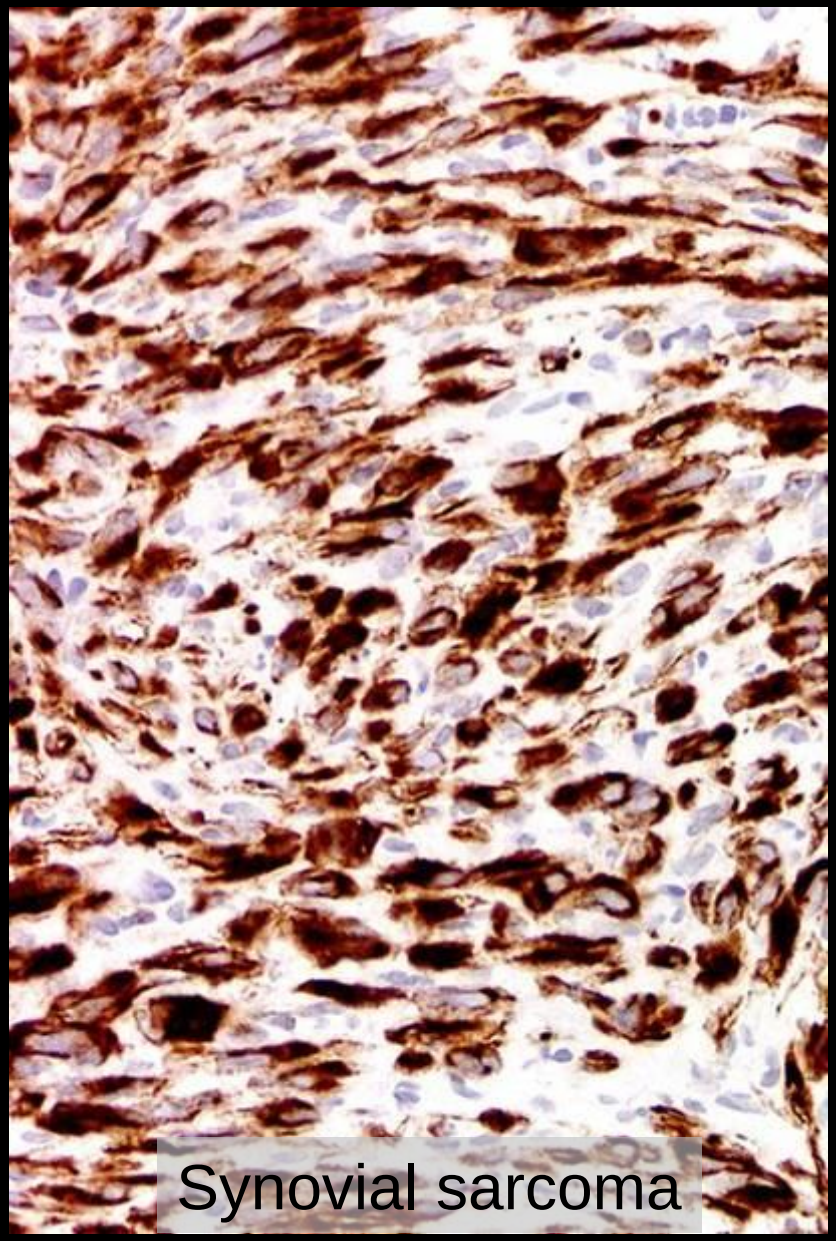
Cytokeratins in malignant mesothelioma



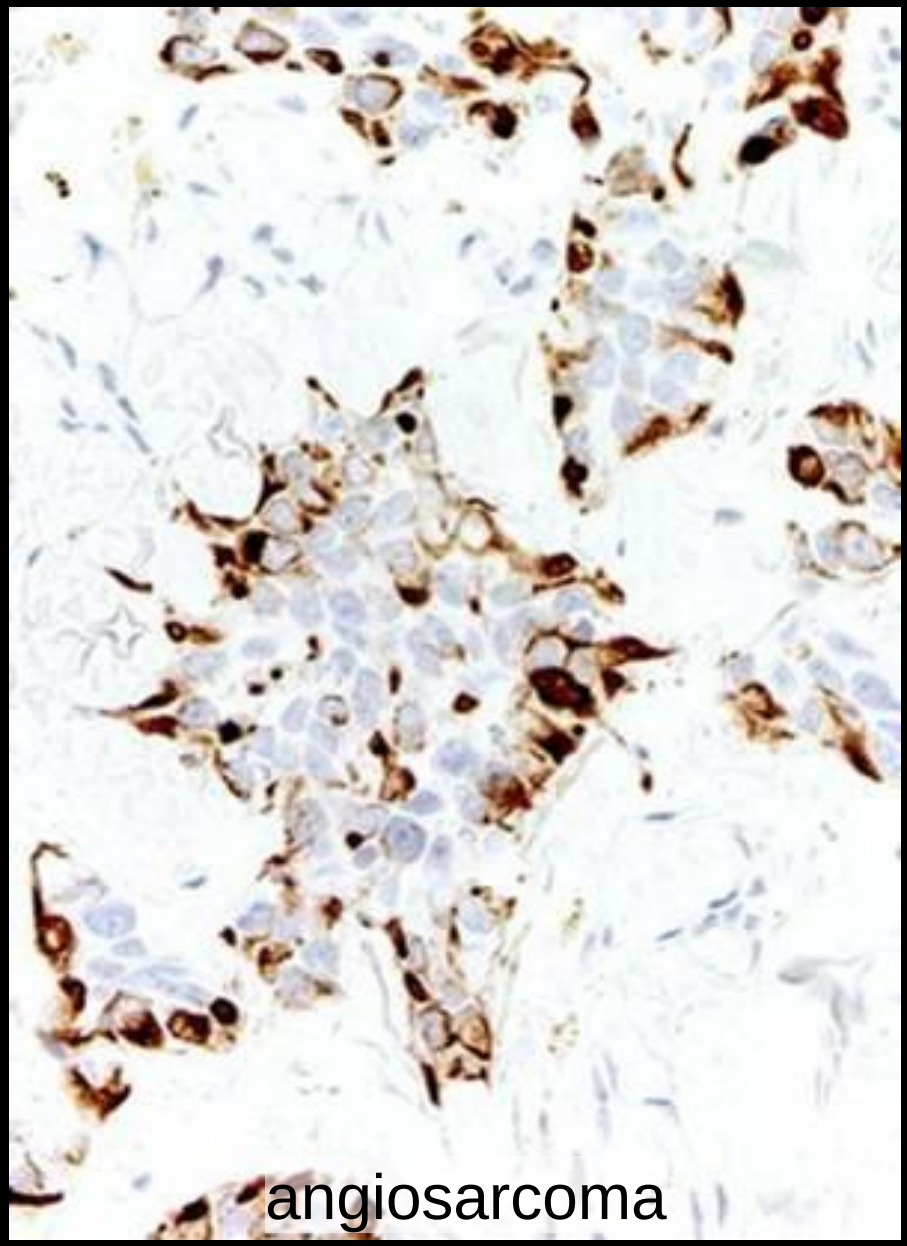
Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

Cytokeratins in sarcomas



Synovial sarcoma

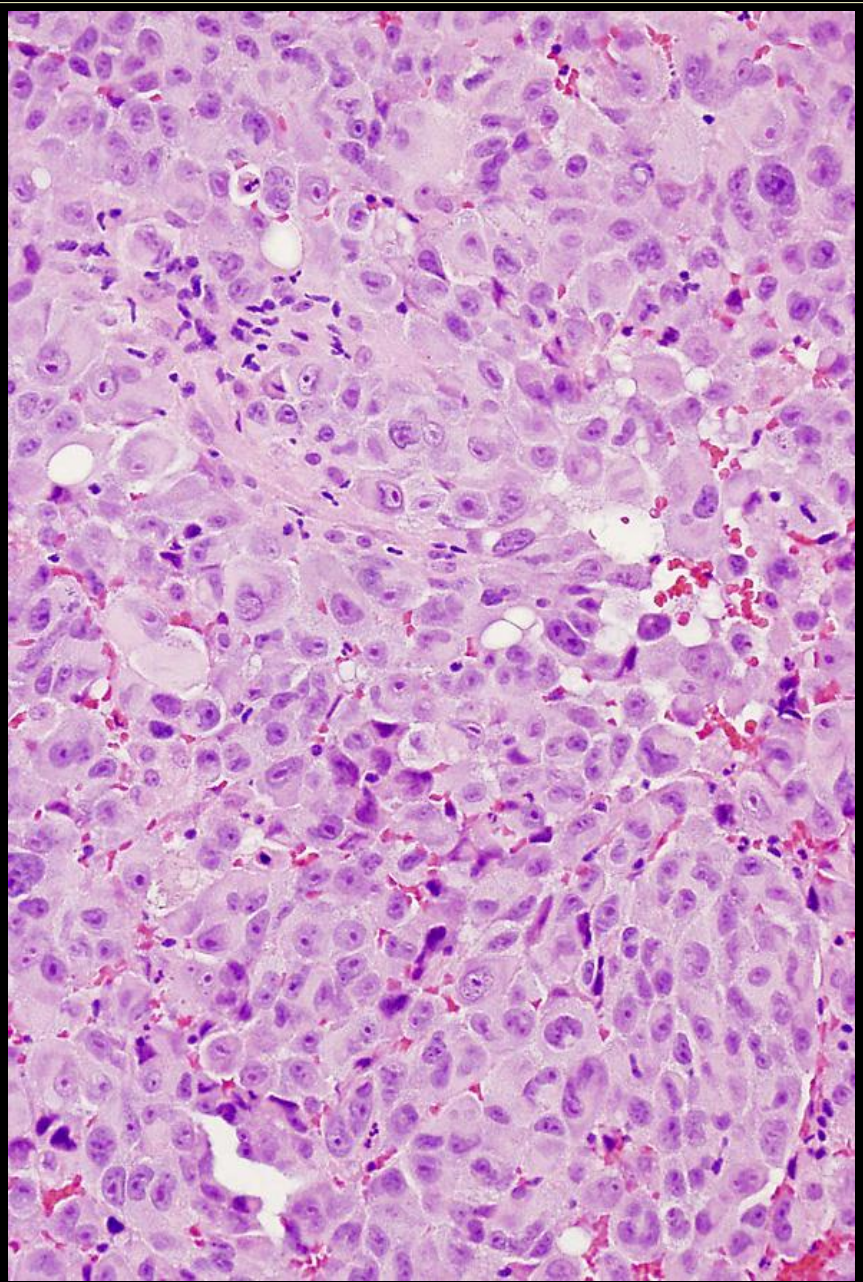


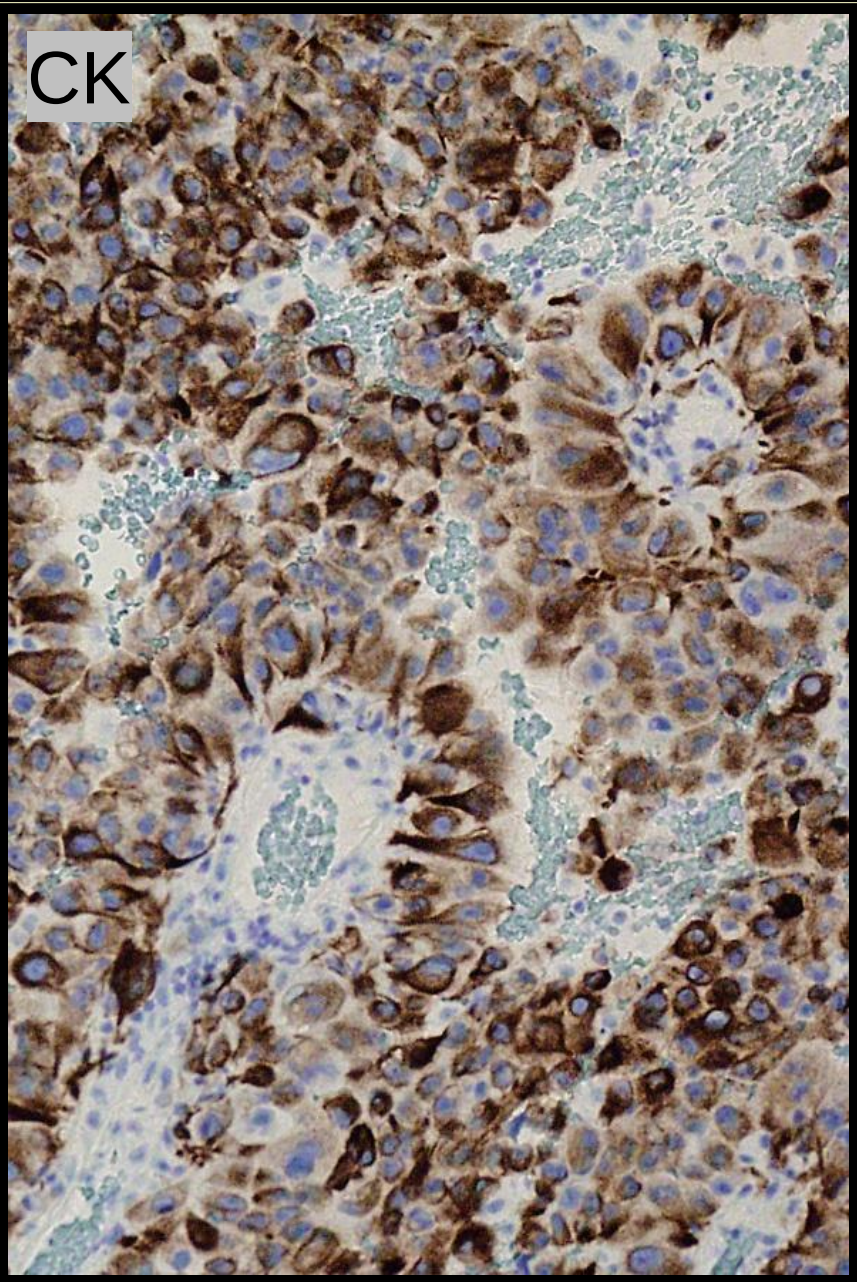
angiosarcoma

Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

Cytokeratins in malignant melanoma

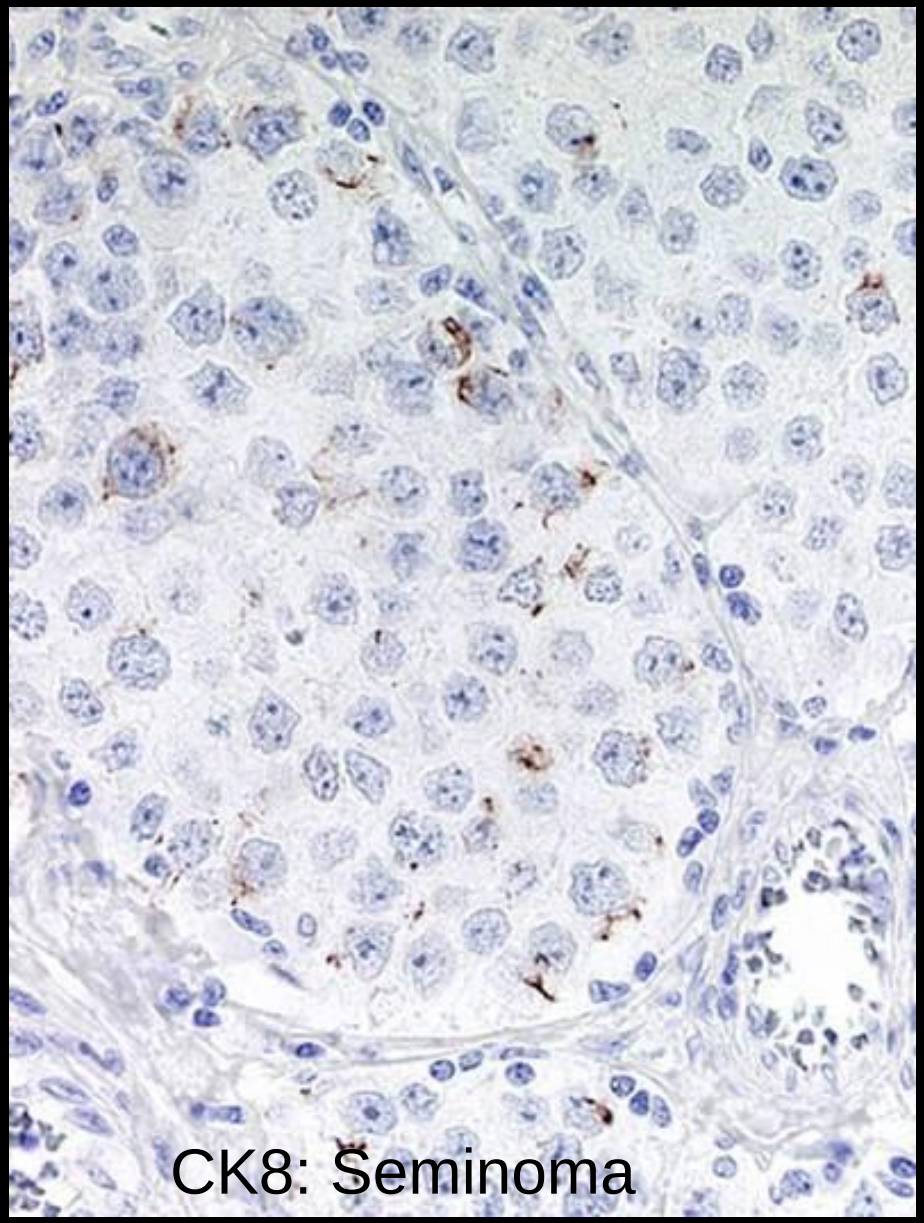




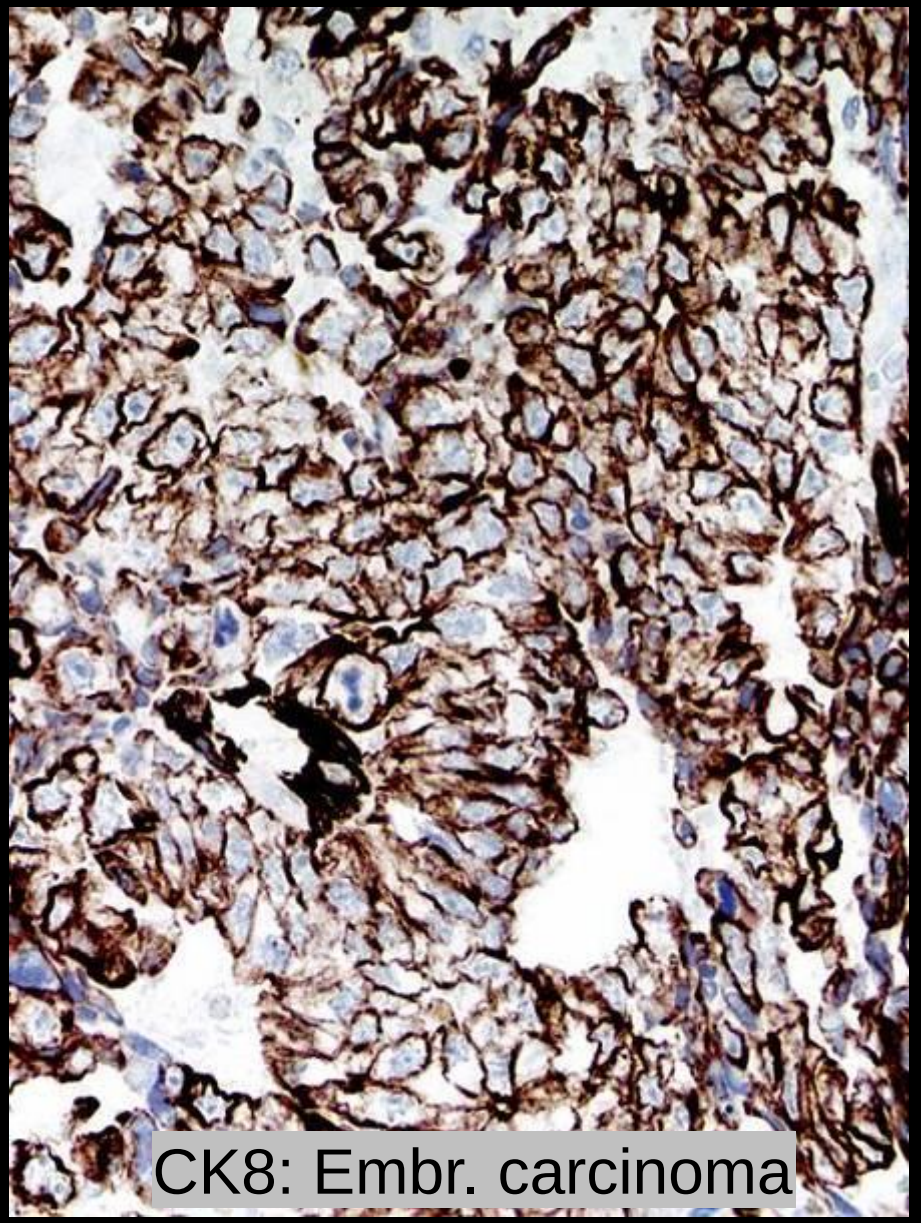
Primary panel for the unknown primary tumour

	CD45	Pan-CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

Cytokeratins in germ cell tumours

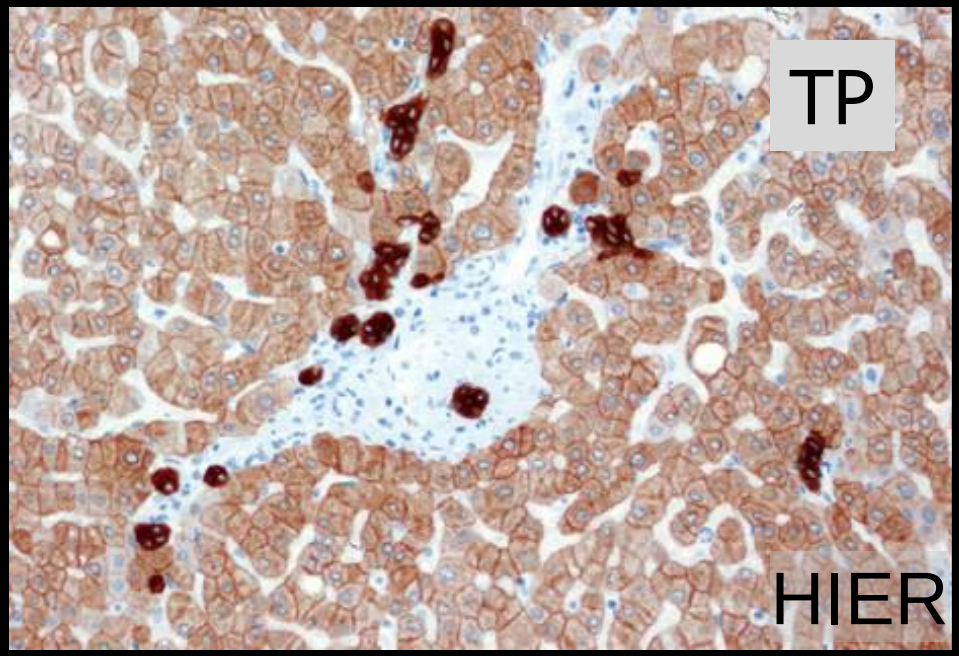


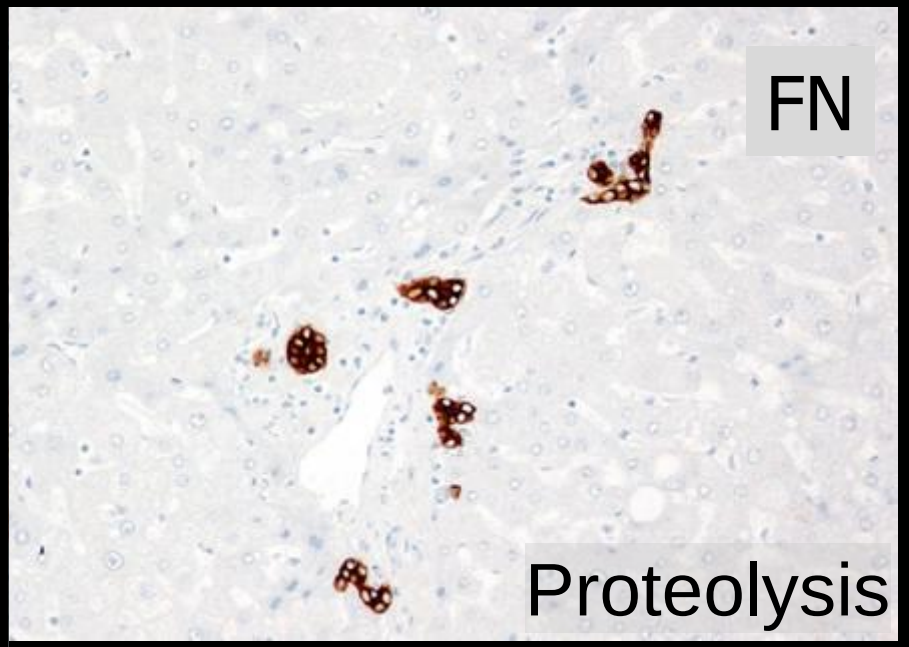
CK8: Seminoma



CK8: Embr. carcinoma

Cytokeratins: proteolysis causes false negativity

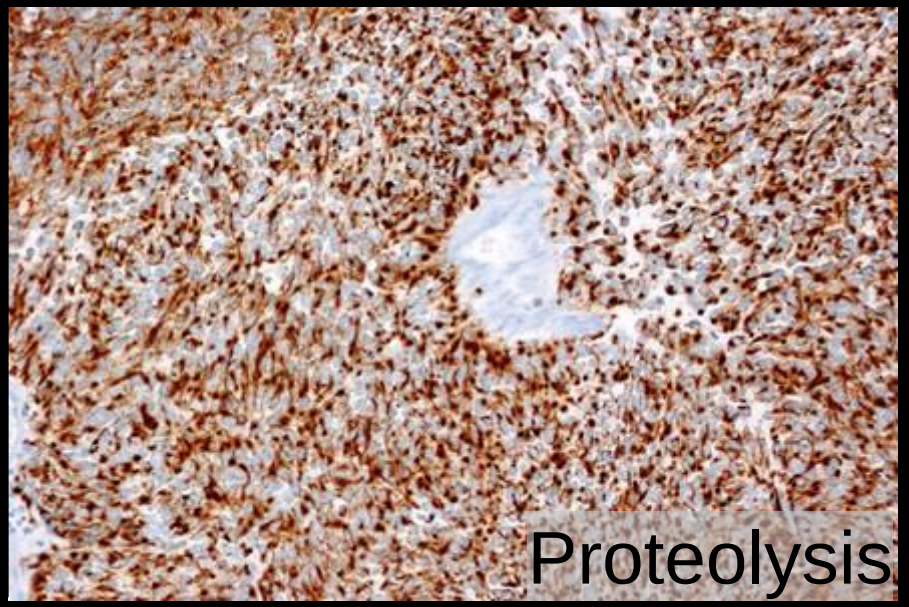




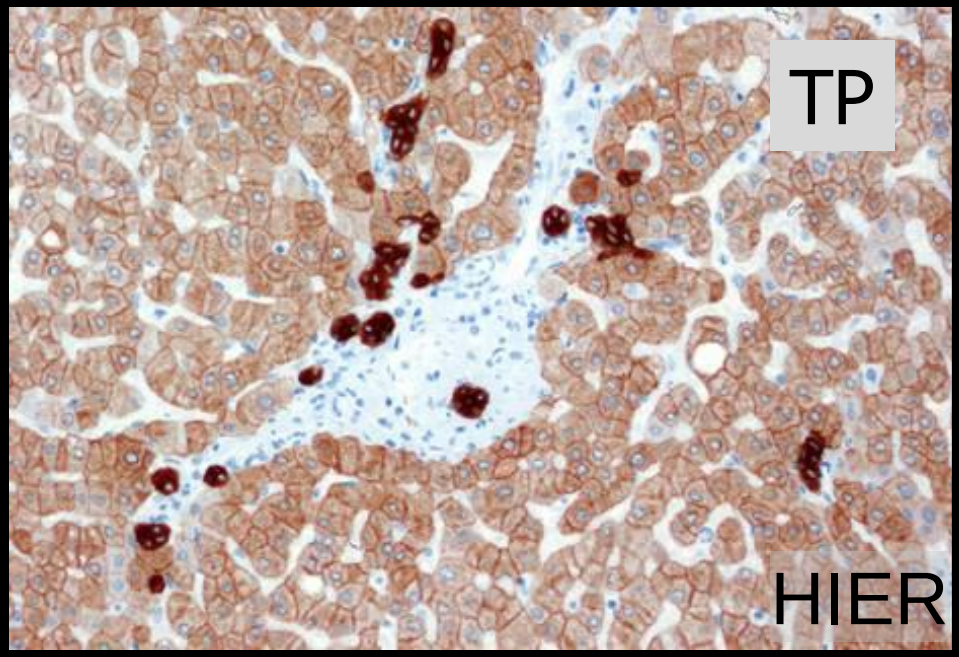
PAN-CK - AE1/AE3 clone cocktail:

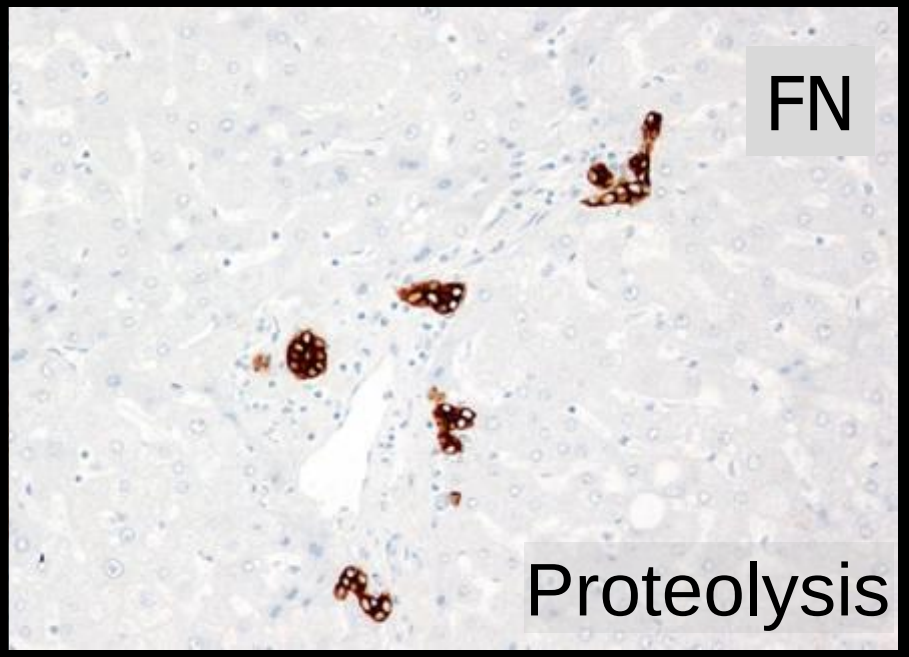
- AE1 detects CK8 after HIER only
- AE1 does not detect CK18
- AE3 neither detects CK8 or CK18

SCLC

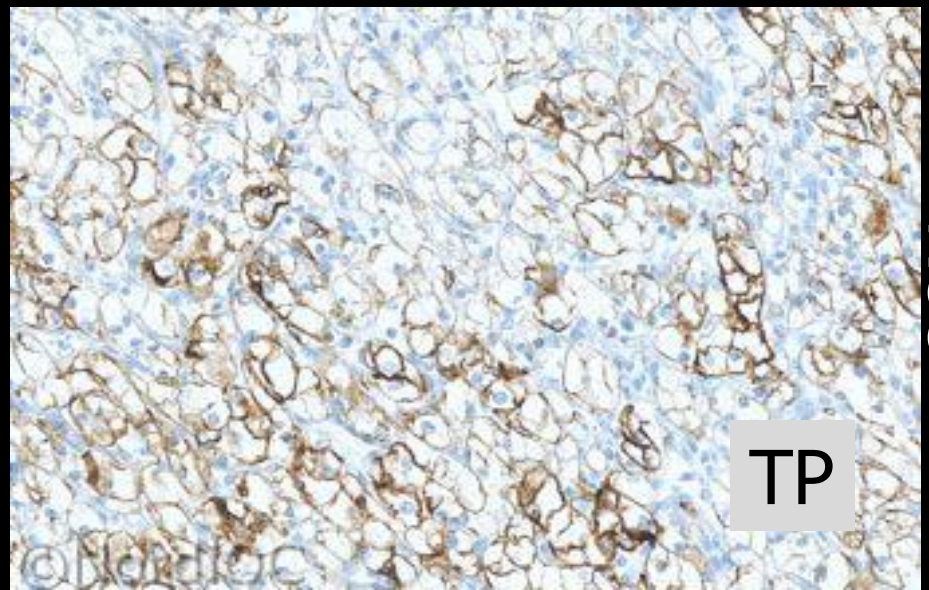


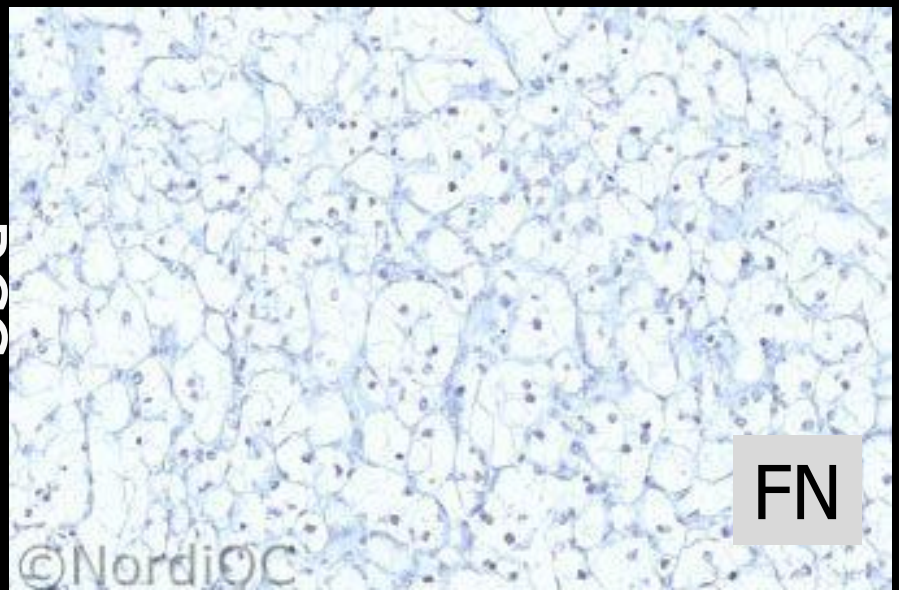
Cytokeratins: retrieval causing false negativity





RCC





Misleading datasheets

Table 1. Recommended Staining Protocols for Anti-Pan Keratin (AE1/AE3/PCK26)

Procedure Type	Platform/Method	
	ES or NexES IHC	BenchMark or BenchMark XT
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None Required	None Required
Enzyme (Protease)	Protease 1, 4 minutes	Protease 1, 4 minutes
Antibody (Primary)	Pan Keratin, approximately 16 minutes	Pan Keratin, approximately 16 minutes
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin, 2 to 4 minutes	Hematoxylin, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

Giving false negative results when only LMW-CKs are present

Misleading datasheets

Fig 2:
Enzymatic and heat pre-treatment:
mild CC1 and Protease 3 for 4 min, CK-Pan incubated for 8 min *ultra*View™ DAB

IHC - NordiQC 2016

Table 2. **Proportion of sufficient results for CK-PAN in the eight NordiQC runs performed**

	Run 8 2003	Run 15 2005	Run 20 2008	Run 24 2008	Run 30 2010	Run 36 2012	Run 41 2014	Run 47 2016
Participants, n=	72	85	103	123	168	202	233	275
Sufficient results	53%	58%	62%	60%	65%	65%	67%	72%

AE1/AE3 : Optimal results **only** obtained by **HIER** in NordiQC runs
AE1/AE3/PCK26: Optimal results mainly obtained by HIER+protelysis

Dako: RTU – HIER
Leica: RTU – Proteolysis
Thermo:
.....

Conc: Proteolysis or HIER
Conc: HIER
Conc: HIER Quanto – Proteolysis UltraVision

Misleading data sheets + Wrong control material used !

Improved datasheets

By 17th October 2014

Table 1. Recommended Staining Protocol for anti-Pan Keratin (AE1/AE3/PCK26) with ultraView Universal DAB Detection Kit on a BenchMark XT instrument.

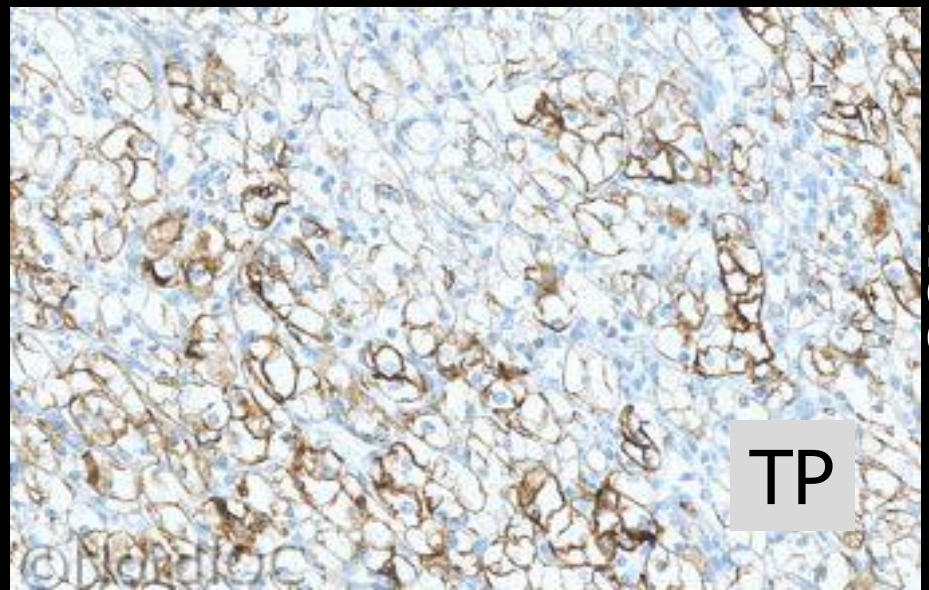
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	Protease 3, 4 minutes
Antibody (Primary)	BenchMark XT instrument 8 minutes, 37° C
ultraBlock	*VENTANA Antibody Diluent with Casein (760-219), 4 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

*Use of VENTANA Antibody Diluent with Casein (760-219) at the ultraBlock step is recommended to reduce staining on smooth muscle

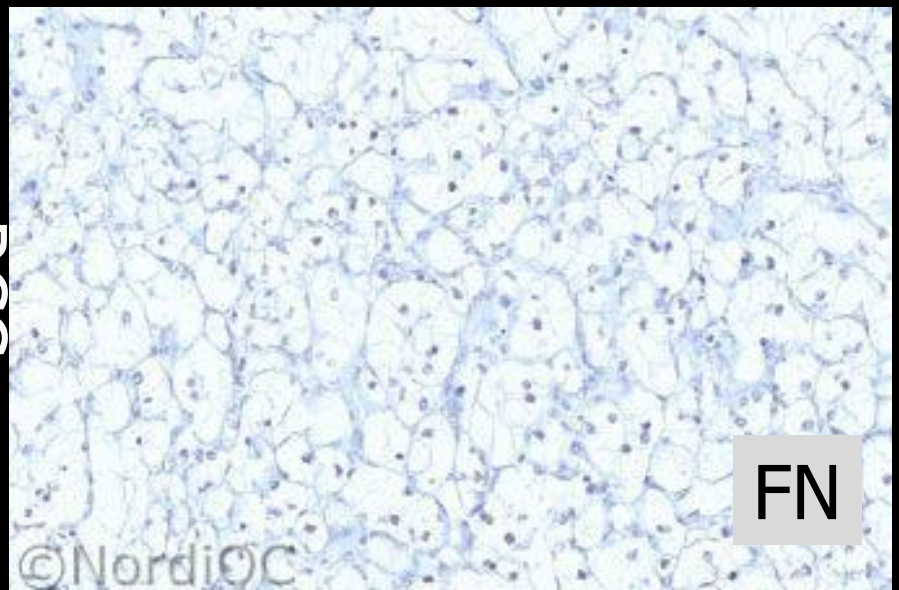
NordiQC run 41 2014

Fra: Galloway, Mary [mailto:Mary.Galloway@fda.hhs.gov]
Sendt: 13. november 2014 01:14
Til: Søren Nielsen / Region Nordjylland
Emne: RE: Changes Made to Package Inserts

Sören,
*Thanks for identifying and alerting us to the issues with **...anti-Pan Keratin**. The package inserts are now changed (see links below).*
I hope we can continue to learn of any future staining problems you may uncover.
Much appreciated!
Mary



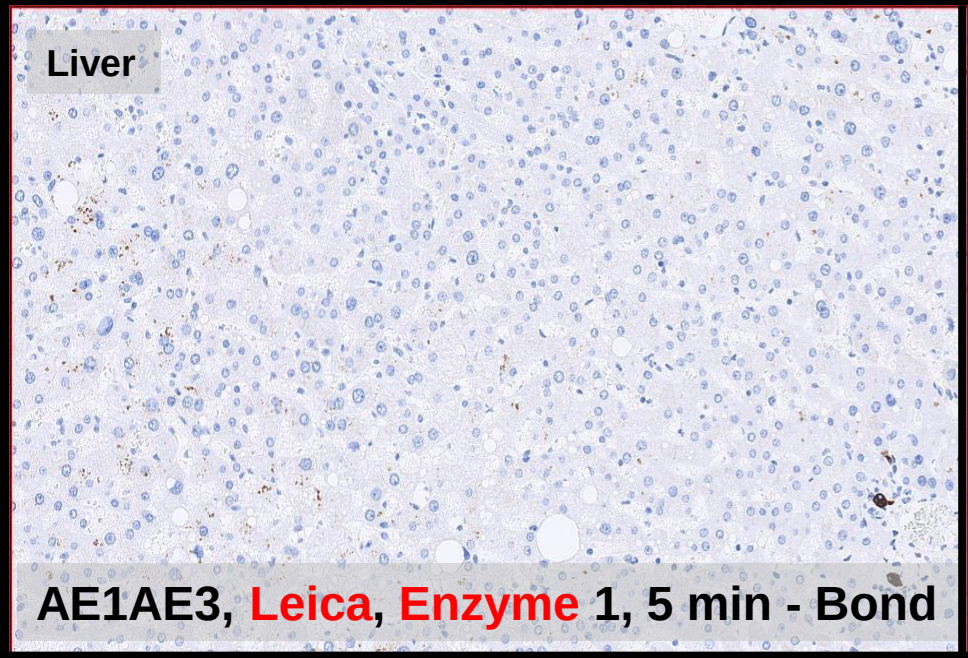
RCC



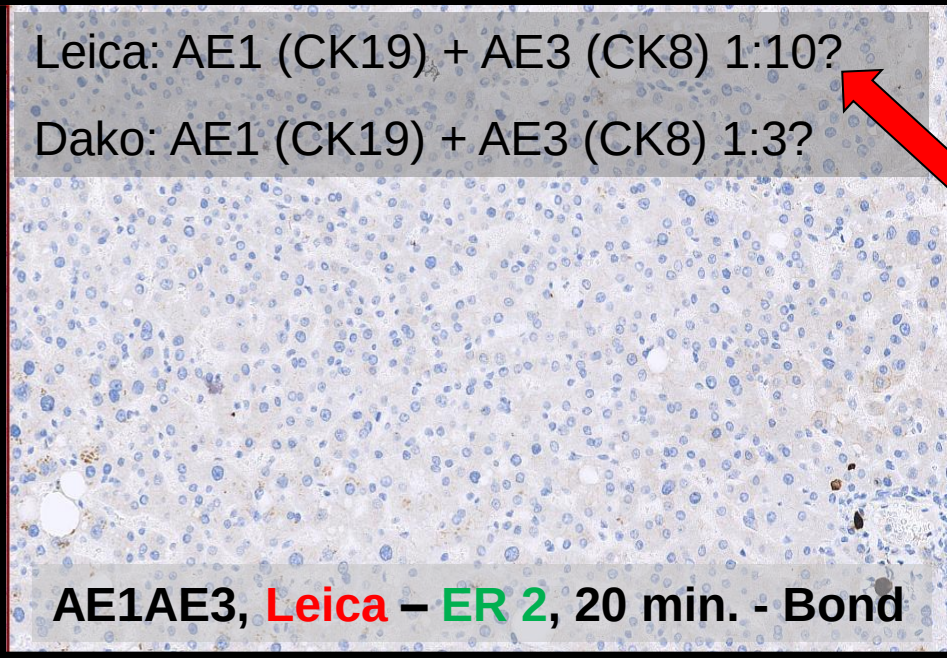
IHC - NordiQC 2016

Ready-To-Use antibodies								
mAb clone cocktail **AE1/AE3 IR053**	36	Dako/Agilent	28	5	2	1	92%	95%
mAb clone cocktail **AE1/AE3 GA053**	19	Dako/Agilent	18	0	1	0	95%	100%
mAb clone cocktail **AE1/AE3 313M-18**	3	Cell Marque	0	1	0	2	-	-
mAb clone cocktail **AE1/AE3 MAD 001000QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone cocktail **AE1/AE3 Kit-0009**	1	Maixin	1	0	0	0	-	-
mAb clone cocktail **AE1/AE3 PA0909**	5	Leica/Novocastra	0	1	3	1	20%	-
mAb clone cocktail **AE1/AE3 RTU-AE1/AE3**	2	Leica/Novocastra	0	0	2	0	-	-
mAb clone cocktail **AE1/AE3/5D3 IP162**	2	Biocare	1	1	0	0	-	-
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595**	62	Ventana/Roche	37	8	5	12	73%	96%
rmAb clone cocktail **EP24/EP67/B22.1/B23.1 MAD-000680QD**	2	Master Diagnostica	0	2	0	0	-	-
Total	275		132	65	43	35	-	
Proportion			48%	24%	16%	12%	72%	

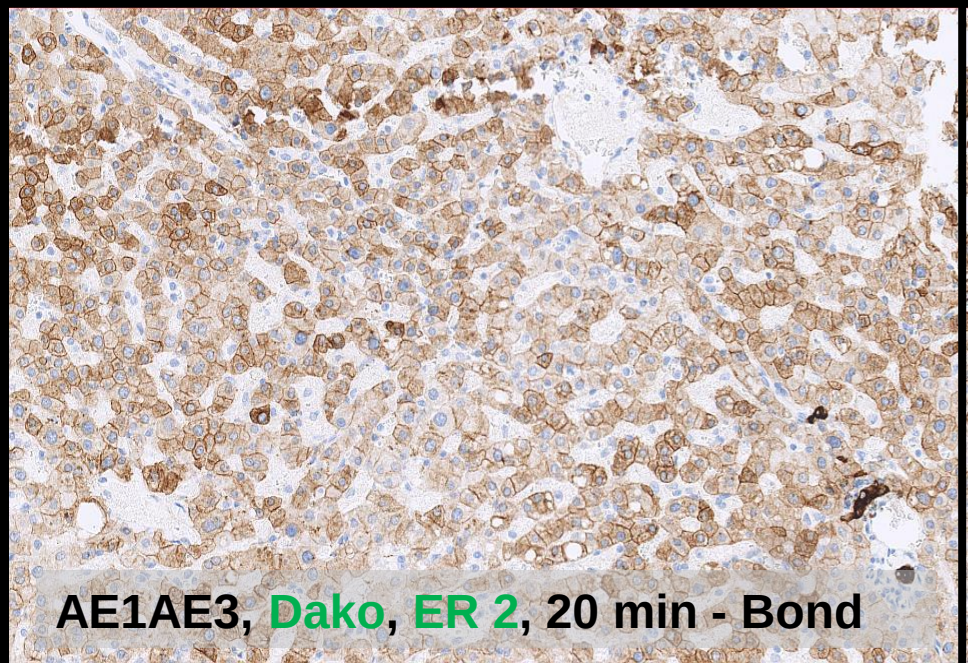
IHC – Controls and CSQI for the primary panel



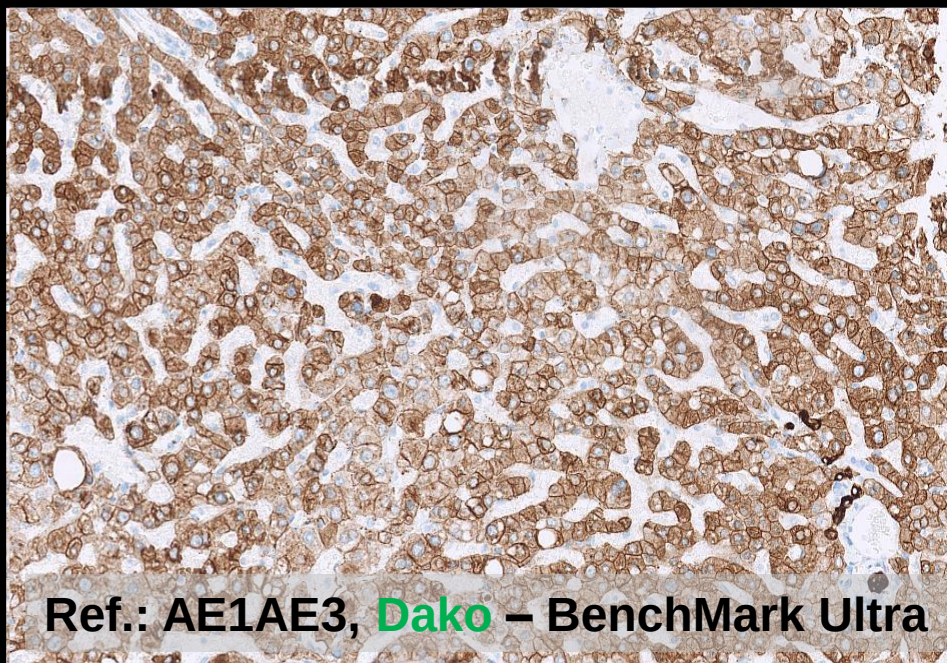
AE1AE3, Leica, Enzyme 1, 5 min - Bond



AE1AE3, Leica – ER 2, 20 min. - Bond



AE1AE3, Dako, ER 2, 20 min - Bond



Ref.: AE1AE3, Dako – BenchMark Ultra

Primary panel for the unknown primary tumour

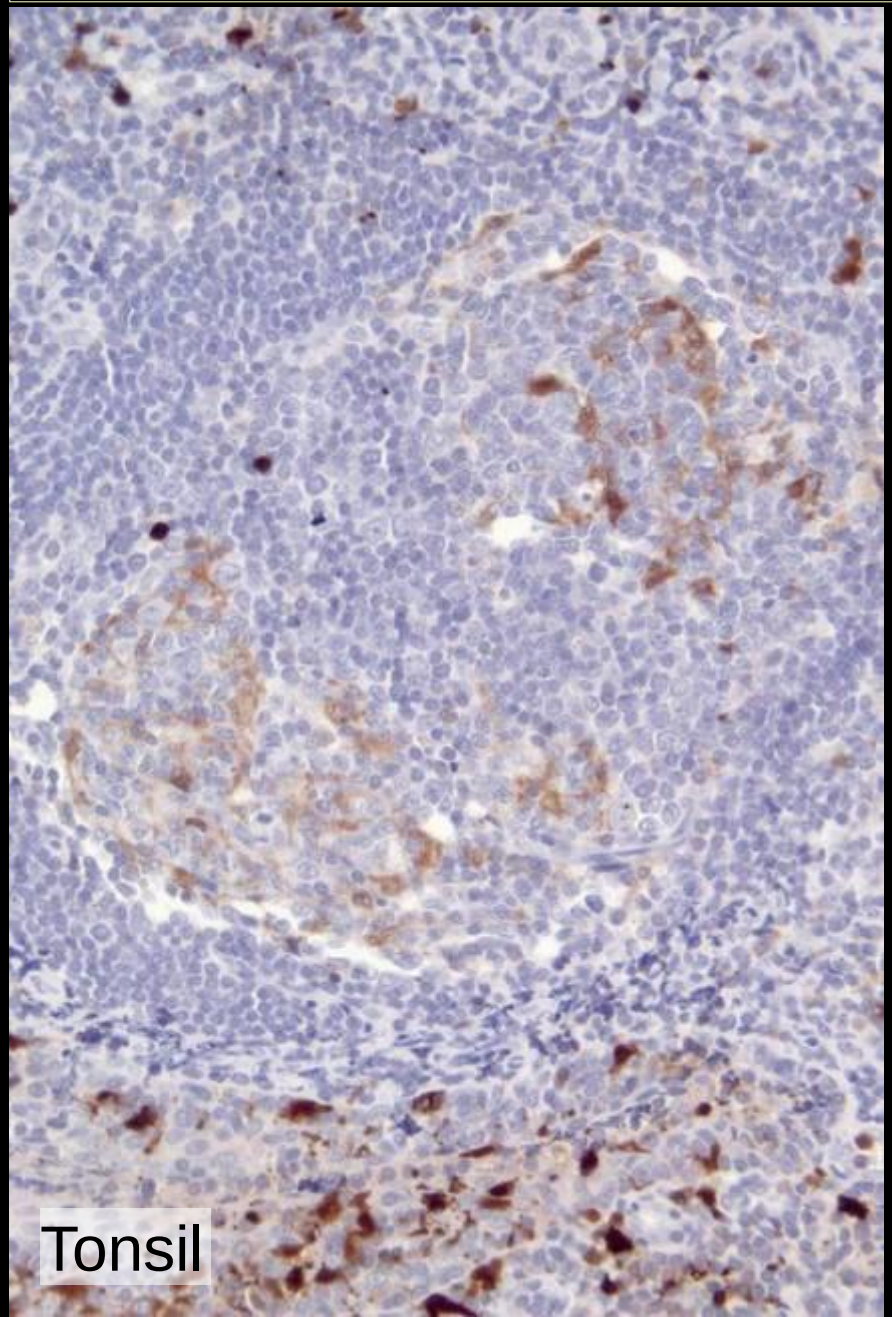
	CD45	CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

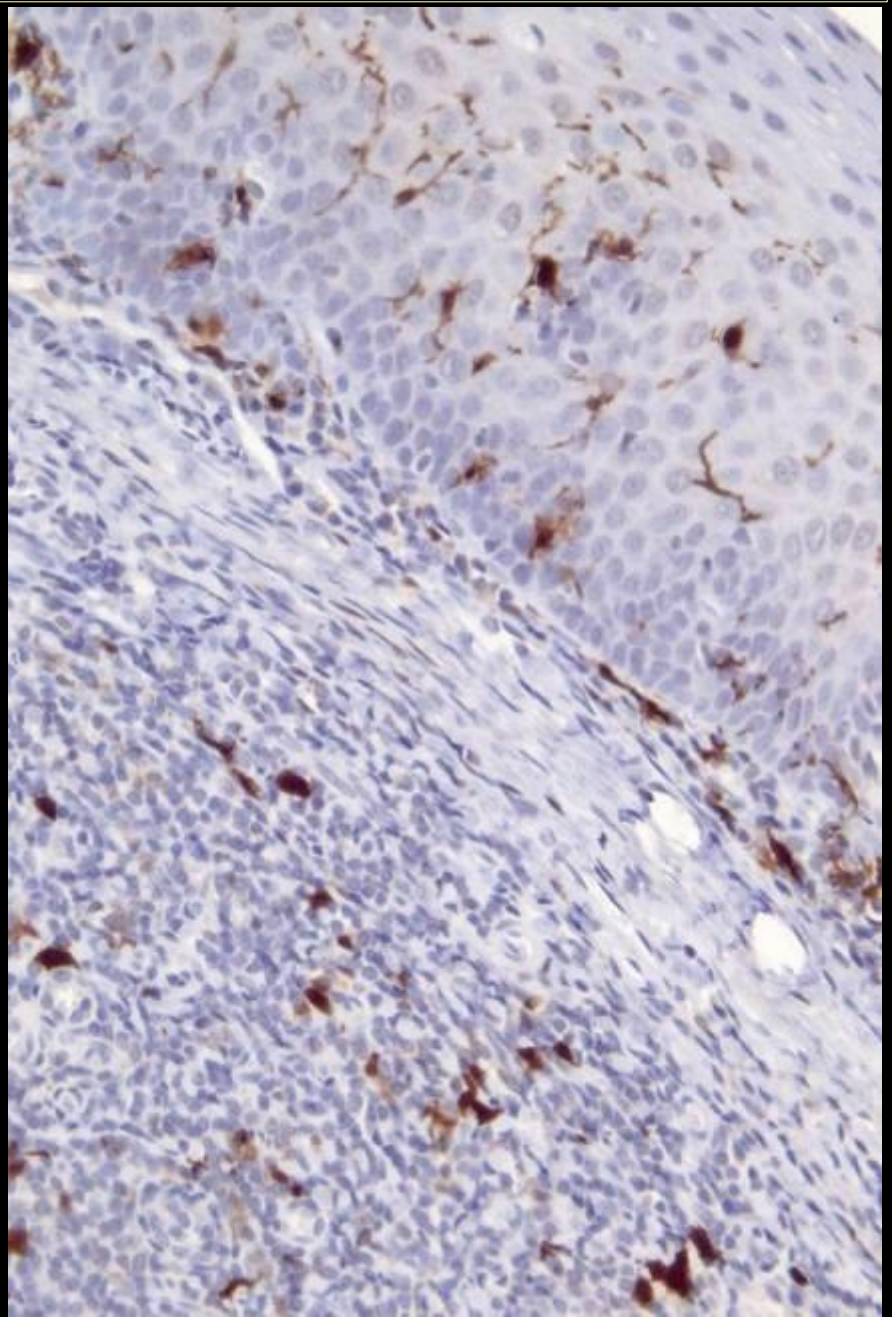
S-100 protein

- Family of acid calcium binding proteins 9/13 kDa
- Located in nuclei, cytoplasm and cell membranes
- at least 10 α-chains and one β-chain creating homo- and heterodimers

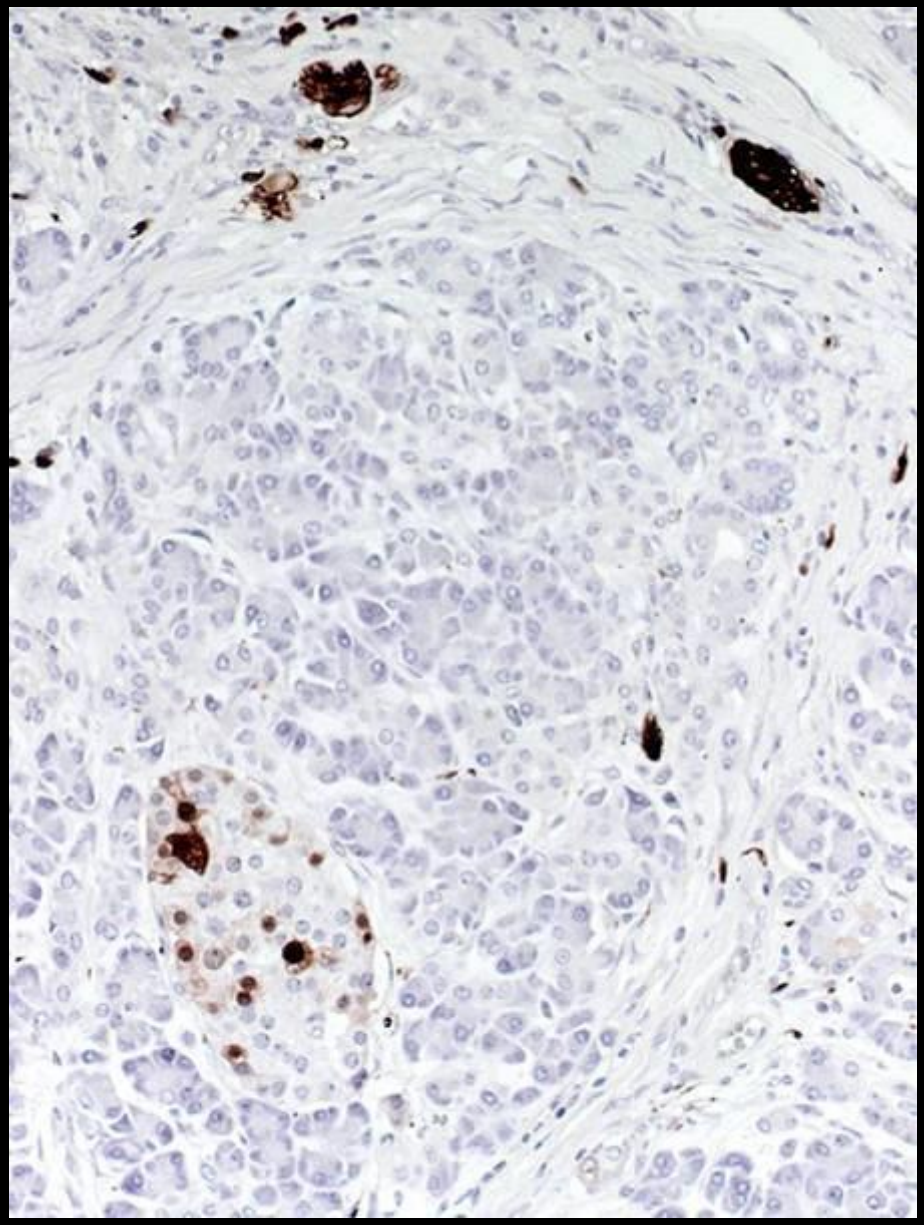
- S-100 β-chain mainly found in
  - Melanocytes
  - Glial cells
  - Langerhans' cells / interdigitating reticulum cells
  - Fat cells
  - Myoepithelial cells
- Polyclonal antibodies primarily detects the β-chain

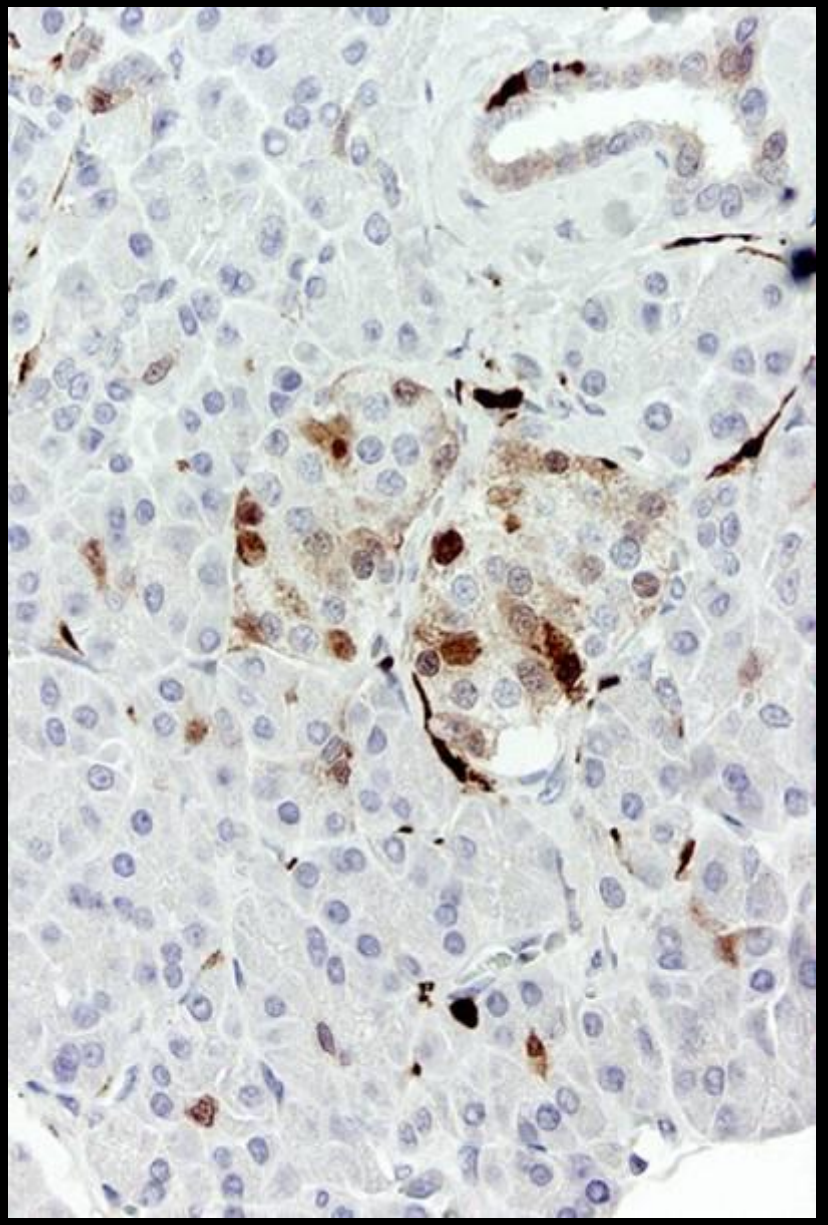
S-100 protein



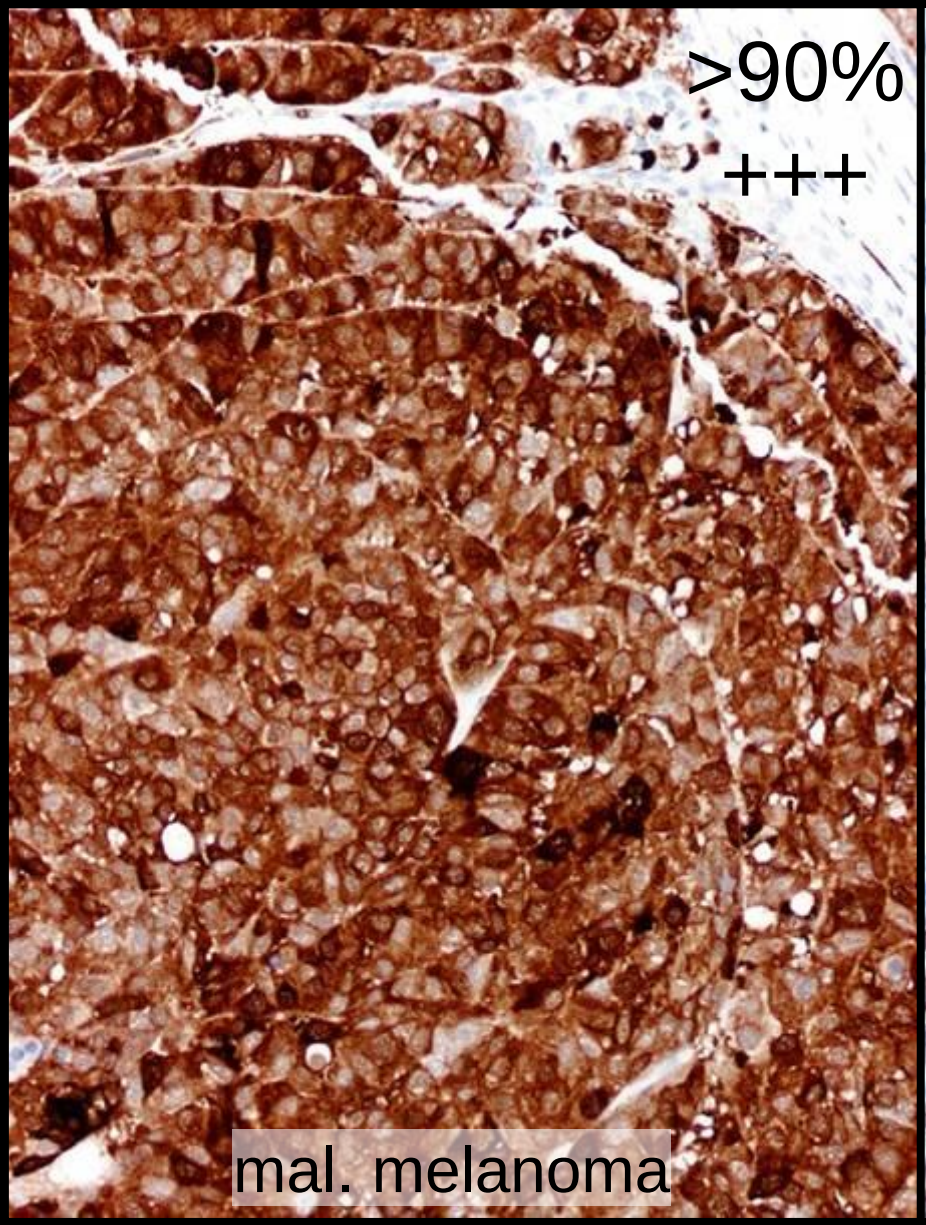


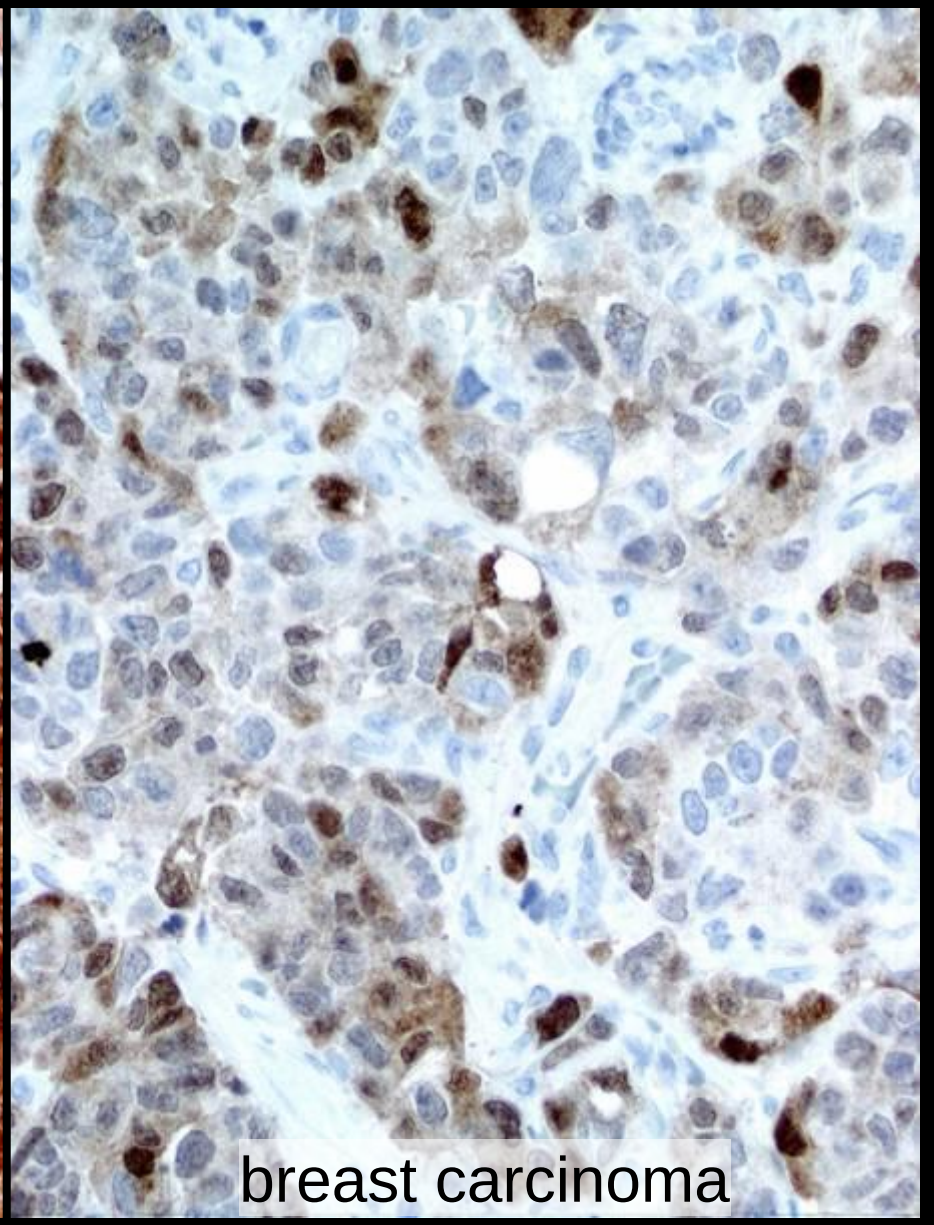
S-100 protein – pancreas





S-100 in malignant tumours





S100 run 52 2018: 299 labs

Table 1. **Antibodies and assessment marks for S100, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
pAb **Z0311**	137	Agilent/Dako	62	60	14	1	89%	97%
Total	299	Conc.+RTU	67	178	49	5	-	
Proportion			23%	59%	16%	2%	82%	

Table 4. **Proportion of sufficient and optimal results for S100 for the most commonly used RTU IHC systems**

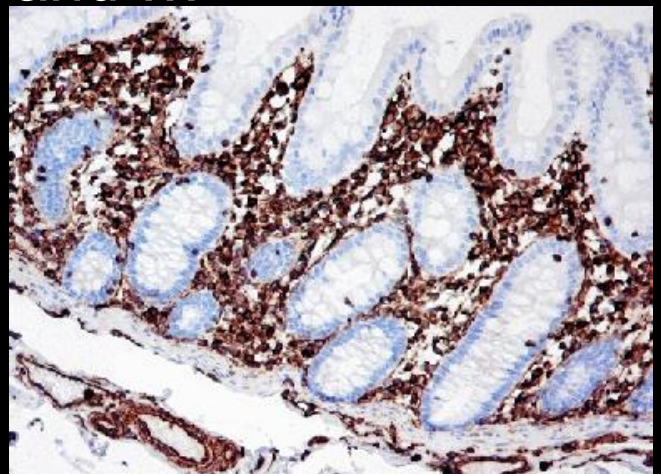
RTU systems 117 labs	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS pAb **IS/IR504**	80% (8/10)	0% (0/10)	88% (14/16)	0% (0/16)
Dako Omnis pAb **GA504**	100% (15/15)	7% (1/15)	83% (5/6)	0% (0/6)
Leica BOND MAX/III pAb **PA0900**	0% (0/0)	0% (0/0)	100% (6/6)	0% (0/6)
VMS Ultra/XT pAb **760-2523**	100% (6/6)	0% (0/6)	77% (17/22)	0% (0/22)
VMS Ultra/XT mAb 4C4.9 **790-2914**	33% (1/3)	0% (0/3)	58% (19/33)	0% (0/33)

Primary panel for the unknown primary tumour

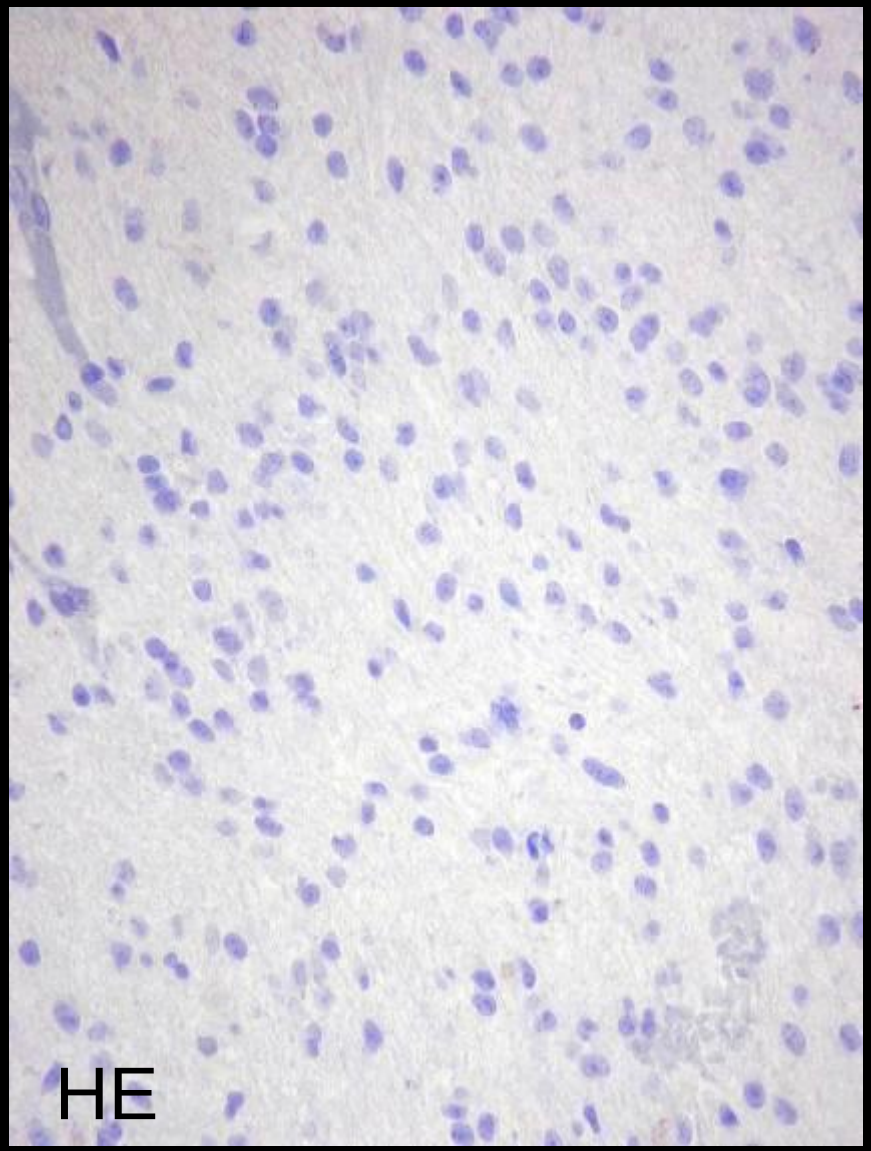
”Real”	CD45	CK	S-100	VIM
Haemato-lymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
Mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
Non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

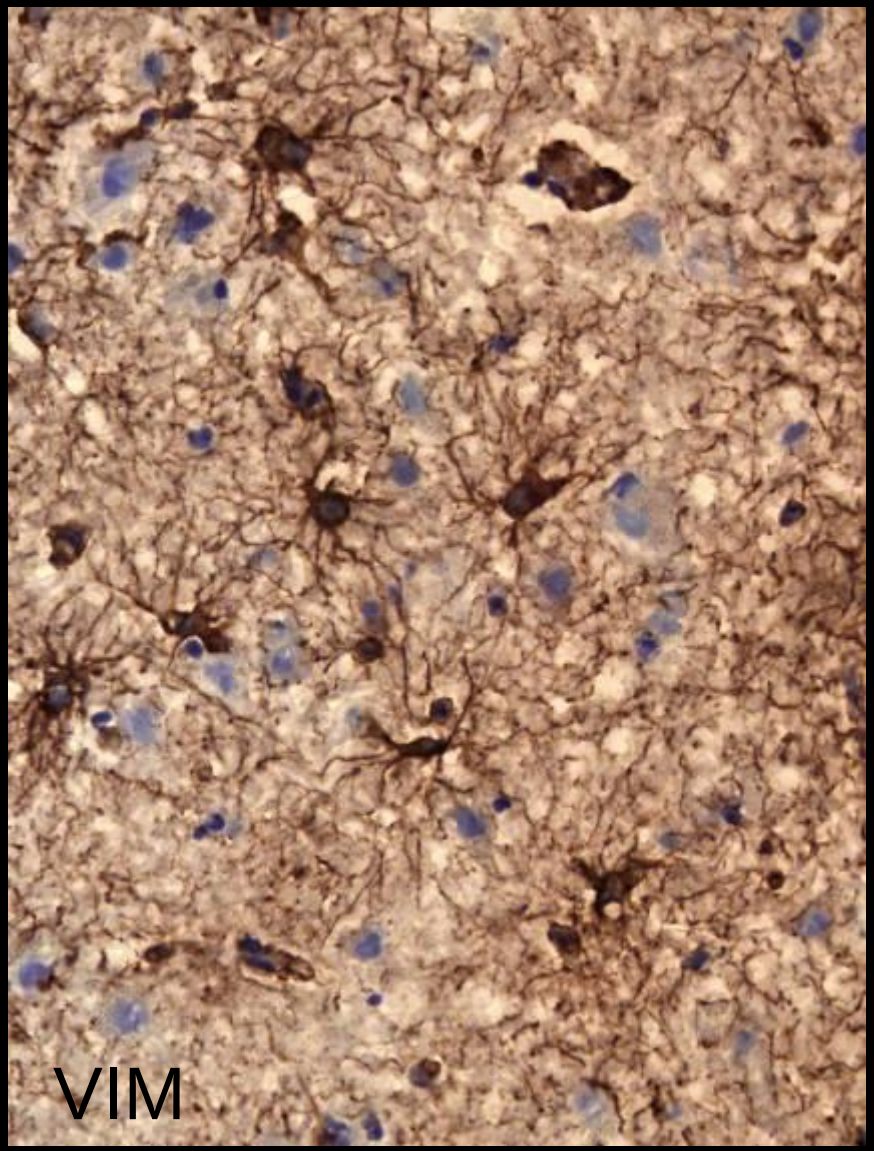
Vimentin

- Cytoplasmic intermediate filament, 57 kDa
- Present in all mesenchymal cells
- Present in early stages of all cells, replaced by other intermediate filaments in most non-mesenchymal cells
- Coexpressed with cytokeratin in some epithelia
  - Endometrium, renal tubules, thyroid gland …
- Coexpressed with cytokeratin in some non-epithelial cells
  - Mesothelium



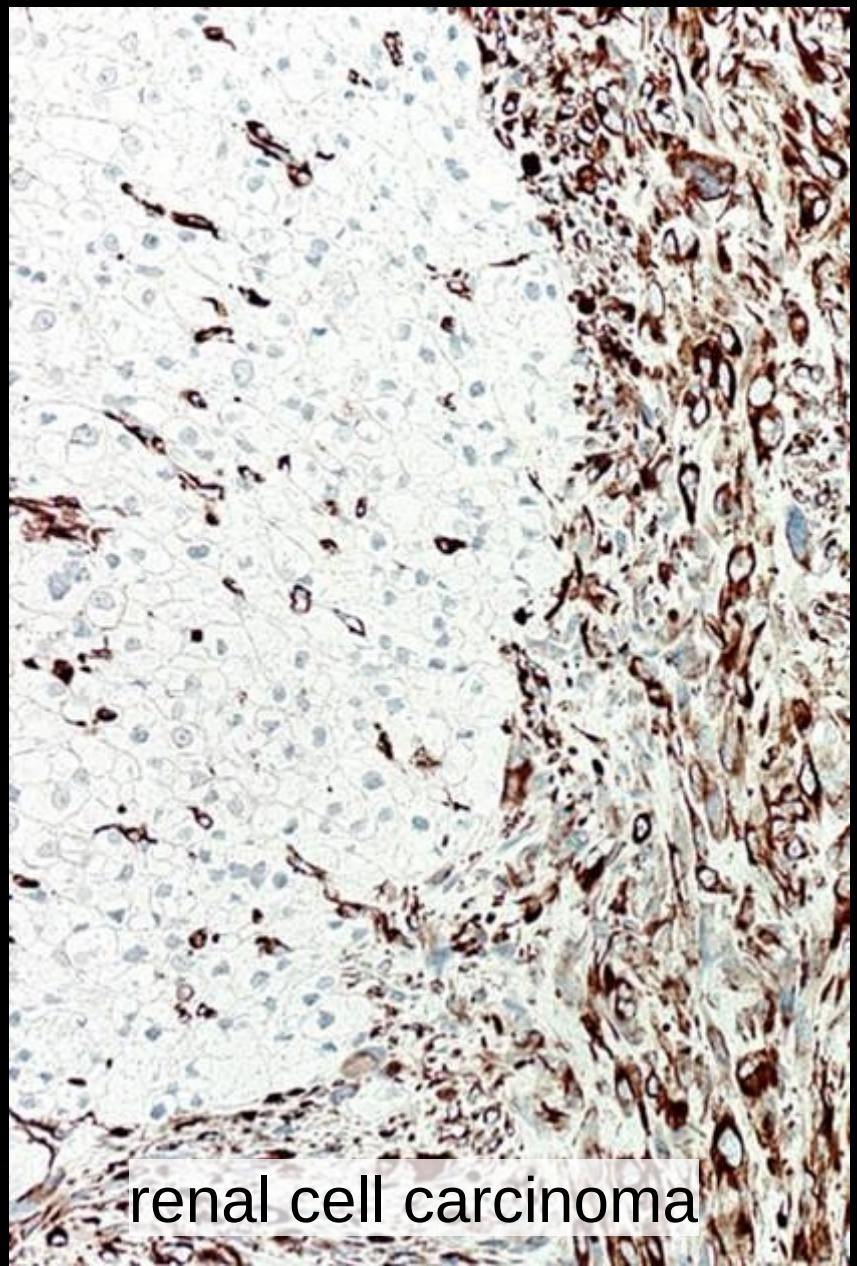
Vimentin in normal tissue



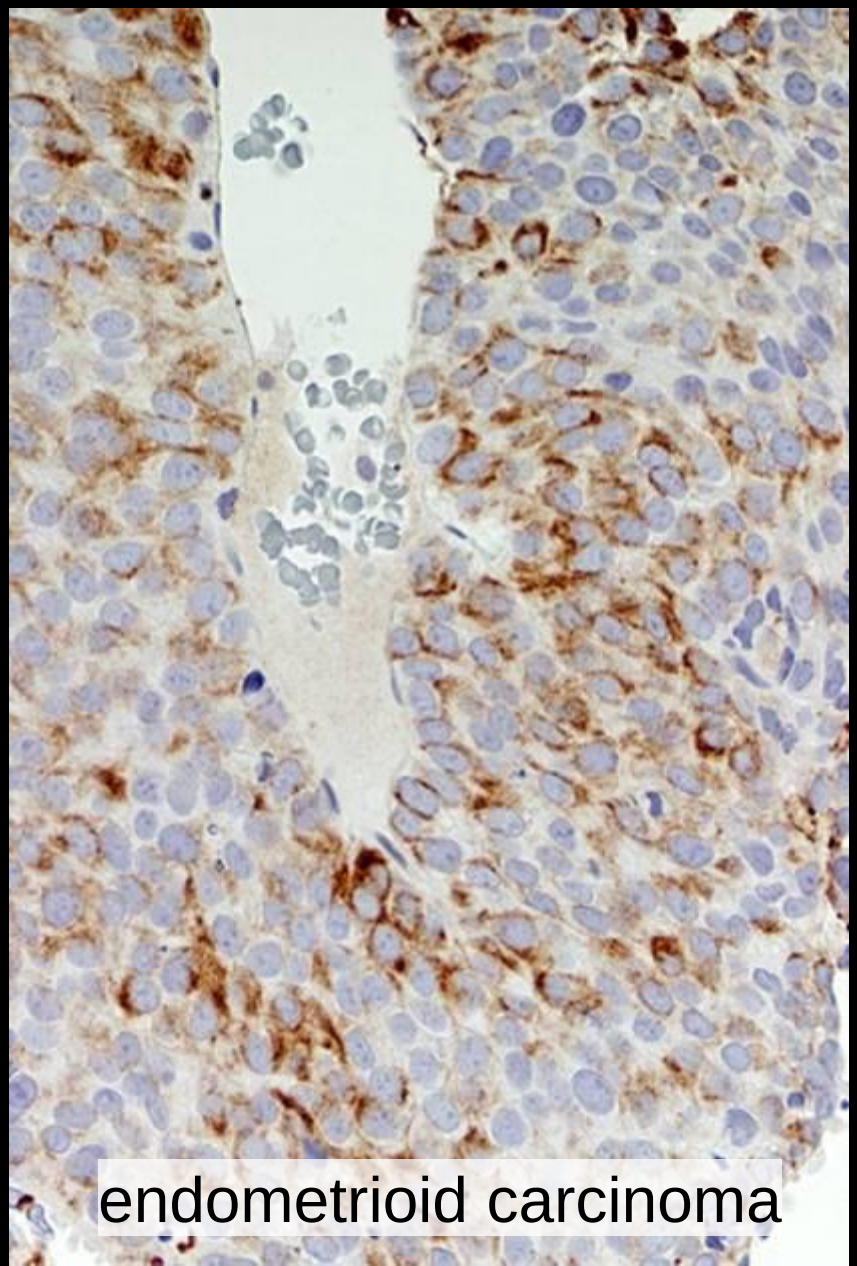


Normal brain

Vimentin in carcinomas

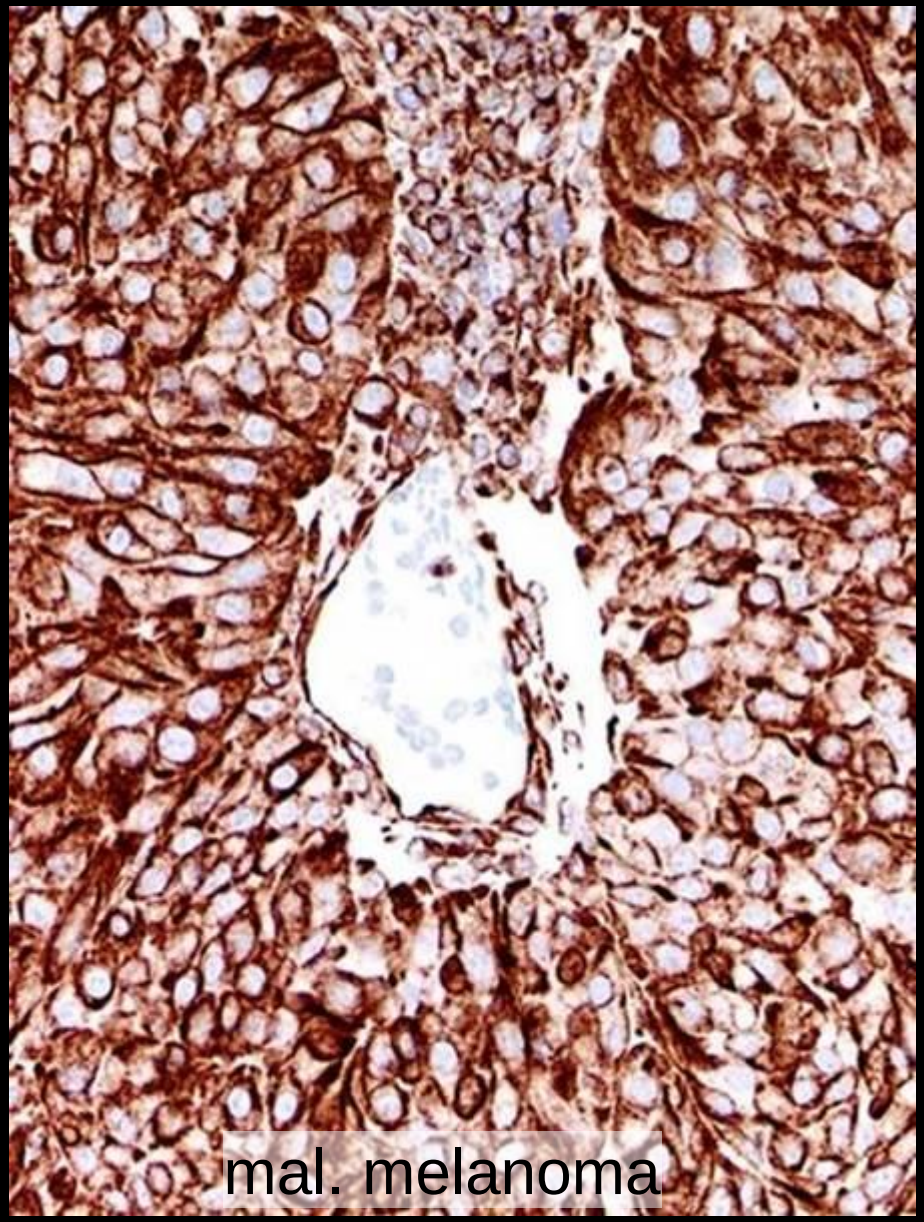


renal cell carcinoma

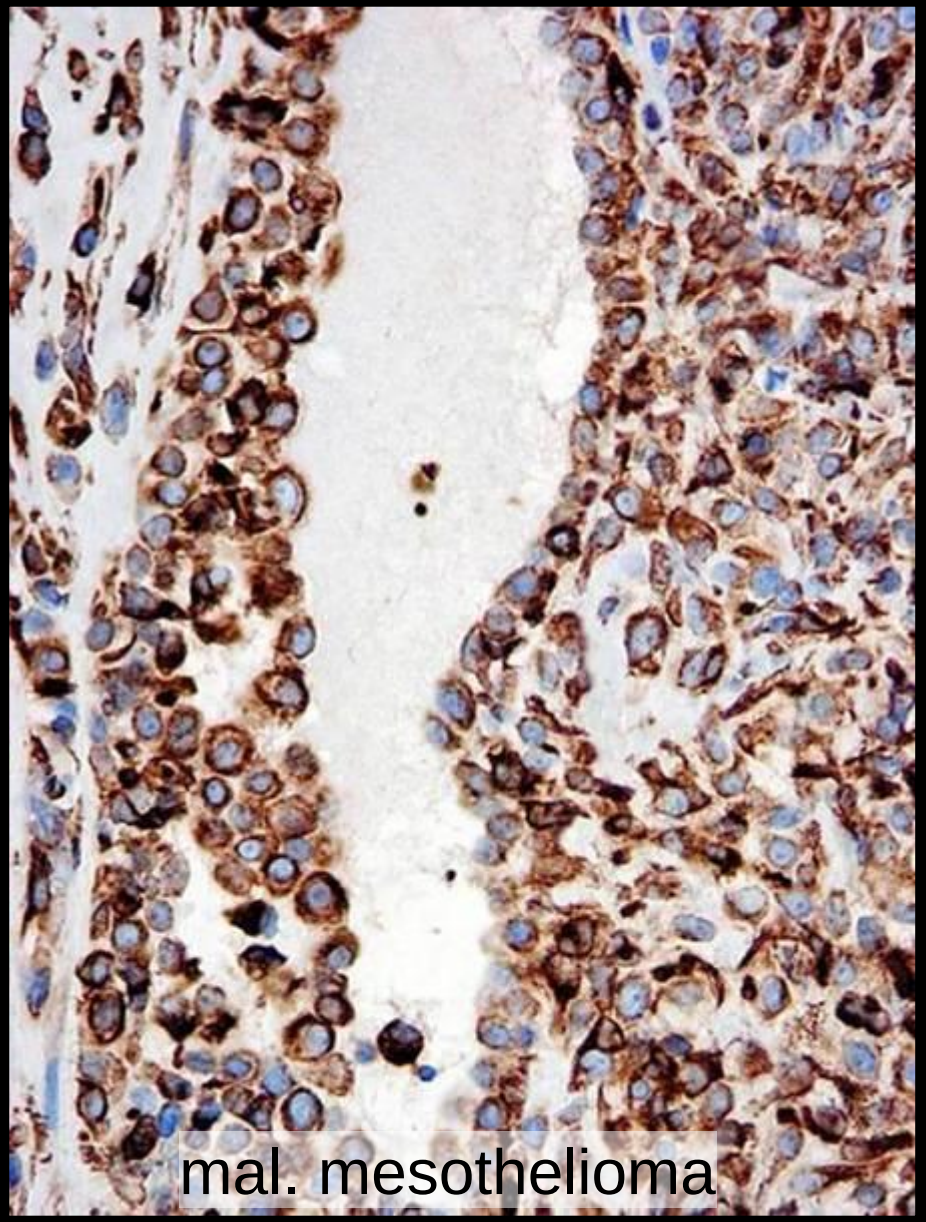


endometrioid carcinoma

Vimentin in non-epithelial tumours



mal. melanoma



mal. mesothelioma

Vimentin run 52 2018: 308 labs

RTUs

Table 4. **Proportion of sufficient and optimal results for VIM for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
159 labs	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/III mAb V9 **PA0640**	3/3	2/3	4/4	3/4
Dako AS mAb V9 **IR630**	92% (11/12)	92% (11/12)	88% (15/17)	82% (14/17)
Dako Omnis mAb V9 **GA630**	100% (16/16)	100% (16/16)	64% (7/11)	45% (5/11)
VMS Ultra/XT/GX mAb V9 **790-2917**	1/1	0/1	72% (71/99)	21% (21/99)

NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 19th–21st 2018

The unknown primary tumour: IHC classification – part I, the primary panel

- Antibody selection, protocol optimization, controls and EQA

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NORTH DENMARK REGION