Pathological diagnosis
Molecular Pathology

Prof. Dr. András Kiss Med. habil., Ph.D., D.Sc.

2nd Department of Pathology

2021. November

Macroscopic pathology


JO. BAPTISTÆ
MORGAGNI
P. P. P. P.
DE SEDIBUS, ET CAUSIS
MORBORUM
PER ANATOMEN INDAGATIS
LIBRI QUINQUE.
DISSECTIONES, ET ANIMADVERSIONES, NUNC PRIMUM EDITAS
COMPLECTUNTUR PROPEMODUM INNUMERAS, MEDICIS,
CHIRURGIS, ANATOMICIS PROFUTURAS.
Multiplex præfixus est Index rerum, & nominum
accuratissimus.
TOMUS PRIMUS
DUOS PRIORES CONTINENS LIBROS.
VENETIIS,
MDCCLXI.
EX TYPOGRAPHIA REMONDINIANA.
SUPERIORUM PERMISSU, AC PRIVILEGIO.

Rokitansky- Vienna
1840
Pathologic Anatomy

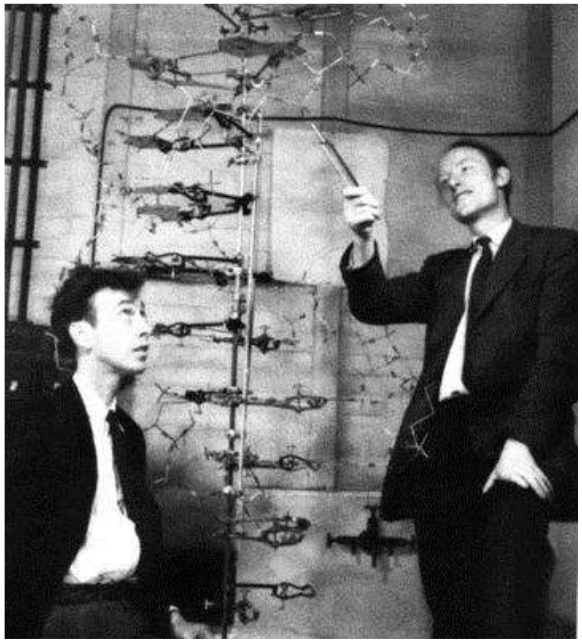
Lobar and broncho-pneumonia

Morgagni,1761

GI diseases

Virchow,1858

„Zellularpathologie”



Watson & Creek, 1953

DNA structure

KOMPLEXITY of Tumor THERAPY, TEAM WORK – systemic

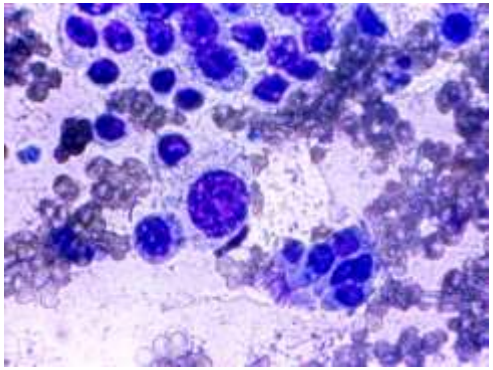
AAA: **A**micability
Availability
Affinity

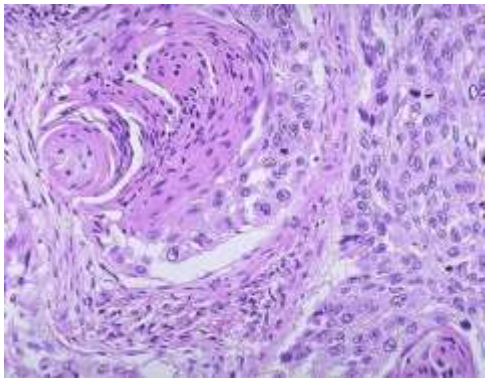
P4

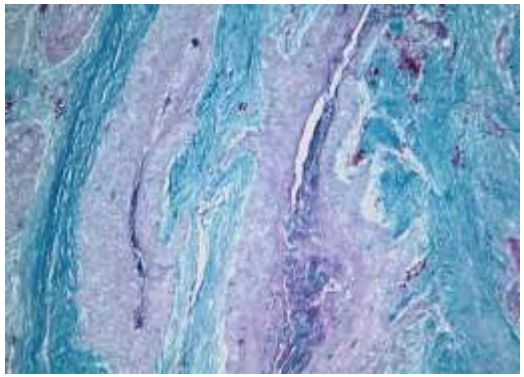
Predictive
Preventive
Personalized
Participatory

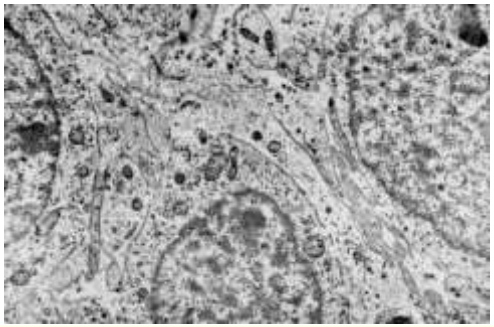
A XX. century technologies

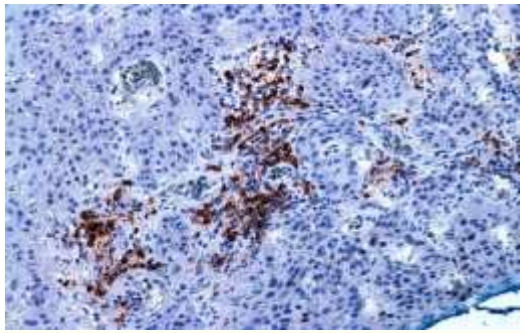
- Macroscopy (Grossing)
- Citology
- Histology
- Citochemistry
- Immuncitochemistry
- Electronmicroscopy
- Molecular biology
- Molecular genetics
- XXI. century.

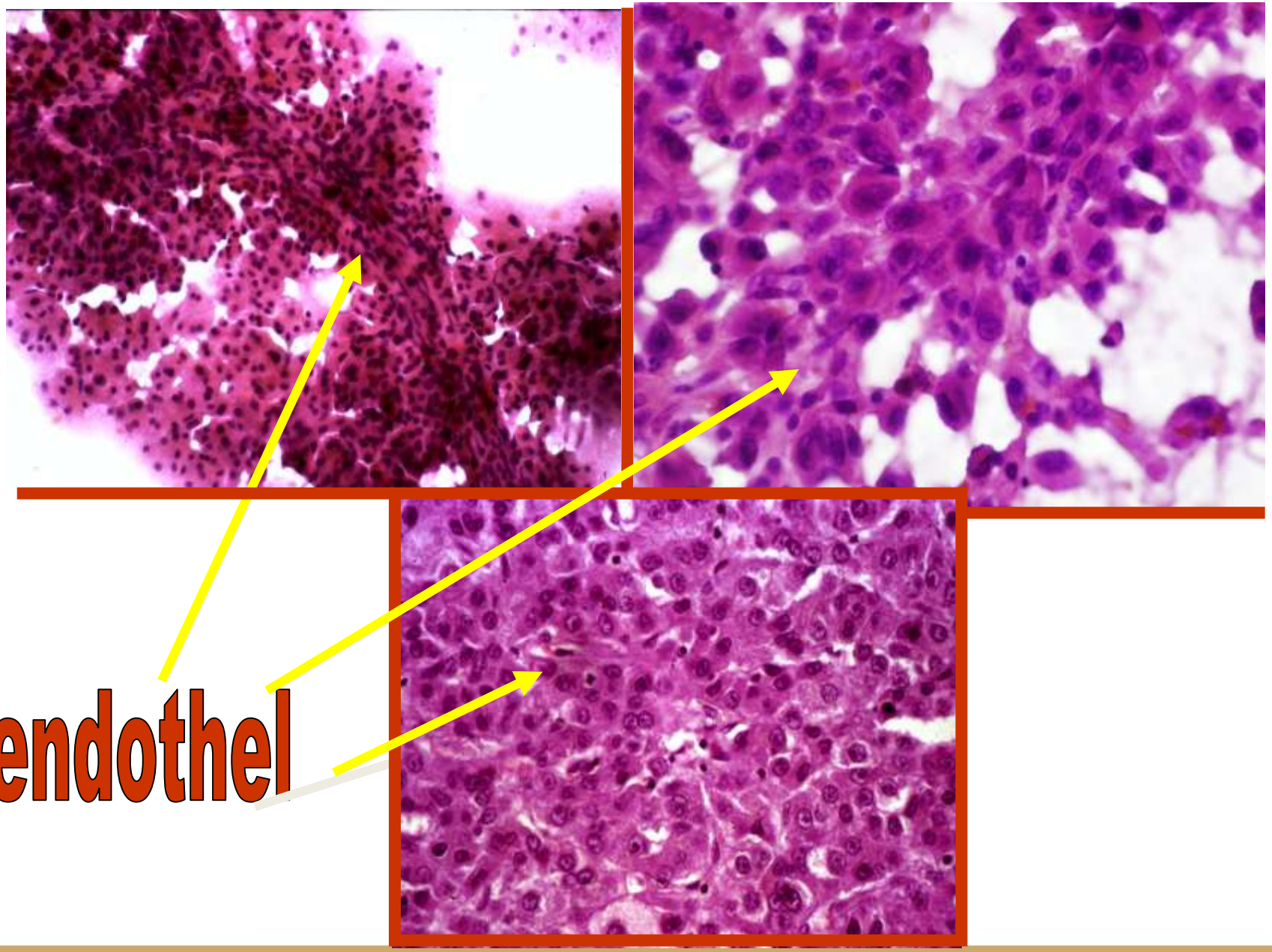


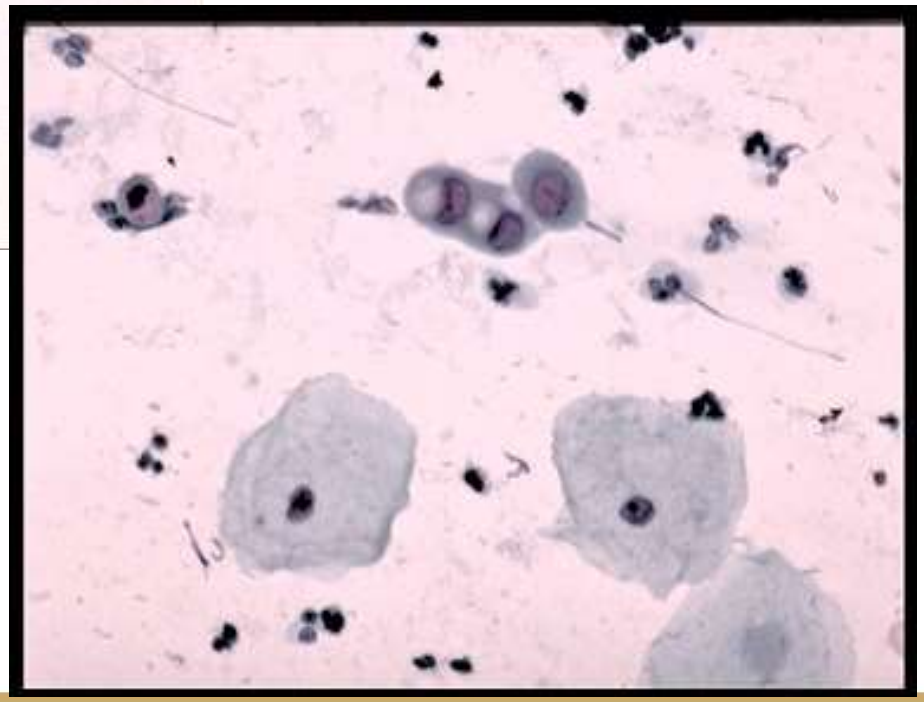


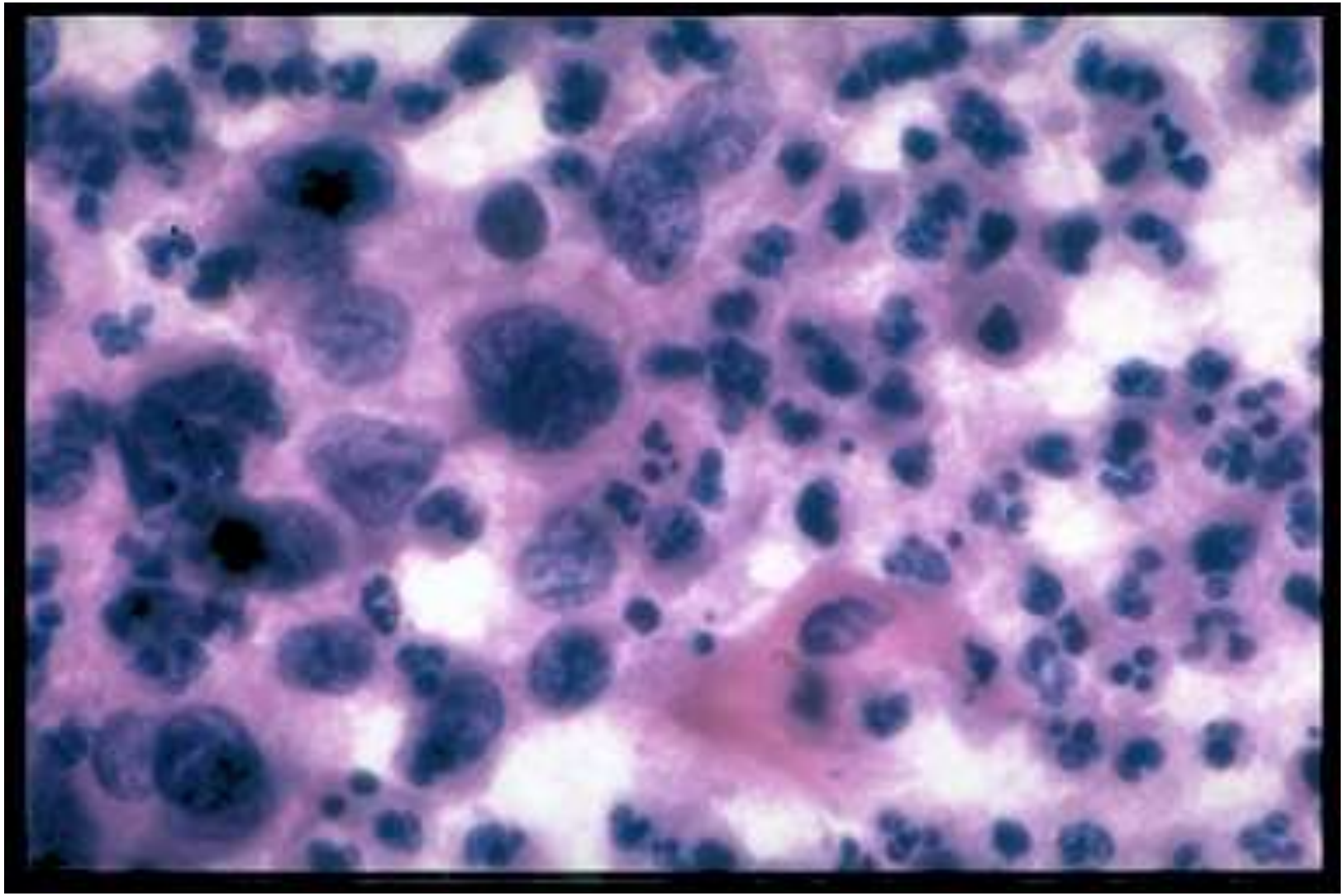


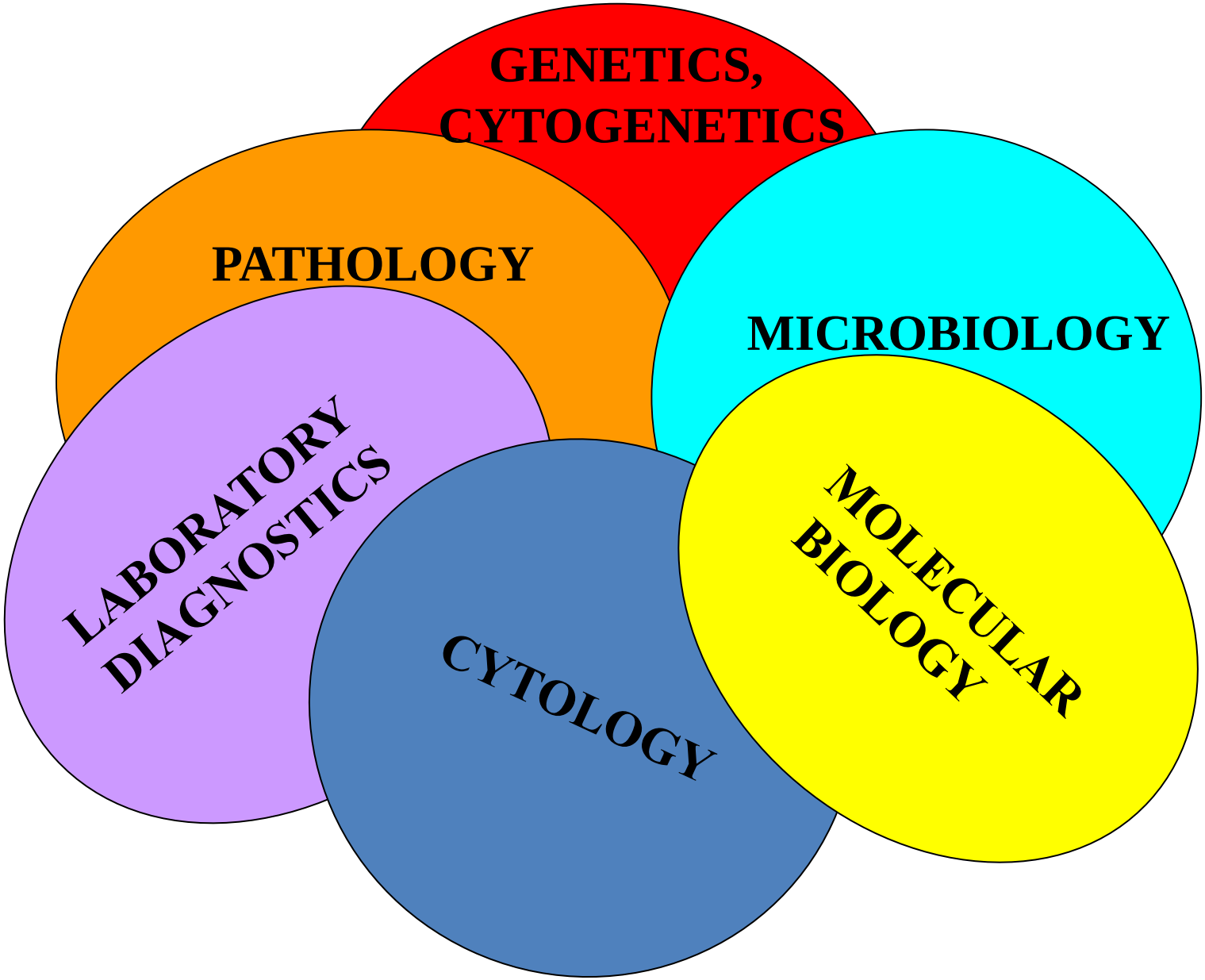





endothel






GENETICS,
CYTOGENETICS
PATHOLOGY
MICROBIOLOGY
LABORATORY
DIAGNOSTICS
CYTOLOGY
MOLECULAR
BIOLOGY

INVESTIGATION of FFPE materials

HE

Special stainings

Immunhistochemistry

In situ hibridisation

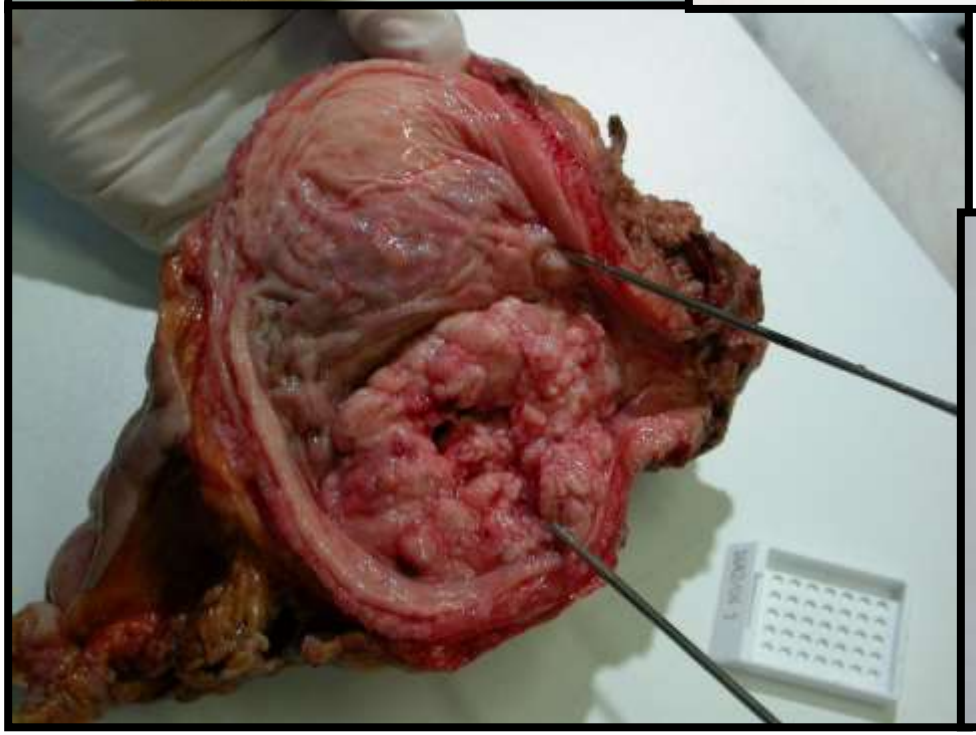
(FISH, CISH, SISH)

DNS based technology

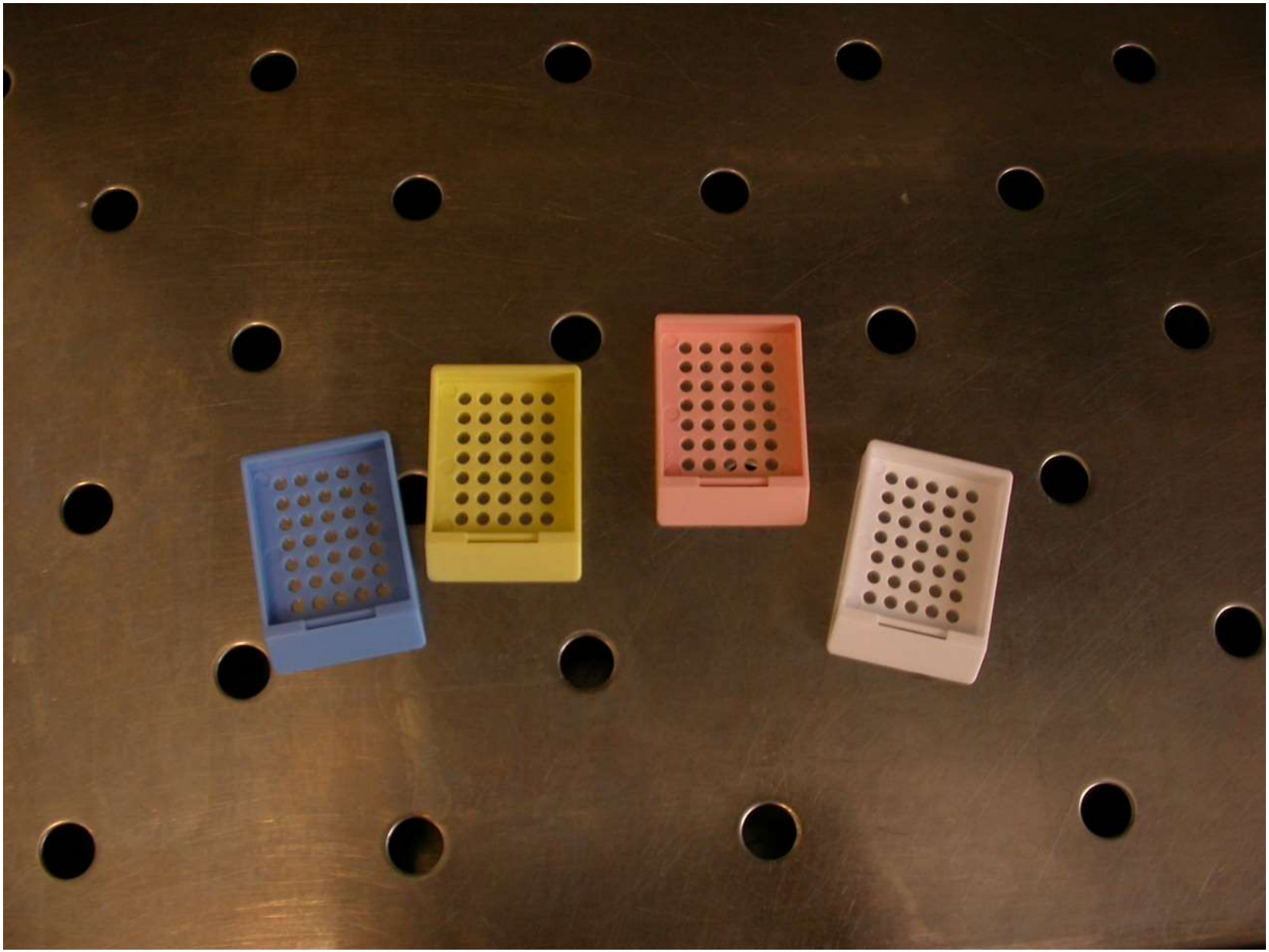
RNS based technology

Protein-based technology

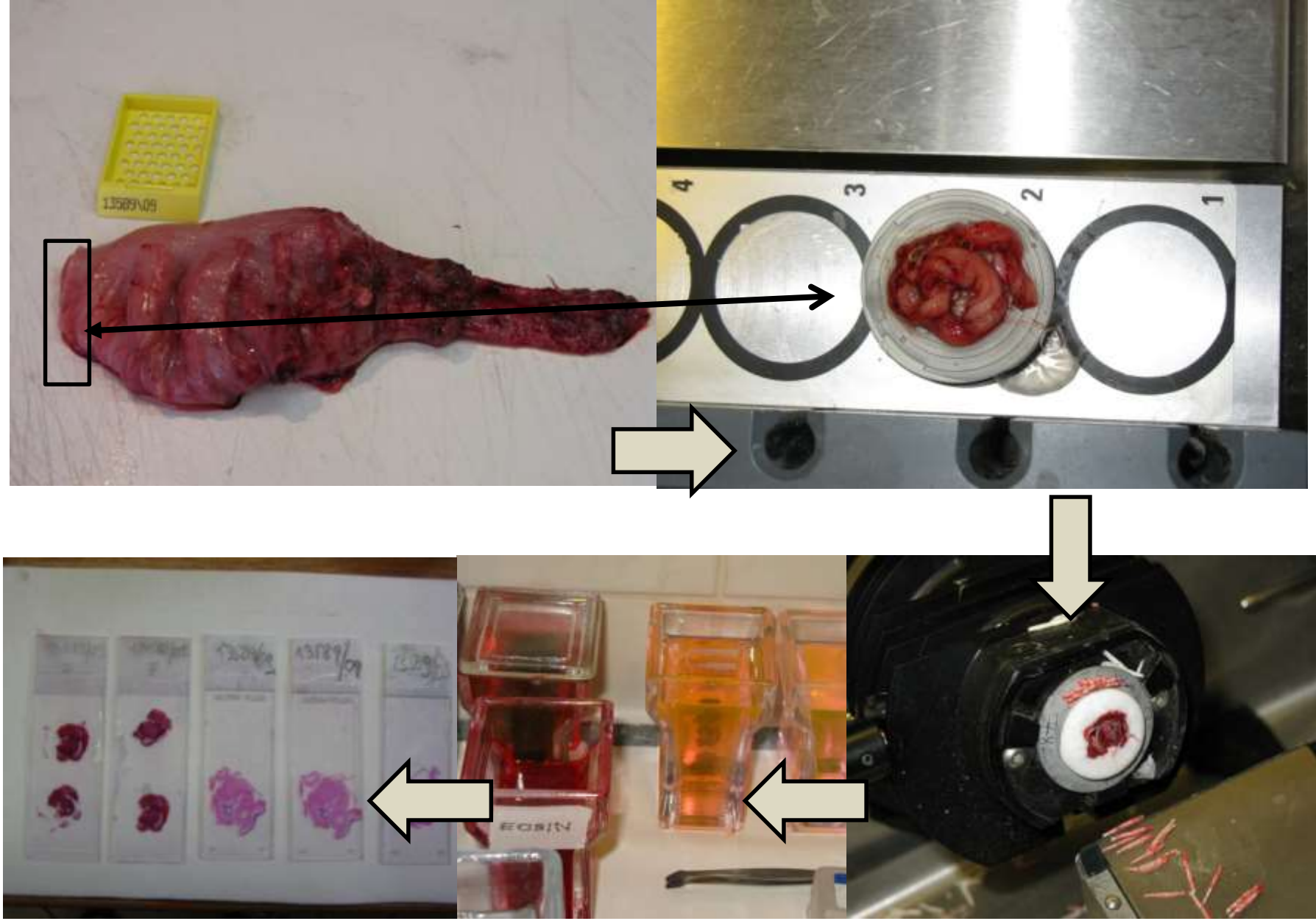

Shandon



Grossing




21:27
24.2°C


4
3
2
1
EOSIN

WAY of SURGICAL RESECTION SPECIMENS

OP Theatre

OPTIMAL TEMPERATURE (20-22°C) | TIME FACTOR (15 min. – 1 hour - hours)

↓

Pathology Dept.

↓

Fixation (24 hrs, 10% buffered formalin

↓

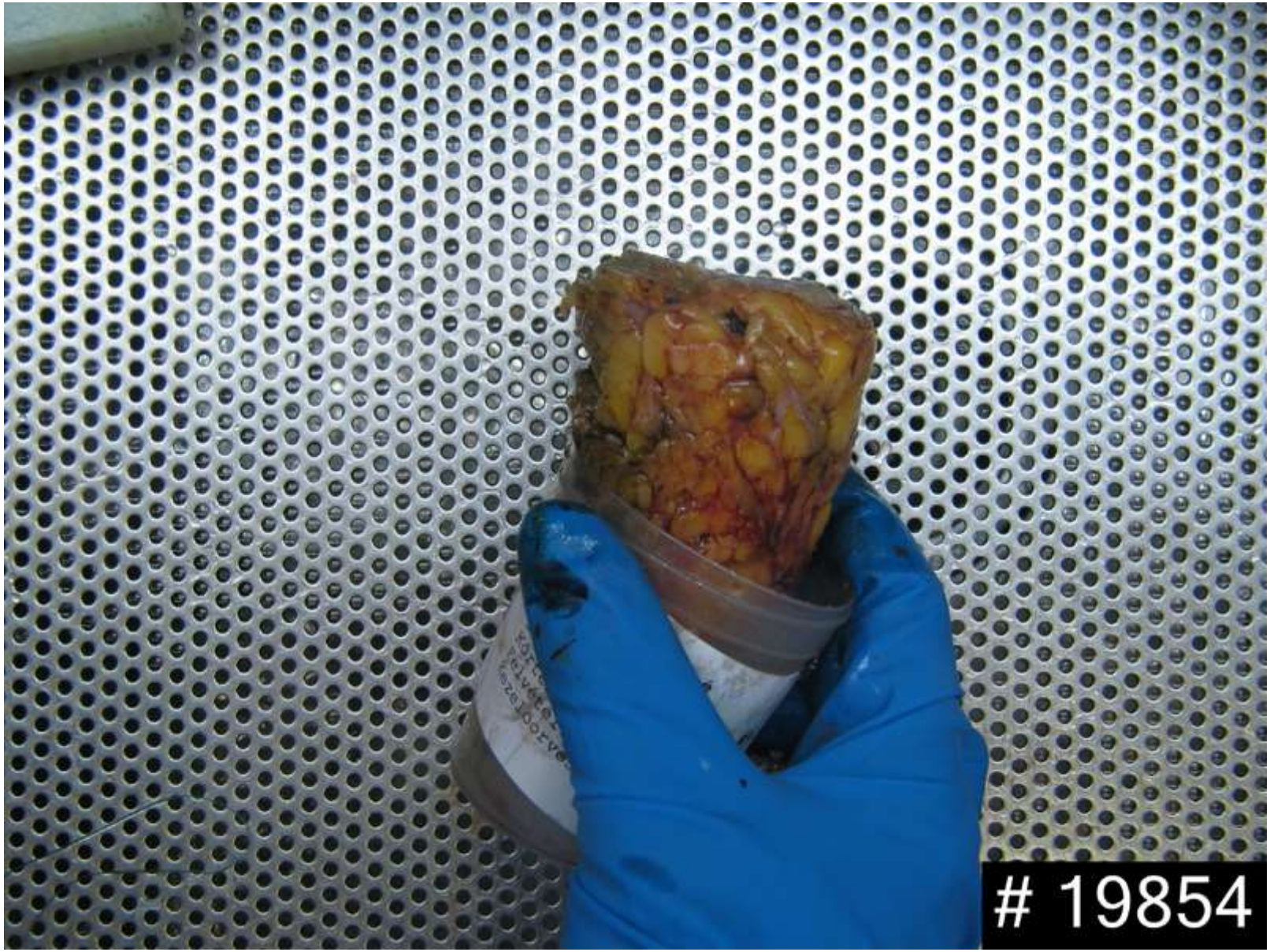
Grossing

↓

Paraffin Embedding

↓

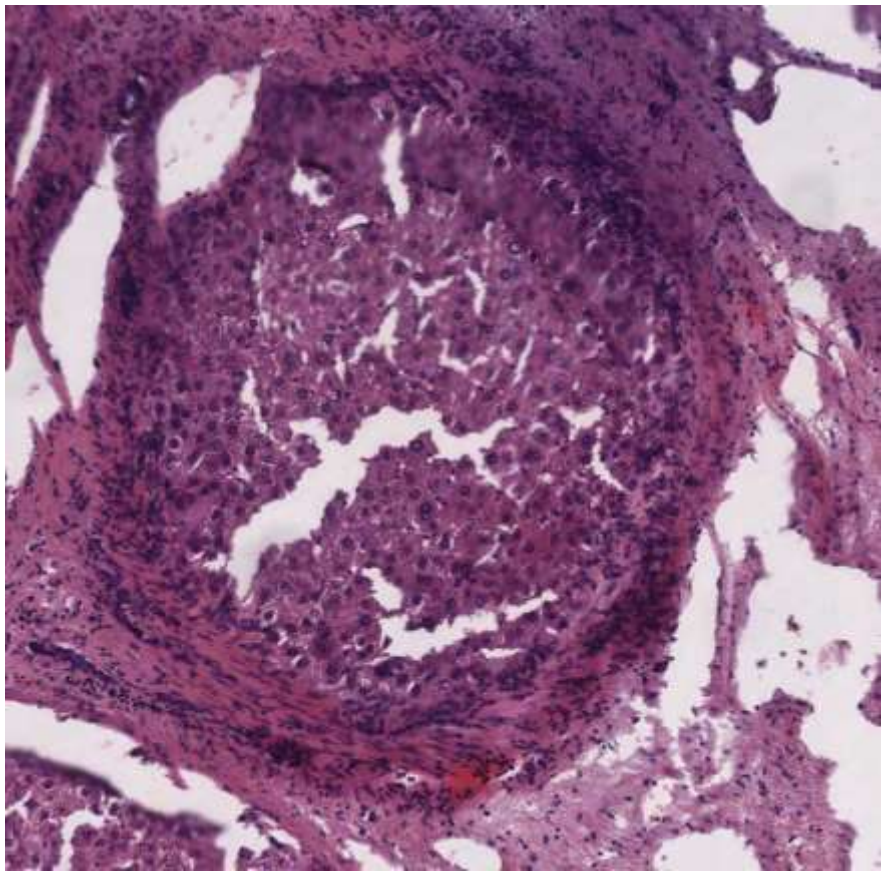
Diagnosis


19854

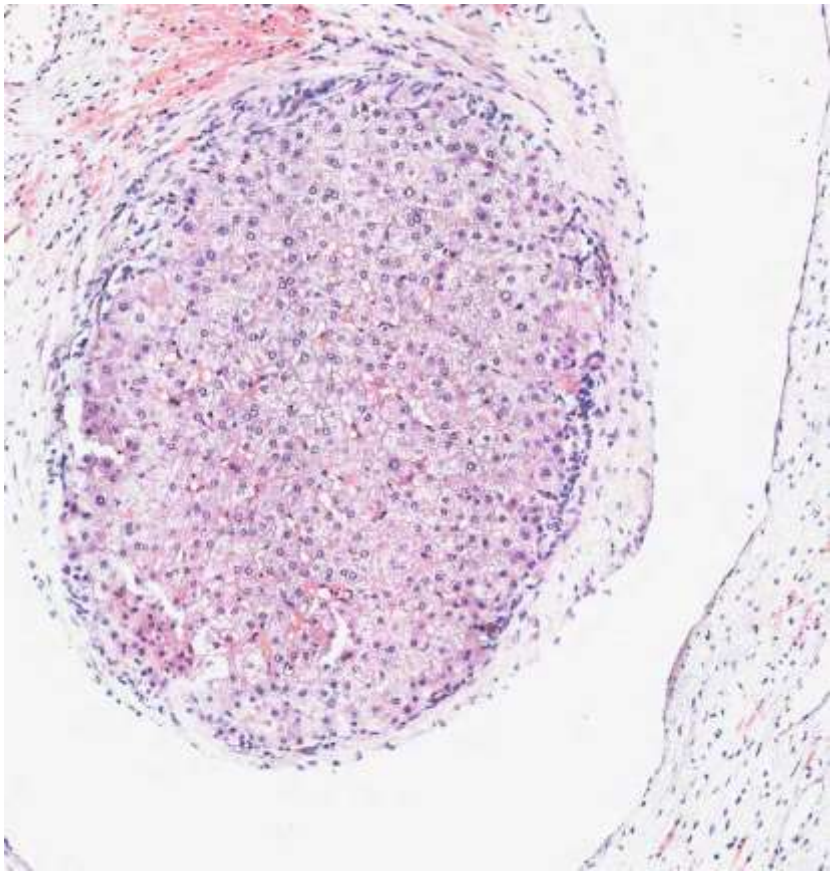

OSPEDALE MAGGIORE DI S GIOVANNI BATTISTA
DELLA
CITTA DI TORINO

Liver specimen. Immersed in Formalin. 24 h.

Inner

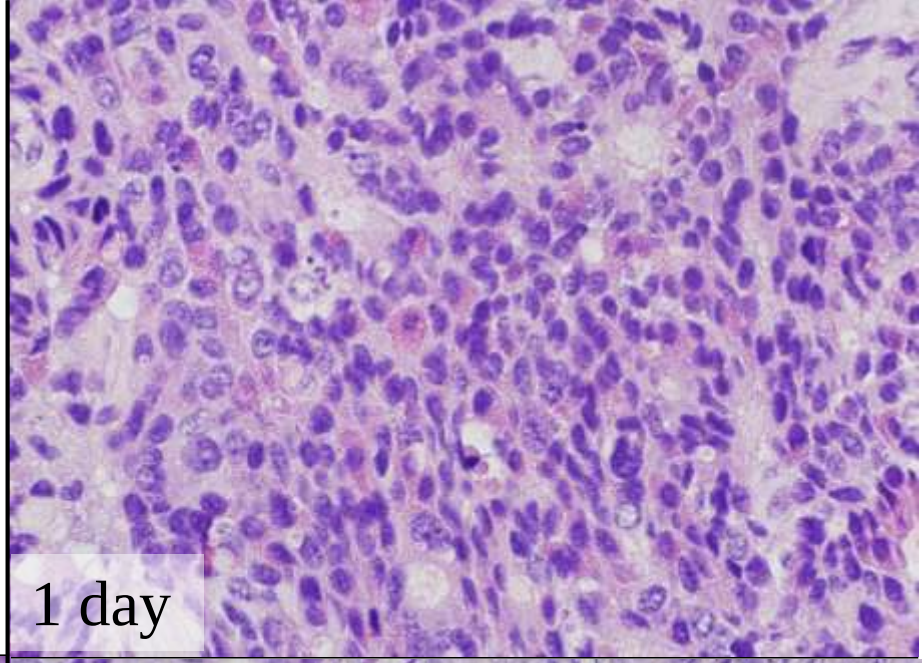


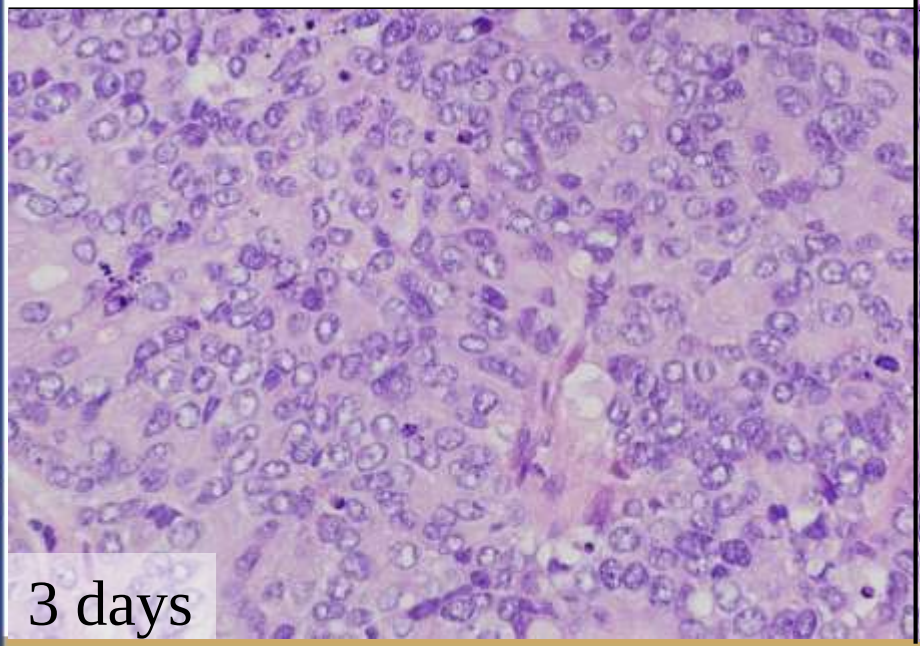
Outer

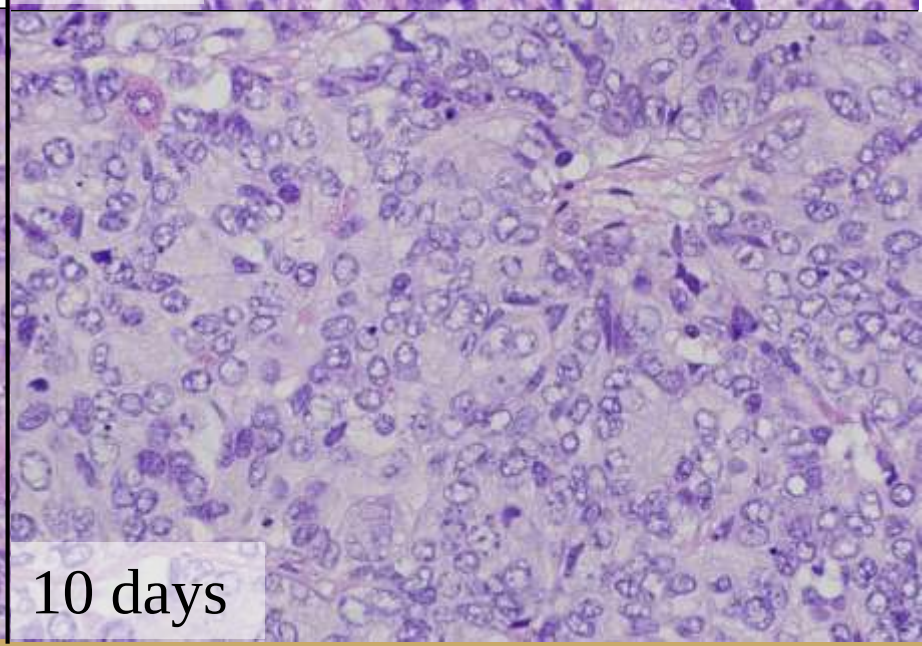


Liver specimen. Immersed in Formalin. 24 h.

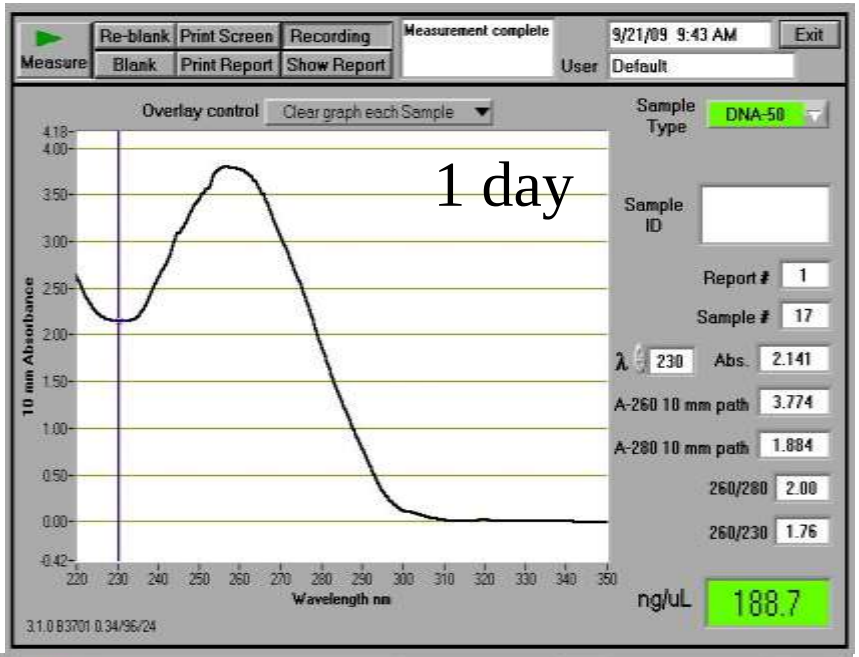
Effect of fixation time on the tissue structure

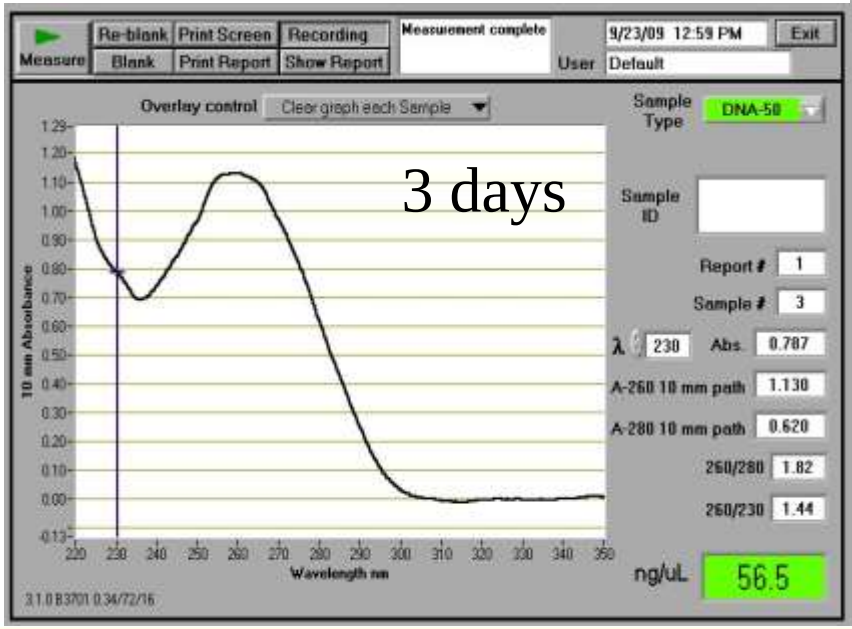


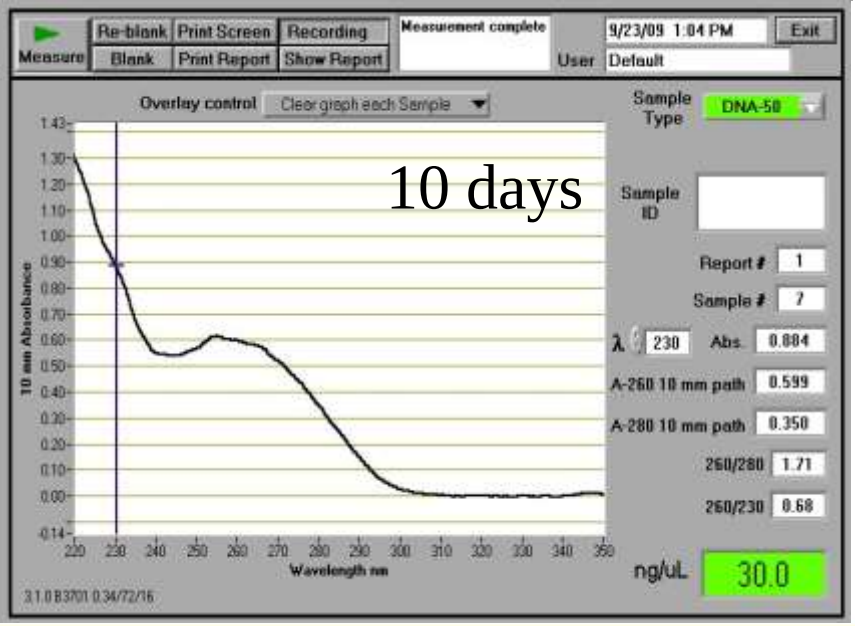




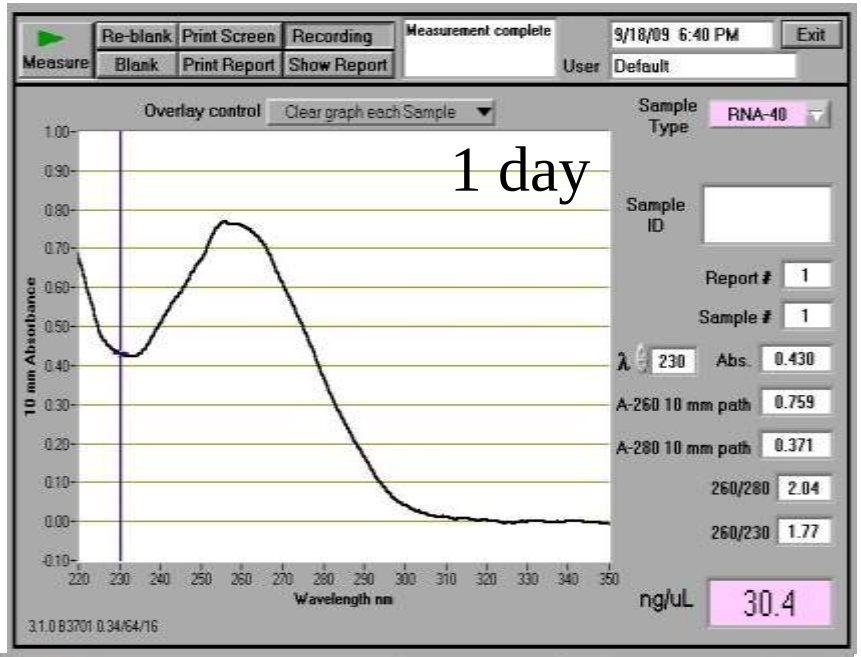
Effect of fixation time on the quality of isolated DNA

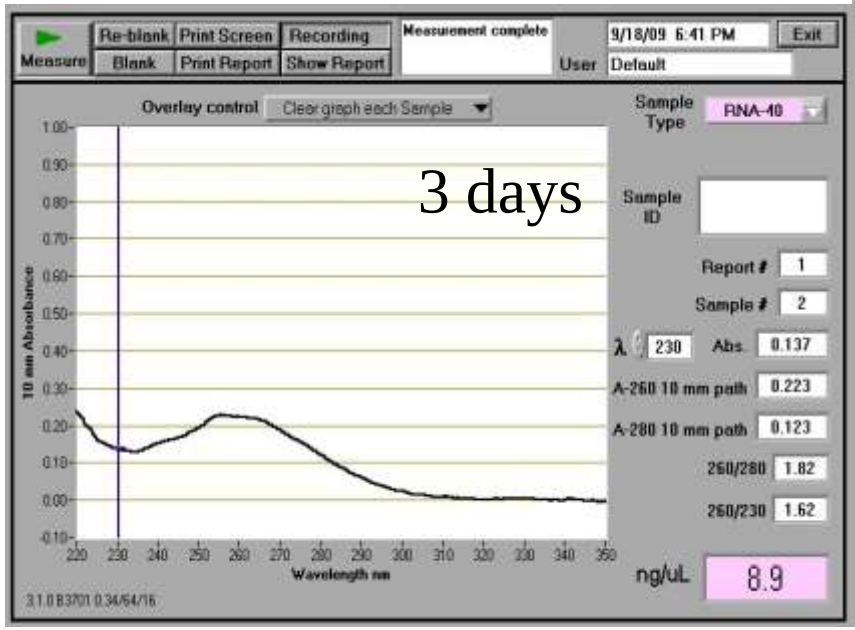


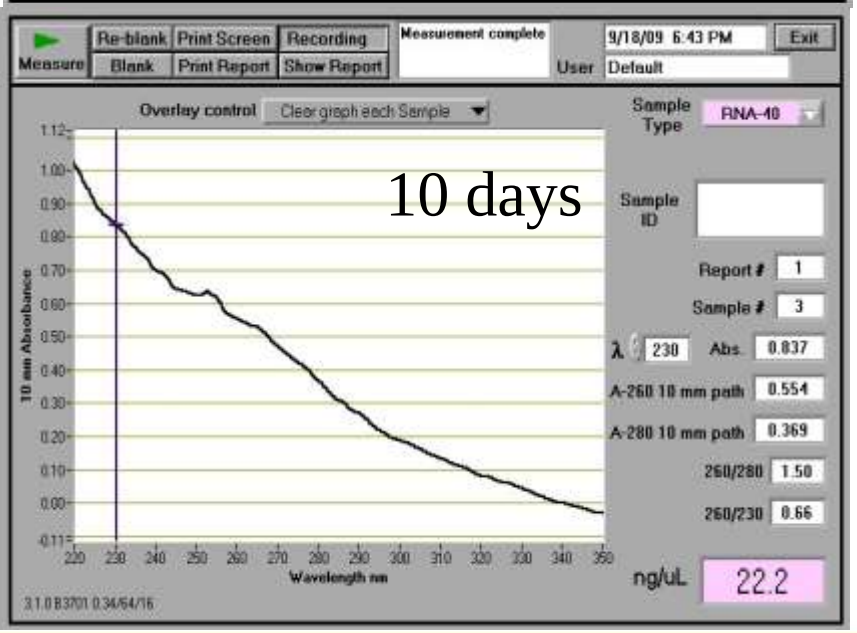




Effect of fixation time on the quality of isolated RNA







MILESTONE TissueSAFE High Vacuum Biospecimens Transfer System ™

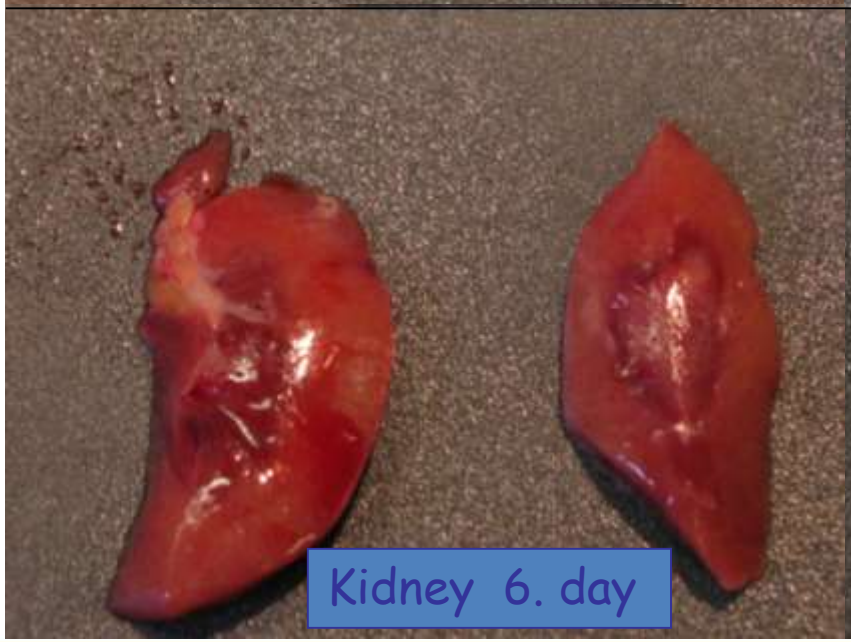
4 C° !!!

~~34 C°~~ !!!




Kidney at arrival (0. day)

Kidney 3. day



Kidney 6. day

Kidney 10. day

FFPE material

Tissue localisation

In vitro

Immunhistochemistry

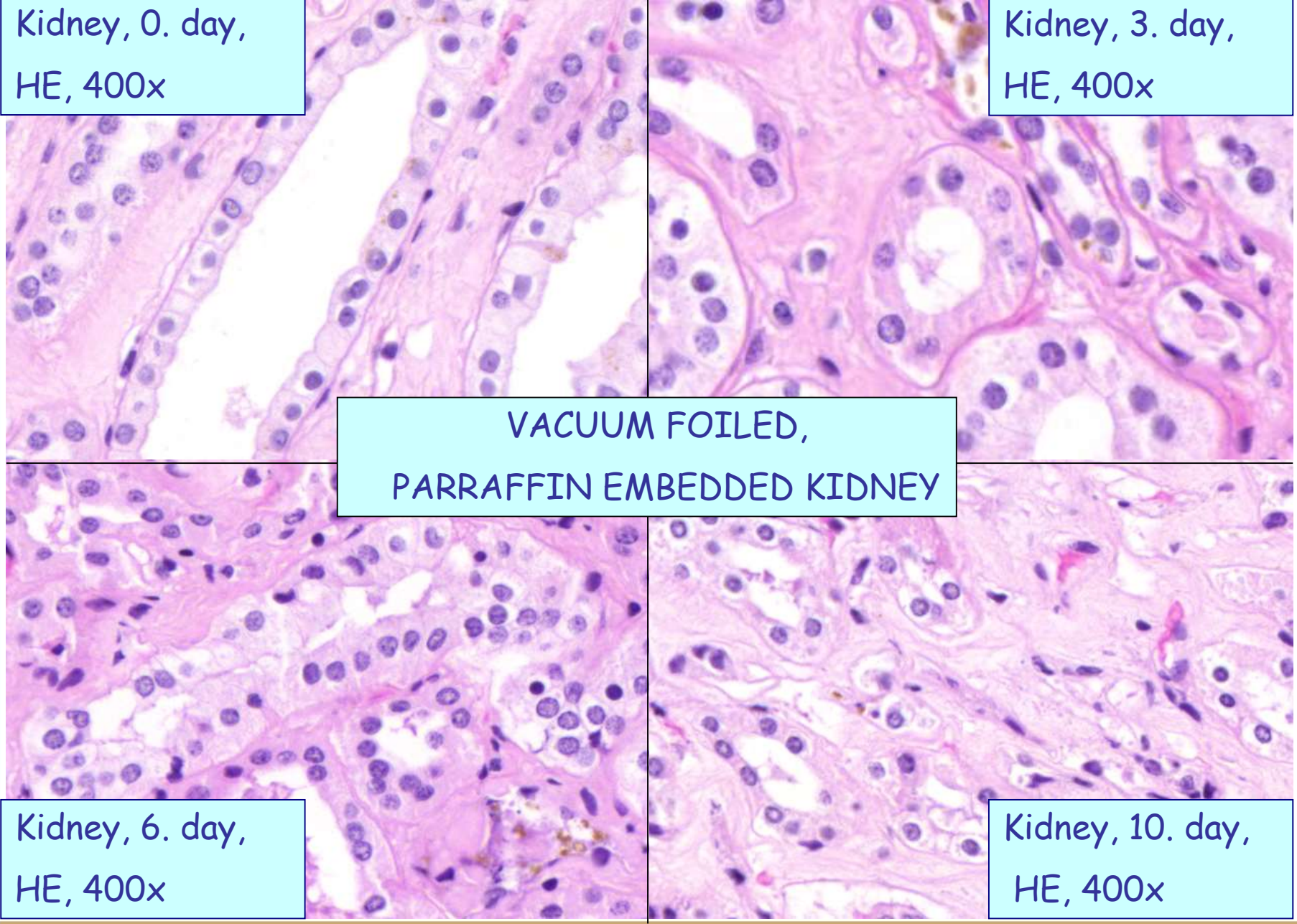
***In situ* hibridisation**

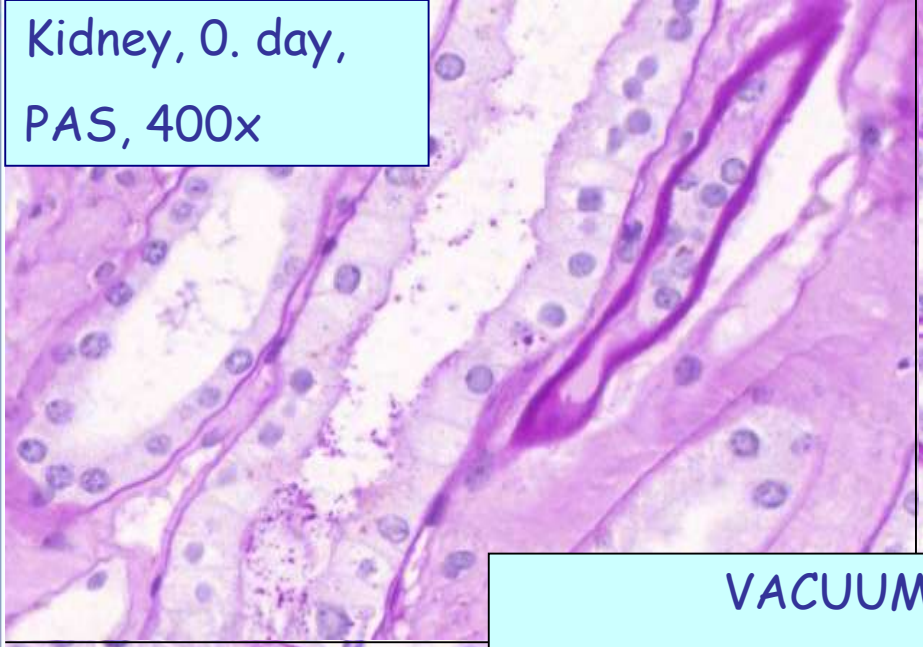
(CISH, FISH)

DNA based techniques

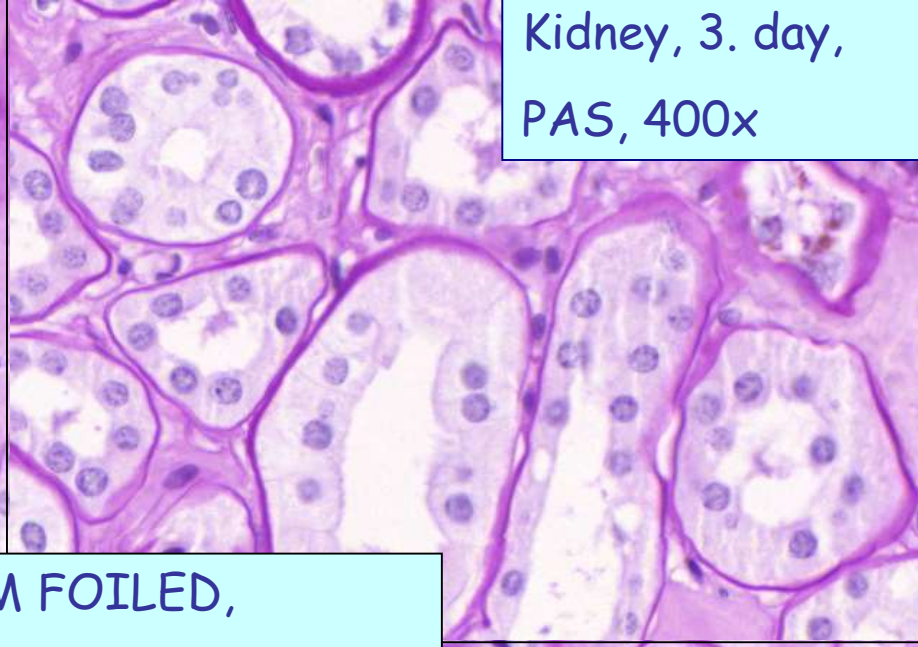
RNA based techniques

Protein based techniques


Kidney, 0. day,
HE, 400x
Kidney, 3. day,
HE, 400x
VACUUM FOILED,
PARRAFFIN EMBEDDED KIDNEY
Kidney, 6. day,
HE, 400x
Kidney, 10. day,
HE, 400x

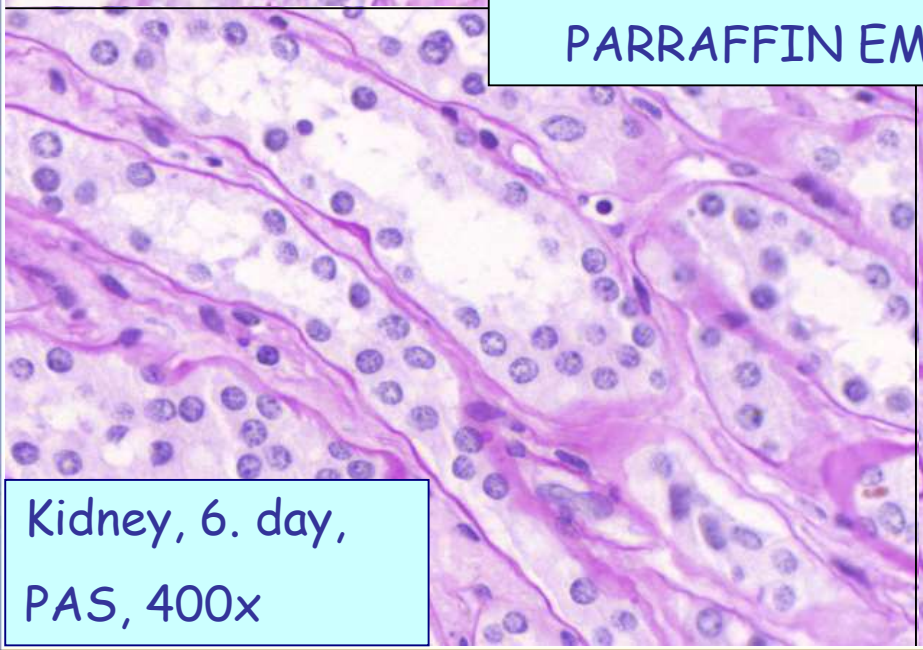


Kidney, 0. day,
PAS, 400x

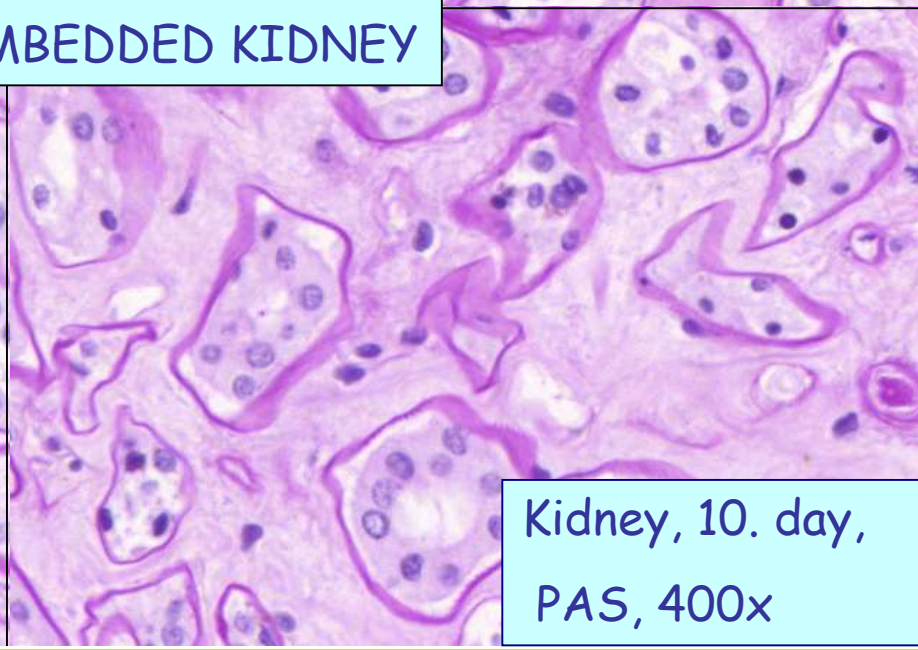


Kidney, 3. day,
PAS, 400x

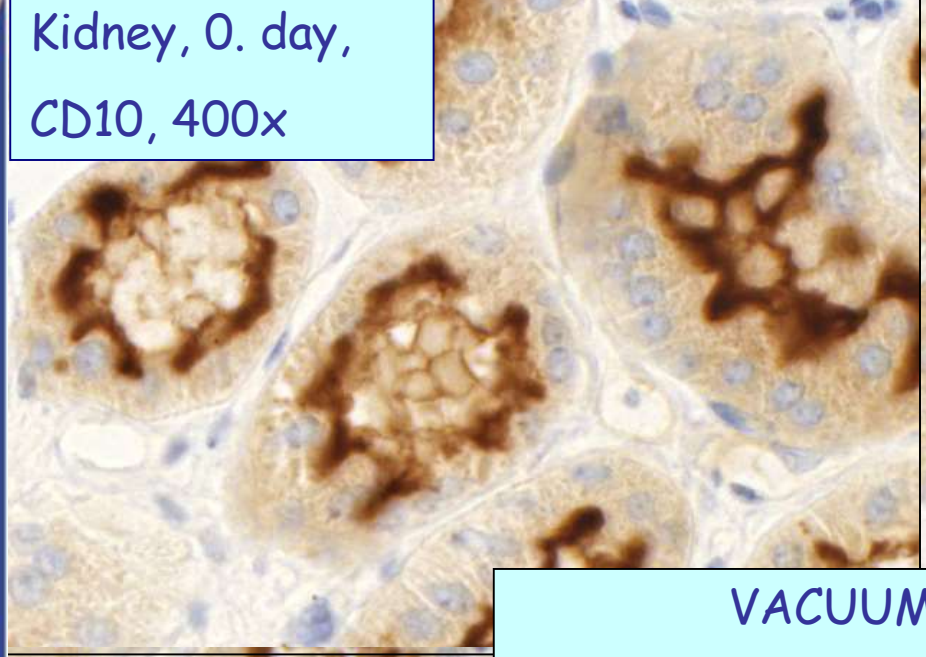
VACUUM FOILED,
PARRAFFIN EMBEDDED KIDNEY



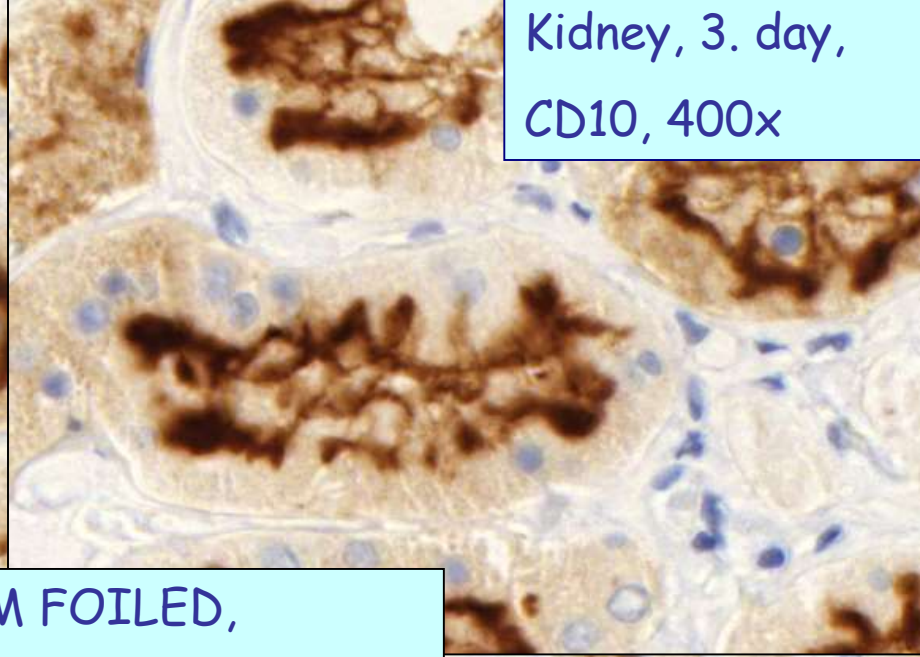
Kidney, 6. day,
PAS, 400x



Kidney, 10. day,
PAS, 400x

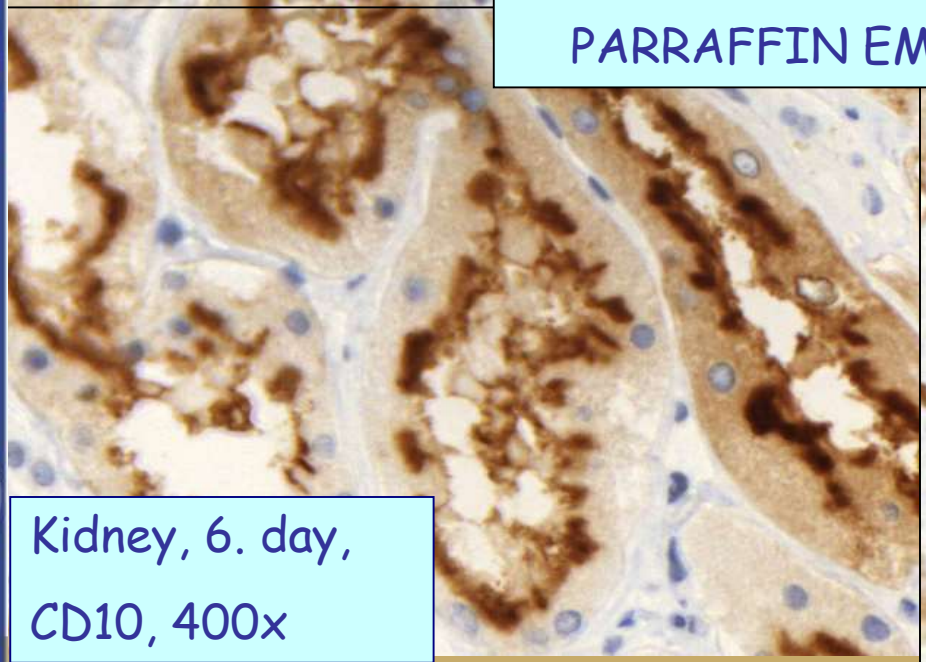


Kidney, 0. day,
CD10, 400x

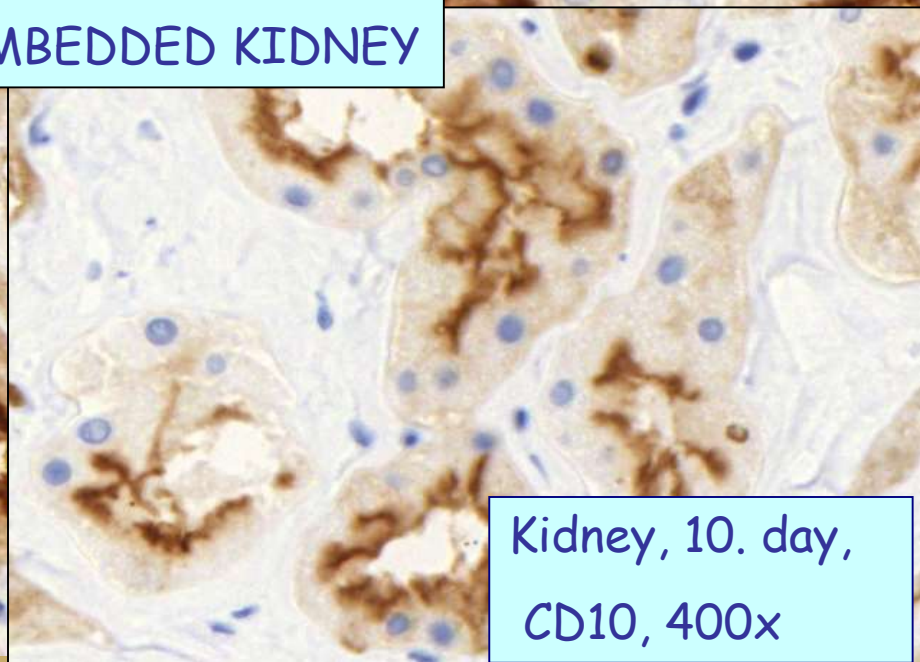


Kidney, 3. day,
CD10, 400x

VACUUM FOILED, PARRAFFIN EMBEDDED KIDNEY

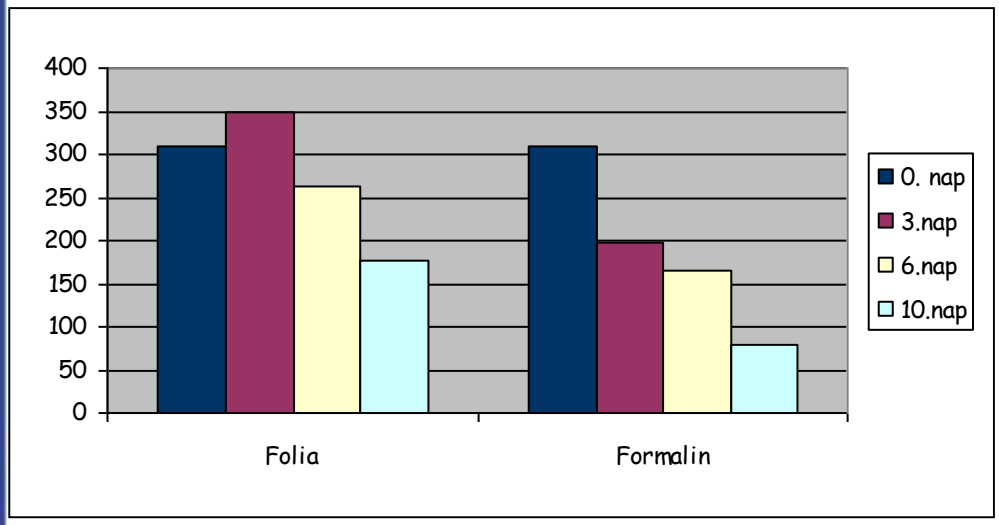


Kidney, 6. day,
CD10, 400x

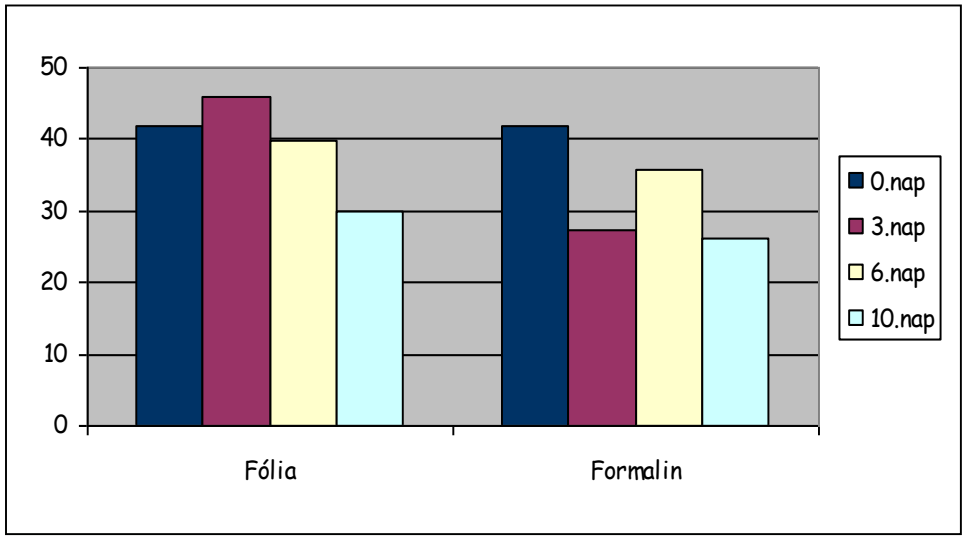


Kidney, 10. day,
CD10, 400x

Effect of vacuum foiling on concentration of isolated RNA and DNA



RNA (ng/µl)

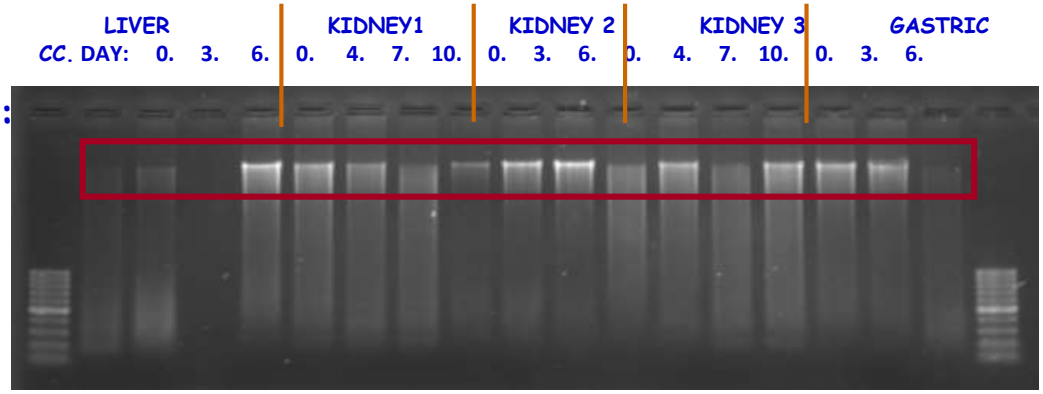


DNA ng/ µl)

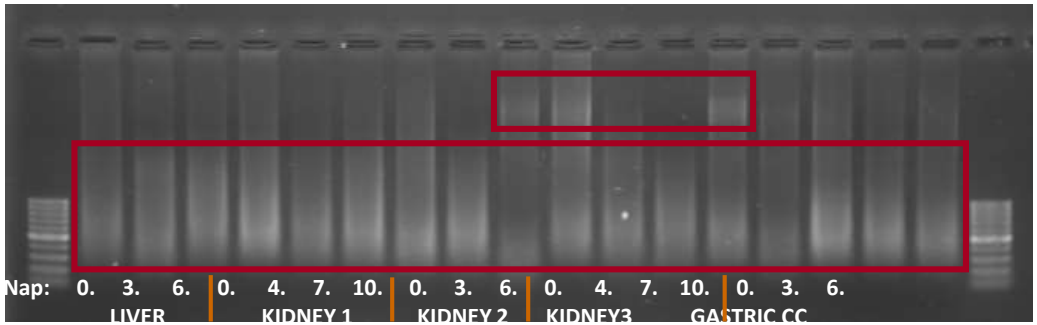
FRAGMENTATION of isolated DNA from vacuum foiled samples

VACUUM, then:

-80C°

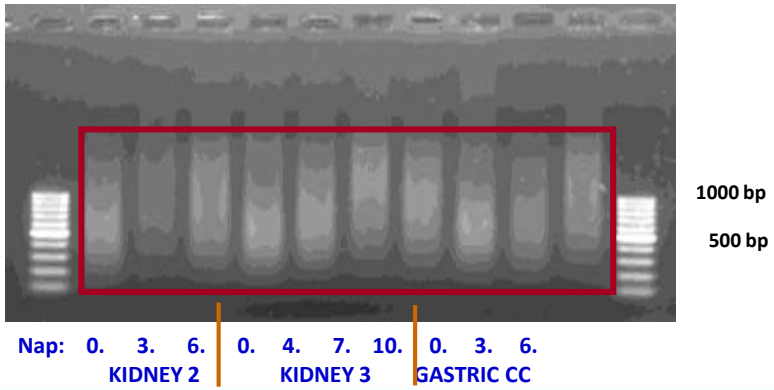


embedding

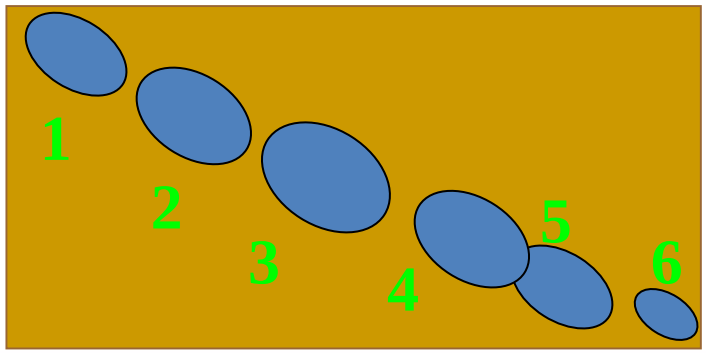


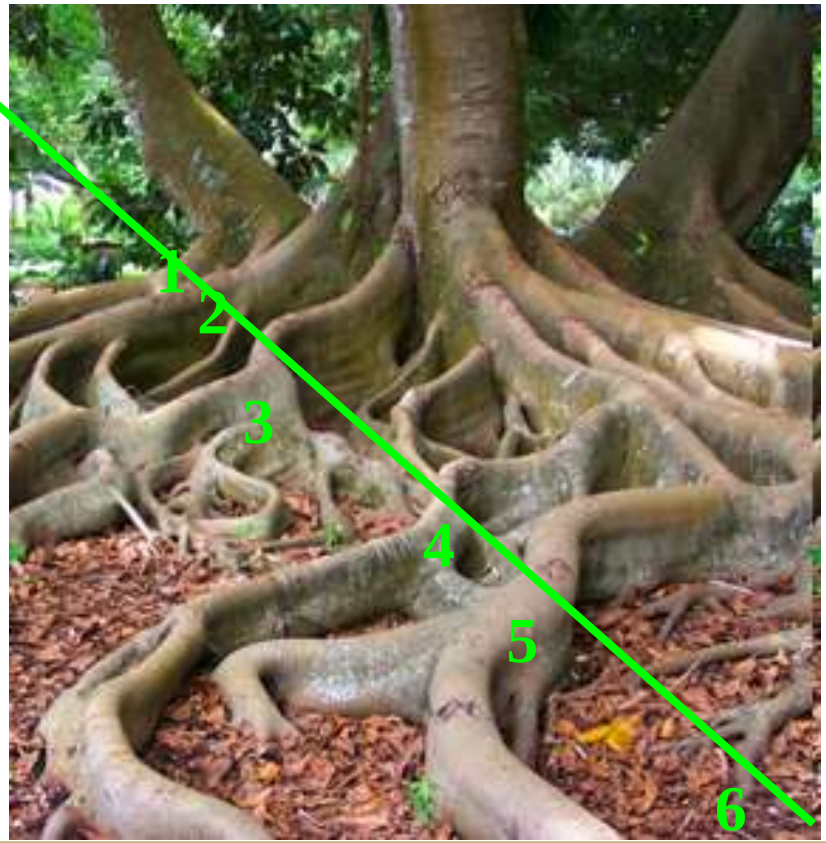
Formalin fixation, then embedding

~0,5 μg DNA/sample, 1.2% gel

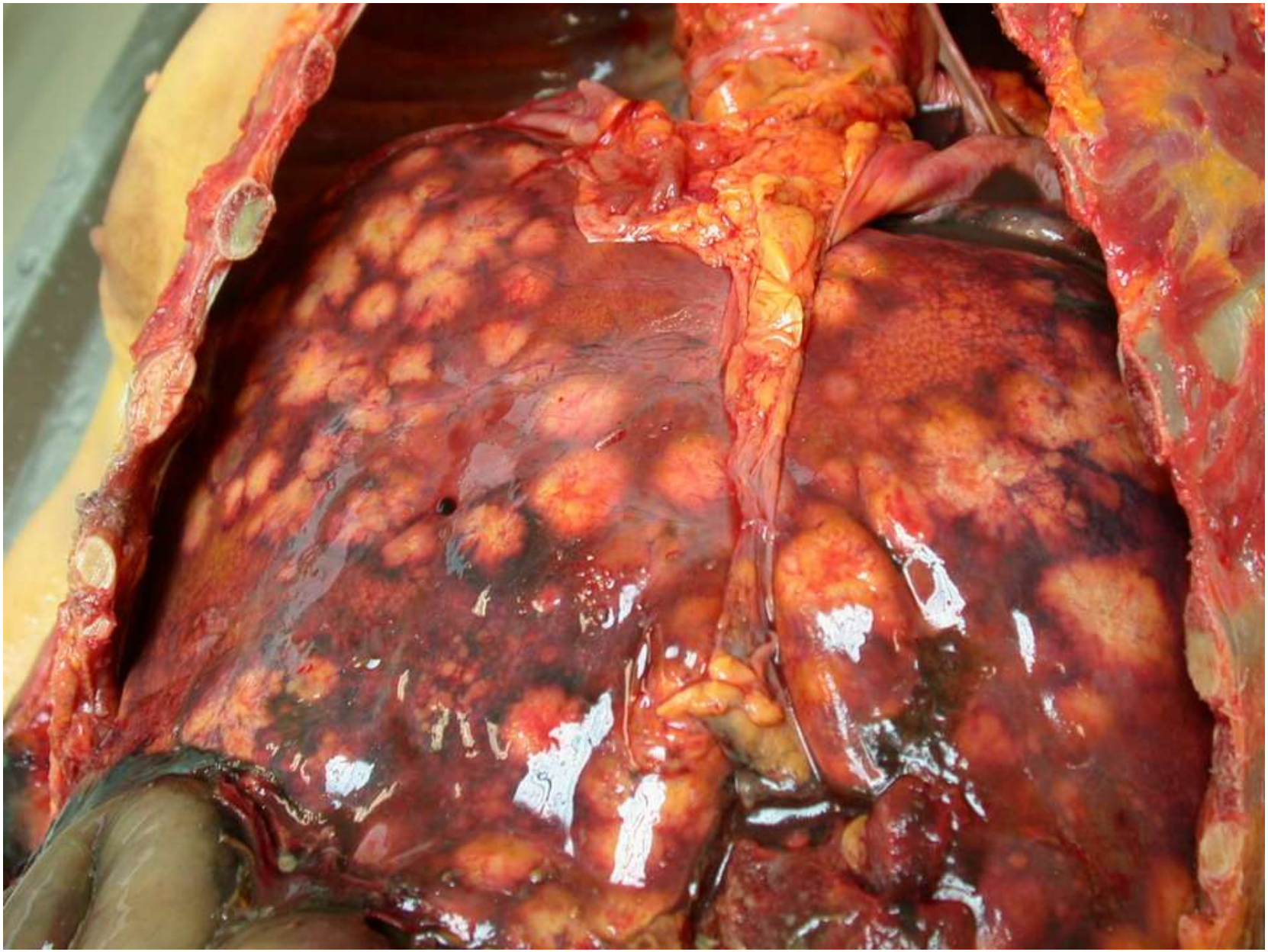


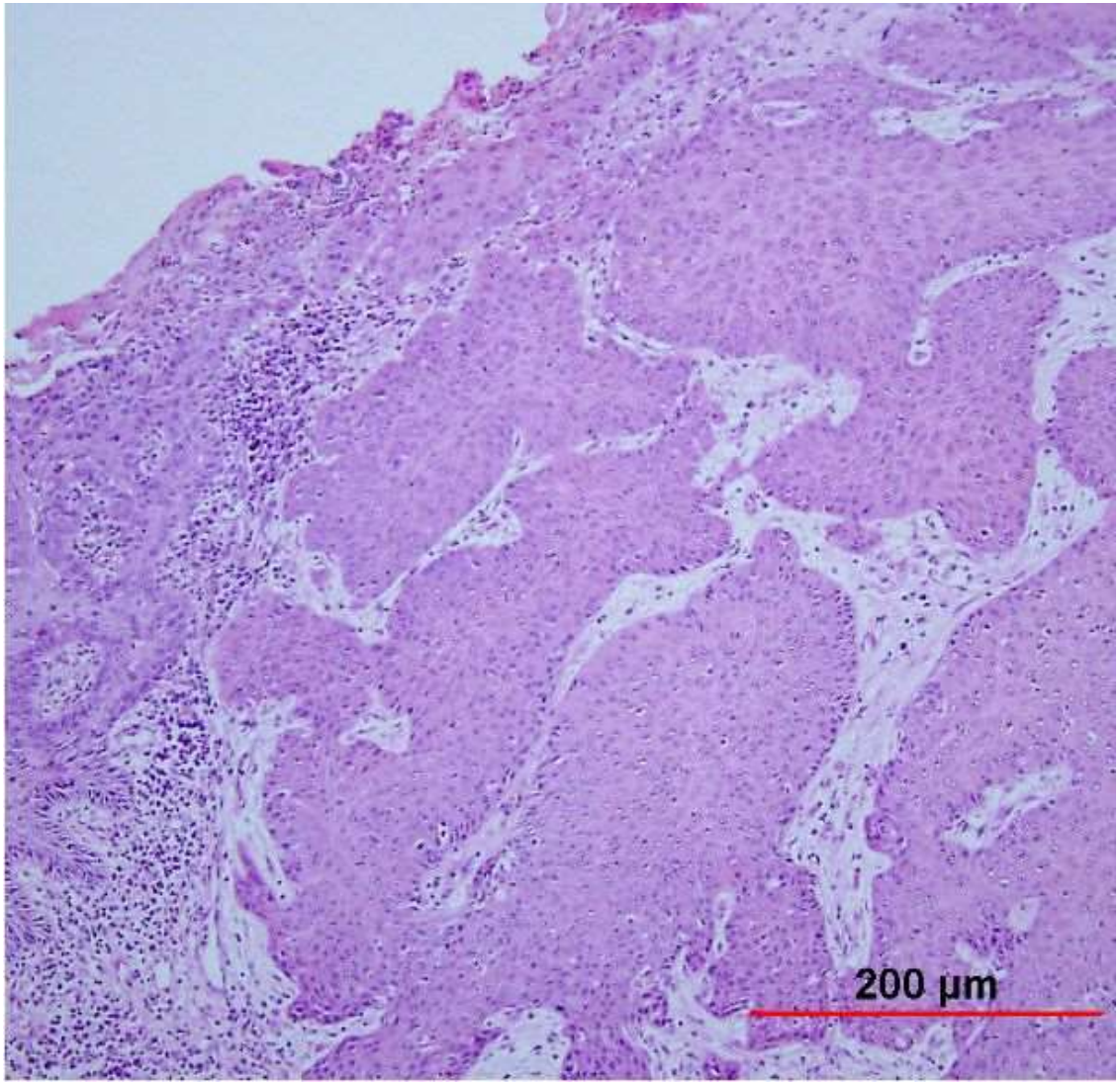
3 D in 2 dimension



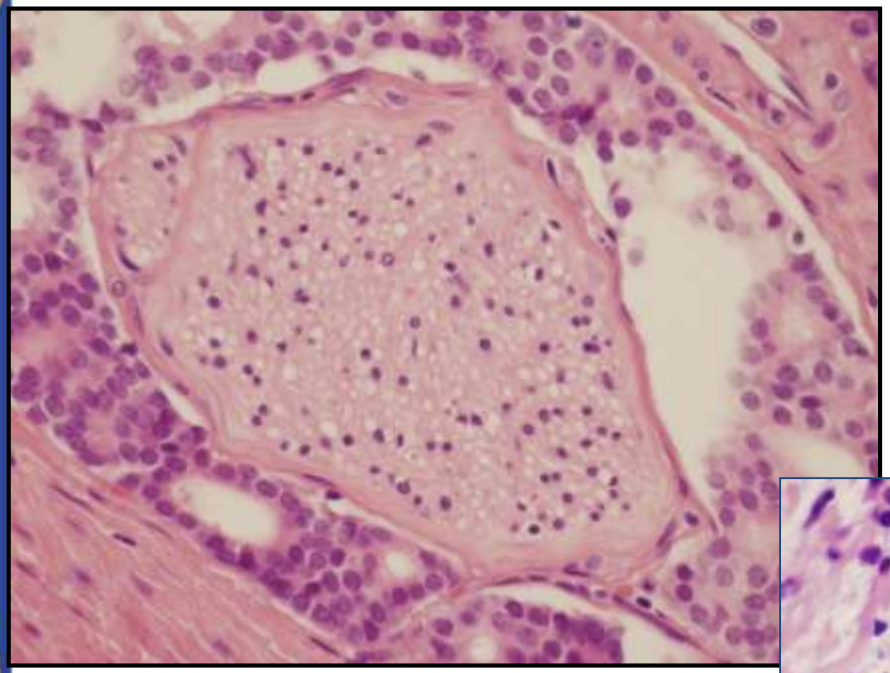


Metastasis



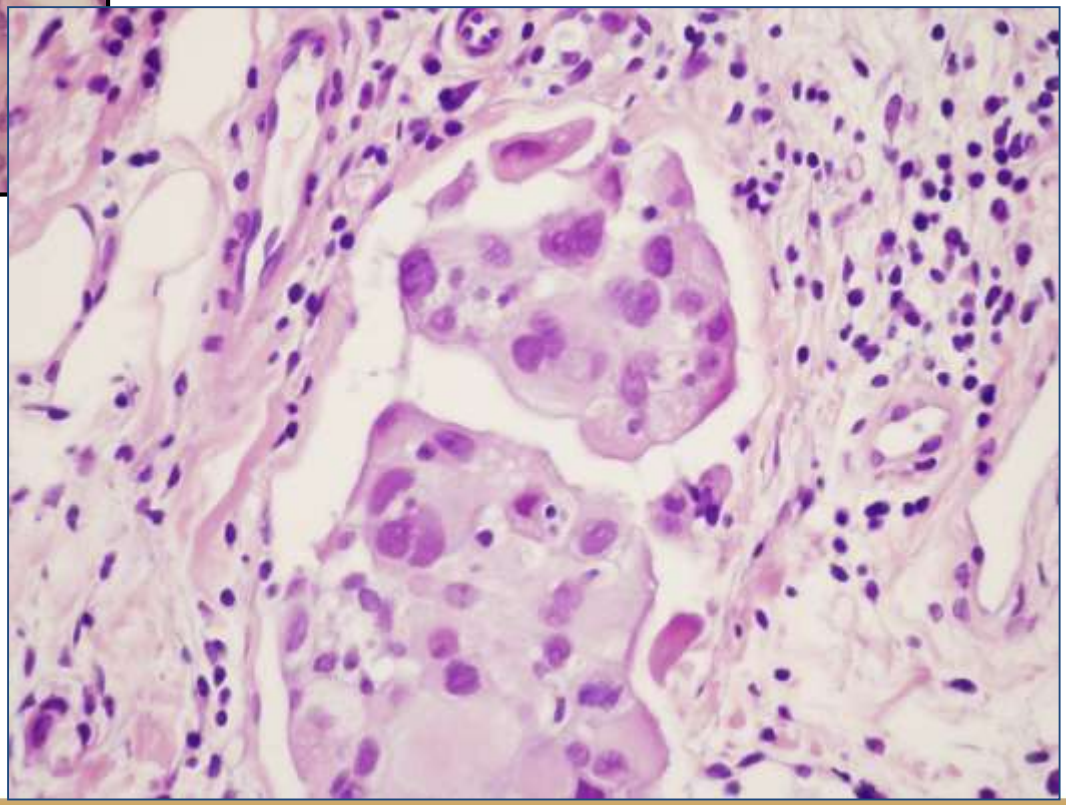


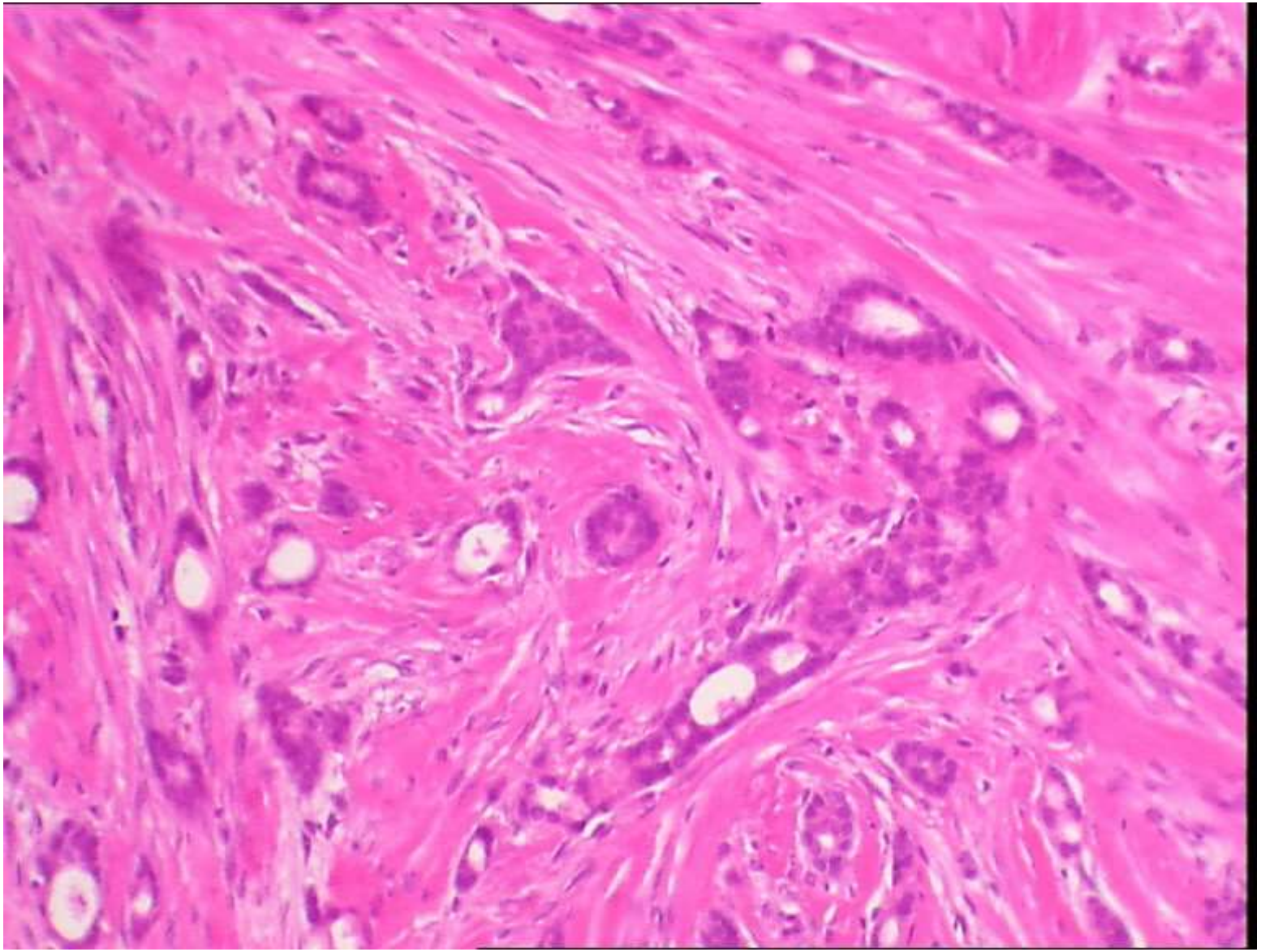
invasive carcinoma -cervix



Perineural invasion

Vascular invasion





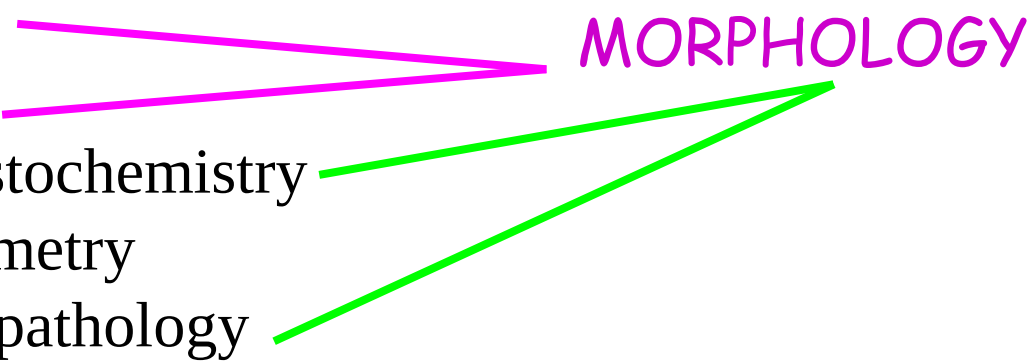
Pathological diagnosis

Laboratory Medicine - Clinical laboratory

Medical Microbiology

Pathology - Anatomical pathology (+laboratory pathology)
- Molecular pathology

Cytology
Histology
Immunohistochemistry
Flow Cytometry
Molecular pathology

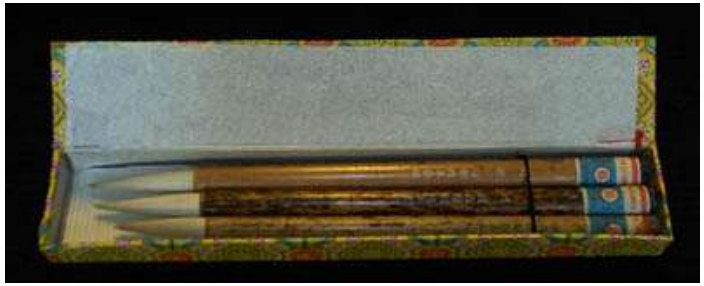


Staining methods

ROUTINE:

- haematoxylin-eosin

- PAS (periodic acid-Schiff)
- Alcianblue + PAS
- Giemsa
- Papanicolau



Special stainings

Masson

Cristall violet

Best

Nissl

Grimelius

Gomori

Schmorl

Gram

Ziehl-Neelsen

Gallyas

Romeis

Van Gieson

Orcein

Grocott

Tri-chrome

Mallory

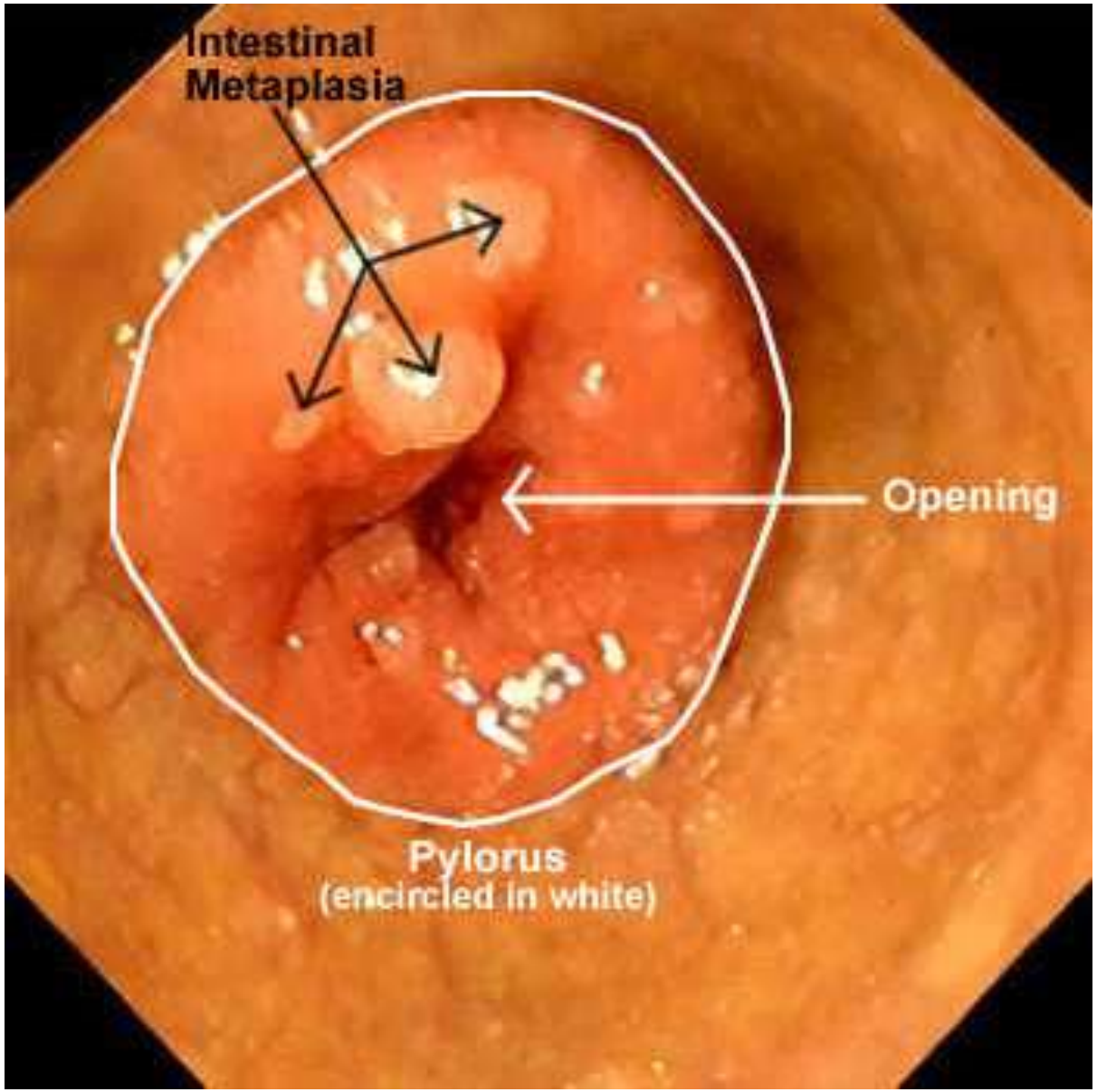
Resorcin-fuchsin

Silver staining

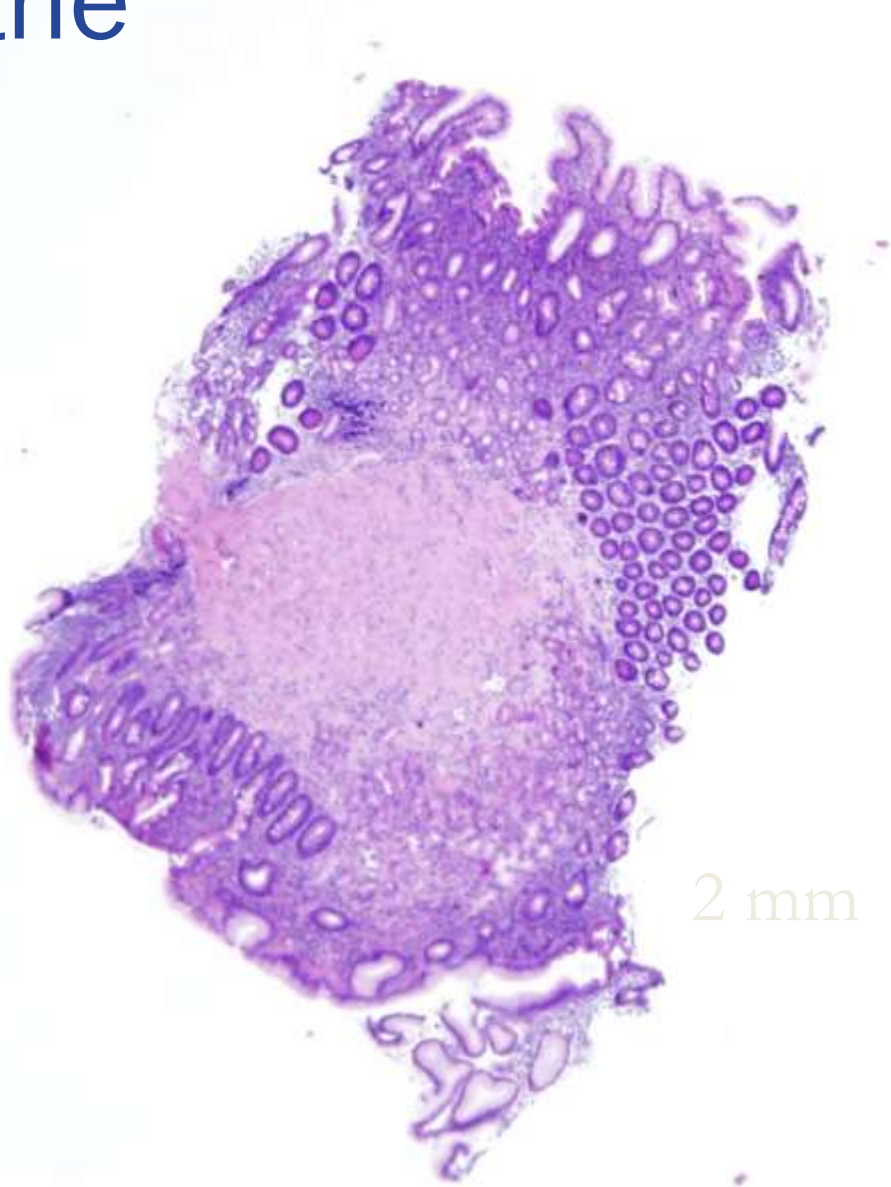
Congo

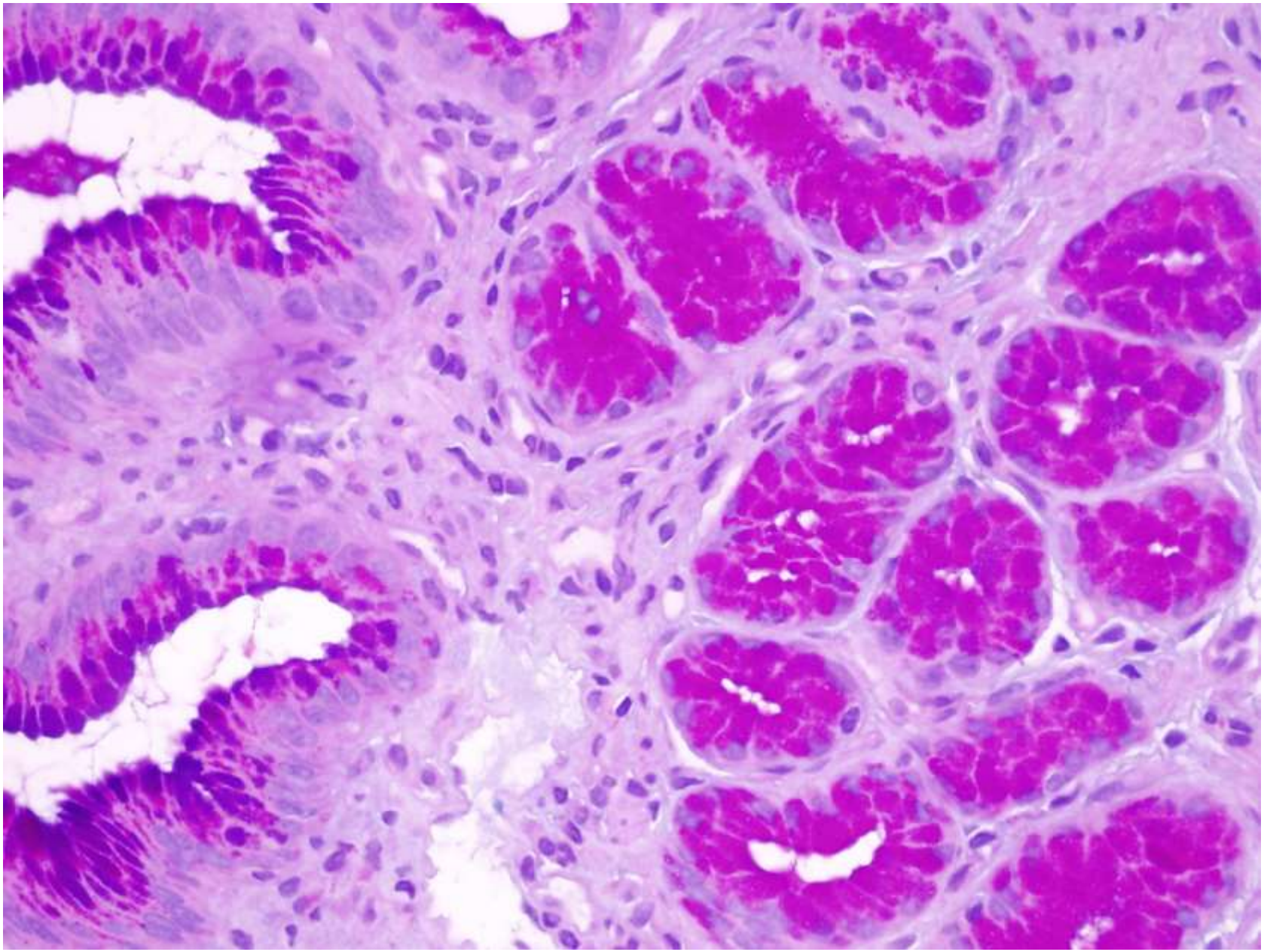
Kossa

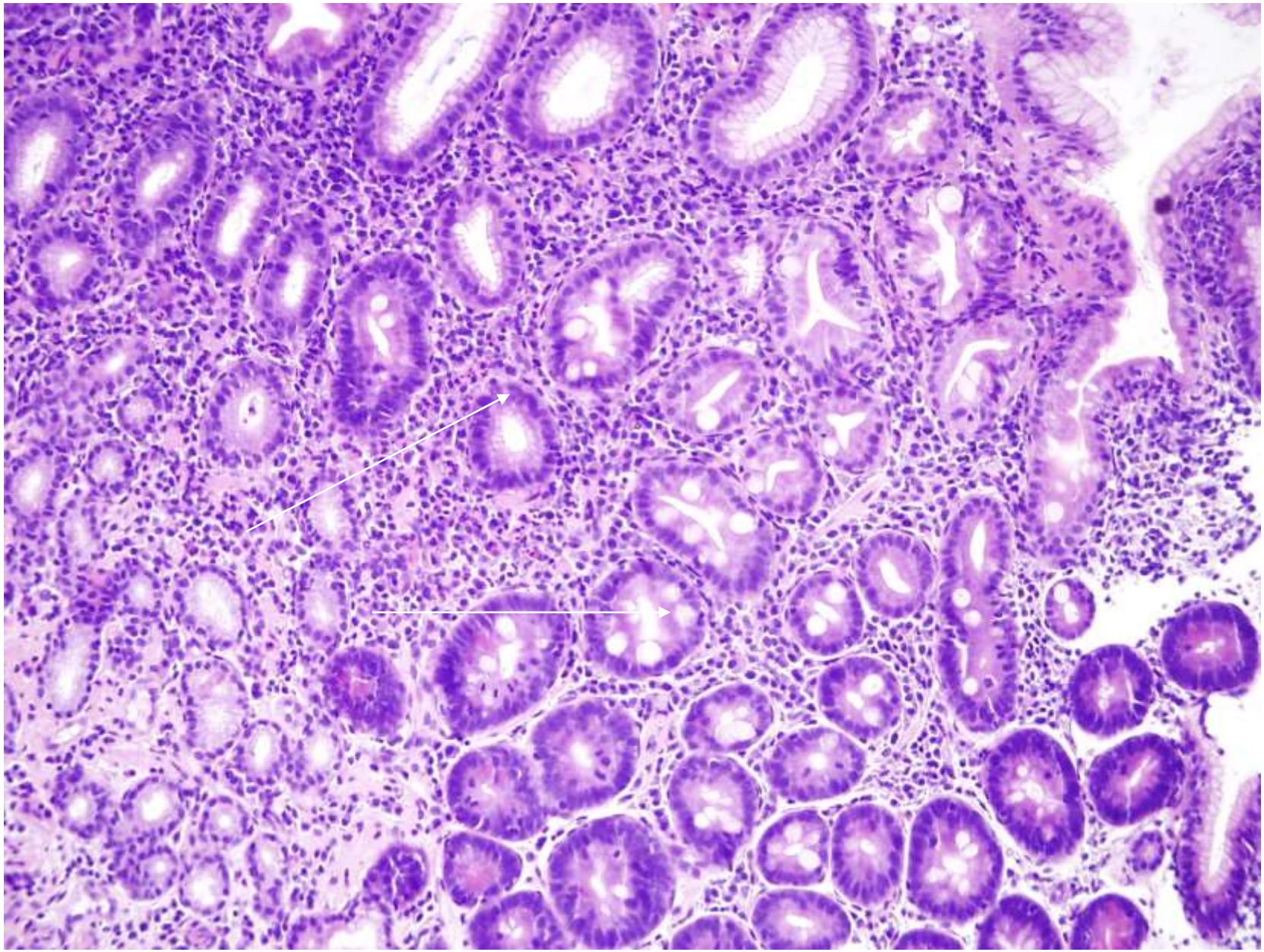
Endoscopy

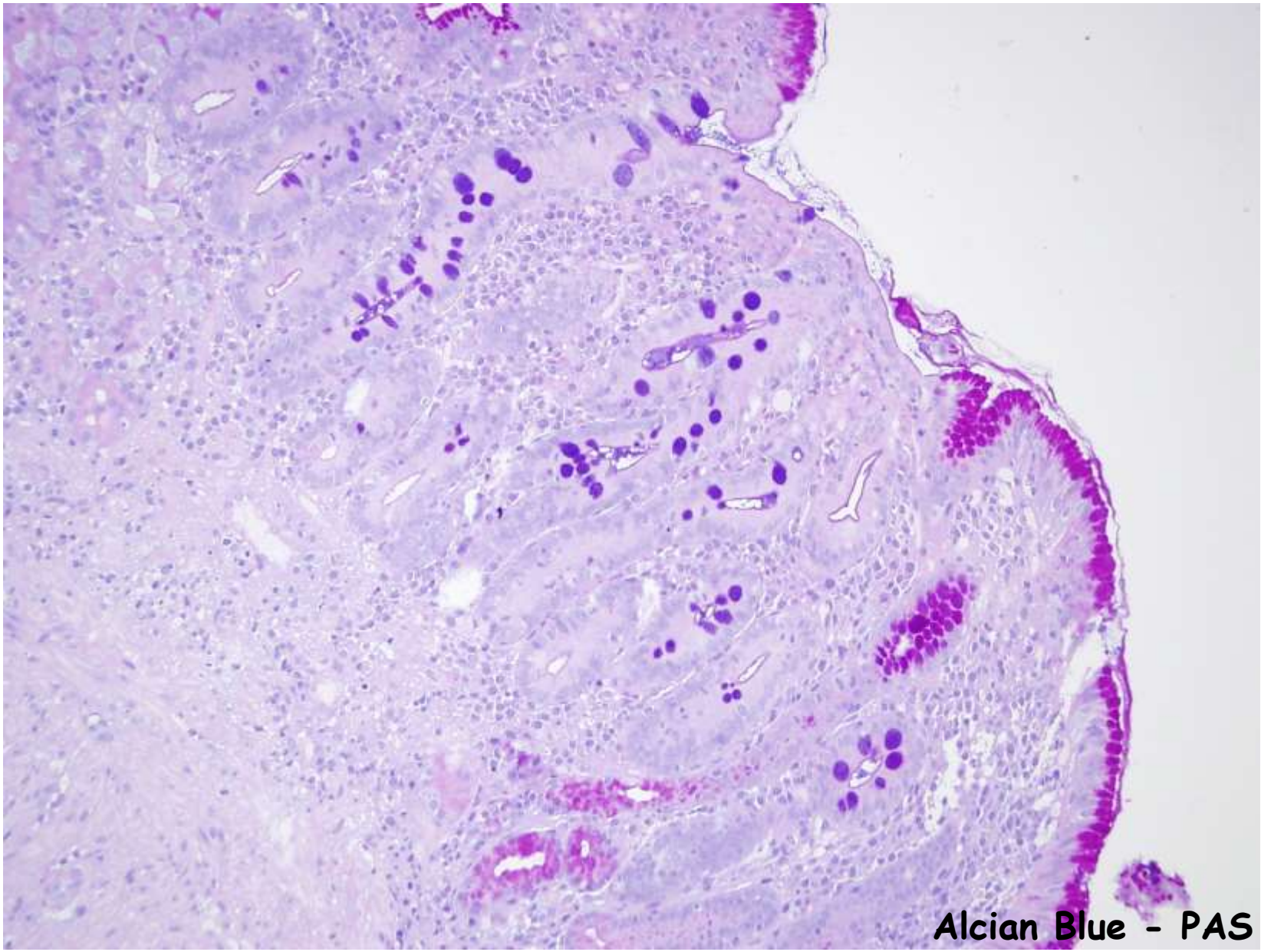

Intestinal
Metaplasia
Opening
Pylorus
(encircled in white)

Biopsy of the stomach

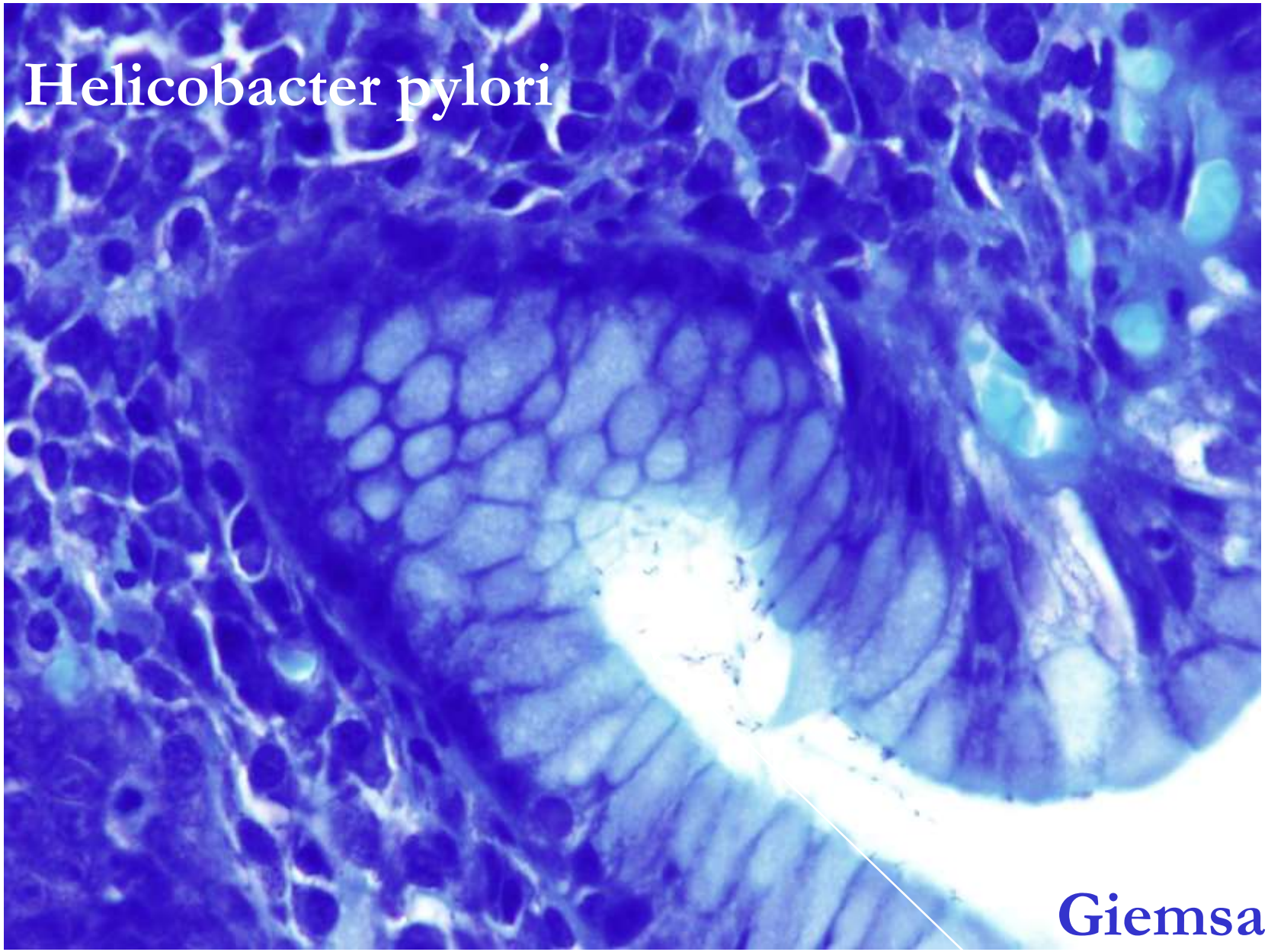






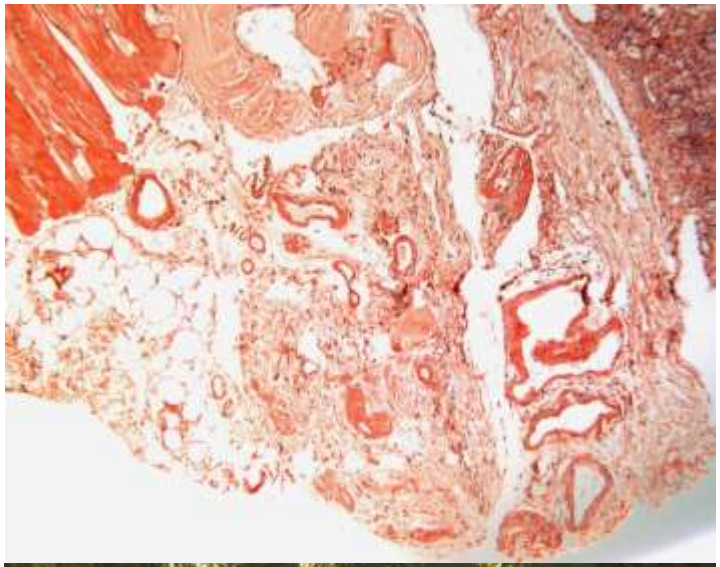

Alcian Blue - PAS

Helicobacter pylori

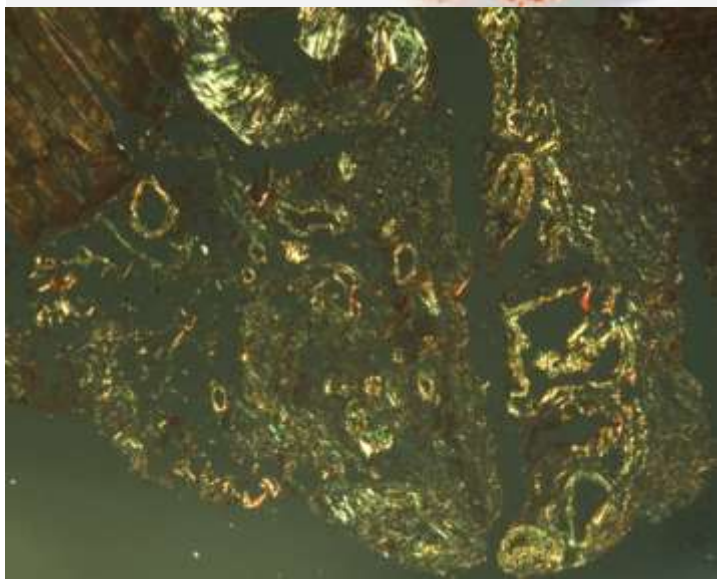


Warthin-Starry staining

Amyloidosis

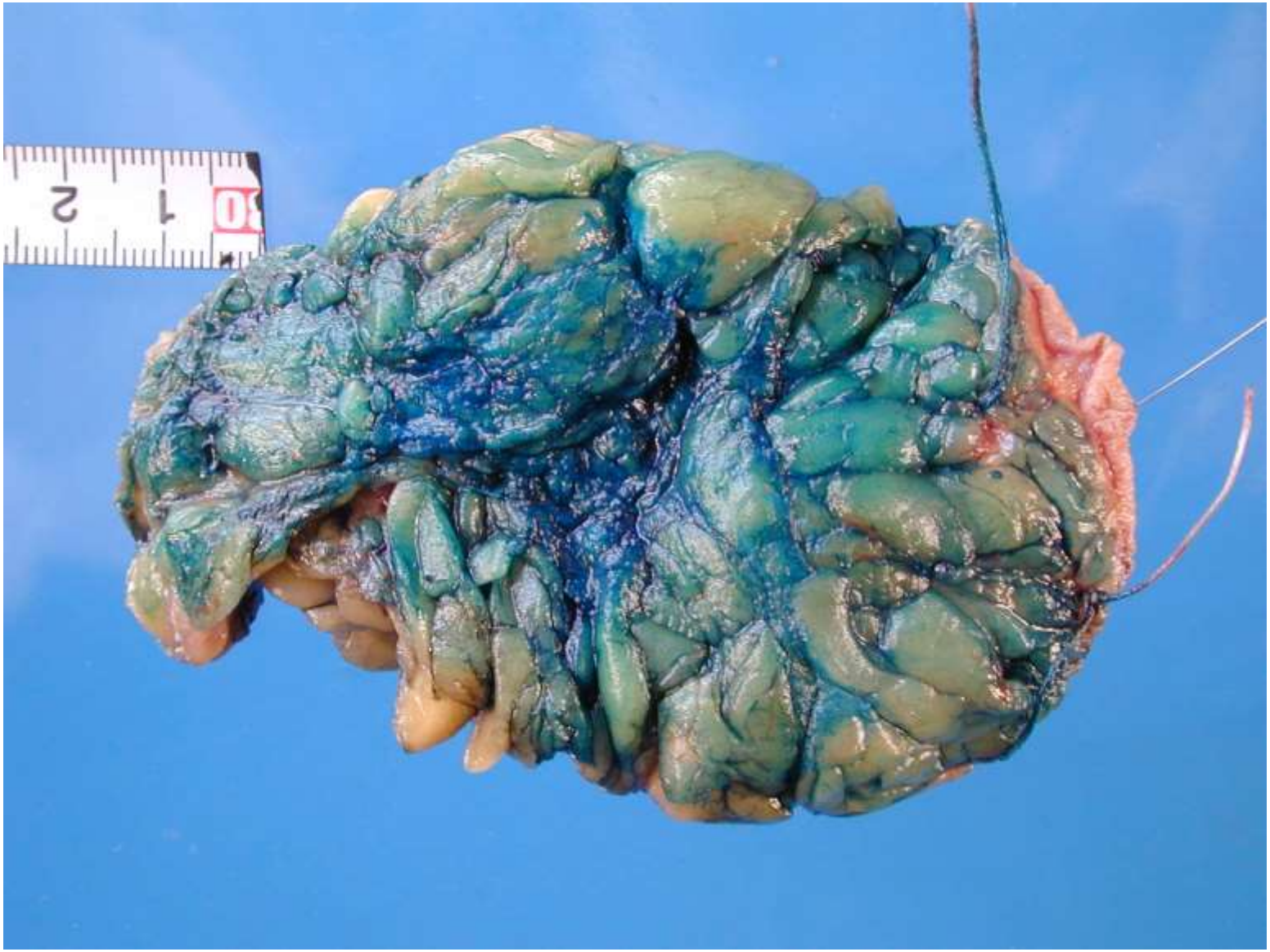


Congo

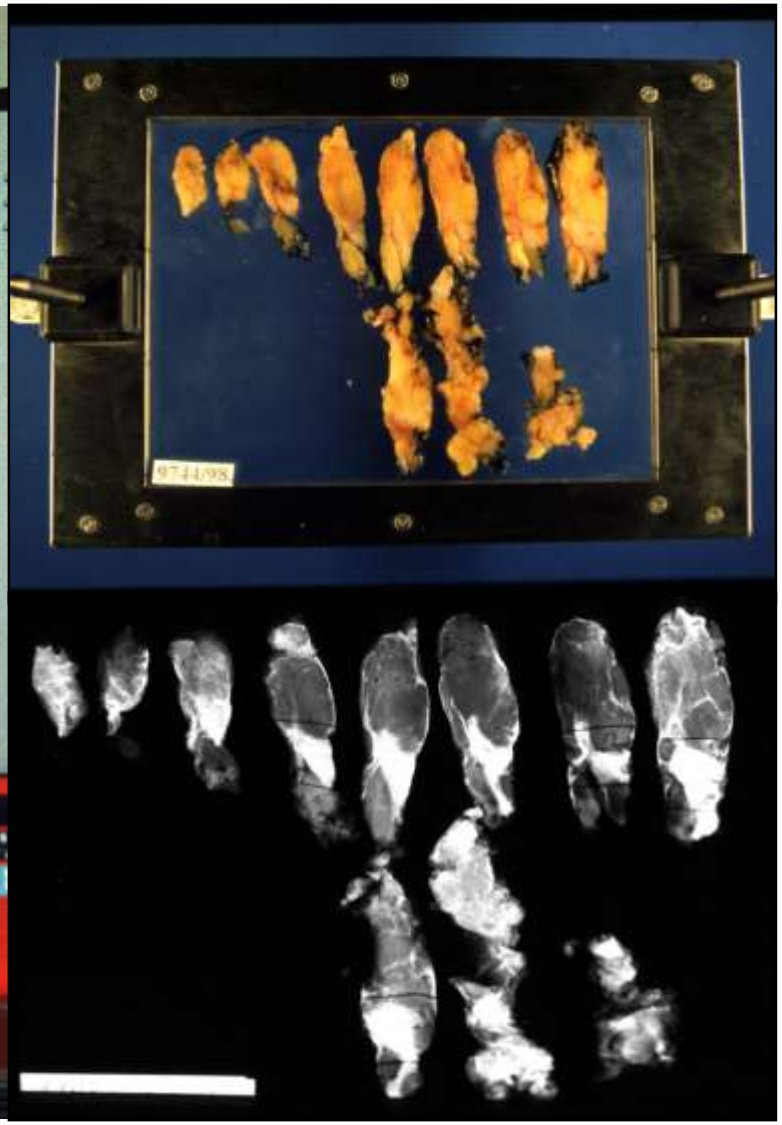


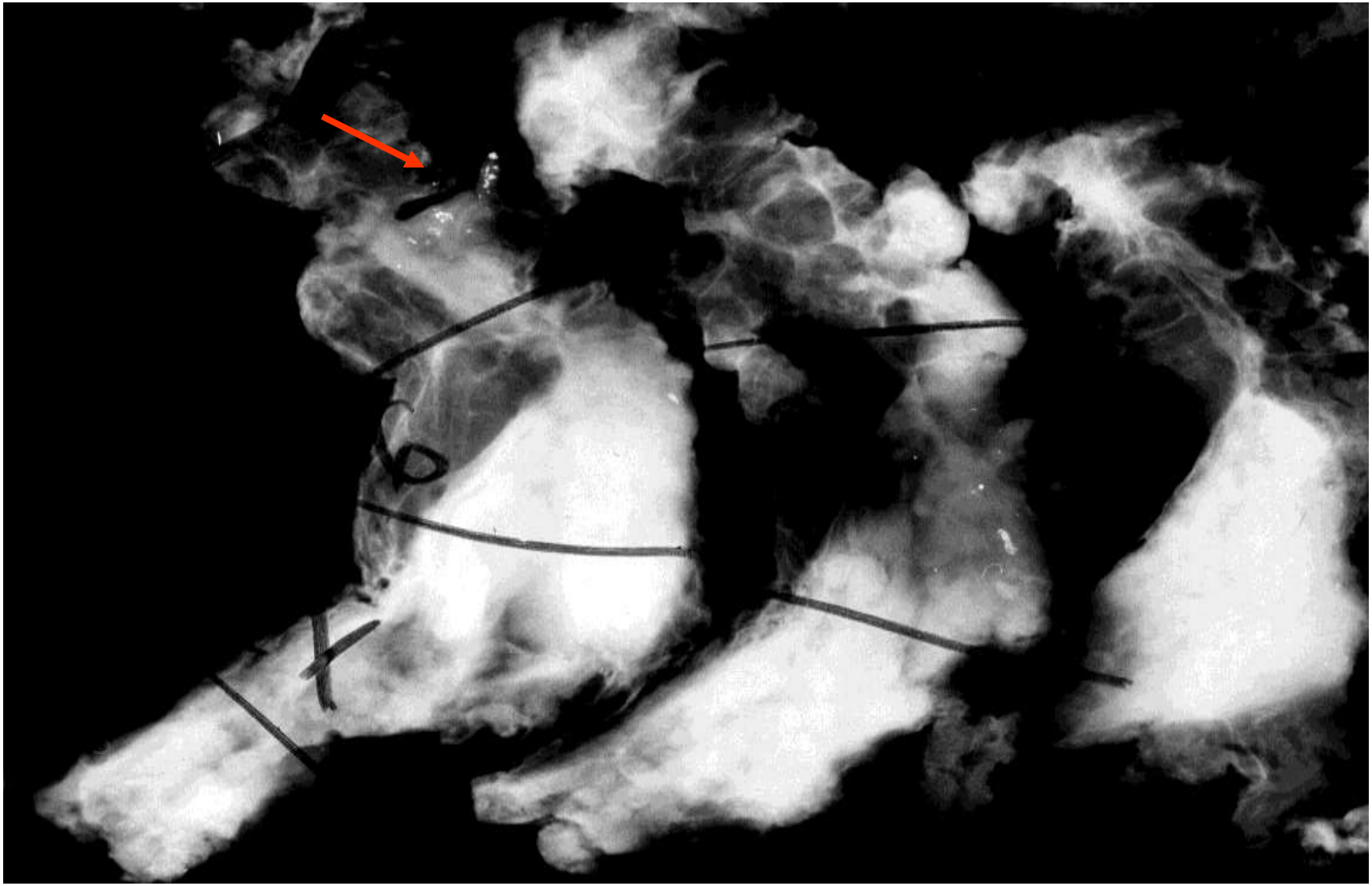
Congo: POLARIZED light

ORIENTATION

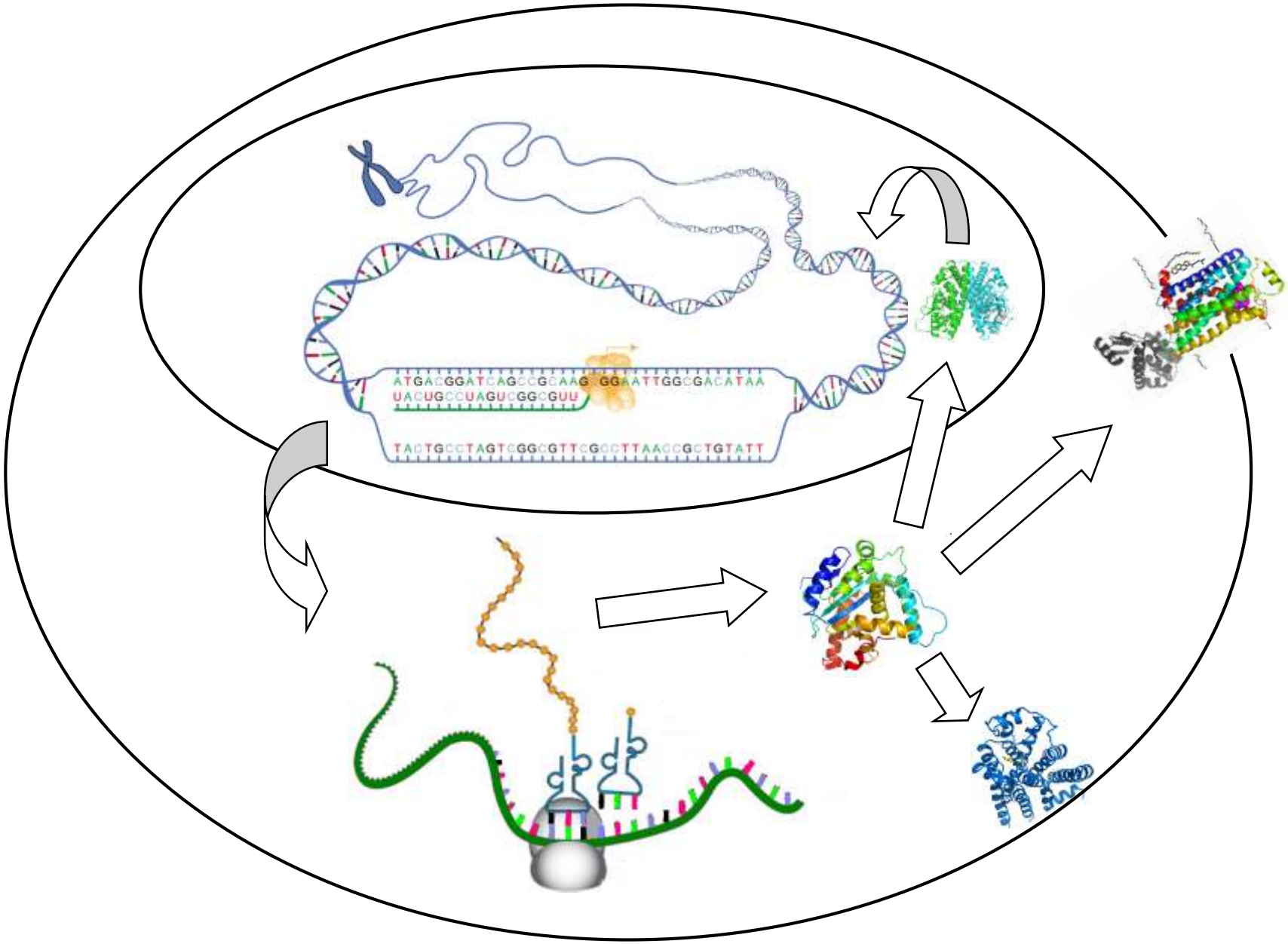



SPECIMEX DC





Specimen-mammography - microcalcification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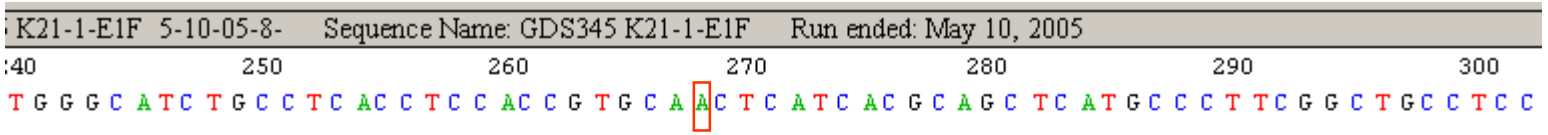
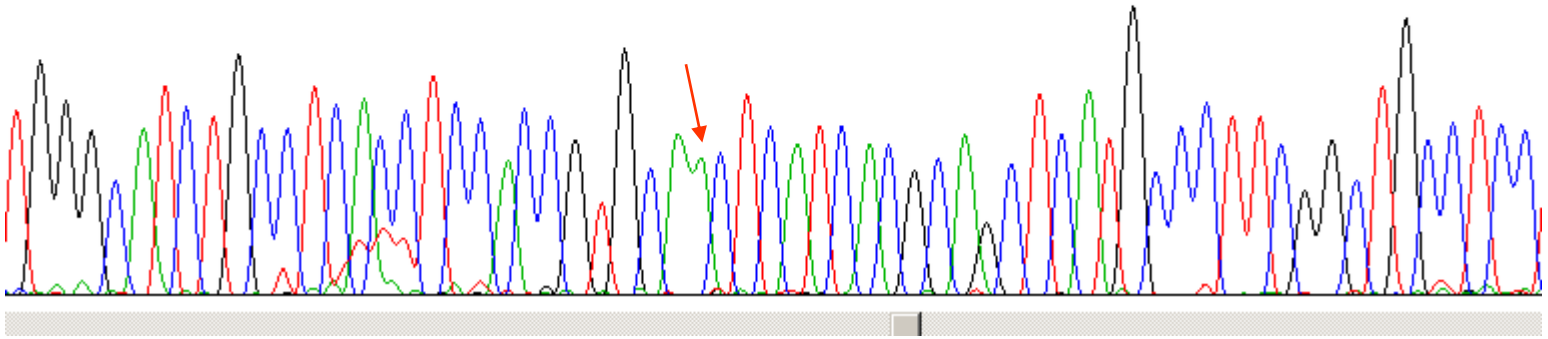
UACUGCCUAGUCGGCGUU
TACTGCCTAGTCGGCGTTCGCCTTAACCGCTGTATT

Genetics in 2 dimension





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             |||| |||||||||||||||||||||||||||||||||||||
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Query: 326  aagacaatattggctcccagtacctgctcaactggtgtgtgcaga
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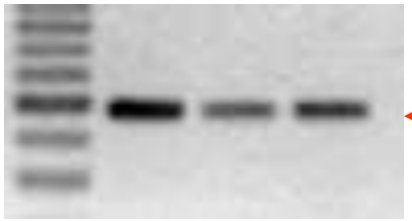
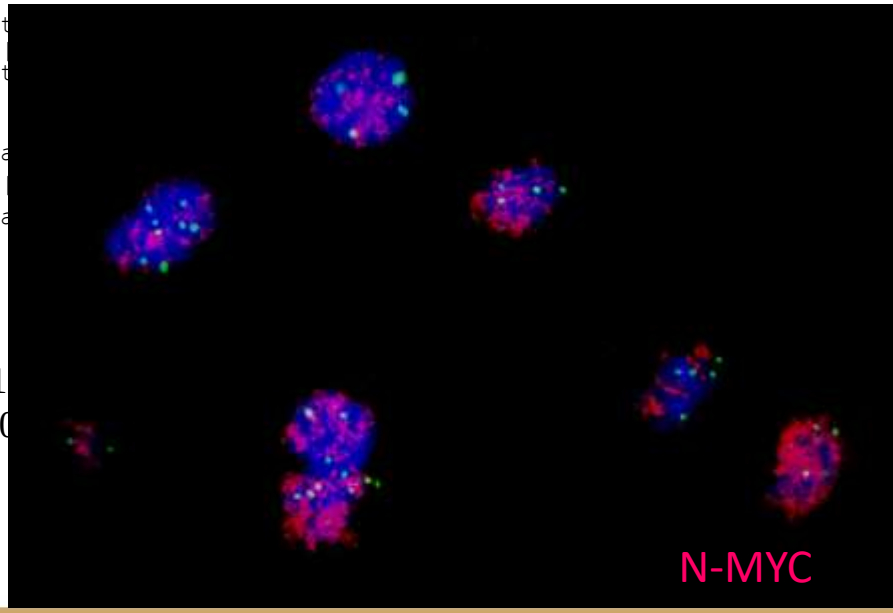


Exon 1
NM_00



Driver oncogene concept (Vogelstein)

- Somaticaly mutated gene
- Mutation provides constitutive (disregulated) function
- Protein product of the mutated gene conquers a key fundamental pathway
- Mutated gene function leads to oncogenesis (transformation potential)

Molecular targeted therapy

- A given cancer has a well defined number of driver oncogenes
- The function of the protein product can be suspended by an antibody or a selective inhibitor
- The antibody or the inhibitor have anticancer activity in clinical trials

MOLECULAR PATHOLOGY
BASICS

Molecular biology ≠ Molecular Pathology

MULTIDISZIPLINARY SCIENCE

Handling of the material (sampling, sample delivery), fixation as well as establishing diagnosis and interpretation of that might need consultation !!

GENERAL COMMENTS

- SPECIFICITY, SENSITIVITY
- What is pathology for (including molecular pathology) ?

Questions ?

What does morphology/ genetic information reveal ?

Developmental disorder ? Inheritable ?

Inflammation ? Acute or chronic ?

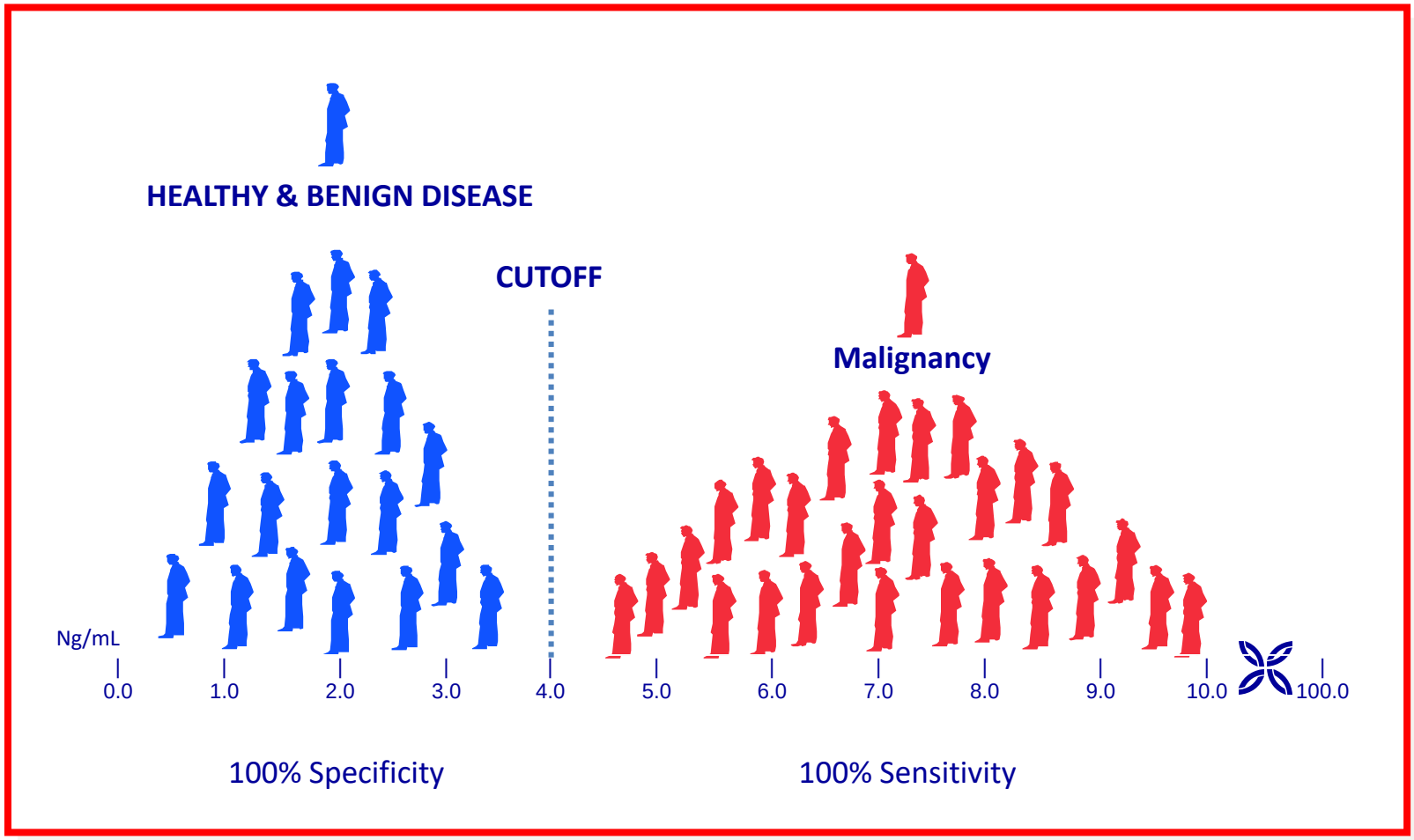
Neoplasia ? Tumor like lesion, benign, **malignant** ?

Therapy / Prognosis / Prediction !

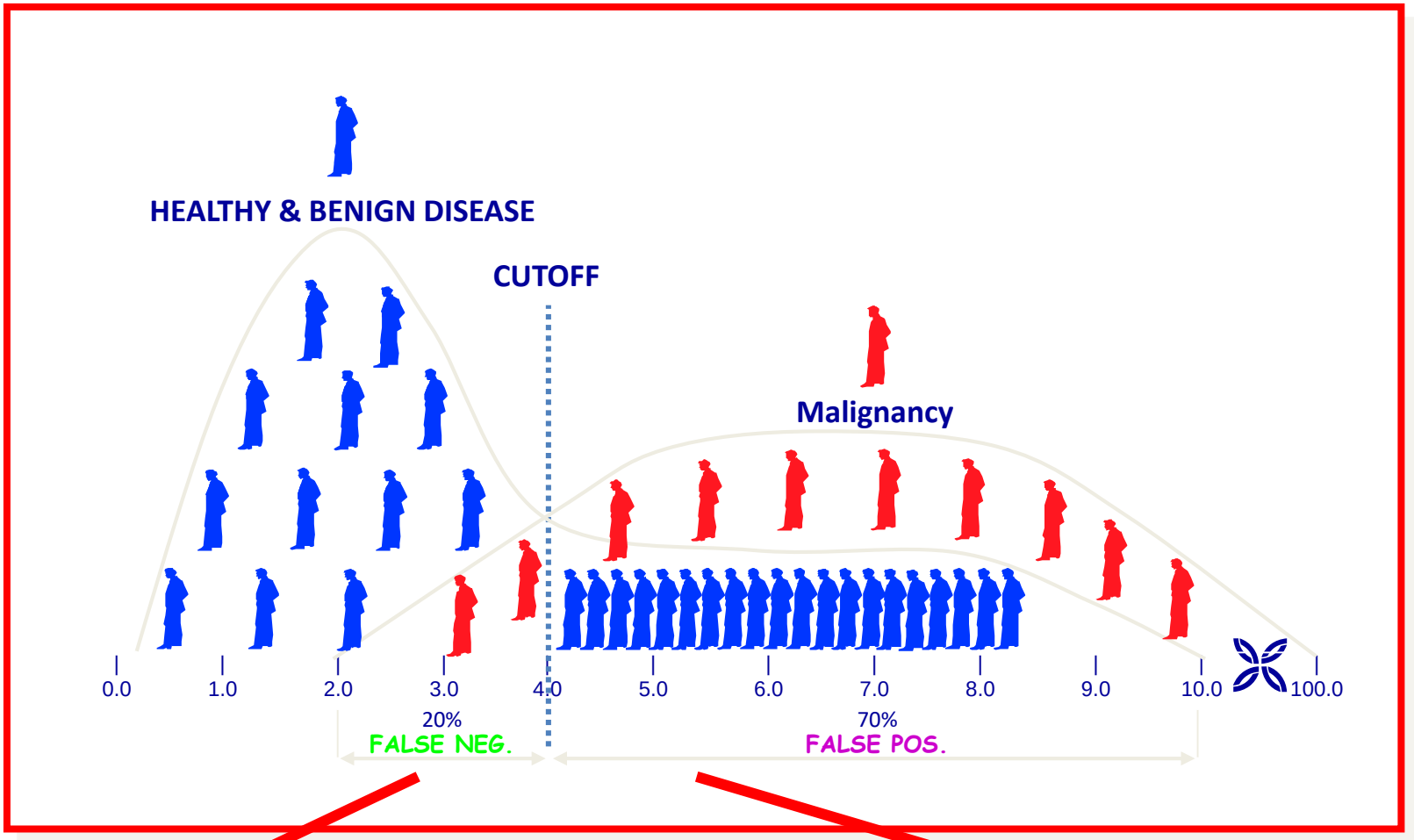
Several fold magnitude in specificity and sensitivy

Powers of Ten, by Charles and Ray Eames

Ideal Marker for Malignant Neoplasia



Reality of Testing



RELEASED
Tumor progresses further

INVESTIGATED FURTHER
FINALLY DISCLOSED

GENERAL COMMENTS

For malignant neoplasias:

Questions ?

Primary or secondary (metastatic) ?

If metastatic: where is the primary ?

Therapy ? Parameters for therapy: grade and stage

These determine the nature of therapy:

Resection only ± Radiotherapy ± Chemoterapy

(preoperative or postoperative)

COMMUNICATION TO THE CLINITIANS !

Surely malignant, surely benign: O.K.

Pathologist does not know: WHY ?

Surgical resection: R0 ?

Resection margins ?

GENERAL COMMENTS

For malignant neoplasias:

Questions ?

Pathologist does not know: WHY ?

Not experienced / trained - theoretically possible – please consult !
(e.g.signature of two board certified pathologist for malignant diagnosis!)
Clinical sample is not appropriate for definitive diagnosis !
Malignant tumor ? Answer: may be ? = NO HELP IN DECISION MAKING
How to have reliable result: New biopsy ? Control ? Anxilliary techniques ?

COMMUNICATION TO THE CLINITIANS !

BEST DIAGNOSIS OF NO VALUE IF NOT COMMUNICATED !!
SPEED and COMMUNICATION are the measures of success !

90's: IMMUNOCHEMISTRY WAS CONSIDERED USEFUL
OTHER TECHNIQUES WERE NOT IMPORTANT

2000's: IMMUNOCHEMISTRY WAS ESSENTIAL
FLOW CYTOMETRY WAS USEFUL
OTHER TECHNIQUES WERE NOT IMPORTANT

RECENT YEARS:

IMMUNOCYTOCHEMISTRY	Almost every dpt.
TUMOR MARKERS	Clin. laboratories
FLOW CYTOMETRY	Path. Centers
PCR	Path. Centers
FISH	Path Centers
IN SITU HYBRIDIZATION	Path Centers
CYTOGENETICS	Spec. centers
TISSUE MICROARRAYS	

INTER-PERSONAL SKILLS

TRIPLE A: AVAILABILITY, AMICABILITY, ABILITY (AFFABILITY)

EMOTIONAL INTELLIGENCE, INTEGRITY

MOTIVATION, SELF STARTERS

PROACTIVELY ENGAGE CLINICIANS

NEED TO UNDERSTAND CLINICIANS' PROBLEMS

NEED TO KNOW HOW TO DO CONSULTATIONS

BECOME INVOLVED

SURGICAL PATHOLOGY DIAGNOSIS

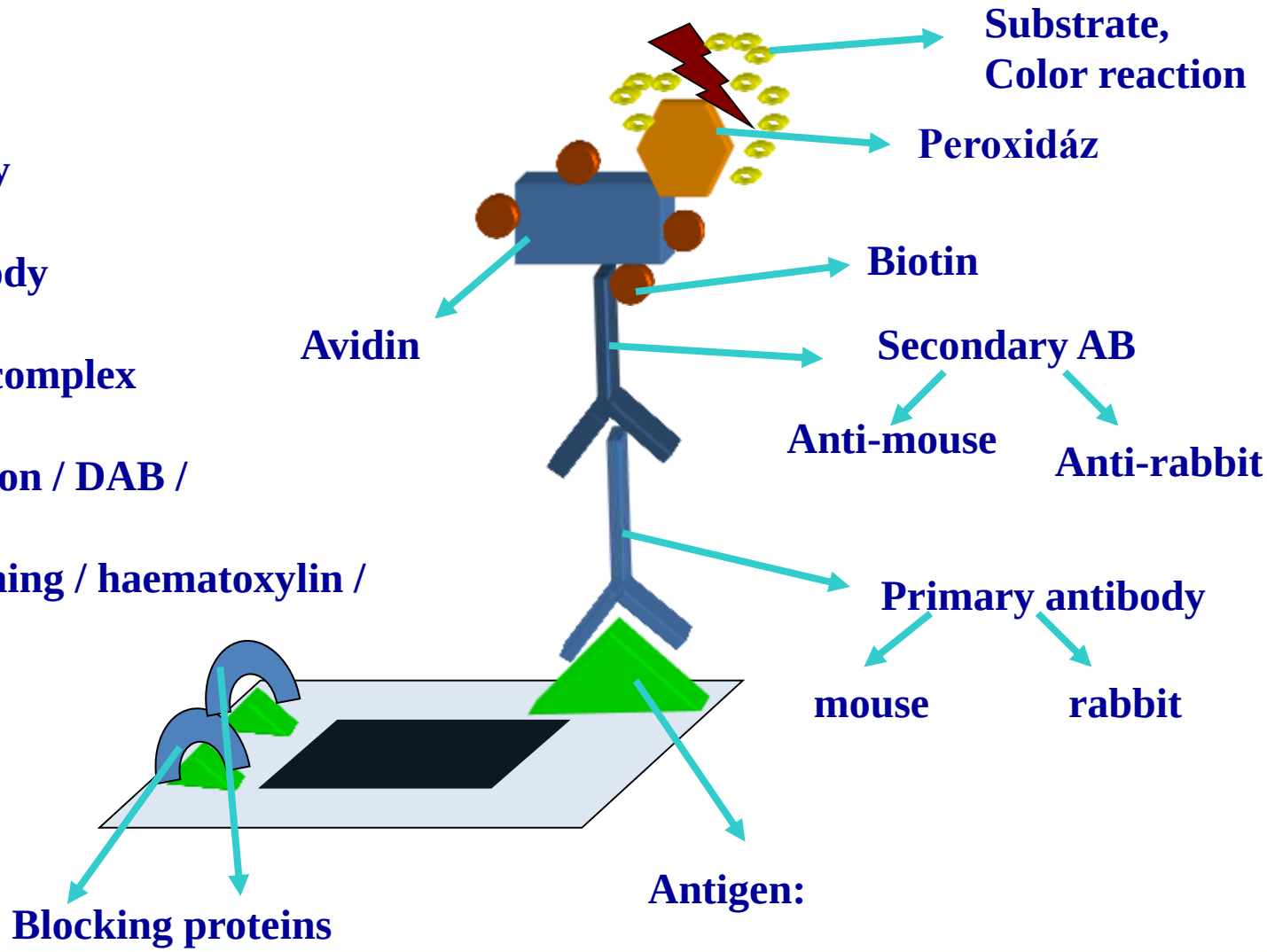
FROZEN SECTION DIAGNOSIS
GROSS DISSECTION
NON-GYN CYTOLOGY
GYN CYTOLOGY (NEW)
FNAB
QUALITY ASSURANCE – ISO

MOLECULAR PATHOLOGY EXPECTATIONS

IMMUNOCYTOCHEMISTRY
INTERPRETIVE ABILITY OF ALL TECHNIQUES

Methods - *Immunhistochemistry*

- Deparaffinization
- Antigene retreaval /microwave owen treatment
- Blocking serum
- Primary antibody
- Secondary antibody
- Avidin - Biotin - complex
- Peroxidase reaction / DAB /
- Background staining / haematoxylin /



The Nobel Prize in Physiology or Medicine 1984
Niels K. Jerne, Georges J.F. Köhler, César Milstein

The Nobel Prize in Physiology or Medicine 1984


Niels K. Jerne


Georges J.F. Köhler


César Milstein

The Nobel Prize in Physiology or Medicine 1984 was awarded jointly to Niels K. Jerne, Georges J.F. Köhler and César Milstein *"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"*.

Diagnostic Markers

Tumor Type	Marker
Epithelial Tumors (Carcinomas)	• Cytokeratin Types • Tissue-specific markers (PSA, TTF-1 usw.)
Mesenchymal Tumors	Tissue specific Markers (e.g. actin, s-100, Faktor VIII)
Hematological Tumoren	CD Proteins (e.g. T/B Cell markers)
Tumoren of unknown origin (Definition of the primary tumors)	Carcinoma = CK + Melanoma = S-100, melan-A + Lymphoma = CD45 (LCA) + only Vimentin + = Sarkom a

Prognostic / predictiv Markers

Prognosis	• Proliferation: Ki-67 • Oncoprotein Mutations, • Accumulations (nukl.): p-53
Prediktiv Markers (targeted tumor therapiy)	• Hormon receptors ER • Growth factor receptors: EGFR, HER2, c-KIT

BASICS in MOLECULAR PATHOLOGY

- **Tumor Diagnostics - IHC**
- **Diagnostics of infectious agents**
- **Diagnostics of genetic diseases**


Lung cancer

PATHOLOGY – LUNG CANCER

Small cell (SCLC)

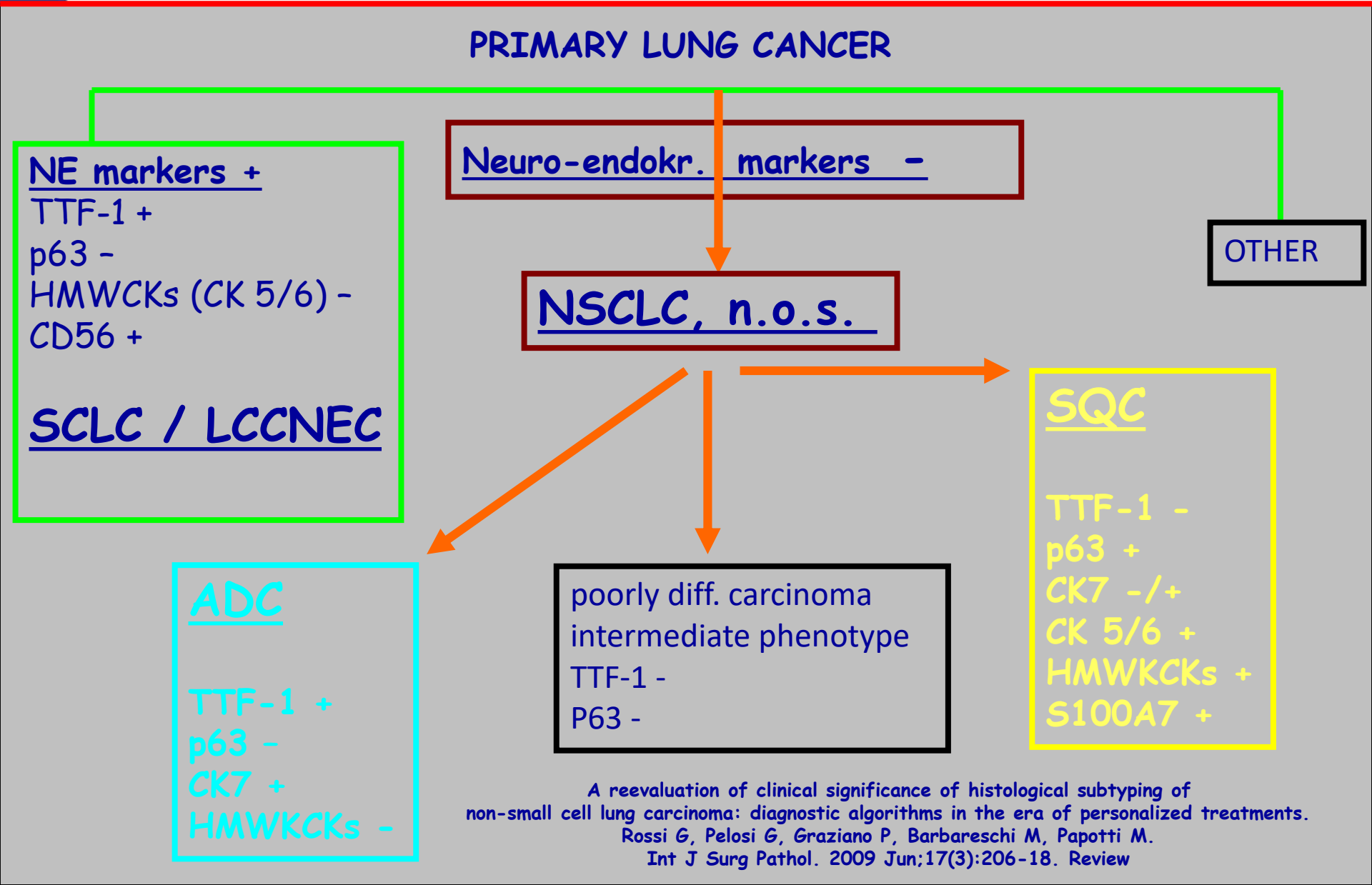
?

Non-small cell (NSCLC)

Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens.
Loo PS, Thomas SC, Nicolson MC, Fyfe MN, Kerr KM.
J Thorac Oncol. 2010 Apr;5(4):442-7.

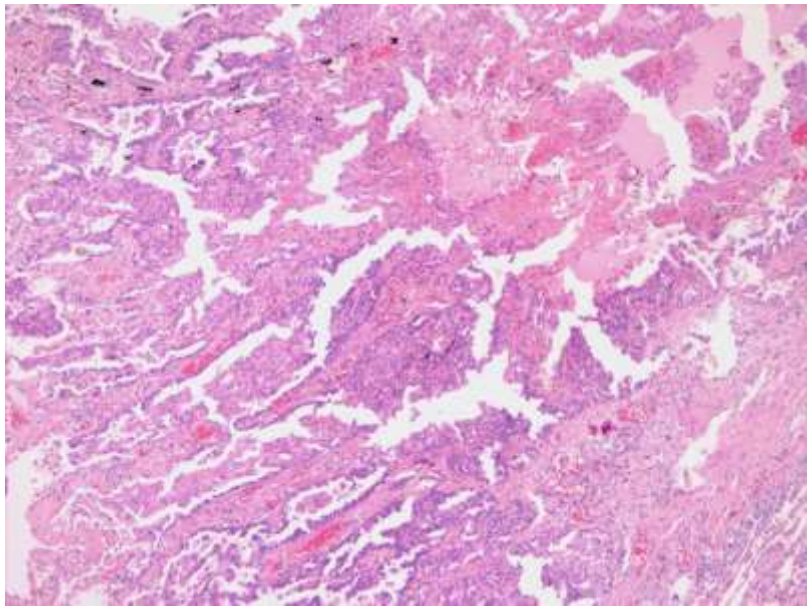
A reevaluation of the clinical significance of histological subtyping of non--small-cell lung carcinoma: diagnostic algorithms in the era of personalized treatments.
Rossi G, Pelosi G, Graziano P, Barbareschi M, Papotti M.
Int J Surg Pathol. 2009 Jun;17(3):206-18. Review.

PATHOLOGY - DIAGNOSTICS

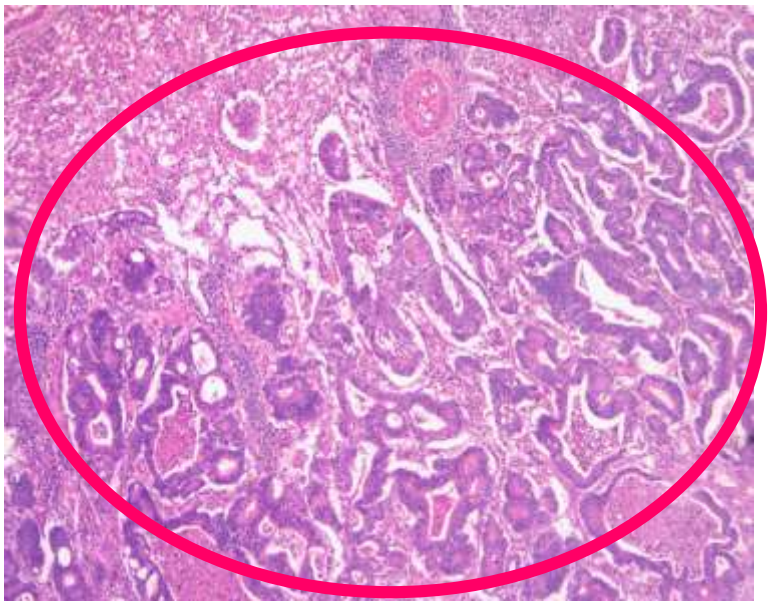


A reevaluation of clinical significance of histological subtyping of non-small cell lung carcinoma: diagnostic algorithms in the era of personalized treatments. Rossi G, Pelosi G, Graziano P, Barbareschi M, Papotti M. Int J Surg Pathol. 2009 Jun;17(3):206-18. Review

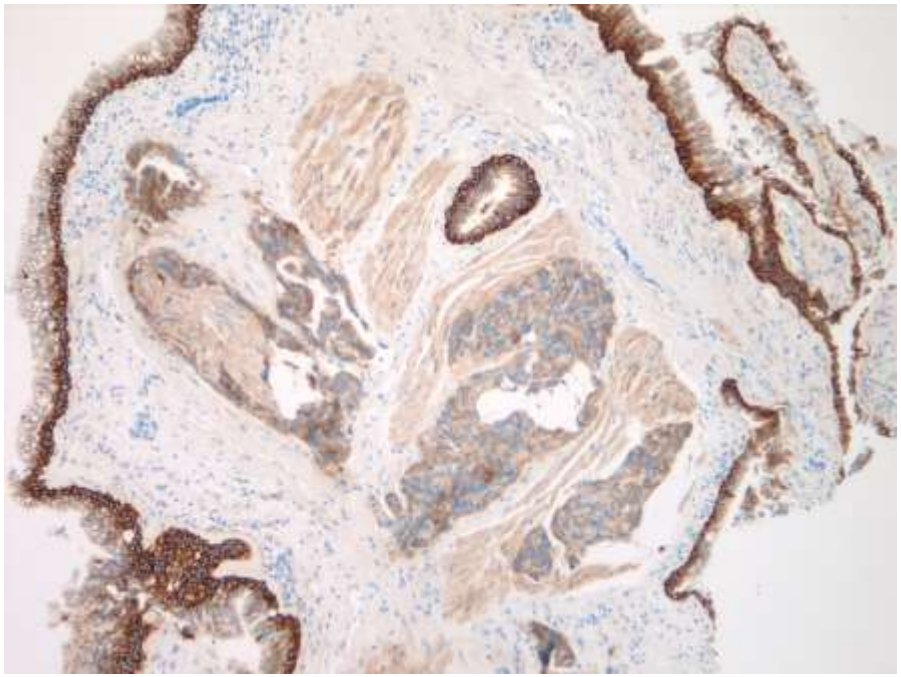
Surgical resection



Macrodissection …..

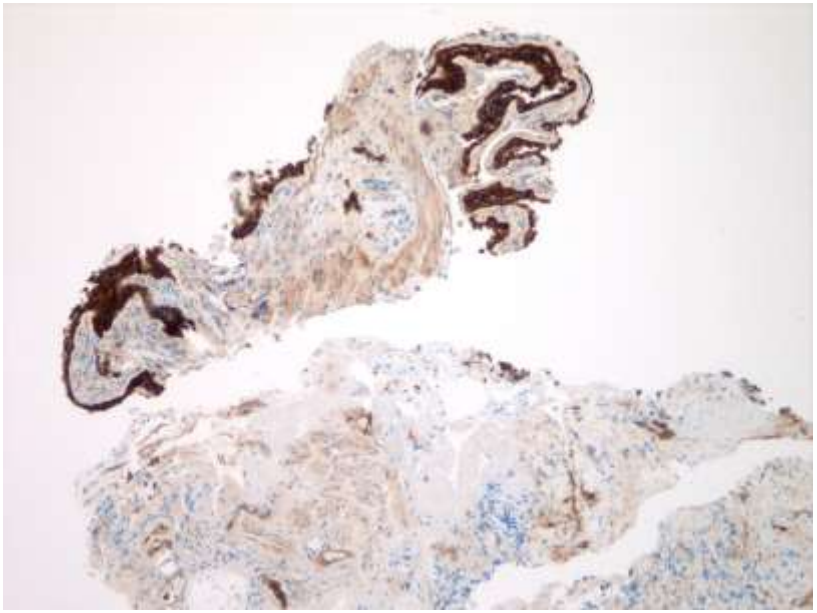


Transbronchial biopsy

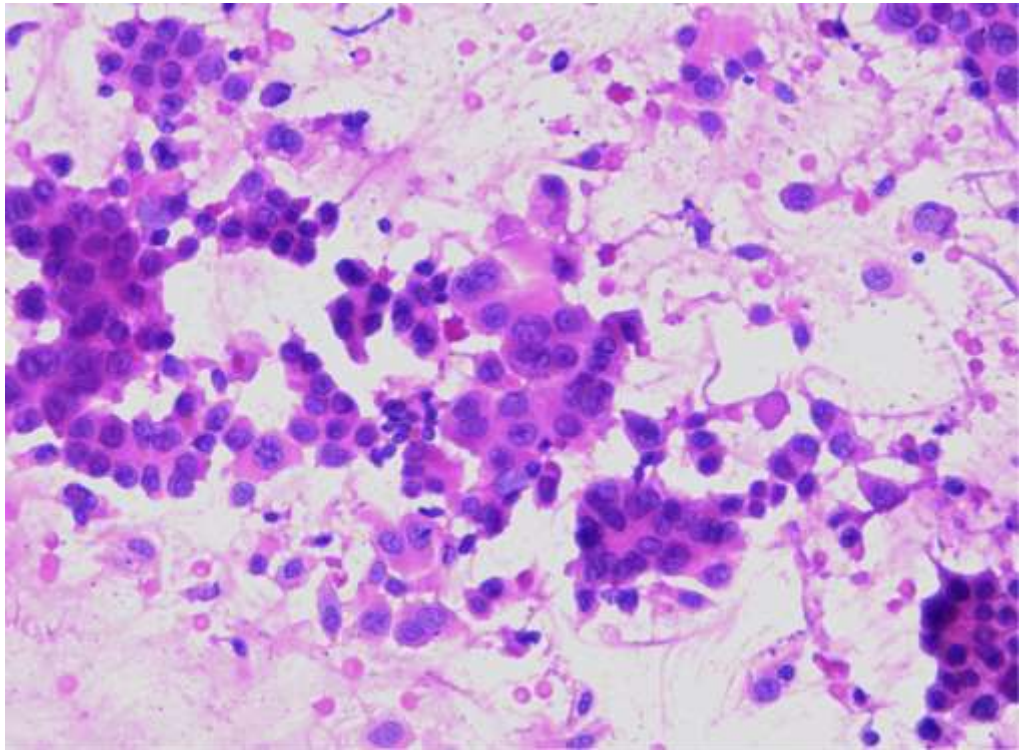


T/N <1:2

T/N<1:8

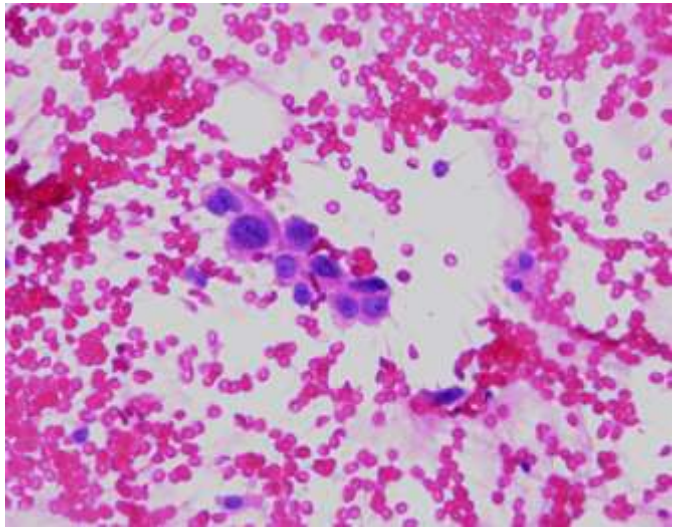


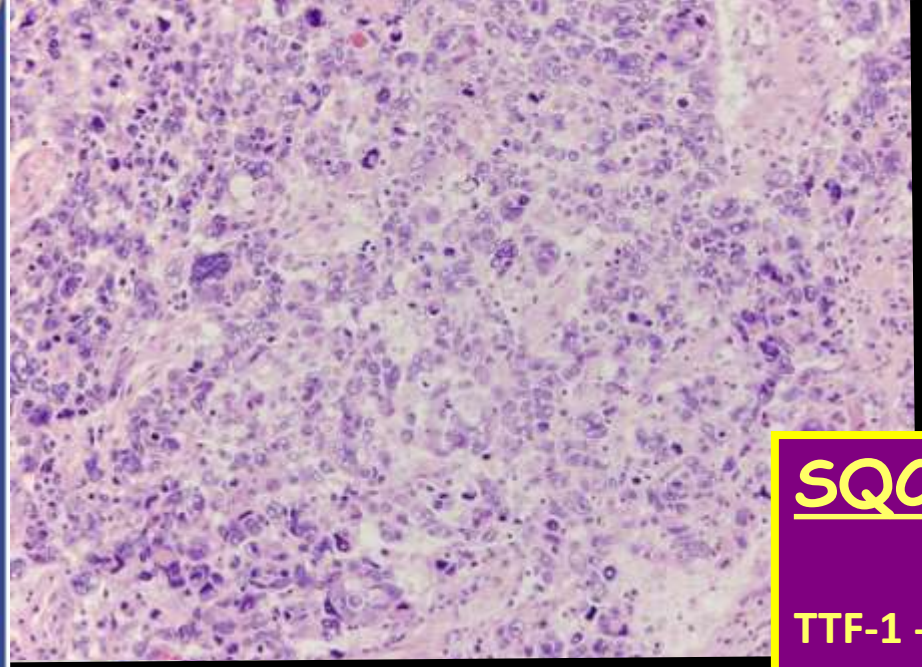
Bronchus brush cytology

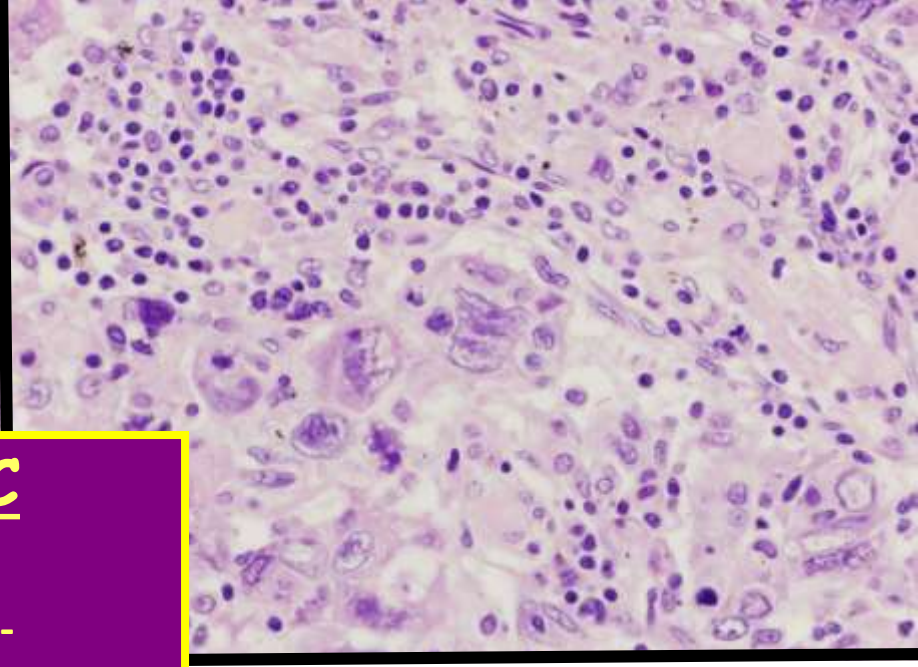


T/N>10/1 (90%)

T/N>1/10 (10%)

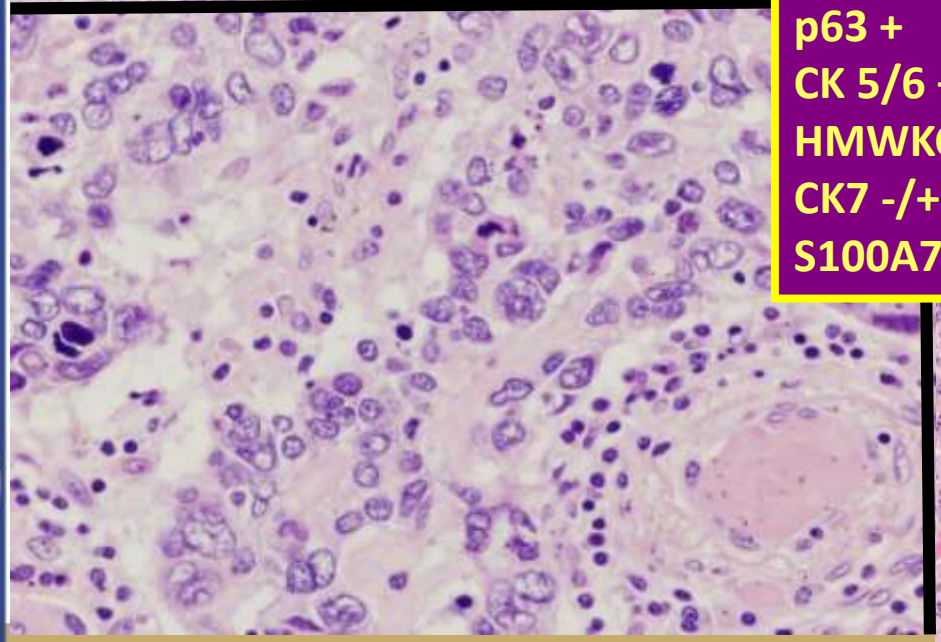


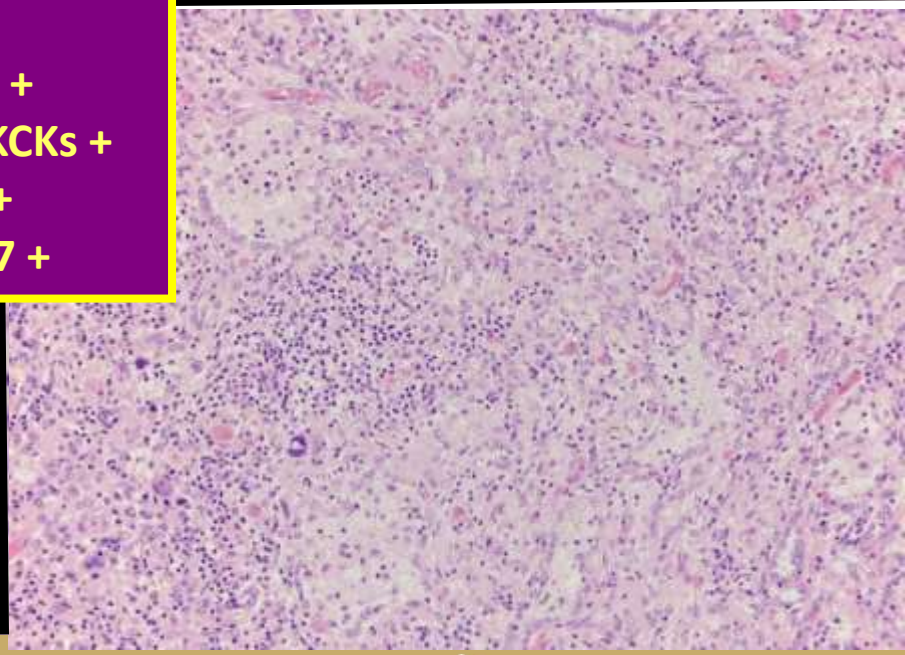


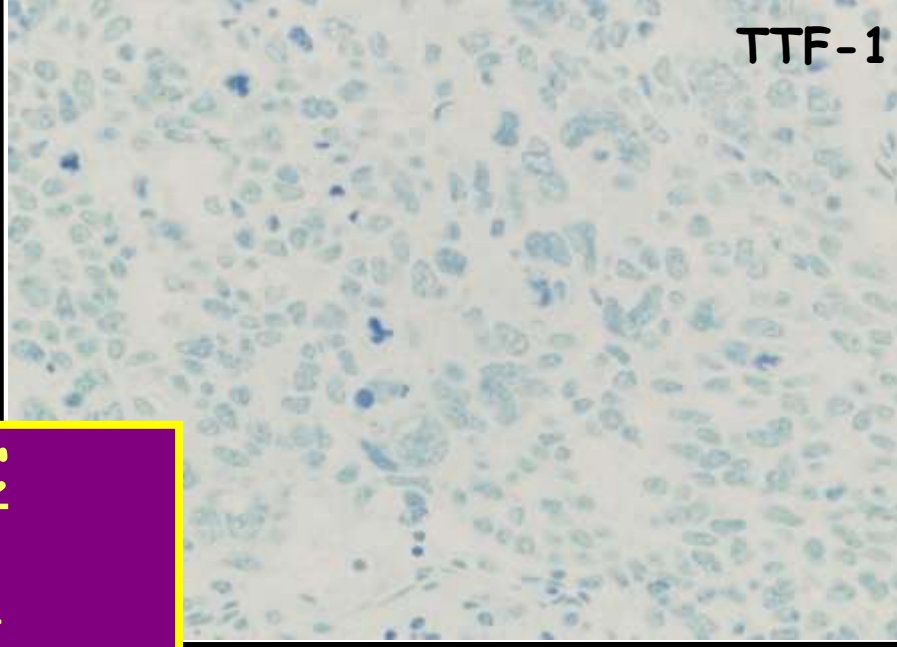


SQC

TTF-1 -
p63 +
CK 5/6 +
HMWKCKs +
CK7 -/+
S100A7 +

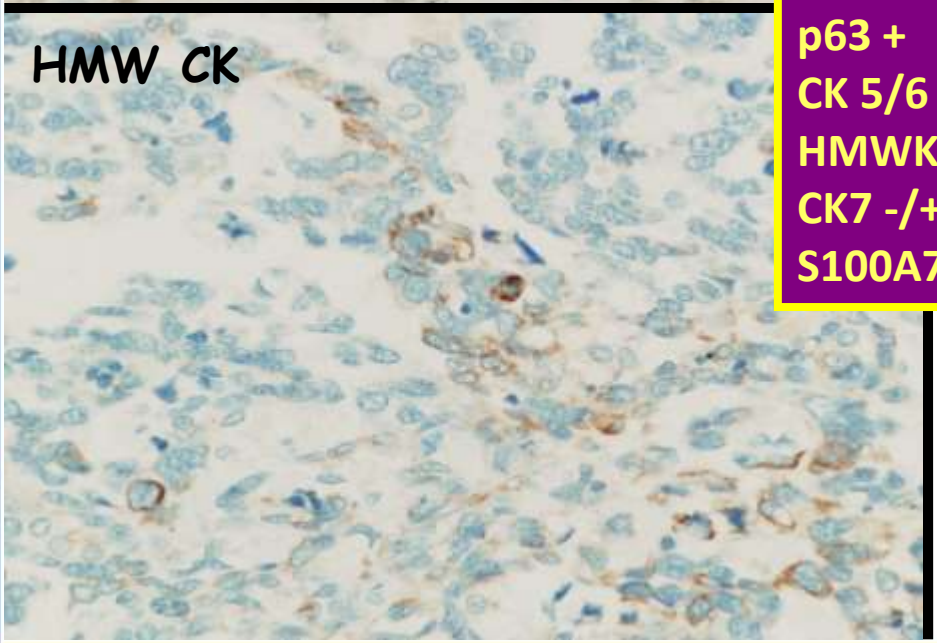


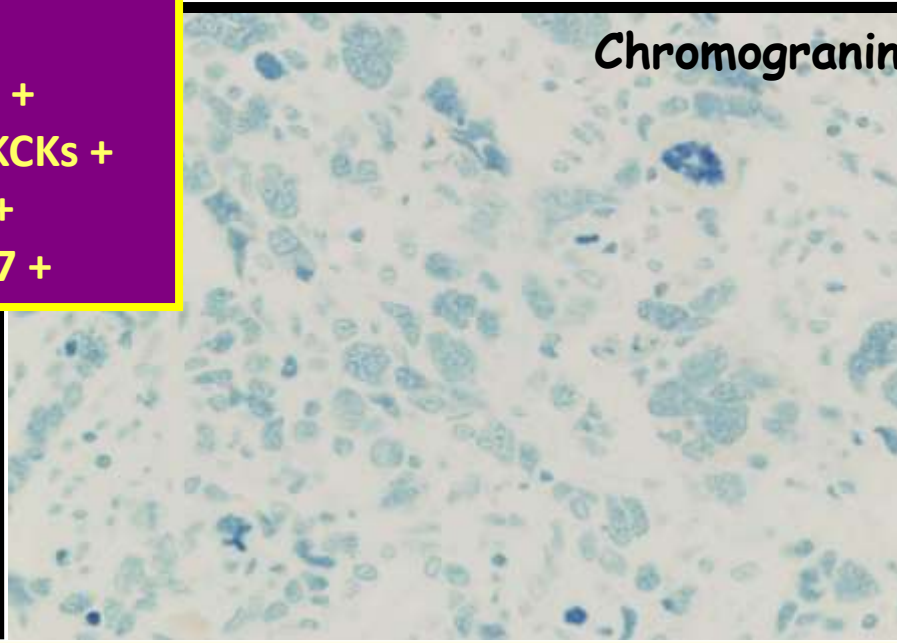


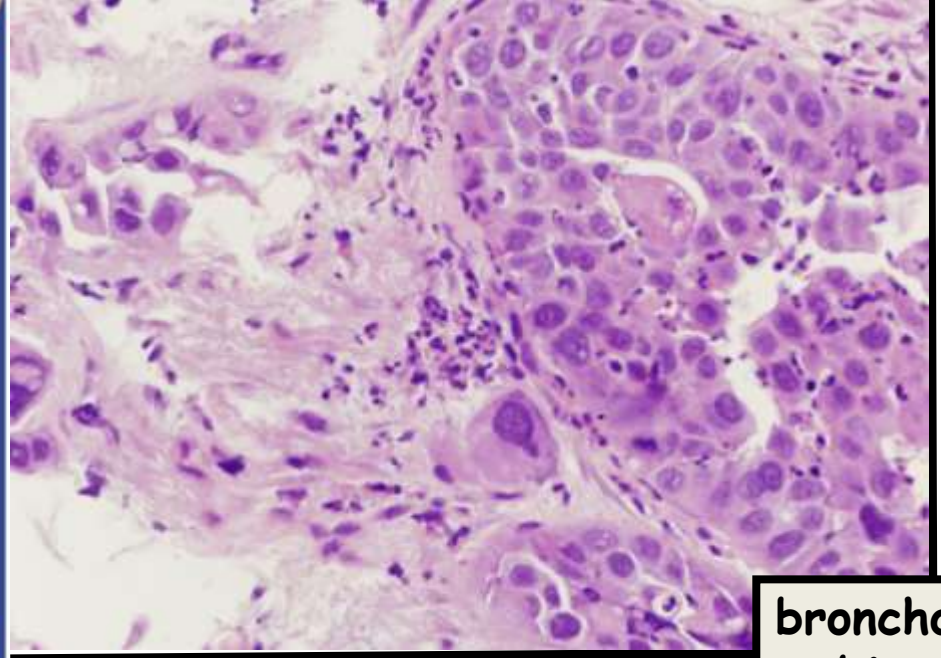


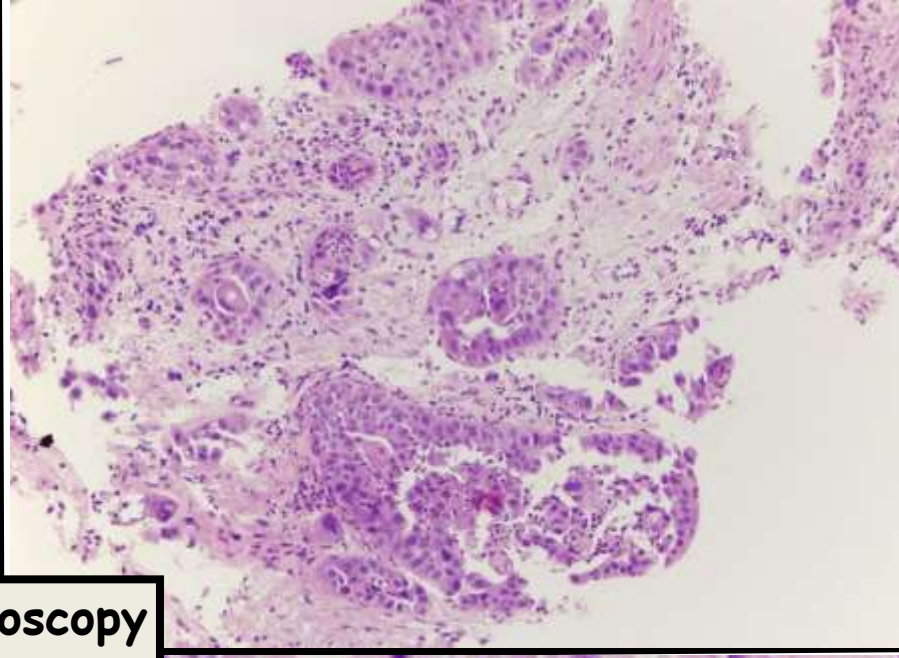
SQC

TTF-1 -
p63 +
CK 5/6 +
HMWKCKs +
CK7 -/+
S100A7 +

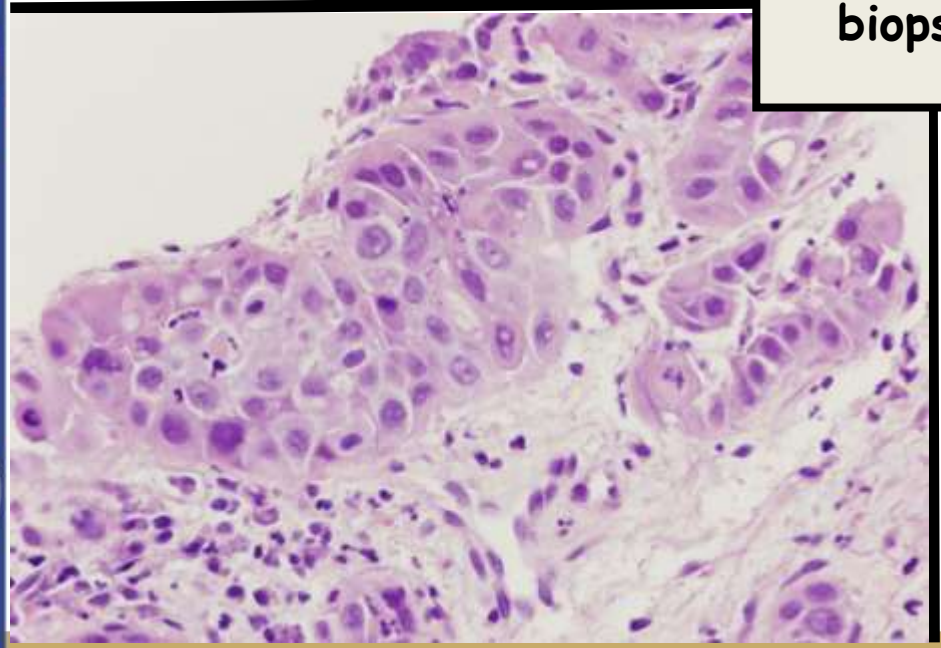


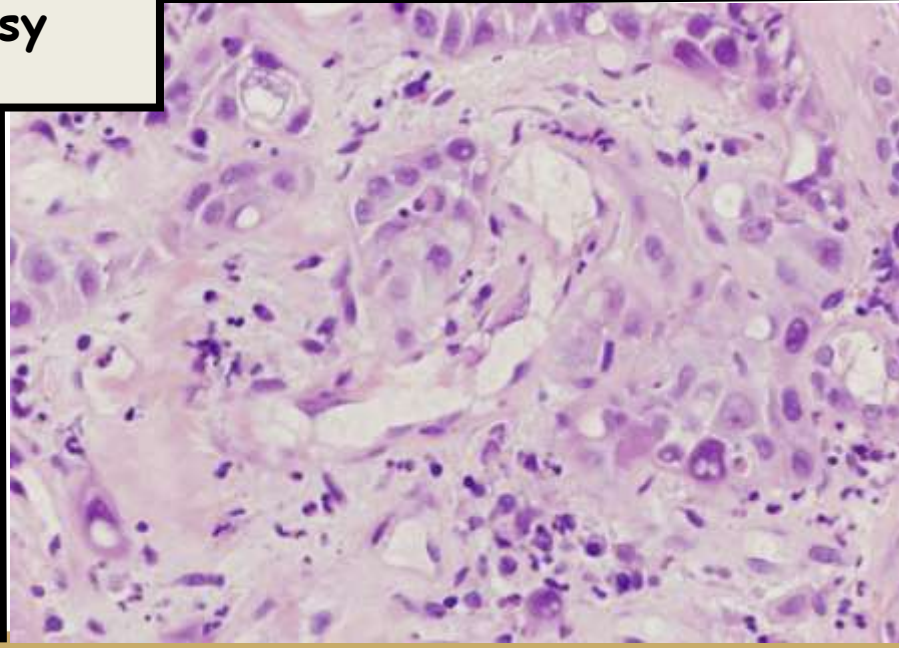


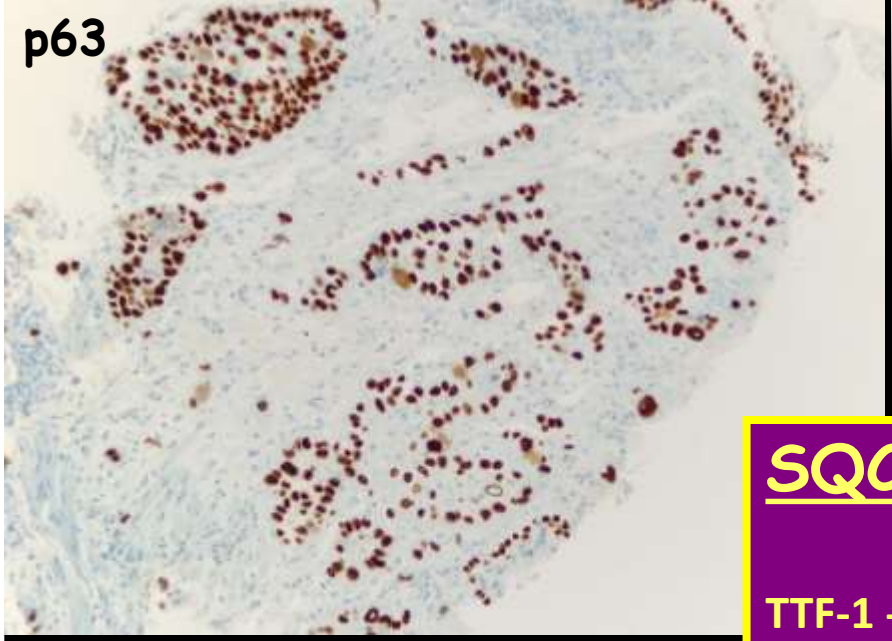


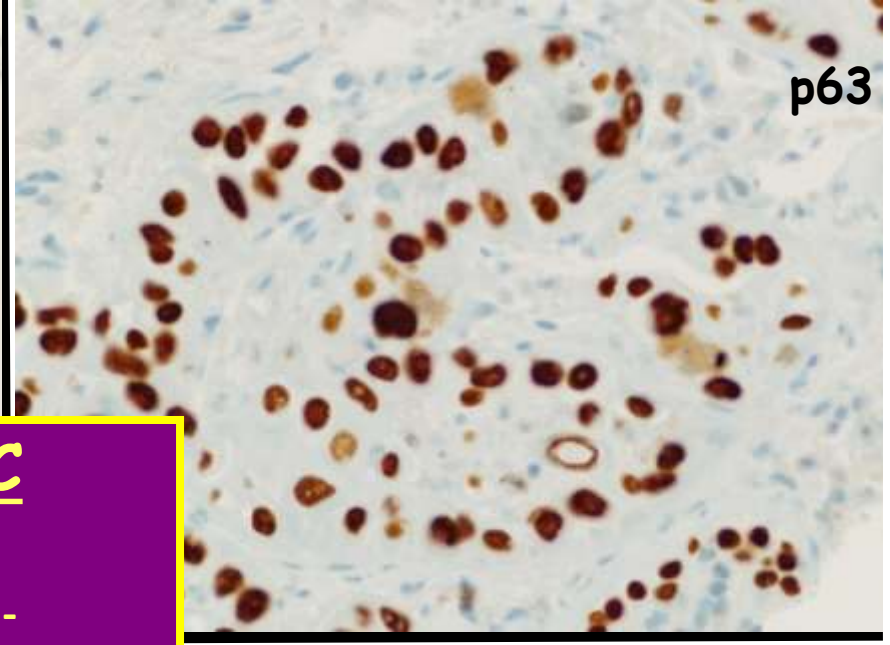


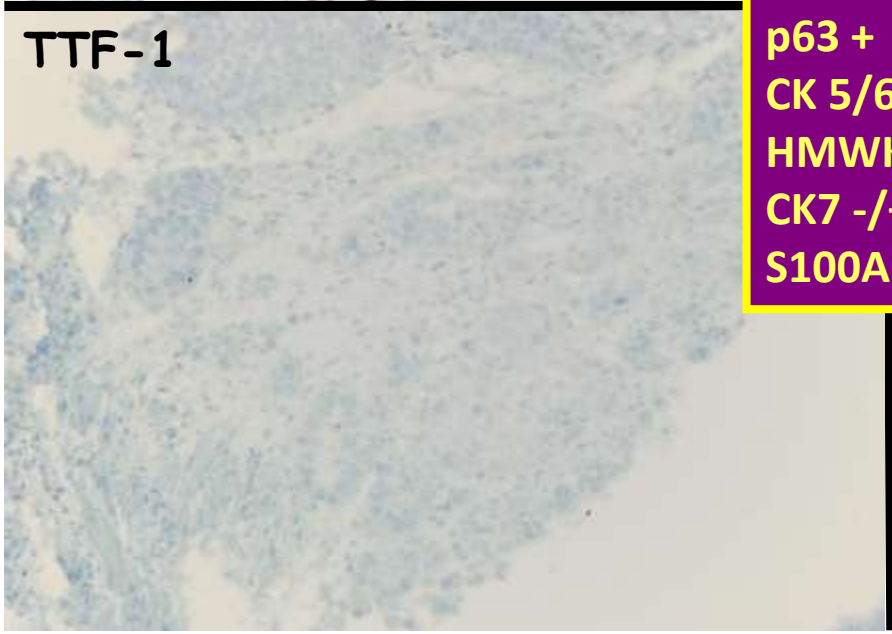
bronchoscopy biopsy

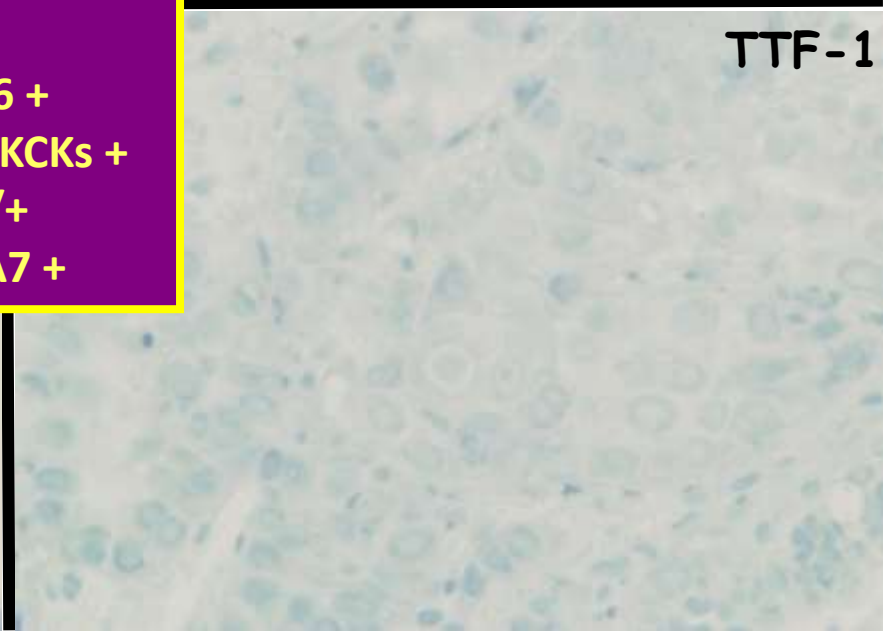






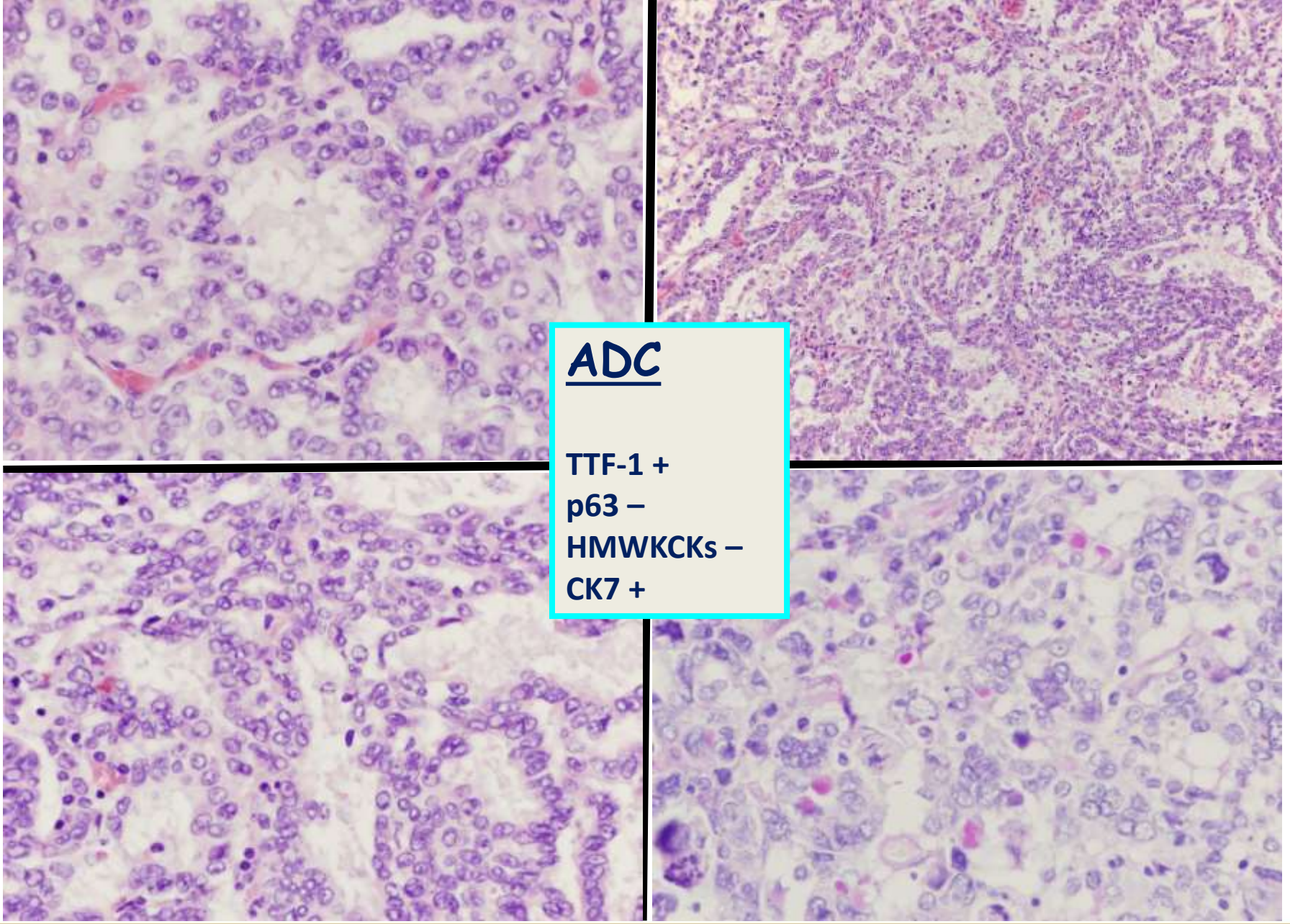


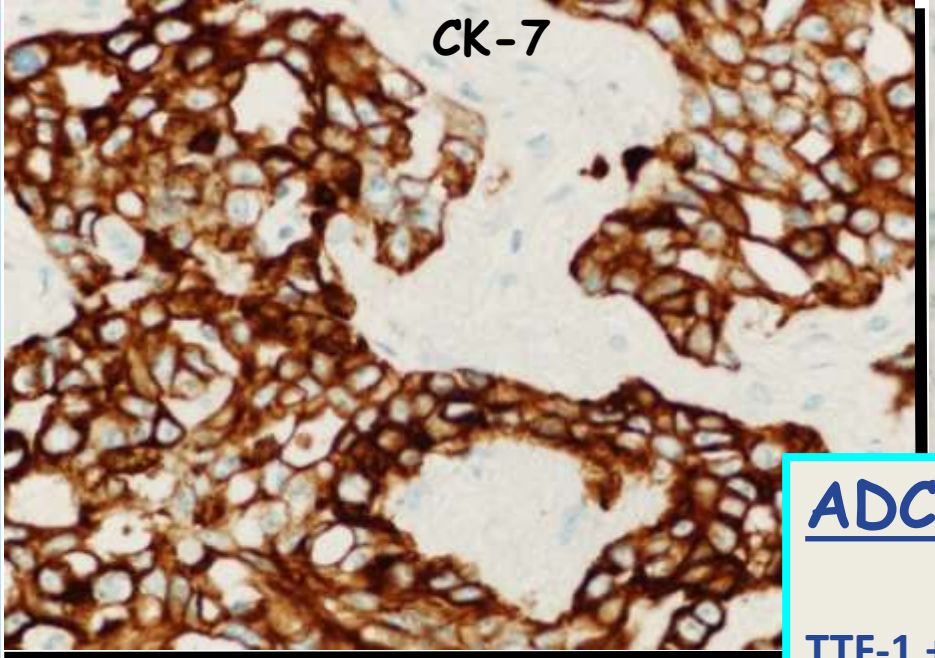


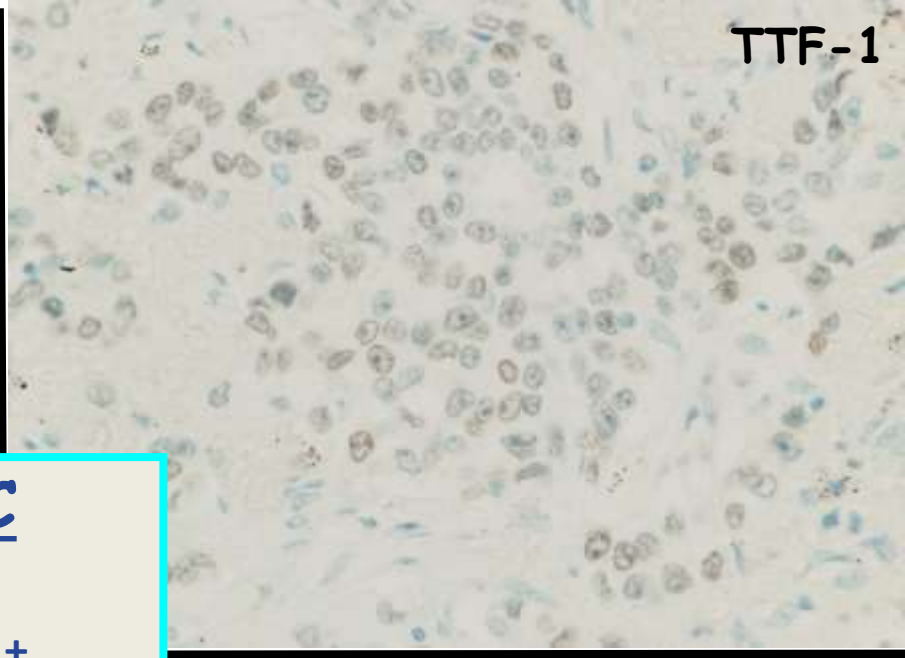


SQC

TTF-1 -
p63 +
CK 5/6 +
HMWKCKs +
CK7 -/+
S100A7 +

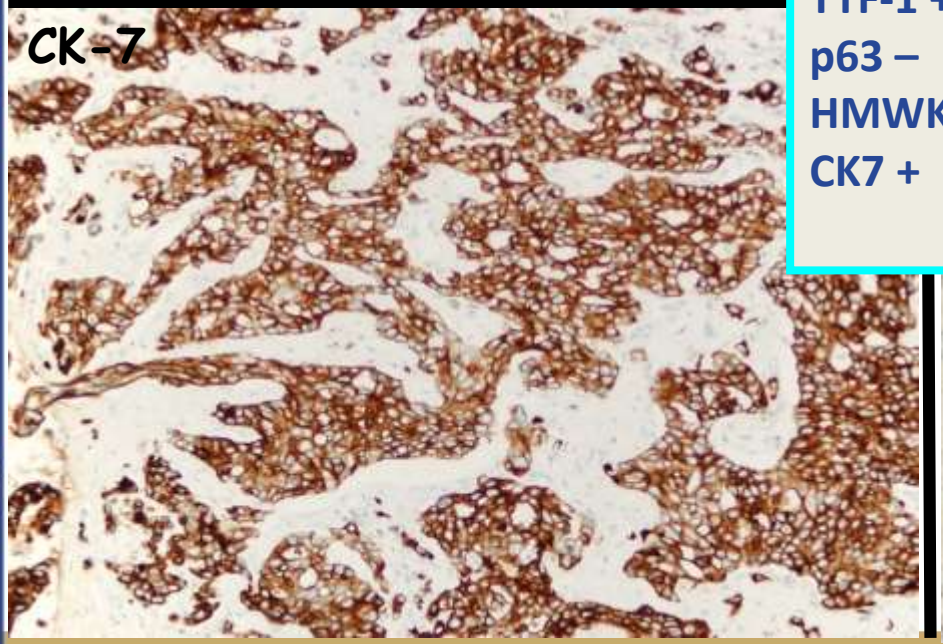

ADC
TTF-1 +
p63 –
HMWKCKs –
CK7 +

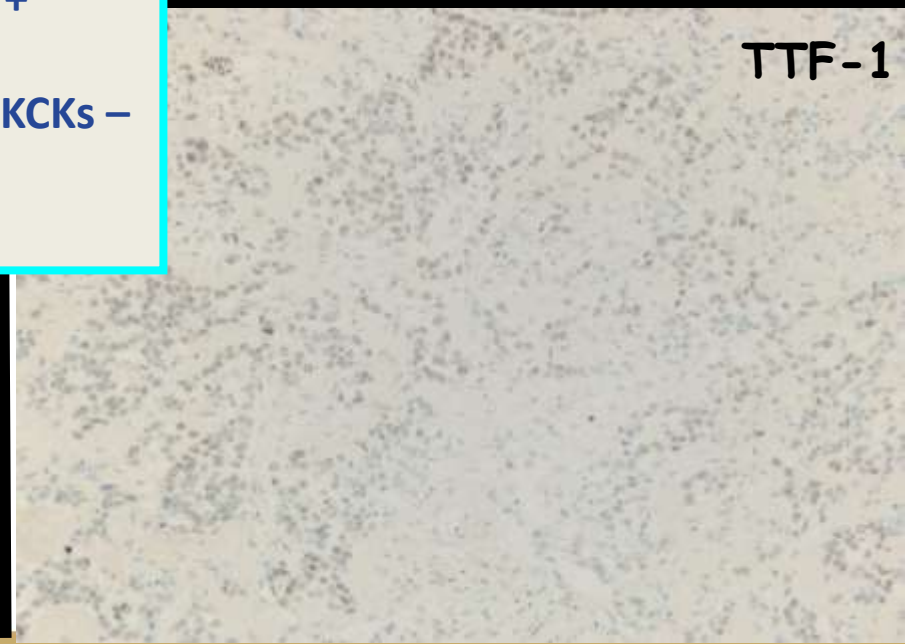


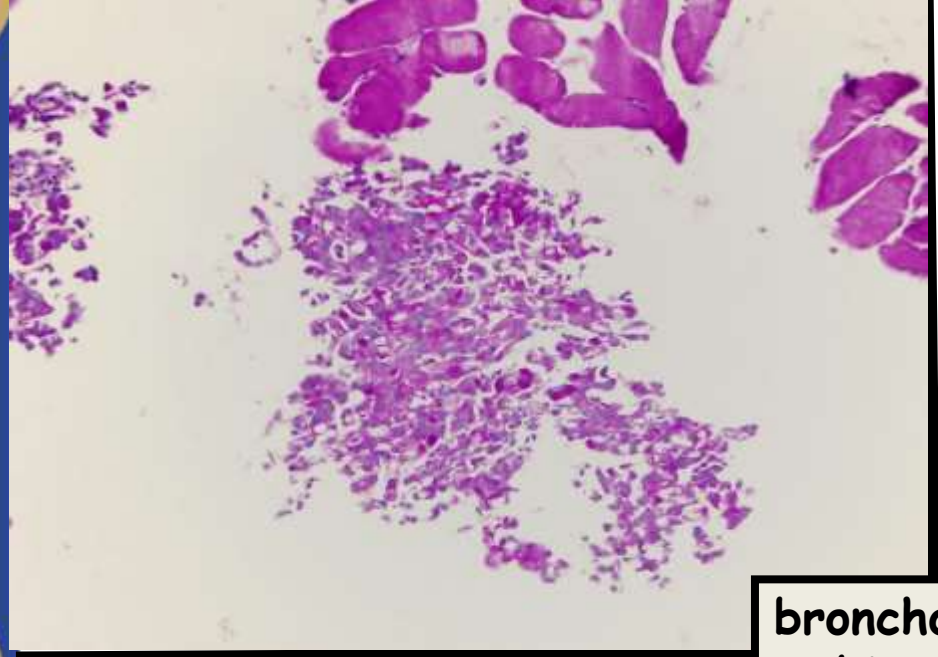


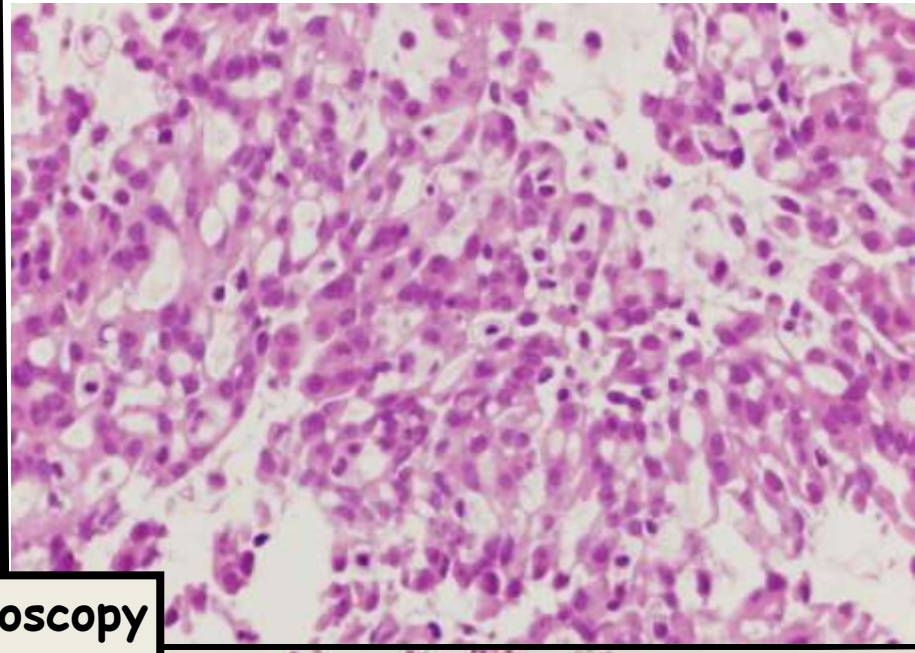
ADC

TTF-1 +
p63 –
HMWKCKs –
CK7 +

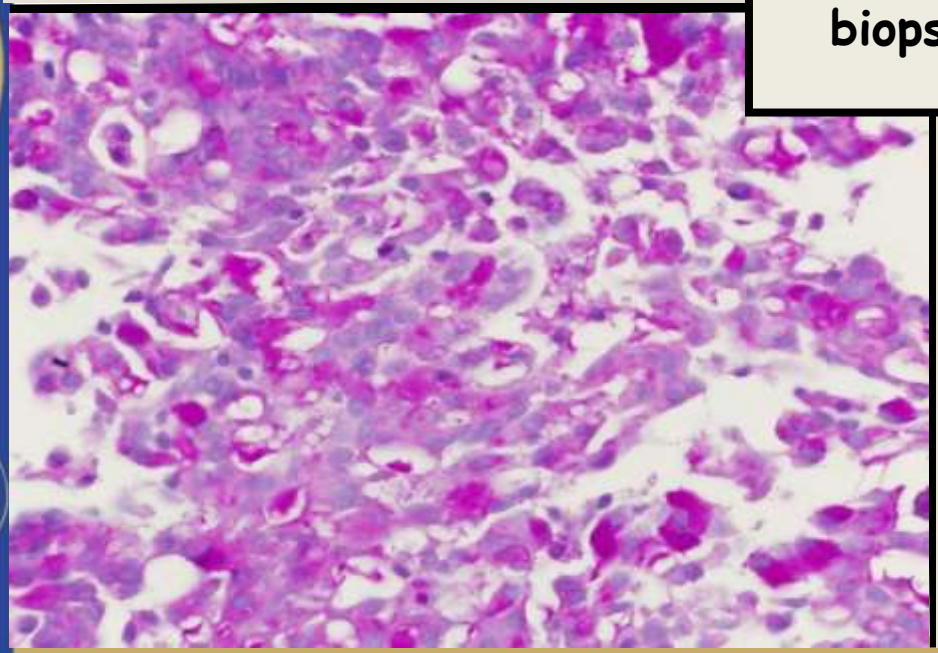


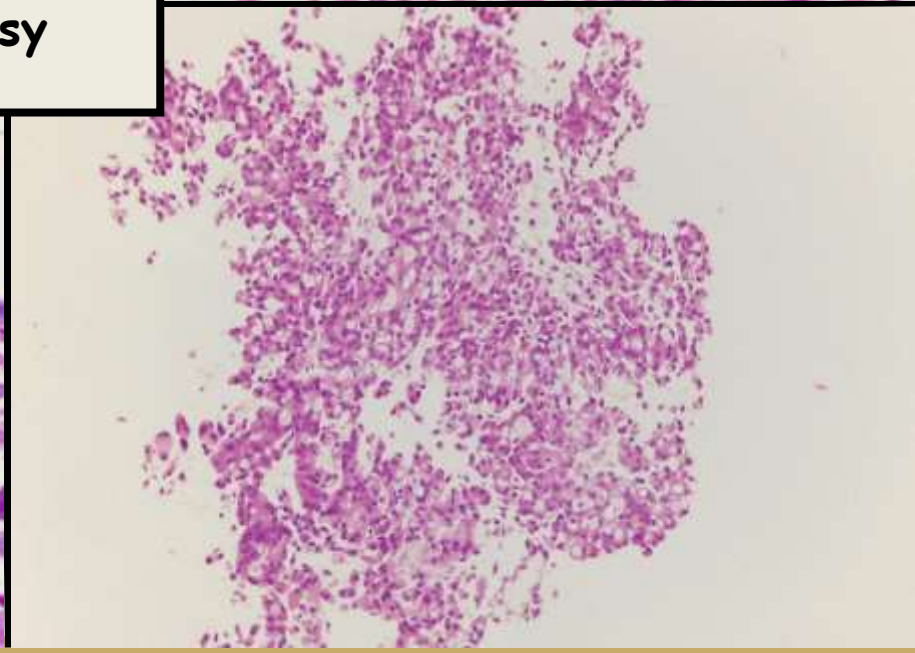


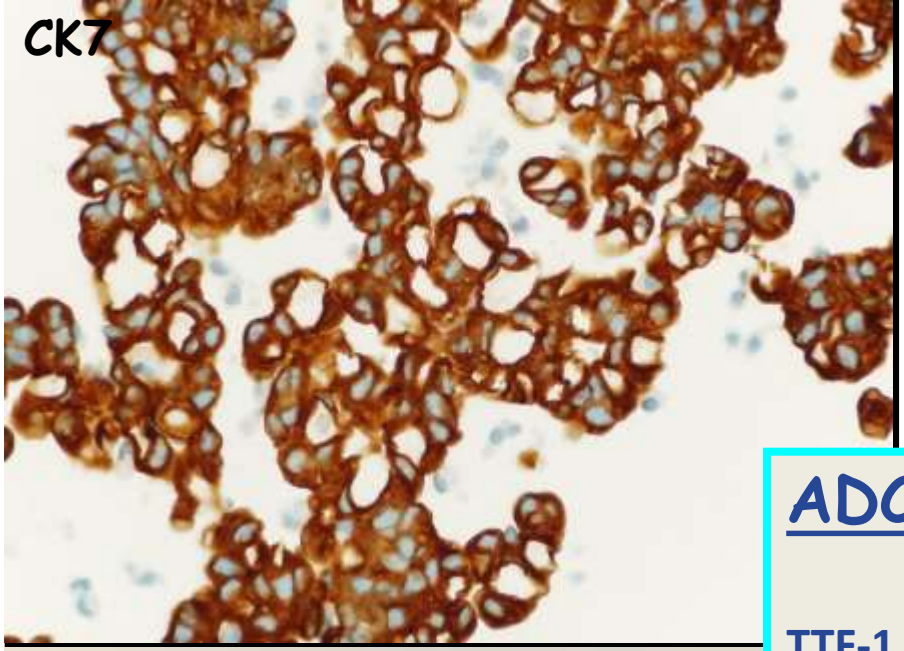


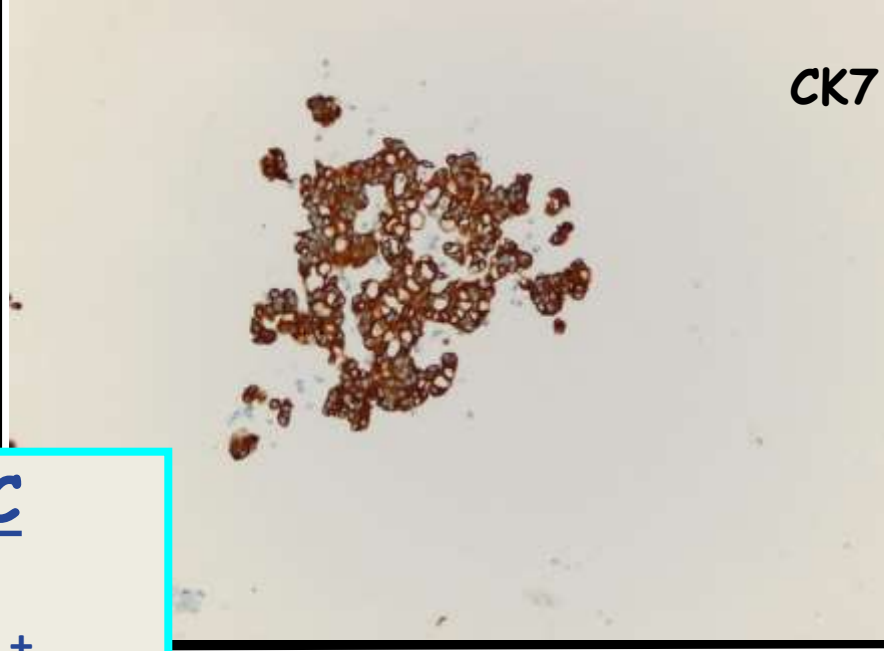


bronchoscopy biopsy



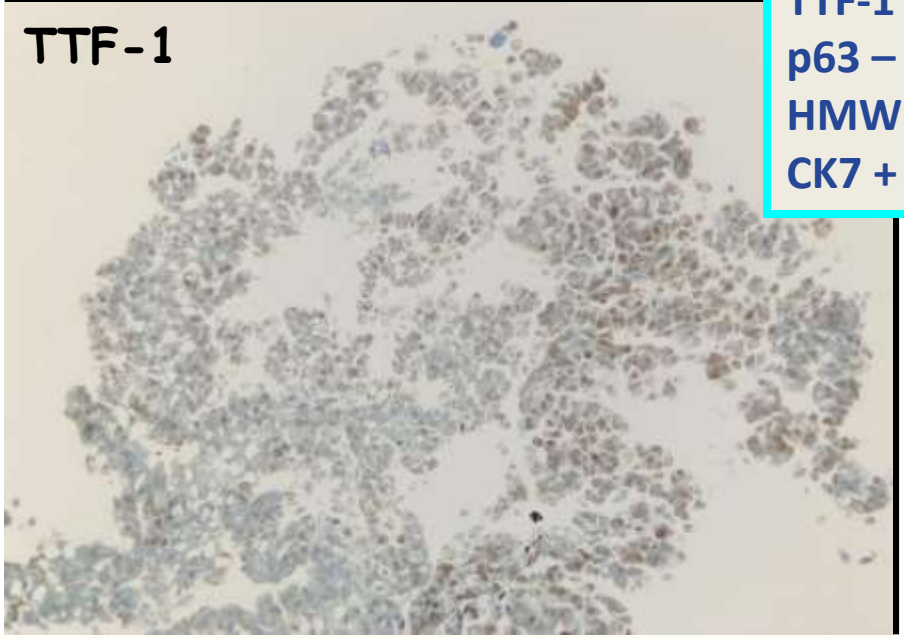


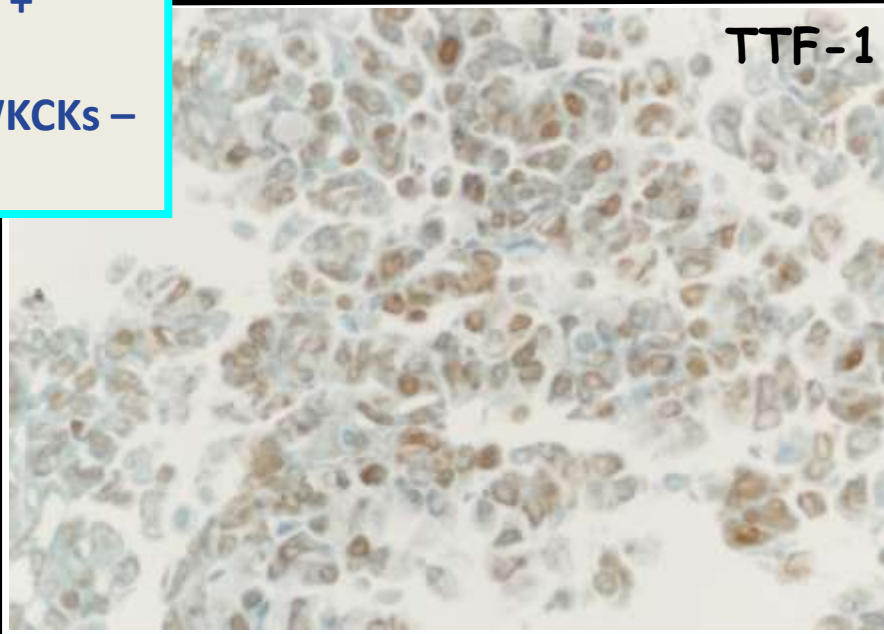




ADC

TTF-1 +
p63 –
HMWKCKs –
CK7 +

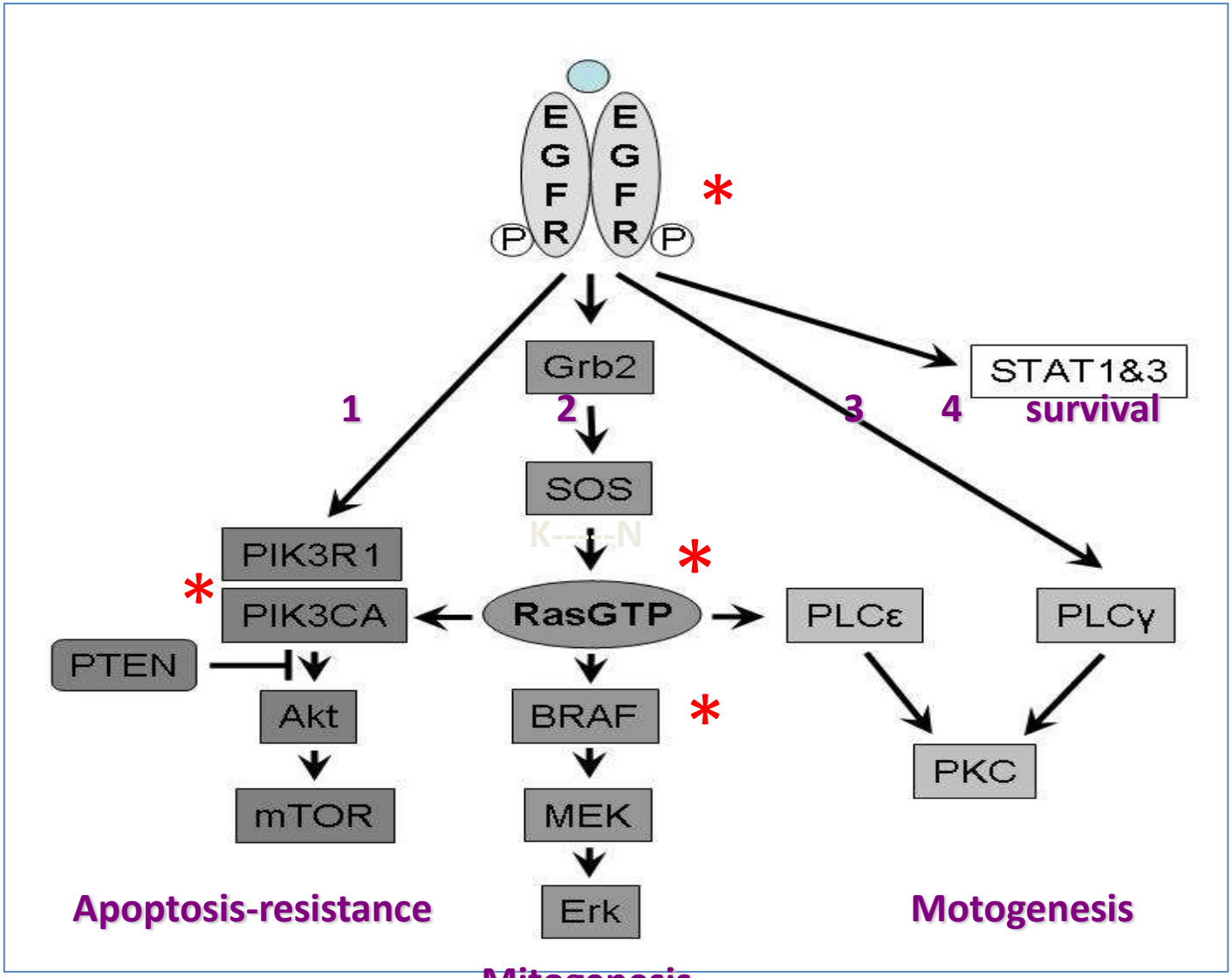




BASICS in MOLECULAR PATHOLOGY

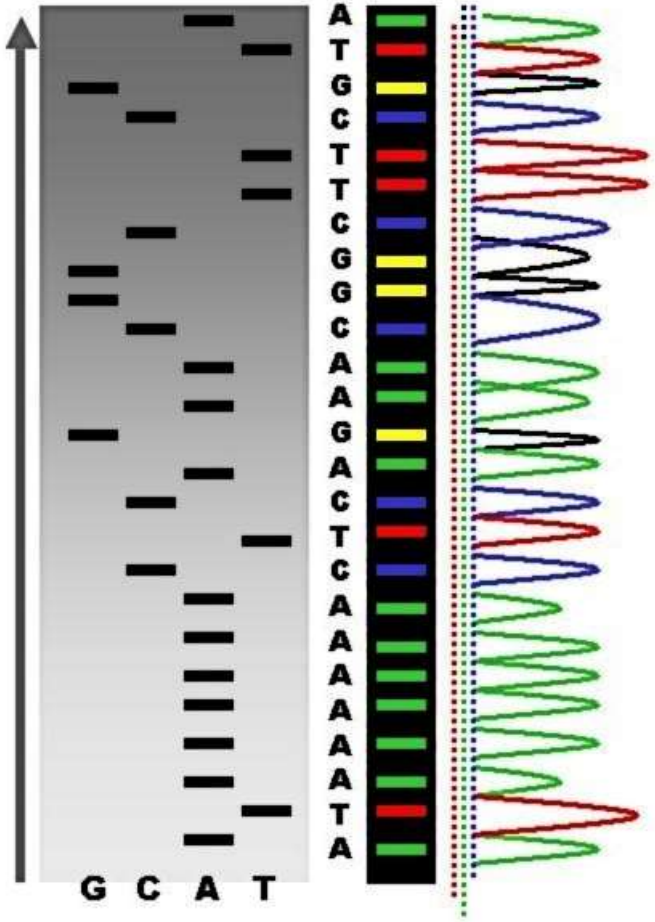
- **Tumor Diagnostics**
- **Diagnostics of infectious agents**
- **Diagnostics of genetic diseases**

EGFR signaling pathway



Next Generation Sequencing. Precision medicine

Myseq: 1000-USD genome



Targeted Therapy – solid tumors

Lung cc.

kRAS WT

erlotinib (TARCEVA -Roche)
EGFR tik
1 % immunh. EGFRpoz.

gefitinib (IRESSA-Astra)
EGFR tik
EGFR WT poz. prediktor

crizotinib (XALKORI -Pfizer)
ALK-EML4 FISH
Tik, ha kRAS, EGFR WT
(MET inhib. as well)

Colon cc.

Panitumumab (VECTIBIX-(Amgen)
EGFR MAB
Kras és nRAS (2. ,3., 4. Exon WT)

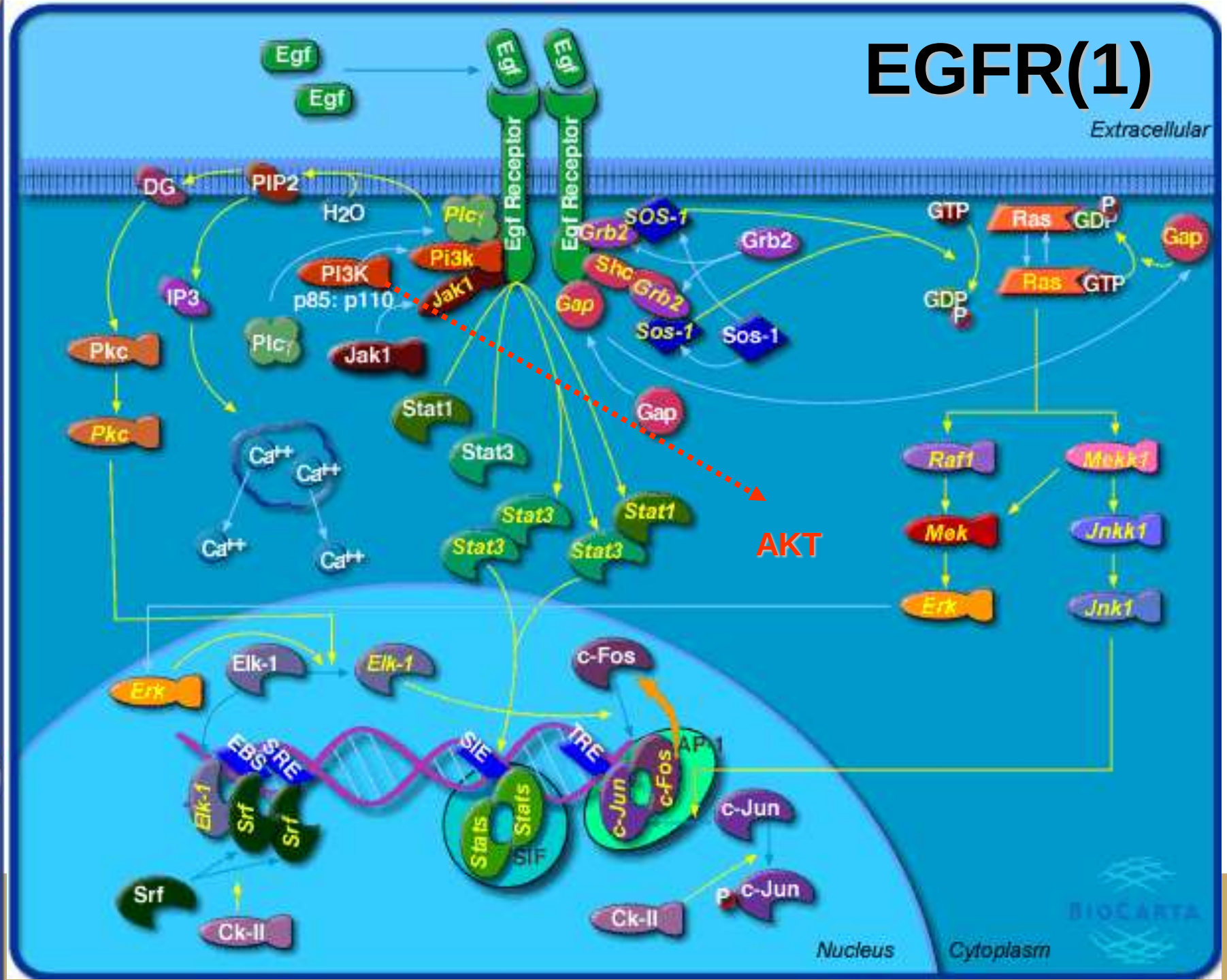
Bevazicumab (AVASTIN-Roche)
MAB, VEGF-A

Head&Neck, Squamous cell cc.

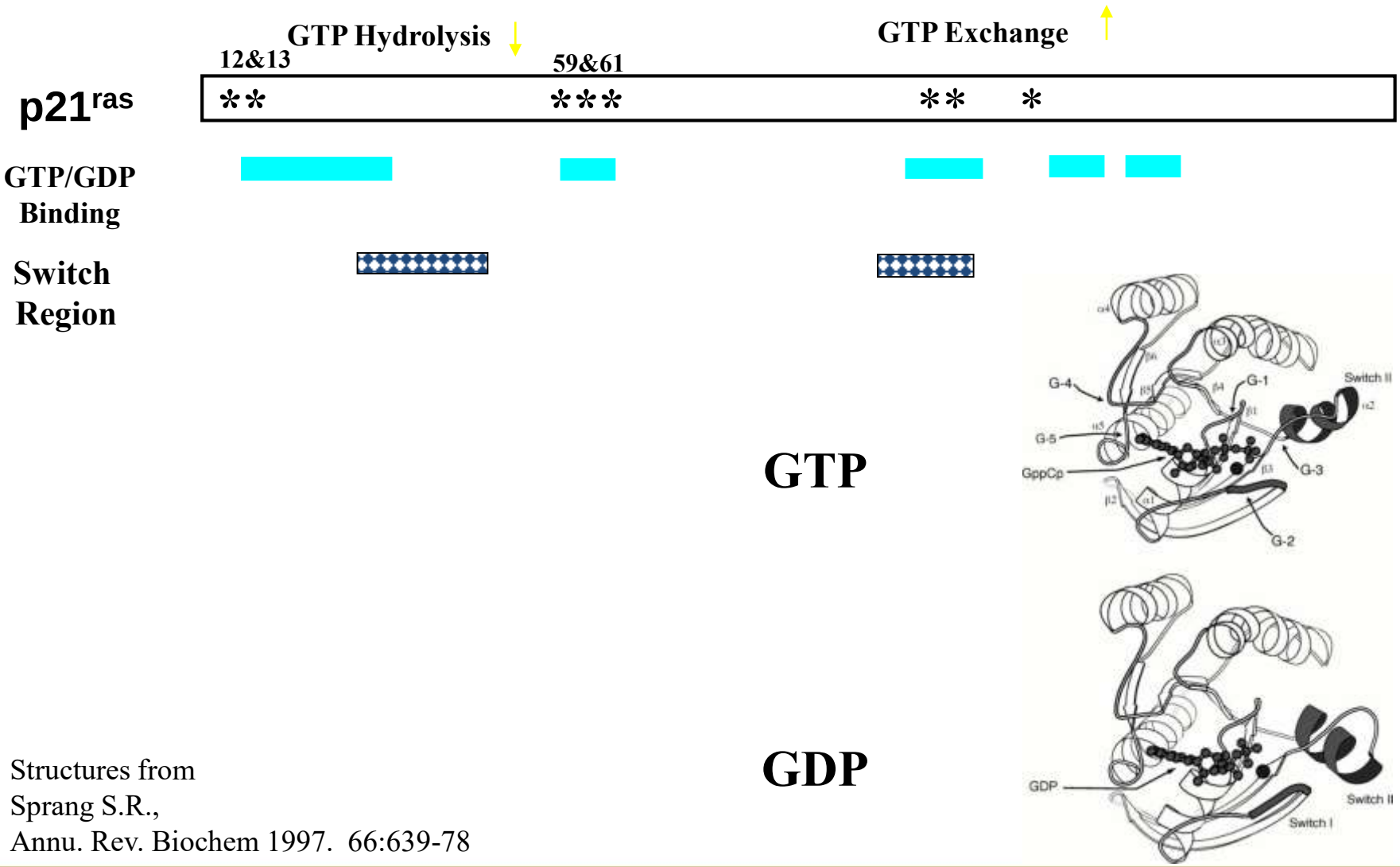
MELANOMA:

vemurafenib (ZELBORAF-Pfizer)
bRAF tik (V-600E mutation neg. predict.)

cetuximab (ERBITUX-Roche)
EGFR MAB

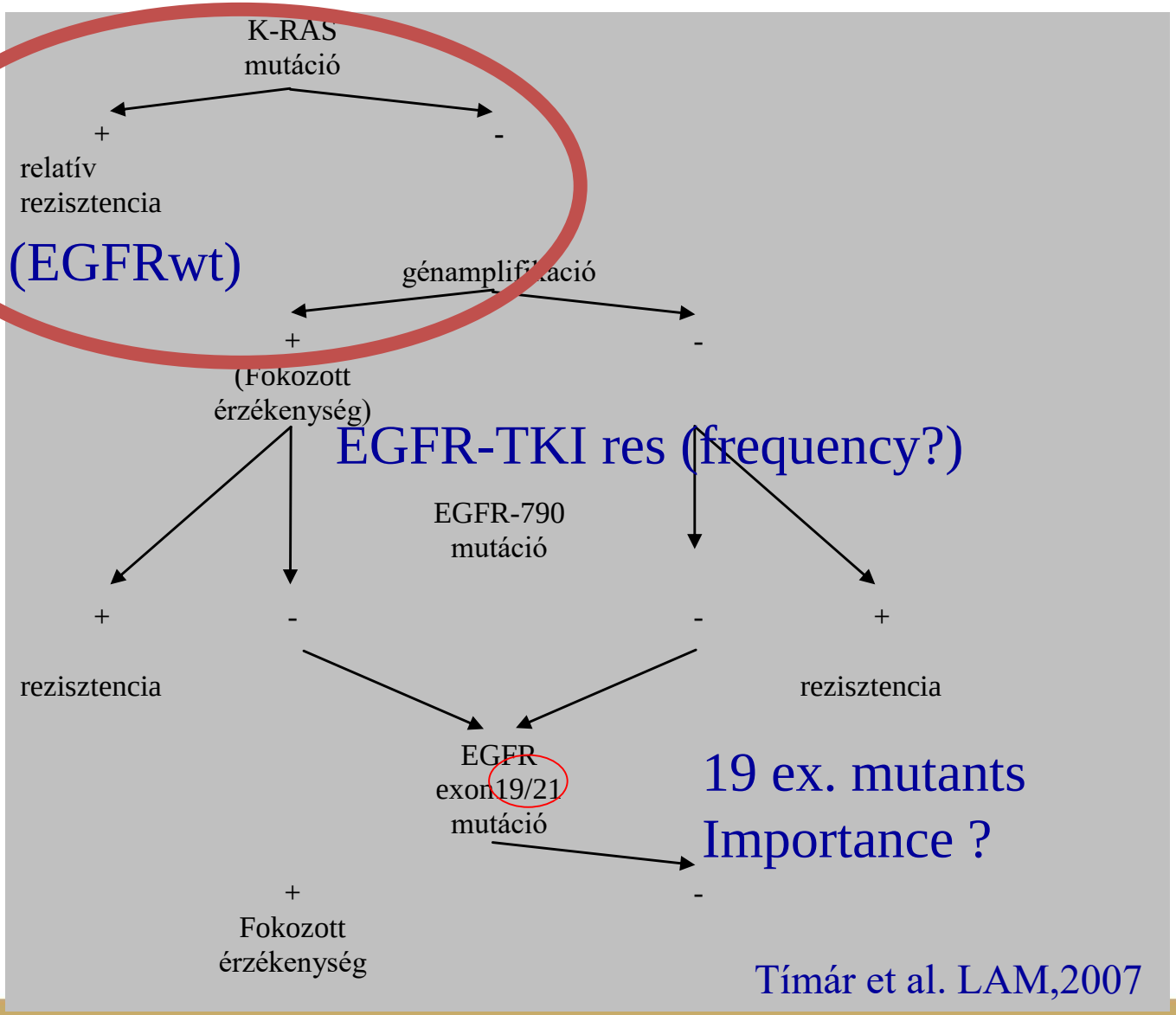

EGFR(1)
Extracellular
Egf
Egf
Egf Receptor
Egf Receptor
DG
PIP2
H2O
Plc
Pi3k
PI3K
p85: p110
Jak1
IP3
Plc
Jak1
Pkc
Pkc
Ca++
Ca++
Ca++
Ca++
Stat1
Stat3
Gap
Grb2
SOS-1
Grb2
Shc
Grb2
Gap
Sos-1
Sos-1
GTP
Ras
GDP
P
Gap
Ras
GTP
GDP
P
AKT
Raf1
Mekk1
Mek
Jnkk1
Erk
Jnk1
Stat3
Stat1
Stat3
Stat3
Elk-1
Elk-1
Erk
c-Fos
EBS
SRE
SIE
IRE
AP1
Elk-1
Srf
Srf
Stats
Stats
SIF
c-Jun
c-Fos
c-Jun
Srf
Ck-II
Ck-II
P
c-Jun
Nucleus
Cytoplasm
BIOCARTA

Activating Ras Mutations



Structures from
Sprang S.R.,
Annu. Rev. Biochem 1997. 66:639-78

Molecular genetic selection: adenocarcinoma



K-*ras* mutation analysis exon 2 codon 12. PCR-RFLP

K-*ras*

Codon 10 11 12 13 14 15

.....GGA **G**CT GGT GGC GTA .....

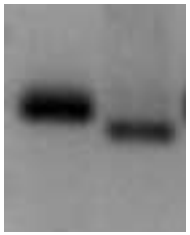
PCR „mismatch"primer

.....GGA **C**CT

*Bst*NI site

Normal .....GGA **C**CT GG....

***Bst*NI splicing**



No *Bst*NI site

Mutant

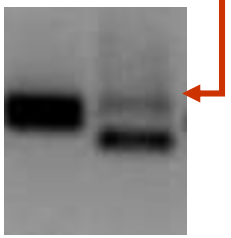
.....GGA **C**CT NG...

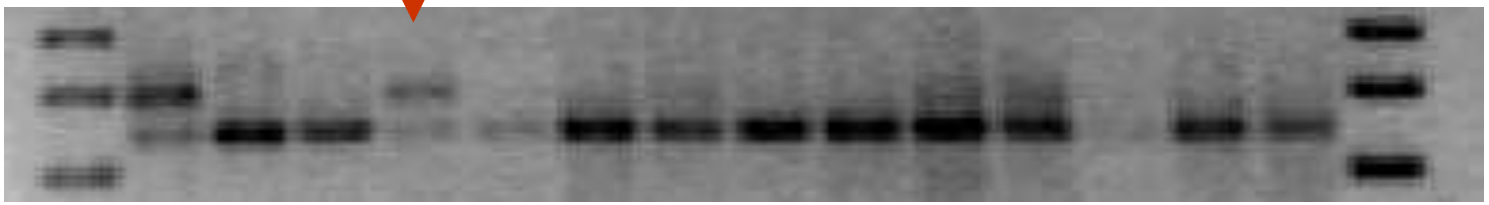
or

.....GGA **C**CT GN...

(N ≠ G)

***NO Bst*NI splicing**





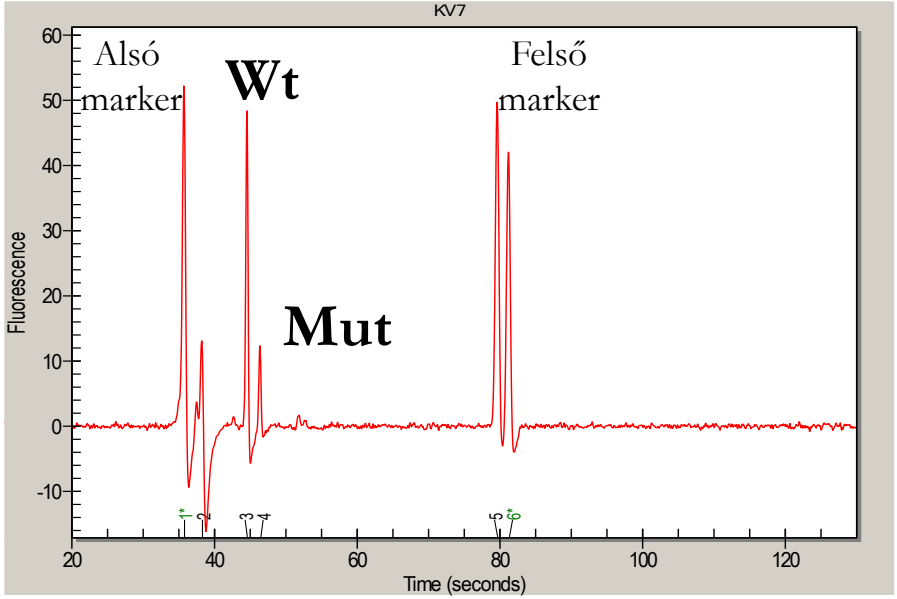
M + - **M**

RFMD (Restriction Fragment Microfluidic based Detection)

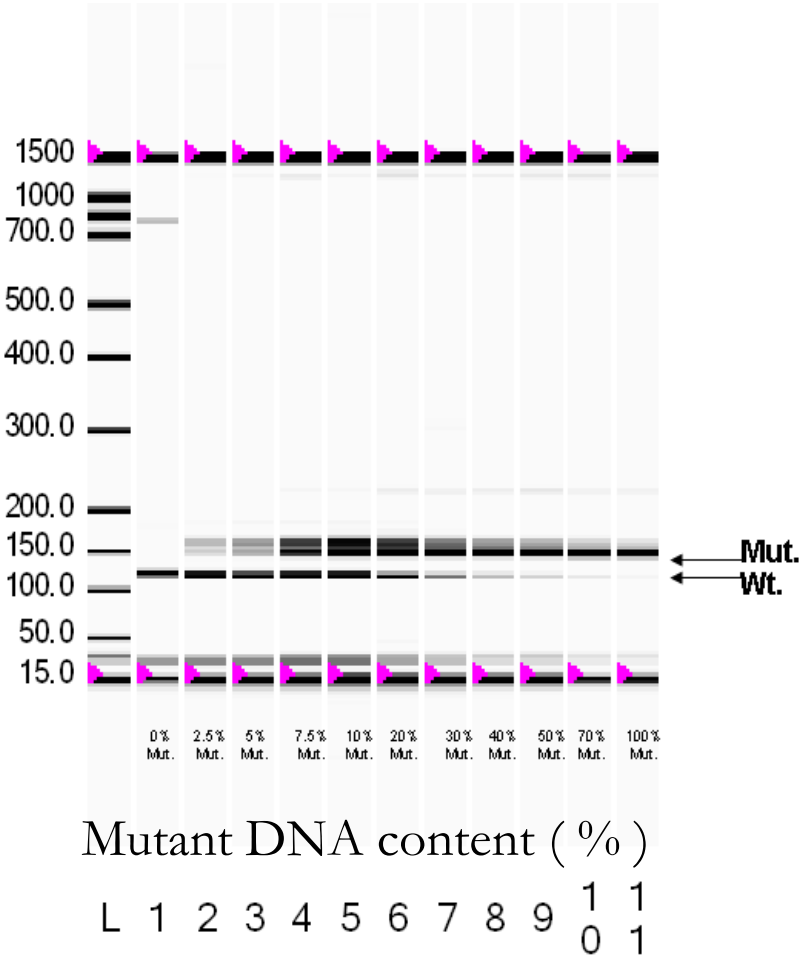
The flurescence labelled and microfluidic based detection is a sensitiv technology to detect the results of restriction fragments

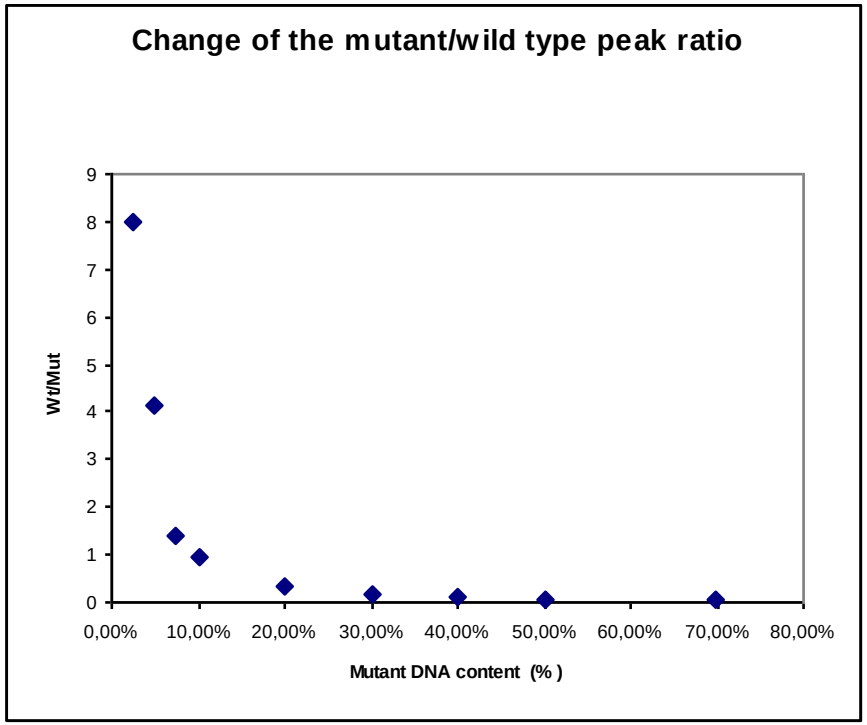
The system produces rapid, reproducible, and reliable separation of nucleic acid samples.

Run time: 40 min/11 samples



Sensitivity of the PCR (RFMD) method





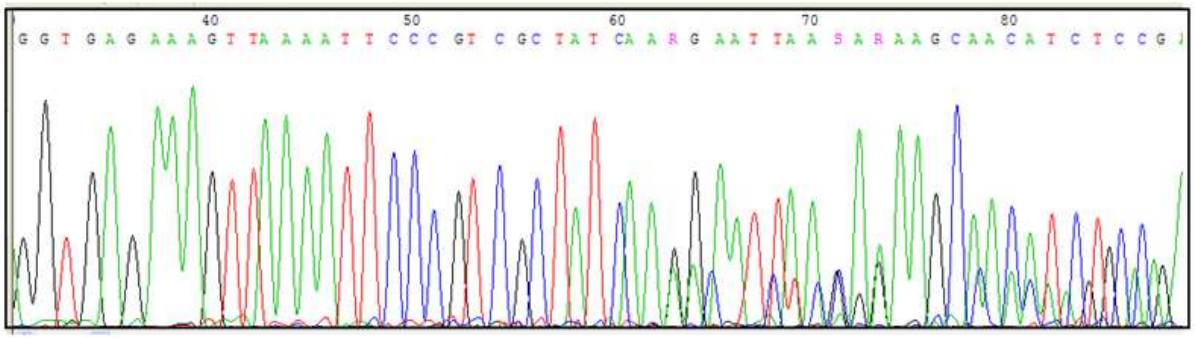
Dilution range was generated from K Ras mutant (H358) and wild type(LCLC) cell line to define the sensitivity of this PCR RFLP method. ***We were able to detect 2% mutant DNA with Experion electrophoresis system***

EGFR-HRM exon 18. 19, 20, 21 sequencing

EGFR mutáció kimutatása

A beteg 5% tumorsejtet tartalamzó citológiai mintájából, DNS-t izoláltunk és a PCR reakcióval történő szekvencia-specifikus felsokszorozást követően elvégeztük az EGFR 18, 19, 20 és 21-es exonjainak és azokat határoló intronszakaszoknak a bidirekcionális szekvenálását. A vizsgálati módszer érzékenysége 20%, specificitása 100%. A 19-es exont határoló intronszakaszban heterozigóta deléciót (del 4831886 - 4831892; ref[NT_033968.6) mutattunk ki.

Vélemény: A 19. és 20. exon közötti intronszakasz heterozigóta deléciós mutációját hordozó adenocarcinoma. **A talált elváltozás nem tartozik a bizonyított, EGFR tirozinkináz domént kódoló szakaszt érintő mutációk körébe.**



Budapest, 2010.05.05.

AIM of Molecular Pathology

To decide diagnostic and therapeutic questions
Hematology: e.g. Philadelphia chromosome (CML)
Soft tissue tumors

To define prognosis: n-myc copy Nr. in neuroblastoma

To define therapeutic TARGETS:
Breast cc.: HER-2 copy Nr. ⇒ HERCEPTIN treatment?
Lung/colon cc.: K-ras mutation ⇒ EGFR inhibitors
EGFR mutation

To identifiy infectious agents, typing:
e.g. HPV, Cytomegalovirus, Chlamydia pn., TBC, Helicobacter pylori – clarythromycin resistency

AIM of Molecular Pathology

To verify/confirm suggested diagnosis, genotyping, to investigate polymorphysmus

CML (Philadelphia chr.), lymphoma typing,

Wilson dis., Haemochromatosis dg.

MRD Minimal Residual Disease !! e.g. Breast cc.- bone marrow, hematological diseases – bone marrow

TOPICS in MOLECULAR PATHOLOGY

1. Lung cancer target therapy sensitvity
EGFR exon 19-21 mutation analysis
K-RAS exon 2, codon 12/13 mutation anaalysis
EML4-ALK fusion gene analysis
EGFR, MET copy number analysis (FISH)

2.Colon cancer target therapy sensitivity
K-RAS and B-RAF mutation analysis
EGFR exon-2-7 mutation anaylsis
EGFR copy number analysis (FISH)
MSI status determination: MLH1 and MSH2 inactivity (IHC)
EGFR, MET copy number (FISH)

3. Breast target therapy sensitivity
HER-2 copy number (FISH)
HER-2 ec-domain deletion test p95

4. Malignant melanoma target therapy sensitivity
B-RAF mutation anaylsis
N-RAS mutation anaylsis (codon 61)
C-KIT mutation anaylsis (exons)
EGFR, MET copy number (FISH)

TOPICS in MOLECULAR PATHOLOGY

5. Neuroblastoma - n-myc copy number

6. Genetic backgroung of kidney developmental disorders - Wilms tumor dg.
WT1 mutation anaylsis

8. Infectious diseases: detection and analysis
HP antibiotics resistency analysis FISH
HPV detectionn – typing (PCR)
. CMV, EBV, TBC detection

9. Hematopathology diagnostics:
e.g. Philadelphia chr. 9-22 transloc. BCL-ABL fusion gene
Polycythemia vera: JAK2 point mutatios
Primary myelofibrosis: JAK2 or MPL mutations
anaplastic large B cell lymphoma: ALK rearrangement
mantle cell lmyphoma: Cyclin D1-IgH fusion gene

10. Soft tissue tumor diagnostics:
Ewing sarcoma: FL1-EWS fsion gene
Liposarcoma: CHOP/TLS fusion gene
Clear cell src: EWS-ATF1 fusion gene

BASICS in MOLECULAR PATHOLOGY

- **Tumor Diagnostics**
- **Diagnostics of infectious agents**
- **Diagnostics of genetic diseases**

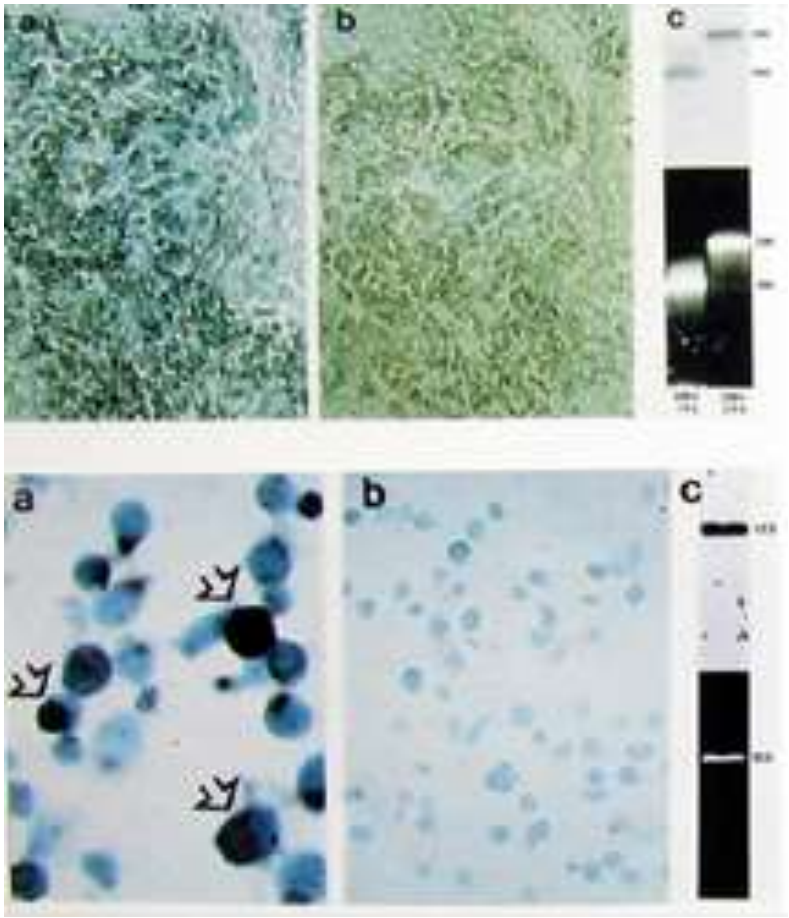
FDA approved clinical methods

Target	Módszer
Cytomegalovírus (CMV)	NASBA, Hybrid capture
Hepatitis C vírus (HCV) (kvalitatív)	TMA, PCR
Hepatitis C vírus (HCV) (kvantitatív)	bDNA
Human immunodeficiency vírus (kvantitatív)	bDNA, NASBA, RTPCR
HIV rezisztencia teszt	Szekvenálás
HBV/HCV/HIV szűrés véradóknál	TMA, PCR, RTPCR
Humán papilloma vírus (HPV)	Hybrid capture
Chlamydia trachomatis (CT)	Hybrid capture, TMA, HPA, PCR
Neisseria gonorrhoeae (NG)	Hybrid capture, TMA, HPA, PCR
Gardnerella, Trichomonas vaginalis, Candida	Hibridizáció
Streptococcus A	HPA
Streptococcus B	HPA, real-time PCR
Legionella pneumophila	SDA
Methicillin Resistant *Staphylococcus aureus*	real-time PCR
Mycobacterium tuberculosis	TMA, PCR

HPA Hybridization Protection Assay **TMA** transcription-mediated amplification
NASBA nucleic acid sequence-based amplification

MOLECULAR PATHOLOGY
in situ PCR

HBV in situ PCR



HIV detection

RT-in situ PCR

Hybridization

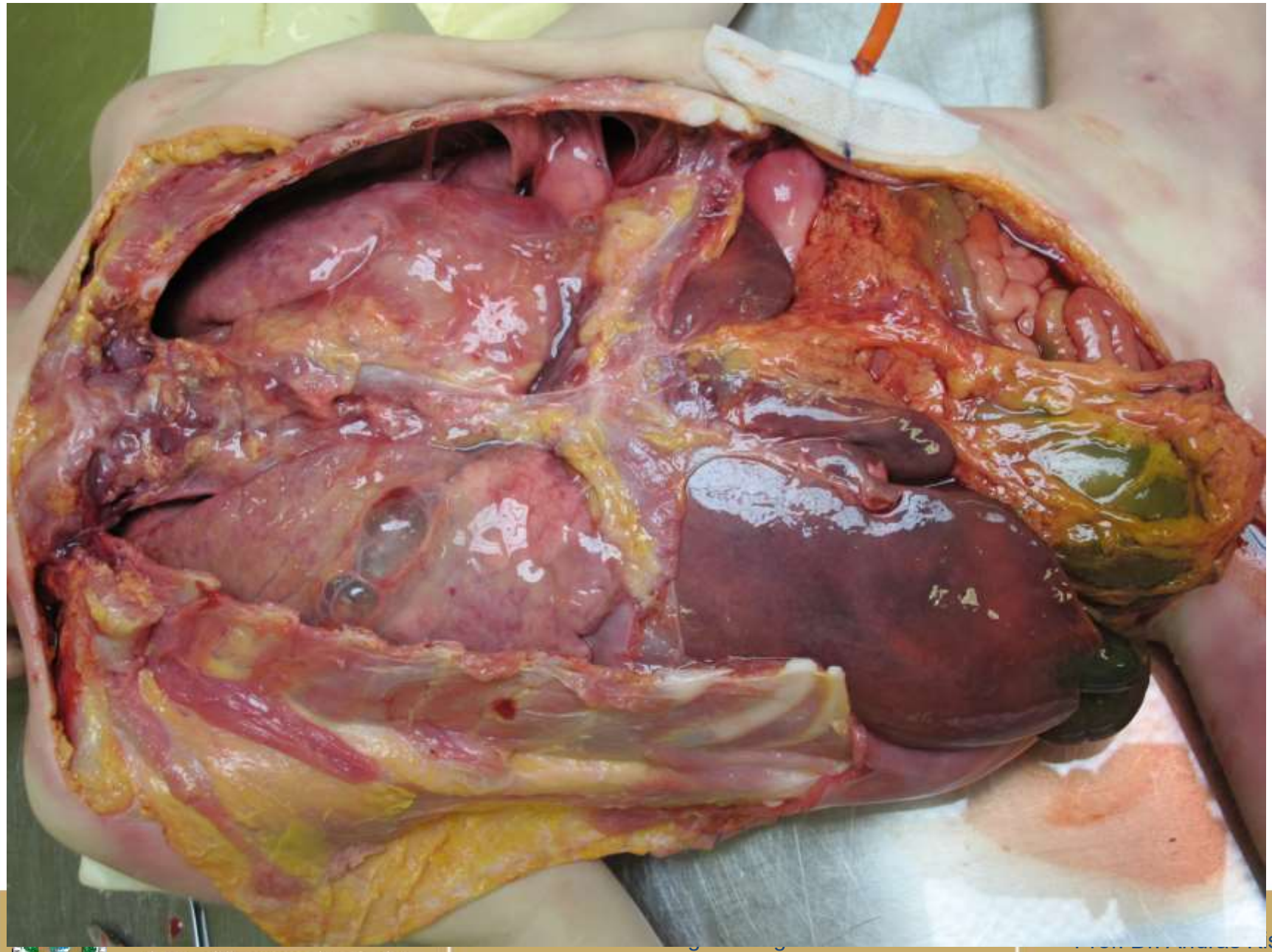
PCR

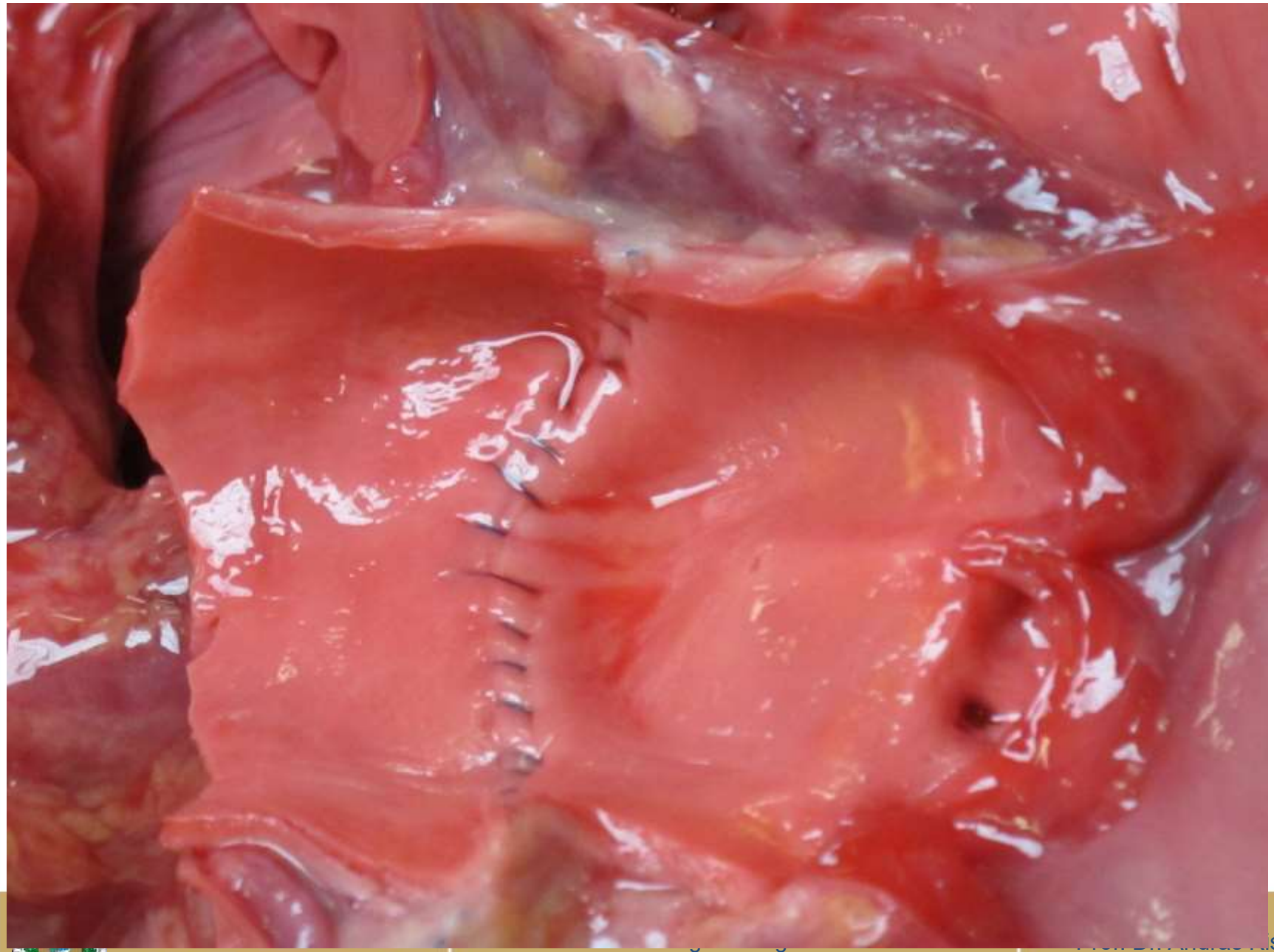
3 years old female

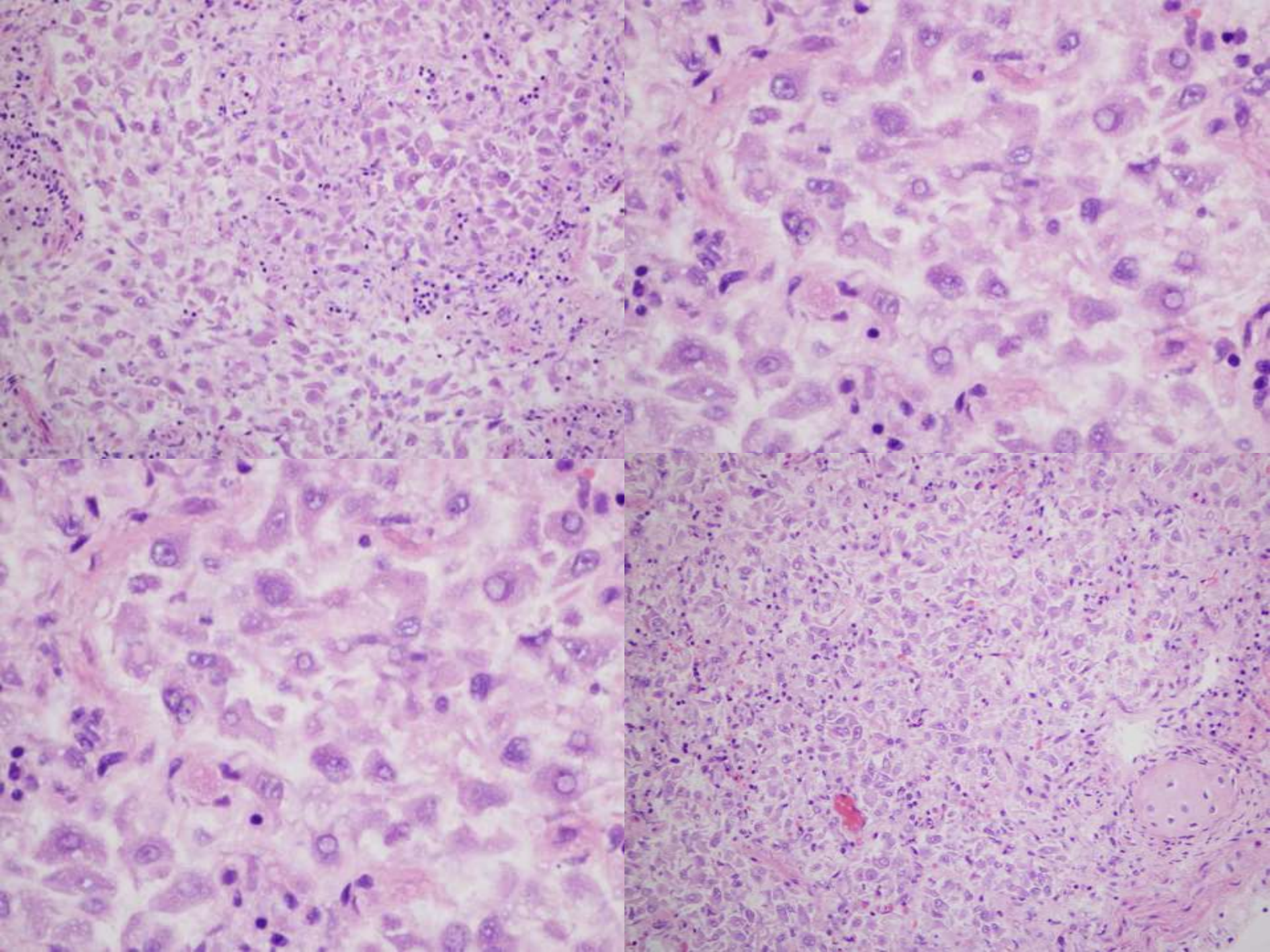
Heart TX : dilatative cardiomyopathy

Clinical diagnosis: Pneumonia
Lung fibrosis
CMV infection – sepsis
Arrhytmia
Respiratory insuffitiency

Pathology: Heart TX status post
Lung fibrosis
Pneumonia
ARDS







49508/10 bjk. LUNG TX

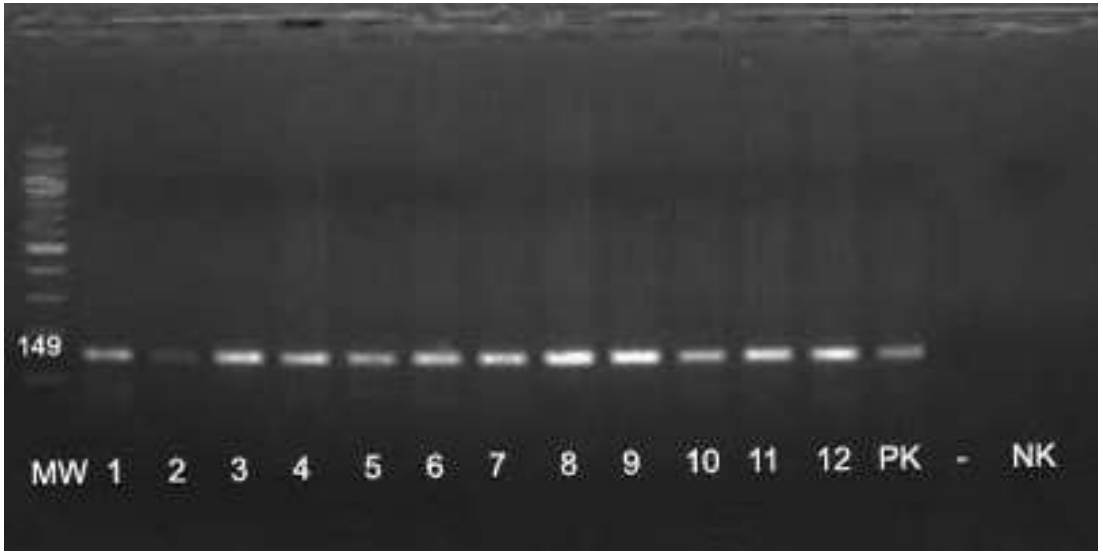
„Semi-sterile" samples :

1. Heart
2. Lung
3. Brain
4. Spleen
5. Liver
6. Adreanl gland
7. Kidney

„ Non sterile" samples

8. Lung
9. Liver
10. Adrenal gland
11. Spleen
12. Kidney

PK- positive control (sequenced verified CMV DNA)
NK- negative control

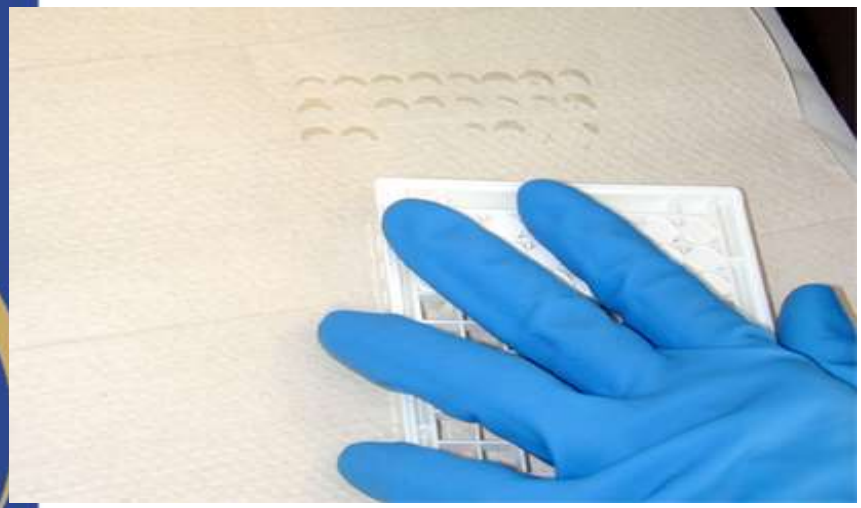


MOLECULAR PATHOLOGY

HPV genotyping

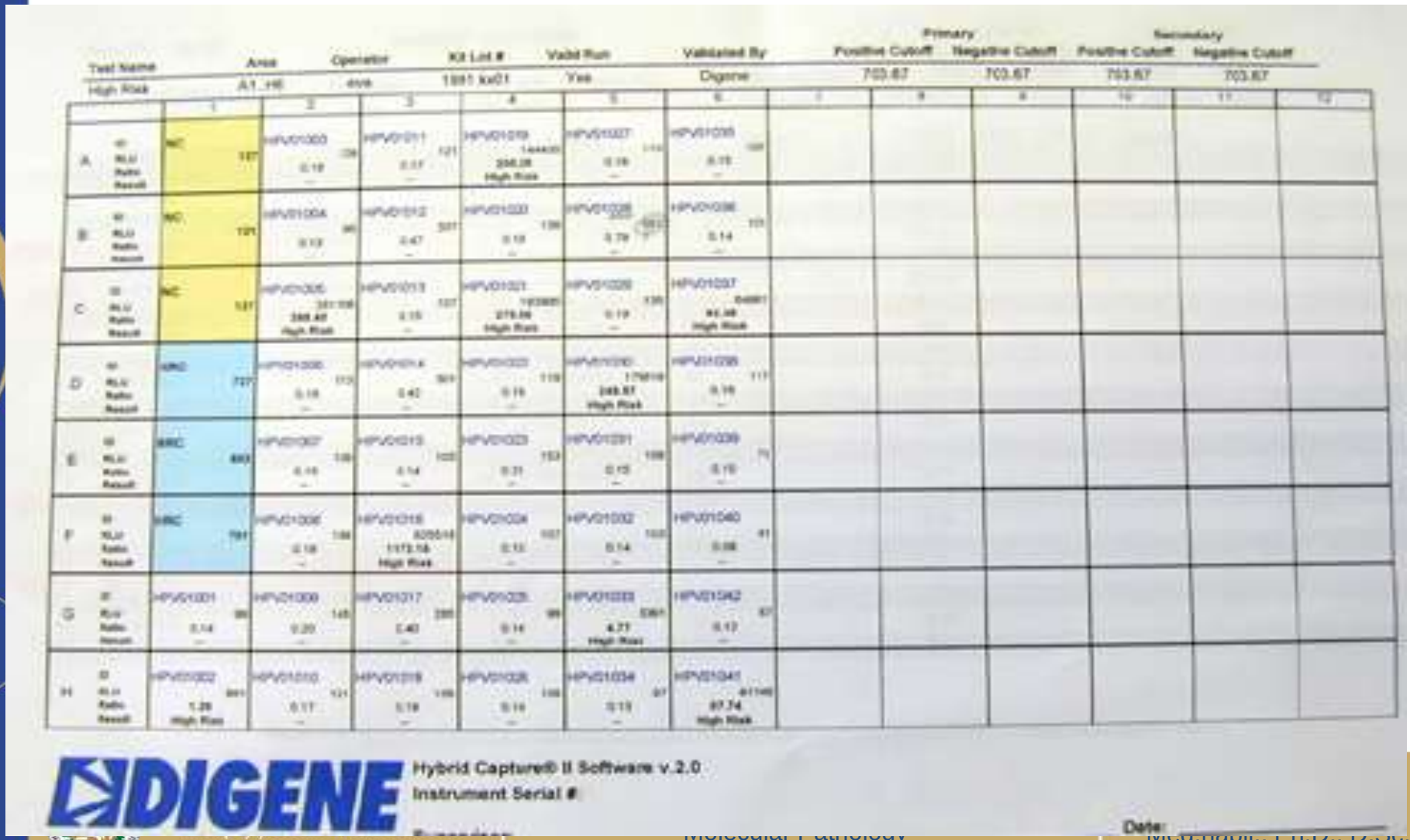
MOLECULAR PATHOLOGY

HPV genotyping



MOLECULAR PATHOLOGY

HPV genotyping



Test Name	Area	Operator	Kit Lot #	Valid Run	Validated By	Primary Positive Cutoff	Primary Negative Cutoff	Secondary Positive Cutoff	Secondary Negative Cutoff
High Risk	A1..H6	dvw	1091 kx01	Yes	Digene	703.67	703.67	703.67	703.67

	1	2	3	4	5	6	7	8	9	10	11	12
A	RLU / Ratio / Result: NC 137	HPV01003 0.18	HPV01011 121 0.17	HPV01019 144435 396.28 High Risk	HPV01027 0.19	HPV01035 0.15						
B	RLU / Ratio / Result: NC 121	HPV01004 0.13	HPV01012 0.47	HPV01020 0.18	HPV01028 0.79	HPV01036 0.14						
C	RLU / Ratio / Result: NC 131	HPV01005 331108 388.47 High Risk	HPV01013 0.15	HPV01021 192885 270.56 High Risk	HPV01029 0.19	HPV01037 84.48 High Risk						
D	RLU / Ratio / Result: NC 727	HPV01006 0.18	HPV01014 0.42	HPV01022 0.19	HPV01030 176816 248.57 High Risk	HPV01038 0.16						
E	RLU / Ratio / Result: NC 883	HPV01007 0.19	HPV01015 0.14	HPV01023 0.21	HPV01031 0.15	HPV01039 0.15						
F	RLU / Ratio / Result: NC 781	HPV01008 0.18	HPV01016 825514 1173.16 High Risk	HPV01024 0.12	HPV01032 0.14	HPV01040 0.08						
G	RLU / Ratio / Result: HPV01001 0.14	HPV01009 0.20	HPV01017 0.40	HPV01025 0.14	HPV01033 4.21 High Risk	HPV01042 0.13						
H	RLU / Ratio / Result: HPV01002 1.28 High Risk	HPV01010 0.17	HPV01018 0.19	HPV01026 0.16	HPV01034 0.19	HPV01041 97.74 High Risk						

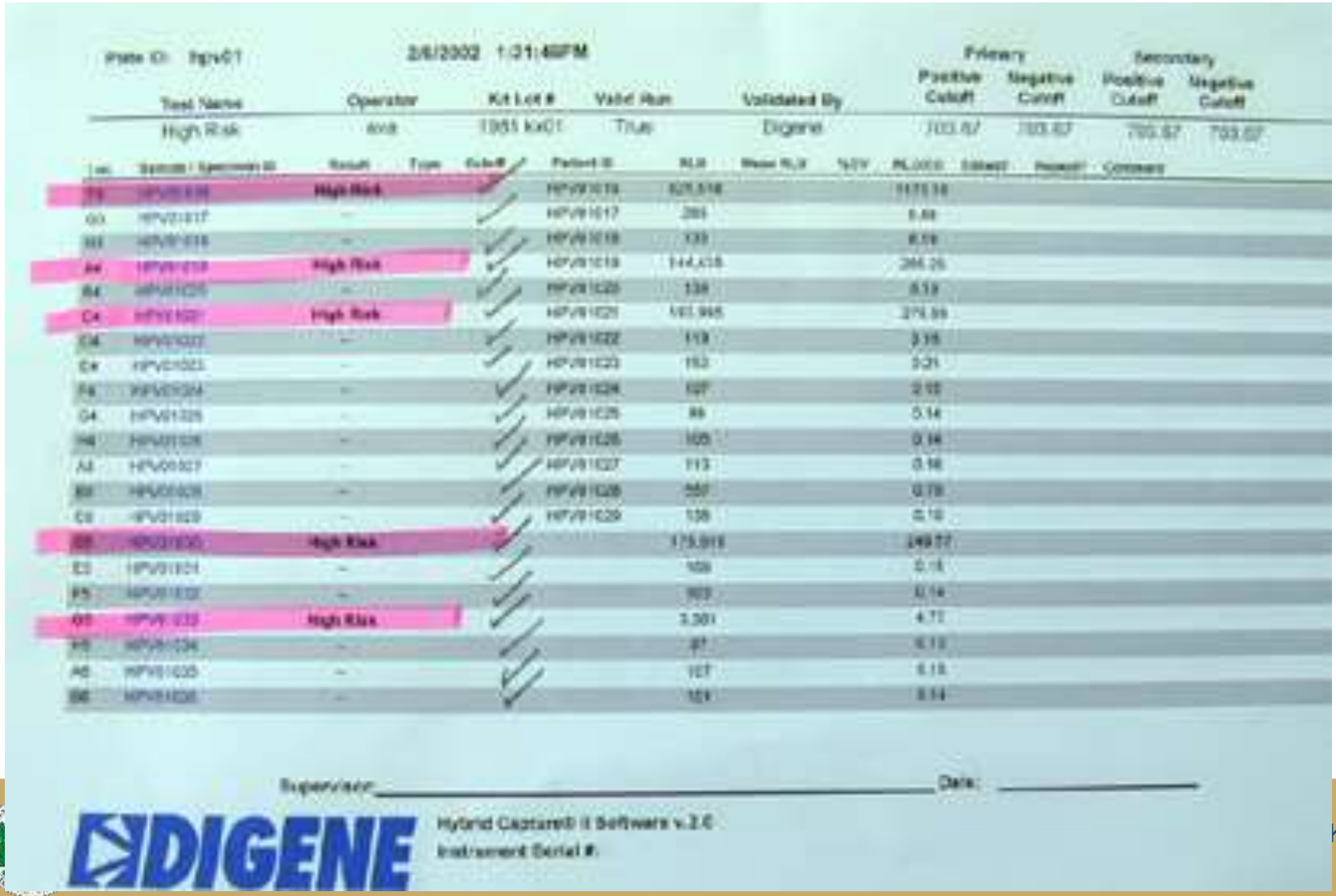
DIGENE Hybrid Capture® II Software v.2.0

Instrument Serial #:

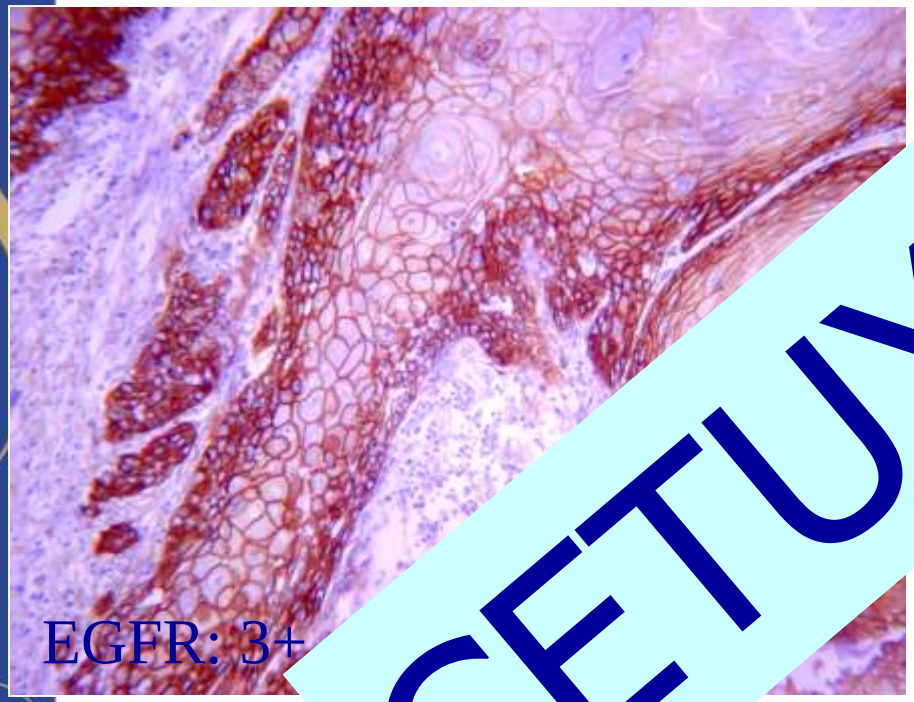
Date:

MOLECULAR PATHOLOGY

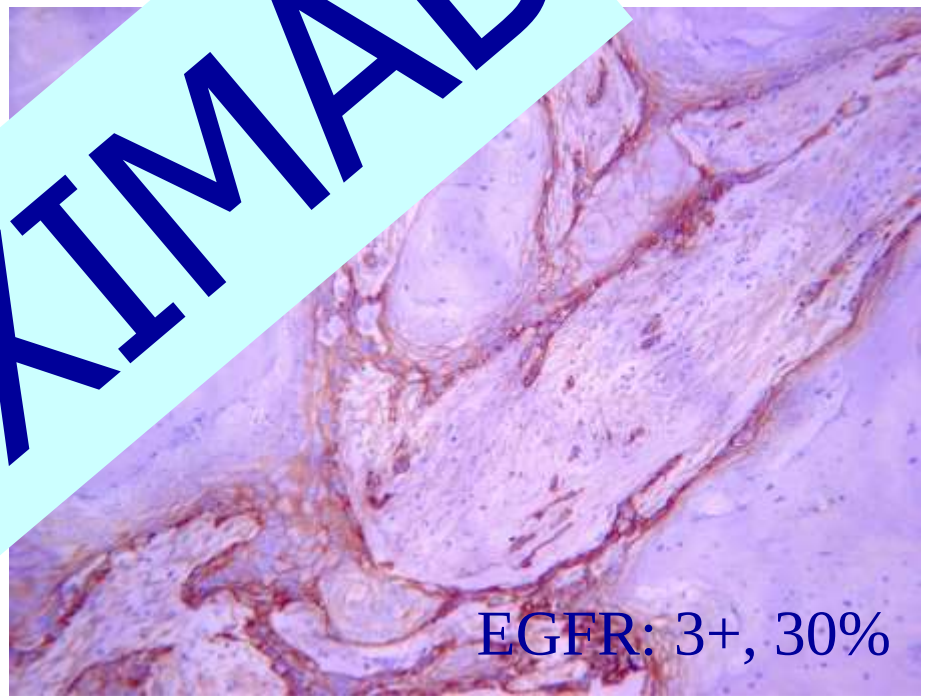
HPV genotyping



EGFR expression in head and neck squamous cell carcinoma (C NFIRM)

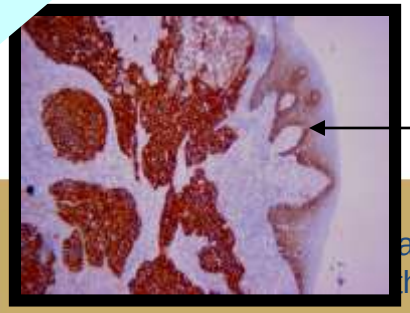


EGFR: 3+



EGFR: 3+, 30%

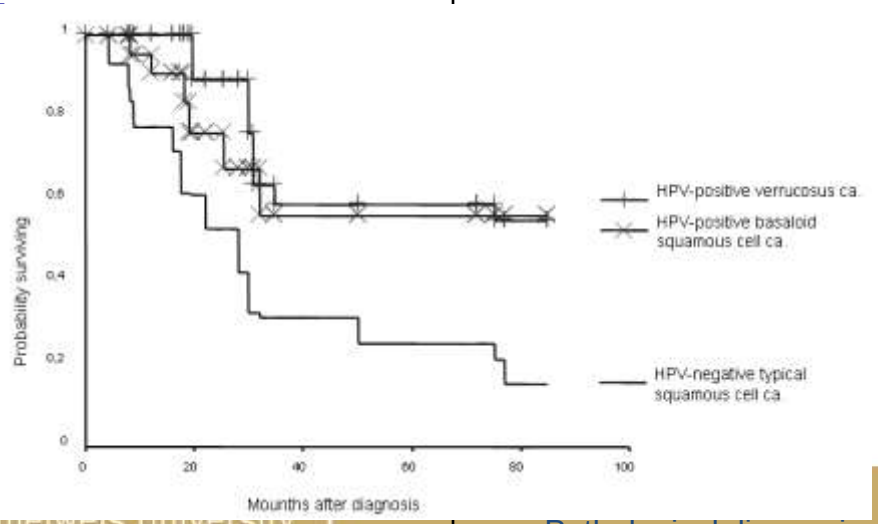
CETUXIMAB



Positive control

HPV and H&N SQCC

Localisation	HPV frequency (%)
Cervix	64.5
Penis	52.4
Oral cavity	48.5
Larinx	35.7
Esophagu	33.3
Cardia	37.0
Lung	16.0



Szentirmay et al. Canc Met Rev 2005

BASIS of MOLECULAR PATHOLOGY

- **Tumor Diagnostics**
- **Diagnostics of infectious agents**
- **Diagnostics of genetic diseases**

Diagnostics of Hereditary diseases with molecular biology methods

„Monogenic" diseases and detection by molecular biology methods

Fehérje típusa	Eltérés típusa	Példa	Gén (lokalizáció)	Mutáció típusa	Kimutatás módszere
Struktúr	Hemoglobinopátia	Sarlósejtes anémia	β-hemoglobin (11p15.5)	missense	Seq, PCR-RFLP
	Kötőszövet rendell.	Marfan szindróma	Fibrillin (15q21.1)	missense	Seq, LA
	Sejtmembrán-assz. feh. diszfunkció	Izomdisztrófia	Dystrophin, DMD (Xp12.2)	deléció	RFLP, MPCR, LA, SB
Sejtfelszíni receptor	Hiperkoleszterinémia	Familiáris hiperkoleszterinémia	LDL-receptor (19p13.2)	deléció, pontmutáció	Seq, Probe amplifikáció
	Anyagcsere	D-vitamin re-zisztens angolkór	D-vitamin receptor (12q12-q14)	pontmutáció	SB, RFLP, Seq
Regulátor	Fibróma	Neurofibromatosis 1 (von Recklinghausen)	Neurofibromin 1 (NF-1) (17q11.2)	missense, frameshift, SS	Seq, LA
	Fibróma	Neurofibromatosis 2 (von Recklinghausen)	Merlin TS (NF-2) 22q12	nonsense, frameshift, SS	LA
	Daganathajlam	Li-Fraumeni	p53 (TP53) 17p13	missense	Seq, SSCP, DGGE
Enzim	Anyagcsere rendellenesség	Alkaptonuria	Homogentizinsav oxidáz (3q21-q23)	missense, frameshift, SS	Seq, SSCP
		Fenilketonúria	PAH (PKU1)	missense, deléció, SS	Seq, ligase chain reaction
	Immundeficiencia	Severe combined immundeficiency	Adenosin deamiláz (20q13.11)	pontmutáció	Seq

SB = Southern blot, MPCR= multiplex PCR
LA = linkage analysis, Seq = szekvenálás

Mitochondrial gene mutations and related clinical syndroms

Mitokondriális gének mutációja következtében létrejövő elváltozások

Rendellenesség	Érintett gén	Kimutatás
Kearnes-Sayre szindróma	Del (2-7 kb)	PCR, PCR-RFLP, SB
Pearson szindróma	Del (?)	PCR-RFLP, SB
Pigmentált retinopátia	tRNS (tyr) deléció	PCR-RFLP, SB
Mitokondriális encefalo-miopátia, laktát acidózissal és sztrókszerű epizódokkal (MELAS)	tRNS (leu) pontmutáció	PCR-RFLP,Seq
Leber-féle opticus neuropátia (LHON)	Cyt6 és URF pontmutáció	PCR-RFLP
Neuropátia, ataxia, retinitis pigmentosa (NARP)	ATPáz VI pontmutáció	PCR-RFLP
MERFF szindróma	tRNS (lys) pontmutáció	PCR-RFLP
Leigh szindróma NARP-pal	ATPáz VI, NADH: ubiquinone oxido-reduktáz alegység mutáció	PCR-RFLP
Mitokondriális DNS depléció szindróma	Timidin kináz gén mutációja	PCR, Seq

Thank you for your attention !